Vedlegg 3: Prosedyre for 16S rRNA amplifisering

Vedlegget beskriver eksakt prosedyre brukt til 16S rRNA amplifikasjon av isolert DNA. Prosedyren ble utarbeidet av Ann-Kristin Tveten ved instituttet for biologiske fag ved NTNU i Ålesund.

1. Thaw all Ion 16S Metagenomics Kit reagents and keep on ice.
2. For each sample, prepare two reactions (one for each of the 2 primer sets). Include one positive and negative control per PCR run. Before you pipet each reagent, vortex for 5 seconds and pulse-spin the reagent tube.

Component	Tube A	Tube B	Negative control
2X Environmental Master Mix	7,5 µl	7,5 µl	7,5 µl
16S Primer Set (10X) (V2-4-8)	1,5 µl		
16S Primer Set (10X) (V3-6, 7-9)		1,5 µl	1,5 µl
DNA template	6 µl	6 µl	
Negative Control (water)			6 µl
Totalt	15 µl	15 µl	15 l

3. Place the tubes or plate in the thermal cycler and run the following program:

Stage	Temperature	Tid
Hold	95°C	10 min
Cycle 19-25 cycles	95°C	30 sek
	58°C	30 sek
	72°C	20 sek
Hold	72°C	7 min
Hold	4°C	∞

4. (Optimal) If samples contain non-microbial DNA, confirm the presence of PCR products (use a Bioanalyzer instrument or 2% agarose gel) before you continue to the purification step.