**PCR and NGS of Glycogene CRISPR Library for checking guide representation**

PCR primers:

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| --- | --- |
| 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.