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Preparation of the sgRNA-Barcode Amplicon Library

PLOS One

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GUIDELINES

This protocol is used to assess the sgRNA-to-barcode linkage in Mosaic-seq. The 3kb fragment can be amplified from either plasmids or genomic DNA extract from cells after lentiviral infection. The fragments are then circularized and used to amplify a smaller amplicon (about 270bp) which is compatible with Illumina sequencing.

OPEN ACCESS

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Protocol status: Working

We use this protocol and it's working

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PROTOCOL integer ID:
11879

PCR to get the 3kb fragment

1 Prepare the following mixture:

Reagent	Vol. (μl)
NEB Next mixture	25
PCR 1 primers + (10 μM)	1 / 1
H ₂ O	Up to 50
Plasmid / gDNA	indicated

T o t a l	5 0
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Note

Use 1000ng genomic DNA or 100ng plasmid DNA for the PCR

2 PCR cycles

98°C 30sec
 98°C 10sec |
 53°C 30sec | 22 or 12 cycles
 72°C 3min |
 72°C 5min

Note

22 cycles for gDNA, 12 cycles for plasmids

3 Gel extract the PCR product:

- Run the PCR products on 1% agarose gel. Include vector control in another lane.
- Excise 3kb fragment and perform MinElute PCR cleanup. Elute in 2 x 10 µL of EB.

PNK treatment to phosphorylate 5' ends

4 Set up the following reaction:

R e a g e n t	V o l. (µ l)
P C R 1 p r o d u c t i n w a t e r	3 9

50 ng PCR product in water	78	
2X Quick ligation buffer	80	
Quick ligation	2	

8 Incubate at 25°C for 2 hours.

25 °C 2 hours

9 Perform MinElute PCR cleanup. Elute in 2x10 µl EB.

PlasmidSafe digestion

10 Setup the following reaction:

R e a g e n t	V o l. (μ l)
D N A i n w a t e r	4 2
2 5 m M A T P	2
1 0 X R e a c t i o n b u f f e r	5
P l a s m i d - S a f e D N a s e	1

11 Incubate at 37C for 30 min.

37 °C 30 min

12 Perform MinElute PCR cleanup. Elute in 2x10ul. Spec the DNA concentration by using Qubit.

2nd PCR

13 Setup the following reaction:

Reagent	Vol. (μl)
KAPA HiFi HS Master Mix	25
BarLi bP5S tagger mix (10 mM)	1
BarLi bP7NX (10 mM)	1

C ir c ul at e d D N A	1 8
W at er	t o 5 0

14 Perform PCR by using the following program:

95°C 3min
 98°C 20sec |
 60°C 15sec | 16 cycles
 72°C 1min |
 72°C 3min

15 Run samples on 1% agarose gel. Excise band at 400bp

16 Spec DNA concentration by Qubit.

17 Run the samples on Tapestation (D1000 tape) to check the final size of the library.