May 07, 2018

Preparation of the sgRNA-Barcode Amplicon Library

PLOS One

DOI
dx.doi.org/10.17504/protocols.io.pufdntn

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DOI: dx.doi.org/10.17504/protocols.io.pufdntn

External link: https://doi.org/10.1371/journal.pone.0198635

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

Manuscript citation:
Xie S, Cooley A, Armendariz D, Zhou P, Hon GC (2018) Frequent sgRNA-barcode recombination in single-cell perturbation assays. PLoS ONE 13(6): e0198635. doi: 10.1371/journal.pone.0198635

Protocol status: Working
We use this protocol and it's working

Created: April 29, 2018

Last Modified: May 07, 2018

Protocol Integer ID: 11879

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.

PCR to get the 3kb fragment

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

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 × 10 µL of EB.

PNK treatment to phosphorylate 5′ ends

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

5 Incubate at 37°C for 30 min.
37 °C 30 min

6 Perform MinElute PCR cleanup. Elute in 2×10ul. Spec the DNA concentration by Qubit.

Self-ligation with Quick Ligase

7 Setup the following reaction:

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

8 Incubate at 25°C for 2 hours.
25 °C 2 hours

9 Perform MinElute PCR cleanup. Elute in 2×10 µl EB.

PlasmidSafe digestion

10 Setup the following reaction:

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

11 Incubate at 37C for 30 min.
37 °C 30 min

12 Perform MinElute PCR cleanup. Elute in 2×10ul. Spec the DNA concentration by using Qubit.

2nd PCR

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

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.