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PCR

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ABSTRACT

50 µL volume PCR protocol

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1 Centrifuge the tubes with the sequences for one minute at 3,000 g x m in the centrifuge

2 Centrifuge for 5 seconds in the microcentrifuge

3 Add nuclease-free water needed to have a final concentration of 10 ng/uL.

4 Vortex, followed by incubating for 20 minutes at 50°C.

5 Mix briefly by vortex and centrifuge for 8 seconds in mini centrifuge.

PCR

6 Add nuclease-free water to the first forward and reverse until a concentration of 100 uM is reached, centrifuged quickly in microcentrifuge.

7 Make dilutions with 10 uL of primers and 90 uL of nuclease-free water to reach a concentration of 10 uM

8 In 0.2 mL Eppendorf tubes make the following mixture.

9

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A	B
Nuclease Free Water	17 uL
Buffer 10X PCR	5 uL
50 mM MgCl2	1.5 uL
10 mM dNTP's	1 uL
10 uM Forward primer	2.5 uL
10 uM Reverse primer	2.5 uL
DNA (25 mg/uL)	20 uL
Taq Platinum Polymerase	0.5 uL
Final Volume	50 uL

10 Perform the next cycle in thermocycler

A	B	C	D
1 Cycle	Initial activation	2 minutes	94ºC
35 Cycles	Denaturation	30 seconds	94ºC
35 Cycles	Alignment	30 seconds	50ºC
35 Cycles	Extension	2 minutes	72ºC
1 Cycle	Final Extension	5 minutes	72ºC
Maintenance	Maintenance	-	4ºC

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