# Library Cell Pool Use Instruction

## hGeCKO Library B#1 in HeLa

## Product Info

Ubigene's CRISPR Library Cell Pool is conducted by utilizing CRISPR iScreen™. Ubigene's CRISPR Library Cell Pool is standardized constructed in batches by firstly obtaining Library Plasmid with high coverage and good uniformity using self-developed high-efficiency competent cells, then packaging the virus using Lentiviral Packaging Kit (#YK-LVP-20) to obtain high-titer CRISPR Library Virus, finally through the exclusive cell pool preparation process, infecting the target cell line with low infect MOI to restrict one virus per cell. Ubigene's CRISPR Library Cell Pools have small batch differences and good reproducibility. Screening on Cell Pools under different pressure conditions can screen target genes suitable for research in different fields.

| Carrier Information | |
|---|---|
| Backbone Map |  |
| Verification Primers | LentiCRISPRv2-F: ATTTCTTGGGTAGTTTGCAGTTT<br>LentiCRISPRv2-R: GACTCGGTGCCACTTTTTCA<br>PCR Fragment: 213 bp<br>he above primers can be used for PCR fragment amplification before library NGS sequencing. The amplified fragments can be purified and used for NGS sequencing. |

| Cell Info | | | |
|---|---|---|---|
| Product Name | hGeCKO Library B#1 in HeLa | | |
| Product Catalog | LIBR-H001B-C300D3 | | |
| Library Type | Human CRISPR Knockout Pooled Library B(1 vector system) | | |
| Morphology | Epithelial-like, adherent | Passage ratio | 1：2-1：4 |
| Antibiotic concentration | Puromycin 1.5μg/mL | Coverage | 300X |
| Culture medium | 90%DMEM+10% FBS<br>Ublist_contentigene didn't use P/S. But client could use P/S after cells grow in good condition after thawing. | | |
| Cryopreservation solution | 50%DMEM+40% FBS+10%DMSO | | |
| Special note | | | |

## Cell Reception

EZ-editor™ CRISPR library cells will be transported with dry ice, upon received, immediately transfer to liquid nitrogen for storage or store briefly (24h) at -80°C freezer, or proceed directly to cell thawing.

Notice: Upon received, please ensure to takes photos of the package, including dry ice and the tubes, and contact us within 24 hrs if any abnormalities such as dry ice has ran out, the cap of the cryovial is dislodged, broken and the cell is contaminated.

## Cell Thawing

1) Preparation: warm up the complete culture medium in 37°C water bath for 30 mins. Transfer the cryopreserved vial from liquid nitrogen to - 80°C freezer, and leave for several minutes to volatilize residual liquid nitrogen; Thawed cell amount are shown below (Table 1).

**Table1 Thawed cell amount**

| gRNA# | Thawed cell amount |
|---|---|
| ≤10,000 | 3.00E+06~5.00E+06 |
| ≤20,000 | 6.00E+06~1.00E+07 |
| ≤30,000 | 9.00E+06~1.50E+07 |
| ≤60,000 | 1.80E+07~3.00E+07 |
| ≤130,000 | 3.90E+07~6.50E+07 |

2) Inside the ultra-clean bench, pipet 6-7 mL of complete medium into a 15 mL centrifuge tube; every 2 vials of the cells use a 15 mL centrifuge tube.

3) Take out the cryopreserved vial from -80℃ freezer and leave in dry ice temporarily, shake slightly before thawing to remove residual dry ice and liquid nitrogen. Then hold the cap with forceps, quickly thaw cells in a 37°C water bath by gently swirling the vial (Note: keep the cap out of the water). In about 1 minute, it would completely thaw;

4) Inside the ultra-clean bench, sterilize the outer surface of the vial by wiping with an alcohol cotton pellet and leave it to dry. Transfer the thawed cells to the prepared centrifuge tube (step 2) by pipette, close the lid, and centrifuge at 1100 rpm for 4 mins at

room temp to collect the cells; Inside the ultra-clean bench, carefully remove and discard the supernatant. Resuspend cell pellet with 3-5mL of fresh complete medium.

5) Mix the cells (15 ml centrifuge tubes) and then transfer to a T75 flask (Prepare the number of T75 flasks based on the total number of cells), label the flask with cell name, date and passage no., incubate the flask in a 37℃, 5%$CO_2$ incubator.

## Cell Passaging

1) As long as the cells are 80%-90% confluent, it is ready to passage. Inside the ultra-clean bench, remove and discard the medium from the flask and briefly rinse the cell 1-2 times with 1×PBS (8-10 mL for T75 flask) to remove residual medium and serum;

2) Add the corresponding volume of trypsin solution (see below table 2 for details) and allow trypsin completely cover the cells, place the flask into the incubator and incubate for 1-2 mins (If cells are hard to digest, allow appropriate extension of incubation), until the majority of the cells become round and non-adherent as observed under the microscope, a large number of cells detached from each side when gently shaking and tapping the flask, terminate trypsin digestion immediately;

3) Add complete medium to stop digestion, the volume is 2 times of trypsin. Then gently pipet the cells several times to allow all cells to be completely detached from the flask;

4) Transfer the cell suspension with a 25 mL pipette into a 50 mL centrifuge tube, rinse the residual cells from the flask using appropriate volume of PBS , then collect and put them together to the centrifuge tube;

5) Centrifuge at 1100 rpm for 4 mins at room temp. After centrifugation, remove and discard the supernatant and resuspend the cells with appropriate amount of complete medium;

6) Cells need to be passaged at appropriate passage ratio, 1:3 for the first passage, increasing the passaging ratio if the cells are grown to confluence within two days, or decreasing the passaging ratio if the cells are not grown to confluence in 3-4 days.

**Table 2. Volume of Trypsin Consumption**

| Size of culture plates/flasks | Trypsin Volume added |
|---|---|
| 6-well plate | 0.2ml |
| T25 | 0.3ml-0.5ml |
| T75 | 1ml-1.5ml |
| T175 | 2ml-2.5ml |

Note: Half of the antibiotic concentration (listed above in *Cell Info*) needs to be added during the passage.

## Cell cryopreservation

1) Same as procedures of cell passaging, inside the ultra-clean bench, digest the cells to a single-cell suspension, and terminate digestion by adding complete medium. All liquid is transferred to a 50 mL centrifuge tube;
2) Same as procedures of cell passaging, inside the ultra-clean bench, digest the cells to a single-cell suspension, and terminate digestion by adding complete medium. All liquid is transferred to a 50 mL centrifuge tube;
3) Centrifuge at 1100 rpm for 4 mins at room temp. After centrifugation, remove and discard the supernatant, and resuspend the cells with 4℃ pre-cooled cryopreservation medium (use the one you usually use in lab, or any commercial cryopreservation solutions are fine), then add cryopreservation medium to adjust to the required density ($5x10^6$-$1x10^7$cells/mL);
4) Aliquot the cell suspension to cryovials as 1 mL/tube, close the lid tightly, and the cryovials should be labeled with the cell name, source, cell passage number, and date of cryopreservation in advance;
5) Place the cryovials in 4°C pre-cooled Freezing Container, then put the container in -80℃ freezers within 15 mins after cell cryopreservation;

6) Stay overnight, transfer the cryovials to liquid nitrogen for long-term storage.

Note: The cryopreserved cell amount of the CRISPR library cell pools is different from that of common cells, and the amount of cryopreserved cells should refer Table 1.

## Drug screening with library cell pool

1) Determine the concentration and duration of drug screening. The screening concentration can be selected according to the commonly used concentration in the literature, or determined according to the IC50, IC80 and other values of the drug acting on the cell; The recommended screening duration is 2-4 weeks.

2) According to the number of experimental group and control group set in the screening experiment (recorded as N), the required cell amount (cell amount =gRNA number *500*N) is calculated.

Note: 500 refers to the 500X fold of library cell coverage, it is recommended the coverage > 500X for each group of cells during drug screening.

3) The library cells should be expanded to the required amount of cells, and the cells should be equally divided into several parts according to the set number of experimental groups.

Note: To ensure the coverage and uniformity of gRNA in the library cells during the drug screening period, the cells should not be passaged too many times, and it is recommended to control it within 5 passages.

4) According to the determined drug screening concentration and screening duration, the library cells of the experimental group is screened, and the cells of the control group should be simultaneously cultured.

5) After drug screening, all cells in the experimental group and cells in the control group with a coverage of no less than 500X are collected.

6) The cell genomes are extracted for downstream NGS library sequencing.

## Relevant products and service

Ubigene 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!