**Genome-scale CRISPR-Cas9 knockout screening**

**Library Description**

The genome-scale library targets more than 18, 000 genes with 185634 guide RNAs. The library is divided into 2 parts (h1 and h2). The h1 and h2 libraries contain in total 10 sgRNAs per gene (5 sgRNAs in each library). The library is in 1 vector (lentiCRISPRv2) format.

**Library Amplification**

Follow these instructions for each library (h1 and h2). Amplify and prepare half-libraries separately as follows:

1. Dilute the library to 50 ng/µL in water or TE (if not already diluted).
2. Test transformation efficiencies of the bacteria by adding 0.5 µL and 1µL of each gRNA library into 25µL highly competent bacteria (XL-10 gold is recommended for chemical transformation; Lucigen Endura cells are recommended for transformation by electroporation). After the recovery period of the transformation, perform dilution plating to determine transformation efficiency using 0.01% and 0.001% of the total reaction.
	1. To ensure no loss of representation, 20 parallel transformations were performed and plate onto 10 plates of 245 mm\* 245mm.
	2. The transformation efficiency must be at least 50-fold greater than the pool size to ensure adequate library representation. If this efficiency is not achieved, discard the bacterial culture and re-transform.
	3. Harvest bacteria prior to reaching stationary phase (after 12-16 hours of growth)
3. Extract plasmid DNA from pelleted bacteria using by GenEluteTM HP Endotoxin-Free Plasmid Maxiprep Kit (Sigma, Catalog #NA0410-1KT). It is critical that transfection-grade DNA is obtained
4. Identify the quality of amplified libraries by sequencing.
First round PCR:
Primer\_R: TCTACTATTCTTTCCCCTGCACTGTACCTGTGGGCGATGTGCGCTCTG
Primer\_F: AATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCG
	1. Using 50 ng plasmids as template. Perform a 50-µL reaction using Q5® High-Fidelity DNA Polymerase (Biolabs®) and then combined the resulting amplicons. Recommended PCR begin with 8 cycles. Run 2% E-Gel (Invitrogen) or do qPCR to decide appropriate number of PCR cycles, where the yield of amplicons are about to saturate. If amplicons are still in an upswing, add 1-2 more cycles. But the number of cycles should not exceed 16.
	2. Perform a second PCR to attach Illumina adaptors and to barcode samples. Perform the second PCR in a 100 µL reaction volume using 2 µL of the product from the first PCR. The thermocycling Conditions is the same as first round PCR.

	Primers for the second PCR:

Cri\_library\_F: AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTTGTGGAAAGGACGAAACACCG

Cri\_library\_index1:

CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCACGTCTACTATTCTTTCCCCTGCACTGTACC

Cri\_library\_index2:

CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGATGTTCTACTATTCTTTCCCCTGCACTGTACC

Followed by Gel purification of the amplicons using Qiagen gel purification kit.

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| **Component** | **100 µL Reaction** |
| 5X Q5 Reaction Buffer | 20 µL |
| 10 mM dNTPs | 2 µL |
| 10 µM Forward Primer | 2 µL |
| 10 µM Reverse Primer | 2 µL |
| Template DNA | variable |
| Q5 High-Fidelity DNA Polymerase | 1 µL |
| Nuclease-Free Water | to 100 µL |

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| **STEP** | **TEMP** | **TIME** |
| Initial Denaturation | 98°C | 30 seconds |
| 8–10 Cycles | 98°C | 10 seconds |
| 68°C | 20 seconds |
| 72°C | 25 seconds |
| Final Extension | 72°C | 2 minutes |
| Hold | 4°C |  |