Instructions for use of Glycogene CRISPR Library

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A. Amplification of Glycogene CRISPR Library

One day before the library amplification

1. Prepare 250 ml LB agar medium with 100 μg/ml Carbenicillin and distribute into one large square dish (245 mm Square Dish, Nunc 240845, 150-200 ml volume) and four, 100 mm circular Petri dishes (~15 ml each).
2. Also prepare 250 ml of LB liquid medium.

On the day of library amplification

3. Prewarm NEB-10-beta Outgrowth medium at 37 ℃. Also, prewarm the LB agar plates at 37 ℃.
4. Place 1-2ul of pooled library DNA (15ng total) in a microcentrifuge tube on ice. (Optional but included in the steps below: if you want to check the electroporation efficiency of the electrocompetent cells, also place 1 μl of 10ng/μl pUC19 plasmid control from the manufacture, in another tube on ice.)
5. Place two electroporation cuvettes (1 mm gap, BioRad #165-2083) on ice.
6. Thaw a vial of NEB-10 Beta electrocompetent cells (NEB C3020K, or an equivalent strain from another vendor) on ice and mix thoroughly by flicking gently.
7. Add 25 μl of the cells to the tube containing library DNA and mix by gently pipetting up and down 2-3 times.
8. Transfer the mixture to the bottom of pre-chilled cuvette without introducing any bubbles and tap gently on the bench to spread the liquid on the bottom. Carefully wipe the outside of cuvette to remove any water or ice.
9. Electroporate following manufacturer's instructions for *E. coli* cells. Typically, time constant should be between 4.8 and 5.1 milliseconds.
10. IMMEDIATELY, add 975 μl prewarmed outgrowth medium to the cuvette, gently mix by pipetting up and down, and transfer to a 15 ml round bottom tube.
11. Repeat steps 7-10 for the pUC19 control plasmid.
12. Shake vigorously at 250 rpm at 37 ℃ for 1 hour.

Plating to check the transformation efficiency

13. Serially dilute the library transformed cells as follows: first dilute 10 ul cells with 990 ul LB medium (100X) dilution. Mix 20 ul of the diluted cells with 980 ul of LB medium to get a 5000X final dilution. Plate 50 μl and 100 μl into two round Petri dishes containing LB agar medium with Carbenicillin. Incubate overnight at 37 ℃.
14. Next day, calculate the colony forming units (cfu) by the following formula: (#colonies x dilution factor x total volume of cells)/plated volume. Divide the cfu by the number of

guides (3974 in this library) to get the approximate fold representation of each guide in the amplified library.

15. Optional from above: Dilute 10 μl of pUC19 transformed cells in 990 μl LB medium and mix well, plate 50 μl and 100 μl into two round Petri dishes containing LB agar medium with Carbenicillin. Incubate at 37 °C overnight and count the colonies next day. Typically, 50 μl plating volume should give about 25-50 colonies to indicate good electroporation efficiency of the competent cells.

Plating and culture of transformed cells for library amplification

16. Uniformly spread the remaining 990 μl library transformed cells on the large LB agar + Carbenicillin plate and incubate overnight at 37 °C.
17. Next day, you should observe a lawn of bacterial colonies on the plate. Add 10 ml LB + Carbenicillin medium on the agar surface and using a cell scraper, gently scrape the colonies off into the liquid. Repeat 2 more times and retrieve all the bacterial growth into a 30 ml pool in a 500 ml or 1 liter bacterial culture flask.
18. Increase the volume to 200 ml with LB + Carbenicillin medium and grow overnight at 250 rpm, 37 °C. The volume of growth medium can be increased if a bigger library yield is desired.
19. Next day, pellet the bacteria and purify the plasmid using a plasmid midi/maxi prep kit of your choice and following manufacturer's instructions.

B. PCR and NGS of Glycogene CRISPR Library for checking guide representation

PCR primers:

U6 TetO NGSlib Fwd	GACGCTCTTCCGATCTTTATATATCCCTATCAGTGATAGAGACACCG
NGSlib Rev	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTTAGTTTGTATGTCTGTTGCTATTATGTCTACTATTCTTTCC
P5 NGS Fwd	AATGATACGGCGACCACCGAGATCTGACGCTCTTCCGATCT
P7 Index NXXX Rev	CAAGCAGAAGACGGCATACGAGATNNNNNNNNGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

PCR 1:
Dilute the library template to 50 ng/μl concentration and set up the following PCR1 reaction.

Nuclease free water	14 μl
Template	1 μl (50 ng)
10 μM U6 TetO NGSlib Fwd	5 μl
10 μM NGSlib Rev	5 μl
NEBNext Ultra II	25 μl

PCR 1 parameters:

Initial Denaturation	98 °C, 30 sec
10 cycles of:	
|Denaturation	98 °C, 10 sec|
|Annealing/Extension	67 °C, 45 sec|
Final Extension	65 °C, 5 min
Hold	4 °C

Check 1 μl of the PCR product on a 2% agarose gel to confirm presence of 307 bp PCR1 product.
Column purify the remaining PCR reaction using Nucleospin Gel and PCR Clean-up kit (Macherey-Nagel 740-609).
Elute the product in 15 μl elution buffer and use for PCR 2 below.

PCR 2:

PCR 1 Template (from above)	15 μl
10 μM P5 NGS Fwd	5 μl
10 μM P7 Index NXXX Rev	5 μl
NEBNext Ultra II	25 μl

PCR 2 parameters:

Initial Denaturation	98 °C, 30 sec
5 cycles of:	
|Denaturation	98 °C, 10 sec|
|Annealing/Extension	65 °C, 45 sec|
Final Extension	65 °C, 5 min
Hold	4 °C

Check 1 μl of the PCR product on a 2% agarose gel to confirm presence of a 364 bp PCR2 product.
Load the remaining PCR reaction on a 1.8% agarose gel. Excise the 364 bp DNA band and purify using Nucleospin Gel and PCR Clean-up kit (Macherey-Nagel 740609.50).
Elute the product in 15 μl elution buffer.
This is the NGS sample for the Glycogene CRISPR library.
The primer U6 TetO NGSlib Fwd can be used as Read 1 primer for a 20 cycle NGS read to evaluate guide representation in the amplified library.