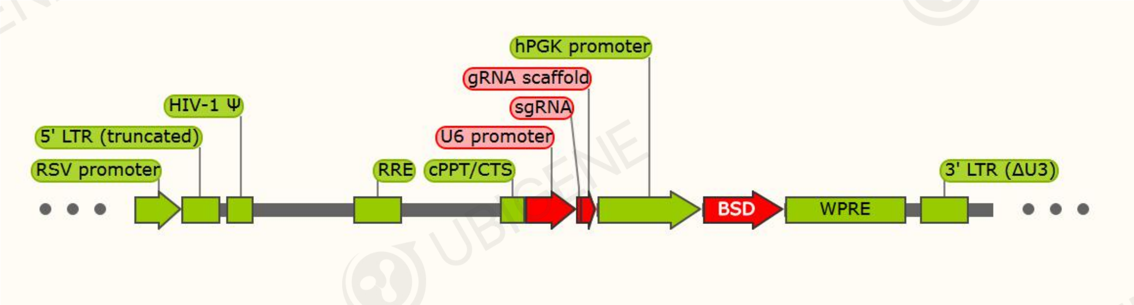
Use Instruction - Mouse CRISPR Knockout Pooled Library A(2 vector system)

Product Info

The CRISPR knockout library targets 20,611 genes across the mouse genome and contains a total of 67,405 knockout plasmid vectors, of which 3 different gRNA vectors are designed for each gene, in addition to 1,000 control vectors targeting intergenic sequences.The library uses YCS-LV006 as the backbone, which is a two-plasmid system that expresses only gRNA, while the Cas9 gene is on a separate vector that needs to be used in conjunction.

Library Details

Product Name	Mouse CRISPR Knockout Pooled Library A(2 vector system)
Product Catalog	LIBR-M015A-P
Product Details	67405gRNAs (gRNA sequences see attachment); Dual-plasmid system; Bsd resistance, blasticidin 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 20611 genes,3gRNAs per gene; 1000non-target control sgRNAs（1000 targeting non-genic sequences）.

Backbone Map	
Verification Primers	YCS-LV006-F：ATTTCTTGGGTAGTTTGCAGTTT YCS-LV006-R：GACTCGGTGCCACTTTTTCA PCR Fragment: 213 bp 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.
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

Add50 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 七 times and get 8 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 8 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 2.02 x 10^7, move on to the next step.If it is less than 2.02 x 10^7, redo this step.

* Note: It is recommended that the number of colonies should be 40000X greater than3.37 x 10^7 to ensure the uniformity of Library gRNA.

2) For the remaining electroporation end products, plate 400 μL/dish (40dishes can be plated in total), then incubate at 37℃ 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

①Determine 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 Bsd 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 3.37 x 10^7 cells from control group; get all the cells from experimental group and the cell amount before cryopreservation should be greater than 3*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

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!