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Stable cell line generation via retrovirus

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Protocol status: Working
We use this protocol and it's working

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Abstract

Production of pseudotyped virus an subsequent transduction.

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Day 0: Thaw fresh cryovial of cells to be transduced

Thaw ~ 1 week prior to planned transduction day, use low passage number if possible. Aim for cells to be 80% confluent on day of transduction.
Recommend seeding four wells of a 12-well plate (100,000 cells/well) the day before transduction (after collecting virus).

Day 1: HEK293T Transfection

1. Prepare 2 80% confluent 10 cm plates of HEK293T for transfection

1. Prepare 3 mL of warm Opti-mem solution.
Add 10 µg of retro/lentiviral transfection plasmid, 10 µg VSV-G plasmid, and 10 µg pCMV-MLV (if retroviral)**[DT1]** **[DT2]** or 10 µg pCMV R8.74 (if lentiviral)

1. Add 90 µL of LT-1 reagent and swirl.

1. Incubate at room temperature for 15 minutes.

1. Add 1.5 mL dropwise to each 10 cm

HEK293T plate.

1. Incubate for 3 days in incubator before harvesting viral particles. If a fluorescent marker was included, it should begin to be visible on days 1,2 post transfection.

SAFETY: After this point all media and plasticware is to be treated as potentially containing viral particles. Decontaminate using 10% bleach for 10 minutes before discarding as red bag waste. Rinse pipettes with 10% bleach before discarding as biohazard waste.

Day 4: Harvest viral particles

1. Collect media from both HEK293T plates, combine into one 20 mL portion in a 50 mL falcon tube. Bleach and discard used HEK293T plate.

1. Spin 50 mL tube containing harvested viral particles in Sorvall for 2 min at 2,000 rpm**[DT3]** **[DT4]** to clarify media and pellet stray cells

1. Aspirate off media without disturbing pellet.

1. Add 6 mL Lenti-X concentrator solution to 18 mL of recovered media (concentrator is 4x). Invert to mix.

1. Incubate in refrigerator 1 H – O/N**[DT5]**

1. Spin 50 mL conical containing viral particles in centrifuge at 1,500 rcf for 45 min

1. Remove 2 mL of supernatant from top of pelleted solution and save.

1. Aspirate off remaining media to recover viral pellet. Bleach excess media.

1. Resuspend retro/lenti pellet in 2 mL of saved medium.

1. Titrate concentrated retro/lenti solution into prepared 12-well containing target cells. Recommend 100-200-400-800 µL titration for 4 total attempts at transfection. Typically 400 or 800 uL will yield ~100% transfection efficiency

1. Return 12-well plate containing transduced cells to incubator.

Day 5: Check on transduction

1. May be able to see fluorescence today.

1. Swap media to fresh.

Day 6-7: Check on transduction

Day 8-:

1. Allow cells to grow until almost confluent on 12-well plate. Trypsinize and replate in a 6-well, allow to grow until confluent, then replate in a 10 cm plate.

1. Split into two 10 cm plates, Freeze one plate down into 4 cryovials the next day to save progress. Label with estimated transduction efficiency if able to measure.

1. Take other plate and select with antibiotics if able to/necessary, or clonally isolate populations if able to/necessary.