IVISbrite lentiviral particles transduction protocol

I. Transduction of target cells

Materials

- 1 vial IVISbrite™ RediFect™ Lentiviral Particles (Part #: CLS960002, CLS960003, or CLS960004) containing 200 µL of lentiviral particles at a concentration of 1 x 10^7 IU/ml stock = 2x10^6 infectious lentiviral particles in 200 µL phosphate buffered saline.
- Polybrene (Hexadimethrine Bromide) or LentiBOOST™ (Sirion Biotech GmbH)
- Complete medium containing 10% fetal bovine serum
- 96 well cell culture plates
- 96 well black cell culture plates
- Mammalian cells to be transduced

Remember that you will be handling and working with samples containing infectious viral particles. Strictly adhere to BSL-2 safety guidelines, including without limitation, always wearing gloves, using filtered tips, and working under a class II biosafety cabinet. Follow the recommended guidelines for all materials containing BSL-2 organisms. For further safety information, please consult with the guidelines provided by your local authorities.

Usually, the maximum expression level won't be reached until 48h after transduction. This is especially true for sensitive cells, where it may be helpful to allow adequate time between stressful events such as splitting and selection. Therefore, we recommend the schedule below:

- Day 1: Seeding
- Day 2: Transduction
- Day 3: Medium exchange
- Day 4: Splitting
- Day 5: Selection start

- **Day 1 - Seeding:** Plate 5,000 wild-type cells per well in complete medium (please check your cell growth medium condition to ensure cell viability) into wells of a 96 well plate (for cell culture). Incubate cells at 37 °C in a humidified 5% CO_2 incubator for 24 hours.
- **Day 2 - Transduction:** Thaw a vial of IVISbrite lentiviral particles on ice. Replace culture medium with 110ul of fresh complete medium containing hexadimethrine bromide (polybrene*) at a final concentration of 4 ug/ml.

* Polybrene enhances transduction of most cell lines. However, some cells are known to be sensitive to Polybrene. For sensitive cells like CD34+ hematopoietic stem cells (HSC), mesenchymal stem cells (MSC), neuronal stem cells, primary T cells, hard-to-transduce murine T cells, NK cells, and fibroblasts it is recommended to use LentiBOOST™ (Sirion Biotech GmbH) instead of Polybrene.

- 0, 5, and 50

Incubate MOI dose trials for 24 hours

0 = Control dose (no viral particles added, Polybrene or LentiBOOST™ respectively)

To determine the total number of Particles to use, multiply the number of cells by the desired MOI.

If the optimal MOI is unknown for the cell line of choice, it is recommended to use a range of MOIs (20-50).

- **Day 3 - Medium Exchange:** Discard the medium which contains virus (decontaminate medium during disposal) and gently wash the cells with fresh medium. Replace the medium with 120 µL of fresh pre-warmed complete culture medium and incubate cells for 24 hours.
- **Day 4 - Splitting:** Passage the cells to a new 96 well plate with 5,000 cells per well. Add fresh complete culture medium for a total volume of 120 µl per well. Passage the control group (MOI of 0) according to the same procedure.
- **Day 5 - Begin Selection:** Puromycin concentration should be optimized for each cell line; typical concentrations range from 2-5 µg/mL. ***CSL960003 Red F-luc GFP does not have puromycin resistance***. From our experience, optimization is achieved by using 3 puromycin concentrations: the one determined within the killing curve, double that concentration, and half of that concentration. (See Section III of this document for puromycin kill curve.)

Note: Puromycin is stable for up to 3 months at room temperature and at least 1 year at 4°C. For optimal stability and long-term storage, aqueous solution aliquots can be stored at -20°C.

- **Day 6 -** Repeat passaging procedure from Day 4. (Save 1 or 2 small flasks or 12 well plate of infected cells as backup if needed).
- **Day 8 -** Seed 5,000 and 1,000 cells/well in media on black cell culture plate. Freeze backup cells and original cells as 1st generation infected cells.
- After 2-4 weeks, thaw a frozen vial of 1st generation infected cells and perform *in vitro* assay for expression of luciferase to ensure stable transfection of healthy stock.
- **Day 10 - and forward:** Perform *in vitro* assay for bioluminescence signal from the expression of RedF-luc luciferase (black cell culture plate) as follows:

Seed 5, 50, 500, 5000 cells/well. Incubate at 37°C for 5-10 minutes before performing bioluminescent imaging.
For *in vitro* bioluminescence assay, final concentration of D-luciferin is 150µg/mL in the media. Image cells (10 to 30 minutes in an IVIS) noting the peak imaging time.

Lentiviral-driven GFP will often have relatively low expression.

Once a good signal is confirmed (>100 photons/cell/sec), seed 50K and 100K cells per well in triplicates, and check the bioluminescence signal at 0, 1, 2, 3, and 4 weeks.

II. Generation of stable, monoclonal new Red F-luc cell lines

To generate homogeneous, monoclonal population of infected cells:

1. Prepare a serial dilution of a cell suspension yielding a final concentration of 1 to 2 cells per 120 µl and plate in 96 well plates. Change media 2 times per week. Mark wells that display good cell growth. Screen wells for duplicates and mark duplicate wells. Incubate cells for 2-8 weeks until they become confluent.
2. Split cells 1:1. Image one plate to determine BLI positive clones, and expand the positive clones from the second plate. Passage cells with flasks, starting from small size flasks. Expand the cells and collect in vials with 2 million cells per vial for freezing/storage as backup.
3. Check the cell viability and *in vitro* assay for expression of luciferase. Choose clones that display the same morphology as the parental culture.
4. Compare the cell growth pattern of parental cells with new Red F-luc cells. After confirmation of the morphology and bioluminescence signal *in vitro*, continue to test *in vivo* growth and signal stability using *in vivo* models.

III. Puromycin titration protocol (kill curve)

****For puromycin resistant cells only. DO NOT perform with Red F-luc GFP cell lines (CLS960003), as these do not have puromycin resistance.** ****

1. Plate 10,000 cells into wells of a 96-well plate with 120 µl fresh media.
2. The next day add puromycin to selected wells. We recommend a broad range, from 0,1 µg/ml to 10 µg/ml.
3. Examine cell viability every 2 days. Keep a record of survival percentage of each puromycin dose.
4. Culture for 10-14 days. Replace the media containing puromycin every 3 days.

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