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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 (Ex: Beckman Coulter Inc. #349946)
- 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 (1 min spin at 1000 rpm, remove viral supernatant leaving small 293T pellet) right before adding to the columns

Assembled column

Inverted column

Assembled bucket

Sterilization of filters:

1. Snap the filter cups into the collection cups, add 30 mL of 70% ethanol to each filter cup, seal with the filter lids and let sit for 5 min. Centrifuge at 4,000 g for 25 min at 30°C.
2. Invert the filter cups in the concentrate cups and centrifuge at 4,000 g for 5 min at 30°C to remove any excess ethanol.
3. 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.
4. 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.
5. Repeat steps 3-4.

Virus concentration:

6. Label the side of the filter cups with the name/condition of the virus.
7. 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 flask(s) into conical tube(s)
        - Clarify virus by centrifuging at 1000 rpm for 1 min
        - Pipette off viral supernatant, being careful to not disturb any cell pellet
        - Filter the virus if necessary with 0.45 uM filter.
8. 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.
9. Sterilize the lids of the filters in a bucket of 70% ethanol and let dry.
10. Reassemble the columns and add up to 60 mL of viral supernatant to each filter cup. Seal with the filter lids and centrifuge at 4,000 g for 1 hr at 30°C.
11. Sterilize concentrate cups in a bucket of 70% ethanol and let dry.

12. Invert the filter cups into the concentrate cups and centrifuge at 1,000 g for 2 min at 30°C to recover the concentrated virus.
13. If concentrating multiple columns of the same virus, combine all of the concentrated virus together, then aliquot.
14. Bleach all filters cups, collection cups, concentrate cups, filter lids, conicals, filters and flasks for at least 20 min.