YCCEH Protocols

LENTIVIRAL PREPARATION (Calcium Phosphate Method)

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*** Lentiviruses are BSL2 reagents and need approval from Institutional Biosafety Committee and appropriate equipment and protective gear per guidelines.***

Reagents:

1.0M HEPES Solution
1.2 gm HEPES
H2O to 5 ml
* Can also be bought directly (e.g., H0887 from Sigma-Aldrich)

0.5M Na2PO4 Solution
0.355gm NaHPO4
H2O to 5 ml

2xHBS Buffer
2.8 ml 5 M NaCl
2.5 ml 1 M HEPES
150 µl 0.5 M Na_2HPO_4
H_2O to 50 mls
adjust pH to 7.05-7.15 (~5 drops 5 M NaOH)
* Can also be bought directly from vendors (J62623-AK from VWR)

Filter sterilize 2x HBS, 1 M $CaCl_2$, and H_2O. Store 2x HBS can be stored at 4° for extended periods of time and can be stored at -20° for long term.

Overview

Calcium Phosphate based preparation of lenti- or retroviral particles can be easily prepared and scaled up when large scale viral preparations are needed (such as for genome-wide libraries). For small-scale preps, the accompanying protocol with Lipofectamine 3000 may be preferable given less variability

HEK 293FT of 293T are commonly used. Cells should be growing in log phase before transfection. Cells can be split a few hours before transfection (we find that this works best) or the evening before transfection. Note that HEK 293T cells acidify the medium to a significant degree—split cells before the medium gets very acidic. These cells do not adhere very well, so use gentle pipetting when transfecting or feeding. Freshly plated, evenly spaced cells at approximately 70% confluence adhere reasonably well and transfect quite well and leave room for some expansion after transfection.

Procedure

Transfection:

For the transfection, mix the DNAs as follows per plate:

- 12 µg provirus plasmid
- 8.7 µg plasmid encoding required HIV proteins
- 5.3 µg plasmid encoding VSVG
- H_2O to 375 µl
- 125 µl 1 M $CaCl_2$

Add 500 µl 2xHBS dropwise with mixing (e.g., bubble the solution using an automatic micro pipet, or, for large volumes, a Pasteur pipette).

Let the mixture stand at room temperature for a minute—the mixture should be slightly cloudy—then overlay plate(s); return plates to incubator after a few minutes, the medium should turn slightly pink.

Feed plates fresh medium 24 hours after transfecting

Harvest Virus

Harvest the medium 48 hr after transfecting

Centrifuge the medium at 6000g or more to pellet cell debris.

Concentrating virus:

The virus in the supernatant can be purified chromatographically using a matrix that binds VSVG or, for large scale preparations, can be pelleted at 150,000g (others suggest lower speed centrifugation, but we have found that this leaves a significant fraction of the virus in the supernatant) for 2 hours, using, for example, an SW28 rotor.

After pelleting, remove the medium—this can be saved for testing the amount of virus remaining in the supernatant.

Resuspend the pellets containing the virus by gentle trituration with an automatic micropipette.

Combine pellets from all of the centrifuge tubes and adjust the volume to approximately 9 ml with PBS.

Filter the mixture using a 10 ml syringe and a 0.45 µ filter. From this point on, maintain sterility.

Transfer the PBS containing the virus to an SW41 tube. Place the tube in a centrifuge bucket that you have sterilized, along with the bucket cap, with 70% EtOH.

Cap the bucket and centrifuge for 2 hours at 41,000g.

Remove the supernatant. Resuspend the virus in PBS by gentle trituration as above using an appropriate volume. We typically resuspend in 100-200 µl.

Store the virus at –80°. The virus is stable to at least several freeze/thaws, but aliquoting is a good idea. We return any unused portion to the –80° freezer for future use.