Ragnhild Lyngved

Somatic embryogenesis in *Cyclamen persicum*

Biological investigations and
educational aspects of cloning

Thesis for the degree of Philosophiae Doctor

Trondheim, November 2008

Norwegian University of Science and Technology
Faculty of Natural Sciences and Technology
Department of Biology

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PREFACE AND ACKNOWLEDGEMENTS

The work presented in this thesis was carried out in 2000-2008 at the Plant Biocentre, Department of Biology, Norwegian University of Science and Technology (NTNU), the Plant Cell Laboratory, Department of Plant and Environmental Sciences, Norwegian University of Life Sciences (UMB) and the Centre de Recherche Public - Gabriel Lippmann (CREBS), Luxembourg. I took two years of maternity leave during this period (2004 and 2007). The project was financially supported by the Research Council of Norway (Project no. 132078/410 and 157603/440), the ALLFORSK Foundation and the Centre de Recherche Public - Gabriel Lippmann. I have also received support from my present employer, Sør-Trøndelag University College (HiST), the Faculty of Teacher and Interpreter Education, which has given me time to write and finish this thesis.

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Ragnhild Lyngved

TABLE OF CONTENTS

Preface and acknowledgements iii
Table of contents v
Abstract vi
List of papers viii
List of appendixes ix

1. INTRODUCTION 1

1.1 BACKGROUND 1
1.2 OUTLINE OF THE THESIS 4
1.3 CYCLAMEN PERSICUM MILL. 5
1.3.1 The genus Cyclamen 5
1.3.2 The species Cyclamen persicum Mill., florist's cyclamen 5
1.3.3 Commercial propagation of C. persicum 6
1.3.4 Embryogenic and non-embryogenic cell lines of C. persicum 7
1.4 SOMATIC EMBRYOGENESIS 8
1.4.1 Induction and development of somatic embryos 9
1.4.2 Bioreactors as a tool for propagation of somatic embryos 12
1.4.3 Gene and protein expression during somatic embryogenesis 15
1.4.4 2-D DIGE as a tool to study somatic embryogenesis 17
1.5 CLONING ~ A CONTROVERSY IN NATURAL SCIENCES 20
1.5.1 Norwegian students knowledge and interests in science 20
1.5.2 Natural sciences as products, processes and social institutions 21
1.5.3 Information, Communication Technologies (ICT) in natural sciences 22
1.5.4 Context-based approaches in natural sciences 22
1.5.5 Viten.no 24
1.5.6 The Viten unit "Cloning plants" 25
1.5.6.1 Aims of the teaching unit 25
1.5.6.2 Description of the teaching unit 25
1.5.6.3 The pedagogical framework behind the unit 29
1.5.6.4 The Viten unit "Cloning" 32

2. AIMS OF THE STUDY 33

3. SUMMARY OF RESULTS AND DISCUSSION 34

3.1 PAPER I: OPTIMIZING GROWTH CONDITIONS FOR PROEMBRYOGENIC MASSES IN BIOREACTORS 34
3.2 PAPER II: CHARACTERIZING EMBRYO-SPECIFIC PROTEINS IN *CYCLAMEN* 36
3.3 PAPER III: INTRODUCING THE ISSUE OF CLONING AND ITS SOCIETAL ASPECTS IN SCIENCE TEACHING 37

4. FUTURE PROSPECTS 39

5. REFERENCES 42

Papers I-III

Appendixes 1-9

ABSTRACT

Cyclamen persicum, one of the most important ornamental plants in the European market, is still propagated via seeds. This generative propagation poses some difficulties, and the ultimate aim is now to produce synthetic seeds via somatic embryogenesis. In order to meet the needs of industrial-scale clonal mass production, quantity and quality modifications of the production system are necessary.

In an attempt to improve the commercial propagation method for *C. persicum*, the influence of four potential growth factors on cell growth and cell viability in bioreactors has been explored. Mathematical models of the potential effects of oxygen concentration, daily mean temperature, the difference between day and night temperature (DIF), and daily light integral to the development of proembryogenic masses in bioreactors were developed.

An understanding of the mechanisms underlying the transition from a somatic cell into an embryogenic cell is also expected to be beneficial in developing more efficient procedures for plant regeneration. In an attempt to gain more information about cellular reorganization and signal transduction during the early stages of embryogenesis, total proteins from isogenic embryogenic and non-embryogenic *Cyclamen* cell lines were characterized. The extracted proteins were separated by two-dimensional differential gel electrophoresis (2-D DIGE) and protein identification was performed using MALDI-TOF-MS.

Somatic embryogenesis forms the basis of cellular totipotency and plant cloning. When it comes to understanding controversial scientific issues like cloning, knowledge of the societal aspects of scientific research is essential. Unfortunately these aspects may still be poorly represented in science teaching. In order to fulfil the responsibility associated with being a molecular biologist, a researcher and a teacher, a context-based digital teaching unit, “Cloning plants”, was designed to introduce the cloning issue and its societal aspects to secondary school students. The research laboratory and the work performed in the present study were used as the context. Norwegian biology students’ learning outcomes and the development of their interest in science from using the unit have been analysed.

The main results obtained and conclusions drawn in the thesis can be summarized as follows:

- Biomass growth, cell viability, and as a consequence embryo production, can be influenced markedly by varying the culture environment in batch cultures. The optimal values for biomass growth were 150% oxygen, 25°C, 1.11 mol m^{-2} day^{-1} and DIF + 10 for the bioreactor system used in this study. The optimal time for cell viability in the bioreactors was 11.3 days, with the following corresponding optimum values for the covariates: 90% oxygen, 20.8°C and 1.10 mol m^{-2} day^{-1}. There was no significant effect of DIF on cell viability. The optimal conditions for cell proliferation as the first step in development of embryos are probably those that give the highest viability (Paper I).

- More than 1200 *Cyclamen* proteins were detected; 943 proteins were common to embryogenic and non-embryogenic lines, of which 205 were differentially expressed, 128 were identified and 27 were proposed as candidates for embryo-specific proteins. The proteins identified were grouped into six functional categories based on their main biological process: cell proliferation (6% of identified proteins), protein processing (14.3%), signal transduction (6.0%), stress response (3.8%), metabolism and energy state (67.7%) and hypothetical function (2.3%). These proteins were discussed according to their functional categories and with regard to their role as metabolic components in the embryogenesis process (Paper II).

- Using the unit "Cloning plants" resulted in a more nuanced understanding of cloning and increased interest in cloning in students. About 80% of the students reported that the realistic context had a positive impact on learning about cloning and 60% reported that they had learned more about scientific research and societal aspects of research on cloning. Students also reported that the use of a context-based approach enhanced their interest in cloning (Paper III).

LIST OF PAPERS

I. Lyngved R, Snipen LG, Iversen T-H, Hvoslef-Eide AK (2008) Influence of potential growth factors on the production of proembryogenic masses of *Cyclamen persicum* Mill. in bioreactors. Sci Hortic 118: 53-59

II. Lyngved R, Renaut J, Hausman J-F, Iversen T-H, Hvoslef-Eide AK (2008) Embryo-specific proteins in *Cyclamen persicum* analysed with 2-D DIGE. J Plant Growth Regul (Online First) http://dx.doi.org/10.1007/s00344-008-9061-8

III. Lyngved R. Learning about cloning: the development of knowledge and interest through an interactive and context-based approach. NorDiNa – In press

Declaration of contribution

Contribution of co-authors:

Lars Gustav Snipen developed the mathematical models (Paper I), Tor-Henning Iversen commented on the manuscripts (Paper I, II), Anne Kathrine Hvoslef-Eide helped in planning the experiments and commented on the manuscripts (Paper I, II), Jenny Renaut helped in performing the 2-D DIGE analyses, did the protein identifications and commented on the manuscript (Paper II), Jean-François Hausman helped in planning the 2-D DIGE analyses (Paper II).

LIST OF APPENDIXES

Appendix 1: Hvoslef-Eide AK, Olsen OAS, Lyngved R, Munster C, Heyerdahl P (2005) Bioreactor design for propagation of somatic embryos. Plant Cell Tiss Org Cult 81: 265-276 (Basis of Paper I)

Appendix 2: Figure 3 and Supplementary Figure, 2-D DIGE (Elements of Paper II)

Appendix 3: Supplementary Table, 2-D DIGE (Element of Paper II)

Appendix 4: Procedure for starting the programme "Cloning plants": Lyngved R, Erlien W, Sørborg Ø (2004) Kloning av planter. http://www.viten.no (Basis of Paper III)

Appendix 5: Information letter to students and parents (Basis of Paper III)

Appendix 6: Questionnaires: Pretest and posttest (Basis of Paper III)

Appendix 7: Interview guide (Basis of Paper III)

Appendix 8: Transcribed interviews (Basis of Paper III)

Appendix 9: Pictures of students working through the teaching unit "Cloning plants" at the Resource Centre for Mathematics, Science and Technology Education at the Norwegian University of Science and Technology (Basis of Paper III)

1. INTRODUCTION

1.1 Background

Somatic embryogenesis is a model system for understanding the earliest developmental events in the life of a higher plant and it is a tool for clonal propagation. *Cyclamen persicum* Mill. (florist's *Cyclamen*) is currently propagated through F1-hybrid seeds, but due to difficulties regarding heterogeneity and high prices for seeds, the ultimate aim is to use somatic embryos. Ongoing research on *C. persicum* mainly concerns optimalization of this new commercial propagation method. Both quantity and quality modifications of the production system are necessary. Insight into the signalling pathways that are required to induce and develop somatic embryos could facilitate optimalization of the propagation process, but at the current time these pathways are far from being known. This doctoral study aims to improve the propagation method, by contributing new data on bioreactor cultivation and signal transduction, in the form of embryo-specific proteins.

As indicated above, research on *C. persicum* is partly governed by societal[1] requirements and wishes, in terms of meeting the demand for cheap seeds and homogenous plants. In addition, the unanswered questions regarding e.g. signalling pathways for embryogenesis illustrate the complexity and uncertainties that are an inseparable part of science at the frontier. In general, scientific uncertainties are not shared with or reported to the general public. As stated by May (2001), and also stressed by Kolstø (2003), Sjøberg (2004), and Osborne and Dillon (2008), the science that students encounter in school deals mainly with crisp certainties. As a participant in a doctoral degree programme, it was important for the present candidate to learn about and act on the various responsibilities involved with being a researcher. According to Fjelland (2003) and UFD (2005), scientists are responsible for revealing uncertainties associated with their results, and they should communicate these uncertainties to involved parties. The uncertainties connected to *Cyclamen* propagation do not have great commercial and societal importance, but illustrate that uncertainties exist in all research areas. Uncertainties connected to the propagation of other plants, particularly food plants such as genetically modified maize or soya, may have other and more important consequences for society. It is therefore important for the general public to be aware of the uncertainties at the scientific frontier.

[1] Throughout the document societal is used rather than social, similar to Ramsden (1994).

With the continuing development of modern biotechnology, the uncertainty aspect is even more important. In his textbook for doctoral students, Fjelland (1999) says that the minimum requirement of our new technological age is that uncertainty is communicated. According to Jonas (1997, 1999), new technologies in science represent a possible risk, and thus new ethical questions and a collective responsibility arise. Researchers in the field should therefore assume a special responsibility in communicating scientific uncertainties to the general public and to younger generations in particular, to enable them to participate in discussions and influence decision making processes (Fjelland 1999). Even though the cloning of ornamental plant species does not represent an ethically problematic area, it is part of the topical and controversial issue of *cloning*, of which new techniques and possibilities continually are debated. Techniques used for cloning plants are easy to illustrate, especially since plant cloning takes place in the wild as well as in laboratories and greenhouses. Plant cloning is thus a natural starting point for learning about cloning, the techniques used in cloning and the controversies that are linked to the general cloning debate. In the application of new technologies and when choosing the direction for future research, scientists and decision makers need to consult the general public to ensure that we have a broad debate prior to decision making (NFR 2004; Ravetz 2004; Myskja 2007; Regulations for the Norwegian Board of Technology[2]). Well-educated and informed citizens ensure a better and more informed debate and a better foundation for decisions.

As the links between science and society have grown, Ziman (1998) points out that scientists have to perform new roles in which ethical considerations can no longer be swept aside. In the report "Europe needs more scientists" (European Commission 2004), the High Level Group on Increasing Human Resources for Science and Technology recommend that scientists need to be trained in communication skills, not just to communicate with other scientists, but especially when communicating with non-scientists. It is also suggested by the Norwegian Government that communication with the general public should be an integrated part of doctoral programmes (UFD 2005). According to the Norwegian ethical guidelines for science and technology, issued by The National Committee for Research Ethics in Science and Technology (NENT 2007), researchers should use different means of communication to reach relevant target groups with information on research results. In

[2] http://www.teknologiradet.no/FullStory.aspx?m=6

addition, the Norwegian Ministry of Education and Research (KUF 1999, UFD 2005) wants a special emphasis on efforts addressed to children and youth. In school, teachers and textbooks should provide a nuanced and realistic picture of research and its consequences, influence and limitations. Furthermore, researchers should publish their results and the consequences of those results in a language that is understandable for non-experts (KUF 1999, UFD 2005).

Students often perceive science as too abstract, and the traditional established content-delivery model of teaching tends to distort student understanding of the nature of both science and knowledge by ignoring the methodological, reasoning and cultural aspects of science (European Commission 2004). Extended dialogue and direct contact between citizens and scientists, schools and research organisations, is necessary in order to promote scientific cultural literacy in society and to help citizens to obtain a better understanding of the role of science and technology in society (European Commission 2004). As pointed out in the Norwegian national curriculum (KD 2006a), science teachers should address all three dimensions of science: science as a product, science as a process and science as a social institution. Societal aspects are by far the most neglected, and are seldom addressed in available teaching materials. This aspect is especially important when it comes to understanding controversial issues such as cloning. The general public needs knowledge about the societal aspects of cloning technology because research activities have an influence on important societal areas such as politics and the economy, because its particular application to humans raises ethical controversies, and because the uncertainties connected to new technologies demand ethical consideration.

In the light of the guidelines and aspects presented above, the present study therefore included a didactic[3] section, directed towards students in upper secondary school. The aim of this didactic work has been to make the results obtained through the somatic embryogenesis studies presented here available to students, to introduce students to cloning technologies in general, to illustrate that science is a process, that new knowledge develops as a result of ongoing research, and that science has a societal dimension. This should prepare the students for participation in future public debates. Computer-based

[3] Here the word didactic is used in a Scandinavian/German tradition, meaning what, how and why to teach and learn, as used in Lijnse (2000) and Sjøberg (2001), as two examples. Traditionally the word didactic in English language has a different meaning.

technologies may help to increase students' understanding of and interest in difficult questions (Roschelle et al. 2000). Consequently, a computer-based interactive approach was chosen for a teaching unit called "Cloning plants", in which the results obtained from and addressed in the embryogenesis study are introduced to upper secondary school students.

1.2 Outline of the thesis

The following sections present a brief introduction of relevant elements in the thesis: background information on *C. persicum* (Section 1.3) and somatic embryogenesis (Section 1.4), educational aspects concerning natural sciences and the controversial issue of cloning (Section 1.5) and information on the "Cloning plants" teaching unit (Section 1.5). The three papers included in this thesis are presented in Chapter 3, with a summary of their results, discussions and conclusions. Different methods have been used in the three papers; hence the methodology is described in each paper. The thesis concludes with copies of the three main articles which form the scientific basis for the work.

1.3 Cyclamen persicum Mill.

1.3.1 The genus *Cyclamen*

The genus *Cyclamen* is traditionally classified in the family *Primulaceae*, but in recent years it has been reclassified in the family *Myrsinaceae*. With 22 species (Grey-Wilson 2003), the genus is relatively small; however, from a horticultural point of view it is an extremely attractive one. All species except one are well established in cultivation and most are easy to cultivate (Grey-Wilson 1988). The species are perennial herbs with a tuberous rootstock, nodding flowers and corolla lobes that are contorted in bud, but reflexed upon anthesis (Figure 1A) (Grey-Wilson 1988). They undergo an annual cycle of growth and rest, usually with the plant dying down to ground level for a part of the year.

Most *Cyclamen* have distinct climatic niches, with the exception of several wide-ranging, geographically expansive species (Yesson and Culham 2006). Their distribution in the wild is focused on the Mediterranean, from sea level to 2400 m (Grey-Wilson 1988). Estimates of the preferred climatic range for each *Cyclamen* species are given by Yesson and Culham (2006).

1.3.2 The species *Cyclamen persicum* Mill., florist's cyclamen

Cyclamen persicum is by far the most widely cultivated species of *Cyclamen* (Grey-Wilson 2003), and is an important ornamental crop in central Europe and Asia (Wiersema 1999). Roughly 200 million *Cyclamen* plants are produced annually on a worldwide basis, of which about 150 million are grown in Europe (Schwenkel 2001a). Cultivars can be brought into flower within nine months from seed, which is one of the secrets of their great success as house plants. None of the *Cyclamen* species flower in their first year in the wild, but take two or more years to do so. *C. persicum* is a tender species requiring frost-free conditions in which to grow and for this reason it is nearly always seen as a pot plant. *C. persicum* is also a very variable species in the wild, a characteristic that has been exploited by florists in the development of horticultural strains (Grey-Wilson 1988).

C. persicum is said to have first been cultivated at Lille in France around 1731, and the original plants apparently had white flowers. Philip Miller in his *Gardeners Dictionary (ed.*

8, no. 3, 1768) first used the epithet *persicum* in referring to the plant as the 'Persian *Cyclamen*' (Grey-Wilson 1988). *C. persicum* is not known from Persia (now Iran), but many plants coming to Europe from the East during the eighteenth century were thought to have originated in Persia. *C. persicum* holds a very isolated place in the genus. Wild forms of *C. persicum* always seem to have 2n = 48 chromosomes. However, cultivars of the species feature various polyploid levels, including 2n = 72, 96 and 136. The distribution of the species in the Mediterranean basin is very fragmented (Figure 1B), which suggests a species of some antiquity (Grey-Wilson 1988). Native species are found in Africa (Algeria, Tunisia), Asia (Cyprus, Israel, Jordan, Lebanon, Syria, Turkey) and Europe (Greece)[4].

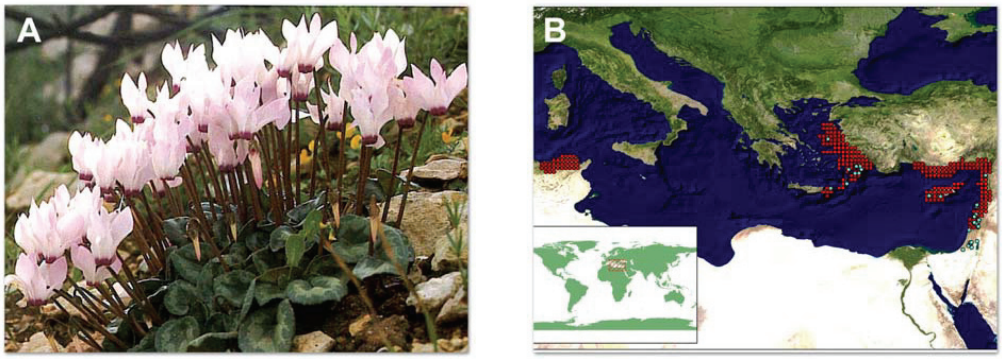

Figure 1 *C. persicum* in its most common form in the wild (A, © Grey-Wilson 2003) and its distribution data (B, © Yesson and Culham 2006). Distribution areas (red points) are extrapolated from the distribution map of Grey-Wilson 2003.

1.3.3 Commercial propagation of *C. persicum*

The cultivars of *C. persicum* are produced mostly through cross-pollination and not by vegetative means. In recent years the trend has been towards uniformity, and F_1 hybrid crosses are much in favour. F_1 hybrids can be repeatedly produced by crossing the same two purebred lines. The relatively few colours of large-flowered *Cyclamen* which are nowadays sold are the products of such crossings (Grey-Wilson 1988). The F_1-hybrid seeds are relatively expensive and sometimes not sufficiently uniform in production (Winkelmann et al. 2000). The high prices thus have facilitated research on an effective system for vegetative (clonal) propagation. Vegetative propagation has been attempted at a

[4] Germplasm Resources Information Network (GRIN) Taxonomy for plants, http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl/?12744

small scale, as there have been a number of obstacles (Geier et al. 1990). Lately, *in vitro* techniques have been established using somatic embryogenesis (Section 1.4) (Otani and Shimada 1991; Kiviharju et al. 1992; Kreuger et al. 1995; Takamura et al. 1995; Takamura and Tanaka 1996; Hvoslef-Eide and Munster 1998; Schwenkel and Winkelmann 1998), and the general aim is now to find a protocol for the production of synthetic seeds that can replace the generative propagation technique currently in use.

In order to match the needs of industrial-scale clonal mass production, quantity and quality modifications of the production system are necessary. To achieve this, suspension or bioreactor culture techniques have been attempted (Hvoslef-Eide and Munster 1998; Hohe et al. 1999a, b), but only to a very limited extent. More recently, the first experiments on desiccation of *Cyclamen* somatic embryos were published (Winkelmann et al. 2004a; Seyring and Hohe 2005). Additionally, early results from the germination of encapsulated somatic embryos (Winkelmann et al. 2004b) and new insights in alginate mixtures for cell encapsulation also exist (Donati et al. 2007), but so far there are still problems with desiccation tolerance (Seyring and Hohe 2005), low germination rates (Winkelmann et al. 2004b) and malformations (Schmidt et al. 2006). Comparative studies on embryo development and germination of zygotic and somatic embryos of C*yclamen* have therefore been conducted (Schmidt et al. 2006) to improve the production of synthetic clonal seeds.

1.3.4 Embryogenic and non-embryogenic cell lines of *C. persicum*

The cell lines of *C. persicum* used in this study were initiated from unpollinated ovules of flower buds from one individual plant of *C. persicum* 'Giganteum' Mill. cv. 'Purple Flamed' (genotype 3738), as described by Schwenkel and Winkelmann (1998). Within this primary culture, various types of callus developed from somatic tissue of the ovules, differing in colour and consistency. From these, one yellow, friable callus (12G) and one brownish, soft callus containing bigger aggregates (VIII) were selected and further subcultured (Winkelmann et al. 1998a, b). When the callus from line VIII was transferred to hormone-free medium, masses of somatic embryos in globular and later stages differentiated. This cell line has retained its embryogenic potency and is regarded as embryogenic. On the other hand, the 12G callus line has never shown any differentiation of somatic embryos, and when transferred to hormone-free medium, this callus continues growing. Line 12G must therefore be regarded as non-embryogenic.

The unique feature of the VIII and 12G cell lines is that they are isogenic (genotypically equal), and hence are derived from the same plant. When these two isogenic cell lines are given precisely the same conditions, they can e.g. be compared in a 2-D gel electrophoresis and the differences can be assigned to the release of their embryogenic potential. This is the closest one can get a controlled experiment in this circumstance, which is why the two cell lines were used in comparative studies in tissue culture laboratories across Europe in the COST 822 and 843 projects, as well as in this doctoral study.

1.4 Somatic embryogenesis

Somatic embryogenesis is defined as a process in which haploid or diploid somatic cells develop into differentiated plants through characteristic embryological stages without fusion of gametes (Williams and Maheswaran 1986). Somatic embryogenesis forms the basis of cellular totipotency that is unique to higher plants. The process can occur naturally and *in vitro*. *In vitro* somatic embryogenesis was first observed in carrot (*Daucus carota*) cell suspensions by Steward et al. (1958) and Reinert (1958). Since then, development of somatic embryos has been shown in a wide range of plant species (Williams and Maheswaran 1986). Somatic embryos are used as a tool for clonal propagation, but also for studying regulation of embryo development. An advantage is that the developmental process of the somatic embryos can be controlled and synchronised, allowing collection of embryos at specific stages. Large quantities of somatic embryos can be produced *in vitro*, making them more amenable to experimentation than their zygotic counterparts. Somatic embryos are also more accessible, and thus more suitable for studying gene expression, particularly in the early stages of embryogenesis (Dodeman et al. 1997). Embryogenic cultures are also an attractive target for genetic modifications. Carrot has remained the primary experimental system for studying somatic embryogenesis (Zimmerman 1993), along with alfalfa (*Medicago sativa*) (Giroux and Pauls 1996), barrel medic (*Medicago truncatula*) and thale cress (*Arabidopsis thaliana*) (Rose and Nolan 2006).

In angiosperms, zygotic embryogenesis begins with the zygote and finishes at the cotyledonary stage. This embryonic phase is crucial for plant development, as it is during this phase that meristems and the shoot-root body pattern are specified (von Arnold et al. 2002). In contrast to zygotic embryogenesis, somatic embryogenesis is an asexual

propagation process where somatic cells differentiate into embryos. Generally, the development of somatic embryos closely resembles that of zygotic embryos both morphologically and temporally, although somatic embryos develop completely outside both the physical constraints and the informational context of maternal tissue (Zimmerman 1993) (Figure 2). The lack of differentiation of endosperm and suspensor tissue in the case of the somatic system are clearly two elements that play a key role in bringing about the successful maturation of the embryo in zygotic embryogenesis (Dodeman et al. 1997).

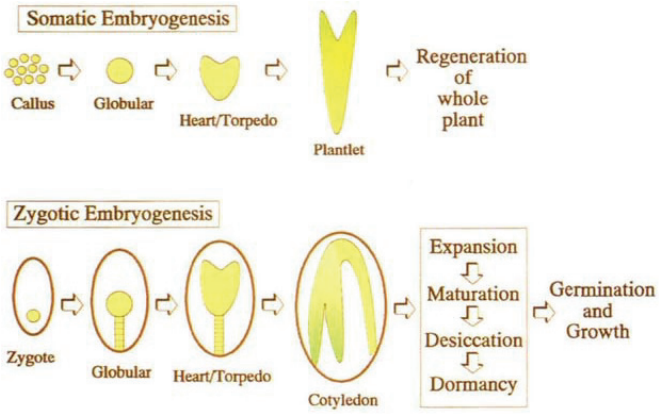

Figure 2 Comparison of somatic and zygotic embryogenesis. © Zimmerman (1993).

Schmidt et al. (2006) found that somatic embryo development in *C. persicum* was accomplished in only 3 weeks, compared to the 17 weeks total development period needed for zygotic embryos. The fact that somatic embryos are missing 14 weeks of development in the somatic pathway compared to their zygotic equivalent leads to a loss of physiological maturation. Moreover, a high proportion (86%) of developmentally aberrant somatic embryos were discovered (Schmidt et al. 2006). Thus, future investigations are necessary to improve the induction of maturity, and to identify and avoid the malformations.

1.4.1 Induction and development of somatic embryos

Somatic embryos can be induced either directly from the explant without an intervening callus phase or indirectly after a callus phase (Williams and Maheswaran 1986; Quiroz-Figueroa et al. 2006). Cells able to undergo embryo development generally appear as

proembryogenic masses (PEMs) (Halperin 1966). According to Williams and Maheswaran (1986), the embryogenic cells are small cells with dense cytoplasmic contents, large nuclei, small vacuoles, a profusion of starch grains, intense RNA synthesis and metabolic activity, all of which are common features for rapidly dividing meristematic cells. This cell morphology may serve as an early marker of embryogenic competence (Fehér et al. 2003).

Embryo development consists of several stages; the globular, heart, torpedo, and cotyledonary stages, and eventually the mature dehydrated embryo (Figure 2). To initiate these stages *in vitro*, regeneration includes five steps (von Arnold et al. 2002), starting with the formation of PEMs, followed by somatic embryo formation, maturation, desiccation and plant regeneration, as follows:

1. **Initiation** of embryogenic cultures by culturing the primary explant on medium supplemented with plant growth regulators (PGRs), mainly auxin but often also cytokinin.
2. **Proliferation** of embryogenic cultures on medium supplemented with PGRs, similar to initiation.
3. **Prematuration** of somatic embryos in medium lacking PGRs; this inhibits proliferation and stimulates somatic embryo formation and early development.
4. **Maturation** of somatic embryos by culturing on medium supplemented with ABA and/or reduced osmotic potential.
5. **Development** of plants on medium lacking PGRs.

Some cells require exposure to exogenous auxin before they are competent to undergo embryogenesis (Zimmerman 1993), but the requirement for auxin or other PGRs is largely determined by the developmental stage of the explant tissue (von Arnold et al. 2002). Once embryogenic cells have been formed, they continue to proliferate, forming PEMs. In general, exogenous auxin is required for the proliferation of PEMs and inhibitory for the development of PEMs into somatic embryos (de Vries et al. 1988). Thus, the removal or decrease in auxin is the trigger of somatic embryo development (Zimmerman 1993), although there are cases where auxin and cytokinin stimulate germination. The involvement of endogenous hormones and PGRs on *in vitro* somatic embryogenesis has been reviewed by Jiménez (2005).

In some species, it is necessary to treat the embryogenic cultures with ABA to stimulate maturation. This is especially important for conifers (von Arnold et al. 2002). During the maturation stage, the storage organs expand, storage products are accumulated and desiccation tolerance is acquired (von Arnold et al. 2002). The quality of the final plants is affected by the treatments given during the *in vitro* phase and during the *ex vitro* establishing phase (Högberg et al. 2001). An understanding of the mechanisms underlying the transition from a somatic cell into an embryogenic cell is also expected to be beneficial to develop more efficient procedures for plant regeneration (Mordhorst et al. 1997). According to Thibaud-Nissen et al. (2003), the arrangement of new cells into organized structures might depend on a genetically controlled balance between cell proliferation and cell death. Helmersson (2007) confirm that programmed cell death is required for embryo differentiation, and hypothesises that genetically aberrant PEMs or embryos are eliminated by programmed cell death. The procedure for *Cyclamen* somatic embryogenesis used in this doctoral study is shown in Figure 3.

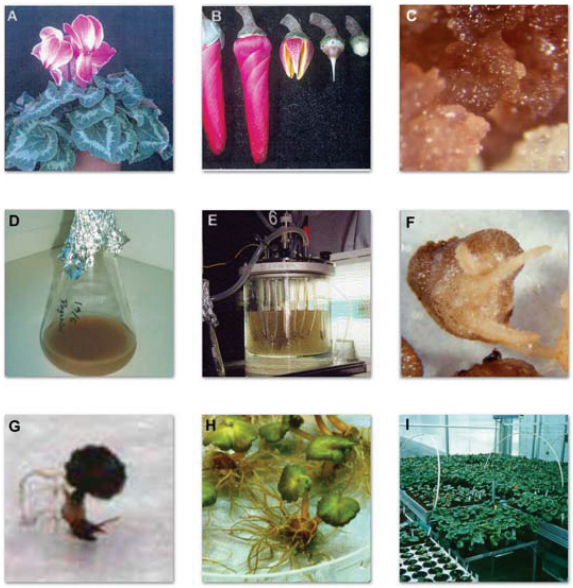

Figure 3 Plant regeneration of *C. persicum* (A) via somatic embryogenesis. A selected *C. persicum* 'Giganteum' Mill. cv. 'Purple Flamed' (genotype 3738) (A) plant was used for the induction of callus cultures. Unpollinated ovules of flower buds (B) turned out to be the most suitable type of explant (Winkelmann et al. 2000), and these were grown on medium containing PGRs, auxin and cytokinin, to form an embryogenic cell line (C). In the presence of PGRs this cell line was proliferated as PEMs in suspension cultures (D) and, more effectively, in bioreactors (E). After withdrawal of PGRs, somatic embryos differentiated, matured, germinated (F) and regenerated into plantlets (G, H, I).

1.4.2 Bioreactors as a tool for propagation of somatic embryos

Bioreactors are usually described in a biochemical context as self-contained, sterile environments that capitalise on liquid nutrient or liquid/air inflow and outflow systems (Paek et al. 2005). In recent years, liquid culture systems based on shoot cultures or somatic embryos have become of increasing interest to commercial micropropagators for some stages of the plant propagation cycle (Hvoslef-Eide and Preil 2005). Commercial plant propagation laboratories have demonstrated that liquid cultures save costs and improve product quality compared with semisolid media culture (Aharoni 2002). Suspension cultures give higher proliferation rates and more synchronized cultures (von Arnold et al. 2002).

For large-scale production of somatic embryos, a bioreactor is one of the most promising ways for scaling-up the system (Ibaraki and Kurata 2001; Gupta and Timmis 2005; Paek et al. 2005). Today a relatively large number and variety of bioreactor systems are available, allowing a rational selection of an appropriate reactor for a given process. The complexity varies from simple devices supplying an arbitrary amount of oxygen, to complex computer-controlled bioreactors that allow for accurate monitoring and control over microenvironmental conditions (agitation, aeration, temperature, pH, gas concentration in headspace or dissolved in the medium, etc). On the basis of mode of operation, a bioreactor may be classified as batch, fed batch or continuous (van't Riet and Tramper 1991) (Figure 4):

- **Batch**: Inoculum is added to fresh medium, growth proceeds without supplementation. No in- and outgoing flows. All that is produced is accumulated.
- **Fed-batch**: Growth medium is added at various intervals, usually to prolong the log phase. Only an ingoing flow. All that is produced is accumulated.
- **Continuous**: Growth medium is added throughout the run, cells and spent medium are simultaneously removed. The inflow equals the outflow.

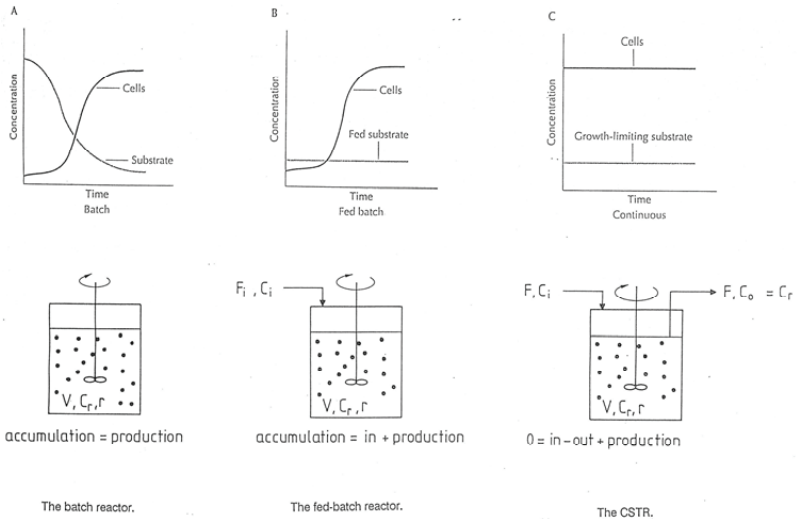

Figure 4 Principles of growth: (A) Batch, (B) Fed-batch, (C) Continuous. © Glick and Pasternak (2003) (progress curves), van't Riet and Tramper (1991) (mass balances).

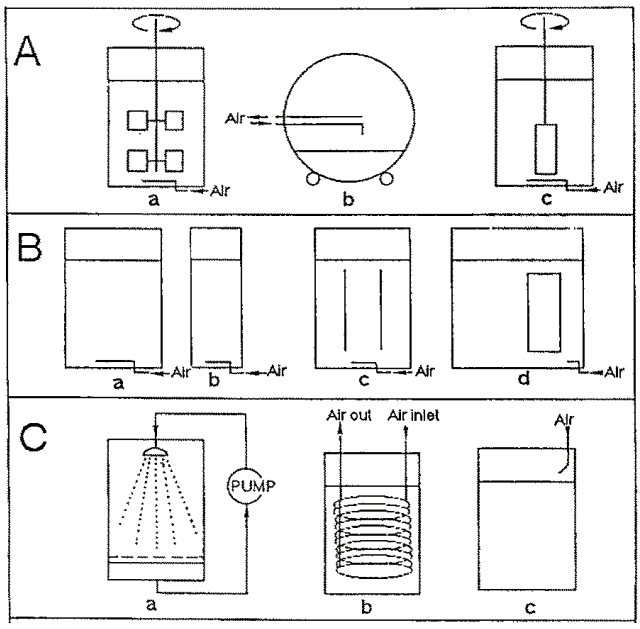

Figure 5 Different types of bioreactors for plant cell, tissue and organs. (A) Mechanically agitated bioreactors, a: aeration-agitation, b: rotating dram, c: spin filter. (B) Pneumatically agitated (air-driven) bioreactors, a: simple aeration, b: bubble column, c: draft tube, d: external loop. (C) Non-agitated bioreactors, a: gaseous phase, b: oxygen permeable membrane aerator, c: overlay aeration. Modified after Takayama and Akita (1994).

Bioreactors for plant cell cultures can be classified by agitation methods and vessel construction (Takayama and Akita 1994) (Figure 5) into:

- **Mechanically agitated bioreactors** (aeration-agitation reactors, rotating drums and spin-filter reactors)
- **Pneumatically agitated (air-driven) bioreactors** (simple aeration reactors, bubble column reactors and air-lift reactors)
- **Non-agitated bioreactors** (gaseous phase (mist) reactors, oxygen permeable membrane reactors, overlay aeration reactors)

The bioreactors on the market have been designed to provide optimal growth for bacteria, yeast and to some extent plant cell cultures for secondary metabolite production. Compared to microbial cells, plant cells are large, tend to form clumps, grow slowly, have a lower oxygen demand and low shear stress tolerance (Taticek et al. 1991) and thus have other requirements for cultivation. In particular, gentle agitation and aeration are important to provide minimum shear forces and oxygen stress. When growing plant cells for production of somatic embryos in bioreactors, the effect of shear forces is more critical than when dealing with cell cultures for secondary metabolites. The challenges of plant propagation in bioreactors have been described in detail by Heyerdahl et al. (1995).

Propagation of somatic embryos in bioreactors is favourable since the embryos are relatively small and uniform in size, and do not require cutting into segments and individual implanting onto media during proliferation. In addition, somatic embryos offer the potential for long-term storage through cryopreservation (Winkelmann et al. 2004c; Gonzalez-Arnao et al. 2008) or desiccation (Winkelmann et al. 2004a; Seyring and Hohe 2005), which facilitates flexibility in scheduling production and transportation and therefore fits large-scale production (Paek et al. 2005). Production of somatic embryos in bioreactors has been reported for a number of species (Paek et al. 2005; Mehrotra et al. 2007), but in commercial use, conifers are the only economically important crop plants for which this technique has been applied (Sutton 2002; Lippert et al. 2005). Many improvements are still needed for practical automated production systems, both mechanical progress and improvements of biological processes, for example with regard to environmental factors in bioreactors (Preil 2005), synchronisation of embryo development, identification of embryo abnormality and overcoming difficulties in acclimatisation

(Ibaraki and Kurata 2001; Paek et al. 2005). Temporary immersion systems, an alternative to bioreactors, are supposed to play a dominant role in future micropropagation of plants (Preil 2005).

The bioreactors used in this doctoral study are batch reactors, which are the most frequently used type of reactors in biotechnological productions (Siebel 1992; Glick and Pasternak 2003). Our batch reactors are self-designed aeration-agitation bioreactors, which are described in detail in Hvoslef-Eide et al. (2005) (Appendix 1). *Cyclamen* may represent a more sensitive species with regard to shear forces and oxygen stress (Hvoslef-Eide 2000). Aeration in our reactors is thus provided via diffusion through silicon tubes, to eliminate the negative effects of air bubbling (foam formation and oxygen stress). The stirring device can be regulated in both speed and direction, which avoids high speed, and thus shear forces are minimized. These two features make our bioreactors gentler for plant cells than various commercial designs.

In this doctoral study, bioreactors and mathematical models were used with the aim of finding the optimal combination of growth factors for the production of large numbers of high-quality proembryogenic masses of *C. persicum*. The existence of six identical reactors provided the possibility to run factorial experiments to uncover interactions between the experimental parameters. By using mathematical models, many dimensions of the cultivation process could be illustrated at the same time. The estimation of optimal conditions from mathematical models may provide insights that can improve the quantity and quality aspects of the production system.

1.4.3 Gene and protein expression during somatic embryogenesis

Several studies have dealt with genetic regulation or changes in protein pattern during somatic embryogenesis, and gymnosperms, angiosperms, monocotyledonous and dicotyledonous plants have been examined, e.g. spruce (Dong and Dunstan 1996a; Lippert et al. 2005; von Arnold et al. 2005), cypress (Sallandrouze et al. 1999), maize (Franz et al. 1989), wheat (Singla et al. 2007), barley (Nielsen and Hansen 1992), rice (Chen and Luthe 1987), orchardgrass (Hahne et al. 1988; Alexandrova and Conger 2002), thale cress (Rose and Nolan 2006), carrot (Fujimura et al. 1980; Sung and Okimoto 1981; de Vries et al. 1988; Hendriks and de Vries 1995; Ko and Kamada 2002), alfalfa (Giroux and Pauls 1996;

Poulsen et al. 1996), barrel medic (Imin et al. 2005; Rose and Nolan 2006), pea (Stirn and Jacobsen 1987), cowpea (Nogueira et al. 2007), sugarcane (Blanco et al. 1997), orange (Gavish et al. 1991), grapevine (Coutos-Thevenot et al. 1992; Marsoni et al. 2008) and birch (Hvoslef-Eide and Corke 1997). It has been estimated that about 3500 different genes are necessary to complete embryo development (von Arnold et al. 2002). However, the signalling pathways, the genes and the proteins that are required to induce and develop somatic embryos are not well defined.

It is generally believed that in the continued presence of auxin, the PEMs in the culture synthesise all the gene products necessary to complete the globular stage of embryogenesis and that the PEMs also contain many other mRNAs and proteins whose continued presence generally inhibits the elaboration of the embryogenesis program. The removal of auxin results in the inactivation of a number of genes as well as synthesis of new gene products, such that the embryogenesis program can now proceed (Zimmerman 1993). Hence, there are clearly opportunities to link the somatic embryogenesis induction genes to hormone inductive requirements, but stresses induced by the preparation and plating of the explant remain important considerations (Fehér et al. 2003). The nature of the connection between stress and the applied hormones needs further understanding (Rose and Nolan 2006). There is also evidence that secreted, soluble signal molecules play an important role in the control of cell differentiation during somatic embryogenesis (de Vries et al. 1988; Gavish et al. 1991; von Arnold et al. 2002).

Numerous genes and proteins have been identified as specifically expressed during somatic embryogenesis, as reviewed by Mordhorst et al. (1997), Chugh and Khurana (2002), Fehér et al. (2003) and Rose and Nolan (2006). Genes have been categorized according to their functions into housekeeping genes, hormone-responsive genes, signal transduction genes, homeobox genes, maturation genes and genes that code for extracellular proteins (Chugh and Khurana 2002). Among the genes and gene products studied are e.g. WUSCHEL (Zuo et al. 2002), BABY BOOM (Boutilier et al. 2002) and LEAFY COTYLEDON genes (Harada 2001), somatic embryo receptor-like kinases (SERKs) (Schmidt et al. 1997; Hecht et al. 2001; Hu et al. 2005), late embryogenesis abundant (LEA) proteins (Zimmerman 1993), heat shock proteins (HSPs) (Pitto et al. 1983; Zimmerman et al. 1989; Györgyey et al. 1991; Dong and Dunstan 1996b), germins (Chugh and Khurana 2002), GTP-binding proteins (Dudits et al. 1995), lipid transfer proteins (LTPs) (Hendriks and de Vries 1995),

arabinogalactan proteins (AGPs) (von Arnold et al. 2002; Quiroz-Figueroa et al. 2006), chitinases and peroxidases (Hendriks and de Vries 1995; von Arnold et al. 2002), to name a few. Studies involving these genes and proteins suggest these are part of a signalling cascade, whose connections are yet to be identified, which lead to embryo formation from somatic cells.

Embryo defective mutants provide an important resource, as do RNAi gene silencing strategies to investigate the role of individual genes (Rose and Nolan 2006). Proteome analysis can be used to increase information on the proteins connected to embryogenesis induction. Increasingly, overlap between stress, defence and development are seen. Fehér et al. (2003) hypothesise that several regulators play key roles through coordinated interactions with hormonal, environmental and developmental signalling pathways. These connections related to signalling pathways might be resolved with the use of microarrays (Rose and Nolan 2006).

Presently, there is limited information about biochemical changes during *Cyclamen* somatic embryogenesis. Recently, Rensing et al. (2005) identified approximately 90 candidate genes that influenced the somatic embryogenesis in *Cyclamen* using EST sequencing, while Winkelmann et al. (2006) have performed proteomic analyses of somatic and zygotic embryos. So far there are no reports available on the comparison between an embryogenic and a non-embryogenic cell line of *Cyclamen*, neither at the level of gene expression nor at the proteome level. Since the PEMs seem to express many of the genes involved in the earliest stages of embryogenesis, PEMs are important to include if one is about to study potentially useful molecular markers for early events in embryogenesis. Comparing gene expression in embryos to that of callus cells, the most common approach to identify somatic embryogenesis related genes (Fehér et al. 2003), will likely eliminate many gene sequences that could represent useful markers (Zimmerman 1993). The isogenic cell lines used in this doctoral study, including one non-embryogenic line, thus represent a useful tool for studying the early events in somatic embryogenesis.

1.4.4 2-D DIGE as a tool to study somatic embryogenesis

Two-dimensional difference gel electrophoresis (2-D DIGE) is an advanced version of classical two-dimensional gel electrophoresis (2D-PAGE) and was first described by Ünlü

et al. (1997). In general, 2-D electrophoresis is widely and increasingly used in proteomics, due to its unparalleled ability to separate thousands of proteins simultaneously (Pennington et al. 1997). The technique is also unique in its ability to detect the level of protein expression, the protein isoforms and the extent to which protein(s) are post-translationally modified, which cannot be predicted from the genome sequence (Pennington et al. 1997; GE Healthcare 2004). Cellular reorganization requires modification and/or removal of unnecessary polypeptides, as well as the proper folding of newly synthesized proteins and protein complexes (Fehér et al. 2003). Signal transduction involves post-translational modifications and protein-protein interactions. Hence, proteomics is a powerful approach for studying signal transduction and cellular reorganization involved in somatic embryogenesis. Because the proteome reflects the expression of the molecules that more directly influence cellular biochemistry, this provides a more accurate representation of cellular state than profiling the expression of mRNAs (Rose et al. 2004).

2-D DIGE was developed to facilitate a direct and reproducible comparison between mixtures of proteins. Its main advantage over the current 2-D PAGE technique is its ability to run different samples on the same gel. This ability is based upon the specific properties of three CyDye DIGE Fluor dyes; Cy2, Cy3 and Cy5. Each sample is labelled specifically with one of the spectrally distinct dyes prior to electrophoresis. CyDye DIGE Fluors are mass- and charge-matched, pH insensitive, spectrally resolvable, highly sensitive, bright and photostable (GE Healthcare 2004). The dyes afford great sensitivity down to 25 pg of a single protein, and a linear response to protein concentration of up to five orders of magnitude (10^5). In comparison, silver stain detects 1-60 ng of protein with a dynamic range of less than two orders of magnitude (GE Healthcare 2005). The CyDyes enable multiplexing of up to three separate protein mixtures on the same 2-D gel (Figure 6). The key benefit is that multiplexing also enables the incorporation of the same internal standard on every gel. The internal standard is a pool of all the samples within the experiment, and therefore contains every protein from every sample. Ensuring that each protein spot has its own internal standard is the only way to remove gel-to-gel variation, thereby significantly increasing accuracy and reproducibility (GE Healthcare 2005). This allows accurate quantification of differences between samples, supported by statistical tests (Lilley and Friedman 2004; GE Healthcare 2005). The 2-D DIGE methodology is currently the only technique to enable accurate standardized quantification (GE Healthcare 2005).

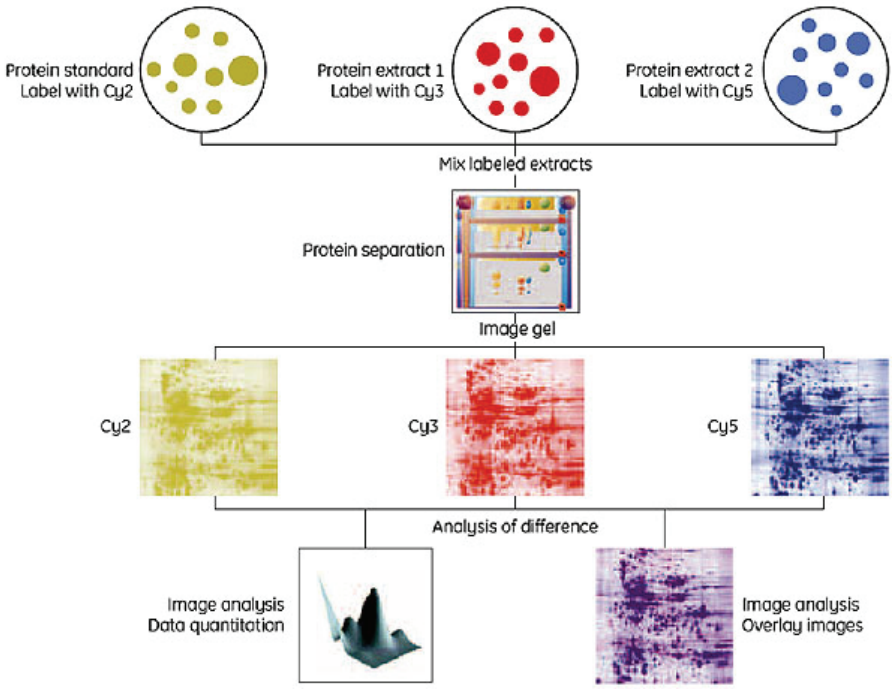

Figure 6 Multiplexing using the CyDye DIGE Fluor minimal dyes. © GE Healthcare (2004)

Whereas proteomics research is quite advanced in humans, animals and yeast, plant proteomics is at a very early stage, and its full potential is far from being fully exploited (Park 2004; Jorrín et al. 2007). An important advance in dicot plant material proteomics has come through the analysis of organelle subproteomes. Of these, the chloroplast, mitochondria and membrane proteomes are by far the best characterized (Agrawal et al. 2005; Jorrín et al. 2007). The so-called 'second generation' proteomic techniques, including 2-D DIGE, are now starting to be successfully applied to plants (Jorrín et al. 2007). Research in somatic embryo development extends over the past twenty years, but much of this work has been physiologically descriptive, or focused on improvements in culturing methodologies (Lippert et al. 2005). More recently, efforts have been made to describe embryogenesis at the molecular level, as shown in Section 1.4.3. Protein expression profiles provide a framework for the investigation of the biology of this complex process as well as opportunities for the practical application of this knowledge.

1.5 Cloning ~ a controversy in natural sciences

Cloning represents a controversial socio-scientific issue, especially with regard to animals and humans, and it is frequently reported in media because of the technological advancements being made in this field. Modern biotechnology, including cloning, is set to become one of the most important scientific and technological revolutions of the twenty-first century (Kirkpatrick et al. 2002). The need for education in this area will therefore become even more important for each individual and for society as a whole (Lappan 2000). It is essential that young people have a well-developed scientific understanding of these processes, enabling them to contribute to public debate and make informed personal decisions (Dawson 2007). In fact, the 2006 Norwegian curriculum gives more attention to biotechnology and cloning than in previous years (KD 2006a).

1.5.1 Norwegian students knowledge and interests in science

In recent years, many Norwegian students have considered the natural sciences to be less attractive and relevant than other disciplines, as shown by a noticeable decline in recruitment to science courses in upper secondary education (KD 2006b) and higher education (OECD 2006). The number of science students, in relative terms, in Norway is below the European average in general (European Commission 2005). Furthermore, Norwegian students in primary and lower secondary school have relatively low scores in international assessments, and they show a steadily declining performance in mathematics and natural sciences (Grønmo et al. 2004; Kjærnsli et al. 2007). To strengthen the country's competence in mathematics, science and technology across the entire educational system and to increase efforts to recruit students to the sciences, the Norwegian Ministry of Education and Research created: "The Joint Promotion of Mathematics, Science and Technology (MST). Strategy 2006 – 2009" (KD 2006b). According to this action plan, it is important that students develop increased awareness regarding the importance of scientific knowledge, that topics for instruction are up-to-date, that instruction is made more relevant and that interaction and cooperation with the country's research institutions is strengthened. According to Osborne et al. (2003), research clearly shows that early childhood experiences serve as a major influence on academic interest. As pointed out by Nergård (2008), the ultimate aim of teaching is to develop a positive attitude towards science among students that leads to continued interest and engagement during the rest of

their lives. She also highlights the fact that negative attitudes towards science may develop when students do not see the relevance of topics that are taught. Hence it is important that teachers use relevant contexts and applications, and that students develop a well-developed scientific understanding, and learn about the three dimensions of scientific subjects.

1.5.2 Natural sciences as products, processes and social institutions

Scientific literacy is increasingly seen as the primary goal of school science (Millar 2007) and understanding the nature of science is an essential component of scientific literacy. According to Sjøberg (2004), science consists of three dimensions: a product, a process and a social institution. The low recruitment and performance in science may be a result of the approaches to teaching science, which traditionally have focused on science as facts and unquestionable knowledge (Kolstø 2003). The process aspects have been given more and more attention in the last few decades, most recently through "The budding researcher" (year 1-10) and "The young biologist" (Biology 1 and 2) (KD 2006a), but the societal aspects[5] are seldom addressed. These aspects are important when it comes to understanding controversial issues such as cloning. Osborne (2000) says: "…'to know science' implies that one knows not only *what* a phenomenon is, but also *how* it relates to other events, *why* it is important and *how* this particular view of the world came to be". To improve students' motivation to learn science and to prepare them to participate in a democratic society, it is important to pay more attention to the last two aspects; the processes and the societal aspects. This is reflected in the primary goal of science education across the EU: to educate students both about the major explanations of the material world that science offers and about the way science works (Osborne and Dillon 2008). Kolstø (2003) claims that school science does not prepare students to understand scientific research and the public debate on scientific issues in society as a whole. He claims that societal processes, including argumentation, are necessary to obtain reliable knowledge. The societal aspects of science can be introduced by giving the students insights into research and the frontiers of science. The European Commission (2007) also

[5] The societal aspects are defined here as those aspects that deal with different actors: those who carry out research, those who make use of research results and those who decide what knowledge should or should not be generated. This includes how and by whom the knowledge should be administered and passed on, how society acts in relation to research, which motivation the researchers have, how free they are, and how society should act with respect to research results.

recommends that improvements in science education should be brought about through new forms of pedagogy: the introduction of inquiry-based approaches in schools.

1.5.3 Information, Communication Technologies (ICT) in natural sciences

ICT allows us to help students access, evaluate and make use of information that connects science to society and decision making processes (Jorde 2003). ICT also allows science teachers to introduce their students to up-to-date studies in science that are often outside the possibilities found in traditional teaching materials, and students are given the opportunity to explore and ask questions about science rather than be passive recipients of information (Jorde 2003). ICT also offers new possibilities for teaching difficult concepts and ideas. Complex systems may be simulated, experiments involving expensive equipment may be animated, controversial topics may be discussed with experts and people outside the immediate classroom, and information may be found linking school science to authentic science research (Jorde 2003). The Norwegian government has a long tradition of supporting the use of ICT in schools (KUF 1996a, 2000; UFD 2004), and digital literacy has now been added to the list of basic skills that learners should develop in all subjects in the national curriculum (KD 2006a). According to Kjærnsli et al. (2007) ICT is less used in mathematics and science, compared to other subjects. An interactive scientific teaching programme that introduce up-to-date knowledge, role models from working life and activities in society should therefore be a tool of interest to teachers.

1.5.4 Context-based approaches in natural sciences

Context-based approaches to science teaching can be used to introduce the societal aspects of science. Campbell et al. (1994) and Ramsden (1997) have shown that such approaches provide pupils with more appealing and more relevant experiences in their science lessons. Nevertheless, it appears that context-based approaches are rarely used. According to Osborne and Dillon (2008), European students often experience that "the science curriculum can appear as a 'catalogue' of discrete ideas, lacking coherence or relevance, with an over-emphasis on content that is often taught in isolation from the kinds of contexts that might provide essential relevance and meaning". In a Swedish study, many pupils did not understand why they were learning science (Lindahl 2003). Many pupils also said that they would not choose science, because they do not understand science the

way it is currently taught. Nevertheless, the pupils did show a greater interest in science content if it was presented in another context, in accordance with the findings of Sjøberg (2002). Hov and van Marion (2004) say that contextualized teaching materials usually are used as a supplement to the more traditional science textbooks. In recent years, however, the use of a context-based approach to science teaching in some courses has attracted international attention: ChemComm, CEPUP and PRIME Science in the United States, the Salters' project in the United Kingdom and PLON in the Netherlands (Ramsden 1997; Barker and Millar 2000). In addition to courses, some curriculum enrichment resources, such as SATIS (Science and Technology in Society)[6], Murder under the Microscope[7] and ParIS (Gräber et al. 2005) use scientific applications and contexts as starting points. One important influence on these context-based developments was the emergence of the 'Science, Technology and Society (STS)' movement (Solomon and Aikenhead 1994). The high degree of interest and enthusiasm that students express for STS instruction indicates that future developments toward such instruction will receive encouragingly positive reaction from most students (Aikenhead 1994).

Comparing a context-based approach with a more traditional approach to high school chemistry, Ramsden (1997) found little difference in levels of understanding key chemical ideas, but there appeared to be some benefits associated with a context-based approach in terms of stimulating pupils' interest in science. There was some evidence to suggest that a context-based approach was the more successful in terms of providing pupils with what they perceived to be a worthwhile experience in their science lessons. This is in accordance with conclusions drawn by Kjærnsli et al. (2007): a focus upon applications of the topics increased interest in and valuation of science. Ramsden (1994) also concluded that the Salters' Science course provided benefits in terms of pupil motivation in lessons. This in turn suggested that the approach advocated in STS-type courses, which puts applications and issues first, is likely to be more motivating for pupils than the more traditional approach of science first, applications afterwards. According to Barker and Millar (2000), the context-related approach in the Salters Advanced Chemistry course was clearly effective in teaching the basic chemical ideas explored; however, some misunderstandings remained difficult to change. Some key areas of chemistry seem to be poorly grasped

[6] The Association for Science Education (ASE). SATIS revisited, http://www.satisrevisited.co.uk
[7] Waterwatch. Murder under the Microscope, http://www.waterwatch.nsw.gov.au/07_murder_microscope

whatever the approach, and it may be that some ideas are difficult and present too great challenge for most pupils (Ramsden 1997). Hughes (2000) found indications that a contextual approach to the teaching of science improves female engagement.

The introduction of context-based approaches and ICT in science teaching may affect the way students learn, by promoting active learning and the societal perspectives of the learning process. According to Hov and van Marion (2004), good contexts should be meaningful, catchy, arouse interest, and lead the students to the needed scientific content knowledge. These contexts should hold the students interest over time, should not mask the professional content and should give students the opportunity to make use of the scientific knowledge in new contexts.

1.5.5 Viten.no

The website http://www.viten.no is designed for science teachers and their students. The site has been developed using free open sourced software and the programmes are designed to present web-based science to students in grades 8-13, with topics taken from geology, mathematics, physics, biology and chemistry. Viten.no is well established in Norwegian schools, as illustrated in Mork (2006a). The Viten programmes are designed according to the Scaffolded Knowledge Integration (SKI)-principles (Linn and Hsi 2000; Mork 2006b), and they share many of the characteristics of STS teaching. The programmes address issues that go beyond the scientific content: they teach about education for citizenship in a scientific context, they teach scientific literacy, about decision making in scientific issues, and about evaluating the presentation of science in the media (Jorde et al. 2003). Furthermore, the Viten framework draws on theories about the importance of communicating with the language and symbols of youth culture (Jorde et al. 2003).

In the new version of viten.no being developed, each programme will be constructed as an assembly of small learning objects that can be reused in other contexts, separately or combined in new ways, and/or in other virtual learning environments. The team developing Viten wants in the new version to make it possible to harness the potential of integrating the content with external resources and tools in a more seamless way. They will still develop programmes with contextual approaches and have no intentions of excluding

pedagogy from the learning objects or encouraging teachers to focus only on the product dimensions.

1.5.6 The Viten unit "Cloning plants"

The Viten unit "Cloning plants" (Appendix 4) was released in August 2004, and as of September 2008 had been used by 4615 students and 518 teachers. Cloning and regulation are the main topics of the unit, and the content was chosen in accordance with the national curriculum for natural science and biology in upper secondary school (KUF 1993; 1996b). The topic of cloning was chosen because it represents a controversial issue that is also of interest to the general public. Molecular biology and regulation have become main issues of research, and the products of this modern research will increasingly have an impact on society as a whole. Actually, biotechnology and cloning have also been given more attention than in previous years in the Norwegian curriculum introduced in 2006 (KD 2006a).

1.5.6.1 Aims of the teaching unit

The unit was designed to introduce the societal aspects of cloning to school science. To be able to understand these societal aspects, the students need content-specific knowledge. Therefore, the teaching unit starts with traditional theories about cloning, but these are presented in a real life context, based upon events in a modern research laboratory. The overall aims of the teaching unit are to:

1. *Increase student knowledge of cloning, regulation and propagation of plants*
2. *Increase student interest in participation in the public debate on cloning*
3. *Increase student interest in research*
4. *Increase student skills and knowledge in how to use modern research methods and instruments*
5. *Increase user insights into frontline research in a modern research milieu*

1.5.6.2 Description of the teaching unit

The teaching unit involves a virtual case in the context of a research laboratory and an international research conference on cloning. The students are assigned roles as participants in a summer school located at the research laboratory. Their mission is to solve

a criminal case (Part 1). The researcher at the laboratory has been cloning plants, and after a burglary in the research laboratory, a valuable powder was found to be missing. At the same time an international research conference on cloning was being held in the city, and traces of powders were found in the hotel rooms of three of the conference participants. The students have to try to solve the case. Their clues are information from the local police (Part 2), the conference material (Part 3), an Internet search on cloning (Part 4), and a guided tour through the laboratory (Part 5) – with the possibility of analysing samples of powder (Part 6). To solve the mystery, the students need to 1) develop an understanding of cloning, 2) assess the statements of the suspects, and 3) carry out analyses of samples of powder in a virtual laboratory. Finally, they need to present their conclusions, based upon scientific knowledge and argumentation (Part 7). The seven parts of the unit are described below and illustrated in Figure 7.

Part 1 Introduction

In the first part of the programme the students are introduced to the burglary and the research laboratory, and are assigned roles and the task. Their five sources of information needed to solve the case (Parts 2-6) are briefly described.

Part 2 Police station

The police investigate the burglary at the research laboratory, but the investigation is dropped because evidence has been destroyed. As the aggrieved party, the researcher has a right to inspect documents. In Part 2, the students are given access to the police report from the burglary, along with reports from judicial examination of the suspects, and analytical findings from confiscated powders, returned from the Criminal Police Centre. The analytical results are returned via fax, but the students do not receive them until 15 minutes after they visit this page the first time. To obtain the analytical results faster, the students have to analyse the samples themselves in the laboratory (Part 6).

Part 3 Conference

At the same time and in the same city as the burglary took place, the "13th International Symposium on Cloning ~ historical review and future prospects" is being held. Part 3 includes the conference programme, pictures from a guided tour of the research laboratory, an article from the newspaper and a radio programme, both of which provided coverage of

the cloning conference by interviewing five participants. The radio programme provides important clues to determine who stole the powder from the laboratory.

Figure 7 Screen shots that illustrate the main parts of the teaching unit.

Part 4 Internet search on cloning

To solve the mystery, one of the fictitious summer school students has conducted an Internet search on cloning. The results from this search are given in Part 4. The students are given a definition of cloning, and are introduced to the different levels of cloning (genes, cells and whole organisms: plants, animals and humans) along with insights on laws, rules and ethical questions. In addition they have to perform five small exercises. The text in this component was constructed on the basis of material published by the

Norwegian Biotechnology Advisory Board[8]. A representative from this advisory board also reviewed the pages.

Part 5 Laboratory – a guided tour

The laboratory researcher's goal is to try to find a metabolic component that regulates the development of plant embryos. She has found an interesting component, but lost most of the existing powder during the burglary. In Part 5, she brings the students through the laboratory, telling them about her research. This context gives the students an introduction to general theory about hormones, proteins, tissue cultures, regeneration and propagation. They are also given two small exercises, a puzzle and a test.

Part 6 Laboratory – a station for analyses

In connection with the burglary, the police confiscated three different powders. These were sent to the Criminal Police Centre for analysis, but the researcher also received test samples as well. The researcher keeps a small portion of the lost powder, which can be used for comparison of samples. In Part 6 the students are allowed to analyse all these samples in two ways: a) spectrophotometric determination of protein content and protein level, b) electrophoretic comparison of proteins. The students can take notes on their laboratory results in notepads.

Part 7 Presentation

To prepare for the closing activity, the presentation, the students are given several questions in Part 7, as well as guidelines for making the presentation. Most of the text in the unit can be copied, and a picture gallery is included, so that the students can spend most of their time on the scientific content.

The rationale behind choosing this approach was to connect scientific processes and societal aspects to scientific information on cloning. The approach aimed at showing students that cloning is an issue of debate in society at large, and that possessing knowledge about this issue is important if one is to be an informed citizen in a democratic society.

[8] http://www.bion.no/tema/kloning.shtml and http://www.bion.no/tema/stamceller.shtml

1.5.6.3 The pedagogical framework behind the unit

"Cloning plants" was designed according to the Scaffolded Knowledge Integration (SKI)-principles (Linn and Hsi 2000; Mork 2006b), which are the same principles as were employed in the other Viten programmes: 1) make science accessible, 2) make thinking visible, 3) help students learn from each other and 4) promote autonomy and lifelong learning. This pedagogical framework is based on theories regarding personal and social constructivism, which are described by Driver et al. (1994) and Leach and Scott (2003). The four principles are discussed below.

SKI-principle 1: Make science accessible

Scientific theories are made more easily accessible when students extend their knowledge by building on their own scientific ideas. In the Internet search on cloning (Part 4) the students must first give their own definition of cloning. The subsequent page, which provides the actual scientific definition, is not available until this question is answered. The aim of this task is to make the students more aware of their own knowledge. Following the Internet search, new aspects of cloning are introduced, and at the end of Part 4, the students are given the opportunity to comment on and correct their first answer. To help students reflect on what they read, different exercises connected to the text pages are included.

Issues related to molecular biology and regulation may be difficult for students to visualize in a school setting, and simulations may represent valuable approaches that make these topics more accessible. Thus, animations or interactive activities have been used to explain the following scientific processes or principles: cloning genes through bacteria and PCR, cloning animals through nuclear transfer (Figure 8), functioning and regulation of enzymes (Figure 8), functioning of hormones and growth regulation by the use of plant hormones. In addition, the teaching unit allows for laboratory analyses to be simulated, and all of the objects associated with each technique are interactive and are required to complete the simulations (Figure 8).

Scientific topics may also be more accessible to students if they feel a personal connection to the issue and are motivated to learn. Hence a context is used where the students are assigned roles and given a task. An exercise is also included about actually cloning their teacher, as an example that has personal relevance. The case is based on a young researcher, and thus their distance to this "world of research" may be reduced. The choice

of design and colours in the Viten unit are based on the popular TV programme “24 hours”, which might be a source of connection for student motivation. The students also work towards a closing activity, creating a presentation (Part 7), which can increase their motivation. At any time teachers may also view student work and provide feedback by using the teacher evaluation tool, included in all Viten programmes. This may motivate revisiting of relevant pages.

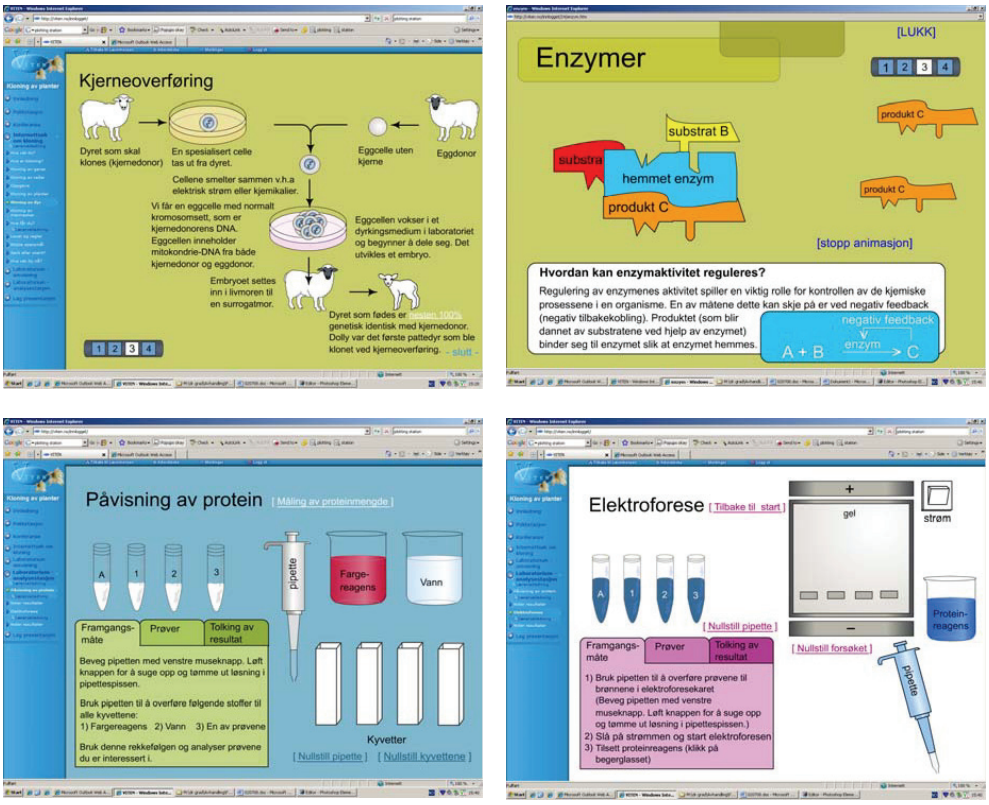

Figure 8 Screen shots that illustrate some of the objects found in the teaching unit.

SKI-principle 2: Make thinking visible

Through the different exercises, the students are asked to explain and reflect upon their ideas, and in this way to visualize their thinking. One task in Part 4 deals with stem cell research and the students have to find arguments for and against using stem cells. In another exercise they have to describe what they need if they want to clone their teacher, along with describing the result of this trial and a comparison of their clone and the teacher with monozygotic twins. In an exercise called “True or false?” the students have to

evaluate ten assertions. At the end of Part 5 there is a puzzle about how the researcher makes her cloned plants and a multiple-choice test where the students have to evaluate alternative statements. Each time this test is started, a new and random sample of questions is generated. Results from laboratory analyses (Part 6) can be made visible on a notepad. In the closing activity the students have to evaluate different explanations, find mistakes and connections, make conclusions and finally visualize their thoughts in a presentation.

SKI-principle 3: Help students learn from each other

This principle is reflected in the programme's social activities, which promote productive interactions between students. The teacher support material recommends that students work in groups of 2-3 students per computer. Working together maximizes use of the social environment, allowing students the opportunity to talk science and discuss their opinions with their peers. The questions in the programme are meant to stimulate argumentation and judgements, so that the students must explain their ideas and ask each other questions. For example, in Part 4, many ethical questions, some of which are provocative, are posed regarding stem cell research, with the aim of starting a discussion among students. Following this, an exercise is given where the students are instructed to write down different arguments for and against the use of stem cells. Holding a discussion as a final closing activity, as recommended in the teacher resources, will create social interactions that enable learners to hear ideas in words of members of diverse cultural groups.

SKI-principle 4: Promote autonomy and lifelong learning

Lifelong learning results when the students are asked to reflect on their own scientific knowledge and points of view, as well as others scientific ideas. Questions that require reflection are included in Part 4. There are also many links to external Internet pages and supplementary information throughout the teaching unit, e.g. about Roslin Institute, cloned human embryos, hormones and enzymes. These are included as inspiration for the students to learn more by themselves, and encourage students to revisit the phenomena in new contexts. Furthermore, as a part of finding the guilty person, the students are trained to search for information and to evaluate arguments or statements. These abilities are a necessity if one is to be able to learn autonomously and to continue to learn over a lifetime. Useful Internet addresses for sites dealing with biotechnology, cloning, proteins and biology in general are included in the background information for teachers. In addition, it

is recommended that the unit be concluded with a discussion, where the students are assigned specific roles and must find arguments that reflect those roles. Guidelines for two kinds of discussions, along with useful Internet addresses, are given in the background information as well. Teachers also have the opportunity to send practical and theoretical questions to the researcher at the laboratory, through a direct link on the main page of the programme. In these ways the unit stimulates lifelong learning both for students and teachers.

1.5.6.4 The Viten unit "Cloning"

The Viten unit "Cloning" was released in October 2004. This unit consists of a small part of the "Cloning plants" unit (Part 4) and has a non-contextual approach. As of September 2008, this programme had been used by 11391 students and 867 teachers. Despite some sources of error related to students and class registration, data from the Viten server show that a greater number of students have worked through this programme, as compared to "Cloning plants" (used by 4615 students). This may be explained by the organizational challenges facing schools. For example, the number of computers at many schools is still too low. Biology teachers have explained that it is difficult to get access to the computer room, so that all the students in the class can work through such a programme together. Some teachers would therefore prefer to use this kind of resource as homework or simply use small parts of the programmes. Even if teachers do choose "Cloning", that does not necessarily mean that they do not teach this issue in context. They might create their own context offline or with the help of other sources.

2. AIMS OF THE STUDY

C. persicum is currently propagated through F1-hybrid seeds, but due to inbreeding depressions, inhomogeneity in some cultivars, manual labour for seed production and high prices for seeds, the use of somatic embryos is preferred and is the ultimate goal. To date the production system has not been sufficiently successful (Seyring and Hohe 2005), and improvements in tissue culture methodology are required. Advancements in somatic embryogenesis could be made both at a macro and micro scale, with new insights into bioreactor growth and signalling pathways. As plant cloning is a part of the socio-scientific and controversial issue of cloning, and as the links between science and society have grown stronger, researchers who work with cloning have a special responsibility with regard to communication of their results to the general public and to the next generation of citizens (Fjelland 1999).

Based on the above, the main goals of this project were:

1. To study interactions between environmental factors for C*yclamen* propagation in bioreactors through the use of mathematical models. More specifically, to determine optimal conditions for biomass growth and cell viability with regard to oxygen level, daily mean temperature, the difference between day and night temperature (DIF), and daily light integral (Paper I).

2. To identify specific proteins produced in embryogenic and non-embryogenic isogenic cell suspensions of *C. persicum* and to describe the differentially accumulating proteins, to obtain new insights into early events in somatic embryogenesis and to determine if there are proteins that might be used as molecular markers for somatic embryogenesis in *C. persicum* (Paper II).

3. To design a context-based digital teaching unit on cloning ("Cloning plants", http://www.viten.no, Appendix 4) in order to introduce the issue of cloning and its societal aspects to secondary school students, and to see whether or not a context-based approach improves the students' knowledge base and interest (Paper III).

3. SUMMARY OF RESULTS AND DISCUSSION

3.1 Paper I: Optimizing growth conditions for proembryogenic masses in bioreactors

This paper presents models of the potential effects of four growth factors on the development of proembryogenic masses of *C. persicum* Mill. in bioreactors. Each factor was observed at three levels; oxygen concentration (50, 100 and 150% of fully oxygen saturated medium without cells), daily mean temperature (15, 20 and 25^{o}C), the difference between day and night temperature (DIF) (+ 10, 0 and – 10) and daily light integral (0, 1.3 and 2.6 mol m^{-2} day^{-1}). Two response variables, biomass growth and cell viability, were measured at day 0, 7, 14, 21 and 28 after start-up.

The effect of varying the oxygen concentration from 50% to 150% on biomass growth can be described using a linear model. Our data showed a positive linear effect from oxygen, resulting in 150% as the optimal value for growth (Figure 1, Paper I). These results are consistent with the results obtained by Hohe et al. (1999a), Tate and Payne (1991) and de Feria et al. (2003), considering our limited concentration interval. On the other hand, our maximum for cell viability, found using the optimal model, was in most cases just below 100%, and 150% O_2 was detrimental to viability (Figure 3 and Figure 4, Paper I). This response is consistent with the results found by Hohe et al. (1999a), and it is not surprising that the oxygen level that nature can provide is the optimal (when the medium was fully saturated with oxygen from the air, 21% oxygen, the condition was defined as 100%). Suppressed viability at the highest concentration may be explained as the effect of toxicity.

Within the temperature range for this study, there was a stable positive linear effect of temperature on biomass growth, giving 25^{o}C as the optimal value (Figure 1, Paper I). However, temperatures higher than 20.8^{o}C appear to be less favourable for cell viability (Figure 5, Paper I). This is in accordance with earlier experiments with the same culture (Hvoslef-Eide et al. 2003), and with a study performed by Hohe et al. (2001), where 24^{o}C was used for cell growth and 18^{o}C for regeneration into embryos. Our results are also in accordance with the finding that 32^{o}C is considered to be heat stress for *Cyclamen* (Oh et al. 2007), and that seed germination of *Cyclamen* is favourable at 15^{o}C, compared to 20^{o}C (Neveur et al. 1986). It appears that the optimal temperature for biomass growth is higher

than for viability and germination. This shows that cell cultures respond much in the same way as whole plants with regards to temperature.

DIF seemed to be more optimal for cell proliferation than constant temperature, whether positive or negative (Figure 2, Paper I). The quadratic term was positive, which gave a maximum effect at the limits. In 75% of the cases, the optimum for biomass growth was the upper limit $D = 10$. This shows that our cell cultures were able to respond to DIF. The physiological basis of the DIF response is still poorly understood (Thingnaes et al. 2003), but it has been shown that gibberellin and auxin, light intensity and light quality may influence the response (Moe and Heins 2000; Thingnaes et al. 2003; Stavang et al. 2005; Bachman and McMahon 2006). There was no significant effect of DIF on cell viability.

A daily light integral between 1.03 and 1.19 mol m^{-2} day^{-1} gave higher biomass growth and cell viability than complete darkness and double the amount of light (Figure 2 and Figure 6, Paper I). This favourable light intensity means about a 12h photoperiod with the OTT bioLIGHTSYSTEMS used in this study. Our result may indicate that the light saturation point of this *Cyclamen* suspension culture was about 1.10 mol m^{-2} day^{-1}. The fact that the biomass growth increased at 1.10 mol m^{-2} day^{-1} compared to darkness in our study may also be explained as a hormone effect. The red/far red ratio of 2.7-2.8 that was used in our study is regarded as normal to high (Kurepin et al. 2007) and provides a relatively high amount of red light. Red light stimulates the formation of an IAA oxidation inhibitor (Hillman and Galston 1957; Mumford et al. 1961) and will therefore favour cell growth. The highest light intensity may be above the saturation point, and therefore detrimental to the cells.

With regard to cell viability, the optimal time in the bioreactors was 11.3 days. Normally, bioreactor batch cultures are diluted every week. Our data sets show that there is not only the potential to save labour costs by waiting longer than the normal seven days, but that the cultures will also have increased viability.

3.2 Paper II: Characterizing embryo-specific proteins in *Cyclamen*

According to Dudits et al. (1995), the primary aim of molecular studies of various embryogenic culture systems is to identify the characteristic regulatory components that generate the embryogenic state. In Paper II, the protein patterns of two isogenic cell lines of *C. persicum* Mill., one embryogenic (E) and one which never has shown any embryogenic capacity (NE) have been compared, using 2-D DIGE and a MALDI-TOF-MS.

After 2-D DIGE separation, more than 1200 *Cyclamen* proteins were detected by digital image analysis over a pH range of 4-7 and a size range of 10-100kDa. Roughly 1000 proteins were common to all samples. The statistical analysis showed that 205 protein spots changed significantly ($p<0.001$) between the two cell lines E and NE (Figure 3, Paper II). Among them, 108 proteins were up-regulated and 97 down-regulated in E versus NE suspensions, and 128 proteins were identified (Table 2, Paper II). Many of the proteins were represented by several spots that matched the same gene sequence but that had different pI and/or M_r (Table 2, Paper II). These proteins were most likely the result of post-translational modification, as can be shown by the position of the spots in the 2-D gel (Supplementary Figure, Appendix 2) and the ion sequences (Supplementary Table, Appendix 3). Furthermore, 27 proteins were unique to the embryogenic cells and 11 proteins to the non-embryogenic cells. Unfortunately only 12 and 4 respectively of these were identified (see Table 2, marked g and h respectively). The unique spots could be considered molecular markers for somatic embryogenesis, although some that have been identified do not indicate rare functions. They could, however, be specific isoforms.

Establishment of embryogenic competence and cell reprogramming is accompanied by e.g. active metabolic changes (Fehér et al. 2003), synthesis of new mRNA molecules (Dudits et al. 1995), posttranslational modification of proteins, protein folding and protein translocation, cell wall and membrane formation (Chugh and Khurana 2002), activation of signalling molecules (Dudits et al. 1995) and stress protein genes (Bond and Schlesinger 1987). Our identified proteins were classified into six functional categories based on their main biological process, as suggested by Marsoni et al. (2008): 1) cell proliferation (6.0% of identified proteins), 2) protein processing (14.3%), 3) signal transduction (6.0%), 4) stress response (3.8%), 5) metabolism and energy state (67.7%), 6) hypothetical function

(2.3%). The largest classes were those representing proteins implicated in protein processing and metabolism. This is in accordance with results from grapevine (Marsoni et al. 2008) and cowpea (Nogueira et al. 2007). In Paper II, the proteins identified are discussed according to their functional categories and brought into the context of somatic embryogenesis, focusing on the proteins that were unique for the embryogenic and non-embryogenic cell lines, as well as some proteins that previously have been discussed with regard to their role as metabolic components in the embryogenesis process.

The 12 embryo-specific proteins that were identified represent different biological functions: cell proliferation, and carbohydrate, amino acid, lipid and energy metabolism. ATPase (spot 1502) (Table 2, Paper II), the most abundant protein, was unique for the embryogenic cell line. This may explain the proembryogenic state of our culture, as described by Smith and Krikorian (1990), but may also mean that the embryogenic cells need more energy for their metabolic changes. Three putative metallophosphatases (spots 65, 89 and 128) (Table 2, Paper II) as well as a molecular chaperone Hsp90 (spot 93) (Table 2, Paper II) were unique for the non-embryogenic cell line. These may prevent differentiation.

3.3 Paper III: Introducing the issue of cloning and its societal aspects in science teaching

The "Cloning plants" teaching unit (Appendix 4) was released in August 2004, and by September 2008 had been used by 4615 students and 518 teachers. Paper III presents the results from a small-scale study where 44 students worked through the "Cloning plants" unit, after which their knowledge and interest outcomes were analysed. As human beings were the sources of data in this study, all measurement, storage and use of data were conducted pursuant to guidelines given by the Data Inspectorate in Norway. Informed consent was obtained through an informational letter to students and parents (Appendix 5). Two students said no, and this was respected. The questionnaires (pretest and posttest) are presented in Appendix 6, the interview guide in Appendix 7, the transcribed interviews in Appendix 8, and pictures of students working through the teaching unit are presented in Appendix 9.

Statistical tests show that the students' knowledge score was significantly higher after using the context-based teaching unit (Figure 1 and 2, Paper III). Initially, the students associated cloning with "copying" something and "Dolly the sheep" was the familiar example, similar to results obtained by Dawson (2007). Afterwards the students knew that cloning occurs at different levels, from genes, to cells, to whole organisms. They increased their understanding of stem cells, and animal and plant cloning. Nevertheless, some appeared to be confused about the difference between cloning and genetic engineering, as was found with Australian and Slovakian students (Dawson and Schibeci 2003; Prokop et al. 2007). Afterwards, 60% agreed that they knew more about the way researchers work. In addition, 72.5% agreed that they had improved their understanding of ethical issues related to cloning and cloning research. "*Good figures and animations*" were particularly valued as instructive.

Students that initially showed low interest increased their interest in cloning (Figure 3, Paper III), and a positive correlation was found between an increase in knowledge and increase in interest (Figure 4, Paper III). Initially many students associated cloning with reproductive cloning and saw no real benefits, as was found by Shepherd et al. (2007). After their exposure to the material, the majority were able to describe both positive and negative aspects of cloning. About 60% agreed that they became more interested in the way researchers work.

Student answers indicated that there might be a connection between their increased knowledge and interest and the context-based approach. About 80% believed that the use of the mystery as a teaching tool had positive effect on their learning, while five students actually claimed that the contextual case distracted their attention from gaining knowledge of cloning. Statements from interviews indicate that the focus on societal aspects of cloning enhanced students' interest in research and cloning. Benefits associated with a context-based approach in terms of stimulating pupils' interest in science and motivation in learning have also been found by Wierstra (1984) and Ramsden (1994, 1997).

4. FUTURE PROSPECTS

The work presented in Paper I and II was initiated as a result of a cooperative effort with the European Union through COST Action 822, "Development of integrated systems for large-scale propagation of elite plants using *in vitro* techniques" (Ríordáin 1997, 2000, 2001; Schwenkel 2001b), and COST Action 843, "Quality enhancement of plant production through tissue culture" (Libiaková and Gajdošová 2005). The ultimate aim of the *Cyclamen* sub-group in these COST actions was to incorporate technological advances in plant cell and tissue culture that would make European production of *Cyclamen* more efficient and cost-competitive (Schwenkel 2001b). The research in this COST group has focused both on quantitative and qualitative aspects of the production system.

Based upon the results from this doctoral study, further work on the improvement of the propagation method for *Cyclamen* should focus on the embryo formation. As shown in Paper I, it is clear that biomass growth and cell viability can be influenced markedly by the changing culture environment in batch cultures. Samples from all the experiments in Paper I have been induced for differentiation and measurements of embryo development have been performed. Analysis of the results, using mathematical models, remains to be done. These results will tell us more about the environments in batch cultures that are favourable for promoting differentiation into embryos. The process format used also requires attention. Multicycle or draw-fill culture technique (Lipsky 1992) and disposable wave bioreactors (Eibl and Eibl 2006) should be considered for embryogenic cultures.

The new insights obtained on protein expression in embryogenic and non-embryogenic *Cyclamen* cultures (Paper II) may give us a more exact understanding of the mechanisms involved during early somatic embryogenesis. Further studies of *Cyclamen* proteomics should include suspension cultures that are induced for differentiation, and their protein expression over time. Such samples have already been collected, but the protein separations were not successful and new separations and identifications remain to be done. Candidate proteins could be analysed in more detail with regard to subcellular localization, because proteins that are bound to or that are a part of the cell wall could be a good basis for developing antibodies. Prefractionation may also be required for identifying components of signal transduction pathways (Tang et al. 2008). The possibility of

antibody-covered beads as a means of cell separation will enable enrichment of embryogenic cells in bioreactors and more synchronous development of embryos. Antibodies against embryo-specific proteins in general can be useful for the identification of appropriate treatments or environments during *in vitro* testing. Secreted proteins can also influence the development of somatic embryos (von Arnold et al. 2002). Extracellular proteins should therefore be investigated to obtain a complete image of the embryogenesis process. In addition to regulation at the level of protein or gene expression, the importance of the physiological state of the cells, the presence of signals that have been secreted or derived from cell walls, as well as endogenous hormones and the interaction of different signalling cascades are evident from many studies (Fehér et al. 2003), and have to be studied in more detail.

Ongoing *Cyclamen* projects in Europe are now addressing desiccation tolerance and encapsulation of somatic embryos and induction of maturity, as well as expression analyses based on the EST library of *Cyclamen* embryogenic tissues that has been recently established (Rensing et al. 2005). cDNA array-analyses have been performed and some interesting results have been verified via real-time PCR (Hohe A, personal communication). Commercial mass propagation of conifers through somatic embryogenesis is already taking place, although relatively new (Lippert et al. 2005). If commercialization of somatic embryo production for ornamental plants is achieved, it is suggested that *Cyclamen* will be among the first to be produced (Hvoslef-Eide AK, personal communication). Among the outcomes of the COST Action 843 was the proposal for a new Action, dealing with the understanding of the fundamental mechanisms underlying the competence for regeneration *in vitro*. A very large number of teams from many countries participated on a proposal, but no financing has been awarded to date.

The results presented here from the classroom study (Paper III) strengthen the assumption that context-based approaches may facilitate learning about societal aspects of scientific topics, and that such approaches improve students' knowledge level and interest as well. To be able to document the effects of learning from interactive and context-based approaches, the students' scores should be compared to students who receive traditional instruction only. Furthermore, the long-term effect of students' learning outcomes could be investigated for the two approaches. It might also be interesting to study whether and to what extent the students' skills are transferable to other cases – whether the two

approaches foster higher-order thinking skills, and whether interactive and context-based learning materials alone would contribute to foster scientific and technological literacy.

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Paper I

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Influence of potential growth factors on the production of proembryogenic masses of *Cyclamen persicum* Mill. in bioreactors

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Somatic embryogenesis
Mathematical modelling

ABSTRACT

Propagation in liquid culture, especially bioreactors, is one possible way to produce clonal propagules of *Cyclamen persicum* Mill. at a low cost. The current propagation method for *C. persicum* is from expensive hybrid seeds. This paper presents models of the potential effects of oxygen concentration, daily mean temperature, the difference between day and night temperature (DIF), and daily light integral on the development of proembryogenic masses of *C. persicum* Mill. in bioreactors. Each of the four growth factors was observed at three levels; oxygen concentration (50, 100 and 150% of fully oxygen saturated medium without cells), daily mean temperature (15, 20 and 25 °C), DIF (+10, 0 and −10) and daily light integral (0, 1.3 and 2.6 mol m^{-2} day^{-1}). Two response variables, biomass growth and cell viability, were measured at day 0, 7, 14, 21 and 28 after start-up. The optimal values for biomass growth were 150% oxygen, 25 °C, 1.11 mol m^{-2} day^{-1} and DIF + 10. There was a stable positive linear effect from temperature. Oxygen showed a similar, but less stable effect. DIF gave maximum effect at the outer levels, and its optimum was the upper level +10. The optimal values for light were between 1.03 and 1.19 mol m^{-2} day^{-1}. The optimal time for cell viability in the bioreactors was 10–11 days. Temperature and daily light integral had stable optima of 20.8 °C and 1.10 mol m^{-2} day^{-1}. For oxygen the optimum was more unstable, but in most cases it was below 100%. There was no significant effect of DIF on cell viability.

1. Introduction

Cyclamen persicum Mill. is an important ornamental crop in central Europe and Asia (Wiersema, 1999). The commercial propagation of cyclamen is currently achieved using seeds (Geier et al., 1990), preferably from F_1-hybrid seeds, although these are relatively expensive and sometimes not sufficiently uniform in production (Winkelmann et al., 2000). Vegetative propagation has been attempted at a small scale, as there have been a number of obstacles (Geier et al., 1990). Later, *in vitro* techniques have been established using somatic embryogenesis (Hvoslef-Eide and Munster, 1998; Winkelmann et al., 2000). One aim of somatic embryogenesis is to find a protocol for the production of synthetic seeds that can replace the generative propagation technique used to date. In order to match the needs of industrial-scale clonal mass production, quantity and quality modifications of the production system are necessary. To achieve this, suspension or bioreactor culture techniques have been attempted (Hvoslef-Eide and Munster, 1998; Hohe et al., 1999a,b), but only to a very limited extent. More recently, the first experiments on desiccation of cyclamen somatic embryos were published (Winkelmann et al., 2004a; Seyring and Hohe, 2005). Early results from the germination of encapsulated somatic embryos (Winkelmann et al., 2004b) and new insights in alginate mixtures for cell encapsulation also exist (Donati et al., 2007), but there are still problems with low germination rates and desiccation tolerance (Winkelmann et al., 2004b; Seyring and Hohe, 2005). Comparative studies of embryo development and germination of zygotic and somatic embryos of cyclamen have therefore been performed (Schmidt et al., 2006).

Proembryogenic masses (PEMs) have been defined as cell lumps able to produce somatic embryos (Halperin, 1966) and consist of small, highly cytoplasmic cells, the embryogenic cells (Ibaraki and Kurata, 2001). The PEMs in suspension cultures will not develop into embryos in presence of high auxin levels (Halperin, 1966) but can proliferate to form large numbers of PEMs. Large-scale production of PEMs can be greatly enhanced by the use of bioreactors (Ibaraki and Kurata, 2001). As part of improving the

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commercial propagation method and improving the *in vitro* mass propagation scheme for *C. persicum*, the influence of four potential growth factors on cell growth and cell viability in bioreactors have been tested.

According to Kurata and Shimazu (2006), there have been several reports on somatic embryo production systems using bioreactors, but they conclude that the effect of physical environmental factors has not been sufficiently investigated in detail. One potentially important growth factor is the gas composition in the culture vessel. The effect of O_2 partial pressure and CO_2 accumulation on cell proliferation and the subsequent regeneration of somatic embryos of *C. persicum* have been reported for flasks and Applikon bioreactors (Hohe et al., 1999a,b). The effect of temperature on somatic embryogenesis of cyclamen was demonstrated by Takamura et al. (1995). Obviously, temperature will also affect biomass growth. Commonly, within the same species, high temperatures give good cell growth, while regeneration is favoured at lower temperatures (for cyclamen, see Schwenkel and Winkelmann, 1998). The effect of changing the temperature during the day is therefore of interest with regard to somatic embryogenesis. Greenhouse crops of cyclamen respond to the difference between day and night temperature (DIF) and daily light integral (Hendriks and Scharpf, 1987), and it is likely that cell suspensions show some kind of response as well. Some reports that light inhibits embryogenesis *in vitro* (Takamura et al., 1995), while others recommend low light intensities (Hohe et al., 2001).

In order to maximize biomass growth and cell viability, some combinations of these forcing factors will be more productive than others. As there are no reports on interactions between environmental factors for cyclamen propagation in bioreactors, the aim of this study was to determine optimal conditions for biomass growth and cell viability with regard to oxygen level, daily mean temperature, DIF and daily light integral. This has been achieved by fitting models, e.g. response surface models, to the data and estimating the optimal combinations from these, which is a common practice in many process industries (Myers and Montgomery, 2002). This use of mathematical models could also be generalized to provide new insights into field studies.

2. Materials and methods

2.1. Suspension cultures and media

The experiments were performed with liquid cultures of the embryogenic cell line 3738-VIII of *C. persicum* Mill. cultivar 'Purple Flamed', obtained through COST822 cooperation. The callus cultures were initiated as described in Schwenkel and Winkelmann (1998) and suspension cultures were established as described by Winkelmann et al. (1998). The cell line was maintained as a long-term suspension culture by subculturing it every 2 weeks in a growth regulator containing medium, as described in Winkelmann et al. (1998). Cultures were maintained in 500 ml Erlenmeyer flasks containing 100 ml medium, and agitated on a rotary shaker (100 rpm) under darkness at 24 °C.

The media used were based on MS medium (Murashige and Skoog, 1962) with modifications according to Schwenkel and Winkelmann (1998). For cell proliferation in Erlenmeyer flasks and bioreactors, the medium was supplemented with 2.0 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.8 mg l^{-1} 6-(γ,γ-dimethylallylamino) purine (2iP).

Prior to inoculating the bioreactors, the suspensions were sieved through a mesh of 1000 μm, collected in a large flask and mixed thoroughly to obtain a homogenous suspension. This homogenous mixture was used as the inoculum for all bioreactors in the experiment, providing identical starting material and conditions. The cells of the suspension mixture were collected on a 100 μm mesh before being distributed in six identical bioreactors.

2.2. Bioreactor conditions

Six 2.0-l capacity bioreactors (Hvoslef-Eide et al., 2005) were used for batch growth studies. The bioreactors were especially designed for plant cell growth, with low shear forces and gentle agitation to produce as little stress as possible, to give proliferating cultures high viability. To optimise production of PEMs, seven bioreactor experiments were performed. In each of the experiments one or two of the following culture parameters was varied: oxygen level (*O*), temperature (*T*), difference between day and night temperature (DIF, *D*, day temperature minus night temperature) and daily light integral (*L*) (see Table 1). The parameters were constant over time during the entire time series of 28 days. Because of infection and technical failure, experiment 2 was repeated twice and experiment 6 once. The repetitions were labelled experiments 3, 4 and 7. Experiments 1 and 5 were not repeated because of clear results that were in accordance with earlier experiments (Hvoslef-Eide et al., 2003).

Bioreactor vessels containing 1.0 l of modified MS medium with hormones were autoclaved for 20 min at 121 °C (110 kPa), cooled to set point temperature and calibrated for oxygen. The oxygen calibration was done by allowing air without extra oxygen enrichment to flow through the bioreactors for 24 h. The medium was then regarded as fully saturated with oxygen from the air (21% oxygen), and this reading was calibrated to 100% at the given temperature. The oxygen electrode was then disconnected and no reading was calibrated to be 0%. The pH electrode was calibrated prior to sterilisation using standard solutions of pH 4 and 7, and a sample of the autoclaved medium. The pH of this sample was measured with an external pH meter and the reading entered into the computer. After oxygen calibration, vessels were inoculated with 30 g of cells (fraction 100–1000 μm, in total 2% packed cell volume (PCV)) and 0.5 l of autoclaved medium.

Table 1
Growth conditions during the seven experiments[a]

Experiments	Oxygen level (*O*, %)	Temperature (*T*, °C[b])	DIF (*D*, °C)	Light integral (*L*, mol m^{-2} day^{-1})
1	50(2), 100(1[c]), 150 (2)	25/15 (2), 15/25 (2), 20 (1[c])	+10 (2), −10 (2), 0 (1[c])	1.3
2	100	25/15 (2[c]), 15/25 (2), 20 (2[c])	+10 (2[c]), −10 (2), 0 (2[c])	1.3
3	100	25/15 (1), 15/25 (1), 20 (2)	+10 (1), −10 (1), 0 (2)	1.3
4	100	25/15 (2[c]), 15/25 (2), 20 (2)	+10 (2[c]), −10 (2), 0 (2)	1.3
5	100	15 (2), 20 (2), 25 (2)	0	1.3
6	100	20	0	0 (2), 1.3 (2[c]), 2.6 (2)
7	100	20	0	0 (3[c]), 1.3 (3)

[a] The digit in parenthesis represents the number of bioreactors in that treatment.
[b] Temperature 25/15 means that the day temperature was 25 °C and the night temperature 15 °C.
[c] Indicates infection or technical failure in one bioreactor, which led to exclusion of that bioreactor.

Bioreactor cultures were incubated as described in Table 1. The four different growth factors were observed at three levels: oxygen level (50, 100 and 150%), temperature (15, 20 and 25 °C), DIF (+10, 0 and −10) and daily light integral (0, 1.3 and 2.6 mol m^{-2} day^{-1}). Stirring speed was set to 30 rpm and the stirring direction was changed every 10 s to obtain gentle agitation with no settlement of cells in quiet zones. DIF treatments were correlated with illumination (OTT bioLIGHTSYSTEMS Inc., Santa Barbara, CA, United States, red/far-red ratio 2.7–2.8). Since cyclamen is a day-neutral species (Moe and Heins, 2000), a day length of 12 h (1.3 mol m^{-2} day^{-1}) was used during alternating diurnal conditions, after Heo et al. (2003). Alternating light conditions were compared with complete darkness (0 mol m^{-2} day^{-1}) and a 24 h photoperiod (2.6 mol m^{-2} day^{-1}).

A two-step-system of proembryogenic mass (PEM) proliferation and subsequent embryo formation (Schwenkel and Winkelmann, 1998) was used in this study. According to Schwenkel and Winkelmann (1998), this protocol results in high regeneration rates, low levels of somaclonal variation, and can be applied to many genotypes.

2.3. Sampling

One sample (75 ml) was removed from each bioreactor at days 0, 7, 14, 21 and 28 after start-up. All samples were analysed in three (experiments 1–5) or five (experiments 6 and 7) replicates with respect to PCV, fresh and dry weight, cell viability and pH. The cells' ability to germinate into somatic embryos was also investigated. This paper focuses on the biomass growth (dry weight) and cell viability.

Dry weight was found after vacuum filtration of 5 ml samples and drying at 70 °C over night. Cell viability was measured using the conventional 2,3,5-triphenyltetrazolium chloride (TTC) assay (Bennett and Loomis, 1949; Towill and Mazur, 1975), as absorbance (490 nm)/mg fresh weight, according to Harding and Benson (1995). Cells were incubated at 28 °C overnight in 0.5% TTC in 0.05 M Na_2HPO_4/KH_2PO_4 buffer with pH 7.4 and 0.05% Tween 80. The red formazan was extracted with 96% ethanol by boiling in a water bath for 5 min, and absorbance of the extract was read at 490 nm using a spectrophotometer (Helios Alpha, Unicam UV–vis Spectrometry).

Gas concentration (O_2 and CO_2) in the headspace was measured off-line by a gas chromatograph (CHROMPACK, Micro GC CP-2002 P, Middelburg, The Netherlands) every week during the experiment, to control the growth conditions. In addition the pH and O_2 concentration in the suspension were measured on-line with autoclavable electrodes.

2.4. Statistical analysis

For both biomass growth and cell viability a response surface modelling approach has been adopted, where the biomass growth rate and the cell viability are considered response surfaces over the four covariates describing the bioreactor conditions (*O*, *T*, *D* and *L*, see Table 1). Response surface models are standard tools for estimating the optimal settings in an industrial production (Khuri and Cornell, 1996). Statistical analysis were performed in Matlab (www.mathworks.com).

2.4.1. Biomass growth

All experiments have been conducted during the first exponential phase of growth, i.e. experiment i in bioreactor j gives:

$$\omega_{t,i,j} = \omega_{o,i,j} \exp(\phi_{i,j} t) \quad (1)$$

where $\omega_{t,0i,j}$ is the biomass at day t from start day 0, and $\phi_{i,j}$ is some growth rate. Using the transform $\mu_{t,i,j} = \log(\omega_{t,i,j})$ gives:

$$\mu_{t,i,j} = \mu_{o,i,j} + \phi_{i,j} t \quad (2)$$

It is assumed that the growth rate can be described by a linear model:

$$\phi_{i,j} = \mathbf{a}_{i,j}\boldsymbol{\theta} \quad (3)$$

where the $(1 \times p)$ row vector $\mathbf{a}_{i,j}$ is the explanatory variable derived from the covariates for experiment i in bioreactor j, and θ is a $(p \times 1)$ vector of unknown parameters. Since each of the four covariates have been varied over three different levels in the data set, the response surface model includes all possible terms up to second-order (quadratic terms and interactions between two and two covariates).

Assembling all biomass data for the time series of experiment i in bioreactor j gives, using Eqs. (2) and (3):

$$\boldsymbol{y}_{i,j} = \mu_{o,i,j}\mathbf{1}_{i,j} + \mathbf{t}_{i,j}\boldsymbol{a}_{i,j}\boldsymbol{\theta} + \boldsymbol{e}_{i,j} \quad (4)$$

where $\mathbf{y}_{i,j}$ is all observed log biomasses, $\mathbf{1}_{i,j}$ is a column with the appropriate number of ones, $\mathbf{t}_{i,j}$ is a column vector of sampling times for each observation and $\mathbf{e}_{i,j}$ is a stochastic error term.

2.4.2. Cell viability

Unlike biomass, cell viability cannot be expected to follow some specified function in time. However, for a set of experimental conditions cell viability must be expected to be some continuous function in time, and a polynomial is used to approximate this. For sample day t in experiment i in bioreactor j the cell viability $\gamma_{t,i,j}$ is

$$\gamma_{t,i,j} = \sum_{k=1}^{q} \mathbf{a}_{i,j}\boldsymbol{\theta}_k \mathrm{t}^k \quad (5)$$

where the polynomial coefficients $\mathbf{a}_{i,j}\theta_k$ are linear combinations of the covariate terms $\mathbf{a}_{i,j}$ as in Eq. (3), except that here there is one linear combination for each polynomial degree.

2.4.3. Model fitting

Only the parameters θ, and θ_k for cell viability are of interest, since they describe the effects of the covariates. The remaining parameters can be seen as nuisance parameters essential in describing the data, but of little interest to this study.

It is highly unlikely that all possible terms, especially higher order terms from Eqs. (4) and (5), reflect significant effects in our data set; hence it is necessary to implement a model selection procedure. A sub-model is any model where some (from none to all) of the covariate terms are excluded. All sub-models that can be restricted using the principle of functional marginality (Peixoto, 1987) have been considered. This means any higher order term is only used as long as its first-order terms are also present in the model. To select the best sub-model, Schwarz's Bayesian information criterion (BIC) has been used, where the sub-model with the smallest BIC score is the optimal (Schwarz, 1978). The BIC score requires a maximum likelihood estimate of all parameters, which is straightforward from (4) or (5) under the assumption of Gaussian independent noise.

2.4.4. Optimal conditions

The optimal conditions are the combination of covariates *O*, *T*, *D* and *L* that maximizes either the biomass growth rate or cell viability, given the optimal sub-models and their estimated parameters θ^* and θ_k^*, $k = 1, \ldots, q$. Let $c = (O, T, D, L)$ represent one set of covariate values. The combination of covariates that maximizes the growth rate from (4) or cell viability from (5) using

the estimated parameters is denoted $\mathbf{c}^*$. This value is restricted so that it is within the span of the data set. Note that for cell viability the optimal point in time must also be found in addition to the other covariates.

These optima clearly depend on parameter estimates; hence they are influenced by the randomness of data. To illustrate the uncertainties a bootstrap approach has been employed. For each bootstrap sample a corresponding optimum was found by re-estimating the parameters for each sample, using the optimal sub-model in every case.

3. Results and discussion

3.1. Biomass growth

The optimal sub-model for biomass growth included the first-order term for all four covariates and the quadratic terms of *L* and *D*, but no interactions. Since there are only first-order effects of *O* and *T*, these covariates will have their optimum at either the upper or lower limits, depending upon the sign of the corresponding parameter. Their estimated effects from the data are shown in Fig. 1.

The effect of varying the oxygen concentration from 50 to 150% on biomass growth can be described using a linear model. The data showed a positive linear effect from oxygen, resulting in 150% as the optimal value for growth (Fig. 1). In 5% of the bootstrap samples the effect of oxygen was negative, with a corresponding optimum at its lower limit $O = 50$. In experiments in Applikon bioreactors with the same cell line of cyclamen, Hohe et al. (1999a) observed a higher growth rate at 50 and 100% oxygen compared to 25 and 200% (data converted from 10, 20, 5 and 40% oxygen partial pressure, as the calibration was performed with pure O_2 by this working group). Tate and Payne (1991) showed that the growth rate of suspension cultures of *Catharanthus roseus* was reduced below 50% and above 350% O_2 (data converted from 10 and 70% oxygen partial pressure, as the calibration was performed with pure O_2). De Feria et al. (2003) produced a higher number of cell aggregates in cell suspensions of *Coffea arabica* when using 80% dissolved oxygen compared to 50%. As reviewed by Preil (1991), oxygen may be a parameter whose optimum concentration can vary significantly among different species. Our results are consistent with the studies mentioned above, considering our limited concentration interval.

For our suspension cultures in bioreactors, 25 °C was optimal for biomass growth (Fig. 1). This result is in accordance with earlier experiments with the same culture (Hvoslef-Eide et al., 2003) and with the recommendations of 24 °C given by Winkelmann et al. (1998). The bootstrap analysis indicates that there was a stable positive linear effect of temperature on growth, i.e. the optimal temperature was $T = 25$ for every case of the 1000 bootstrap samples. This means that temperatures higher than 25 °C might give even higher biomass growth. However, temperatures above 20.8 °C appear to be less favourable for viability (Fig. 5). In general, 25 °C is regarded as a high temperature for cyclamen. This fact has been documented by Oh et al. (2007) and Park et al. (2007), whom considered 32 °C to be heat stress and 12 °C to be a low temperature for *C. persicum* cv. Metis Scarlet Red.

An unconstrained optimum can only be present if there are some second-order terms with corresponding negative parameter estimate. This was the case for *L*, while the quadratic term of *D* had a small but positive estimated parameter. The estimated effects of these two covariates are illustrated in Fig. 2.

For our cell suspensions, positive DIF (+10) gave the highest biomass growth (Fig. 2), which shows that cell cultures were able to respond to DIF. For *D* the quadratic term was positive, which gave a maximum effect at the limits. In other words, DIF seemed to be more optimal for cell proliferation than constant temperature, whether positive or negative (Fig. 2). In 75% of the cases its optimum was the upper limit $D = 10$ and in 25% of the cases, at the lower limit $D = -10$. The fact that both positive and negative DIF gave similar responses may be due to the heterotrophy of the cultures and the undifferentiated cells. It has been shown that a wide range of species among short-day plants, long-day plants and day-neutral plants respond very strongly and similarly to DIF (Moe and Heins, 2000). However, the physiological basis of the DIF response is still poorly understood (Thingnaes et al., 2003). DIF exerts its growth effects via two basic cellular processes, cell division and cell elongation (Strøm and Moe, 1997); gibberellin and auxin might affect both of these processes (Thingnaes et al., 2003; Stavang et al., 2005). Light intensity and light quality may also influence the response (Moe and Heins, 2000; Bachman and McMahon, 2006). Active phytochrome, P_{fr}, seems to be required for proper negative DIF response and far red light seems to increase tissue sensitivity to gibberellic acid or gibberellic acid metabolism (Moe and Heins, 2000; Patil et al., 2003). Recently it has been suggested that transcriptional regulation of gibberellic acid-deactivation is an important mechanism mediating thermoperiodic stem elongation, and that light is required for the

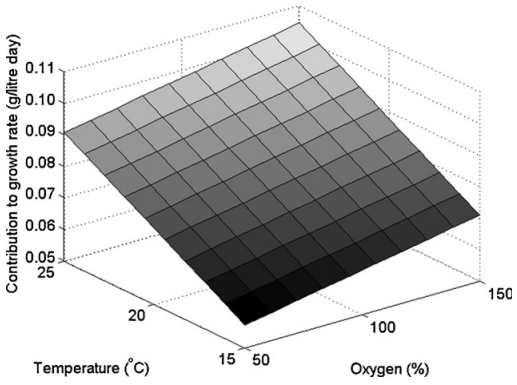

Fig. 1. Estimated effects of daily mean temperature (*T*, °C) and oxygen concentration (*O*, %) on biomass growth (g l^{-1} day^{-1}). The values for optimal growth are 25 °C and 150% oxygen.

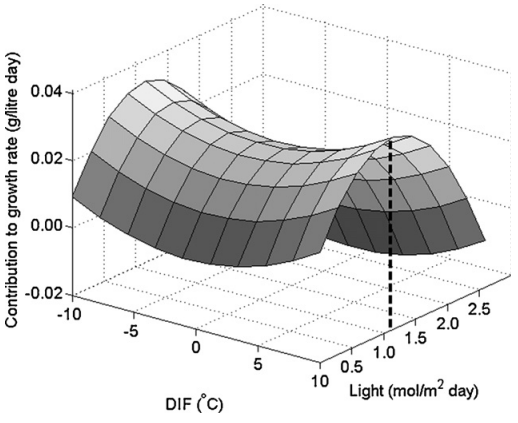

Fig. 2. Estimated effects of daily light integral (*L*, mol m^{-2} day^{-1}) and DIF (*D*, °C) on biomass growth (g l^{-1} day^{-1}). The values for optimal growth are 1.11 mol m^{-2} day^{-1} and DIF + 10.

deactivation of gibberellin (Stavang et al., 2005, 2007). During DIF treatments in this study, the cultures were grown under low irradiance of 1.3 mol m^{-2} day^{-1} (R/FR photon ratio of 2.7–2.8). This seemed to be a sufficient light intensity and quality for increasing cell division.

The only covariate with an unconstrained maximum was light. The optimal model constructed in this study suggests a daily light integral between 1.03 and 1.19 mol m^{-2} day^{-1} (Fig. 2). In 90% of the bootstrap cases the optimal value for *L* was within this interval. This means about a 12-h photoperiod with the OTT bioLIGHTSYSTEMS. Complete darkness, as well as double the amount of light gave less cell growth. This result may indicate that the light saturation point of this cyclamen suspension culture was about 1.10 mol m^{-2} day^{-1}. Most cell cultures are kept in the dark (Hvoslef-Eide, 2000) and it has been reported that light inhibits callus growth of cyclamen (Geier et al., 1990; Karam and Al-Majathoub, 2000). On the other hand, Jacques et al. (2007) recently reported the highest biomass productivity of *Panax vietnamensis* in bioreactors under continuous light. Light quality influences both the callus production of herbaceous and woody plant species (Hvoslef-Eide and Sæbø, 1991) and embryo formation in *C. persicum* (Hvoslef-Eide and Munster, 1998). Red and far red light enhances callus growth, callus quality and enhances suspension establishment, while blue light inhibits the required development compared to darkness (Hvoslef-Eide and Sæbø, 1991). Hvoslef-Eide (2000) proposed that it is blue light that inhibits callus production by promoting cell differentiation instead. The fact that the biomass growth increased at 1.10 mol m^{-2} day^{-1} compared to darkness in our study may be explained as a hormone effect. According to the light quality definitions used by Kurepin et al. (2007), the red/far red ratio of 2.7–2.8 is regarded as normal to high and therefore provides a relatively high amount of red light. Red light was reported to stimulate the formation of an IAA oxidation inhibitor (Hillman and Galston, 1957; Mumford et al., 1961) and will therefore favour cell growth. The effect was reversed by near infrared light (Hillman and Galston, 1957). Blue light may also enhance IAA destruction (Chee, 1986). The light source used in this study, OTT bioLIGHTSYSTEMS, has a light spectrum as close to natural daylight as possible. It is possible that the amount of blue light reaches a threshold at the highest irradiance and may be the reason why the highest amount of light, 2.6 mol m^{-2} day^{-1}, resulted in reduced growth compared to 1.10 mol m^{-2} day^{-1}. The highest light intensity may also be above the saturation point, and therefore detrimental to the cells.

3.2. Cell viability

For cell viability, the first-order and quadratic term for covariates *O*, *T* and *L* were included in the optimal model, i.e. there was no significant effect of *D*, and no interactions. The optimal time had to be sought together with the optimal conditions for the bioreactors and a third-degree polynomial in time was optimal. The data show that the optimal time in the bioreactors was 11.3 days, with the following corresponding optimum values for the covariates: *O* = 90, *T* = 20.8 and *L* = 1.10 (Fig. 3).

Fig. 3 shows a sampling of histograms for the optima found from the 1000 bootstrap samples, using the optimal model. Clearly, the optimal time in the bioreactors splits into two separate groups. In the majority of cases (829 of 1000) the optimal time was around 10–11 days (black bars), while for the remaining data sets the optimum was somewhere between 20 and 28 days (grey bars). According to Hohe et al. (2001), the optimal period of liquid culture for globular embryo production is 3 weeks. This type of culture is undertaken in a medium where hormones are removed to induce somatic embryos. In such a culture, cell growth slows and cell

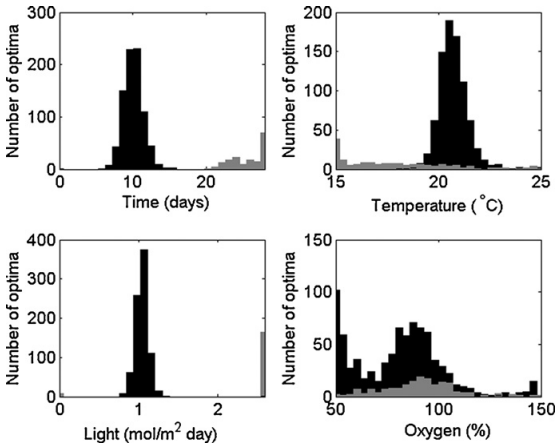

Fig. 3. Distribution of maximum cell viability for time (days), temperature (°C), light (mol m^{-2} day^{-1}) and oxygen (%) using the bootstrap approach (1000 bootstrap samples). Black bars indicate cases where maximum cell viability was reached in an early stage (before 16–18 days) and grey bars indicate a late stage (see upper left panel).

differentiation begins, and hence cell density does not increase. In our cultures reported here, the cells were grown in a medium containing hormones at all times. In these types of batch cultures, cell growth will usually follow an exponential curve, with the decrease in growth probably caused by exploitation of the medium, as shown by Huang et al. (1993) in somatic embryos of *Daucus carota*. Normally, bioreactor batch cultures are diluted every week. With regard to cell viability, our data sets gave an optimal time of 10–11 days. This shows that there is not only the potential to save labour costs by waiting longer than the normal 7 days, but that the cultures will also have increased viability.

The other three histograms in Fig. 3 show how the corresponding optima for the covariates varied. The majority group indicates that *T* and *L* have stable optima. In 90% of these cases in this group, *T* was between 19.9 and 22, and *L* was between 0.96 and 1.22. For *O* the optimum was more unstable, but in most cases it was below *O* = 100.

Air saturation of 90% seems to be optimal for cyclamen cell viability (Fig. 4). This response is consistent with results of Hohe et al. (1999a), who observed highest viability at 100% oxygen for *C.*

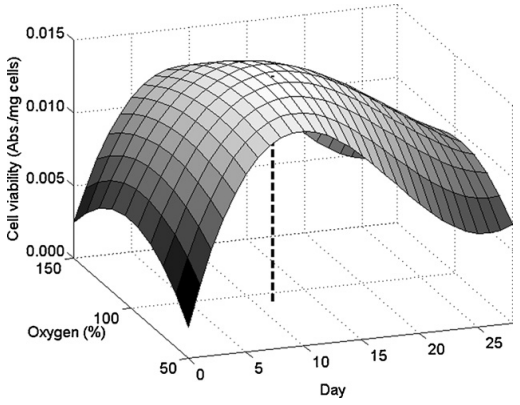

Fig. 4. Estimated effects of oxygen (*O*, %) on cell viability (Abs., mg $cells^{-1}$). The maximum is found after 11.3 days, with 90%.

persicum Mill. line 3738-VIII (data converted from 20% oxygen partial pressure, as the calibration was performed with pure O_2). They also found that 200% O_2 (data converted from 40%, as the calibration was performed with pure O_2) was detrimental to cell proliferation and cell viability, and attributed this result to the medium acidification due to the pH-control. Our maximum, found using the optimal model, was in most cases below 100%, and 150% O_2 was detrimental to viability (Fig. 3). Suppressed viability at the highest concentration may be explained as the effect of toxicity. Medium acidification did not occur in our study (data not shown). Shear damage as a consequence of high gas supply may explain reduced growth or viability in bioreactors with bubble aeration systems. In our bioreactors, oxygen was provided bubble-free through thin silicone tubing loops, so that the shear damage was minimized and very little cell debris was observed.

Within the temperature range for this study, the biomass growth increased with increasing temperature, but temperatures higher than 20.8 °C were not favourable regarding cell viability (Fig. 5). This is in accordance with a study performed by Hohe et al. (2001), where 24 °C was used for cell growth and 18 °C for regeneration into embryos. This result is also similar to that obtained by Neveur et al. (1986), who reported that seed germination of *C. persicum* was optimal at 15 °C and did not occur above 20 °C. From these studies it appears that the optimal temperature for biomass growth is higher than for viability and germination. This shows that cell cultures respond much in the same way as whole plants with regards to temperature.

A daily light integral between 1.03 and 1.19 mol m^{-2} day^{-1} gave higher cell viability than complete darkness and double the amount of light (Fig. 6), as was found with biomass growth. This is in contrast to the results of Cheon et al. (2006), who found that a high photosynthetic photon flux (17.3 and 25.9 mol m^{-2} day^{-1}) was required to promote growth, flower initiation and development of *C. persicum* 'Metis Scarlet Red'. This shows that greenhouse crops of cyclamen tolerate and need more light than cell cultures, possibly because a plant is a complex structure of differentiated cells that require energy for transport and metabolism in addition to just cell growth. The minority group (171 out of 1000 cases), which had an optimum towards the end of the experiment, achieved this optimum with either maximum or minimum *L*. Temperature and oxygen seemed to be unimportant in this case, and took on almost any value. The reason for this effect can be seen in Fig. 6. The estimated response to light had some very non-linear effects near the end of the time series. It might be that the cells need more light after a long period of culture, as the biomass increases and the cells block each other's light. It is difficult, however, to understand why darkness would result in better viability than 1.10 mol m^{-2} day^{-1}, the maximum found after 11.3 days. This, or these other non-linear effects may have arisen from an artefact in our data.

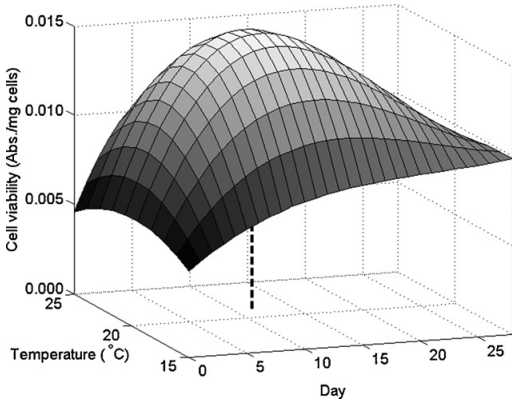

Fig. 5. Estimated effects of temperature (*T*, °C) on cell viability (Abs., mg $cells^{-1}$). The maximum is found after 11.3 days, with 20.8 °C.

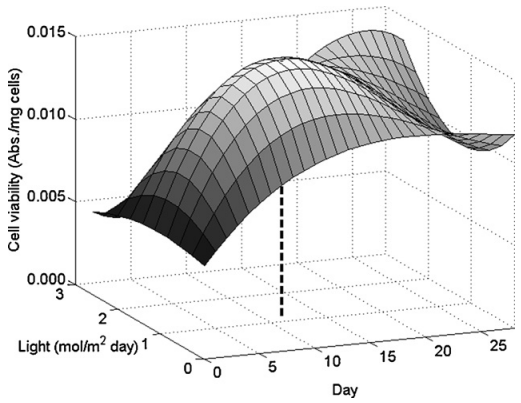

Fig. 6. Estimated effects of light (*L*, mol m^{-2} day^{-1}) on cell viability (Abs., mg $cells^{-1}$). The maximum is found after 11.3 days, with 1.10 mol m^{-2} day^{-1}.

As part of improving the commercial propagation method for cyclamen, more attention should be paid to the subsequent embryo formation. The process format used also requires attention. Lipsky (1992) reported that the multicycle or draw–fill culture technique gives an increase of 1.5–2 in biomass productivity compared with a batch culture. Such a system has been considered to be one of the most economical culture methods (Lipsky, 1989) and has been reported for a number of cultures (Lipsky, 1992). According to Eibl and Eibl (2006), disposable wave bioreactors should also be considered for embryogenic cultures.

4. Conclusion

It is clear that cell viability, and as a consequence embryo production, can be influenced markedly by the changing culture environment in batch cultures. Maximum biomass growth was achieved with 25 °C, 150% oxygen, 1.11 mol m^{-2} day^{-1} and positive DIF (+10) for the bioreactor system used in this study. The optimal conditions for cell proliferation as the first step in development of embryos are probably those that give the highest viability. To obtain maximum viability for the bioreactor system employed in our experiments, the duration of bioreactor culture should be about 11 days, with a temperature of 20.8 °C, oxygen concentration of 90% and a daily light integral of 1.10 mol m^{-2} day^{-1}.

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The authors would like to acknowledge cooperation with the European Union through COST822 and the gift of a *C. persicum* Mill. line 3738 cell culture from Dr. Hans-Georg Schwenkel, Erfurt, Germany. We also acknowledge invaluable assistance with the growth of cyclamen cultures over the years from Tone I. Melby, a technician at the Plant Cell Laboratory, as well as from Tom Ringstad, a sectional engineer at Norwegian University of Life Sciences, who has been invaluable with his technical support at all times. We are grateful to Lisbeth Mehli, associate professor at Sør-Trøndelag University College, for her critical reading of the

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CORRECTIONS TO PAPER I

During the processing of the pdf-file by the Springer Correction Team some errors were accidentally introduced into the text, specifically in Section 2.4, Statistical analyses (page 55):

2.4.1, line 4: "where $\omega_{t,0i,j}$ is the biomass at day t from start day 0, and ϕ_{ij} is some"
should read "where $\omega_{t,i,j}$ is the biomass at day t from start day 0, and $\phi_{i,j}$ is some"

2.4.1, line 10: "where the $(1 \times p)$ row vector $\mathbf{a}_{ij}$ is the explanatory variable derived"
should read "where the $(1 \times p)$ row vector $\mathbf{a}_{i,j}$ is the explanatory variable derived"

2.4.1, line 11: "from the covariates for experiment i in bioreactor j, and θ is a"
should read "from the covariates for experiment i in bioreactor j, and $\boldsymbol{\theta}$ is a"

2.4.1, line 20-22: "where $\mathbf{y}_{ij}$ is all observed log biomasses, $\mathbf{1}_{ij}$ is a column with the appropriate number of ones, $\mathbf{t}_{ij}$ is a column vector of sampling times for each observation and $\mathbf{e}_{ij}$ is a stochastic error term."
should read "where $\mathbf{y}_{i,j}$ is all observed log biomasses, $\mathbf{1}_{i,j}$ is a column with the appropriate number of ones, $\mathbf{t}_{i,j}$ is a column vector of sampling times for each observation and $\mathbf{e}_{i,j}$ is a stochastic error term."

2.4.2, line 7-8: "where the polynomial coefficients $\mathbf{a}_{ij}\theta_k$ are linear combinations of the covariate terms $\mathbf{a}_{ij}$ as in Eq. (3), except that here there is one"
should read "where the polynomial coefficients $\mathbf{a}_{i,j}\boldsymbol{\theta}_k$ are linear combinations of the covariate terms $\mathbf{a}_{i,j}$ as in Eq. (3), except that here there is one"

2.4.3, line 1: "Only the parameters θ, and θ_k for cell viability are of interest,"
should read "Only the parameters $\boldsymbol{\theta}$, and $\boldsymbol{\theta}_k$ for cell viability, are of interest,"

2.4.4, line 4: "parameters θ^* and θ_k^*, $k = 1, ..., q$. Let $c = (O, T, D, L)$ represent"
should read "parameters $\boldsymbol{\theta}^*$ and $\boldsymbol{\theta}_k^*$, $k = 1, ..., q$. Let $\boldsymbol{c} = (O, T, D, L)$ represent"

Paper II

Is not included due to copyright

Paper III

Is not included due to copyright

Appendix 1

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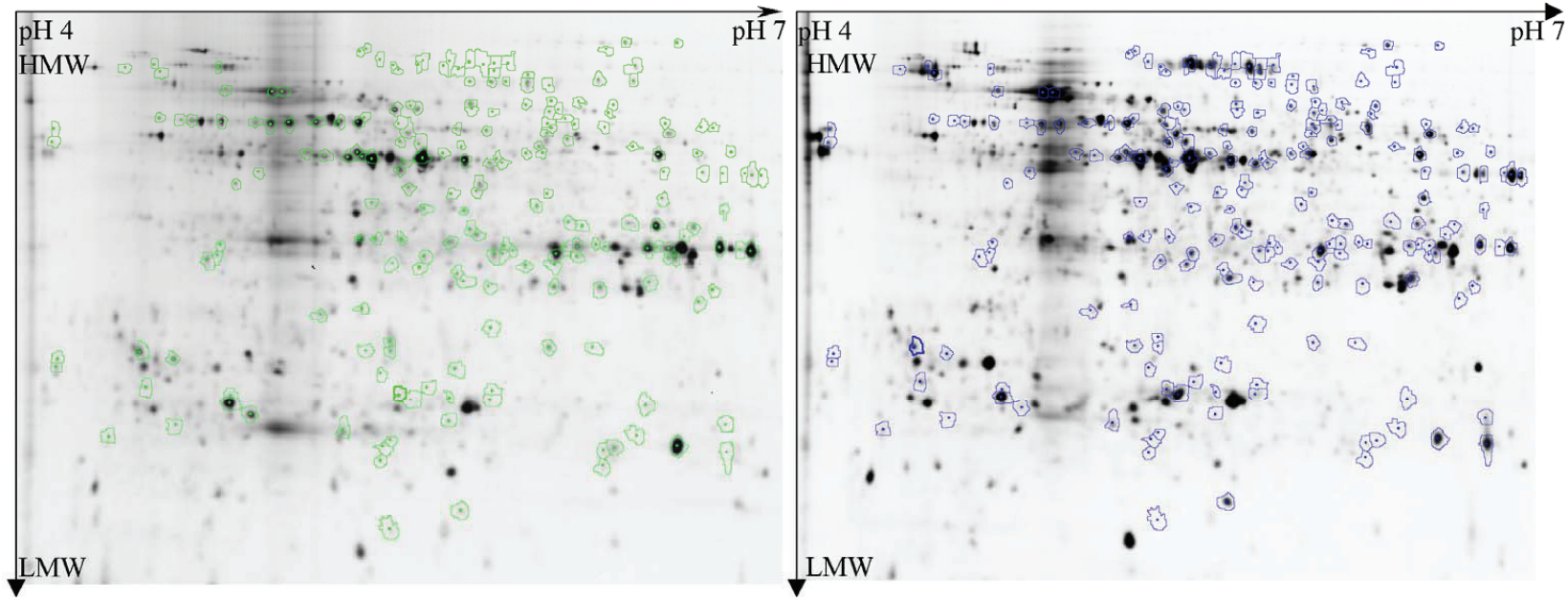
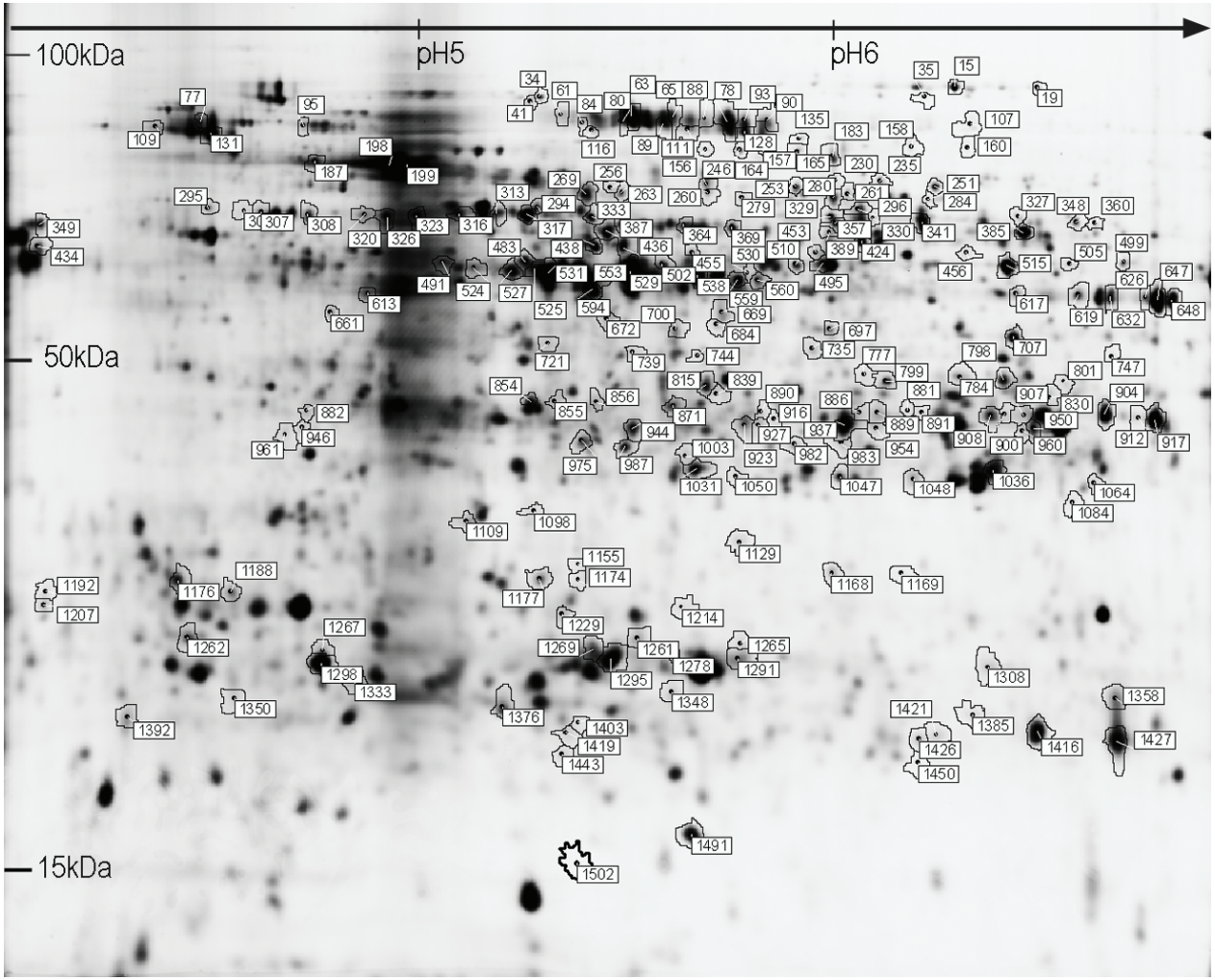
Appendix 2

Figure 3, Paper II
Bidimensional gels on a pH 4-7. The left shows an example image from the embryogenic cells, while the right shows an example from the non-embryogenic cells.



Supplementary Figure

Bidimensional gel with spot numbers. Circled spots were differentially expressed in embryogenic and non-embryogenic cells. These spots were picked from the gel and analyzed using mass spectrometry. Protein identification data are presented in the Supplementary Table (Appendix 3).



Appendix 3

Supplementary Table
Results from protein identification and database search

PART I Spots with increased abundance in embryogenic cells (E). Blue: identified, Yellow: not identified.

Spot	Ratio	t-test	GI accession	Name	Species	Number of peptide matched	Sequences of ions (Score)	Estimated pI	Estimated Mr	Theoretical pI	Theoretical Mr	Sum of ions scores	Protein score (+manual ion score)
95	**-2,88**	**4,3E-05**	38154489	molecular chaperone Hsp90-1	*Lycopersicon esculentum*	38	K.RAPFDLFDTK.K (48); K.ADLVNNLGTIAR.S (38); K.HFSVEGQLEFK.A (67); K.GIVDSEDLPLNISR.E (74); R.KPEEITKEEYAAFYK.S (116)	4,68	82	4,98	80,16	343	456
107	**-3,89**	**1,2E-04**						6,38	82				
135	**-2,16**	**5,5E-05**	52353232	putative metallophosphatase	*Lupinus luteus*	8	K.SSPYPGQDSLQR.V (33); R.SPAGTLTFTR.N(6); R.DPGFIHTSFLR.G (22);	5,94	80	6,10	71,00	61	(61)
156	**-2,45**	**8,1E-06**						5,70	78				
157	**-4,48**	**1,0E-06**	4586600	transketolase	*Cicer arietinum*	15	K.ANSYSVHGAALGAK.E (33); R.KKLPNLPGTSIEGVEK.G (27); K.ALPTYTPETPADATR.N (25)	5,85	78	5,85	17,13	85	55 (+58)
158	**-3,29**	**3,9E-05**						6,23	78				
160	**-4,39**	**1,5E-06**						6,37	78				
164	**-2,10**	**9,0E-05**	38154489	molecular chaperone Hsp90-1	*Lycopersicon esculentum*	17	K.RAPFDLFDTK.K (11); R.KPEEITKEEYAAFYK.S (40); K.EKFEGLCKVIK.D (20)	5,79	78	4,98	80,16	71	77 (+20)
165	**-4,44**	**5,4E-06**	21664287	heat shock protein 70	*Oryza sativa (indica cultivar-group)*	18	R.TTPSYVGFTDSER.L (11); K.NAVVTVPAYFNDSQR.Q (29); R.IINEPTAAAIAYGLDKK.A (57)	5,94	77	5,17	71,00	97	129
246	**-1,85**	**5,0E-05**	15221564	stress-inducible protein, putative	*Arabidopsis thaliana*	8	K.AIELDDEDISFITNR.A (88); K.ELEQKEYFDPK.L (53); R.GDLTPEELKER.Q (15)	5,71	72	5,85	64,52	156	274

256	**-1,59**	**3,7E-05**	544242	Endoplasmin homolog precursor (GRP94 homolog)	*Hordeum vulgare*	20	K.FWNEFGK.S + kynurenin (W) (22); K.GLVDSDTLPLNVSR.E (22); R.ELISNASDALDKIR.F (15); R.NSAEKFEFQAEVSR.L (29);	5,46	71	4,86	92,92	88	79 (+29)
263	**-2,42**	**3,1E-05**	3914394	2,3-bisphosphoglycerate-independent phosphoglycerate mutase (Phosphoglyceromutase) (BPG-independent)	*Mesembryanthemum crystallinum*	28	K.FGHVTFFWNGNR.S + kynurenin (W) (39); K.ALEYEDFDKFDR.V (86); R.HYLVSPPEIDR.T (46); K.AVGLPTEDDMGNSEVGHNALGAGR.I (39); K.IQILTSHTLEPVPIAIGGPGLAPGVR.F (85)	5,49	70	5,39	61,18	295	231 (+131)
269	**-1,69**	**2,4E-05**						5,40	70				
295	**-1,50**	**6,4E-04**	461736	Chaperonin CPN60-2, mitochondrial precursor (HSP60-2)	*Cucurbita maxima*	24	R.NVVIEQSYGAPK.V (68); K.CELDDPLILIHEK.K (86); R.GYISPYFITNTK.N (73); K.TLYNELEVVEGMKLDR.G (20); R.MISTSEEIAQVGTISANGER.E (53); K.QVANATNDVAGDGTTCATVLTR.A(17);	4,44	67	6,28	61,13	317	266 (+110)
305	**-2,99**	**9,2E-05**						4,53	67				
307	**-2,46**	**1,4E-07**	15238328	SCPL42 (serine carboxypeptidase-like 42); serine carboxypeptidase	*Arabidopsis thaliana*	7	R.ALHLFSSFVR.G (56); R.GAAHMVPYAQPSR.A (17)	4,58	67	6,60	52,87	73	89

308	**-2,18**	**4,8E-05**	461836	Chaperonin CPN60-2, mitochondrial precursor (HSP60-2)	*Cucurbita maxima*	23	R.NVVIEQSYGAPK.V (43); R.GYISPYFITNTK.N (36); K.AAVEEGIVPGGGVALLYATK.E (43); K.QVANATNDVAGDGTTCATVLTR.A (1)	4,70	67	6,28	61,13	123	102 (+79)
313	**-2,32**	**7,1E-05**	24637539	heat shock protein 60	*Prunus dulcis*	27	R.NVVIEQSWGAPK.V (2); K.TLYNELEVVEGMK.L (12); R.AAVEEGIVPGGGVALLYATK.E (64); K.TLYNELEVVEGMKLDR.G (2); R.MISTSEEIAQVGTISANGER.E (2); R.SSIELSTSDYDKEKLQER.L (33); K.QRPLLIVAEDVESEALATLIINK.L (12);	5,18	67	5,26	57,76	127	154 (+66)
316	**-2,61**	**7,8E-05**	683502	protein phosphatase 2A 65 kDa regulatory subunit	*Arabidopsis thaliana*	19	R.LAAGEWFTAR.V + kynurenin (W) (52); R.YMVANQLYELCEAVGPEPTR.T (25); K.LLEPQDCVQHILPVIVNFSQDK.S (75); K.TIRPSLVELAEDPDVDVR.F (56)	5,08	67	4,94	65,58	208	183 (+131)
317	**-1,59**	**1,7E-04**						5,25	67				
320	**-1,82**	**3,3E-05**	24637539	heat shock protein 60	*Prunus dulcis*	25	R.NVVIEQSWGAPK.V (1); K.TLYNELEVVEGMK.L (12); R.AAVEEGIVPGGGVALLYATK.E (79); K.TLYNELEVVEGMKLDR.G (24); R.MISTSEEIAQVGTISANGER.E (42); K.QRPLLIVAEDVESEALATLIINK.L (45);	4,84	67	5,26	57,76	203	189 (+122)
323	**-2,76**	**4,9E-04**	15225438	malic enzyme/ oxidoreductase, acting on NADH or NADPH,	*Arabidopsis thaliana*	14	R.LHDRNETMYYK.V (10); K.IVVAGAGSAGIGVLNAAR.K (23); K.LLKDPLYLGLQEHR.L (41); R.WPHVIVQFEDFQSK.W (55); R.MFNDDVQGTAGVAIAGLLGAVR.A (32)	4,97	67	5,31	69,66	161	134 (+41)

				NAD or NADP as acceptor									
326	**-1,92**	**3,4E-05**	226027	acetolactate synthase SuRB	*Nicotiana sp.*	7	K.HNYLVLNVEDIPR.I (60); R.MIGTDAFQETPIVEVTR.S (28)	4,90	67	6,67	72,43	88	99
389	**-1,62**	**2,1E-04**	2554835	Chain I, Acetohydroxy Acid Isomeroreductase Complexed With Nadph, Magnesium And Inhibitor Ipoha (N-Hydroxy-N-Isopropyloxamate).	*Spinacia oleracea*	12	R.DLFPLLPDAFK.G (66); R.VNLAGHEEYIVR.G (20); R.GGRDLFPLLPDAFK.G (45); R.GVSFMVDNCSTTAR.L (16); K.EKINLAGHDEYIVR.G (51); K.EVNGAGINSSFAVHQDVDGR.A (146)	6,02	64	5,52	56,99	344	166 (+236)
453	**-1,88**	**6,6E-04**						5,99	61				
483	**-2,53**	**4,6E-06**	145328282	ALDH6B2 (Aldehyde dehydrogenase 6B2)	*Arabidopsis thaliana*	5	K.ASFAGDLNFYGK.A (55); K.NHGVVLPDANVDATLNALLAAGFGAAGQR.C (81)	5,25	60	5,71	53,41	136	(136)
491	**-1,88**	**2,2E-04**	15233891	(VACUOLAR ATP SYNTHASE SUBUNIT B2); hydrogen ion transporting ATP synthase, rotational mechanism	*Arabidopsis thaliana*	35	K.YQEIVNIR.L (39); R.QIYPPINVLPSLSR.L (61); R.VTLFLNLANDPTIER.I (74); R.GYPGYMYTDLATIYER.A (16); K.IPLFSAAGLPHNEIAAQICR.Q (95); K.AVVGEEALSSEDLLYLEFLDKFER.K (112); R.IFNGSGKPIDNGPPILPEAYLDISGSSINPSER.T (50)	5,04	60	5,03	54,30	447	619

505	**-1,96**	**7,3E-06**	48093459	putative UDP-glucose dehydrogenase 2	*Nicotiana tabacum*	22	K.LAANAFLAQR.I (37); K.AADLTYWESAAR.M (23); K.FLNASVGFGGSCFQK.D (51); K.CPSIEVAVVDISVPR.I (33); K.ISIYDPQVTEDQIQR.D (34); K.GINFQILSNPEFLAEGTAIEDLFKPDR.V (26)	6,63	60	5,92	58,24	204	191 (+34)
515	**-1,60**	**9,4E-06**	15228319	ALDH2B4 (ALDEHYDE DEHYDROGENASE 2); aldehyde dehydrogenase/ oxidoreductase	*Arabidopsis thaliana*	*16*	K.GIYSLNNYLQIK.A (73); R.EKGIYSLNNYLQIK.A (74); R.TGEVIAHVAEGDAEDINR.A (120)	6,47	59	7,11	58,59	267	175
524	**-3,29**	**7,1E-04**						5,12	59				
525	**-2,44**	**1,6E-04**						5,27	59				
527	**-1,95**	**1,3E-05**						5,21	59				
529	**-1,88**	**5,8E-05**	17402533	UDP-glucose pyrophosphorylase	*Nicotiana tabacum*	14	K.SGFINLVAR.Y (45); R.LVIDDFLPLPSK.G (52); K.VQLLEIAQVPDEHVNEFK.S (89); K.YANSNIEIHTFNQSQYPR.L (123)	5,51	59	7,12	41,03	309	244 (+97)
530	**-3,70**	**1,4E-04**	32493114	putative glutamate decarboxylase	*Glycine max*	15	K.EGLEKTGRFSIVSK.D (28); K.GSSQVIAQYYQLIR.L (19)	5,81	59	5,67	51,27	47	82
531	**-1,58**	**3,8E-05**						5,31	58				
538	**-1,66**	**1,4E-04**						5,68	58				

661	**-1,69**	**8,7E-04**	23197864	26S proteasome AAA-ATPase subunit RPT5a	*Arabidopsis thaliana*	32	K.AVCVEAGMLALRR.D (7); K.LAGPQLVQMFIGDGAK.L (44); K.RFDSEVSGDREVQR.T (68); R.KMNVHPDVNFEELAR.S (69); K.QIQELVEAIVLPMTHKER.F (23); R.DATEVNHEDFNEGIIQVQAK.K (61)	4,75	54	4,95	47,53	272	353
669	**-1,82**	**3,3E-05**	149390699	arginosuccinate synthase	*Oryza sativa (indica cultivar-group)*	6	K.YAELVYAGR.W (37); -.GVYETPGGTILFTAVR.E (28)	5,75	54	6,23	12,68	65	81
672	**-3,00**	**6,6E-05**	15238328	SCPL42 (serine carboxypeptidase-like 42); serine carboxypeptidase	*Arabidopsis thaliana*	9	-.QLYHSLGSR.T (23); R.ALHLFSSFVR.G (39); R.GAAHMVPYAQPSR.A (2);	5,46	53	6,60	52,87	64	81
684	**-1,80**	**1,2E-04**	14423688	enolase	*Hevea brasiliensis*	9	K.VNQIGSVTESIEAVK.M (78); R.IEEELGAEAVYAGASFR.K (101); K.LAMQEFMILPVGASSFK.E (2); K.IVLPVPAFNVINGGSHAGNK.L (87); R.SGETEDTFIADLSVGLATGQIK.T (94); K.YGQDATNVGDEGGFAPNIQENR.K (56)	5,73	52	5,57	47,83	418	175 (+223)
721	**-3,14**	**5,0E-04**	5731259	alanine:glyoxylate aminotransferase 2 homolog	*Arabidopsis thaliana*	11	K.GGLHGNVFR.I (16); K.DLQKRHDIIGDVR.G (4); K.AGGVCIADEVQTGFGR.T (67); K.IQFNTFGGNPVCSAGGLAVLR.V (10)	5,31	50	7,70	51,94	97	125
735	**-2,05**	**4,9E-05**						5,98	49				
739	**-3,38**	**3,2E-04**						5,52	49				
744	**-7,81**	**5,2E-05**						5,68	49				

777	**-2,71**	**1,7E-04**	33415263	enolase	*Gossypium barbadense*	18	K.MGVEVYHHLK.S (21); K.MGVEVYHHLKSVIKK.K (81); K.LAMQEFMILPVGASSFK.E (40); K.LVLPVPAFNVINGGSHAGNK.L (69); R.SGETEDTFIADLSVGLATGQIK.T (95)	6,11	46	6,16	47,73	306	237 (+102)
784	**-1,92**	**1,4E-05**						6,35	46				
798	**-4,33**	**4,0E-08**						6,46	46				
801	**-5,01**	**2,4E-04**	12229785	Glutamate dehydrogenase A (GDH A)	*Nicotiana plumbaginifolia*	22	R.MGAFTLGVNR.V (18); K.DDGTLVSYVGFR.V (75); K.VECTIPKDDGTLVSYVGFR.V (130); K.FHGYSPAVVTGKPIDLGGSLGR.D (75); K.YIIEAANHPTDPEADEILSKK.G (41); R.YHPEVDLDEVNALAQLMTWK.T + kynurenin (W) (32)	6,61	45	6,61	44,80	371	274 (+116)
830	**-2,40**	**2,1E-04**	12229785	Glutamate dehydrogenase A (GDH A)	*Nicotiana plumbaginifolia*	19	K.SLLIPFR.E (13); K.SLLIPFREIK.V (29); K.VECTIPKDDGTLVSYVGFR.V (65); K.FHGYSPAVVTGKPIDLGGSLGR.D (40); R.YHPEVDLDEVNALAQLMTWK.T + kynurenin (W) (37)	6,58	44	6,61	44,80	184	221 (+40)
839	**-7,27**	**3,9E-05**	1709449	Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial precursor (PDHE1-A).	*Pisum sativum*	13	K.GFGVEAFGADRK.E (52); K.NGPIILEMDTYR.Y (33); R.YHGHSMSDPGSTYR.T (22); K.ESPMPEPSELFTNVYVK.G (22)	5,73	44	8,02	43,53	129	107 (74)
854	**-1,78**	**1,2E-04**	1389768	phosphoribosyl anthranilate transferase	*Arabidopsis thaliana*	8	R.AKGETYEEIVGLAR.A (61); R.AIGGSGVLVSPLTYIFDEEPPR.W (22)	5,26	44	6,96	46,36	83	70 (+22)

				1									
855	**-3,41**	**1,9E-05**						5,32	44				
856	**-2,65**	**2,1E-05**	121485004	cytosolic phosphoglycerate kinase	*Helianthus annuus*	15	K.YSLKPLVPR.L (9); K.ELDYLVGAVSNPK.K + Pyro-glu (N-term E) (19); K.VNVLILGGGMIFTFYKA.- (9); K.LASLADLYVNDAFGTAHR.A (47)	5,43	44	5,82	42,30	84	113
871	**-2,12**	**2,3E-04**						5,62	43				
881	**-3,83**	**9,7E-04**						6,22	43				
886	**-2,73**	**2,4E-04**	90820120	UDP-glucose pyrophosphorylase	*Cucumis melo*	9	R.LVIDDFLPLPSK.G (35); K.GILGYTEDDVVSTDFVGDSR.S (78)	6,09	42	6,69	52,06	113	104 (+35)
889	**-4,84**	**1,6E-06**						6,14	42				
890	**-3,14**	**1,4E-05**	34582497	Alpha-1,4-glucan-protein synthase [UDP-forming] (UDP-glucose:protein transglucosylase) (UPTG)	*Pisum sativum*	16	K.TGLPYIWHSK.A + kynurenin (W) (40); R.ELIGPAMYFGLMGDGQPIGR.Y (16); K.NLLSPSTPFFFNTLYDPYR.E (8)	5,85	42	5,73	41,57	64	112
891	**-5,35**	**2,8E-05**						6,25	42				
900	**-5,05**	**2,3E-05**	25453061	Isovaleryl-CoA dehydrogenase 2, mitochondrial precursor (IVD 2)	*Solanum tuberosum*	15	K.LYEIGAGTSEIRR.M (34); K.GVYVMMSGLDLER.L (3); R.EQFGRPIGEFQFIQGK.L (67); R.QREQFGRPIGEFQFIQGK.L (8); R.ASGSVGLSYGAHSNLCINQLVR.H (23); R.ATQVALQAIQCLGGNGYVNEYPTGR.F (59)	6,46	42	6,13	43,97	194	121 (+101)

904	**-3,81**	**5,7E-06**	34099812	glyceraldehyde-3-phosphate dehydrogenase	*Panax ginseng*	11	K.TVDGPSMKDWR.G + kynurenin (W) (27); K.TVDGPSMKDWR.G + DOUBLE Ox (W) (22); R.VPTVDVSVVDLTAR.L (78); K.GILGYTEDDVVSTDFLGDSR.S (145)	6,72	42	6,40	31,81	272	155 (+127)
907	**-7,15**	**9,8E-06**	120666	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic	*Antirrhinum majus*	25	K.TLLFGEKPVTVFGIR.N (82); K.LVSWYDNEWGYSTR.V + 2 kynurenin (W) (67); K.LVSWYDNEWGYSTR.V + DOUBLE Ox (W); kynurenin (W) (28); K.KATYEQIKAAIKEESEGK.L (2); K.GILGYTEDDVVSTDFVGDSR.S (144); K.LKGILGYTEDDVVSTDFVGDSR.S (161)	6,51	42	8,30	36,69	484	442 (+82)
908	**-4,84**	**1,3E-06**	120666	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic	*Antirrhinum majus*	6	K.TLLFGEKPVTVFGIR.N (18); K.GILGYTEDDVVSTDFVGDSR.S (82); K.GILGYTEDDVVSTDFIGDSR.S (32)	6,43	42	8,30	36,69	132	(132)
912	**-3,38**	**1,4E-04**						6,80	42				
916	**-4,79**	**2,1E-04**						5,88	42				
917	**-3,30**	**2,2E-07**	120666	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic	*Antirrhinum majus*	19	K.VLPQLNGKLTGMSFR.V (25); R.VPTVDVSVVDLTVRLEK.K (2); K.KATYEQIKAAIKEESEGK.L (1); K.GILGYTEDDVVSTDFVGDSR.S (79); K.LKGILGYTEDDVVSTDFVGDSR.S (30)	6,84	42	8,30	36,69	137	165
923	**-2,08**	**8,0E-05**						5,80	41				
927	**-1,94**	**4,0E-04**						5,84	41				
937	**-2,01**	**1,9E-04**						6,06	41				
946	**-2,55**	**9,2E-05**	120666	Glyceraldehyde-3-phosphate dehydrogen	*Antirrhinum majus*	22	K.TLLFGEKPVTVFGIR.N (50); K.FVSWYDNEWGYSNR.V + 2 kynurenin (W) (37); K.GILGYTEDDVVSTDFVGDSR.S (113);	4,68	41	8,30	36,69	277	184 (+164)

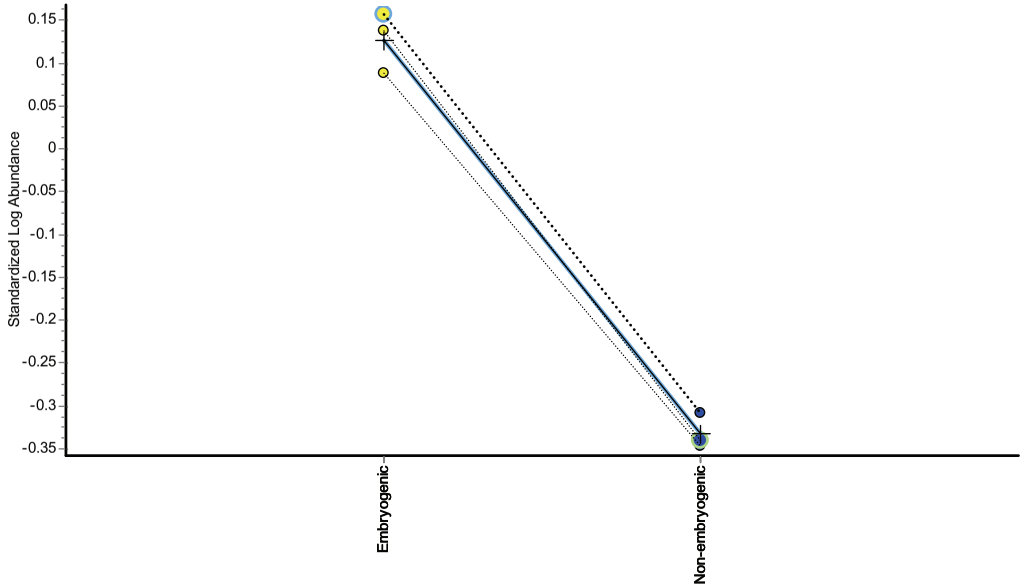
				ase, cytosolic			K.GILGYTEDDVVSTDFLGDSR.S (77)						
950	**-2,49**	**2,8E-05**	12585316	Phosphoglucomutase, cytoplasmic (Glucose phosphomutase) (PGM).	*Solanum tuberosum*	10	R.YLFEDGSR.L (23); R.LYIEQYEKDPAK.T (48); R.LYIEQYEKDPAKTGR.D (10)	6,54	41	6,01	63,47		108
954	**-2,52**	**1,4E-04**						6,14	41				
960	**-3,47**	**2,5E-05**	30689469	SSI2; acyl-[acyl-carrier protein] desaturase	*Arabidopsis thaliana*	24	R.ATFISHGNTAR.Q (39); R.KAQDYVCGLPPR.I (44); K.TIQYLIGSGMDPR.T (11); R.HGDLLNKYLYLSGR.V (29); R.TENNPYLGFIYTSFQER.A (55); K.LAQICGTIAADEKRHETAYTK.I (4); K.SWQPQDFLPDPASDGFEDQVR.E + kynurenin (W) (30)	6,51	41	6,19	45,65	212	236 (+44)
961	**-6,18**	**5,8E-07**	120666	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic	*Antirrhinum majus*	13	K.VLPQLNGKLTGMSFR.V (23); K.GILGYTEDDVVSTDFVGDSR.S (76)	4,64	40	8,30	36,69	99	109
982	**-2,10**	**7,1E-05**	120666	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic	*Antirrhinum majus*	26	K.IKIGINGFGR.I (50); K.TLLFGEKPVTVFGIR.N (69); K.LVSWYDNEWGYSTR.V + 2 kynurenin (W) (93); K.LVSWYDNEWGYSTR.V + DOUBLE Ox (W); kynurenin (W) (71); K.YDSVHGQWKHHELKVK.D + DOUBLE Ox (W) (5); K.GILGYTEDDVVSTDFVGDSR.S (175)	5,93	39	8,30	36,69	463	335 (+124)

983	**-2,32**	**5,5E-04**	399333	Cysteine synthase, chloroplast precursor	*Capsicum annuum*	11	K.LIAVVFPSFGER.Y (34); K.TVLVEPTSGNTGIGLAFIAASR .G (48)	6,05	39	5,22	39,98	82	(97)
1003	**-2,65**	**8,5E-04**						5,65	39				
1047	**-1,58**	**2,9E-04**						6,05	37				
1048	**-2,37**	**8,4E-05**						6,23	37				
1050	**-1,76**	**4,6E-04**	114421	ATP synthase subunit beta, mitochondrial precursor	*Nicotiana plumbaginifolia*	17	K.VVDLLAPYQR.G (44); K.AHGGFSVFAGVGER.T (50); R.VLNTGSPITVPVGR.A (32)	5,78	37	5,95	59,86		156
1084	**-3,08**	**4,4E-06**	114421	ATP synthase subunit beta, mitochondrial precursor	*Nicotiana plumbaginifolia*	21	K.VVDLLAPYQR.G (66); K.AHGGFSVFAGVGER.T (102); R.VLNTGSPITVPVGR.A (47); R.FTQANSEVSALLGR.I (95); K.CALVYGQMNEPPGAR.A (16); R.LVLEVAQHLGENMVR.T (29); R.IPSAVGYQPTLATDLGGLQER .I (92)	6,63	35	5,95	59,86	447	501
1098	**-1,80**	**5,7E-04**	135460	Tubulin beta-2 chain (Beta-2 tubulin)	*Zea mays*	23	R.YLTASAMFR.G (13); K.LAVNLIPFPR.L (36); R.FPGQLNSDLR.K (21); R.VSEQFTAMFR.R (15); R.FPGQLNSDLRK.L (14); R.RVSEQFTAMFR.R (14)	5,27	34	4,82	49,88	113	207
1109	**-2,23**	**1,1E-04**	5668671	Beta-tubulin	*Zinnia elegans*	22	R.YLTASAIYR.G (34); K.LAVNLIPFPR.L (33); R.FPGQLNSDLR.K (23); R.VSEQFTAMFR.R (20); R.FPGQLNSDLRK.L (27); R.RVSEQFTAMFR.R (21); K.NSSYFVEWIPNNVK.S + kynurenin (W) (9)	5,10	33	4,68	49,39	167	243
1129	**-2,18**	**7,6E-06**	15241168	TUA3	*Arabidopsis thaliana*	11	R.SLDIERPTYTNLNR.L (31); R.IHFMLSSYAPVISAAK.A (53)	5,79	31	4,95	49,65	84	78
1155	**-10,28**	**1,1E-06**						5,38	29				
1168	**-2,36**	**4,7E-05**						6,03	29				

1169	**-2,13**	**1,7E-04**						6,20	39				
1174	**-5,89**	**1,6E-06**						5,38	29				
1176	**-2,13**	**2,7E-05**	12585489	UTP--glucose-1-phosphate uridylyltransferase (UDP-glucose pyrophosphorylase) (UDPGP) (UGPase)	*Musa acuminata*	5	K.SGFINLVAR.Y (41); K.ECCLALENVASALSLR.D + Glu->pyro-Glu (N-term E) (58); K.YANSNIEIHTFNQSQYPR.L (66)	4,37	29	5,48	51,36	165	65 (+107)
1188	**-2,49**	**4,9E-05**	38564727	putative alpha-soluble NSF attachment protein	*Helianthus annuus*	9	K.IAQFSAQLEQYQK.A (47); K.QKIAQFSAQLEQYQK.A (21)	4,50	28	5,12	32,66	68	74
1192	**-2,44**	**7,1E-04**	3202034	root catalase	*Mesembryanthemum crystallinum*	17	R.FSTVIHER.G (31); K.SHIQEXWR.I + kynurenin (W) (7); R.APGVQTPVIVR.F (25); R.GPILLEDYHLLEK.I (37); R.EGNFDLVGNNFPVFFNR.D (36)	4,03	28	7,39	57,04	136	151 (+32)
1207	**-2,31**	**1,6E-05**						4,03	27				
1214	**-3,31**	**1,7E-04**						5,64	27				
1267	**-1,66**	**2,5E-04**						4,73	25				
1298	**-1,57**	**6,1E-05**						4,73	24				
1308	**-1,82**	**7,7E-04**	110278805	14-3-3-like protein GF14-D (G-box factor 14-3-3 homolog D)	*Oryza sativa (japonica group)*	11	R.NLLSVAYKNVIGAR.R (27); K.AAQDIALADLAPTHPIR.L (54)	6,42	24	4,83	29,26	81	85
1333	**-5,57**	**1,4E-04**						4,82	23				

1348	**-1,51**	**3,0E-04**	28627542	putative ascorbate peroxidase	*Capsicum annuum*	12	R.EDKPEPPVEGR.L (45); K.GSDHLRDVFVK.Q (39); K.ALLSDPAFRPLVEK.Y (44); R.LPDATKGSDHLRDVFVK.Q (29); K.YAADEDAFFADYTEAHLK.L (113)	5,62	22	5,43	27,49	270	168 (+157)
1350	**-2,00**	**1,1E-04**						4,51	22				
1376	**-1,55**	**7,6E-04**	15228818	ATFER3; binding / ferric iron binding	*Arabidopsis thaliana*	6	R.LLNLHAVASR.S (48); K.ISEYVSQLRR.L (46); K.FFKESSVEER.E (10)	5,19	22	5,54	28,84	104	(104)
1403	**-4,56**	**4,3E-05**						5,38	21				
1416	**-4,35**	**1,6E-06**	78128515	ferritin	*Chorispora bungeana*	11	R.LLNLHAVASR.S (31); K.ISEYVSQLR.R (23); K.ISEYVSQLRR.L (51)	6,55	20	5,44	29,17	105	67 (+31)
1419	**-1,59**	**4,3E-04**						5,35	20				
1421	**-4,57**	**6,0E-07**						6,29	20				
1426	**-1,95**	**1,2E-05**						6,25	20				
1443	**-1,98**	**1,5E-04**	21664287	heat shock protein 70	*Oryza sativa (indica cultivar-group)*	13	R.TTPSYVGFTDSER.L (19); K.NAVVTVPAYFNDSQR.Q (37)	5,34	20	5,17	71,00	56	73
1450	**-5,82**	**2,6E-05**	6094439	Translationally-controlled tumor protein homolog (TCTP)	*Fragaria x ananassa*	12	K.VVDIVDTFR.L (31); R.LQEQPPFDKK.Q (23); K.EGATNPTFLYFGHGLK.E (70)	6,24	19	4,37	19,04	124	163
1502	**-27,77**	**9,0E-05**	357982	ATPase alpha,F1	*Arachis hypogaea*	17	R.GIRPAINVGLSVSR.V (27); K.QVCGSLKLELAQYR.E (46); K.QILVIYAAVNGFCDR.M (63); R.EVAAFAQFGSDLDAATQALLNR.G (64)	5,38	15	5,84	55,08	200	223

Example: Spot 95



PART II Spots with increased abundance in non-embryogenic cells (NE). Blue: identified, Yellow: not identified.

Spot	Ratio	t-test	GI accession	Name	Species	Number of peptide matched	Sequences of ions (Score)	Estimated pI	Estimated Mr	Theoretical pI	Theoretical Mr	Sum of ions scores	Protein score (+manual ion score)
15	**1,59**	**2,7E-04**	30407706	aconitase	*Lycopersicon pennellii*	13	R.IDKLPYSIR.I (14); K.LVEIPFKPAR.V (24); K.FYSLPALNDPR.I (47)	6,34	99	5,83	97,98	85	124
19	**1,72**	**2,3E-04**						6,55	91				
34	**1,85**	**1,4E-04**						5,28	90				
35	**1,76**	**3,6E-05**						6,26	89				
41	**4,36**	**5,7E-04**	15232776	CDC48 (CELL DIVISION CYCLE 48); ATP binding / ATPase/ hydrolase/ nucleoside - triphosphatase	*Arabidopsis thaliana*	23	K.GILLYGPPGSGK.T (46); R.RIVSQLLTLMDGLKSR.A (2); K.YQAFAQTLQQSR.G (37); R.KYQAFAQTLQQSR.G (36); R.LDQLIYIPLPDEESR.Y (0)	5,19	89	5,13	89,39	121	156
61	**8,67**	**5,4E-04**	52353232	putative metallophosphatase	*Lupinus luteus*	7	K.SSPYPGQDSLQR.V (19); R.DPGFIHTSFLK.E (38); R.SPAGTLTFTR.N (12)	5,34	86	6,10	71,00	69	(69)
63	**18,19**	**3,4E-04**						5,52	86				
65	**12,99**	**4,1E-04**	52353232	putative metallophosphatase	*Lupinus luteus*	8	K.FQLINQR.A (29); R.DPGFIHTSFLK.E (82); R.SPAGTLTFTR.N (49); K.SSPYPGQDSLQR.V (39)	5,62	85	6,10	71,00	199	111 (+88)
77	**9,50**	**1,2E-04**						4,42	85				

78	**21,03**	**1,8E-05**						5,76	85				
80	**17,19**	**4,1E-04**	42568444	hydrolase/ protein serine/threonine phosphatase	*Arabidopsis thaliana*	5	R.DPGFIHTSFLK.E (51); R.SPAGTLTFTR.N (18); K.SSPYPGQDSLQR.V (36)	5,50	84	5,99	68,90	105	60 (+105)
84	**9,76**	**4,0E-04**	52353232	putative metallophosphatase	*Lupinus luteus*	9	K.FQLINQR.A (27); R.DPGFIHTSFLK.E (74); K.SSPYPGQDSLQR.V (52); K.QPWLIFAAHR.V + kynurenin (W) (16)	5,39	84	6,10	71,00	169	111 (+68)
88	**11,30**	**1,4E-05**						5,70	84				
89	**13,18**	**8,4E-07**	52353232	putative metallophosphatase	*Lupinus luteus*	13	K.FQIINQR.A (37); R.SPAGTLTFTR.N (41); K.SSPYPGQDSLQR.V (45)	5,60	84	6,10	71,00	123	101 (+37)
90	**7,63**	**6,9E-04**	52353232	putative metallophosphatase	*Lupinus luteus*	9	K.FQLINQR.A (28); R.DPGFIHTSFLK.E (78); K.SSPYPGQDSLQR.V (22); K.QPWLIFAAHR.V + kynurenin (W) (35)	5,86	84	6,10	71,00	163	106 (+57)
93	**16,39**	**5,0E-06**	38154489	molecular chaperone Hsp90-1	*Lycopersicon esculentum*	29	K.RAPFDLFDTK.K (20); K.ADLVNNLGTIAR.S (20); K.HFSVEGQLEFK.A (58); K.GIVDSEDLPLNISR.E (38)	5,80	84	4,98	80,16	136	296
109	**3,02**	**6,0E-04**	18025340	alpha-L-arabinofuranosidase/ beta-D-xylosidase isoenzyme ARA-I	*Hordeum vulgare*	4	R.LGFFDGDPR.Q (29); -.DTFQPPFK.S (27)	4,31	83	5,59	81,99	56	(56)
111	**12,00**	**4,8E-04**						5,66	83				

116	**6,61**	**3,8E-06**	52353232	putative metallophosphatase	*Lupinus luteus*	12	R.SPAGTLTFTR.N (13); K.SSPYPGQDSLQR.V (47); K.FQIINQR.A (21)	5,42	83	6,10	71,00	81	73 (+21)
128	**11,69**	**2,8E-05**	52353232	putative metallophosphatase	*Lupinus luteus*	9	K.FQLINQR.A (26); R.DPGFIHTSFLK.E (85); K.SSPYPGQDSLQR.V (50)	5,80	83	6,10	71,00	161	122 (+50)
131	**25,61**	**3,9E-05**						4,45	82				
183	**5,76**	**1,9E-04**	18025340	alpha-L-arabinofuranosidase/beta-D-xylosidase isoenzyme ARA-I	*Hordeum vulgare*	6	R.LGFFDGDPR.Q (22); K.HYTAYDVDNWK.G + kynurenin (W) (52)	6,03	78	5,59	81,99	74	94
187	**1,97**	**8,6E-04**	21664287	heat shock protein 70	*Oryza sativa (indica cultivar-group)*	22	K.NALENYAYNMR.N (8); R.TTPSYVGFTDSER.L (98); K.NAVVTVPAYFNDSQR.Q (24); R.IINEPTAAAIAYGLDKK.A (73)	4,71	77	5,17	71,00	203	254
198	**1,64**	**5,4E-04**	762844	Hsc70	*Lycopersicon esculentum*	30	R.MVNHFVQEFK.R (52); K.NALENYAYNMR.N (20); R.TTPSYVAFTDTER.L (45); R.ARFEELNMDLFR.K (10); K.NAVVTVPAYFNDSQR.Q (43); R.IINEPTAAAIAYGLDKK.I (87)	4,90	77	5,18	71,52	257	364
199	**2,84**	**8,2E-04**	38325815	heat shock protein 70-3	*Nicotiana tabacum*	33	R.MVNHFVQEFK.R (52); K.NALENYAYNMR.N (29); R.TTPSYVAFTDTER.L (54); R.ARFEELNMDLFR.K (17); K.NAVVTVPAYFNDSQR.Q (44); R.IINEPTAAAIAYGLDKK.I (85)	4,95	77	5,14	70,99	281	407

230	**2,30**	**1,4E-04**	399940	Heat shock 70 kDa protein, mitochondrial precursor	*Phaseolus vulgaris*	30	R.IAGLDVQR.I (19); K.HLNITLTR.S (57); K.AVITVPAYFNDAQR.Q (72); K.SQVFSTAADNQTQVGIK.V (140); K.GVNPDEAVAMGAAIQGGILR.G (65); R.TTPSVVAFNQKGELLVGTPAK.R (45)	6,03	72	5,95	72,54	398	522
235	**2,76**	**3,7E-04**	300265	HSP68=68 kda heat-stress DnaK homolog	*Lycopersicon peruvianum*	16	K.DRLALQR.L (5); K.HLNITLTR.S (57); K.EVDEVLLVGGMTR.V (38); K.SQVFSTAADNQTQVGIK.V (140); K.GVNPDEAVAMGAAIQGGILR.G (65); R.IAGLDVQR.I (18); K.AVITVPAYFNDAQR.Q (72)	6,15	72	5,20	62,38	395	355 (+90)
251	**3,80**	**2,5E-05**	41018257	Heat shock protein STI (Stress-inducible protein) (GmSTI)	*Glycine max*	15	K.ELEQQEYFDPK.L (73); R.GDLTPEELKER.Q (27); K.ELEQQEYFDPK.L (19); K.AIELDDEDISFITNR.A (84)	6,29	73	5,81	63,59	203	77 (+130)
253	**2,53**	**1,7E-05**						5,93	73				
260	**1,51**	**6,2E-04**						5,71	72				
261	**1,86**	**7,7E-04**	15240075	SDH1-1 (Succinate dehydrogenase 1-1)	*Arabidopsis thaliana*	19	R.LGANSLLDIVVFGR.A (81); K.AVIELENYGLPFSR.T (64); R.ASSTILATGGYGR.A (14)	6,06	72	5,86	69,66	159	191 (+14)
279	**2,66**	**7,3E-04**	3914394	2,3-bisphosphoglycerate-independent phosphogl	*Mesembryanthemum crystallinum*	13	K.ALEYEDFDKFDR.V (34); K.AVGPIVDGDAVVTLNFR.A (27); R.HYLVSPPEIDR.T (19)	5,80	71	5,39	61,18	80	80 (+19)

				ycerate mutase (Phosphoglyceromutase)									
280	**1,87**	**3,0E-05**	3914394	2,3-bisphosphoglycerate-independent phosphoglycerate mutase (Phosphoglyceromutase)	*Mesembryanthemum crystallinum*	19	K.FGHVTFFWNGNR.S + kynurenin (W) (10); K.ALEYEDFDKFDR.V (30); K.AVGPIVDGDAVVTLNFR.A (42)	6,03	71	5,39	61,18	82	120
284	**2,46**	**3,1E-05**						6,27	71				
294	**1,75**	**1,4E-06**	461736	Chaperonin CPN60-2, mitochondrial precursor (HSP60-2)	*Cucurbita maxima*	23	R.NVVIEQSYGAPK.V (59); R.GYISPYFITNTK.N (45); K.AAVEEGIVPGGGVALLYATK.E (83); K.TLYNELEVVEGMKLDR.G (6); R.MISTSEEIAQVGTISANGER.E (17); R.SSIELSTSDYDKEKLQER.L (22)	5,28	70	6,28	61,13	232	124 (+173)
296	**5,74**	**1,2E-05**						6,09	69				
327	**1,87**	**1,5E-04**						6,50	68				
329	**2,87**	**1,1E-05**	3820612	herbicide resistant acetolactate synthase precursor	*Bassia scoparia*	14	R.HEQGGIFAAEGYAR.A (38); R.FDDRVTGKLEAFASR.A (38); R.MIGTDAFQETPIVEVTR.S (30)	6,02	68	6,96	72,43	106	111

330	**1,51**	**3,4E-04**	2506277	RuBisCO large subunit-binding protein subunit beta, chloroplast precursor (60 kDa chaperonin subuni	*Pisum sativum*	25	R.GYISPYFVTDSEK.M (71); K.AAVEEGIVVGGGCTLLR.L (72); K.SAENSLYVVEGMQFDR.G (28); K.LSGGVAVIQVGAQTETELKEK.K (70); K.TNDLAGDGTTTSVVLAQGLIAEGVK.V (86); R.SAENALYVVEGMQFDR.G (12); R.DLINVLEDAIR.G (68)	6,13	68	5,85	62,98	407	393 (+80)
333	**3,68**	**1,4E-04**	15238328	SCPL42 (serine carboxypeptidase-like 42)	*Arabidopsis thaliana*	4	R.ALHLFSSFVR.G (73); GAAHMVPYAQPSR (22)	5,42	68	6,60	52,87	95	(95)
341	**8,85**	**7,4E-06**						6,25	67				
348	**4,21**	**3,8E-05**	15238328	SCPL42 (serine carboxypeptidase-like 42)	*Arabidopsis thaliana*	9	R.ALHLFSSFVR.G (67); GAAHMVPYAQPSR (34)	6,64	67	6,60	52,87	101	152 (+155)
349	**2,52**	**5,0E-04**	15235730	phosphoenolpyruvate carboxykinase (ATP)	*Arabidopsis thaliana*	18	R.AAYPIEFIPNAK.I (47); R.YTHYMTSSTSVDLNLAR.R (22); K.FGTVLENVVFDEHYR.E (64); K.GSPNIEMDEHTFLVNR.E (22);	4,02	67	6,61	73,41	155	79 (+108)
357	**2,10**	**1,6E-04**						6,03	67				
360	**1,52**	**2,3E-04**	11131631	Calreticulin precursor	*Beta vulgaris*	3	K.YVGIELWQVK.S + kynurenin (W) (32); R.FDDGWESR.W + kynurenin (W) (15); R.FYAISAEFPEFSNKDK.T (15)	6,69	67	4,45	48,14	62	(62)
364	**1,55**	**1,1E-05**						5,65	67				

369	**1,78**	**8,2E-05**	266346	Ketol-acid reductoisomerase, chloroplast precursor (Acetohydroxy-acid reductoisomerase)	*Spinacia oleracea*	12	K.EVNGAGINSSFAVHQDVDGR.A (86); K.INLAGHDEYIVR.G (49); K.EKINLAGHDEYIVR.G (12); R.DLFPLLPDAFK.G (39); R.GGRDLFPLLPDAFK.G (19)	5,77	67	6,54	63,75	205	94 (+119)
385	**1,99**	**1,0E-04**	15238328	SCPL42 (serine carboxypeptidase-like 42)	*Arabidopsis thaliana*	3	R.ALHLFSSFVR.G (53); R.GAAHMVPYAQPSR.A (20)	6,51	66	6,60	52,87	73	72 (+20)
387	**1,87**	**6,8E-04**	2506277	RuBisCO large subunit-binding protein subunit beta, chloroplast precursor	*Pisum sativum*	5	K.VSDEVPFLADLKPSGK.Y (65); R.GYLSPYFVTDSEK.M (26)	5,45	66	5,85	62,98	91	(91)
424	**1,90**	**8,5E-04**						6,10	65				
434	**2,09**	**5,8E-04**	18424599	ALDH12A1 (Aldehyde dehydrogenase 12A1);	*Arabidopsis thaliana*	9	K.SYQQAFGEVYVTQK.F (40); R.SFAVPGNHLGQQSHGFR.W (37)	4,01	64	6,26	61,77	77	(77)
436	**1,64**	**4,3E-04**	15229546	dehydratase family	*Arabidopsis thaliana*	11	K.VSDQVPFLADLKPSGK.Y (82); K.WTPPPHKAARGALWKYTK.L (26)	5,50	64	5,85	64,91	108	106 (+26)

438	**3,74**	**1,7E-05**	1009712	calreticulin	*Arabidopsis thaliana*	26	K.YVGLELWQVK.S + kynurenin (W) (34); K.LKYVGLELWQVK.S + kynurenin (W) (19); R.FYAISAEFPEFSNKDK.T (54); K.FGGDTPYSIMFGPDICGYSTK.K (35); K.APMIDNPDFKDDPDIYVFPK.L (0)	5,42	64	4,37	46,58	142	198
455	**1,74**	**2,2E-04**						5,67	63				
456	**3,96**	**1,9E-05**	114404	ATP synthase subunit alpha, mitochondrial	*Helianthus annuus*	13	R.VVDALGVPIDGR.G (25); R.EAFPGDVFYLHSR.L (51)	6,37	63	6,02	55,49	76	81
495	**1,96**	**3,1E-04**	6094228	Adenosylhomocysteinase (S-adenosyl-L-homocysteine hydrolase) (AdoHcyase)	*Mesembryanthemum crystallinum*	18	K.SKFDNLYGCR.H (61); R.AEFGPAQPFKGAR.I (57); K.DQADYISVPVEGPYKPAHYRY.- (68)	6,00	61	5,75	53,18	186	166 (+57)
499	**2,15**	**8,0E-04**	6094413	Seryl-tRNA synthetase (Serine--tRNA ligase) (SerRS)	*Helianthus annuus*	18	R.QFELEQLR.K + Pyro-glu (N-term Q) (24); R.YAGYSSCFRK.E (18); R.ELVSCSNCTDYQSR.K (29)	6,76	61	6,51	50,10	71	71
502	**1,78**	**9,9E-05**	1743354	aldehyde dehydrogenase (NAD+)	*Nicotiana tabacum*	11	R.TGEVIAHVAEGDAEDINR.A (88); K.AFDEGPWPKMNAYERSK.I + kynurenin (W) (72)	5,60	61	6,90	59,34	160	101 (+72)

510	**1,76**	**5,0E-04**	6979698	dehydrin	*Vaccinium corymbosum*	14	R.QDQQRGEYK.Q + Pyro-glu (N-term Q) (31); K.EGGWLMDKVKDK.I + kynurenin (W) (25)	5,93	61	6,67	22,95	56	70
553	**3,70**	**5,9E-06**	13430632	putative glutathione reductase	*Arabidopsis thaliana*	6	K.KILVYGASYGPELEDAR.N (55); K.SSLQNLCNLR.M (30)	5,44	59	6,36	53,84	85	(80)
559	**1,96**	**1,6E-04**	15241704	UDP-glucose 6-dehydrogenase	*Arabidopsis thaliana*	11	K.LAANAFLAQR.I (44); K.FLNASVGFGGSCFQK.D (72)	5,78	59	5,60	53,10	116	133
560	**1,75**	**2,4E-04**	33415263	enolase	*Gossypium barbadense*	19	K.LAMQEFMILPVGASSFK.E (29); K.LVLPVPAFNVINGGSHAGNK.L (71); R.SGETEDTFIADLSVGLATGQIK.T (166); R.IEEELGAEAVYAGASFR.K (87); K.HAGWGVMASHR.S + kynurenin (W) (17); K.YGQDATNVGDEGGFAPNIQENR.K (106)	5,84	59	6,16	47,73	476	299 (+210)
594	**1,63**	**3,8E-05**	266567	Mitochondrial-processing peptidase alpha subunit, mitochondrial precursor (Alpha-MPP) (Ubiquinol-cytochrome-c reductase subunit II).	*Solanum tuberosum*	6	R.QILTYGKR.K (55); K.SVYVGGDYR.C (40)	5,42	58	5,71	54,68	95	(95)

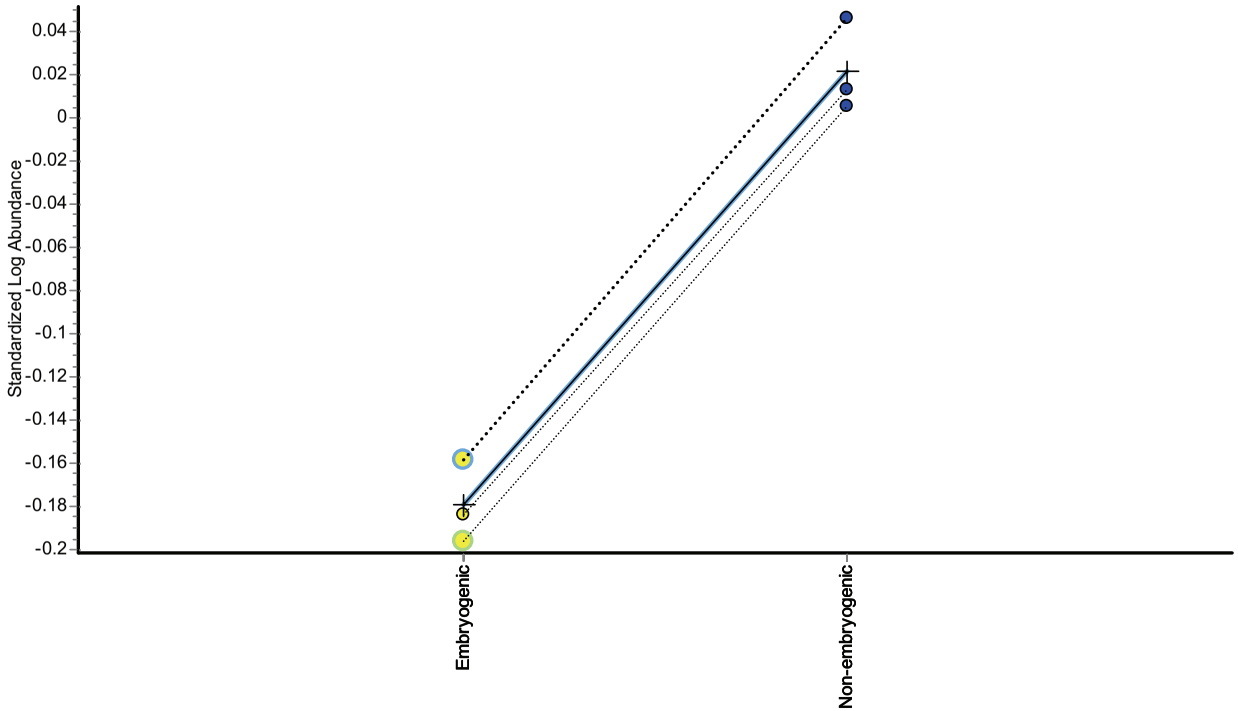
613	**2,32**	**3,4E-04**	4049354	glycine hydroxymethyltransferase (EC 2.1.2.1)-like protein	*Arabidopsis thaliana*	4	K.LIIAGASAYPR.D (42); R.YYGGNEFIDQIENLCRSR.A (30)	4,85	57	8,13	50,74	72	(72)
617	**2,26**	**4,4E-04**	464849	Tubulin alpha chain	*Prunus dulcis*	26	R.SLDIERPTYTNLNR.L (27); R.AVFVDLEPTVIDEVR.T (91); R.IHFMLSSYAPVISAEK.A (48); R.AVFLDLEPTVIDEVR.T (84); K.TVGGGDDAFNTFFSETGAGK.H (113); R.QLFHPEQLISGKEDAANNFAR.G (114)	6,49	57	4,92	49,53	477	467 (+84)
619	**2,14**	**5,7E-06**	4049354	glycine hydroxymethyltransferase (EC 2.1.2.1)-like protein	*Arabidopsis thaliana*	4	R.YYGGNEFIDQIENLCRSR.A (57) ; K.LIIAGASAYPR.D (54)	6,65	57	8,13	50,74	111	(111)
626	**5,17**	**9,6E-07**	15236375	SHM4 (SERINE HYDROXYMETHYLTRANSFERASE 4)	*Arabidopsis thaliana*	4	R.YYGGNEFIDQIENLCRSR.A (38); K.LIIAGASAYPR.D (46)	6,82	57	6,80	51,72	84	(84)
632	**6,98**	**6,8E-07**						6,73	57				
647	**7,10**	**1,5E-04**	15320419	monodehydroascorbate reductase	*Spinacia oleracea*	12	K.VPGIFAIGDVAAFPLK.M (96); R.LPGFHTCVGSGGER.L (69); R.VFEYEGSPR.K (48)	6,85	56	6,65	54,01	213	120 (+117)

648	**7,78**	**7,7E-08**						6,89	56				
697	**2,59**	**1,0E-04**						6,02	53				
700	**1,90**	**7,4E-04**	9759413	beta-ureidopropionase	*Arabidopsis thaliana*	11	K.IAVNICYGR.H (22); K.KYNMVIVSPILER.D (7); R.NAAIANSYFVGSINR.V (85); R.VGDFNESTYYMEGNTGHPVFETAFGK.I (12)	5,63	53	5,97	45,17	126	133 (+19)
707	**3,25**	**1,2E-05**	3775985	RNA helicase	*Arabidopsis thaliana*	15	K.RKVDYLSEK.M (19); R.GFKDQIYDVYR.Y (24); R.GIDVQQVSLVINYDLPNNR.E (33)	6,49	52	5,52	44,32		71 (+33)
747	**2,15**	**7,2E-04**	58013197	actin	*Isatis tinctoria*	16	R.AVFPSIVGRPR.H (22); K.NYELPDGQVITIGAER.F (17); R.VAPEEHPVLLTEAPLNPK.A (12)	6,73	49	5,31	41,82	51	125 (+4)
799	**2,62**	**3,4E-04**	3023685	Enolase (2-phosphoglycerate dehydratase) (2-phospho-D-glycerate hydro-lyase)	*Alnus glutinosa*	15	K.LVLPVPAFNVINGGSHAGNK.L (77); R.SGETEDTFIADLSVGLATGQIK.T (118); R.IEEELGADAIYAGASFR.A (84); K.YGQDATNVGDEGGFAPNIQENR.K (74)	6,16	47	5,41	47,60	353	221 (+158)
815	**1,57**	**4,1E-05**	90820120	UDP-glucose pyrophosphorylase	*Cucumis melo*	4	R.LVIDDFLPLPSK.G (41); K.DGWYPPGHGDVFPSLR.N + kynurenin (W) (34)	5,71	45	6,69	52,06	75	(75)
882	**1,86**	**2,9E-04**	18418013	GLN1;4; glutamate-ammonia ligase	*Arabidopsis thaliana*	13	R.HKEHIAAYGEGNER.R (59); K.HETADINTFLWGVANR.G + kynurenin (W) (65); R.GNNILVMCDAYTPAGEPIPTNKR.H (31)	4,69	43	5,12	38,99	155	184
944	**1,72**	**7,8E-04**						5,52	41				

975	**3,14**	**1,8E-04**	6319167	branched-chain amino acid aminotransferase	*Solanum tuberosum*	2	K.EGLAPINLVIETEMHR.A (83); K.CSQGENFSKGELQR.F (22)	5,39	40	5,43	40,92	105	(105)
987	**1,84**	**7,5E-04**	113624	Fructose-bisphosphate aldolase, cytoplasmic isozyme	*Spinacia oleracea*	11	K.AQEAFLAR.C (50); K.GTVELAGTNGETTTQGLDGLAQR.C (141); K.VDKGTVELAGTNGETTTQGLDGLAQR.C (84)	5,50	39	5,96	38,47	275	243 (+50)
1031	**3,75**	**1,2E-05**	2204236	enoyl-ACP reductase	*Nicotiana tabacum*	16	K.VLAFEAGR.K (17); K.VLAFEAGRK.G (41); R.VNTISAGPLR.S (33); K.VYPLDAVYDSPEDVPEDVK.G (50); K.HFVPIMNPGGTTISLTYIASER.I (5)	5,68	37	8,88	41,78	146	137 (+38)
1036	**2,46**	**3,7E-05**	126896	Malate dehydrogenase, mitochondrial precursor	*Citrullus lanatus*	15	K.LFGVTTLDVVR.A (16); R.DDLFNINAGIVK.S (100); K.KLFGVTTLDVVR.A (46); K.AGTYDEKKLFGVTTLDVVR.A (31)	6,43	37	8,88	36,20	193	235
1064	**1,54**	**7,0E-04**						6,69	36				
1177	**2,10**	**3,0E-05**	58201024	thiamine biosynthetic enzyme	*Picrorhiza kurrooa*	15	K.LLARPNVK.L (27); K.HAALFTSTIMSK.L (103); R.MGPTFGAMMISGQK.A (54); K.IVVSSCGHDGPFGATGVK.R (37); K.IVVSSCGHDGPFGATGVKR.L (46)	5,28	29	5,03	37,48	267	308
1229	**1,76**	**6,7E-04**						5,34	27				

1261	**1,86**	**2,6E-05**	30407706	aconitase	*Lycopersicon pennellii*	9	R.IDKLPYSIR.I (32); K.LVEIPFKPAR.V (28); K.DSPAARYLMERGVDR.R (1); R.SENAVQANMELEFQR.N (6)	5,53	26	5,83	97,98	67	67 (+28)
1262	**2,04**	**4,0E-06**						4,39	25				
1265	**2,68**	**2,0E-06**	58013197	actin	*Isatis tinctoria*	20	R.GYSFTTTAER.E (17); K.SYELPDGQVITIGAER.F (83); K.NYELPDGQVITIGAER.F (41); K.SSSSVEKNYELPDGQVITIGAER.F (57); R.TTGIVLDSGDGVSHTVPIYEGYALPHAILR.L (61)	5,79	25	5,31	41,82	259	236 (+90)
1269	**2,15**	**4,2E-04**	15241245	RAN2; GTP binding	*Arabidopsis thaliana*	10	K.AKQVTFHR.K (26); K.SNYNFEKPFLYLAR.K (25)	5,42	25	6,38	25,06	51	58 (+25)
1278	**2,12**	**7,9E-04**	15241245	RAN2; GTP binding	*Arabidopsis thaliana*	14	R.HLTGEFEK.K (4); K.AKQVTFHR.K (14); K.NLQYYEISAK.S (20); K.SNYNFEKPFLYLAR.K (33)	5,62	24	6,38	25,06	71	131
1291	**2,51**	**3,2E-04**	15241245	RAN2; GTP binding	*Arabidopsis thaliana*	17	R.HLTGEFEK.K (23); K.AKQVTFHR.K (24); K.NLQYYEISAK.S (36); K.SNYNFEKPFLYLAR.K (43)	5,79	24	6,38	25,06	126	206
1295	**5,57**	**6,7E-05**						5,46	24				
1358	**3,09**	**1,2E-04**						6,74	22				
1385	**2,00**	**2,2E-04**						6,38	21				
1392	**1,57**	**8,2E-04**						4,24	21				
1427	**14,93**	**1,3E-04**						6,75	20				
1491	**2,46**	**4,5E-06**	30025966	heat shock protein 70 (fragment)	*Nicotiana tabacum*	9	R.TTPSYVGFTDSER.L (14); K.NAVVTVPAYFNDSQR.Q (43)	5,67	17	5,17	70,88	57	71

Example: Spot 15



Appendix 4

Lyngved R, Erlien W, Sørborg Ø (2004) Kloning av planter.
Available at http://www.viten.no (see below)

Procedure for starting the programme "Cloning plants":

1. Start your Internet browser and open the page http://www.viten.no (Figure 1).

Figure 1 Main page Viten.no

2. Be sure that your computer has Macromedia Flashplayer installed and that your Internet browser allows pop-up windows on viten.no. You can check these requirements via the link "Les mer" on the main page http://www.viten.no:

3. Choose "Lærerregistrering", fill in the registration form and press "Registrer meg". You will automatically receive a username.

4. Log in on the main page with the username you were assigned and the password you chose.

5. Choose the Viten programme "Kloning av planter" (see Figure 2). A brief description of the programme will appear.

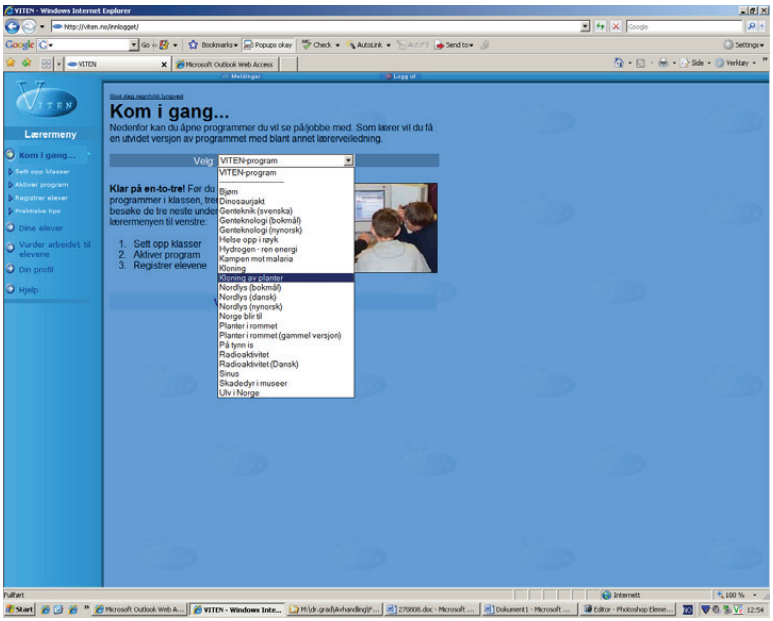


Figure 2 Available Viten programmes

6. Press "Åpne program". Information about the programme will appear (Figure 3) and you will find all the information you need to start the programme.

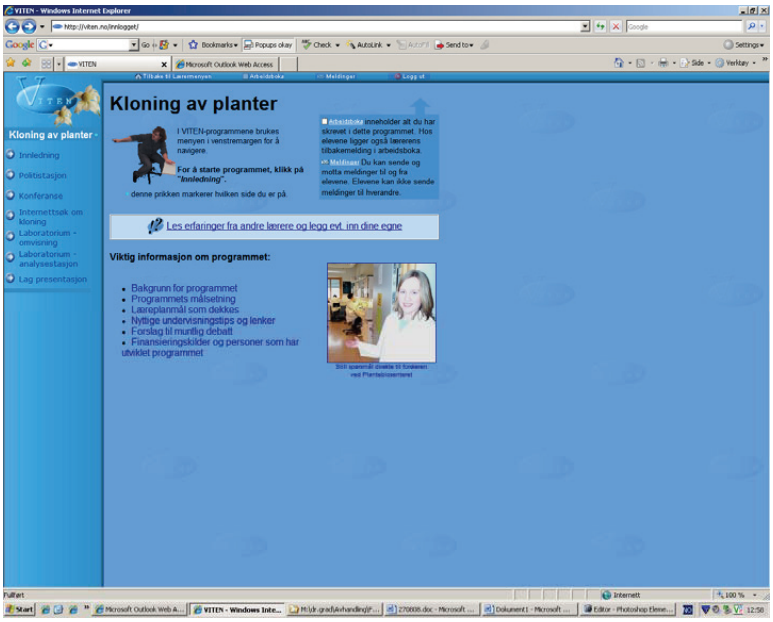


Figure 3 Start page "Kloning av planter"

Appendix 5

NTNU
Norges teknisk-naturvitenskapelige universitet

Program for lærerutdanning (PLU)

Elever og foresatte

Doktorgradsstipendiat
Ragnhild Lyngved
Telefon 73590173
E-post Ragnhild.Lyngved@plu.ntnu.no

Dato: 05.11.2003

"Viten - nettbasert undervisning i realfag"

NTNU, Universitetet i Oslo og University of Berkeley, California, har gått sammen om å utvikle internettbaserte undervisningsprogrammer i realfag. Undertegnede har fått midler fra Norges forskningsråd til å utvikle et undervisningsprogram om kloning, og til å evaluere hvordan dette programmet fungerer i skolen. Arbeidet skal etter hvert munne ut i en doktorgradsavhandling. Deres videregående skole er en av flere skoler som er valgt ut til å være med på forskningsdelen av prosjektet.

Vi skal undersøke hva elevene lærer, og forsøke å få et innblikk i hvordan de lærer ved å bruke Internett i undervisningen. For å få svar på slike spørsmål er vi avhengig av å registrere diskusjoner mellom elever når de sitter ved datamaskinene. I den sammenheng ønsker vi å gjøre lydopptak av samtaler mellom elevene når de jobber. For at vi skal ha nytte av lydopptakene må vi også vite hva elevene ser på skjermen når de diskuterer. Vi ønsker derfor å plassere videokamera bak elevene og filme dataskjermen mens elevene jobber. Vi ønsker også å intervjue en gruppe elever og ta lydopptak av disse gruppeintervjuene.

Vi ber med dette om tillatelse til å foreta lyd- og videoregistrering, og trenger elevens og foresattes samtykke. Vi ber derfor om at **både** elev og foresatte skriver under den vedlagte avtalen.

All registrering, lagring og bruk blir gjort i henhold til Datatilsynets retningslinjer. All informasjon vil bli anonymisert, og kan ikke føres tilbake til den enkelte elev. Det er bare prosjektledelsen som vil se og høre opptakene.

Med vennlig hilsen

Ragnhild Lyngved
Doktorgradsstipendiat

Peter van Marion
Prosjektansvarlig/veileder

NTNU
Norges teknisk-naturvitenskapelige universitet

Avtale

Elevens navn

	Ja	Nei
Undertegnede godtar at det blir gjort lyd- og video-opptak, samt tatt bilder med digitalt kamera i forbindelse med prosjektet "Viten – nettbasert undervisning i realfag".		
Undertegnede godtar at det blir tatt lydopptak av intervjuer i forbindelse med prosjektet "Viten – nettbasert undervisning i realfag".		
Undertegnede godtar at bilder tatt med digitalt kamera kan benyttes på prosjektets hjemmesider (http://viten.no), og ved presentasjoner av resultater fra prosjektet. **Kun bilder** vil bli brukt. De vil **ikke** bli koblet til navn, skole eller hjemsted. (På neste side ser dere hva slags type bilder det er snakk om).		

Opptak, bruk og lagring av opptakene vil bli foretatt i henhold til Datatilsynets retningslinjer. Ved publisering av resultatene fra prosjektet vil informasjon ikke kunne føres tilbake til den enkelte deltaker på opptakene.

Dato Sted

Elevens underskrift

Foresattes underskrift

Eksempler på digitale bilder med elever i undervisningssituasjon.

Appendix 6

Pre-test: Kloning

Navn:	Dato:	Gutt:
Skole:	Klasse:	Jente:

Nedenfor er det en del spørsmål. Du må svare på alle spørsmålene selv om du ikke er sikker på svaret. Legg merke til at noen spørsmål inneholder to eller flere deler.

1. Hva er kloning?

2. Kan du gi noen eksempler på kloning? Hvilke?

3. Hva er stamceller?

4. Hva kan stamceller brukes til?

5. Vet du om noen måter dyr kan klones på?

Hvis ja, forklar hvordan!

6. Kan du nevne noen planter som kan klone seg selv i naturen?

Forklar hvordan!

7. Hva kan plantehormoner brukes til?

8. Hvilke tanker gjør du deg om kloning?

9. Hvilke risikoer kan det være forbundet med kloning? Begrunn svaret ditt.

10. Kan du huske å ha sett, hørt eller lest noe om kloning i nyhetsbildet? Fortell hva det gjaldt og hva du kan huske fra det.

Sett kryss foran det du mener er riktig svar:

11. Hvilke av disse formeringsobjektene representerer kloning? Sett ett kryss.
- ❑ **Solsikkefrø**
- ❑ **Settepoteter**
- ❑ **Plommesteiner**

12. Hvordan ble sauen Dolly klonet?
- ❑ **En spesialisert celle fra dyret som skulle klones ble stimulert med hormoner og satt inn i en livmor**
- ❑ **En cellekjerne fra dyret som skulle klones ble satt inn i en spesialisert kjerneløs kroppscelle som ble dyrket fram til et embryo**
- ❑ **En kroppscelle fra dyret som skulle klones ble smeltet sammen med en kjerneløs ubefruktet eggcelle**

13. Hvilket signalsystem er viktigst hos planter?
- ❑ **Hormonsystemet**
- ❑ **Nervesystemet**
- ❑ **Rotsystemet**

14. Hvilken påstand er riktig?
- ❑ **Proteiner styrer alle prosesser i cellene**
- ❑ **Alle proteiner er enzymer**
- ❑ **Proteiner er bygd opp av aminosyrer og nukleinsyrer**

15. Hvordan virker hormoner? Sett ett kryss.
- ❑ **Hormoner virker på alle celler, vev og organer**
- ❑ **Hormoner virker bare på celler med bestemte reseptorer**
- ❑ **Hormoner virker som vekststimulatorer på bestemte celler**

16. Hvilken påstand er feil?
- ❑ **Auxin får stiklinger til å lage røtter**
- ❑ **Eten får frukt til å modnes**
- ❑ **Cytokinin hemmer celledeling**

17. Hva vil du gjøre for å få planter av ensartet kvalitet? Sett ett kryss.
- ❑ **Ta vev fra en plante og dyrke det i reagensrør**
- ❑ **Dyrke fram planter fra frø av samme morplante**
- ❑ **Så frø i veksthus under kontrollerte klimaforhold**

Post-test: Kloning

Navn:	Dato:	Gutt:
Skole:	Klasse:	Jente:

Nedenfor er det en del spørsmål. Du må svare på alle spørsmålene selv om du ikke er sikker på svaret. Legg merke til at noen spørsmål inneholder to eller flere deler.

1. Hva er kloning?

2. Kan du gi noen eksempler på kloning? Hvilke?

3. Hva er stamceller?

4. Hva kan stamceller brukes til?

5. Vet du om noen måter dyr kan klones på?

Hvis ja, forklar hvordan!

6. Kan du nevne noen planter som kan klone seg selv i naturen?

Forklar hvordan!

7. Hva kan plantehormoner brukes til?

8. Hvilke tanker gjør du deg om kloning?

9. Hvilke risikoer kan det være forbundet med kloning? Begrunn svaret ditt.

11. Kan du huske å ha sett, hørt eller lest noe om kloning i nyhetsbildet? Fortell hva det gjaldt og hva du kan huske fra det.

Sett kryss foran det du mener er riktig svar:

11. Hvilke av disse formeringsobjektene representerer kloning? Sett ett kryss.
- ❑ **Solsikkefrø**
- ❑ **Settepoteter**
- ❑ **Plommesteiner**

12. Hvordan ble sauen Dolly klonet?
- ❑ **En spesialisert celle fra dyret som skulle klones ble stimulert med hormoner og satt inn i en livmor**
- ❑ **En cellekjerne fra dyret som skulle klones ble satt inn i en spesialisert kjerneløs kroppscelle som ble dyrket fram til et embryo**
- ❑ **En kroppscelle fra dyret som skulle klones ble smeltet sammen med en kjerneløs ubefruktet eggcelle**

13. Hvilket signalsystem er viktigst hos planter?
- ❑ **Hormonsystemet**
- ❑ **Nervesystemet**
- ❑ **Rotsystemet**

14. Hvilken påstand er riktig?
- ❑ **Proteiner styrer alle prosesser i cellene**
- ❑ **Alle proteiner er enzymer**
- ❑ **Proteiner er bygd opp av aminosyrer og nukleinsyrer**

15. Hvordan virker hormoner? Sett ett kryss.
- ❑ **Hormoner virker på alle celler, vev og organer**
- ❑ **Hormoner virker bare på celler med bestemte reseptorer**
- ❑ **Hormoner virker som vekststimulatorer på bestemte celler**

16. Hvilken påstand er feil?
- ❑ **Auxin får stiklinger til å lage røtter**
- ❑ **Eten får frukt til å modnes**
- ❑ **Cytokinin hemmer celledeling**

17. Hva vil du gjøre for å få planter av ensartet kvalitet? Sett ett kryss.
- ❑ **Ta vev fra en plante og dyrke det i reagensrør**
- ❑ **Dyrke fram planter fra frø av samme morplante**
- ❑ **Så frø i veksthus under kontrollerte klimaforhold**

Sett ett kryss på en skala fra 1- 6, der 1 er helt uenig og 6 er helt enig.

18. Hvor enig er du i følgende påstander?

	1	2	3	4	5	6
a. Arbeidet med programmet Kloning har gitt meg større kunnskap om kloning	☐	☐	☐	☐	☐	☐
b. Arbeidet med programmet Kloning har ført til at jeg er blitt mer interessert i kloning	☐	☐	☐	☐	☐	☐

Dersom du svarte 1, 2 eller 3 på spørsmål 18 a …… Gå videre til spørsmål 19
Dersom du svarte 4, 5 eller 6 på spørsmål 18 a …… Gå videre til spørsmål 20

19. Hvor enig eller uenig er du i følgende påstander?
Jeg har ikke fått så mye kunnskap om kloning fordi:

	1	2	3	4	5	6
a. det hele var satt i en virkelighetsnær sammenheng	☐	☐	☐	☐	☐	☐
b. det var IKT-basert	☐	☐	☐	☐	☐	☐
c. det var dårlige forklaringer	☐	☐	☐	☐	☐	☐
d. det var dårlige figurer og animasjoner	☐	☐	☐	☐	☐	☐
e. det var dårlige spørsmål og oppgaver	☐	☐	☐	☐	☐	☐
f. programmet var lagt opp slik at en skulle løse en kriminalgåte	☐	☐	☐	☐	☐	☐

20. Hvor enig eller uenig er du i følgende påstander?
Jeg har fått en del kunnskap om kloning spesielt fordi:

	1	2	3	4	5	6
a. det hele var satt i en virkelighetsnær sammenheng	☐	☐	☐	☐	☐	☐
b. det var IKT-basert	☐	☐	☐	☐	☐	☐
c. det var enkle og gode forklaringer	☐	☐	☐	☐	☐	☐
d. det var gode figurer og animasjoner	☐	☐	☐	☐	☐	☐
e. det var gode spørsmål og oppgaver	☐	☐	☐	☐	☐	☐
f. programmet var lagt opp slik at en skulle løse en kriminalgåte	☐	☐	☐	☐	☐	☐

Dersom du svarte 1, 2 eller 3 på spørsmål 18 b …… Gå videre til spørsmål 21
Dersom du svarte 4, 5 eller 6 på spørsmål 18 b …… Gå videre til spørsmål 22

21. Hvor enig eller uenig er du i følgende påstander?
Jeg har ikke blitt noe mer interessert i kloning fordi:

	1	2	3	4	5	6
a. det hele var satt i en virkelighetsnær sammenheng	☐	☐	☐	☐	☐	☐
b. det var IKT-basert	☐	☐	☐	☐	☐	☐
c. det var dårlige forklaringer	☐	☐	☐	☐	☐	☐
d. det var dårlige figurer og animasjoner	☐	☐	☐	☐	☐	☐
e. det var dårlige spørsmål og oppgaver	☐	☐	☐	☐	☐	☐
f. programmet var lagt opp slik at en skulle løse en kriminalgåte	☐	☐	☐	☐	☐	☐

22. Hvor enig eller uenig er du i følgende påstander?
Jeg har blitt mer interessert i kloning spesielt fordi:

	1	2	3	4	5	6
a. det hele var satt i en virkelighetsnær sammenheng	☐	☐	☐	☐	☐	☐
b. det var IKT-basert	☐	☐	☐	☐	☐	☐
c. det var enkle og gode forklaringer	☐	☐	☐	☐	☐	☐
d. det var gode figurer og animasjoner	☐	☐	☐	☐	☐	☐
e. det var gode spørsmål og oppgaver	☐	☐	☐	☐	☐	☐
f. programmet var lagt opp slik at en skulle løse en kriminalgåte	☐	☐	☐	☐	☐	☐

Alle skal besvare dette siste spørsmålet:

23. Hvor enig er du i følgende påstander? 1 betyr helt uenig; 6 betyr helt enig.

	1	2	3	4	5	6
a. Jeg synes at det var spennende å jobbe med kloningsprogrammet	☐	☐	☐	☐	☐	☐
b. Jeg synes at arbeidet med klonings-programmet var lærerikt	☐	☐	☐	☐	☐	☐
c. Jeg vet mer om kloning nå enn tidligere	☐	☐	☐	☐	☐	☐
d. Jeg forstår mer om de biologiske prosessene som er sentrale i forbindelse med kloning enn tidligere	☐	☐	☐	☐	☐	☐
e. Jeg har større forståelse for de etiske momentene som er forbundet med kloning og forskningen forbundet med kloning enn tidligere.	☐	☐	☐	☐	☐	☐
f. Jeg synes det er lettere å ta stilling til de etiske problemstillingene som er forbundet med kloning og moderne molekylærbiologisk forskning enn tidligere	☐	☐	☐	☐	☐	☐
g. Jeg har større kunnskap om hvordan moderne forskere jobber enn jeg hadde før	☐	☐	☐	☐	☐	☐
h. Jeg er mer interessert i kloning nå enn jeg var før	☐	☐	☐	☐	☐	☐
i. Jeg følger mer med i nyhetsoppslag om kloning enn før	☐	☐	☐	☐	☐	☐
j. Jeg leser oftere enn før oppslag og artikler som gjelder kloning i aviser, tidsskrifter, nettaviser etc.	☐	☐	☐	☐	☐	☐
k. Jeg er mer interessert i hvordan forskere jobber nå enn jeg var før	☐	☐	☐	☐	☐	☐

Appendix 7

Spørsmål til gruppeintervju

Intervjuguide for Ragnhild Lyngved
– før utprøving av "Kloning av planter"

- **Kort informasjon om programmet og opplegget, påpeke betydningen av å snakke med samme elever både før og etter utprøving.**

- **Jeg stille 8-10 spørsmål. Henvende meg til 1 person først (varierer til hvem). De andre kan deretter fritt komme med sine kommentarer, synspunkter**

- **Ikke "intervju", men samtale / diskusjon**

- **Henvender meg til dere vha tall (hver person får et tall istf navn) – anonymt**

1. Først vil jeg at dere tenker litt på ordet kloning. Hva tenker dere på (er det første som slår dere) når dere hører dette ordet? Hva forbinder du med ordet kloning?

2. Har dere hørt om Dolly? Hvordan ble Dolly klonet?

3. Har du noen følelse knyttet til kloning generelt? (nøytral, positiv, negativ)
   Hvilken? Hvorfor ikke?
   Hvorfor nettopp dette?
   Hvor har dere hørt om nettopp dette?
   Har dere hentet det fra noe sted? (venner, familie, eget hode, nyhetsbildet…?)

4. Er det etisk forsvarlig å klone dyr? Hvorfor? Hvorfor ikke?
   Eksempler på hvorfor? Hvorfor ikke?
   Eksempler på når det evt kan være forsvarlig?

   (Hvorfor ønsker noen å klone dyr?
   - Av forskningshensyn
   - For å reprodusere individer med spesielt ønskede gener (utryddingstruede/genmodifiserte)
   - For å klone døde kjæledyr)

5. Er det etisk forsvarlig å klone mennesker? Hvorfor ikke?

6. Synes du at temaet kloning er interessant? Hvorfor? Hvorfor ikke?

7. Synes du at det er interessant å lære om hvordan forskere jobber?
   (evt "sånne forskere som jobber med kloning")
   Hvorfor? Hvorfor ikke?

8. Har du noen tanker om det å bruke Internettbaserte undervisningsprogrammer i undervisningen?
   Har dere gjort det før?

   Hvis ja;
   Hvordan virket det? Lærerrikt? Verdt å bruke tid på? Faglig utbytte?
   Fremmet det interessen for temaet?

   Hvis nei;
   Ønske om å bruke slike program/Internett i undervisningen?
   Hvorfor? Hvorfor ikke?

Spørsmål til gruppeintervju

Intervjuguide for Ragnhild Lyngved
– etter utprøving av "Kloning av planter"

1. Først vil jeg at dere tenker litt på ordet kloning igjen. Hva tenker dere på (er det første som slår dere) når dere hører dette ordet? Hva forbinder du med ordet kloning? Endring siden sist?

2. Vet dere nå hvordan Dolly ble klonet?

3. Synes du at du har hatt faglig utbytte av programmet?

4. Hva konkret har du lært mer om? Hvorfor? Var det noe i programmet som gjorde at du lærte om det?

5. Har du noen følelse knyttet til kloning generelt? (nøytral, positiv, negativ) Endring?
   Hvilken? Hvorfor ikke?
   Hvorfor nettopp dette?
   Hvor har dere hørt om nettopp dette?
   Har dere hentet det fra noe sted? (venner, familie, eget hode, nyhetsbildet…?)

6. Er det etisk forsvarlig å klone dyr? Hvorfor? Hvorfor ikke?
   Eksempler på hvorfor? Hvorfor ikke?
   Eksempler på når det evt kan være forsvarlig?

   (Hvorfor ønsker noen å klone dyr?
   - Av forskningshensyn
   - For å reprodusere individer med spesielt ønskede gener (utryddingstruede/genmodifiserte)
   - For å klone døde kjæledyr)

7. Er det etisk forsvarlig å klone mennesker? Hvorfor ikke?
   (usikre konsekvenser, press, identitet)

8. Synes du nå at temaet kloning er interessant? Hvorfor? Hvorfor ikke?

9. Synes du at det er interessant å lære om hvordan forskere jobber?
   (evt "sånne forskere som jobber med kloning")
   Hvorfor? Hvorfor ikke? Endring?

10. Synes du at det ble mer interessant å lære om kloning når du samtidig fikk innblikk i hvordan forskere jobber? Var det interessant å lære om kloning "via en forsker"?
    Hvorfor? (ledende spørsmål, begrunnelse av "hvorfor" er spesielt viktig!)
    Hadde det noe å si at teoristoffet var satt inn i en virkelighetsnær sammenheng?
    På hvilken måte hadde det noe å si?

11. Har dere nå andre tanker om det å bruke Internettbaserte undervisningsprogrammer i undervisningen?

    Hvis ja;
    Hvilke?
    Hvordan virket det? Lærerrikt? Verdt å bruke tid på? Faglig utbytte?
    Fremmet det interessen for temaet?
    Hvorfor tror dere det hadde denne effekten?

    Hvis nei;
    Hvorfor?

Appendix 8

GRUPPEINTERVJU 2BI
FØR UTPRØVING AV PROGRAMMET

E = Elev
R = Ragnhild
4 elever: E21 (student x, deltok ikke i utprøvingen på grunn av sykdom), E22 (student 16), E23 (student 12), E24 (student 15) (Betegnelsene student 12, 15 og 16 brukes i Paper III)

… betyr at eleven tenker en stund

1. **R: Først vil jeg gjerne høre hva dere tenker når dere hører ordet kloning? Hva er det som slår dere først?**

   E21(student x): Tukling med gener. Det er det jeg tenker på.

   E22 (student 16): Jeg tenker på sauen Dolly.

   R: Hva var det?
   E22 (student 16): Det var den første klonede sauen, tror jeg.

   R: Vet du noe mer om sauen Dolly?
   E22 (student 16): Jeg tror at hun er død. Jeg vet ikke noe mer.

   E23 (student 12): Samme som tukling med gener, i dårlig film tenker jeg på. Det er ofte ”world”, ”the clone”, som ”Star Wars” og sånn.

   E24 (student 15): Noe om det samme, om menneske kloning.

2. **R: Har alle hørt om Dolly?**
   Alle svarer ja.

   R: Kan dere forklare hvordan Dolly ble klonet?
   E24 (student 15): Det er jo sånt at… tar de ikke noen kjønnsceller? Jeg vet ikke hvordan de kloner. Men jeg tenker meg frem til det liksom at de tok kanskje kjønnsceller fra en hannsau og en hunnsau, jeg vet ikke hva de heter, de parer en zygote sikkert og til slutt utviklet det seg sikkert til Dolly. Jeg vet ikke. Men de tok jo... Siden det er kloning var den helt lik den originalen, for å si det sånn. De tok sikkert gener fra den. Jeg er ikke helt sikker.

   R: Vet 23 noe mer?
   E23 (student 12): Det var noe sånn at de tok DNA for å si det sånn, fra den originale sauen, så manipulerer de befruktningen og noe sånn.

   E21(student x): Det er noe sånn at de tar en celle fra morsauen, og så lager de en helt lik sau.

   R: Jeg skal ikke røpe noe mer om det fordi dere skal lære det på mandag.

3. **R: Hvis dere tenker på kloning, har dere noen følelse knyttet til det? (positiv, negativ eller nøytral)**
   E22 (student 16): Jeg er ganske nøytral til det. Jeg er ikke så mye for at de skal begynne å klone mennesker. Det synes jeg ikke så mye om.

   R: 21?
   E21(student x): Jeg leste det : det var ganske lenge siden. Det var noe om Madonna, at de kunne klone et likt menneske som Madonna, ut fra et hårstrå. Det går litt lang da, når de begynner med sånt. Det tror jeg ikke er så positiv innvirkning på samfunnet heller. Det er litt skummelt egentlig.

   R: Generelt, eller bare om mennesker?
   E21(student x): Egentlig generelt.

   R: 23?
   E23 (student 12): Jeg er positiv fordi du kan få donor organer som hjerter og sånn. Sånn sett er det veldig positivt hvis du kan klone menneskeorganer. Det blir kjedelig hvis du skal drive på og få de samme

menneskene som man har hatt. Hvis du skal drive på å klone Hitler og sånn, så er jeg selvfølgelig ikke positiv.

R: 24?
E24 (student 15): Jeg er veldig enig med det de har sagt. Og jeg er ganske nøytral til det, jeg også. Jeg mener at det kan være rett for eksempel hvis det går an å klone… Nei, glem det! Nei, helt enig med 23.

R: De tankene, er de noe som dere har tenkt ut selv eller har dere blitt påvirket av medier, foreldre, venner, eller andre ting?
E23 (student 12): Litt av alt kanskje. Det har vært mye på media, som har blitt diskutert. Hjemme også. Så du gjør deg jo til slutt dine egne tanker, om det er positivt eller negativt, ut av hva du har fått med deg av informasjon.

R: 24?
E24 (student 15): Enig

R: 22?
E22 (student 16): Enig med han, medier har påvirket meg ihvertfall.

R: 21?
E21 (student x): Det er sånn at du får masse inntrykk fra forskjellige plasser. Du tenker litt selv og så gjør deg opp din egen mening. Men jeg har blitt litt påvirket, tror jeg.

4. **R: Hva synes dere: å klone dyr, er det etisk forsvarlig?**
E21(student x): Jeg stiller dyr på lik linje med mennesker, så jeg mener akkurat det samme som det jeg synes om menneskekloning

R: Kan du utdype hvorfor?
E21 (student x): Jeg synes at det blir litt feil. Men, for eksempel, hvis du tar… jeg husker noe som jeg har sett, en okse som ser så forferdelig ut fordi mennesker hadde tuklet med gener og sånn der, for at den skal få mer kjøtt og for at det blir bedre for menneske å leve, mer mat. Jeg synes at det blir litt feil. Men også for eksempel med kloning av dyr. Får du nok mat til en stor befolkning, kan det være bra, men jeg synes ikke likevel...

R: 22?
E22 (student 16): Jeg har ikke tenkt så mye på det før, men jeg stiller det på lik linje som hos mennesker.

R: Er det ingen eksempler på at det kan være etisk forsvarlig eller ikke?
E22 (student 16): Som 21 sa at du kan klone dyr for å få kjøtt og sånn der.

R: 23?
E23 (student 12): Det er veldig positiv hvis du kan få tilbake dyr som er utryddet, for eksempel DODO eller Mammut eller sånne ting. Men hvis du bare skal fortsette å klone dyr som vi har mange av nå, for eksempel sauen Dolly og sånn, så det blir mer for å teste ut, for det skal jo være sånn variasjon i arten og sånn. For bare oppdrett blir det galt, men for få tilbake ting som er utryddet synes jeg at det er litt spennende.

R: 24?
E24 (student 15): Jeg synes at dyr kan man klone hvis det går til et bra formål, for å si det sånn. Hvis de bruker det til å mate folk i fattige land, i Afrika for eksempel, slik som sultehjelp, så synes jeg at det kan gå. Jeg er ikke imot det. Men hvis det blir som overdriving, og de kloner for folk for I-Land for eksempel så er jeg ikke helt enig. Men jeg stiller óg dyr på lik linje som mennesker.

5. **R: Kan du, 24, si mer om hvorfor eller hvorfor ikke det er etisk forsvarlig å klone mennesker?**
E24 (student 15): Jeg vet ikke, men hvis for eksempel et klonet menneskebarn vokser opp, så tilhører det ikke noen for å si det sånn. Den har ikke foreldre. Den kommer sikkert til å knytte bånd til andre folk men den ville bare være et eksperimentelt forsøk, da. Den ble ikke laget av kjærlighet for å si det sånn.

R: 23, Har du noen tanker om hvorfor ikke mennesker skulle bli klonet, hvis du synes det?
E23 (student 12): Jeg har også sett at mennesker kan bli klonet av flere grunner egentlig. men hvis det klones personer som allerede er oppegående, for å si det sånn, er jeg veldig negativ fordi du mister identiteten din. Den andre personen kan gå rundt og gjøre ting og så kan du for skylda etterpå.

R: Andre ting 22?
E22 (student 16): Nei.

R: Og 21?
E21 (student x): Jeg vil bare si noe på det med kloning av dyr. Jeg tenker på den oksen som jeg tok som eksempel. Jeg tror ikke at de har det så veldig godt der de går. Det tror jeg ikke. Jeg er ganske enig med det 24 og 23 sa om kloning av mennesker.

6. **R: Synes dere at temaet kloning er interessant? Hvorfor? Hvorfor ikke? 22?**
E22 (student 16): Det er ganske interessant egentlig fordi det er noe nytt på en måte. Jeg har ikke hørt så mye om det, egentlig. Jeg synes det er litt interessant. Måten de gjør det på og hvorfor de gjør det.

R: 23?
E23 (student 12): Det er en ny sjanse for de som har mistet barna sine. Det var snakk om at det var noen i Amerika selvfølgelig, som hadde mistet ungen sin og skulle få klonet en ny. Det er kanskje litt bisart, men hvis det er det de mener som skal til ... er det positivt for deres del.

R: Så du synes det er interessant for deg?
E23 (student 12): Det er spennende, sånn sett. Det kan du sikkert snakke om fram og tilbake, for og mot og sånn. Det er spennende å følge med på utviklingen.

R: 21, Er det interessant for deg?
E21 (student x): Ja, det er egentlig ganske interessant å følge med, for du lærer mer om oss mennesker også om dyr. Hvilke grenser de har.

R: 24?
E24 (student 15): Jeg synes det er interessant, og spesielt hvis det fører til noe positivt, da.

7. **R: Synes du at det er interessant å vite mer om forskningen, for eksempel forskning på kloning? Hvordan forskere jobber?**
R: 24?
E24 (student 15): Jo, kanskje det! … Jeg kan ikke så veldig mye om det nå. Jeg synes det er interessant hvis det er å vite mer.

R: 23?
E23 (student 12): Det blir sånn blandet, det blir på samme måte som å lære om hvordan brannmenn eller politimenn jobber. Hvis du skal velge sånn videre karriere utvikling, er det litt morsomt å lære samtidig hvordan de gjør og jobber med det.

R: 22?
E22 (student 16): Det er interessant for å tenke litt om det. Artig å lære noe nytt på en måte.

R: 21?
E21 (student x): Jeg vil gjerne lære om hvordan de gjør det, hvordan de jobber. Jeg har ikke en så sterk mening om det, heller.

R: Det har kanskje litt å gjøre med hva slags retning en velger, hvis man tenker å gå i denne retningen så er det interessant, som 23 sa.

8. **R: Et siste spørsmål. Har dere gjort dere noen tanker om det å bruke Internettbaserte undervisningsprogrammer i undervisningen? Det å bruke Internett? 21?**
E21 (student x): Nei, egentlig ikke.

R: Har du brukt det noen gang? Har du brukt det i biologi?
E21 (student x): Nei.

E23 (student 12): Tenker du på at vi lærer gjennom Internett?
R: Ja.

E23 (student 12): Eller i prosjekt og sånn, vi bruker Internett for å finne informasjon og sånn.

R: I Biologi, og?

E23 (student 12): Ja vel ….
E21 (student x): Vi tok ikke så ….

E23 (student 12): Vi har ikke hatt så mye prosjekt, sånn sett… Det er greit hvis du skal finne noe du leter etter, informasjon om et spesielt emne, så er det veldig greit å bruke Internett, som en kilde, eller noe sånn.

R: Er det noe som fremmer interessen for temaet, eller er det bare å bruke det som et leksikon, et oppslagssverk? Eller kunne du tenke deg at læreren bruker det mer i undervisningen i klassen?
E23 (student 12): Sånn sett, det er en ny læremåte, og nye læremåter er stort sett alltid spennende. Det blir som innføring av å se film i undervisning og sånn. Det blir spennende. Det kan være en ny måte å få elevene til å følge med bedre i timene, i stedet for vanlig tavleundervisning. Sånn sett så høres det jo bra ut.

R: 22, Har du tenkt å bruke det?
E22 (student 16): Ja, man blir lei av den gamle læremåten.

R: 24, Tror du at det kunne være lærerikt, interessant?
E24 (student 15): Ja, det er vel det.

R: Hvorfor, eventuelt?
E24 (student 15): Det er jo nytt, da. Og alt nytt er alltid spennende, så. Vi var nå med på noe lignende ting i fjor, i naturfaget, da vi var på viten.no i naturfaget.

R: Ja, det var brenselcelleprosjektet?
E24 (student 15): Det var ganske interessant, synes jeg.

R: Det gjelder både 21 og 22, eller alle? Var 23 med også?
E23 (student 12): Ja.

R: Så dere synes dere hadde noe igjen for det?
E21(student x): Det er ikke alt du…du finner jo alltid mer stoff på Internett enn det som står i boka. Artig å lære nye ting og mer.

E22 (student 16): Det skjer kanskje at det er flere sider av en sak. Med flere kilder og sånt. Det kan være nyttig.

GRUPPEINTERVJU 2BI
ETTER UTPRØVING AV PROGRAMMET

E = Elev
R = Ragnhild
3 elever: E22 (student 16), E23 (student 12), E24 (student 15)
(Betegnelsene student 12, 15 og 16 brukes i Paper III)
E21 var borte på grunn av sykdom

… betyr at eleven tenker en stund

1. **R: Hvis dere starter med å tenke på kloning i dag, har dere noen andre assosiasjoner enn sist?**
   E22 (student 16): Jeg tenker på plantekloning, dyrekloning og menneskekloning, ja.

   R: I forhold til sist, så var det mer?
   E22 (student 16): Jeg tenkte på… Hva tenkte jeg på? Da var det bare menneskekloning jeg tenkte på, tror jeg.

   E23 (student 12): Det var mye det samme at det på en måte. Det var bare å tenke på mennesker, om det var kloning og sånn, og Dolly selvfølgelig, og nå er det mer bredt utover planter.

   E24 (student 15): Jeg vil nå si også at jeg fikk vite at det går an å klone celler og organer, også forskjellige ting. Jeg trodde bare at liksom hele mennesker og hele dyr ...det er bredere perspektiv.

   R: Det det gjøres mest av er egentlig kloning på et lavere nivå, også gener.
   E24 (student 15): Og bakterier.

2. **R: Kan dere fortelle meg nå om hvordan Dolly ble klonet?**
   E24 (student 15): Jeg tror jeg husker spesielt fra den animasjonen som bildene viser den sauen som skulle bli klonet. Jeg tror at de tok en kroppscelle fra den, og så tok man ei eggcelle fra den donorsauen, som jeg kaller det. Og så tok man og fjernet kjernen derifra tror jeg, fra eggcellen, og satt sammen, tror jeg, eggcellen og den kjerneløse eggcellen og kroppscellen. Og så satt man det sikkert sammen med ei livmor til den sauen som skulle bære det fram, liksom. Det husker jeg mest av ihvertfall.

   R: 23 nikker, høres det riktig ut?
   E23 (student 12): Det er stort sett det som jeg husker. Cirka.

   E22 (student 16): Jeg husker ikke noe mer om det.

   R: Må det være tre sauer for å utføre kjerneoverføring, for å få fram et lite dyr?
   E24 (student 15): Jeg vet ikke. Kanskje to, tre. Ihvertfall ikke mindre enn to.

   R: Fordi?
   E24 (student 15): Du må jo... Du kan ikke ha... Du må ha DNA til det dyret som skal bli klonet, og det kan ikke, liksom, føde seg selv, hvis du skjønner, hvis du putter det sammen med ei eggcelle. Eller kan det? Det tror jeg ikke, jeg, ihvertfall. Jeg vet ikke hvordan jeg skal forklare det. Jeg tror ikke at det går an.

   R: Hva tror 22?
   E22 (student 16): Ikke peiling.

   E23 (student 12): Jeg tror det må tre dyr til, fordi hvis det er bare dem to, så blir det på en måte de som er stammen til det nye dyret eventuelt. Det blir jo hvis det er ett av de to første som skal bære fram altså den nye klonen, så blir det nesten innavl.

   R: ….så kan dere se at det faktisk kan være bare en sau, hvis det er en hunnsau. Da kan du få ei eggcelle derfra og så kan du smelte den sammen med en annen kroppcelle. Da blir det 100 prosent likt. Hvis du bruker to donorer kan du si, en som du tar ei eggcelle fra og en annen… så får du med mitokondrie-DNA sammen med ei eggcelle, så da blir det ikke 100 prosent likt som den du tok kroppscella fra.

3. **R: Synes dere at dere har hatt noe faglig utbytte av programmet?**
   E22 (student 16): I hvert fall har jeg lært litt mer.

4. **R: Kan du si litt mer om det?**
E22 (student 16): Spesielt da vi skulle påvise proteinene i det her programmet, så jeg lærte. Jeg fikk et annet syn på det, på en måte.

R: Kan du si noe om hvorfor akkurat det?
E22 (student 16): Jeg visste ikke at de gjorde det på den måten. Jeg lærte litt mer om hvordan forskere jobber og sånn, enn jeg gjorde før.

E23 (student 12): Som 22 sa, at det var lab-analysene, det var på en måte spennende, fordi det var noe som du skulle gjøre. Det var ikke bare lesing eller å se en film. Du jobbet med det selv. Det er kanskje det som jeg synes var spennende. Det som jeg lærte mest av kanskje.

R: Synes du at du lærte noe i det hele tatt av noe annet?
E23 (student 12): Ja, selvfølgelig å komme seg fram til løsningen på mysteriet. Du måtte jo vite, eller få med noe av det andre som stod der også, og sånn sett jo lærte vi noe av det.

R: Det kunne du ikke fra før?
E23 (student 12): Nei, langt i fra.

R: 24, Synes du at du har lært noe?
E24 (student 15): Selvfølgelig, jeg synes at det var et veldig lærerikt program. Spesielt når det var med animasjoner. Når det viste oss hvordan det og det ble utført, og sånt. Ja.

R: Er det noen spesielle animasjoner du tenker på?
E24 (student 15): Den med sauen.

R: Den husker du veldig godt. Mener alle at det er de tingene der dere måtte gjøre noe praktisk eller så bevegelser, …var det der dere lærte noe først og fremst?
E22 (student 16): Jeg lærte ting...
E?: De intervjuene, bare radio intervjuene. Jeg synes at det var artigere enn å lese. Det var noe annet enn å bare sitte og lese på fakta, på en måte. Jeg synes at det var som noe annet.

R: Hva synes du at du har lært derfra?
E22 (student 16): Jeg kommer ikke på noe nå, jeg tenkte etterpå ihvertfall at jeg lærte litt.

5. **R: Hvis dere går tilbake på det med følelsen dere hadde for kloning, positiv eller negativ. Har den holdningen endret seg siden sist?**
E23 (student 12): Egentlig ikke. Det har ikke endret seg noe særlig. (*Mobiltelefonen gjør at det blir umulig å forstå*)

R: Andre eksempler på hvorfor du er negativt innstilt til det?
E23 (student 12): Nei, egentlig ikke.

R: Kommer du på noen eksempler på hvorfor det kan være positivt?
E23 (student 12): Med kloning?

6. **R: For eksempel med å klone dyr?**
E23 (student 12): Nei.

E24 (student 15): For eksempel dyr som kan være utrydningstruet, de kan hjelpe dem å komme tilbake, for å si det sånn. Du lager mer av dem. Det er også positivt det at du kan klone, prøve å klone menneskeorganer til for eksempel pasienter som trenger det, som er hjertesyke eller noe sånn. Det synes jeg er veldig nyttig. Men når det gjelder å klone hele mennesker så sier jeg at jeg er helt imot det, liksom.

7. **R: Har du noe flere eksempler på hvorfor, eller hva det er som er galt med å klone mennesker, konkret?**
E24 (student 15): Jo, jeg tenker liksom på menneskebarn liksom når de vokser opp liksom. De har ikke noe mor eller noe far, liksom, som har født dem ordentlig for å si det sånn. Også liksom at han tenker sikkert: var jeg bare en følge av et eksperiment eller? Jeg vet ikke. Føler ikke tilhørighet til noe, liksom. Etiske spørsmål.

E23 (student 12): Det blir vel litt sånn som… Det blir ikke noe annerledes egentlig enn sånn kunstig befruktning, et prøverørsbarn. Egentlig bør det være litt det samme. Du stammer fra noen, i bunn og grunn. Sånn sett er tilhørlighet og sånn på samme måte som prøverørsbarn. Jeg tror ikke at hvis du vokser opp og vet at du er et prøverørsbarn, så eventuelt i fremtida kommer det ikke til å bli mye annerledes, ja at du er en klone. Det blir som om foreldrene sier: Ja, du ble oppkalt etter bestefaren din. Nå kan du heller kanskje si at: Ja, du er klonen til bestefaren din. I framtida så blir det vel egentlig den samme tingen. Det blir ikke noe negativt, sånn sett.

R: 22, Er du enig med 23?
E22 (student 16): Ja, jeg er enig med det.

R: 24, Er du enig?
E24 (student 15): Prøverørsbarn, det ser ikke helt likt ut som... Det er ikke identisk med noe på mennesket. Det blir et nytt individ, da. Tror du ikke det?

E23 (student 12): Jo

E24 (student 15): At vi klonet barn, vi klonet for eksempel fordi barna våres døde før oss og vi vil ha det tilbake, fordi at du… Å klone deg fordi du ligner på dem. Så jeg er ikke helt enig.

E23 (student 12): Hvis du vokser opp og får vite at ja, nei, du ble klonet fordi at den forrige ungen vår den døde i en bilulykke sånn at vi vil ha den tilbake. Da føler jeg meg litt mistilpass, selvfølgelig. Men hvis det er kloning fordi det blir mer for å finne en ideell unge, eller familiemedlem, eller noe sånt framfor at det blir klonet noen som har dødd eller har gått i fra deg, da. Hvis du først skal klone noen som har forsvunnet blir det jo en feil hvis du sier: Nei, du er bare som en reserve, forsøk nummer to, liksom. Da blir du jo selvfølgelig skadet både psykisk og fysisk.

R: Tror du at det nye individet blir helt likt som det gamle?
E24 (student 15): Det blir ikke.

E22 (student 16): Det er noe med miljøet da, der du vokser opp. Du blir påvirket av samfunnet og miljø.

R: Det var en misforståelse at noen trodde at det blir helt likt. Men det fysiske utseendet blir nokså likt.
E22 (student 16): Ja.

8. R: Synes dere at temaet kloning er mer interessant nå enn før dere prøvde programmet?
E24 (student 15): Ja

R: Hadde det vært det samme om du hadde lest om kloning i ei bok?
E24 (student 15): Egentlig ikke.

R: Kan du si hvorfor?
E24 (student 15): Liksom hvis du leser det i ei bok, liksom bare: Hå ja. Liksom ganske tørt stoff hvis du ikke har bilder som kan vise deg for eksempel hvordan det skjer, og eksempler på det og sånt.

E24 (student 15): Det blir mer interessant blir det. Hvis vi har bilder og animasjoner og sånne tegninger. Kan ikke utdype mer om det. Det er ihvertfall mer interessant enn å lese.

R: Hva synes 22?
E22 (student 16): Jeg er helt enig, faktisk.

R: Hva er det som gjør det mer interessant enn ei bok?
E22 (student 16): Jeg synes at det er veldig kjedelig å bare sitte og lese og lese. Men hvis det... Jeg vet ikke. Det er bare noe annet når du ser animasjoner. Det bare blir noe annet. Når du skal løse et mysterium selv og du har blitt mer interessert, det kan få deg til å bli mer engasjert enn å bare lese det.

R: Så det har med den saken å gjøre, egentlig, ikke bare at det er animasjoner…?
E22 (student 16): Hum Hum.

R: Hva synes 23?

E23 (student 12): Om jeg har blitt mer interessert? Det høres kanskje litt skuffende ut om jeg sier nei. Sånn sett er det at kloning er et veldig spennende tema. Så jeg er kanskje ikke blitt mer interessert. Men jeg forholder meg like interessert som jeg var før. Sånn sett. Det er spennende å følge med på utviklingen.

R: Tror du at du kommer til å følge med mer enn før?
E23 (student 12): Nei, kanskje ikke. Hvis det først dukker opp noe i nyhetsbildet om kloning, så leser jeg det egentlig uansett før eller etter at jeg har hatt dette her. Men nå risikerer jeg faktisk å forstå hva som står der da. ...i forhold til tidligere. Sånn sett har jeg lærte noe.

R: Kan du si noe om hvorfor du har lært noe?
E23 (student 12): Så det er… Jeg har litt vanskeligheter for å gjengi ordrett det som jeg fikk med meg i programmet. Men hvis jeg ser det i en sammenheng så tror jeg at det er lettere for meg å skjønne det som står her, når jeg har blitt forklart det i et sånt program. Men tidligere hvis jeg hadde lest i naturfagsboka, og det var bare tørt kjedelig stoff, så…

9. **R: Synes dere at det var interessant å lære om hvordan forskerne jobber?**
E22 (student 16): Ja, som jeg sa før, det er alltid artig å vite hvordan de kommer fram til noe. Jeg fikk jo vite det på en ganske bra måte gjennom dette programmet.

R: Synes du det ble mer interessant å lære om kloning? Ved at du fikk det gjennom den forskeren?
E22 (student 16): Ja, mer interessant. Nei, kanskje ikke mer interessant, men sånn på lik linje som jeg gjorde før egentlig.

10. **R: Synes du, 23, at det blir mer interessant å lære om kloning gjennom en forsker?**
E23 (student 12): Mer troverdig ihvertfall, enn at lille Ole skal stå på gata og si: Hei, visste du at kloning skjer ved… En sånn banan som du hopper fram og tilbake på skjermen og sånt. Det blir mer troverdig at det er forskeren som står der og forklarer. Det blir mer lettere å sette seg inn i situasjonen. Når det er… det virker mer reelt. Sånn sett var det mer interessant å følge med på.

R: Hva sier 24, synes du at det var interessant å lære om hvordan forskerne jobber?
E24 (student 15): Det var litt, synes jeg, jeg er enig med det 23 sa.

R: Synes du at det er noen endring fra sist, holdninger som du hadde fra før?
E24 (student 15): Jeg vet ikke. Jeg visste ikke så mye om hvordan forskerne jobber med det. Nå har jeg større innsikt i hvordan det foregår.

R: Er det det som er interessant, det å lære om hvordan forskerne jobber, eller er det det at du lettere lærte om kloning?
E24 (student 15): Det at du lettere lærer om kloning, da, ved hjelp av forskeren.

R: Klarer du å si noe mer om hva det er som gjorde at du lærte lettere?
E24 (student 15): Det at det er troverdig som 23 sa. Det er jo folk som har jobbet mye med det og kan det de kan liksom.

E23 (student 12): Det virker hvert fall.

11. **R: Har dere nå andre tanker om det å bruke Internett i undervisningen enn før dere startet?**
E22 (student 16): Som jeg sa før vi startet, at jeg kommer til å bli… at jeg kommer til å være mer engasjert i å lære mer, for at det er Internett enn bare vanlig tavleundervisning, og det står jeg for ennå. Det er mer interessant. Det er det. Helt klart.

R: Var det noe spesielt med programmet som gjorde det? Eller kunne det vært Internett generelt?
E22 (student 16): Det er litt kjedelig å surfe. Ja, men det programmet vi fikk var ganske artig.... å løse selv og sånn der.

R: Kan du si mer om det i programmet?
E22 (student 16): At du fikk et oppdrag, da. At vi måtte finne ting selv gjennom det de sa.

E23 (student 12): Opp… særlig det la jeg merke til. Det var sånn nedtelling, eller opptelling. Tv serien 24, det var sånn å fange opp oppmerksomheten ganske fort. At det ble liksom kanskje tørt stoff, men satt i en mer action-sammenheng, og bil som skrenser, litt sånn happening, overvåkningskamera, tyverier og greier.

På laben har vi mistet et rør med hvitt pulver, finn det. Det blir mye spennende på en måte. Den kriminelle handlingen og sånn. Oppbygning sånn sett var kjempebra.

R: 24, Er du enig?
E24 (student 15): Helt enig.

R: 24, Er du enig at et sånt program er bedre enn å bare surfe som 22 sa?
E24 (student 15): Det tror jeg. Noe som har sammenheng liksom, ikke bare at du går på en ting, og ... og sånn. Noe som går i rekkefølge for å si det sånn.

R: Var det noen andre ting enn oppbygningen som du synes var lærerikt og bra?
E24 (student 15): Ja det var jo det.

R: Som du sa litt før, at det var animasjoner og tegninger?
E24 (student 15): Jo, det mer animasjoner, at det kom fram sånn. At du skulle diskutere, at det kommer fram et spørsmål og du skulle liksom tenke gjennom det og så kom det neste spørsmålet og tenke gjennom det, og diskutere og sånt. Det synes jeg var litt bra.

R: Oppgavene?
E24 (student 15): At du får oppgaver underveis, liksom. Jeg synes at det er ganske bra.

R: Kan du si noe om hva du synes var mindre bra?
E24 (student 15): … *Hun tenker*

R: Var det noe som gjorde at du ikke lærte noe, synes du?
E24 (student 15): Jeg vet ikke. Kanskje at noen ord ikke var forklart liksom, i begynnelsen, så at du skulle skjønne hva det var. For eksempel det med embryo, jeg visste ikke hva det var. Det stod noe, det stod ganske mye om det før du kom ned til det punktet hvor det stod hva det var, liksom. Men ellers var det ikke mye dårlig.

R: Kan de andre peke på noe som kunne vært gjort annerledes?
E23 (student 12): Kanskje, for å komme fram til løsningen på gåta. Det var kanskje ikke alt stoffet som stod der som var like relevant for å løse saken. Det var bra, jo, gjennomført med animasjon og sånn, men det var kanskje litt mye som var urelevant for å løse gåta, som du egentlig ikke trengte.

E22 (student 16): Jeg er enig med 23.

R: Jeg har ikke flere spørsmål, hvis dere ikke lurer på noe?
Alle sier nei.

GRUPPEINTERVJU 3BI
FØR UTPRØVING AV PROGRAMMET

E = Elev
R = Ragnhild
4 elever: E31 (student 20), E32 (student 22), E33 (student 32), E34 (student 21)
(Betegnelsene student 20, 21, 22 og 32 brukes i Paper III)

… betyr at eleven tenker en stund

1. **R: Først vil jeg gjerne høre hva dere tenker på hvis dere hører ordet kloning. Hva er det første som slår dere, hva forbinder dere med kloning?**
   E31 (student 20): Kopiering.

   R: Kopiering av noe spesielt?
   E31 (student 20): Celler, dyr

   E32 (student 22): Jeg tenker på sauen Dolly. Det er ganske typisk sikkert.

   R: Kan du si noe mer om den sauen?
   E32 (student 22): Hun ble nå kopiert, kan du si. Det var noe med cellekjernen som ble puttet inni eggceller, som ble identiske. Det var to individer som ble helt like, liksom.

   E33 (student 32): Jeg tenker på Dolly, og jeg tenker på planter og sånn, som kan kopieres. Som stiklinger og sånn. Jeg hadde noen spørsmål på prøven, da visste jeg ikke noe om det, så jeg spurte læreren. Og etter det tenker jeg mer på det. Poteter.

   E34 (student 21): Jeg tenker også først og fremst på kopiering av et individ, da et menneske, først og fremst. Fordi det har vært så mye snakk om det i medier og sånn. Det er det som dukker opp først.

   E33 (student 32): …Jeg har hørt om en italiensk forsker som hadde klonet et menneske. Men de ville ikke si noe mer om det fordi det er forbudt.

   R: Det har ihvertfall ikke kommet fram noe enda i denne saken. Jeg har ventet på det. Har dere andre hørt noe mer?
   E32 (student 22): Det er ganske mange som har hørt om at de har sagt at de har fått til å klone et menneske, men så hører man ikke noe mer etter det.

   R: Var det flere enn 32 som har hørt om sauen Dolly?
   Alle svarer ja

2. **R: Vet dere hvordan Dolly ble klonet? 32 sa litt. Kan dere andre føye til noe mer?**
   E33 (student 32): Jeg tror at de tok ut fra en voksen sau. Så tok dem ut DNA fra cellekjernen og satt inn en celle som de hadde tatt ut cellekjernen fra før. Det var en eggcelle, tror jeg. Også plasserte dem det inni i livmora til en voksen sau tror jeg. Også utviklet den seg til å bli et foster, til det ble født.

   E32 (student 22): Var det ikke ei celle fra juret?

   E33 (student 32): Ja, det var det.

   E32 (student 22): De hadde tatt ut cellekjernen. Og så hadde dem plassert cellekjernen, eller hadde tatt ut cellekjernen fra den kjernecella. Og så plasserte de kjernen fra juret inni eggcella.

   E33 (student 32): Ja, det er DNA fra juret.

   R: 34, Har du noe mer å si?
   E34 (student 21): Det var jo blitt klonet. Men at Dolly var den første som faktisk levde noen år, som en vellykket kloning.

   E31 (student 20): Nei…

   R: Er du enig i det som ble sagt?

E31 (student 20): Ja.

3. **R: Når dere hører ordet kloning, har dere da noen følelse generelt for kloning (positiv, negativ eller nøytral)? Hvis dere hører begrepet?**
E34 (student 21): Jeg er ganske negativ til det. Det er negativt da. Jeg mener at de ikke vet, forskerne ikke vet nok om kloning til at dem kan tukle med det. Det er ganske mye etiske spørsmål rundt det. Det er ganske så farlig. Mest det etiske som …

R: Kan du komme med noen eksempler på et etisk problem?
E34 (student 21): Det at vi skal ha det perfekte. At det blir at de som ikke er perfekte bare kommer til å dø ut. Det er ikke noe bra eksempel men. Jeg har ikke noe sånn veldig konkret, men.

R: Ja, men det er helt greit.
E32 (student 22): Hvis du vil ha to stykker som er helt like, like hverandre, som skal gå rundt på jorda. Jeg synes det er ganske … egentlig. Fordi at hvis alle sammen skal bli like tilslutt, kan ikke det gå bra. Det er jo liksom, det å tukle litt med naturen, jeg synes det ikke er helt bra. Egentlig ikke, ihvertfall hvis det skal brukes til mennesker. Men jeg lurer på om mat og sånn, hvis de blir klonet. Jeg vet ikke om det er helt riktig heller. Det er sikkert nyttig på en måte, kanskje. Det blir mat fordi det blir mindre problem å få tak i, å skaffe seg den. Men jeg vet ikke om det blir …

E34 (student 21): Men samtidig så er det veldig positivt med kloning i den forstand når du kan bruke organer og sånt hvis det er noe som er skadet og må byttes, og sånt. Da er det veldig positivt.

R: 31, Har du noen følelse knyttet til kloning?
E31 (student 20): Nei, men menneske og dyrekloning, det er jeg generelt negativ til, da. Men som E34 sier om organer og sånt, det ser jeg noe fint i. Mat har jeg ikke gjort meg noe formening om.

E33 (student 32): Jeg synes det er ganske negativt. Det som er litt skummelt med kloning er hvis noe er lov, så er det lett å gå videre og tøye grensene enda mer. Mer og mer blir lovlig. Så hvis man først begynner å si at noe er greit, så blir det så lett at mer og mer blir tillatt.

E32 (student 22): Det blir mer og mer interessant å fortsette videre.

E33 (student 32): Ja. Så, men det om organer, er ikke så positivt. Det kan jo også misbrukes. Det meste kan jo misbrukes. Men om mennesker synes jeg det er helt forferdelig.

R: De synspunktene som dere har, er det noe som dere har tenkt ut selv, at det er helt egne meninger eller har dere blitt påvirket av medier, familier, venner?
E31 (student 20): Jeg tror sikkert at media har påvirket meg. Det er der hvor jeg hørte om kloning og sånn, utenom kritikken. For min del det har litt å si.

E32 (student 22): Det er i media vi hører mest om det. Det er jo sånn jeg har plukket det opp ihvertfall. Men meninga mi om det, den har jeg gjort meg opp ut i fra det som jeg har hørt, og kanskje det som jeg har pratet med foreldrene mine om liksom.

E33 (student 32): Jeg har hørt mye liksom fra media. Det er liksom hvilke forskjellige type medier jeg hørte det fra. Det er ikke alle medier som liksom skriver så mye om det etiske rundt det. Det er nesten bare om hva som skjer og sånn. Mens om media føler jeg liksom problematikken rundt det liksom, litt mer om det, ja etiske og sånt.Jeg har ikke hørt så veldig mye om det, ikke så kjempe mye. Det er jo egentlig så fjernt på en måte. Det høres så uvirkelig ut at man kan klone et menneske. Jeg synes at det er så utenkelig på en måte. Men det er jo noe som er aktuelt i dagens samfunn. Det kommer til å bli mer aktuelt ihvertfall, tror jeg. Jeg har vel gjort meg opp en egen mening, men jeg tror ikke at jeg kan nok om det. Men jeg er imot det.

E34 (student 21): Jeg har blitt påvirket ganske mye av media, men jeg synes at de legger frem veldig mye av de etiske spørsmålene. Fordi det er det jeg hører om. Jeg hører ikke så mye om hva som skjer. Så det jeg har hørt er negativt. Jeg har heller ikke hørt noe veldig mye positivt om det. Så det har jo sikkert påvirket meg. Jeg tror uansett hvor mye positiv jeg hører, så vil jeg stå negativt. Foreldrene mine tror jeg ikke har hatt en så stor betydning for mitt synspunkt på det.

4. **R: Er alle enig om at det ikke er etisk forsvarlig å klone dyr?**

E34 (student 21): Det kommer an på hva de skal bruke dette klonede dyret til. Skal de for eksempel bruke det til et organ, eller noe sånn hjelp, medisinsk hjelp, eller noe sånt, da synes jeg at det forsvarlig, men ikke hvis det er for å få et avkom som er supert og det beste.

E33 (student 32): Jeg synes kanskje på en måte at det kanskje ikke er så farlig, men bare at det er noe skummelt med det. Det er det som vi sa i sted, at jeg er redd for utviklingen som kan skje. Når de først har startet med dyr, så er det ganske lett å gå over til mennesker. Derfor mener jeg kanskje egentlig at det ikke bør gjøres. De har jo holdt på en del med det. Men de er ikke så vellykkede, de forsøkene deres. Sauen Dolly levde ikke noe særlig lenge. De har veldig mye mislykkede forsøk bak seg. Det er litt skummelt, ja!

E32 (student 22): Egentlig er jeg enig med 34. Det kan… ikke så lenge det brukes til noe bra, men samtidig så vet jeg ikke om dyret selv tar noen skade av det, eller eventuelt hvem som får det organet og hvordan det fungerer, og sånt. Det er liksom at det… De må prøve det ut for å få det til. Det er det som jeg synes er skummelt, dette med prøvekaniner. Hvem skal være prøvekaniner? For da må de involvere mennesker som prøvekaniner. Det er ille nok at de bruker dyr, synes jeg. Og så skal de begynne å bruke mennesker. Da synes jeg at det begynner å gå litt for langt egentlig.

E31 (student 20): Jeg tror at jeg sier meg enig med de andre. Samme som 33. Hvis de begynner med dyr og det utvikler seg. Så hvem vet hvor det ender liksom.

5. **R: Det neste spørsmålet mitt var: er det etisk forsvarlig å klone mennesker? Dere har allerede svart på det og dere var ganske engasjerte.**
E34 (student 21): Det går ganske mye på menneskeverdet, når de begynner å klone folk og sånt. Hvert individ er unik og sånt. Hvis de skal begynne å fordoble og begynne å tulle med dette, så... jeg vet ikke hvor realistisk det er at de klarer å klone et helt menneske, så at det klarer å bli helt oppegående som de andre. Kan de klare det? Jeg vet ikke. De klarer det ikke nå, tror jeg. Men vi så hvordan det var med sauen, eller hvor fort hvor mye problemer den fikk. Sånn sett er det kanskje ikke realistisk. Men hvis det kommer til å skje i framtida, så går det veldig mye ut over menneskeverdet. Hvilke oppfatninger de har av mennesket, og sånt. Det er ganske skummelt.

E32 (student 22): Det blir kunstig. Du lager et kunstig menneske eller en kunstig … det er liksom. Det er ikke noe naturlig med det …jeg føler at de tukler med naturen. De er borti ting som de egentlig ikke skulle vært borti. Det er det jeg føler ihvertfall.

R: 34 eller 31, Har dere noen argumenter om hvorfor eller hvorfor ikke klone et menneske?
E34 (student 21): Det kommer masse spørsmål. Hvem som skal klones og hvem som skal få leve på en måte. Det er på en måte sånn, hva heter det, å perfeksjonere liksom. Det er vel kanskje ikke det rette.

E32 (student 22): En ting til. Hvis du har mistet et barn for eksempel. Å prøve å klone det på nytt, fordi du skal ha den samme ungen om igjen, fordi det er den eneste du har. Det kommer ikke til å bli det samme likevel, liksom. Nei, det er så mye, nei. Jeg er ikke for at mennesker skal bli klonet. Da går det galt.

E31 (student 20): Jeg har ikke noe å tilføye, tror ikke.

6. **R: 31, Synes du at temaet kloning er interessant?**
E31 (student 20): Ja, jeg har ikke hørt så veldig mye om det i media og sånn. Det lille vi har lært i 3Bi nå. Selvfølgelig hvis det blir… Ja, hvis de begynner å klone mennesker, så kunne det være kjekt å vite noe mer om det, hvordan det gjøres og sånt.

E32 (student 22): Det er veldig interessant å lære om det.

R: Hvorfor?
E32 (student 22): Fordi det er noe som er helt nytt, som er nytt for nesten alle. Det er ordentlig artig når man oppdager noe nytt, finner opp noe nytt, men samtidig er det litt skummelt. Det er ganske interessant å høre om hva forskerne egentlig klarer å finne ut, hva de klarer å gjøre med kunnskapen sin.

R: 33, Er det interessant for deg?
E33 (student 32): Ja, jeg synes det er veldig interessant. Men jeg ser for meg at jeg tror det er viktig at vi lærer noe om det, slik at vi på en måte kan lære hvilke konsekvenser det egentlig har, og sånt. Er de negative til det, hvilke skadelige konsekvenser det kan ha, slik at vi kan prøve å få stoppet det på en måte. I hvert fall stoppe det hvis de skulle komme til å klone mennesket, og klone dyr…

E34 (student 21): Jeg synes også at det er interessant.

R: Fordi?
E34 (student 21): Fordi at det er noe helt nytt som ingen vet så mye om. Til og med de som vet mest, de vet ikke alt de heller. Det er liksom på forskningsstadiet enda. Det er jo interessant å finne, eller få se hva som kommer til å skje, eller hva de kommer til å finne ut til slutt. Og at det kanskje kan bidra i utviklingen, både positivt og negativt, det er nå spennende.

7. R: 34, Synes du at det er interessant å lære om hvordan forskere jobber? Eventuelt hvorfor, hvorfor ikke?
E34 (student 21): Hvordan forskere jobber?

R: For eksempel hvordan forskere jobber med kloning?
E34 (student 21): Interessant å?

R: Interessant å lære om hvordan de jobber? Hva de jobber med?
E34 (student 21): Jeg har egentlig ikke hørt noe om det, så jeg kan kanskje ikke si så mye om det. Men det er kanskje noe å ha lært litt om det, fordi det er ganske omfangende, så sikkert, litt, ja. Vanskelig, eller kjed… eller det kan bli litt mye. Jeg regner med at det er en god del som er inne i bildet her. Men selvfølgelig vil jeg vite mer om hva de gjør. Jeg har ikke hørt noen ting om det.

R: E33, Er det interessant å lære om forskning?
E33 (student 32): Ja, jeg synes det er ganske interessant, men, ja. Det høres interessant ut. Men jeg tror ikke jeg hadde vært så interessert i å høre om de som holder på og kloner mennesker og sånt, fordi det synes jeg er teit. Men det er så rart at de får det til, det er så smått det som de holder på med, på en måte, så det er rart hvordan de får det til. Mye av det de holder på med synes jeg er negativt. Men masse kunne ha vært interessant, ja.

R: 31?
E31 (student 20): Jo, både og. Hovedtrekkene, kanskje, men ikke sånn kvik kvik kvik på en måte (*Hun banker på bordet samtidig*). Men bare sånn en viss oversikt, det kunne sikkert vært interessant.

E32 (student 22): Jeg synes det er spennende å høre hvordan de jobber. For det jeg har ikke så mye peiling på det, som de driver med egentlig, men hvis det er på et sånt generelt, sånn at de allmenne kunne skjønne det, så er det ganske artig. Jeg har kjemi og jeg synes at det er litt artig å holde på med kjemikalier, og sånn når det blir litt sånne artige farger. Og hvis det smeller litt og sånn, så synes jeg at det er litt artig. De sitter og pirker litt i ting, og forsker for å finne ut ting. De har funnet ut så mye allerede. Jeg synes egentlig at det er ganske spennende.

8. R: Jeg begynner med 32. Har du gjort deg noen tanker om det å bruke Internettbaserte undervisningsprogrammer i undervisningen? Hva synes du eventuelt om det?
E32 (student 22): Vi så litt på disse gensidene i forrige timen på Internett. Det å bruke det som… bruke Internett hele tida som undervisning det vet jeg ikke om det er helt… Det kan være at det har litt effekt på noen. Alle er ikke likeens fortsatt så, det virker sikkert til en viss grad, kan jeg tenke meg. Det at du får sitte der og jobbe selv og må tenke selv for å finne ut ting, men samtidig så har du liksom det opplegget du må forholde deg til. Læreren kan ofte komme med litt sånne bakgrunnskunnskaper og litt sånn ekstra input liksom, innimellom sånn, men hvis du har den Internett undervisningen så begrenser det deg nesten, ihvertfall tenker jeg.

R: 33?
E33 (student 32): Jeg synes at…Vi snakket litt om temaet først og så så vi på de sidene. Og det synes jeg var ganske bra, for da fikk vi repetert det, og sett det på en annen måte, og kanskje koblet litt flere sanser, og husket litt bedre kanskje. Det er ganske pussig å få bruke Internett til noe bra liksom og. Men jeg synes, sånn kloning kanskje kan være et vanskelig tema, så kan det være bra tror jeg. (A*vbrytelse, en person kommer inn*). Så tror jeg at det kan være bra å bruke Internett. Det er jo slik at folk lærer forskjellig som 32 sa. Så det kan være forskjellige måter som folk tar det til seg på. Noen kan jo… skikkelig bra fakta av det. Det er sikkert det du skal finne ut. *Hun ler.*

R: 31, Har du noen tenker om det?
E31 (student 20): Ja, folk er forskjellige, tar til seg læring på forskjellige måter. For min del… jeg vet ikke egentlig hvor mye nytte jeg hadde av det. Da i så fall måtte de ha gått gjennom stoffet først, som 33 sa, at

gå gjennom først og kikke på det etterpå, når du har litt bakgrunnsinformasjon. Men å bare gå rett på det, da tror jeg at det ikke hadde gått så veldig bra, hvert fall ikke for min del.

R: 34?
E34 (student 21): Ja … Jeg er enig med resten og. Men også at for min del er jeg ganske redd for å begynne å hoppe på Internett hvis jeg ikke har noen bakgrunnsstoff først på et program. For at jeg kanskje er litt redd for å få feil informasjon, eller at det skal forvirre meg, eller noe sånt. Jeg vet ikke hvor pålitelige disse sidene er. Så da ønsker jeg først å få undervisning av læreren der jeg kan stille spørsmål. Jeg vet at det er liksom, at det er en politisk bakgrunn. Eller at det er riktig det hun forteller meg på en måte. Så jeg er litt skeptisk til det å gå rett på et program på Internett, men selvfølgelig er det veldig spennende å gjøre noe anna utenom skoleboka. Det er litt artigere å se fordi du får fram litt mer bilder og innlevelse på en måte. For eksempel hvis jeg skal ha en prøve nå, om kloning, så tror jeg kanskje at jeg kommer til å gå inn på Internett å se litt. Vi har hatt en del sånn. Har vi ikke? Det er veldig fint å repetere og sånt.

R: Hva brukte dere Internett til, i biologi for eksempel? Du snakket om gensider?
E31 (student 20): Gensider som Universitet i Bergen som hadde laget.

R: Er det Gensidene? De kjenner jeg til.
E31 (student 20): Ja, det var jo bra det. Noen som snakket før 32, at det var…. Jeg har glemt det.

E34 (student 21): Vi har vel hatt en del sånn i kjemien også, sånn Internett. Jeg synes jeg husker et sånt program.

R: Var det et eget program, undervisningsprogram, eller?
E32 (student 22): Vi satt her og han holdt på å trykke på tavla. Noe á la de her Gensidene hvor vi fikk se hvordan ting ble satt sammen og fungerte sammen. Det tror jeg faktisk.

E34 (student 21): Vi har nå sett en del og sånn. Det er ganske mer spennende enn tørt stoff da, men du må ha det tørre stoffet først da, synes jeg.

E32 (student 22): Jeg synes at de gjør det enklere å se det for seg da, når du får enkle bilder som viser deg hvordan ting fungerer, så gjør det at jeg har et visst bilde i hodet mitt, og forstår det lettere nesten. Men så er det dette med å stille spørsmål. Når du får kunnskap om en ting, og lærer ting, så er det naturlig at jeg har lyst å spørre om det og kanskje vite mer litt utenfor, i hvert fall. At du arbeider deg opp en mening oppe i hodet ditt og ønsker å stille spørsmål. Og det er ikke mulig hvis du begrenser deg til Internett for eksempel. Da kan du jo ikke spørre Internett om det da, hva vil det her si liksom...

E33 (student 32): I så fall skulle det være spørre, sett mer og spørre liksom. At det hadde vært sånn informativt. At det har vært nytt stoff om forskning og sånt. Hva som er nytt akkurat nå. Det med kloning og sånt, det kunne ha… Det er ikke alltid at læreren har så mye peiling heller da. … men jeg har inntrykk av at du har fått høre mer om det og hvordan det praktiseres i hverdagen på en måte, ikke bare at det her er kloning, men sånn at vi ikke får vite hva de bruker det til. Vi har hørt om sauen Dolly, da. Men jeg håper at vi slipper å høre om hvordan de gjør det på mennesker.
Alle ler.

E34 (student 21): Jeg skulle ha ønsket også at det var flere sånne der tester i forbindelse med de her programmene, sånn at du kunne teste deg selv etterpå om du hadde skjønt noe og sånt, da eventuelt spørre læreren. Sånn at du vet hvor du står, etter å ha lest disse sidene. Om du har fått det inn. Det er det ikke på alle sidene vi har vært inne på ihvertfall.

R: Har 31 noe å tilføre?
E31 (student 20): Nei

GRUPPEINTERVJU 3BI
ETTER UTPRØVING AV PROGRAMMET

E = Elev
R = Ragnhild
3 elever: E31(student 20), E32 (student 22), E33 (student 32)
(Betegnelsene student 20, 22 og 32 brukes i Paper III)
E34 (student 21) var borte på grunn av religionsprøve

… betyr at eleven tenker en stund

1. **R: Først spør jeg igjen: Hvis dere tenker på kloning i dag, hva assosierer dere med kloning nå?**
E32 (student 22): Mer enn i det siste ihvertfall, for forrige gang var det bare Dolly, men nå tenker jeg litt i banen av at planter gjør det hele tida. At jordbærplanten hadde utløpere der hvor de vokser. Dem er jo like mora. Potetplanten har jo stiklinger, de kloner seg på en måte. Så det ble mer enn det som jeg hadde tenkt før, akkurat Dolly. Jeg hadde aldri sett på kloning før. Det var litt artig.

   E33 (student 32): Ja, jeg tenker både på planter, på dyr. Litt av det samme. Fortsatt er det bare sauen som kommer, men litt det med planter og.

   E31 (student 20): Ja, det samme. Det er Dolly som kommer først, men plantene visste jeg ikke så mye om. Nå har jeg skjønt at de også holder på å klone seg selv.

   R: Tenker du bare på Dolly og planter? Eller på andre ting også?
E31 (student 20): Nei, kloning og gener generelt, egentlig. Men Dolly er liksom eksempelet på kloning, på en måte.

2. **R: Kan dere si meg hvordan Dolly ble klonet?**
E33 (student 32): Ja, de tok ut kjernen på en celle fra juret til en sau. Og så tok de den og plasserte denne kjernen inni ei eggcelle. Ei ubefruktet eggcelle, tror jeg, men det er sikkert noe feil, da. Så puttet de den inni, og så plasserte de den i livmora på en surrogatmor. Det var at de ble sammen med mitokondrier. Det ble ikke helt likt. Den fikk mitokondrier fra den surrogatmora, så den ble ikke helt identisk lik på grunn av det. Jeg vet ikke helt.

   R: 31, Er du enig, eller har du en annen oppfatning?
E31 (student 20): Nei, jeg er forsåvidt enig. Det var vel ei vanlig celle, så tok de ut kjernen, og så kom den andre sauen og så tok de eggceller der, og så puttet de dem inn i en tredje sau, ja.

   R: Må det vær tre sauer?
E31 (student 20): Ja, det må vel det. Det vet jeg ikke helt.

   E33 (student 32): Det kan vel være to da. Hvis de puttet de i den samme igjen liksom, for da får de kanskje de samme mitokondriene.

   E32 (student 22): De må ihvertfall være to, for eggcella og den jurcella ble jo tatt fra to forskjellige individer.
E?: Ja, det må være to forskjellige.

   E33 (student 32): Det trenger kanskje ikke det. De kan jo ta samme eggcella.

   E32 (student 22): Blir det ikke noe sånn rart? Innavl eller noe sånt tull? Så at det blir veldig misdannet. At det bare dør. Jeg vet ikke. Hvis det blir for mye av det samme DNA.

   E33 (student 32): Det blir jo det uansett på en måte. For eggcella, den har ikke noe DNA på en måte. Den kan ikke komme med noe av det. Eller? Eggceller uten kjerne, har fremdeles DNA, nei, den har jo ikke det.

   E32 (student 22): Jeg blir forvirret.

   R: 32, Hvordan kan du forklare kjerneoverføring?
E32 (student 22): De tok nå… En del nye jurceller, tok ut kjernen derfra. Tok ut kjernen fra eggcella og så tok de cellekjernen fra jureceller, og puttet inni eggcellen. Det er vel det det går ut på.

E33 (student 32): Det stemmer det.

R: Som det stod i programmet: de tok ikke direkte ut kjernen av jurcella, men de tok kjernen fra eggcella og så smeltet de den kjerneløse eggcella sammen med jurcella. Så det blir i praksis det samme.
E33 (student 32): Jeg husker når vi hadde et spørsmål om det, at de smeltet dem.

R: Så står det også at du trenger ikke å ha mer enn én sau, hvis det er en hunn-sau. Da blir det 100% likt, da får du mitokondrie-DNA også fra den.
E33 (student 32): Er det DNA i mitokondrier?

R: Ja.
E33 (student 32): Er det det?

R: Litt
E33 (student 32): OK.

R: Jeg tror at dere har fått litt mer klarhet i det.
E33 (student 32): Dersom det på en måte bare er én sau, da. Nå husker jeg på det. Jeg tror jeg husker at jeg har lest det, men det festet seg ikke før du sa det nå. Eller når du prøvde å hinte frampå "Er det nå én…"

3. **R: Neste spørsmål: Synes du at du har hatt faglig utbytte av programmet?**
E31 (student 20): Ikke så veldig mye, synes jeg. Jeg har vært mer opphengt i det der med å finne hvem tyven var. For min egen del lite grann, men ikke noe veldig. Å lete etter tyven og finne ut hvem det kunne være, men noe fikk jeg.

4. **R: Hva noe?**
E31 (student 20): Ja , litt om hva kloner var og hvordan det foregikk. Planter og gener, at ikke bare dyr blir klonet.

R: Kan du si hvorfor det ble mye historie?
E31 (student 20): Jeg vet ikke helt altså.

R: 32, Synes du at du har lært noe?
E32 (student 22): Ja, det synes jeg. Men det som er, er at det ble veldig mye på en gang skulle jeg til å si. Det er det som gjør at du blir litt forvirret. Så mye fagstoff på en gang som du skal ta inn. Om ikke kunne, men ihvertfall å ha litt peiling på det. Da er det lett at du blander og litt sånn forskjellig. Men jeg har jo litt utbytte av det. Absolutt.

R: Har du noen eksempler? På hva du har lært?
E32 (student 22): Mye av det samme som E31. Men det at plantehormoner ikke har proteiner. Det har jeg og lært! Og at planter bare har 5 forskjellige hormoner eller noe sånn, mens vi mennesker har over 50.

E33 (student 32): Vi lærer det på slutten av 2Bi.

E32 (student 22): Mot slutten. *Hun ler.* Og at de fleste hormoner hos mennesker består av proteiner, ikke alle men de fleste. Jeg lærte litt om hormoner, hvordan de virker, litt om kloning og planter.

E33 (student 32): Det var litt mer om det etiske. Det med spørsmålene rundt det om vi skal klone mennesker. Om hva. Jeg har ikke tenkt akkurat i den retningen, jeg har jo tenkt på det, men det har vært bevisstgjørende, hvordan det psykiske problemet som man kan få, dem som blir klonet. Også, ja. Det blir om forskning. Det som var knyttet til forskning på en måte. Det var litt større forskjell mellom… i og med den konferansen, at forskerne ble samlet at det hjelper noe mer.

E32 (student 22): Det som overrasket meg var at de hadde klonet en gris og en mus. Jeg hadde ikke fått med meg i det hele tatt at de har klonet gris og mus. Så er det bare at de ikke var vellykket, eller er det så lite at de ikke sier noe om det? Dolly ble det masse styr om. Det er litt rart at vi ikke hørte om det.

E33 (student 32): Jeg fikk ikke vite akkurat hvordan vitenskapsmenn kloner planter, men. Det var veldig mye snakk om cytokjemien. Det stod noe om hva de kunne bruke det til, men er det reelt? Bruker de det i kloning av planter?

R: Ja.
E33 (student 32): De gjør det? For det er sånne plantehormoner som de kan bruke liksom i kloninga.

R: Vevsformering for eksempel, det er det samme som kloning av planter. Da bruker du det hele tiden.
E33 (student 32): Ja. Ok.
E33 (student 32): Jeg husker at de hadde sånn maskin som de brukte, som de hadde når de skulle klone for å få en optimal temperatur eller noe sånn. Eller få en optimal tilværelse for det de skulle klone.

R: 32 og 33 har ihvertfall lært en del, synes dere. Kan dere peke på hvorfor dere har lært noe gjennom det programmet? Hva i programmet var det som gjorde at dere lærte det? Er det noe spesielt med programmet?

E32 (student 22): Det er litt artig. Du må bruke den kunnskapen du tilegna deg i løpet av den tida du sitter der, du må bruke den til noe. Du må liksom kunne det i praksis. Det er ikke så ofte du trenger om å kunne i praksis når du er i et klasserom, liksom. Da får du inn den kunnskapen, om hvordan det fungerer, så har du bare det ene eksempelet som står i boka for eksempel. Du lærer ikke å bruke det utenfor. Jeg synes at det er litt artig at du måtte bruke det du hadde av kunnskapen fra før og kanskje tilegnet deg nye da du satt her. At det blir liksom, at hele settingen blir artig. Det er ikke noe sånn…, ja.

R: Så du mener at du trengte å kunne det for å løse den saken, at det var det som gjorde det litt lettere… 33, har du noen mening om det?

E33 (student 32): Som 32 sa, det var... Så var det litt annerledes, da. Du ble kanskje litt "klar" på slutten, liksom, du ble litt sliten. Det kunne kanskje vært en idé hvis det hadde vært delt i to liksom. Bare lese gjennom hele opplegget, å prøve og lese fakta og sånt, først. Så kunne de kanskje neste gang begynne å løse det. Bare så de kunne få litt tid å tenke på det. Det var veldig bra laga. Veldig reelt på en måte. Så det var bra.

R: Jeg sa at det var ønskelig at dere skulle få komme to ganger, at utprøvingen ble delt på to dager. Men det er ikke så lett å få til i praksis når dere må få fri fra skolen og komme ens ærend. Så derfor ble det sånn. Jeg skjønner veldig godt at dere ble slitne.

5. **R: Har dere den samme følelsen til kloning nå som sist? Hvis dere tenker på kloning generelt, er dere negativt innstilt eller positivt? Har holdningen endret seg?**
E31 (student 20): Det har egentlig ikke forandra seg så veldig mye. Det med dyr og mennesker er kanskje litt negativt.

6. **R: Har du noen eksempler hvorfor du er negativ til det?**
E31 (student 20): Hvis det blir veldig utbredt, hvor det skal ende til slutt. Hvis du bare kan gå og klone i hytt og pine, så…. Og tukle litt med naturen egentlig

7. **R: 33, Du fortalte litt mer om noen aspekter ved kloning av mennesker?**
E33 (student 32): Ja, med det psykiske og sånt. Og det skaper mye om … jeg tror hvis … det er fort mulig at det blir reelt det med menneskekloning, man ser det allerede i dag, så det er noe som man må løse nå. Jeg tror at det kan bli et veldig sånn der kynisk samfunn på en måte, som går i den retningen. Og det må være veldig vanskelig for dem som blir klonet, sånn psykisk, og de kan få mindreverdighetskomplekser, kanskje. Jeg er negativ til det, fortsatt.

R: Har du andre holdninger til dyr? Har du noen eksempler? Hvorfor kan noen tenke seg å klone dyr?
E33 (student 32): Jeg synes det blir vanskelig, det med dyr, egentlig. På en måte kan det være greit, men på en annen måte synes jeg at det er litt skummelt, fordi det kan være med å bygge ned til at det blir lettere å begynne med menneskekloning. Man ser det allerede nå da, at man forsker på dyr. Og så begynner man på mennesker, liksom. Så det kan være liksom det skumle med dyrekloning. Men ellers, ja. Uansett så synes jeg at de ikke bør gjøre det på dyr, heller.

R: Så ingen positive argumenter for kloning?
E33 (student 32): Det er positivt, det er noe med positive medisinske årsaker.

R: 32, Har du noen eksempler på at kloning av dyr kan være ønskelig? I hvilke sammenhenger?

E32 (student 22): Mer mat, da. Eventuelt hvis du får til å klone samme dyr flere ganger, det sier seg selv at det blir mer mat, og mindre sult egentlig. Jeg synes at det er en fordel. Jeg synes fortsatt at det er skummelt med kloning også, for at mennesker som prøvekanin, og dyr for den saks skyld, og… jeg synes ikke det er helt bra, egentlig ikke. For at du på en måte… du utsetter både den personen som blir klonet og den som er klonen ganske stort psykisk, som 33 sier. Store psykiske belastninger. Si nå at en familie som har mistet et barn, og skal klone det, så at det blir akkurat likeens … Det individ som vokser opp, selv om det er genetisk likt, kommer ikke til å bli det samme. Det vokser opp i ei litt anna tid. Reglene må forandre seg hele tida. Så det er liksom, det blir stor forskjell på individer fordi om de er genetisk like.

E33 (student 32): Jeg er imot dyrekloning, jeg og. *De ler.*

8. **R: Synes dere at temaet kloning er blitt mer eller mindre interessant enn sist dere snakka sammen? Kan dere si noe om hvorfor?**
E33 (student 32): Jeg synes at det er litt mer interessant fordi jeg har jobbet med det, jeg merker det. Litt, ihvertfall.

R: Tror du at du hadde hatt den samme følelsen hvis du hadde lest om det i ei bok?
E33 (student 32): Kanskje ikke, jeg har jo lest om det i biologiboka, da. Liksom, ja. Men jeg har følelsen av at det er litt fjernt likevel. Men etter at vi fikk se, og vi fikk komme inn i det, at det er forskere som holder på med det, og at det er en skikkelig stor konferanse rundt og at det egentlig er reelt. Det bør være litt mer interessant. For at i boka, alt står i bøker, du må lese bøker. Litt mer interessant av den grunn.

R: Det er gjerne sånn at desto mer du setter deg inn i et stoff, uansett om du leser det eller hva, så blir det jo mer interessant. Men hva tror 32?
E32 (student 22): Jeg synes det blir mer interessant når du lærer mer om det. Når du tilhenger deg nye kunnskaper så får du også flere spørsmål, mer du lurer på. Mer du har lyst til å finne ut. Så interessen den blir pirra litegrann når du lærer noe nytt.

R: Men var det noe med programmet som gjorde at det var mer interessant enn å lese om det i ei bok?
E32 (student 22): Det er fortsatt at du må bruke kunnskapen din selv om du ikke… Vi er jo ikke eksperter på kloning. Men likevel klarer vi å bruke det lille vi hadde og det som vi har tilgang til. Klare å løse mysteriet om det, liksom. Så jeg synes at det var litt artig, du trengte ikke å være forsker for å forstå det liksom. Å klare å finne ut ting, tenke sånn cirka i de samme banene som det forskerne må gjøre. Det var litt artig.

R: 31, Synes du at programmet var interessant?
E31 (student 20): Ja litegrann, men det var kanskje…Jeg hadde sikkert syntes at det var interessant å lese om det i ei bok og. Men det var kanskje litt bedre. Jeg fikk bilder og ikke bare på svart-hvitt.

E32 (student 22): Og det var interessant at det det var litt mer unge forskere som holdt på. Det virket som om det var en yngre forsker som holdt på og at det var liksom nytt. Ikke bare gamle fyrer med langt, hvitt skjegg, briller og mage. *De ler.*

9. **R: 31, Synes du at det var interessant å lære om hvordan forskere jobber?**
E31 (student 20): Ja, for å få en viss forståelse for noe, men ikke sånn veldig dypt inn i det. Men bare en sånn liten oversikt, sånn at du har noe å gå etter, men ikke sånn veldig.

10. **R: Synes du at temaet kloning ble mer interessant da du lærte om det via forskerne?**
E31 (student 20): Ja, det synes jeg. Det med bilder, og det visuelle, på en måte, ikke bare svart-hvitt.

R: 32, Synes du det ble interessant å lære om kloning samtidig som du fikk innblikk i hvordan forskerne jobber?
E32 (student 22): Ja, fordi du får det på en måte litt fra en annen synsvinkel. Fra før har du det på en måte litt fra media sin påvirkning og litt fra foreldre kanskje. Litt fra bare folk du hører snakke om det, så gjør du kanskje opp din egen mening. Snakker veldig lite om hva forskerne driver på med, hva de synes og mener om det. De ser litt annerledes på det enn det vi andre gjør. Jeg synes fortsatt at det er litt interessant å høre hvordan forskere jobber. Litt småpirk og sånn, litt spennende.

R: Betyr det at du kan tenke deg å bli forsker selv?
E32 (student 22): Jeg vet ikke. Jeg har ikke helt klart for meg hva jeg skal bli. Men å være forsker? Jeg synes kjemi er veldig artig, men jeg vet ikke hvor mye det kan relateres mot det å være forsker.

11. R: Har dere noen andre tanker i dag om det å bruke Internett i undervisningen?
E31 (student 20): Jeg har ikke det. Jeg må sette meg å lese først og høre eventuelt, når jeg skal begynne på et mysterium som det her. Jeg må eventuelt lese i boka først, og så begynne på Internett bare for å få repetert. Men ikke bare å gå rett på det. Det har ikke noe utbytte for meg ihvertfall.

R: I det programmet så fant du jo teorien som står i boka, men likevel så…
E31 (student 20): Jeg må ha satt meg inn i det lite grann kort og så eventuelt gå på det. Da tror jeg at det blir mye lettere for min egen del.

R: Hva synes du 33?
E33 (student 32): Jeg synes det var veldig greit at vi hadde litt bakgrunnskunnskap før vi kom liksom. Det var greit, ellers hadde det blitt mye mer arbeid med stoffet. Det hadde ihvertfall vært nødvendig å sette av to ganger. Jeg tror det hadde vært fullt mulig å lære det bare ved å lese sånn. For det er viktig med repetisjon etterpå. At en snakker om det og stiller spørsmål om det, og bruker det for at en husker. Fordi på data går det ofte veldig fort. Og så kan det gå fort forbi liksom. Så det er viktig å snakke om det etterpå.

R: Hadde det hjulpet hvis dere hadde hatt lengre tid, fordelt over flere ganger?
E33 (student 32): Ja, det tror jeg. Jeg tror det er fullt mulig å lære det på data, men det spørs veldig på det som 31 sa. Folk er veldig forskjellige, hvordan de lærer. Men det er veldig nyttig for mange, tror jeg, bare å bli opplyste om det. Jeg var liksom opplyst om at det fantes et program. Eller også illustrasjoner på data.

E32 (student 22): Ja, det er mulig fortsatt. Det er ikke så dumt egentlig, men det er som 31 sier at det er kjekt å ha noen knagger før du går inn sånn at du kjenner igjen noe av stoffet du lærer om. Så det fester seg litt bedre hvis du har noen få stikkord i bakhodet på forhånd. Også det med at du kan stille spørsmål, du har noen som kan svare på dem liksom. Jeg synes fortsatt at læreren… at ikke Internett bør ta helt av. Det er litt begrenset, synes jeg. Liksom at du bare har det de sier som står der, får ikke noe kunnskap ut over det. Hvis du trenger forklaring på noe, kan du ikke spørre om å få forklaring fordi det står som det står. Å ha læreren tilgjengelig for å kunne spørre og grave litt og sånn. Det hadde vært kjekt. Men det er jo absolutt gjennomførbart.

R: Du kan jo bruke programmet på mange måter…
E33 (student 32): Bra at læreren sitter og kan skrive meldinger, det var bra.

R: Var det noe spesielt med programmet, innholdet eller hvordan ting var laget, som dere likte? Eller noe annet?
E33 (student 32): Det var litt kult, da. Det var ikke så gammeldags. Layouten var litt ny, på en måte. Litt moderne. Sånne bilder, bra modeller, illustrasjoner og sånt. Det var bra.

E32 (student 22): Det er nytt at det blir en annen måte å lære ting på som du ikke har vært bort i før, som gjør det enda litt mer interessant. Jeg skulle si en ting til, men jeg glemte det. Kan du si spørsmålet en gang til, så kanskje jeg kommer på det?

R: Var det noe med programmet som dere syntes var spesielt bra?
E32 (student 22): At situasjonen ble såpass virkelighetsnær som den ble, at det ikke var noen romvesener og sånn. Det var såpass konkret, og så var du i et miljø som du for så vidt kjenner ganske godt, du forholdt deg til Trondheim by, der du bor og det blir liksom gripelig.

R: Kan dere si kort om det var noe som dere ikke likte?
E33 (student 32): Jeg synes at det var lengden. Vi ble sittende så lenge, du ble så ”klar”. Men du sa at vi kunne gjøre det i to ganger.

E31 (student 20): Ikke noe sånn at det var dårlig, men det passer ikke helt for meg, å sitte der så lenge, men ikke sånn konkret.

E32 (student 22): Det samme. Det med tida. Det var så mye over såpass begrenset tidsrom at det ble nesten overbelastning på hjernen. Det blir liksom at det å sitte foran en dataskjerm i tre timer, det kjentes ut som sandpapir i øynene da vi sluttet. Det er det eneste som er litt negativt. Men det er bare å dele, som du sa at det skulle ha vært.

Appendix 9

Students working through the teaching unit “Cloning plants” at the Resource Centre for Mathematics, Science and Technology Education at the Norwegian University of Science and Technology.

Doctoral theses in Biology
Norwegian University of Science and Technology
Department of Biology

Year	Name	Degree	Title
1974	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos. Zoology	Breeding events of birds in relation to spring temperature and environmental phenology.
1978	Egil Sakshaug	Dr.philos Botany	"The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton"
1980	Arnfinn Langeland	Dr. philos. Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake.
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1982	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana*
1982	Dag Dolmen	Dr. philos. Zoology	Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation.
1984	Eivin Røskaft	Dr. philos. Zoology	Sociobiological studies of the rook *Corvus frugilegus.*
1984	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats
1984	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exosed to occupational air pollution. An evaluation of the AM-test
1985	Jarle Mork	Dr. philos. Zoology	Biochemical genetic studies in fish.
1985	John Solem	Dr. philos. Zoology	Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains.
1985	Randi E. Reinertsen	Dr. philos. Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds.
1986	Bernt-Erik Sæther	Dr. philos. Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach.
1986	Torleif Holthe	Dr. philos. Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna.
1987	Helene Lampe	Dr. scient. Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires.
1987	Olav Hogstad	Dr. philos. Zoology	Winter survival strategies of the Willow tit *Parus montanus*.
1987	Jarle Inge Holten	Dr. philos Botany	Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway

1987	Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium*
1987	Bjørn Åge Tømmerås	Dr. scient. Zoology	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction.
1988	Hans Christian Pedersen	Dr. philos. Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care.
1988	Tor G. Heggberget	Dr. philos. Zoology	Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure.
1988	Marianne V. Nielsen	Dr. scient. Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*).
1988	Ole Kristian Berg	Dr. scient. Zoology	The formation of landlocked Atlantic salmon (*Salmo salar* L.).
1989	John W. Jensen	Dr. philos. Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth.
1989	Helga J. Vivås	Dr. scient. Zoology	Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces*.
1989	Reidar Andersen	Dr. scient. Zoology	Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation.
1989	Kurt Ingar Draget	Dr. scient Botany	Alginate gel media for plant tissue culture,
1990	Bengt Finstad	Dr. scient. Zoology	Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season.
1990	Hege Johannesen	Dr. scient. Zoology	Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung.
1990	Åse Krøkje	Dr. scient Botany	The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test
1990	Arne Johan Jensen	Dr. philos. Zoology	Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmion (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams.
1990	Tor Jørgen Almaas	Dr. scient. Zoology	Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues.
1990	Magne Husby	Dr. scient. Zoology	Breeding strategies in birds: Experiments with the Magpie *Pica pica*.
1991	Tor Kvam	Dr. scient. Zoology	Population biology of the European lynx (*Lynx lynx*) in Norway.
1991	Jan Henning L'Abêe Lund	Dr. philos. Zoology	Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular.
1991	Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands
1991	Else Marie Løbersli	Dr. scient Botany	Soil acidification and metal uptake in plants
1991	Trond Nordtug	Dr. scient. Zoology	Reflctometric studies of photomechanical adaptation in superposition eyes of arthropods.

1991	Thyra Solem	Dr. scient Botany	Age, origin and development of blanket mires in Central Norway
1991	Odd Terje Sandlund	Dr. philos. Zoology	The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism.
1991	Nina Jonsson	Dr. philos.	Aspects of migration and spawning in salmonids.
1991	Atle Bones	Dr. scient Botany	Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase)
1992	Torgrim Breiehagen	Dr. scient. Zoology	Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher.
1992	Anne Kjersti Bakken	Dr. scient Botany	The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.)
1992	Tycho Anker-Nilssen	Dr. scient. Zoology	Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica*
1992	Bjørn Munro Jenssen	Dr. philos. Zoology	Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks.
1992	Arne Vollan Aarset	Dr. philos. Zoology	The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans.
1993	Geir Slupphaug	Dr. scient Botany	Regulation and expression of uracil-DNA glycosylase and O^6-methylguanine-DNA methyltransferase in mammalian cells
1993	Tor Fredrik Næsje	Dr. scient. Zoology	Habitat shifts in coregonids.
1993	Yngvar Asbjørn Olsen	Dr. scient. Zoology	Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels ans some secondary effects.
1993	Bård Pedersen	Dr. scient Botany	Theoretical studies of life history evolution in modular and clonal organisms
1993	Ole Petter Thangstad	Dr. scient Botany	Molecular studies of myrosinase in Brassicaceae
1993	Thrine L. M. Heggberget	Dr. scient. Zoology	Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra*.
1993	Kjetil Bevanger	Dr. scient. Zoology	Avian interactions with utility structures, a biological approach.
1993	Kåre Haugan	Dr. scient Bothany	Mutations in the replication control gene trfA of the broad host-range plasmid RK2
1994	Peder Fiske	Dr. scient. Zoology	Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek.
1994	Kjell Inge Reitan	Dr. scient Botany	Nutritional effects of algae in first-feeding of marine fish larvae
1994	Nils Røv	Dr. scient. Zoology	Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo*.
1994	Annette-Susanne Hoepfner	Dr. scient Botany	Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.)
1994	Inga Elise Bruteig	Dr. scient Bothany	Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers
1994	Geir Johnsen	Dr. scient Botany	Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses

1994	Morten Bakken	Dr. scient. Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes*.
1994	Arne Moksnes	Dr. philos. Zoology	Host adaptations towards brood parasitism by the Cockoo.
1994	Solveig Bakken	Dr. scient Bothany	Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply
1995	Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions.
1995	Hanne Christensen	Dr. scient. Zoology	Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision*.
1995	Svein Håkon Lorentsen	Dr. scient. Zoology	Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition.
1995	Chris Jørgen Jensen	Dr. scient. Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
1995	Martha Kold Bakkevig	Dr. scient. Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport.
1995	Vidar Moen	Dr. scient. Zoology	Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations.
1995	Hans Haavardsholm Blom	Dr. philos Bothany	A revision of the *Schistidium apocarpum* complex in Norway and Sweden.
1996	Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; inpact fish-bacterial interactions on growth and survival of larvae.
1996	Ola Ugedal	Dr. scient. Zoology	Radiocesium turnover in freshwater fishes
1996	Ingibjørg Einarsdottir	Dr. scient. Zoology	Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines.
1996	Christina M. S. Pereira	Dr. scient. Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation.
1996	Jan Fredrik Børseth	Dr. scient. Zoology	The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics.
1996	Gunnar Henriksen	Dr. scient. Zoology	Status of Grey seal *Halichoerus grypus* and Harbour seal *Phoca vitulina* in the Barents sea region.
1997	Gunvor Øie	Dr. scient Bothany	Eevalution of rotifer *Brachionus plicatilis* quality in early first feeding of turbot *Scophtalmus maximus* L. larvae.
1997	Håkon Holien	Dr. scient Botany	Studies of lichens in spurce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters.
1997	Ole Reitan	Dr. scient. Zoology	Responses of birds to habitat disturbance due to damming.
1997	Jon Arne Grøttum	Dr. scient. Zoology	Physiological effects of reduced water quality on fish in aquaculture.
1997	Per Gustav Thingstad	Dr. scient. Zoology	Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher.

1997	Torgeir Nygård	Dr. scient. Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as Biomonitors.
1997	Signe Nybø	Dr. scient. Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway.
1997	Atle Wibe	Dr. scient. Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry.
1997	Rolv Lundheim	Dr. scient. Zoology	Adaptive and incidental biological ice nucleators.
1997	Arild Magne Landa	Dr. scient. Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation.
1997	Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius*.
1997	Jarle Tufto	Dr. scient. Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models
1997	Trygve Hesthagen	Dr. philos. Zoology	Population responces of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters
1997	Trygve Sigholt	Dr. philos. Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
1997	Jan Østnes	Dr. scient. Zoology	Cold sensation in adult and neonate birds
1998	Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins.
1998	Thor Harald Ringsby	Dr. scient. Zoology	Variation in space and time: The biology of a House sparrow metapopulation
1998	Erling Johan Solberg	Dr. scient. Zoology	Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment
1998	Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity.
1998	Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro.
1998	Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservtaion biological approach.
1998	Bente Gunnveig Berg	Dr. scient. Zoology	Encoding of pheromone information in two related moth species
1999	Kristian Overskaug	Dr. scient. Zoology	Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach
1999	Hans Kristen Stenøien	Dr. scient Bothany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)
1999	Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway.
1999	Ingvar Stenberg	Dr. scient. Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos*

1999	Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis.
1999	Trina Falck Galloway	Dr. scient. Zoology	Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.)
1999	Torbjørn Forseth	Dr. scient. Zoology	Bioenergetics in ecological and life history studies of fishes.
1999	Marianne Giæver	Dr. scient. Zoology	Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gradus morhua*) in the North-East Atlantic
1999	Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus*.
1999	Ingrid Bysveen Mjølnerød	Dr. scient. Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques
1999	Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from *Brassica napus* hypocotyls cultivated under various g-forces
1999	Stein-Are Sæther	Dr. philos. Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
1999	Katrine Wangen Rustad	Dr. scient. Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease
1999	Per Terje Smiseth	Dr. scient. Zoology	Social evolution in monogamous families: mate choice and conflicts over parental care in the Bluethroat (*Luscinia s. svecica*)
1999	Gunnbjørn Bremset	Dr. scient. Zoology	Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
1999	Frode Ødegaard	Dr. scient. Zoology	Host spesificity as parameter in estimates of arhrophod species richness
1999	Sonja Andersen	Dr. scient Bothany	Expressional and functional analyses of human, secretory phospholipase A2
2000	Ingrid Salvesen, I	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
2000	Ingar Jostein Øien	Dr. scient. Zoology	The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race
2000	Pavlos Makridis	Dr. scient Botany	Methods for the microbial econtrol of live food used for the rearing of marine fish larvae
2000	Sigbjørn Stokke	Dr. scient. Zoology	Sexual segregation in the African elephant (*Loxodonta africana*)
2000	Odd A. Gulseth	Dr. philos. Zoology	Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
2000	Pål A. Olsvik	Dr. scient. Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway
2000	Sigurd Einum	Dr. scient. Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size

2001	Jan Ove Evjemo	Dr. scient. Zoology	Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species
2001	Olga Hilmo	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forset systems
2001	Ingebrigt Uglem	Dr. scient. Zoology	Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.)
2001	Bård Gunnar Stokke	Dr. scient. Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
2002	Ronny Aanes	Dr. scient	Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*)
2002	Mariann Sandsund	Dr. scient. Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
2002	Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
2002	Frank Rosell	Dr. scient. Zoology	The function of scent marking in beaver (*Castor fiber*)
2002	Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A_2 in Monocytes During Atherosclerosis Development
2002	Terje Thun	Dr.philos Biology	Dendrochronological constructions of Norwegian conifer chronologies providing dating of historical material
2002	Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth
2002	Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
2002	Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and
2002	Henrik Jensen	Dr. scient Biology	Causes and consequenses of individual variation in fitness-related traits in house sparrows
2003	Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
2003	Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L.
2003	Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
2003	Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears
2003	Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana
2003	Marit Stranden	Dr.scient Biology	Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*)
2003	Kristian Hassel	Dr.scient Biology	Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum*
2003	David Alexander Rae	Dr.scient Biology	Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments
2003	Åsa A Borg	Dr.scient Biology	Sex roles and reproductive behaviour in gobies and guppies: a female perspective
2003	Eldar Åsgard Bendiksen	Dr.scient Biology	Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo Salar* L.) parr and smolt

2004	Torkild Bakken	Dr.scient Biology	A revision of Nereidinae (Polychaeta, Nereididae)
2004	Ingar Pareliussen	Dr.scient Biology	Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar
2004	Tore Brembu	Dr.scient Biology	Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in *Arabidopsis thaliana*
2004	Liv S. Nilsen	Dr.scient Biology	Coastal heath vegetation on central Norway; recent past, present state and future possibilities
2004	Hanne T. Skiri	Dr.scient Biology	Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species (*Heliothis virescens, Helicoverpa armigera* and *Helicoverpa assulta*).
2004	Lene Østby	Dr.scient Biology	Cytochrome P4501A (CYP1A) induction and DNA adducts as biomarkers for organic pollution in the natural environment
2004	Emmanuel J. Gerreta	Dr. philos Biology	The Importance of Water Quality and Quantity in the Tropical Ecosystems, Tanzania
2004	Linda Dalen	Dr.scient Biology	Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming
2004	Lisbeth Mehli	Dr.scient Biology	Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry (*Fragaria* x *ananassa*): characterisation and induction of the gene following fruit infection by *Botrytis cinerea*
2004	Børge Moe	Dr.scient Biology	Energy-Allocation in Avian Nestlings Facing Short-Term Food Shortage
2005	Matilde Skogen Chauton	Dr.scient Biology	Metabolic profiling and species discrimination from High-Resolution Magic Angle Spinning NMR analysis of whole-cell samples
2005	Sten Karlsson	Dr.scient Biology	Dynamics of Genetic Polymorphisms
2005	Terje Bongard	Dr.scient Biology	Life History strategies, mate choice, and parental investment among Norwegians over a 300-year period
2005	Tonette Røstelien	PhD Biology	Functional characterisation of olfactory receptor neurone types in heliothine moths
2005	Erlend Kristiansen	Dr.scient Biology	Studies on antifreeze proteins
2005	Eugen G. Sørmo	Dr.scient Biology	Organochlorine pollutants in grey seal (*Halichoerus grypus*) pups and their impact on plasma thyrid hormone and vitamin A concentrations.
2005	Christian Westad	Dr.scient Biology	Motor control of the upper trapezius
2005	Lasse Mork Olsen	PhD Biology	Interactions between marine osmo- and phagotrophs in different physicochemical environments
2005	Åslaug Viken	PhD Biology	Implications of mate choice for the management of small populations
2005	Ariaya Hymete Sahle Dingle	PhD Biology	Investigation of the biological activities and chemical constituents of selected *Echinops* spp. growing in Ethiopia
2005	Anders Gravbrøt Finstad	PhD Biology	Salmonid fishes in a changing climate: The winter challenge
2005	Shimane Washington Makabu	PhD Biology	Interactions between woody plants, elephants and other browsers in the Chobe Riverfront, Botswana

2005	Kjartan Østbye	Dr.scient Biology	The European whitefish *Coregonus lavaretus* (L.) species complex: historical contingency and adaptive radiation
2006	Kari Mette Murvoll	PhD Biology	Levels and effects of persistent organic pollutans (POPs) in seabirds Retinoids and α-tocopherol – potential biomakers of POPs in birds?
2006	Ivar Herfindal	Dr.scient Biology	Life history consequences of environmental variation along ecological gradients in northern ungulates
2006	Nils Egil Tokle	Phd Biology	Are the ubiquitous marine copepods limited by food or predation? Experimental and field-based studies with main focus on *Calanus finmarchicus*
2006	Jan Ove Gjershaug	Dr.philos Biology	Taxonomy and conservation status of some booted eagles in south-east Asia
2006	Jon Kristian Skei	Dr.scient Biology	Conservation biology and acidification problems in the breeding habitat of amphibians in Norway
2006	Johanna Järnegren	PhD Biology	Acesta Oophaga and Acesta Excavata – a study of hidden biodiversity
2006	Bjørn Henrik Hansen	PhD Biology	Metal-mediated oxidative stress responses in brown trout (*Salmo trutta*) from mining contaminated rivers in Central Norway
2006	Vidar Grøtan	PhD Biology	Temporal and spatial effects of climate fluctuations on population dynamics of vertebrates
2006	Jafari R Kideghesho	phD Biology	Wildlife conservation and local land use conflicts in western Serengeti, Corridor Tanzania
2006	Anna Maria Billing	PhD Biology	Reproductive decisions in the sex role reversed pipefish *Syngnathus typhle*: when and how to invest in reproduction
2006	Henrik Pärn	PhD Biology	Female ornaments and reproductive biology in the bluethroat
2006	Anders J. Fjellheim	PhD Biology	Selection and administration of probiotic bacteria to marine fish larvae
2006	P. Andreas Svensson	phD Biology	Female coloration, egg carotenoids and reproductive success: gobies as a model system
2007	Sindre A. Pedersen	PhD Biology	Metal binding proteins and antifreeze proteins in the beetle *Tenebrio molitor* - a study on possible competition for the semi-essential amino acid cysteine
2007	Kasper Hancke	PhD Biology	Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae
2007	Tomas Holmern	PhD Biology	Bushmeat hunting in the western Serengeti: Implications for community-based conservation
2007	Kari Jørgensen	PhD Biology	Functional tracing of gustatory receptor neurons in the CNS and chemosensory learning in the moth *Heliothis virescens*
2007	Stig Ulland	PhD Biology	Functional Characterisation of Olfactory Receptor Neurons in the Cabbage Moth, */Mamestra Brassicae/* L. (Lepidoptera, Noctuidae). Gas Chromatography Linked to Single Cell Recordings and Mass Spectrometry
2007	Snorre Henriksen	PhD Biology	Spatial and temporal variation in herbivore resources at northern latitudes
2007	Roelof Frans May	PhD Biology	Spatial Ecology of Wolverines in Scandinavia

2007	Vedasto Gabriel Ndibalema	PhD Biology	Demographic variation, distribution and habitat use between wildebeest sub-populations in the Serengeti National Park, Tanzania
2007	Julius William Nyahongo	PhD Biology	Depredation of Livestock by wild Carnivores and Illegal Utilization of Natural Resources by Humans in the Western Serengeti, Tanzania
2007	Shombe Ntaraluka Hassan	PhD Biology	Effects of fire on large herbivores and their forage resources in Serengeti, Tanzania
2007	Per-Arvid Wold	PhD Biology	Functional development and response to dietary treatment in larval Atlantic cod *(Gadus morhua* L.) Focus on formulated diets and early weaning
2007	Anne Skjetne Mortensen	PhD Biology	Toxicogenomics of Aryl Hydrocarbon- and Estrogen Receptor Interactions in Fish: Mechanisms and Profiling of Gene Expression Patterns in Chemical Mixture Exposure Scenarios
2008	Brage Bremset Hansen	PhD Biology	The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and its food base: plant-herbivore interactions in a high-arctic ecosystem
2008	Jiska van Dijk	PhD Biology	Wolverine foraging strategies in a multiple-use landscape
2008	Flora John Magige	PhD Biology	The ecology and behaviour of the Masai Ostrich (Struthio camelus massaicus) in the Serengeti Ecosystem, Tanzania
2008	Bernt Rønning	PhD Biology	Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, */Taeniopygia guttata/*
2008	Sølvi Wehn	PhD Biology	Biodiversity dynamics in semi-natural mountain landscapes. - A study of consequences of changed agricultural practices in Eastern Jotunheimen
2008	Trond Moxness Kortner	PhD Biology	"The Role of Androgens on previtellogenic oocyte growth in Atlantic cod (*Gadus morhu/*): Identification and patterns of differentially expressed genes in relation to Stereological Evaluations"
2008	Katarina Mariann Jørgensen	Dr.Scient Biology	The role of platelet activating factor in activation of growth arrested keratinocytes and re-epithelialisation
2008	Tommy Jørstad	PhD Biology	Statistical Modelling of Gene Expression Data
2008	Anna Kusnierczyk	PhD Bilogy	*Arabidopsis thaliana* Responses to Aphid Infestation
2008	Jussi Evertsen	PhD Biology	Herbivore sacoglossans with photosynthetic chloroplasts
2008	John Eilif Hermansen	PhD Biology	Mediating ecological interests between locals and globals by means of indicators. A study attributed to the asymmetry between stakeholders of tropical forest at Mt. Kilimanjaro, Tanzania