SYNBIO TECHNOLOGIES

苏州泓迅生物科技股份有限公司

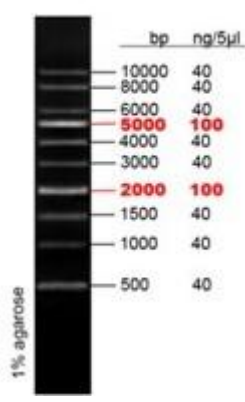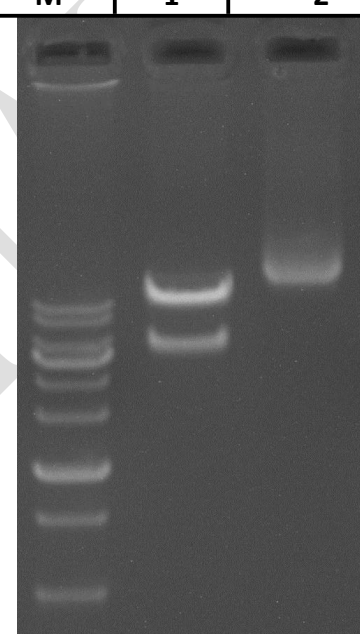| *SERVICE TYPE: LIBRARY CONSTRUCT*	SYNBIO TECHNOLOGIES
# Certificate of Analysis	9 Deerpark Dr., Suite J-25 Monmouth Junction, NJ 08852 Tel: +1 732-230-3003 Fax: +1 609-228-5911 Inquiries: quote@synbio-tech.com
Project ID: chicken	

Construct Information			
Library Name	sgRNA library	**Gene Length**	20bp
Cloning Vector	lentiCRISPR v2	**Construct Resistant**	Amp
Cloning Strategy	BsmBI and BsmBI	**Request Library Size**	1×10^7
Plasmid Maxi Preparation		**Endotoxin Free**	<100 EU/ mg

Item		Description	Result
Library size		Library size= dilution ratio × colony counts	1×10^7 pass
		sgRNA Number	49639
Sequencing	Colony No. for Sanger sequence	**Sample size** (>20)	32
	correct rate	**Accuracy** (>70%)	83.3% pass
	NGS analysis	**Match** (>95%)	99.97%
		Skew ratio (<15)	10
		Undetected guides (<5%)	0.03
DNA Quantity and Quality		Yield (by A260)	

	Concentration	ng/ul
	Purity (A260/A280 = 1.8 - 2.0)	pass
	Dissolution buffer	ddH$_2$O

M	1	2

Restriction Digestion Map

1kb ladder

bp	ng/5µl
10000	40
8000	40
6000	40
5000	100
4000	40
3000	40
2000	100
1500	40
1000	40
500	40

1% agarose

Lane M: DNA Marker
Lane 1: Plasmid digested by KpnI and BamHI

Lane 2: Plasmid DNA

Certified by: G.s Huang Date: 07/29/2022   **Checked by: W.q Liu Date: 07/29/2022**

Certificate of Analysis Description：

1、 **Library Size(>100 x)：** To ensure no loss of representation, sufficient colonies (100 x representations) were harvested before plasmid DNA extraction. It is has been reported that the variance of sgRNA remained largely uncorrelated with the cloning representation when the cloning representation exceed 60 bacterial transform per sgRNA.

2、 **Correct Rate (>0.7):** We sequence 20 randomly isolated clones and guarantee more than 14 clones have the correct gRNA sequences.

3、 **Undetected guides(<0.05)** : Greater than 95 % of sgRNAs were represented with at least one sequencing read in NGS analysis

4、 **Skew ratio (<15):** The difference in representation between the 90_{th} and 10_{th} percentile sgRNAs was always less than 15-fold.

References:

[1] Abigail Read, Shaojian Gao2, Eric Batchelor and Ji Luo. Flexible CRISPR library construction using parallel oligonucleotide retrieval. Nucleic Acids Research, 2017, Vol. 45, No. 11

[2] Neville E. Sanjana, Ophir Shalem and Feng Zhang. Improved vectors and genome-wide libraries for CRISPR screening. Nature Methods, 2014, VOL.11 NO.8

Frequently asked questions:

1. Should I use vector with or without Cas9 for CRISPR-mediated screening?

It depends on many factors. Using a Cas9-containing backbone readily allows screening in any cell line without prior modification of the cell line. However, much less recombination during plasmid amplification and higher viral titers (typically 20- to 100-fold higher) during viral packaging can be achieved by using smaller vectors lacking Cas9. In general, we recommend users who plan to conduct screens across multiple cell lines should clone sgRNA libraries into a Cas9-containing vector. In contrast, those seeking to perform screens across multiple conditions in a single cell line should first derive a Cas9-expressing clone.[1]

2. Should I add the PAM sequence to the oligo when cloning my target spacers into the vector backbone?

There is no need to add the NGG PAM sequence. Because the 'NGG' of the PAM is used to select your genomic target, you need to make sure the NGG immediately follows your target on the genome but NOT on the oligo.

3. Can I transfect my lentiviral library directly into cells?

Direct transfection of cells with the lentiviral library may facilitate expression of your gene of interest, but there are a number of complications. When using retrovirus such as lentivirus or MMLV, the viral genome can integrate into the host cell genome so that genes carried on virus can be stably expressed. By contrast, transfected plasmids library

only have transient expression in the cells. Additionally, direct transfection of plasmids library is non-uniform (some cells contain many copies while others carry very few or none). If the viral vector is used to directly transfect cells, the 5' LTR promoter activity will activate, distort, or even inhibit expression of downstream gene(s) within the lentiviral vector. While the promoter activity of the 5' LTR is inactivated after transduction of cells with the packaged lentivirus. We therefore recommend that lentiviral library be used for production of live virus, and not for direct transfection of cells.

4. How to minimize plasmid library recombined?

The use of Stbl3 cells and incubation of bacteria at 30˚C are both intended to minimize recombination of the lentiviral plasmid library. However, if a substantial fraction of the amplified plasmid library is recombined, as assessed by gel electrophoresis, it might be advisable to grow the transformation products on agar plates rather than in liquid culture.

References:

[1] Tim Wang, Eric S. Lander, and David M. Sabatini. Single Guide RNA Library Design and Construction. Cold Spring Harbor Laboratory Press. 2018.

[2] Neville E. Sanjana, Ophir Shalem and Feng Zhang. Improved vectors and genome-wide libraries for CRISPR screening. Nature Methods. 2014. VOL.11 NO.8