TF ORF barcode library PCR for NGS

Introduction

PCR protocol for amplification of TF ORF barcodes from plasmid or gDNA library for NGS. For additional details on using the TF ORF library for screening, please refer to Joung et al, "Genome-scale CRISPR-Cas9 knockout and transcriptional activation screening," Nature Protocols 2017.

Materials

Forward Primers

- ORF-NGS-F1:
  AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTAAGTAGAGTTGGCTTTATATATCTTGTGGAAAGGACGA
- ORF-NGS-F2:
  AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATCATGCTTATTGGCTTTATATATCTTGTGGAAAGGACGA
- ORF-NGS-F3:
  AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATGCACATCTTTGGCTTTATATATCTTGTGGAAAGGACGA
- ORF-NGS-F4:
  AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATTGCTCGACTTGGCTTTATATATCTTGTGGAAAGGACGA
- ORF-NGS-F5:
  AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGATAGCAATTCTTGGCTTTATATATCTTGTGGAAAGGACGA
- ORF-NGS-F6:
  AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATCGATAGTTGCTTTTGGCTTTATATATCTTGTGGAAAGGACGA
- ORF-NGS-F7:
  AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATCGATCCAGTTAGTTGGCTTTATATATCTTGTGGAAAGGACGA
- ORF-NGS-F8:
  AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATCGATTTGAGCCTTTGGCTTTATATATCTTGTGGAAAGGACGA
- ORF-NGS-F9:
  AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTACGATCGATACACGATCTTGGCTTTATATATCTTGTGGAAAGGACGA
- ORF-NGS-F10:
  AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTACGATCGATGGTCCAGATTGGCTTTATATATCTTGTGGAAAGGACGA

Reverse Primers (only need 1 primer per condition, barcodes are bolded)

- ORF-NGS-R1:
  CAAGCAGAAGACGGCATACGAGAT**TCGCCTTG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAAGCAGCGTATCCACATAGCGT

- ORF-NGS-R2: CAAGCAGAAGACGGCATACGAGAT**ATAGCGTC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAAGCAGCGTATCCACATAGCGT
- ORF-NGS-R3: CAAGCAGAAGACGGCATACGAGAT**GAAGAAGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAAGCAGCGTATCCACATAGCGT
- ORF-NGS-R4: CAAGCAGAAGACGGCATACGAGAT**ATTCTAGG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAAGCAGCGTATCCACATAGCGT
- ORF-NGS-R5: CAAGCAGAAGACGGCATACGAGAT**CGTTACCA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAAGCAGCGTATCCACATAGCGT
- ORF-NGS-R6: CAAGCAGAAGACGGCATACGAGAT**GTCTGATG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAAGCAGCGTATCCACATAGCGT
- ORF-NGS-R7: CAAGCAGAAGACGGCATACGAGAT**TTACGCAC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAAGCAGCGTATCCACATAGCGT
- ORF-NGS-R8: CAAGCAGAAGACGGCATACGAGAT**TTGAATAG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAAGCAGCGTATCCACATAGCGT
- ORF-NGS-R9: CAAGCAGAAGACGGCATACGAGAT**TCCTTGGT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAAGCAGCGTATCCACATAGCGT
- ORF-NGS-R10: CAAGCAGAAGACGGCATACGAGAT**ACAGGTAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAAGCAGCGTATCCACATAGCGT
- ORF-NGS-R11: CAAGCAGAAGACGGCATACGAGAT**AGGTAAGG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAAGCAGCGTATCCACATAGCGT
- ORF-NGS-R12: CAAGCAGAAGACGGCATACGAGAT**AACAATGG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAAGCAGCGTATCCACATAGCGT

- 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-NGS-F primers at equimolar ratio to produce ORF-NGS-F (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-NGS-F (pooled) and a unique ORF-NGS-R for each condition according to the following table. For amplifying from plasmid library, use 20ng of plasmid per reaction as template. For amplifying from gDNA, use 1ug-3ug of gDNA per reaction as template. Use a different ORF-NGS-R primer for each condition, and scale up the number of reactions according to the scale of the screen.

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-NGS-F (pooled)**	10 uM	1.25	0.25 uM
4	**ORF-NGS-R (unique)**	10 uM	1.25	0.25 uM
5	**Template**	20 ng/uL	1	0.4 ng/uL
6	**H2O**		21.5	
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	25 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.