

Vedlegg 4: Prosedyre for vask med AMPure XP beads

Prosedyren beskriver vasketrinn med AMPure XP Beads. Denne ble brukt 3 ganger gjennom forberedelsen av prøvene. Ulike mengder beads ble brukt ved de ulike punktene i prosessen, mengdene er spesifisert i beskrivelsen av metodene. Prosedyren ble utarbeidet av Ann-Kristin Tveten ved instituttet for biologiske fag ved NTNU i Ålesund.

1. Add 54 μl (1.8X volume) of AMPure XP Beads to the sample (30 μl) and mix by pipetting up and down.
2. Incubate for 5 minutes at room temperature.
3. Pulse spin the tube and place in a magnetic rack for approximately 2—3 minutes until the beads have collected to the side of the tube and the solution is clear. Carefully remove and discard the supernatant without disturbing the beads.
4. Keep the tube on the magnet and add 200 μl freshly prepared 70% ethanol. Incubate at room temperature, for 30 seconds, and carefully remove and discard the supernatant.
5. Repeat step 4 once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.
6. Keeping the tube in the magnetic rack, with the cap open, air dry the beads for up to 5 minutes at room temperature.

Caution: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack, they are too dry.

7. Remove the tube from the magnet. Resuspend the beads in 20 μl of sterile 0.1X TE (volume may be adjusted for specific gel-based size selection protocol). Incubate for 2 minutes at room temperature.
8. Pulse-spin the tube and return to the magnet, until the beads have collected to the side of the tube and the solution is clear.
9. Transfer approximately 15 μl (or desired volume) of the supernatant to a clean tube, being careful not to transfer any beads.