Plasmid Prep Protocol for Lentiviral Clones

Lentiviral clones can give low yields especially when performing maxi-preps as these plasmids tend to be more delicate and are more susceptible to unwanted recombination events. The following protocols are recommended for lentiviral plasmid preparations.

Additional recommended products	Quantity/volume	Catalog number
GeneCopoeia GCI-L3 Chemically Competent *E. coli* Cells	10 tubes	CC003*

*Larger sizes available.

Mini-prep protocol for lentiviral clones

1. **Day 1:** If transformation is needed- Transform GeneCopoeia GCI-L3 competent cells or a Stbl3 equivalent strain in the afternoon.

   Note: GCI-L3/Stbl3 exhibit lower recombination rates.

2. **Day 2:** Screen for the positive clones by PCR (optional).

3. Inoculate one of the positive colonies in 5 ml LB culture medium in a 50-ml tube and shake at 37℃, 200 rpm overnight (14 -18 hours).

   Note: If more than 20 µg of plasmid is needed, inoculate one colony in 10-15 ml LB culture medium in a 50-ml tube. Using 2-3 mini-prep columns, and pool the plasmid eluted from the columns into one tube.

4. **Day 3:** Perform mini-preps in the morning.

   Note: Qiagen's Mini-prep kits are recommended.

Maxi-prep protocol for lentiviral clones

1. **Day 1:** If transformation is needed, transform GeneCopoeia GCI-L3 competent cells or a Stbl3 equivalent strain in the afternoon.

   Note: GCI-L3/Stbl3 exhibit lower recombination rates.

2. **Day 2:** Screen for the positive clones by PCR (optional).

3. Pick a positive colony from the transformation plate and inoculate in 5-10 ml LB culture medium in a 50-ml tube.

4. Shake at 37℃, 250 rpm for 6-8 hours, spin down the bacteria at 4000 rpm for 10 minutes, rinse the pellet with 10-20 ml fresh LB, suspend the bacteria pellets in 5-10 ml LB and inoculate into 400 ml LB medium.

   Note: Test different incubation time for the 5 -10 ml culture to ensure the bacteria are in their log phase of growth before inoculating 400 ml culture.

5. **Day 3:** Proceed with the maxi-prep.

   Note: It's not recommended to pick a colony from the transformation plate and inoculate in 400 ml culture directly as it will produce low yield due to poor growth. Therefore, the two-step inoculation is recommended, in which the log phase bacteria from a smaller culture is further inoculated in a larger culture.

To prevent low plasmid yield, the following is also recommended:

1. Test different incubation temperatures: 37℃, 26℃, and 18℃ for instance.

2. Gently process the plasmid DNA during the purification and avoid vortex if possible.

   Note: The sequence composition of the lentiviral plasmid could make the plasmid more vulnerable to harsh treatment which may impact the yield.

Appendix

SOC medium

2.0 g	Bacto-Tryptone
0.5 g	Bacto-Yeast extract
1 ml	1M NaCl
0.25 ml	1M KCl
1 ml	1M $MgCl_2$-$6H_2O$, filter sterilize
1 ml	2M Glucose, filter sterilize
1 ml	1M $MgSO_4$-$7H_2O$, filter sterilize

Add ddH_2O up to 100 ml

Add Bacto-Tryptone, Bacto-Yeast extract, NaCl, and KCl to 97 ml of ddH_2O. Stir to dissolve. Autoclave and cool to room temperature. Add each Mg stock to a final concentration of 10mM, and add 2M Glucose to a final concentration of 20mM. Filter the entire medium through a 0.2-μm filter. The pH should be around 7.0.

LB plates (per liter)

10.0 g	Bacto-Tryptone
5.0 g	Bacto-Yeast extract
5.0 g	NaCl
15.0 g	Agar

Adjust the pH to 7.0 with NaOH (~200 µl 5M NaOH). Autoclave (keep the top loosened to allow steam to vent) and allow to cool to 50℃ before adding antibiotics. Mix well and pour LB media into plates.

LB Medium (per liter)

10.0 g	Bacto-Tryptone
5.0 g	Bacto-Yeast extract
5.0 g	NaCl

Adjust the pH to 7.0 with NaOH (~200 µl 5M NaOH). Autoclave (keep the top loosened to allow steam to vent).

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