Specific reagent: **Lipofectamine 2000** or **3000**

Important: Make a special bin for material in contact with viruses. Take an old medium box and add ~30mL of Anyosime 3X (special lenti).

The protocol gives indication for 1 well (yields approximately 3 mL of virus).

[4 days protocol version]

Day 1

Morning: Prepare the transfection mix (for each well)

- 200 µL **OptiMEM** + 4 µL **Lipofectamine 2000** (2µL Lipo / 1 µg DNA).

- Vortex, let incubate 1 hr RT.

Add DNA (total 2 µg) to the mix. Vortex well each tube after thawing.

- 0.3 µg pCMV\_VSVG / dMD2G (envelope protein) **PLA 422** [Current stock c=1.38ug/ul]

- 0.8 µg PsPax2 (RT, capside, integrase) **PLA 421** [Current stock c=0.66ug/ul]

- 0.9 µg pTRIP or pLenti6 or **any plasmid containing the LTR sequences and the genes to be incorporated in the lentivector**.

- Vortex, let incubate 10 min RT.

Plate **293FT cells** in 1 well: 2mL of cells at 800 000 cells/mL so 1.6e6 cells (in DMEM 61965026 Gibco).

Passage number must be < 20 !

Add the mix on the cells in suspension ! Mix gently.

Afternoon: > 4h later, change medium and replace with 3 mL RPMI (+PS +genta) **OR** Target cells culture medium.

-> Lenti bin !

*NB: RPMI is required when planning to infect primary cells with the virus that will be produced and not a problem when planning to infect HeLa. RPMI induces a higher titer than DMEM. If the target cells do not support RPMI, you may consider producing the virus in the medium they need.*

Day 2 (Ideally in the afternoon)

Virus harvest: Aspirate medium with syringe and expel through 0.45 µm filter. /!\ Don't pipette up-and down because viral particles are fragile ! -> Lenti bin !

Virus addition: Add the virus (2mL either fresh or freshly thawed, 1mL can be enough) + DMEM and adapted amount of **protamin** to enhance infection (stock at 1000X, add protamin at 3x (2x) if total V = 3mL (2mL)).

(It is also possible to put medium again on the 293FT for another harvest at day 3)

Day 3 (Ideally in the morning)

Wash the target cells twice with PBS and renew the medium. -> Lenti bin !

Day 4

Check that the transduction worked.

Viral Transduction

A stable cell line of these 3T3 ATCC-2023 fibroblasts expressing LifeAct-EGFP was produced by lentiviral transduction followed by FACS sorting.

I prepared a mix of OptiMEM (200µL, Thermofischer), Lipofectamin 2000 (4µL, Thermofischer) and DNA (2µg, including viral DNA and the plasmid of interest). I plated 1.6 million HEK-293FT cells (gift from the lab of Nicolas Manel) in 2mL of DMEM (ref 61965059, Thermofischer) and supplemented with the mix. 4 hours later I replaced the medium with 3mL of the 3T3 culture medium.

The next day I harvested the viruses by aspiring the medium with a syringe and filtered it with a 0.45 µm membrane. I added 1 mL of virus solution in three wells of a 6-wells plate containing cultures of 3T3 ATCC-2023 fibroblasts at different concentrations (around 10, 25 and 50% confluency).

The third day I washed the 3T3 cells twice with PBS and renewed the medium. On the fourth day I assessed in which of the three wells the cells had the most fluorescence signal while retaining a normal phenotype. I amplified these cells and proceeded to a FACS sorting to keep only cells within a narrow window of LifeAct-EGFP expression. These cells were amplified and frozen.

Supplementary informations:

The 2 µg DNA include:

- 0.3 µg of pMD2.G — envelope protein (gift from Francois-Xavier Gobert, Institut Curie)

- 0.8 µg of PsPax2 — reverse transcriptase, capside, integrase (gift from Francois-Xavier Gobert, Institut Curie)

- 0.9 µg of the plasmid of interest — pLenti Lifeact-EGFP BlastR (Addgene Plasmid #84383)