# Re-amplification of CRISPRa and CRISPRi libraries

**Pei-Chun Lin**

## Abstract

Re-amplification of CRISPRa and CRISPRi Libraries

**CRISPRi/a sublibraries**

| | | | Dilute to 50 ng/ul | | |
|---|---|---|---|---|---|
| | | sgRNA # | [ul] | MegaX [ul] | Large plate # |
| TssLib_Sub1 | Apoptosis+Cancer+Other_Cancer | 37354 | 3 | 36 | 1 |
| TssLib_Sub2 | Drug_Targets+Kinase_Phosphatase | 28340 | 2 | 24 | 1 |
| TssLib_Sub3 | Gene_Expression | 28430 | 2 | 24 | 1 |
| TssLib_Sub4 | Membrane_Proteins | 13417 | 1 | 12 | 1 |
| TssLib_Sub5 | Stress_Proteostasis | 34556 | 3 | 36 | 1 |
| TssLib_Sub6 | Trafficking+Mitochondria+Motility | 26621 | 2 | 24 | 1 |
| TssLib_Sub7 | Unassigned | 24000 | 2 | 24 | 1 |


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## Guidelines

Reference: Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation Giltert LA et al. Cell. 2014 Oct 23; 159(3):647-61.doi:10.1016/j.cell2014.09.029.PMID:25307932
http://www.cell.com/cell/abstract/S0092-8674(14)01178-7

## Protocol

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

**Step 2.**
Electroporate the library

**Step 3.**
Pre-chill 0.1 cm cuvettes, megaX cells, 10% glycerol on ice

**Step 4.**

**CRISPRi/a sublibraries**

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**Step 5.**
Follow the table for the amounts of sub-library plasmid DNA and MegaX competent cells, mix gently and incubate on ice for 30 min

REAGENTS
MegaX DH10B C6400-03 by Thermo Scientific

DURATION
00:30:00

**Step 6.**
Add pre-chilled 10% glycerol to the MageX-library mix for a final 75 ul, transfer the mix to a prechilled 0.1 cm cuvette

**Step 7.**
Electroporate at 2.0 kV, 200 ohms, 25 uF (Gene Pulser Xcell, Bio-rad)

**Step 8.**
Transfer cells to a culture tube

**Step 9.**
Use 1 ml pipettes and gel loading tips

**Step 10.**
Wash cells out gently with 300 ul S.O.C. twice (total 600 ul)

AMOUNT
300 µl Additional info:

PROTOCOL

**. SOC Media**

CONTACT: New England Biolabs
**Step 10.1.**
SOB Media

PROTOCOL

**. SOB Media**

CONTACT: New England Biolabs
**Step 1.1.**
2% tryptone

**Step 1.2.**

0.5% yeast extract

**Step 1.3.**
10 mM NaCl

**Step 1.4.**
2.5 mM KCl

**Step 1.5.**
10 mM MgCl2

**Step 1.6.**
10 mM MgSO4

**Step 10.2.**
20 mM glucose

**Step 11.**
Incubate at 37°C, 250 rpm, 1.5 hour

DURATION
01:30:00

**Step 12.**
Plate the transformations

**Step 13.**
Plate all in one large square plate per sub-library, use autoclaved beads

**Step 14.**
Incubate at 37°C for 18 hours

DURATION
18:00:00

**Step 15.**
Collect all colonies with LB and do one maxiprep per plate, elute in 500 ul EB; an ideal concentration is about 23 ug/ul

## Sequencing

**Step 16.**
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

## Sequencing

**Step 17.**

| | index primer |
|---|---|
| CRISPRi | oCRISPRi TSS_1 |
| CRISPRa | oCRISPRi TSS_2 |

| | x3<br><br>uL |
|---|---|
| Library (400ng/ul) | 1 |
| 5x Q5 Buffer | 20 |
| 5x Q5 GC Buffer | 20 |
| dNTPs (10mM) | 2 |
| Index Primer (10uM) | 2.5 |
| Common Primer (10uM) | 2.5 |
| Q5 Hoststart polymerase | 1 |
| H2O | 51 |

PCR
1, 98C 30s
2, 98C 15s
3, 58C 15s
4, 72C 15s
5, 2-4x23 cycles
6, 72C 10 m
7, 12C hold

## Sequencing

**Step 18.**
After PCR, combine the 3 tubes and proceed to purify the PCR product (size 270 bp)

## Sequencing

**Step 19.**
Sequencing primer, oCRISPRi TSS_seq V2, gtgtgttttgagactataaGtatcccttggagaaCCAcctTGTTGG