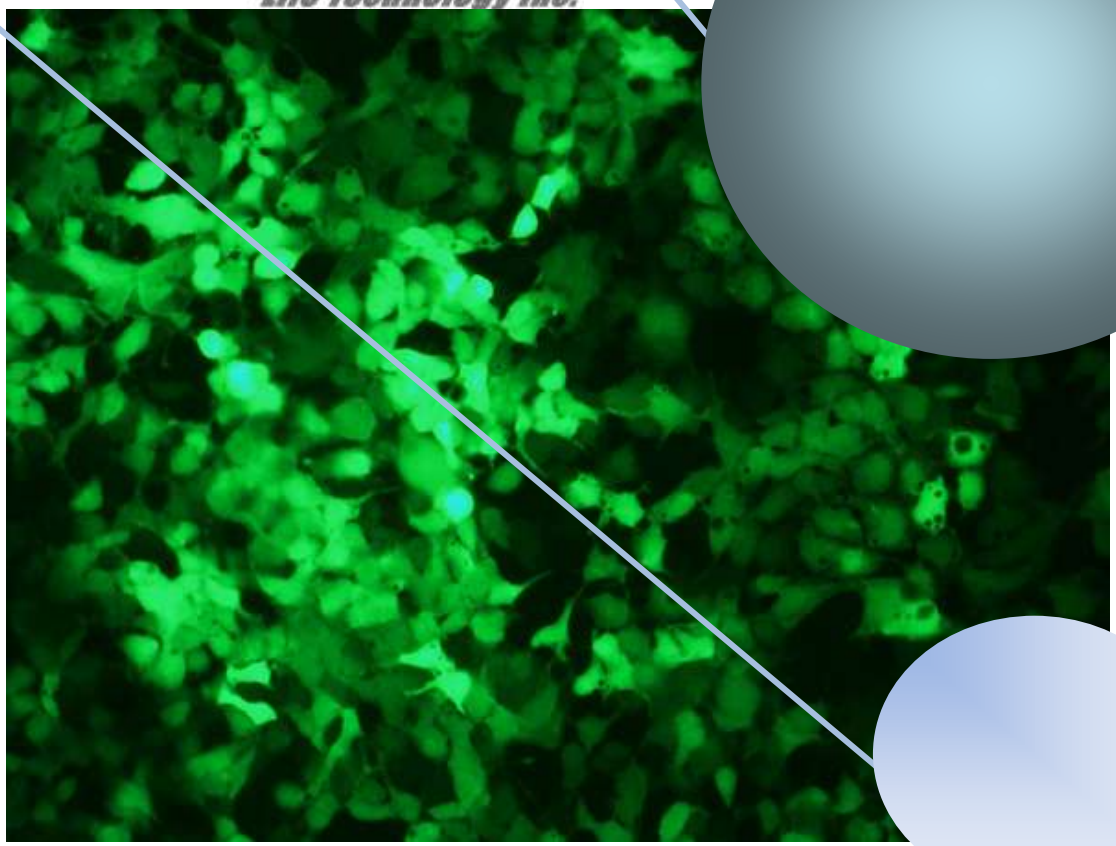

Instructions:

shRNA Lentivirus Plasmids

ATCGbio Life Technology Inc. provides very effective or function-validated shRNA (short-hairpin RNA) in lentivirus plasmid.

- **High specificity (at least 4 mismatches in 19 nt stem)**
- **High target accessibility (calculated based on RNA structure)**
- **High loading efficiency of guide strand (high 5'-3' energy disparity)**
- **Accurate position cut and loading to RISC (newly developed loop structure)**
- **Based on above features, our shRNA plasmids have highly effective.**
- **The basal construct is based on the third generation design.**
- **It expresses GFP and hygromycine resistance gene product.**

Contents

Overview **2**
Tips and Protocol to Handle and Amplify Viral Plasmids **2**
Tips 2
Viruses Production **3**
Infection and Assessing the Effects of Gene knockdown **3**
Contact information **4**

FOR RESEARCH USE ONLY
Not for use in clinical or diagnosis purpose
Important Notice:
Laboratory workers handling pathogenic lentiviruses, recombinant lentiviral vectors, naturally or experimentally infected laboratory animals, or clinical specimens potentially infected with lentiviruses. Diagnostic specimens that contain human blood, body fluids or tissues can be handled and manipulated at the BSL-2 level. BSL-2 is also appropriate for generating and using lentiviral vectors, and handling animals and animal tissues, blood, body fluids and cell lines that are infected with lentivirus vectors. When you practice recombinant lentiviral vectors, please following the requirements outlined in *CDC Biosafety in Microbiological and Biomedical Laboratories*, 5th edition, the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, latest edition.

Instructions for shRNA Lentivirus Plasmids

Overview

Package contains:
1. Negative-control shRNA lentivirus plasmid 20 µl (~50 ng/µl) × 1
2. Pre-made 20 µl (~50 ng/µl) × 1
3. Or Custom-made shRNA lentivirus plasmid 20 µl (~50 ng/µl) × 2

Lentivirus plasmids are shipped in 0.5 ml tube, and users are free to amplify. Upon receiving the tubes, keep it at 4 °C till amplification.

ATCGbio Life Technology Inc. provides very effective (Custom-made) or function-validated (Pre-made) shRNA (short-hairpin RNA) in lentivirus plasmid. Once virus is created, infected cells express shRNA (driven by human U6 promoter) to knock-down specific mRNA* or non-coding RNA. Our shRNAs are designed by our proprietary design scheme having the following features.

1. High specificity (at least 4 mismatches in 19 nt stem)
2. High target accessibility (calculated based on RNA structure)
3. High loading efficiency of guide strand (high 5'-3' energy disparity)
4. Accurate position cut and loading to RISC (newly developed loop structure)
5. Based on above features, our shRNA plasmids have highly effective
6. The basal construct is based on the third generation design.
7. It expresses GFP and hygromycine resistance gene product.

* shRNA works on mRNA which is not always correlated with protein levels. In case of protein expression is largely regulated by protein degradation (e.g. ubiquitination-proteasome system), mRNA levels may not be critical point to regulate protein expression.

Tips and Protocol to Handle and Amplify Viral Plasmids

Tips

Virus plasmids are very prone to mutation in *E.Coli* resulting a short size products, typically, about 3-4 Kb. But, it is very easy to avoid such problem if you practice the following suggestions.

1. Decrease the incubation temperature (both on agar plate and in LB medium) to 32-34 °C. This is the most effective way to avoid mutation. Plasmids still can grow almost normally under this condition, so there is no trade-off.
2. Use virus-stable *E.Coli* such as Stbl3®. Avoid DH5alpha. Most common mistake is using DH5alpha *E.Coli* to amplify lentivirus plasmid under 37 °C. We also recommend to use our VP-Easy Kit (VP1001) which provides reagents making chemically competent *E.coli* suitable for viral plasmid

amplification.

3. Amplify in higher culture volume of LB medium than the volume usually recommended by plasmid purification kit. Typically, we suggested that using 250 ml LB for midi-scale column purification kit instead of the recommended culture volume 40-100 ml; and using 10 ml but not 4 ml of suspension, lysis and neutralization solution. The washing and elution volume are kept the same as original described in the protocol of midi-scale plasmid purification kit. By doing this, plasmid concentration in isopropanol step is high enough to be effectively precipitated.
4. Keep plasmid in TE buffer at 4 °C. With airtight tube, the plasmid is stable for years.
5. Do not vortex the plasmid solution vigorously in order to avoid mechanical damage.

Protocol

Keep in mind above precautions, please follow the next protocol.

1. Centrifuge the tube containing shRNA lentivirus plasmid, and take and transform 1-2 µl plasmid to recommended *E.Coli*.
2. Grow on LB agar ampicillin plate overnight.
3. Pick-up 2-3 colonies and grow it in mini-prep scale (2 ml) LB medium.
4. Purify plasmid and run on 0.7-1% DNA gel.
5. The plasmid should migrate at about 10 kb**.
6. If you see the plasmid migrates at 3-5 kb, that is mutation.
7. Choose one of right-size plasmid and amplify in 250 ml LB with 100 µg/ml ampicillin.
8. Purify the plasmid and re-suspend in 300 µl TE buffer. This scale of amplification should yield at least 100-300 µg DNA.

**Restriction enzyme analysis is not necessary since the insert is too small to detect.

Viruses Production

Our lentivirus plasmid is based on the third generation structure, so gag-pol, rev and VSVG expressing plasmids are required for lentivirus package in 293T cell. It also expresses GFP and hygromycin-resistance gene. To produce high titer lentivirus, we recommend to use only 3 µg plasmid in our Lentivirus Production Kit (LT1001). The kit also provides lentivirus purification and concentration reagents that are successfully used to infect the primary cells which are not compatible with the infection by using crude lentivirus in high-glucose DMEM (medium used to produce lentivirus).

Infection and Assessing the Effects of Gene knockdown

Lentivirus can infect the cells regardless of cell cycle and incorporate shRNA sequence into genome. If the target cells are compatible with DMEM base medium, user can directly infect the cell. For example, for the cell growing in 6-well plates, infection should be done at 20-30% cell confluence with 1 ml medium by adding 50-100 µl viral medium (in case that virus is prepared by using our lentiviral

production kit). Polybrene, final concentration at 2-8 μg/ml, will increase efficiency 2-fold. The knock-down effects can be seen in 2-3 days by RT-PCR and/or westernblot. If user need check the knock-down effects by RT- real time PCR, user can order our GFP RT-real time PCR primer sets. If user choose to make gene knock-down cell-line, 3 days after infection, incubate the cells with hygromycin final concentration at 50 μg/ml overnight and re-plate the cells next day to multiple wells or a bigger plate. Then grow the cells with 100 μg/ml hygromycin. To establish stable gene knock-down cell-lines, cloning procedure is recommended.

References

1. *Y. Ido, et al.* PLoS ONE, 2012 Apr 07(4): e35092

2. *Lan F, et al.* J Biol Chem. 2008 Oct 10;283(41):27628-35

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Ordering information:

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