**Lentiviral vector production in 293FT – Version 1: Alice**

Specific reagent: **TransIT** (MirusBio, MIR6603 to MIR6606 depending of number of tubes), optimized for lenti transfection.

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).

[6 days protocol version] Day 0

Plate **293FT** at 500 000 cells/well in 1 mL DMEM.

***or***

[5 days protocol version] Day 1

Morning: Plate **293FT** at 800 000 cells/well in 1 mL DMEM. Passage number must be < 20 !

Afternoon: Prepare the transfection mix (for each well)

- 200 µL **OptiMEM** + 8 µL **Transit**

- Vortex, let incubate 1 hr RT.

Add DNA (total 3 µg) to the mix. Vortex well each tube after thawing.

- 0,4 µg pCMV\_VSVG / dMD2G (envelope protein) **PLA 422** [Current stock c=1.38ug/ul]

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

- 1,6 µg pTRIP or pLenti6 or **any plasmid containing the LTR sequences and the genes to be incorporated in the lentivector**.

- Vortex, 30 min RT

- Add 200 µL of the final mix per well.

Day 2 (Ideally in the morning)

Change medium and replace with 3 mL RPMI (+PS +genta) / 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 3 (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 4 (Ideally in the morning)

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

Day 5

Check that the transduction worked.

**Lentiviral vector production in 293FT – Version 2: Mathieu**

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** or **3000** (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** in 1 well: 2mL of cells at 800 000 cells/mL so 1.6e6 cells (in DMEM).

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.