Transduction of Cultured Cells with Recombinant Lentiviral Particles

Samantha C. Faber

University of North Carolina

Eva C.M. Vitucci

University of North Carolina

Shaun D. McCullough (✉ mccullough.shaun@epa.gov)

US Environmental Protection Agency <https://orcid.org/0000-0001-6660-346X>

Method Article

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Abstract

This protocol describes the transduction of cultured cells with recombinant lentivirus expressing fluorescent markers.

The attached methods document is a formal version of the information included here.

Disclaimer: The contents of this article have been reviewed by the US Environmental Protection Agency and approved for publication and do not necessarily represent Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendations for use.

Introduction

Reagents

Target cell growth medium

100X penicillin/streptomycin solution (Gibco #15140-122)

Dulbecco's Phosphate Buffered Saline (DPBS, Gibco #14190-144)

Trypsin (Gibco #25200-056)

Polybrene (hexadimethrine bromide, Sigma #107689)

Equipment

Biosafety cabinet

Humidified tissue culture incubator with 5% CO₂

Tissue culture dishes

100mm plates (TPP #93100),

150mm plates (TPP #93150),

6-well plates (Corning #3516),

OR 12-well plates (Corning #3512)

Vacuum-driven bottle filter with 0.2 µm pore

Pipetaid

Serological pipettes

50mL conical tubes

15mL conical tubes

5mL polypropylene round bottom tube for flow cytometry (Falcon #352063)

Tabletop centrifuge

Pipettes

Filter tips (low retention)

Hemocytometer or automated cell counting device

Millex-HV Syringe Filter Unit, 0.45 μm (MilliporeSigma #SLHV033RB)

Millex-HV Syringe Filter Unit, 0.22 μm (MilliporeSigma #SLGV033RS)

10-mL syringe only (BD #309604)

BioExpress GM Series Balance (BioExpress #B-1850)

Procedure

Day 0: Prepare Lentiviral Transduction Plates

1. Plate cells for transduction
 - a. Plate at a density that will reach 80% confluency in ~24hrs (time of transduction).
 - i. 12-well plate:
 1. 16HBE: Plate 1 mL at 5.25×10^5 cells/mL
 2. IMR90: Plate 1 mL at 6.4×10^4 cells/mL
 3. HULEC: Plate 1 mL at 8×10^4 cells/mL
 4. H441: Plate 1 mL at 2.0×10^5 cells/mL
 - ii. **NOTE:** Plate cell in three wells of a separate multi-well plate to be counted at time of transduction for use in determining the amount of viral supernatant to use for transduction.

- iii. **NOTE:** Plate three additional wells for a no viral transduction control
- iv. **NOTE:** Use collagen coated plates
- 2. Incubate cells in a humidified incubator at 37 °C with 5% CO₂ for 24hrs.

Day 1: Determine Cell Density & Transduce Cells

- 1. Determine Cell Density
 - a. Warm appropriate cell culture medium and DPBS in 37°C water bath, and warm trypsin at room temperature
 - b. Aspirate cell culture media of designated counting wells
 - c. Rinse aspirated wells with DPBS
 - i. 12-well plate: Add 1 mL DPBS / well
 - d. Aspirate PBS from each well and add trypsin. Incubate for 7 minutes at 37 °C
 - i. 12-well plate: Add 200uL trypsin / well
 - e. Neutralize trypsin by the addition of culture media to each well. Triturate each cell suspension to break up cell clumps. Transfer each cell suspension to a separate labeled 15mL tube/well.
 - i. 12-well plate: Add 1 mL culture media / well
 - f. Rinse each well with additional culture media for collection of remaining cells. Transfer to matched 15mL tube. Vortex to mix cell suspension.
 - i. 12-well plate: Rinse with 1 mL of culture media / well
 - g. Add an appropriate amount of DPBS to a new Eppendorf tube for each cell suspension to be counted. Add appropriate amount of each cell suspension to matched Eppendorf tube to make a 1:1 dilution.
 - i. 12-well plate: Mix 50 µL DPBS with 50 µL cell suspension
 - h. Vortex each cell-PBS mix and count via hemocytometer to calculate cell number / well.
 - i. **NOTE:** This cell density value will be used to establish the number of cells in the wells designated for transduction.
- 2. Transduce cells with recombinant lentivirus

- a. Make stock solution of polybrene
 - i. Weigh approximately 100 mg of polybrene (it will be challenging to weight exactly 100 mg due to the variable size of polybrene crystals) and add a volume of tissue culture grade water to prepare a stock solution at a concentration of 10 mg/mL.
 - ii. Filter sterilize in hood and store at 4 °C
 - iii. **NOTE:** polybrene solution expires one month after preparation.
- b. Make optimized polybrene dilution from 10 mg/mL stock in appropriate amount of growth medium in 15 mL conical tube. Mix well by vortexing.
 - i. **NOTE:** The optimal concentration of polybrene was determined by testing dilutions ranging from 0 to 10 µg/mL for maximal increase in viral transduction efficiency.
 - 1. 16HBE: 2 µg/mL
 - 2. IMR90: 2 µg/mL
 - 3. HULEC: 8 µg/mL
 - 4. H441: 2 µg/mL
- c. Thaw aliquot(s) of recombinant lentiviral particles at room temperature
- d. Make aliquots of growth medium with X mg/mL polybrene for each well to be transduced in 50 mL conical tubes.
 - i. 12-well plate: 800 µL of polybrene dilution / well to be transduced
 - ii. **NOTE:** Include additional polybrene aliquots to be used as a negative (no virus) transduction control
- e. Add optimized volume of viral particles to each polybrene dilution aliquot designated for viral transduction. Mix well by vortexing.
 - i. **NOTE:** To determine optimal concentration of virus to be used for transduction, perform a viral titration assay with serial dilution from 1:400 to 1:12800. Typically, using an MOI of 3, when the viral preparation has been tittered on the target cell type, results in a transduced population that has >90% fluorescent-positive cells.
- f. Remove plates designated for viral transduction from incubator and aspirate media from wells.
 - i. Aspirate media in a manner that minimizes cell-air contact time
 - ii. Add polybrene-virus mix or polybrene-no virus to appropriate wells

1. 12-well plate: 800 μ L of appropriate mix / well
3. Incubate in a humidified incubator at 37 °C with 5% CO₂ for 24 hrs.

Day 2: Replace growth media

1. In the morning (~9:00 AM) aspirate the transduction medium into a flask containing bleach.
2. Rinse wells with DPBS and aspirate the DPBS. Repeat twice for a total of three DPBS washes.
 - a. 12-well plate: 1mL PBS / well
3. Add fresh growth medium to each plate of transduced cells.
 - a. 12-well plate: 1mL of growth media / well
4. Incubate cells in a humidified incubator at 37 °C with 5% CO₂ for 48 hours

Day 3: Flow Cytometry & Microscope Verification

1. Visualize fluorescent cells and/or quantify the percentage of fluorescent-positive cells by flow cytometry.

Troubleshooting

Time Taken

Anticipated Results

References

Acknowledgements

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [McCulloughMethodsLentiviralTransduction.docx](#)