

PCR protocol for CASEU

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Aim

PCR the extracted DNA for CASEU

Materials

- PCR plates 2

Procedures

Preparation steps:

- ☐ master mix in 96-well plate
- ☐ PCR plates 2
- ☐ Mastermix

Table 1: CASEU PCR master mix.

Reagent	WellVolume	TotalVolume
ddH2O	23.5	5076
5X HF buffer	10.0	2160
dNTPs (10mM)	1.0	216
Phusion	0.5	108
Total	35.0	7560

Table 2: CASEU PCR reagents with master mix.

Reagent	WellVolume	TotalVolume
PCR master mix	35	7560
27F primer (3uM)	5	1080
1492R primer (3uM)	5	1080
teamplate DNA polymerase	5	1080

- Label 2 PCR plates.
- Make **1080 uL** of 3uM of each primer (32.4 uL 100 uM stock + 1047.6 uL ddH₂O).
- Premix the PCR reagents (total 7.56 mL) in a 50 mL falcon tube.
- Use mP200 set at **35 uL** to dispense 35 uL of PCR master mix into each well of 2 PCR plates. This premix can stay at room temperature.
- Right before starting the PCR reaction, use mP20 set at **5 uL** to add primers.
- Use mP20 set at **5 uL** to add DNA. Cover the PCR plates with clear PCR films.
- Use the program “CASEU” in “16S” folder. See the table below for PCR cycle.
- Store PCR plates in -20C freezer.