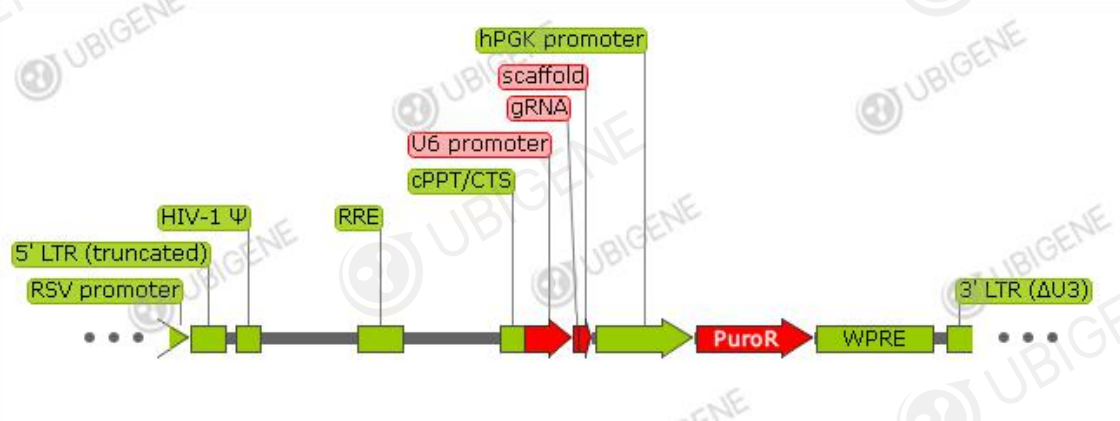
Use Instruction - CRISPR Knockout Library Virus

Product Info

Ubigene's CRISPR Library Virus is conducted by utilizing CRISPR iScreen™. Ubigene's Library Virus with high titer is obtained by firstly obtaining Library Plasmid with high coverage and good uniformity, then packaging the virus using Lentiviral Packaging Kit (#YK-LVP-20), collecting supernatant and eventually concentrating. Ubigene's CRISPR Library Virus can be directly used for the construction of CRISPR library stable cell pool, directly omitting the tedious and complex library plasmid amplification process.

Product Details

Product Name	Human UBDUB CRISPR Knockout Library
Product Catalog	LIBR-H024-LV
Product Details	9274gRNAs (gRNA sequences see attachment); Dual-plasmid system; Puro resistance, puromycin can be used for antibiotic screening upon cell infection; Plasmids paired with 3rd lentivirus packaging system can be directly used for virus packaging. * It is recommended to use Ubigene's Lentiviral Packaging Kit(Cat# YK-LVP-05). Targeting 1500 genes,6 gRNAs per gene; 1000 non-target control sgRNAs（1000 targeting non-genic sequences）

Backbone Map	
Verification Primers	YKO-LV004-F：ATTTCTTGGGTAGTTTGCAGTTT YKO-LV004-R：GACTCGGTGCCACTTTTTCA PCR Fragment：212 bp 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.
Titer	≥1.00E+08TU/ml
Storage Condition	-80℃

Product Reception

1) Ubigene's CRISPR knockout library lentivirus product is transported with dry ice. Upon receiving, lentivirus should be stored at -80℃ and avoid repeated thawing and freezing.
2) Lentivirus can be stable for at least 6 months (stored at -80℃). If the storage time exceeds 6 months, it is recommended to test the virus titer again before use.
3) The infection reagent Polybrene is delivered with the lentivirus and should be stored at -20℃.

CRISPR Knockout Library Lentivirus Use Instruction

1. Definition:

MOI: MOI (Multiple Of Infection) is defined as as the number of infectious viral particles per cell. For different kinds of cells from different sources, the optimal MOI varies. Generally, MOI that can achieve 80% infection efficiency and will not negatively affect the cell condition would be the best MOI. For susceptible cells, the MOI is 1~10. For cells that are more difficult to be infected, MOI of 20 or higher may be required.

Infect MOI: It is defined as the ratio of the number of virus and cells when 30% of cells are infected with virus. This value will be affected by the virus batch, so it is recommended to use this batch of virus for preliminary experiment before library virus infection, and then conduct formal experiment. If using another batch of virus, it is necessary to repeat the preliminary experiment to explore the new infect MOI.

Polybrene: It is an infection reagent with a common concentration of 5~8 μg/ml. Polybrene can enhance the combination of lentivirus and cell membrane by neutralizing the interaction between charges, to improve the transduction efficiency of virus. However, Polybrene is toxic to some cells, and different cells have different sensitivity to Polybrene. If necessary, several working concentrations can be set to test the toxicity of Polybrene to target cells. The concentration of Polybrene provided by Ubigene is 0.5 mg/ml. If necessary, it can be diluted with PBS or culture medium during use.

Determine infect MOI

Plate the cells to a 12-well plate one day advanced, making the cells well plate one day advanced, making the cells will be 30-50% confluent at the time of transduction.

Digest the cells from 2 wells and count the cells, calculate the average numbers of cells from each well, as N.

Calculate the required virus volume according to the virus titer T (TU/ml) and cell amount N. The calculation formula is: V (μL) =1000×MOI×N/T. For example, if MOI=10, the virus titer is 3× 108 TU/mL, the cell amount is 200,000, and the added virus volume should be 6.7 μL.

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 2	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	——	——

3.Transduction of target cells with library virus (Construction of library cell pool)

①Determine the amount of cells and virus:

Cell amount = gRNA# × gRNA coverage / 30%

* gRNA coverage varies from 100 to 500 fold. Genome-wide library coverage is recommended to be 500 fold.

②**Determine the amount of virus:**

Virus amount = cell amount × infect MOI (Determined from preliminary experiment)

Lentivirus transduction and Antibiotic screening:

1) One day before lentivirus transduction, prepare a sufficient amount of cells with 30-50% confluency.

For example, if infecting 293T with human genome-wide knockout library A, infect MOI=0.3, gRNA coverage of 500X, the required cell amount is (65383×500)/30%=1.09E+08. Given that the cell amount of 293T in each T175 flask with 30% confluency is 5.5E+6, the number of flasks with cells required for transduction is approximately 20. In addition, an additional flask with cells is required for antibiotic screening control.。

2) On the day of transduction, take 1 flask with cells, digest the cells to single-cell suspension and count the cells.

3) According to the cell count, calculate the number of flasks corresponding to the required cell amount for transduction, mark the flasks and add Polybrene to each flask.

4) Take the library lentivirus from the refrigerator, thaw the lentivirus on ice, and mix the virus gently by pipetting. Calculate the required amount of virus according to cell amount and infect MOI, add the library virus into the cells and mix gently, and then put it back to the incubator allowing infection.

5) 24H after virus transduction, remove medium containing virus and Polybrene, and replace with fresh complete culture medium.

6) 48H after virus transduction, add corresponding antibiotics for screening until the cells in control group all die.

Note: In order to obtain better antibiotic screening results, it is suggested to carry out antibiotic screening preliminary experiment, to test WT cells with different concentrations of antibiotics, make a kill curve, and select a antibiotic concentration that can completely kill untransduced cells without affecting successfully transduced cells. The following table lists the antibiotic screening concentration and duration for 4 common antibiotics.

Antibiotic	Puromycin	Blasticidin	Hygromycin B	G418
Common	1~10 μg/mL	5~30 μg/mL	100~500 μg/mL	400~1000 μg/mL
Screening duration	2~3 day	7~10 day	3~5 day	4~7 day

7) After the antibiotic screening is completed, half of the antibiotic concentration needs to be added during the cell expansion. Take one library cell pool for NGS validation to detect gRNA coverage. (The cell amount of one library cell pool = gRNA number × coverage)

8) After getting sufficient cell amount, follow regular procedure to cryopreserve the library cell pool.

Safety instructions for CRISPR library lentivirus use

The lentivirus produced by Ubigene belongs to the third-generation lentiviral packaging system. The 3 'LTR of its genome is mutated to form the "self inactivation" (SIN), which means it will not produce new offspring viruses after the virus genome is integrated into the cell genome. Thus, it is safe to use in vitro experiment. However, the virus still has the ability to infect human primary cells, which has potential biological hazard. Ubigene recommends that you shall wear protective equipment such as experimental clothes, masks and gloves according to the BSL-2 safety protection level, and use the biosafety cabinet for the experiment when operating the virus. The pipette tip, centrifuge tube, culture plate, waste liquid and other items that have been in contact with the virus can be sterilized (virus inactivation) by conventional sterilization procedures (121℃, 20 minutes).

Common problem

1.What is gRNA coverage?

When constructing stable cell pools, gRNA coverage refers to the fold relationship between the total number of cells and the number of gRNAs after library virus transduction and antibiotic screening.

For example, given a library with 65383 gRNAs, if a gRNA coverage of 500X is required, it is necessary to ensure that the number of cells after library virus transduction and antibiotic screening is greater than 3.27E+07. Due to the selection of infection MOI based on the remaining 30% of cells after antibiotic screening, the initial cell count at the time of infection should be 3.27E+07/30%, which is 1.09E+08.

2.How to prepare NGS sequencing samples?

The categories of NGS sequencing samples can be cells, genomes, PCR products, or constructed sequencing libraries.

1) **Cell sample**

Resuspend the cells in PBS, centrifuge and discard the supernatant to obtain cell pellet, and send them in dry ice package. The total number of cells should be greater than or equal to the number of gRNAs multiplied by the coverage.

2) **Genome**

Prepare the cell pellet according to the standards of cell samples, and then extract the genome with the kit. The concentration and total amount of genome are provided according to the requirements of sequencing company.

3) **PCR product**

The PCR product is to amplify the gRNA of the library with gRNA amplification primers (excluding sequencing adapters and sequencing primers) after extracting the genome. It needs to be purified, and then provide the corresponding concentration and total amount of PCR purified products according to the requirements of the sequencing company.

Requirements for PCR products to Ubigene for NGS: total amount > 1.5 μg, concentration > 60 ng/μL, fragment size between 250 and 280.

4) **Sequencing Library**

This option is not recommended, and the self-established library may have low matching with the sequencing platform.

3.gRNA amplification primer sequences

Library products purchased from Ubigene will have corresponding library use instructions, which contain gRNA amplification primer sequences.

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!