Murine CD8 T cell transduction

Kristin Anderson[1]

[1]University of Washington, Fred Hutchinson Cancer Research Center

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Kristin Anderson

ABSTRACT

CD8 T cells can be transduced to express genes transferred by retroviral transduction. This protocol outlines the steps for generating retrovirus from PLAT-E packaging cells to transduce primary murine CD8 T cells isolated from donor mouse spleens.

In brief: The ectropic retroviral packaging cell line, Platinum (Plat)-E cells (Cell Biolabs, Inc.), were plated ($2.2x10^6$ cells) in PLAT-E media on 10 mm culture plates. After 24 hours, Plat-E cells were transfected with DNA encoding the desired TCR and/or construct using the Effectene transfection kit (Qiagen). At 48 hours, media was replaced with T cell media. On days 2 and 3 post-transfection (at 72 and 96 hours) virus-containing supernatant was collected. Splenic T cells isolated from P14 Thy1.1+ female mice were stimulated with a-CD3e (clone: 145-2C11) and a-CD28 (clone: 37.51 (both from BD Pharmingen) and IL-2 (50 IU/ml Aldesleukin, UW Pharmacy), and transduced with retroviral supernatant by spinfection in polybrene (5mg/mL, 90 minutes at 1000*xg*) at 24 and 48 hours after T cell activation.

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MATERIALS TEXT

MATERIALS

⊠ DPBS (no Ca, no Mg) **Thermofisher Catalog #14190144**

⊠ Purified Hamster Anti-Mouse CD3e (Clone 145-2C11) **BD Biosciences Catalog #553058**

⊠ Purified Hamster Anti-Mouse CD28 (Clone 37.51) **BD Biosciences Catalog #553295**

⊠ IL-2 (Aldesleukin) **Contributed by users**

⊠ Effectene Transfection Reagent **Qiagen Catalog #301427**

⊠ PLAT-E (Platinum-E) ectropic retroviral packaging cell line **Cell Biolabs, Inc Catalog #RV-101**

⊠ 0.05% Trypsin-EDTA (1x) **Gibco - Thermo Fisher Catalog #25300-054**

⊠ ACK Lysing Buffer **Gibco - Thermo Fisher Catalog #A10492-01**

⊠ Falcon 5mL polypropylene round-bottom tube **Corning Catalog #352063**

⊠ Falcon 50 mL polypropylene conical tube **Corning Catalog #352070**

⊠ 15 mL polypropylene centrifuge tube **Corning Catalog #430791**

⊠ Tissue Culture Dish **Techno Plastic Products (tpp) Catalog #93100**

⊠ T25 cell culture flask, canted neck, 0.2um vent vap **Corning Catalog #430639**

⊠ Paraformaldehyde, 16% w/v aq. soln, methanol free liquid **Alfa Aesar Catalog #43368**

⊠ Falcon cell strainer, 70um, nylon **Corning Catalog #352350**

⊠ BD 10 mL syringe, Luer-Lok Tip **BD Biosciences Catalog #302995**

⊠ Nalgene syringe filter, 0.45um (surfactant free cellulose acetate membrane) **Thermo Scientific Catalog #723-9945**

SAFETY WARNINGS

Retroviral supernatant requires BSL-2 precautions. Work in a biosafety cabinet and wear proper PPE.

Day 0: Prepare PLAT-E cells for transfection

1 **1.** Gently remove PLAT-E media and rinse PLAT-E cells with 1x DPBS.
2. Gently remove PBS and add 0.05% Trypsin. Trypsinize at 37C for 1-2 minutes or until cells being to lift off the plate with a gentle tap.

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3. Dilute and inactivate trypsin with 10ml PLAT-E media.Transfer cells to a conical tube, spin down @ 369*xg* for 4-5 minutes at 4C and remove supernatant.
4. Resuspend PLAT-E cells in 10mL PLAT-E media. Count cells. Make new 10 cm plates with 2.2e6 cells per plate in 10ml PLAT-E media.
Cells:
Quadrants:
Dilution:
Calculation of concentration:
Calculation of cells to add:
Volume of cells per plate:
Media per plate:
5. Return plates to 37C incubator.
DNA to transduce:
Number of plates prepared:

Day 1: Transfect PLAT-E cells

2 **1.** Thaw tubes of DNA. And prepare 5mL polypropylene tubes (1/plate).
2. Add 300 uL EC Buffer.
3. Add 2ug DNA to EC Buffer.
Record DNA amounts to add for each construct:
4. Add 16uL of Enhancer and vortex for 1 second.
5. Incubate 5 min at Room Temp.
6. Add 60uL of Effectene. Vortex for 10 seconds.
7. Incubate 10 min at Room Temp.
8. Aspirate media from PLAT-E plates. Add 7mL of T cell media to each plate.
9. Add 3 mL of T cell media to each tube of Effectene mixture. Pipette up and down to mix, then add slowly (dropwise) to each PLAT-E plate.
10. Swirl gently to mix and return to 37C incubator overnight.

Day 2: Change PLAT-E media and prepare T cells for transduction

3 **1.** Aspirate media from PLAT-E plates and replace with 10.5 mL T cell media**.**
2. Place the plates in the 32C incubator overnight.

3. Harvest T cells by smashing donor spleens through a 40um filter. Rinse and transfer to a 15 or 50 mL tube.
Number of spleens:
Donor mice information:
4. Spin down T cells down @369xg for 4-5 minutes at 4C.
5. Aspirate media and lyse spleens with 2ml/spleen ACK Lysis buffer for 2 minutes.
6. Dilute out the ACK Buffer with equal or greater volume of T cell media. Spin down T cells down @ 369*xg* for 4-5 minutes at 4C.
7. Resuspend pellet in T cell media.
Volume of T cells:
8. Add 1ug/ml of anti-CD3 and anti-CD28 (e.g. 20ul of Ab per 10 mL of splenocytes).
Anti-CD3 added:
Anti-CD28 added:
9. Add 50 IU/mL of IL-2 (e.g. 10ul of Ab per 10 mL splenocytes).
IL-2 added:
10. Transfer 10mL of splenocytes per T25 flask and place in 37C incubator overnight.

Day 3: Transduction 1

4 **1.** Transfer T cells into 50mL tubes (need 1 tube for each independent viral transduction**.** Number of tubes:
2. Harvest virus from PLAT-E plates and filter through 0.45um Nalgene Syringe Filter.
3. Add 10.5 mL T cell media per PLAT-E plate and return to 32C incubator overnight.
4. Resuspend T cells in filtered viral supernatant. Calculate volume of each tube.
Volumes:
5. Add IL-2 at 50 IU/mL and Polybrene at 5ug/mL.
Volume of IL-2 added to each tube:

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Volume of Polybrene added to each tube:

6. Plate T cell/virus mixture in 12 well plates (Add approximately 1.5-2 mL per well). Balance the plates by weight. Spin @ 1000*xg* for 90 minutes at 30C.
7. Place plates in 37C incubator overnight.

Day 4: Transduction 2

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1. Collect T cells from wells, combine similar wells into 50 mL tube, and spin down @1350 RPM for 4-5 min at 4C.
2. Harvest virus from PLAT-E plates and filter through 0.45um Nalgene Syringe Filter.
3. Resuspend T cells in filtered viral supernatant. Calculate volume of each tube.

   Volumes:
4. Add IL-2 at 50 IU/mL and Polybrene at 5ug/mL.

   Volume of IL-2 added to each tube:

   Volume of Polybrene added to each tube:
5. Plate T cell/virus mixture in 12 well plates (~1.5-2.5 mL per well). Balance the plates by weight. Spin @ 1000*xg* for 90 minutes at 30C.
6. Place plates in 37C incubator overnight.

Day 6: Add media (as needed) and IL-2

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1. If the wells are starting to change from oragne to yellow in color, add 1-2 mL of T cell media per well of cells.
2. Add 50 IU/ml IL-2 per well.

Day 7 or 8: Screen T cells for transduction efficiency

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1. Remove a small sample of cells for screening.
2. Continue to add media (ass needed) and IL-2 (50 IU/ml) every 48 hours.

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