BROAD INSTITUTE | GENETIC PERTURBATION PLATFORM

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Protocol: Lentivirus concentration protocol

Brief Description:

This protocol describes a method to concentrate lentiviral preps using spin columns. A range of 50 to 200-fold concentration and a volume of 300-500 uL per 60 mL of unconcentrated viral supernatant have been obtained.

Materials & Instrumentation

- Centrifuge with swinging buckets able to reach 4,000 g that can maintain a temperature of 30°C
- Centrifuge buckets able to hold filters
- Centrifuge bucket snap-on lids (for safety) (Ex: VWR #5000226124)
- Centricon Plus-70 Centrifugal Filter, Ultracel-PL Membrane, 3kDa (Sigma Aldrich #UFC700308)
- Autoclaved Milli-Q water
- 70% ethanol
- 10% bleach
- Lentivirus stock, harvested out of flasks and clarified (spin 1 min at 233 g, remove viral supernatant leaving small 293T pellet) right before adding to the columns.

Sterilization of filters:

Sterilization of the filters must occur immediately prior to concentrating the lentivirus to avoid membranes becoming dry once wetted.

1. Always assemble and disassemble the filter columns and centrifuge buckets within the biosafety hood to maintain sterility.

2. Snap the filter cups into the collection cups and place the assembled column into the centrifuge bucket to stabilize. Add 30 mL of 70% ethanol to each filter cup, cover with the filter lids and press firmly to seal tightly. Let sit for 5 min at room temperature, then centrifuge at 4,000 g for 25 min at 30°C.

3. Invert the filter cups in the concentrate cups, being careful not to touch the tips of the filter, and centrifuge at 4,000 g for 5 min at 30°C to remove any excess ethanol.

4. Reassemble the columns and add 20 mL of autoclaved Milli-Q water to each filter cup. Seal with the filter lids and centrifuge at 4,000 g for 10 min at 30°C to wash the filters.

5. Invert the filter cups in the concentrate cups and centrifuge at 4,000 g for 5 min at 30°C to remove any excess water.

6. Repeat steps 4-5.

Virus concentration:

7. Label the side of the filter cups with the name/condition of the virus.

8. Reassemble the columns and add 60 mL of autoclaved Milli-Q water to each filter cup. Seal with the filter lids and centrifuge at 4,000 g for 1 hr at 30°C to hydrate the filters.

- During this spin harvest the virus from the flasks:
    - Pipette off virus from the flasks into conical tubes.
    - Clarify the virus by centrifuging at 233 g for 1 min.
    - Pipette off the viral supernatant into new conical tubes, being careful to not disturb any cell pellet.
    - The virus may be filtered with a 0.45 uM filter to avoid carrying over any 293T (optional)

9. Invert the filter cups in the concentrate cups and centrifuge at 4,000 g for 5 min at 30°C to remove any excess water.

10. Sterilize the lids of the filters with 70% ethanol and let dry.

11. Reassemble the columns and carefully add up to 60 mL of stock virus to each filter cup. Seal with the filter lids and centrifuge at 4,000 g for 1 hr at 30°C.

12. Sterilize concentrate cups with 70% ethanol and let dry.

13. Invert the filter cups into the concentrate cups and centrifuge 1,000 g for 2

min at 30°C to recover the concentrated virus.

14. If concentrating multiple columns of the same virus, combine all of the concentrated virus together, then aliquot.

15. Bleach all filters cups, collection cups, concentrate cups, filter lids, conicals, filters and flasks for at least 20 min.