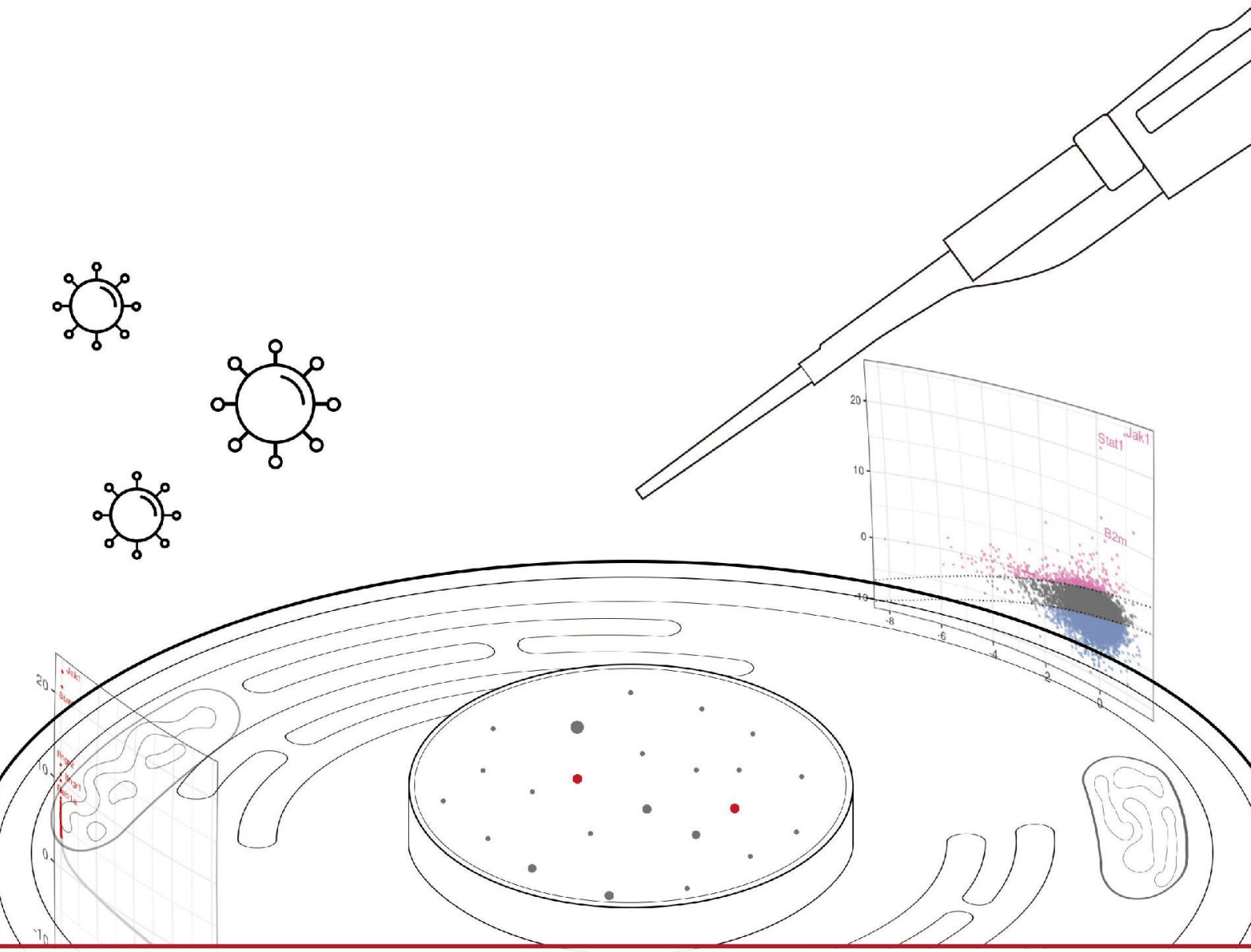
UBIGENE Make genome editing easier

CRISPR Library

Instructions



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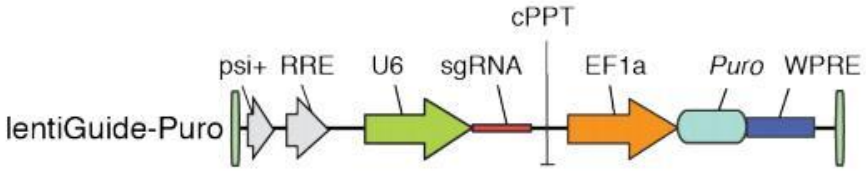
Use Instruction - Human GeCKO v2 genome-wide plasmid library (Dual-plasmid system)

Product Info

This Human GeCKO v2 genome-wide plasmid library (Dual-plasmid system) is applicable for human genome-wide knockout and gene screening, containing over 100,000 knockout vectors targeting all exons of the whole genome. It consists of two half-libraries, library A and library B, each half-library contains 3 gRNAs per gene. So libraries A and B together contain 6 gRNAs per gene. Libraries A and B can be used in combination or alone as per requirement. The vector backbone of the library is LentiGuide-Puro backbone which is the dual-plasmid system, that is, the library construct only expresses the gRNA, while Cas9 will be on a separate plasmid and should be used together.

Library Details

Product Name	Human GeCKO v2 genome-wide plasmid library (Dual-plasmid system)
Product Catalog	YKO-Libr-H002A, YKO-Libr-H002B
Product Details	123,411 gRNAs (gRNA sequences see attachment); Dual-plasmid system, its virus yield would be higher than of single-plasmid system; Puromycin 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)	
	Library A	65,383 gRNAs; Targeting 19,050 genes, 3 gRNAs per gene; Targeting 1,864 miRNAs, 4 gRNAs per miRNA; 1,000 non-target control sgRNAs
	Library B	58,028 gRNAs; Targeting 19,050 genes, 3 gRNAs per gene; 1,000 non-target control sgRNAs
Backbone Map		
Verification Primers	LentiGuide-Puro-F: ATTTCTTGGGTAGTTTGCAGTTT LentiGuide-Puro-R: GACTCGGTGCCACTTTTTCA	
Product Specifications	Ready-to-use, endotoxin-free, maxiprep plasmids, verified by Next Generation Sequencing, with coverage>99% and uniformity<10.	

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℃.

Part 2. Library Plasmid Amplification

1. Library plasmid electroporation

Add 100 ng library plasmid to 25 μL electrocompetent cells with transformation efficiency ≥10^9 cfu/ug, 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 three times and get 4 electroporation end products, share the tubes at 37℃, 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 μL dilution onto a 10 cm Petri dish and incubate plates at 32℃ for 14 hours. Count the colonies in the dish. If the number of colonies is 40000X greater than 6 x 10^6, move on to the next step. If it is less than 6 x 10^6, redo this step.

* Note: It is recommended that the number of colonies should be 40000X greater than 2 x 10^7 to ensure the uniformity of Library gRNA

2) For the remaining electroporation end products, plate 400 μL/dish (20 dishes can be plated in total), then incubate at 32℃ for 14 hours.

3. Collect transformation products

1) Add 500 μL LB medium to each dish, use spreader to scrape the plates and collect the bacteria to a 50 mL centrifuge tube

2) Repeat the steps for all plates

3) 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 QIAGEN, MACHEREY-NAGEL, etc (e.g. EndoFree Plasmid Mega Kit from QIAGEN)

Part 3. Library Screen

1. Construction of Cas9 stable expression cell line

Before using the dual-plasmid system library, it is required to construct a Cas9 stable expression cell line. Ubigene can provide Cas9-expressing viruses with Hygromycin or Blasticidin resistance.

2. Transduction of Cas9 stable expression cell line with library virus

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 (MOI=0.3 in some literatures actually refers to the

amount of virus corresponding to the virus infection of 30% cells).

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	——	——

2) Transduction with library virus

① Determine the amount of cells and virus

$$\text{Cell amount} = \frac{\text{gRNA\#} \times \text{gRNA coverage}}{30\%}$$

* gRNA coverage>500 fold

Virus amount= cell amount × infect MOI

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

③ Use library virus infect the target cells, upon puromycin screening, divide the screened cells into experimental group and control group. Add target drugs to the experimental group for screening, upon screening, collect 5 x 10^7 cells and 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

Ubigene provides 35+ 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!