**Amplification and screening**

1. Transform the plasmid library into Trans1-T1 competent cells (TransGen CD501) or other chemically competent cells/electrocompetent cells with a minimum coverage of 200×.

2. Add the recovered bacteria into pre-warmed LB+amp (ampicillin, 50 μg/ml) medium, and incubated at 37 °C overnight with shaking.

3. Mix the bacterial culture thoroughly and extract the library plasmids using EndoFree Plasmid Maxi Kit (QIAGEN 12362).

4. Generate the lentivirus of the sgRNA library by co-transfection of the library plasmids with two viral packaging plasmids pR8.74 and pVSVG (Addgene, Inc.) as a proportion of 10:10:1 into HEK293T cells using the X-tremeGENE HP DNA transfection reagent (Roche).

5. Infect the library cells with the sgRNA library viruses at a low MOI of no more than 0.3 (at least 400× coverage) and perform the following screening.

**PCR for NGS**

The primers used to amplify the sgRNA-coding regions for deep sequencing are as following: Primer-F (5’- TATCTTGTGGAAAGGACGAAACACC -3’); Primer-R (5’- AATACGGTTATCCACGCGGC -3’).

The sgRNA-coding regions can be PCR-amplified using the primers above by TransTaq HiFi DNA Polymerase (TransGen AP131-13), and further purified with DNA Clean & Concentrator-25 (Zymo Research Corporation D4034). The purified PCR product will be prepared for deep sequencing verification using NEBNext Ultra DNA Library Prep Kit for Illumina (NEB E7370L).