Lentiviral Transduction of CRC Organoids and CAFs

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Introduction

Lentiviral transduction is an efficient method to introduce transgenes into mammalian cells for robust and stable expression. The possibility to express reporter proteins in organoids and cancer-associated fibroblasts (CAFs) using lentiviral vectors has increased the opportunities for the design of patient-derived cell assays. Handling of lentiviral constructs requires appropriate permissions and laboratory infrastructures to work under *Biosafety level 2* conditions.

Purpose

This standard operating procedure (SOP) describes the procedures for lentiviral transduction of CRC organoids and CAFs. Virus production and titration is performed as described in *Michels et al., 2020, Cell Stem Cell 26, 1–11*.

1. Equipment, materials and chemicals

1.1 Equipment

- Cell culture incubator adjusted at 37 °C and 5 % CO2
- Centrifuge (Rotanta 460R, Hettich)
- Evos Fl microscope with 2X, 4X, 10X objectives
- Pipettes with sterile tips
- Pump and aspiration pipettes
- Water bath or heating block
- Pipette boy

1.2 Materials

- 50-ml Falcons
- 5 ml Eppendorf tubes
- Sterile 6 cm and 10 cm cell culture dishes
- Sterile 12-well cell culture suspension plates (Greiner, #665102)
- 5- and 10-ml pipets
- Parafilm M

1.3 Chemicals

- Matrigel™ (Corning, #356231)
- Y-27632 Rho kinase inhibitor (Biotrend, #Y5301)
- PBS (Thermo Fisher, # 10010015)
- 0.1 % gelatin in PBS (Sigma-Aldrich, #G2500-100G)
- Trypsin (Thermo Fisher, #12605-010)
- Polybrene Transfection Reagent (Sigma-Aldrich, #TR-1003-G)
- Puromycin (InvivoGen, #ant-pr-1)
- Accutase (Life Technologies, #A1110501)

2. Medium recipes

2.1 Washing medium (WM)

Component	Concentration (stock)	Amount
DMEM (Thermo Fisher, #31966-047)	1x	500 ml
Bovine Albumin Fraction (Thermo Fisher, #15260-037)	7.5%	2.65 ml
Pent/Strep (Thermo Fisher, #15140-122)	100x	5 ml

2.2 Advanced DMEM+++

Component	Concentration (stock)	Amount
Advanced DMEM/F12 (Thermo Fisher, #12634-028)	1x	500 ml
Glutamax (Thermo Fisher, #35050038)	100x	5 ml
HEPES (Thermo Fisher, #15630056)	1M (100x)	5 ml
Pent/Strep (Thermo Fisher, #15140-122)	100x	5 ml

2.3 Medium for tumor organoid expansion

Component	Concentration (stock)	Amount
Advanced DMEM +++	1x	35 ml
*R-spondin (in house prepared CM)	1x	10 ml, 20% (v/v)
**Noggin (in house prepared CM)	1x	5 ml, 10% (v/v)
B-27 (Thermo Fisher, # 12587010)	50x	1 ml
N-acetylcysteine (Sigma-Aldrich, #A9165)	5M	125 µl
Epidermal growth factor (Peprotech, #AF-100-15)	500 µg/ml	5 µl
ALK5 inhibitor A83-01 (Tocris, #2939/10)	500 µM	50 µl
p38 inhibitor SB202190 (Sigma-Aldrich, #S7076)	30 mM	16.6 µl

**R-spondin1-conditioned medium was obtained from R-spondin producing cells (293T-HA-RspoI-Fc, from Calvin Kuo lab) and used under approved MTA.*

*** Noggin conditioned medium was obtained from Noggin producing cells (HEK293-mNoggin-Fc, from Hans Clevers lab) and used under approved MTA.*

2.3 Medium for tumor fibroblast expansion

Component	Concentration (stock)	Amount
DMEM (Thermo Fisher, #31966-047)	1x	500 ml
Fetal Bovine Serum (Sigma-Aldrich, #F7524)	10x	60 ml
Pent/Strep (Thermo Fisher, #15140-122)	100x	5ml

3. Transduction of organoids

- Thaw the virus (200 µl/transduction) and Matrigel on ice. Use 1 full well of organoids that was passaged at least 5 days before transduction.
- Remove the medium, add 1 ml of cold washing medium (WM) and resuspend the Matrigel drops using a 1000 µl pipette.
- Collect the organoids in a 5 ml Eppendorf tube.
- Mechanically shear the organoids using a 10 µl tip on top of a 1000 µl tip.
- Vigorously pipette up and down 20-30 times.
- Fill up with cold WM and spin down at 1200 rpm for 5 min at 4 °C.
- Remove the supernatant of the pelleted organoids.
- Add 1 mL of Accutase and pipette up and down a few times.
- Incubate for 3 min at 37 °C in a water bath or heating block.
- Pipette up and down and check under the microscope if most cells are singularized.

- Fill up the tube with WM and pellet the organoids at 1500 rpm for 5 min at 4°C.
- Remove the supernatant and resuspend the cell pellet in 1 ml of Advanced DMEM+++ medium (adv DMEM).
- Divide the organoid suspension in 750 µl for the transduction and 250 µl for the control in a 12 well suspension plate.
- To the transduction add 200 µl of virus and 50 µl of adv DMEM medium.
- To the control add 750 µl of adv DMEM medium.
- In both wells add 1:1000 Y-27632 Rho kinase inhibitor and 1:1000 Polybrene.
- Wrap the plate with parafilm and centrifuge at 2200 rpm for 1 h at 32 °C.
- Remove the parafilm and place the plate in the incubator for 3 h.
- Collect each well in a 5 ml Eppendorf tube with 5 ml of WM and centrifuge at 1500 rpm for 5 min at 4 °C.
- Repeat the washing step 2 times.
- Remove the supernatant and add 20 µl of adv DMEM medium.
- Resuspend the transduction mix with 80 µl Matrigel and make 8 drops of 10 µl (4 drops/well) in a 12 well suspension plate.
- Resuspend the control in 40 µl Matrigel and make 4 drops of 10 µl (2 drops/well). In one well add Puromycin and in the other not.
- Place the plate in the incubator for 20-30 min.
- Add 1 ml of pre-warmed organoid medium, supplemented with 1:1000 Y-27632 Rho kinase inhibitor/well and place it back in the incubator.
- After 3 days start selection with 1:1000 Puromycin (leave one control well without Puromycin).
- Usually, the organoids are ready for passaging after 1-2 weeks.

4. Transduction of fibroblasts

- Thaw the virus on ice (200 µl/transduction). Usually use one full 10 cm culture dish with fibroblasts for 5 transduction reactions.
- Add 1 ml of 0.1 % gelatin in PBS to five 6 cm culture dishes and let them solidify for 20-30 min in the incubator.
- Remove the medium from the fibroblasts, add 3 ml of PBS and remove it as well.

- Add 1 ml of Trypsin and place it back in the incubator for 4-5 min.
- After 4 min check under the microscope if the cells started to detach and collect with 4 ml of fibroblast medium in a 5 ml Eppendorf tube.
- Centrifuge at 1500 rpm for 5 min at 4 °C and remove the supernatant.
- Collect the cell pellet in 1 ml fibroblast medium.
- Take up the gelatin from the 6 cm dishes and add 3.6 ml of pre-warmed fibroblast medium with 1:1000 Polybrene.
- In each plate add 200µl of the fibroblasts and 200 µl of virus and place them back in the incubator.
- After 5-6 h remove the medium and add 4 ml of fibroblast medium (check before if fibroblasts are attached).
- After 3 days start selection with 1:4000 Puromycin.
- When the plates are confluent, split the 5 plates in 2 of 10 cm gelatin-coated dishes. After each passage slowly increase the Puromycin concentration to 1:2000.