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Virus Production and Transduction Protocol for Retroviral plasmids

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We use this protocol and it's working

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Abstract

This protocol outlines the generation of retroviral particles using HEK293T cells and the transduction of target cells to establish stable expression lines. The procedure includes virus production, harvesting, and spin infection for efficient gene delivery.

Materials

- HEK293T cells
- Retroviral plasmids
- Packaging plasmids
- VSV-G envelope plasmid
- XtremeGene9 transfection reagent (Sigma)
- DMEM
- Fetal bovine serum (FBS), heat-inactivated
- Polybrene

Day 1: Seeding HEK293T cells

1 Plate 1.5 million cells (or 2 million if evening) into 6cm-dishes (NOT 6-well plates).

2 Use normal medium: DMEM (10% heat-inactivated fetal serum + 1% P/S).

Day 2, end of day (4-6pm): Transfect HEK293T cells

3 Add 1 ug of maxiprepped plasmid of your interest (carrying the construct you want to integrate into cells) to eppendorf tube.

4 Next, add 900ng of the retrovirus or lentivirus pol/gag and 150ng of VSVg DNA.

5 Add 130 ul DMEM (without serum).

6 X-tremeGENE™ 9 DNA Transfection Reagent (Roche Diagnostics GmbH, #06365779001), vortex it very well.

7 Add 6 ul of X-tremeGENE reagent (1:3) to the tube (step 5), mix by pipetting.

8 Incubate for 20 min at room temperature (in the hood).

9 Add to HEK293T cells dropwise.

10 Swirl plate to mix.

Day 3, change media of transfected HEK293T cells

11 At morning (16 hours after transfection) aspirate media (from step 10).

12 Add 5ml of DMEM media (30% heat-inactivated fetal serum + 1% P/S).

Day 4, ~ 3pm (36 hours later):

13 Collect media from HEK293T plates into 15mL falcon tubes = virus.

14 Throw all waste away into bleach container.

15 Centrifuge virus at 300g or 1000 RPM for 5 min.

16 Aliquot virus (Supernatant) into eppendorf tubes (1mL/tube).

17 Use virus to infect cells immediately or store virus at -80°C.

Transduction

18 Prepare 6-well plate for infections, label wells (include no infection control for each cell line)

19 Thaw virus in 37°C water bath

20 Trypsinize, harvest and count target cells.

21 Add 5ug to 10ug of polybrene per ml (final concentration) transfection reagent (EMD Millipore Corp. #TR-1003-G) to each well where infection will take place

22 Add 1 or 2 million target cells / well (including control wells.)

23 Mix virus by vortexing or swirling.

24 Add 100-250 ul of virus (Based on virus efficiency) to each well except the control wells.

25 Top up each well with media to 3 mL total volume.

26 Take plate to the virus centrifuge to perform spin infection: 2200 RPM, 45 min at 37°C.

27 Put plate to cell incubator after spinning.

Selection

28 Aspirate media (from step 27)

29 Optional: Wash the cells with PBS and trypsinize. Transfer the cells, including control samples, to a 10 cm dish containing 10 mL of complete growth medium.

30 Add selection antibiotic (for example: 1:1000 of 10mg/ml stock Blasticidin or 1:1000 of 2mg/ml stock Puromycin)