TF ORF barcode dial out PCR (10X)

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- Forward primers
  - ORF-perturb-NGS-F: AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
- Reverse primers
  - ORF-perturb-NGS-R1: CAAGCAGAAGACGGCATACGAGATTCGCCTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAGTAGAGTTGGCTTTATATATCTTGTGGAAAGGACGA
  - ORF-perturb-NGS-R2: CAAGCAGAAGACGGCATACGAGATATAGCGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCATGCTTATTGGCTTTATATATCTTGTGGAAAGGACGA
  - ORF-perturb-NGS-R3: CAAGCAGAAGACGGCATACGAGATGAAGAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGCACATCTTTGGCTTTATATATCTTGTGGAAAGGACGA
  - ORF-perturb-NGS-R4: CAAGCAGAAGACGGCATACGAGATATTCTAGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGATTGCTCGACTTGGCTTTATATATCTTGTGGAAAGGACGA
  - ORF-perturb-NGS-R5: CAAGCAGAAGACGGCATACGAGATCGTTACCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCGATAGCAATTCTTGGCTTTATATATCTTGTGGAAAGGACGA
  - ORF-perturb-NGS-R6: CAAGCAGAAGACGGCATACGAGATGTCTGATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCGATAGTTGCTTTTGGCTTTATATATCTTGTGGAAAGGACGA
  - ORF-perturb-NGS-R7: CAAGCAGAAGACGGCATACGAGATTTACGCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATCGATCCAGTTAGTTGGCTTTATATATCTTGTGGAAAGGACGA
  - ORF-perturb-NGS-R8: CAAGCAGAAGACGGCATACGAGATTTGAATAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGATCGATTTGAGCCTTTGGCTTTATATATCTTGTGGAAAGGACGA
- NEBNext High Fidelity PCR Master Mix, 2× (New England BioLabs, cat. no. M0541)

Procedure

Library PCR for NGS

1. We have provided NGS primers that amplify the TF ORF barcode region with Illumina adaptor sequences. Pool the 10 ORF-perturb-NGS-R primers at equimolar ratio to produce ORF-perturb-NGS-R (pooled). These primers stagger the resulting PCR amplicons and diversify the library.

2. To prepare the TF ORF barcode library for NGS, set up a reaction using the ORF-pertrub-NGS-F and ORF-perturb-NGS-F (pooled) for each 10X channel according to the following table.

NEBNext PCR reaction components

	A	B	C	D
1	**Component**	**Starting concentration**	**Amount per reaction (uL)**	**Final concentration**
2	**NEBNext PCR Master Mix**	2X	25	1X
3	**ORF-perturb-NGS-F**	10 uM	1.25	0.25 uM
4	**ORF-perturb-NGS-R (pooled)**	10 uM	1.25	0.25 uM
5	**WTA cDNA template**	10-20 ng/uL	2	0.4-0.8 ng/uL
6	**H2O**		20.5	
7			50	

3. Perform a PCR using the following cycling conditions:

NEBNext PCR Cycle Conditions

	A	B	C	D
1	Step	Temperature	Duration	Cycles
2	Initial denaturation	98 ºC	3 min	1
3	Denaturation	98 ºC	10 sec	22
4	Annealing	60 ºC	10 sec	
5	Extension	72 ºC	40 s	
6	Final extension	72 ºC	2 min	1

4. After the reaction is complete, proceed according to the "Next-generation sequencing of the amplified sgRNA library to determine sgRNA distribution" section in Joung, Nature Protocols 2017.