Re-amplification of CRISPRa and CRISPRi libraries (v1.0)Reference: Genome-Scale CRISPR-Mediated Control of Gene Repression and A

IGI

Abstract

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Protocol

Step 1.
Dilute each sub-library to 50 ng/ul in water or EB

Step 2.
Electroporate the library a Pre-chill 01 cm cuvettes, megaX cells, 10% glycerol on ice b Follow the table above for the amounts of sub-library plasmid DNA and MegaX competent cells, mix gently and incubate on ice for 30 min c Add pre-chilled 10% glycerol to the MageX-library mix for a final 75 ul, transfer the mix to a prechilled 01 cm cuvette d Electroporate at 20 kV, 200 ohms, 25 uF (Gene Pulser Xcell, Bio-rad) e Transfer cells to a culture tube i Use 1 ml pipettes and gel loading tips ii Wash cells out gently with 300 ul SOC twice (total 600 ul) f Incubate at 37oC, 250 rpm, 15 hour

Step 3.
Plate the transformations a Plate all in one large square plate per sub-library, use autoclaved beads b Incubate at 37oC for 18 hours

Step 4.
Collect all colonies with LB and do one maxiprep per plate, elute in 500 ul EB; an ideal concentration is about 23 ug/ul

Step 5.
To sequence the library, you can PCR the sgRNA region with the following primers (a common 3’ primer, with 2 different 5’ primers for CRISPRa or CRISPRi) • CRISPRi TSS common 3' caagcagaagacggcatacgaCGACTCGGTGCCACTTTTTC • oCRISPRi TSS_1 (TruSeq Index 12 CTTGTA), aatgatacggcgaccaccgaGATCGGAAGAGCACACGTCTGAACTCCAGTCACCTTGTAgcacaaaaggaaactcaccct • oCRISPRi TSS_2 (TruSeq Index 6 GCCAAT) aatgatacggcgaccaccgaGATCGGAAGAGCACACGTCTGAACTCCAGTCACGCCAATgcacaaaaggaaactcaccct a Pool sub-libraries proportionally (based on the number of sgRNAs) to have the CRISPRa or CRISPRi library, measure the pooled concentration and dilute it to 400 ng/ul for PCR b Run 3 tubes of 100 ul PCR reactions

Step 6.

library 400ng/ul	1
5x Q5 BUFFER	20

5X GC BUFFER	20
dNTPs 10mM	2
Index primer 10uM	2.5
Common Primer 10uM	2.5
Q5 Hotstart polymerase	1
ddH2O	51