Library Cell Pool Use Instruction

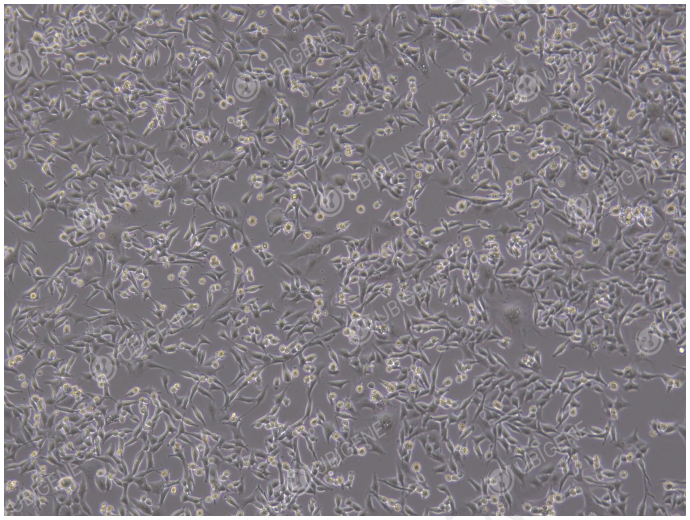
mGeCKO Library AB#1 in MC38

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.

Cell Info			
Product Name	mGeCKO Library AB#1 in MC38		
Product Catalog	LIBR-M003AB-C		
Library Type	Mouse CRISPR Knockout Pooled Library A+B(1 vector system)		
Morphology	Epithelial-like, Mixed: adherent and suspensio	Passage ratio	1:2-1:4
Antibiotic concentration	Puromycin 1.5μg/mL	Coverage	300x
Culture medium	90% DMEM+10%FBS 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 Image



Cell Reception

EZ-editor^{TM} 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 T175 flask (Prepare the number of T175 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%CO2 incubator.

Cell Passaging

The cells can be passaged when they have grown to the required density. The passaging of

suspension cells can be divided into the following two cases:

Half-exchange: Use half-exchange passaging when the cells are in good condition, with less cell debris and no yellowing of the medium;

1. Blow the cells in the culture flask evenly in the ultra-clean table and take 20μL of cells for cell counting;

2. According to the counting results, aspirate part of the cell suspension, adjust the cell density to $2x10^5$~$4.0x10^5$ cells/mL within the culture, depending on the cell culture density of the cell culture in different specifications of the culture flask.

Full exchange: Use full exchange when the cells are in good condition, there are more cell fragments and the medium turns yellow;

1. Transfer the culture solution to a 15mL centrifuge tube or 50mL centrifuge tube and centrifuge at 1100rpm for 4 minutes in an ultra-clean bench;

2. Discard the supernatant, aspirate 1mL of complete medium to resuspend the cell precipitate with a single channel pipette, and 20μL of cell suspension for cell counting;

3. According to the results of cell counting, adjust the cell density to $2x10^5$~$4.0x10^5$ cells/mL within the culture, depending on the volume of cell culture in the cell culture in different sizes of culture flasks. Place in 5% CO2, 37 ℃ cell culture incubator culture can be.

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) Transfer cells from culture flasks to 50 mL centrifuge tubes in an ultra-clean bench as per cell passaging, and centrifuge at 1100 rpm for 4 minutes at room temperature;

2) After centrifugation, open the lid and pour off the supernatant, resuspend the cells with 1~2 mL of 4°C pre-cooled cryopreservative, blow the mixture with a pipette to mix it well, and take 20 μL for cell counting, followed by the addition of the cryopreservative to adjust to the density of about $5x10^6$ cells/mL.

3) Dispense the cell suspension into cryopreservation tubes at an average of 1 mL/tube, screw the caps tightly, and the cryopreservation tubes should be labeled with the cell name, source, cell generation, quantity, and date of cryopreservation in advance;

4) Place the cryopreservation tubes in a programmed cooling box pre-cooled at 4°C and place the programmed cooling box in an ultra-low temperature refrigerator within 15 minutes of the end of freezing;

5) After overnight, transfer the frozen cells to a liquid nitrogen tank for 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

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.

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.

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.

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.

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.

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!