

DNA extraction from sludge

1. Take 900ul of sludge with a tip having a large orifice into a 2ml Eppendorf tube (sterile)
2. Centrifuge sludge sample at 4550xg for 10 minutes, remove supernatant, take 250mg of the pellet in the MoBio bead tube
3. Extract DNA using MoBio kit according to the manufacturers instructions and 10 minutes of bead beating
4. Elution with 50-100ul of C6 buffer
5. Check quality of DNA in agarose gel (0.8-1%)

PCR: Use of Phusion high fidelity polymerase

PCR for bacterial community

Primers:

M13-16S-IA-FL: 5'-**CACGACGTTGTAAAACGACCATGCTGCCTCCCGTAGGAGT**-3'

16S-IA-RL: 5'-**CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGAGTTTGATCCTGGCTCAG**-3'

	MMx1 (ul)	Programme	
Phusion 5x buffer	4	95C	5 min
MgCl ₂	1.4	98C	20 sec
dNTP	0.6 (10mM)	55C	20 sec
BAC_16Sf	0.6 (10uM)	72C	30 sec
BAC_16Sr	0.6 (10uM)	72C	10 min
Phusion Pol	0.2		
H ₂ O	To 20ul		

PCR for archaeal community

Primers: (ARC-344f: 5'-ACGGGGYGCAGCAGGCGCGA-3'; ARC-915r: 5'-GTGCTCCCCCGCCAATTCCT-3')

454 and M13 attached to primer:

ARC16SM13FL: 5'-**CACGACGTTGTAAAACGACACGGGGYGCAGCAGGCGCGA**-3'

ARC16S_915_RL: 5'-**CCTATCCCCTGTGTGCCTTGGCAGTCTCAGGTGCTCCCCCGCCAATTCCT**-3'

	MMx1 (ul)	Programme	
Phusion 5x buffer	4	95C	5 min
MgCl ₂	1.4	98C	20 sec
dNTP	0.6 (10mM)	55C	20 sec
ARC_16Sf	0.6 (10uM)	72C	30 sec
ARC_16Sr	0.6 (10uM)	72C	10 min
Phusion Pol	0.2		
H ₂ O	To 20ul		