

PCR protocol for CASEU

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2021-02-11

Aim

PCR the extracted DNA for CASEU

Procedures

Preparation steps:

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

Reagent	WellVolume	TotalVolume
PCR master mix	35	7560
27F primer (3uM)	5	1080
1492R primer (3uM)	5	1080
DNA polymerase	5	1080
Total	50	10800

- ☐ Label 2 PCR plates. For duplicated PCR.
- ☐ 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 to dispense 35 μ L 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 to add 5 μ L of primers.
- ☐ Use mP20 to add 5 μ L of 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.

Table 3: CASEU PCR cycle.

Step	Temperature	Duration
Initial denaturation	98 C	30 seconds
Amplification (30 cycles)	98 C	30 seconds
	50 C	30 seconds
	72 C	90 seconds
Final extension	72 C	10 minutes
Storage	4 C	Forever