

## Use Instruction - Lin Human miRNA CRISPR Knockout Library

### Product Info

The CRISPR knockout library targets 1,594 miRNA across the human genome and contains a total of 8,382 knockout plasmid vectors, of which 5 different gRNA vectors are designed for miRNA, in addition to 1,000 control vectors targeting intergenic sequences. The library uses YCS-LV001 as the backbone, which is a single-plasmid system that expresses both gRNA and Cas9 gene and can be used directly.

### Library Details

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Product Name    | Lin Human miRNA CRISPR Knockout Library                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Product Catalog | LIBR-H055-P                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Product Details | <p>8382gRNAs (gRNA sequences see attachment);</p> <p>Single-plasmid system, can be directly used for library screening without first constructing Cas9 stable cell lines;</p> <p>Puro resistance, puromycin can be used for antibiotic screening upon cell infection;</p> <p>Plasmids paired with 3rd lentivirus packaging system can be directly used for virus packaging.</p> <p>* It is recommended to use Ubigene's Lentiviral Packaging Kit(Cat# YK-LVP-05)</p> <p>Targeting 1594 genes, 5gRNAs per gene;</p> <p>1000 non-target control sgRNAs (1000 targeting non-genic sequences) .</p> |

|                        |                                                                                                                                                                                                                                                                                         |
|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Backbone Map           |                                                                                                                                                                                                                                                                                         |
| Verification Primers   | <p>YCS-LV001-F: ATTTCTTGGGTAGTTTGCAGTTT</p> <p>YCS-LV001-R: GACTCGGTGCCACTTTTCA</p> <p>PCR Fragment: 213 bp</p> <p>The above primers can be used for PCR fragment amplification before library NGS sequencing. The amplified fragments can be purified and used for NGS sequencing.</p> |
| Product Specifications | <p>Ready-to-use, endotoxin-free, maxiprep plasmids, verified by Next Generation Sequencing, with coverage&gt;99% and uniformity&lt;10.</p>                                                                                                                                              |

## Product Use Instruction

### Part 1. Lentivirus Packaging

Mix library plasmid constructs with 3rd generation lentiviral packaging constructs to be co-transfected into 293T cells (Recommend: Ubigene's 293T cell line specialized for virus packaging, cat#YC-A006). 48 or 72 hours upon transfection, collect lentiviral supernatant and the virus can be used upon concentration. The virus should be stored at -80°C.

### Part 2. Library Plasmid Amplification

#### 1. Library plasmid electroporation

Add 50 ng library plasmid to 25 μL electrocompetent cells with transformation efficiency  $\geq 10^9$  cfu/μg, electroporate cells as per electroporation parameters. Upon electroporation, add 975 μL recovery medium, mix well and transfer to a tube, then add 1 ml recovery medium to the tube and mix well again. Repeat above steps for 3 times and get 4 electroporation end products, share the tubes at 37°C, 250 rpm for 1 hour.

#### 2. Culture of amplified library and calculation of transformation efficiency

1) Mix the 4 tubes of electroporation end products and take 10 μL and dilute with 990 μL

recovery medium. Plate 20  $\mu$ L dilution onto a 10 cm Petri dish and incubate plates at 32°C for 14 hours. Count the colonies in the dish. If the number of colonies is 40000X greater than  $2.51 \times 10^6$ , move on to the next step. If it is less than  $2.51 \times 10^6$ , redo this step.

**\* Note: It is recommended that the number of colonies should be 40000X greater than  $4.19 \times 10^6$  to ensure the uniformity of Library gRNA.**

2) For the remaining electroporation end products, plate 400  $\mu$ L/dish (20 dishes can be plated in total), then incubate at 37°C for overnight.

3. Collect transformation products

1) Collect the bacteria to a 50 mL centrifuge tube.

2) Centrifuge tubes to pellet bacteria, decant LB and weigh pellet (bacteria).

4. Maxiprep

Maxiprep the plasmid DNA according to the instruction for maxiprep kit, it is recommended to use endofree maxiprep kit from well-known, commercial brands such as QIAGEN and MACHEREY-NAGEL. (E.g. EndoFree Plasmid Mega Kit from QIAGEN)

### Part 3. Library Screen

1. Determine infect MOI

Dilute the library virus into different gradients, such as MOI=0.3, 0.5, 1, 5, 10, 30, 100 to infect the target cells (the cell confluency is 30-50%). Each gradient needs to be set with 2 wells. After 48 hours of infection, add puromycin according to the settings in the table below for screening, and stop antibiotic screening when all cells in the blank group (cells not infected with virus) die. The MOI with a survival rate of 30% after antibiotic screening is the virus infection condition for the library screening experiment, that is, infect MOI.

| Group#               | MOI | Antibiotic screening | Cell amount upon antibiotic screening | Survival rate upon antibiotic screening |
|----------------------|-----|----------------------|---------------------------------------|-----------------------------------------|
| Experimental group 1 | 0.3 | Yes                  | N1                                    | N1/M1                                   |
| Experimental group 1 | 0.5 | Yes                  | N2                                    | N2/M2                                   |
| Experimental group 3 | 1   | Yes                  | N3                                    | N3/M3                                   |

|                         |     |     |    |       |
|-------------------------|-----|-----|----|-------|
| Experimental group 4    | 5   | Yes | N4 | N4/M4 |
| Experimental group 5    | 10  | Yes | N5 | N5/M5 |
| Experimental group 6    | 30  | Yes | N6 | N6/M6 |
| Experimental group 7    | 100 | Yes | N7 | N7/M7 |
| Infection blank group 1 | 0.3 | No  | M1 | --    |
| Infection blank group 2 | 0.5 | No  | M2 | --    |
| Infection blank group 3 | 1   | No  | M3 | --    |
| Infection blank group 4 | 5   | No  | M4 | --    |
| Infection blank group 5 | 10  | No  | M5 | --    |
| Infection blank group 6 | 30  | No  | M6 | --    |
| Infection blank group 7 | 100 | No  | M7 | --    |
| Blank group             | 0   | Yes | -- | --    |

## 2. Transduction with library virus

① Determine the amount of cells and virus

Cell amount = gRNA# × gRNA coverage / 30% \* gRNA coverage > 500 fold

Virus amount = cell amount × infect MOI

②Expand the cells according to the cell amount calculated in step ①, and prepare sufficient virus.

③Use library virus to infect the target cells, upon Puro screening, divide the screened cells into experimental group and control group. Add target drugs to the experimental group for screening, upon screening, collect cells respectively from experimental group and control group (It is recommended to get at least  $4.19 \times 10^6$  cells from control group; get all the cells from experimental group and the cell amount before cryopreservation should be greater than  $1 \times 10^6$ ). Perform genome extraction for Next Generation Sequencing, and then compare and analyze the gRNAs of the experimental group and the control group.

## Relevant products and service

Ubigen provides off-shelf libraries including Human/Mouse genome-wide plasmid library and some sub-libraries, and one-stop customized screening services for CRISPR-KO, CRISPRa, and CRISPRi including high-throughput sgRNA library construction, virus packaging, cell infection, drug screening, NGS sequencing, and data analysis, etc. Multiple deliverables fulfill different research needs!