Preparation of the sgRNA-Barcode Amplicon Library

Shiqi Xie,Anne Cooley,Gary Hon

Abstract

Citation: Shiqi Xie,Anne Cooley,Gary Hon Preparation of the sgRNA-Barcode Amplicon Library. **protocols.io** dx.doi.org/10.17504/protocols.io.pufdntn
Published: 07 May 2018

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 lentivirual infection. The fragments are then circularized and used to amplify a smaller amplicon (about 270bp) which is compatible with Illumina sequencing.

Protocol

PCR to get the 3kb fragment

Step 1.

Prepare the following mixture:

Reagent	Vol.(µl)
NEBNext mixture	25
PCR1 primers +/- (10µM)	1/1
H2O	Up to 50
Plasmid / gDNA	indicated
Total	50

NOTES
Shiqi Xie 29 Apr 2018

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

PCR to get the 3kb fragment

Step 2.

PCR cycles

98°C 30sec

98°C 10sec |

53°C 30sec | 22 or 12 cycles

72°C 3min |

72°C 5min

NOTES

Shiqi Xie 29 Apr 2018

22 cycles for gDNA, 12 cycles for plasmids

PCR to get the 3kb fragment

Step 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

Step 4.

Set up the following reaction:

Reagent	Vol.(µl)
PCR1 product in water	39
10X T4 PNK buffer	5
10 mM ATP	5
T4 PNK	1

PNK treatment to phosphorylate 5’ ends

Step 5.

Incubate at 37˚C for 30 min.

TEMPERATURE

37 °C Additional info: 30 min

PNK treatment to phosphorylate 5’ ends

Step 6.

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

Self-ligation with Quick Ligase

Step 7.

Setup the following reaction:

Reagent	Vol.(µl)
50 ng PCR1 product in water	78
2X Quick ligase buffer	80
Quick ligase	2

Self-ligation with Quick Ligase

Step 8.

Incubate at 25°C for 2 hours.

TEMPERATURE

25 °C Additional info: 2 hours

Self-ligation with Quick Ligase

Step 9.

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

PlasmidSafe digestion

Step 10.

Setup the following reaction:

Reagent	Vol.(µl)
DNA in water	42
25 mM ATP	2
10X Reaction buffer	5
Plasmid-Safe DNase	1

PlasmidSafe digestion

Step 11.

Incubate at 37C for 30 min.

TEMPERATURE

37 °C Additional info: 30 min

PlasmidSafe digestion

Step 12.

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

2nd PCR

Step 13.

Setup the following reaction:

Reagent	Vol.(µl)
KAPA HiFi HS MasterMix	25
BarLib P5 Stagger mix (10 mM)	1
BarLib P7 N7XX (10 mM)	1
Circulated DNA	18

Water	to 50

2nd PCR

Step 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	

2nd PCR

Step 15.

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

2nd PCR

Step 16.

Spec DNA concentration by Qubit.

2nd PCR

Step 17.

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