Amplifying the target genomic region by PCR

In 1 collection

Sep 06, 2022

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ABSTRACT

This protocol describes a procedure for amplifying targeted genomic regions using a PCR reaction

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MATERIALS TEXT

Item	Vendor	Catalog #
dNTP	NEB	N0447L
10x HF buffer	NEB	B0518S
Phusion DNA polymerase	NEB	M0530S
Microseal® 'B' Adhesive Seals	Biorad	MSB1001
96-well PCR plate	GeneMate	T-3152-1

1 For a 150bp paired-end or single-read sequencing experiment, design primers in a way that the region of interest, like CRISPR cutting site or specific mutations, locates within 130bp from at least one of the primers

2 Add NGS adapters to 5’ end of primers following the instructions of your local NGS facility

3 20 µl PCR reaction for each sample

3.1 PCR Reaction - setup

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A	B
Ultrapure H2O	10.6 µl
5x HF buffer	4 µl
2.5 mM dNTP	1.6 µl
10 µM primer Forward	0.5 µl
10 µM primer Reverse	0.5 µl
DMSO	0.6 µl
Titan DNA polymerase or Phusion	0.2 µl
Crude cell lysate	2 µl

4 Calculate the amount of each component needed for all samples, also count a negative control and positive control

5 In a pre-PCR area, mix all components except the crude cell lysate to a microcentrifuge tube or a 15 ml conical tube to make master mix

6 Aliquot 18 µl to each 200 µl microcentrifuge tube or each well in a 96-well PCR plate. Use reservoir and multi-channel pipet if there are many samples.

7 Add 2 µl crude cell lysate to the reaction. In one reaction, use 2 µl H2O instead as negative control. In another reaction, use 2 µl previously validated crude cell lysate as positive control.

8 Cap the tubes or seal the plates properly with an adhesive seal.

9 Shake the tubes or plates vigorously to mix

10 Briefly spin the samples

11 Use the following touch-down PCR protocol in a thermocycler

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11.1 Touch-down PCR protocol

A	B
98°C	3min
98°C	30s
70°C (touch down, 1°C/cycle)	30s
72°C	30s
Go to 2	12 cycles in total
98°C	30s
58°C	30s
72°C	30s
Go to 6	23 cycles in total
72°C	7min
4°C or 12°C	forever

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