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16S PCR

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ABSTRACT

A protocol for 16S PCR as outlined by the HUCK Genomics Core.

PROTOCOL CITATION

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MATERIALS TEXT

a. Invitrogen Platinum SuperFi Master Mix
b. 515f Parrada Primer at 10uM
c. 806R Apprill Primer at 10uM
d. Sterile PCR H2O
e. PCR Plate/Tube Rack
f. Adhesive Film
g. 96-well PCR Plate or 8-well PCR tube (depending on number of samples)
h. Pipette Tips (10ul, 20ul, 100ul, 1000ul)
i. Micropipettes (P10, P20, P100, P1000)
j. VWR marker
k. Sterile Centrifuge Tubes

Before You Begin

1 Prepare your workspace

1. Turn on PCR Clean Hood blower and white light.
2. Clean the cabinet with 70% ethanol, including the work surface, walls, and glass.
3. Turn the dial and run the UV light for 15 minutes.

> Do not trust the glass to protect you from UV exposure. You can be in a different part of the lab while it is running, but do not loiter in front of the cabinet.

4. Once the timer is up and the UV light turns off, you are ready to begin.
5. Clean all materials with 70% ethanol before putting them into the cabinet.

2 Gather materials

Place your materials on the bench next to the PCR Clean hood. Clean lab bench well with 70% ethanol before use.

a. Invitrogen Platinum SuperFi Master Mix
b. 515f Parrada Primer at 10uM
c. 806R Apprill Primer at 10uM
d. Sterile PCR H2O
e. PCR Plate/Tube Rack
f. Adhesive Film
g. 96-well PCR Plate or 8-well PCR tube (depending on number of samples)
h. Pipette Tips (10ul, 20ul, 100ul, 1000ul)
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j. VWR marker
k. Sterile Centrifuge Tubes

Master Mix Preparation

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2m

1. In a sterile centrifuge tube labeled “Master Mix/MM” combine:

10 µL */sample Invitrogen Platinum SuperFi Master Mix,*

0.4 µL */sample 515F Parrada Primer,*

0.4 µL */sample 806R Apprill Primer, and*

7.2 µL */sample Sterile PCR H2O.*

Prepare enough master mix for 100 samples if running a full plate:

2. Vortex and spin down master mix when complete. **12500 rpm, 25°C, 00:02:00**

A	B
In Sterile 2mL Centrifuge Tube mix: (100rxns)	
Invitrogen Platinum SuperFi Master Mix	1mL (1000uL)
515F Parrada Primer (10uM)	40uL
806R Apprill Primer (10uM)	40uL
Sterile PCR Water	720uL

PCR Reaction

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1. Add 18 uL of Master Mix to each PCR tube.
2. Add 2 uL of the extracted DNA to each respective PCR tube. Be sure to follow the plate map, create a new PCR plate map, and/or label tubes.
3. Run Thermocycler Program:
4. When cycles are complete, centrifuge tubes. Tubes can then be stored at 4°C until ready to run gel or AMPure.

A	B	C
Thermocycler Program		
Cycle 1	2 minutes	98°C
Cycle 2	10 seconds	98°C
Cycle 3	20 seconds	56.5°C
Cycle 4	15 seconds	72°C
Repeat Cycles 2-4 (20-25X)		
Cycle 5	5 minutes	72°C
Hold at 4°C		