Vedlegg 5: Prosedyre for bibliotekstillaging av 16S amplifiserte fragment

Prosedyren beskriver fremgangsmåten brukt til bibliotekstillaging. Her inngår delene «End repair», «Nick ligate og barcode adaptor» og «Amplify library». Det ble gjort vasketrinn etter vedlegg 4 etter adaptor ligering og amplifisering av biblioteksmolekyl. Prosedyren ble utarbeidet av Ann-Kristin Tveten ved instituttet for biologiske fag ved NTNU i Ålesund.

1. **End repair**

   NEB library kit and Ion Xpress Barcode adapters 1-16 kit (Cat.no. 4471250) was used to make the library and purify the barcoded library product.

   1. Load the following components in a tube and mix thoroughly by pipettor:

Components	Volume
Pooled amplicons	12 µl
Nuclease free water	13,5 µl
5X end repair buffer	3 µl
End repair enzyme	1,5 µl
Total volume	30 µl

   2. Incubate for 20 minutes at 25°C followed by 10 minutes at 70°C, hold at 4°C.

2. **Nick ligate og barcode adaptor**

   3. Load the following components to the sample in a PCR tube to make the barcoded library, using the Ion Xpress Barcode Adapters 1-16 kit (Cat.no. 4471250):

Components	Volume
DNA (sample from 1)	30 µl
10X ligase buffer	5 µl
Ion P1 adapter (barcoded libraries)	1,5 µl
Ion Xpress barcode X (one for each sample)	1 µl
Nuclease-free water	8,5 µl
DNA ligase	3 µl
Nick repair polymerase	1 µl
Total volume	50 µl

IMPORTANT: Be careful not to cross-contaminate when handling barcode adapters. When making a barcoded library, use both Ion PI adapter and the desired Ion Xpress Barcode X.

4. When all the reagents are added to the sample, place the tubes in a thermal cycler with the following program:

Stage	Temperatur	Tid
Hold	25°C	15 min
Hold	65°C	5 min
Hold	4°C	∞*

*= This is not a stopping point, continue with the procedure.

3. Amplify library

Ion Plus fragment library kit can be used to amplify the library.

5. Load the following reagents to the tube containing the library:

Components	Volume
Un- amplified library from C2	13 µl
Platinum PCR superMix High Fidelity	25 µl
Library Amplification Primer Mix	2 µl
ddH2O	10 µl
Total volume	50 l

6. Place PCR tubes into a thermal cycler and run the following program:

Stage	Step	Temperature	Time
Holding	Denature	98°C	30 sec
12 cycles (50 ng of input)	Denature Anneal Extend	98°C 58°C 65°C	10 sec 30 sec 30 sec
Holding	-	65°C	5 min
Holding	-	4°C	Hold for up to 1 hour