Transfection protocols for HeapRG cells

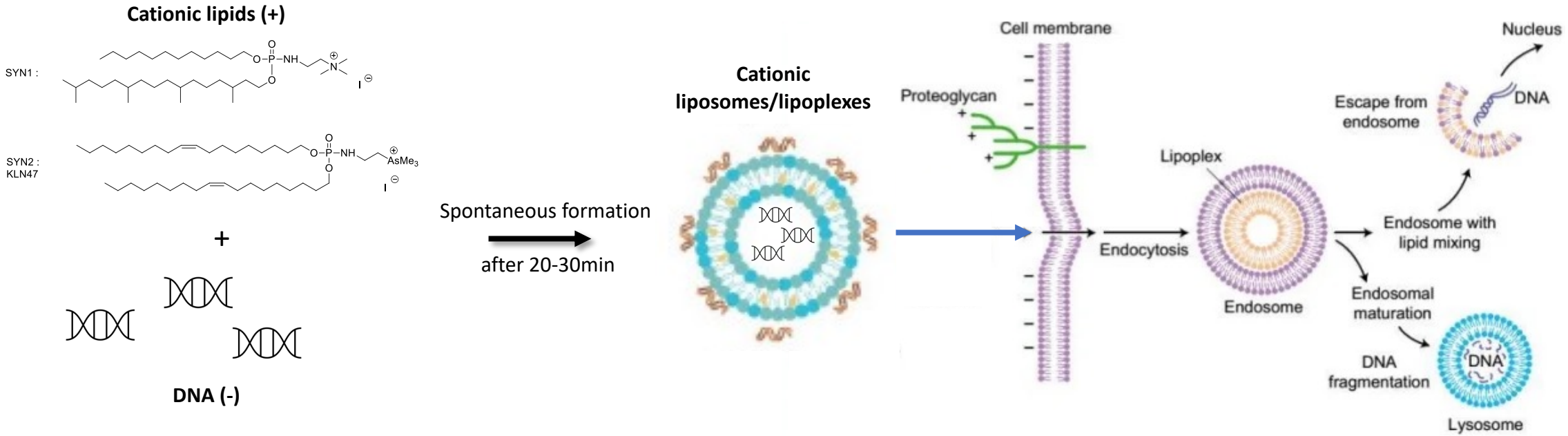
Technical meeting

03/03/2022

Hugo Coppens

Different technologies to achieve enforced expression :

➢ Lipofection (synthetic nanovectors for transient transfection)



Technical optimisation:

- Modulation of the ratio (R) between lipofectant Reagent : DNA
  - R between 1:1 and 4:1
  - Cells should be at 70-80% confluency max.

https://www.slideshare.net/nasimarshadi/transfection-method

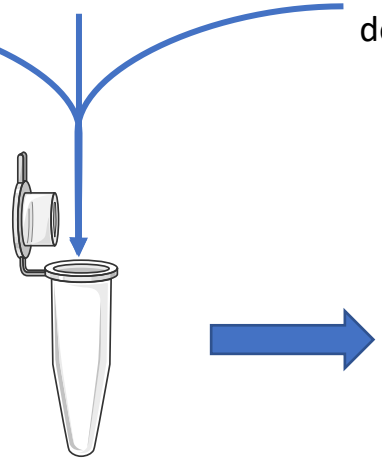
Protocol for lipofection of progenitor HepaRG cells :

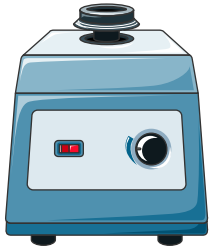
For cells in MW24:

1. 2μg DNA/well

2. Qsp 100μL Opti-MEM™

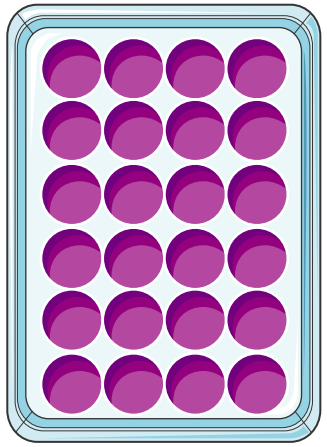
3. Lipofectant solution (volume dependant of the choosen ratio)





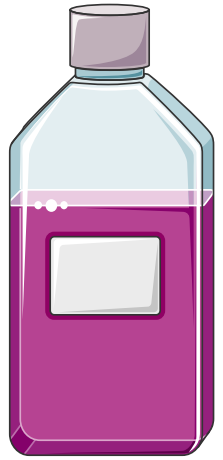
Vortex well for 20-30sec

Wait 20 – 30min



Apply mixture on the cells

J +1



Renew with fresh medium the next morning

⚠ Critical point : Formation of lipoplexes require serum free conditions (Opti-MEM™)

Protocol for lipofection of progenitor HepaRG cells :

Toxicity :

- Plamsid length should not exceed 10Kb
- Purity of the plasmid extraction (A260/A280 ratio of 1.7–1.9)
- Remove antibiotics from the cells medium
- Too high amount of transfection reagent

Low transfection rate :

- Too low amount of transfection reagent
- Remove SVF from the medium during the overnight transfection

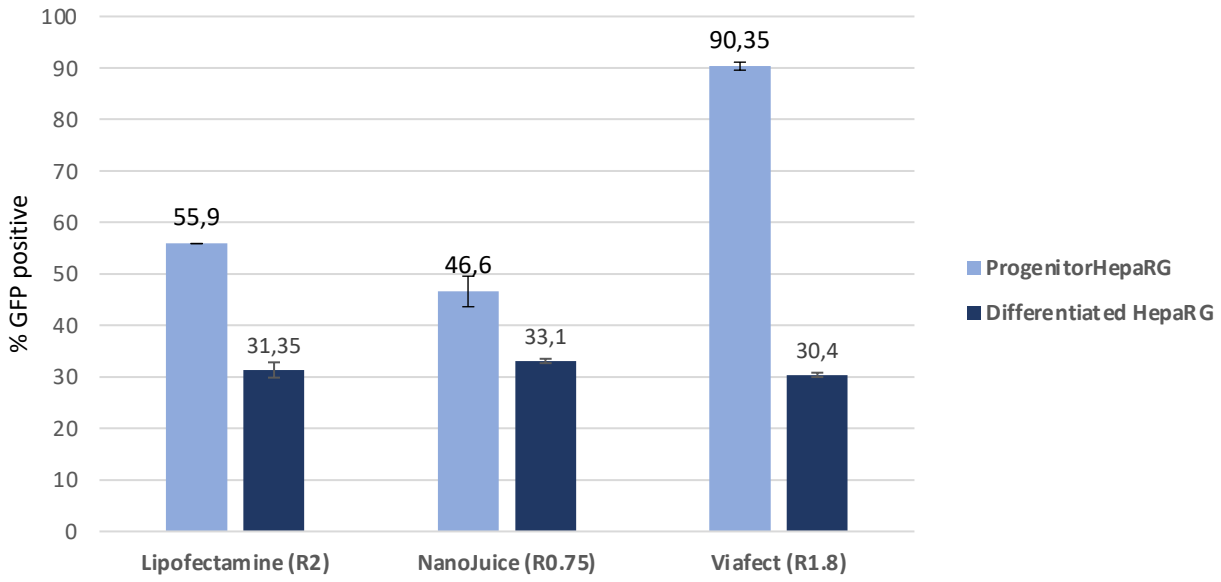
- Lipofectamine™ 3000 Transfection Reagent (0.1mL) : 101€ ou (0.75mL) : 632€
  Invitrogen **Ref: L3000001**

- ViaFect™ Transfection Reagent (0.75mL) : 346€
  Promega **Ref: E4981**

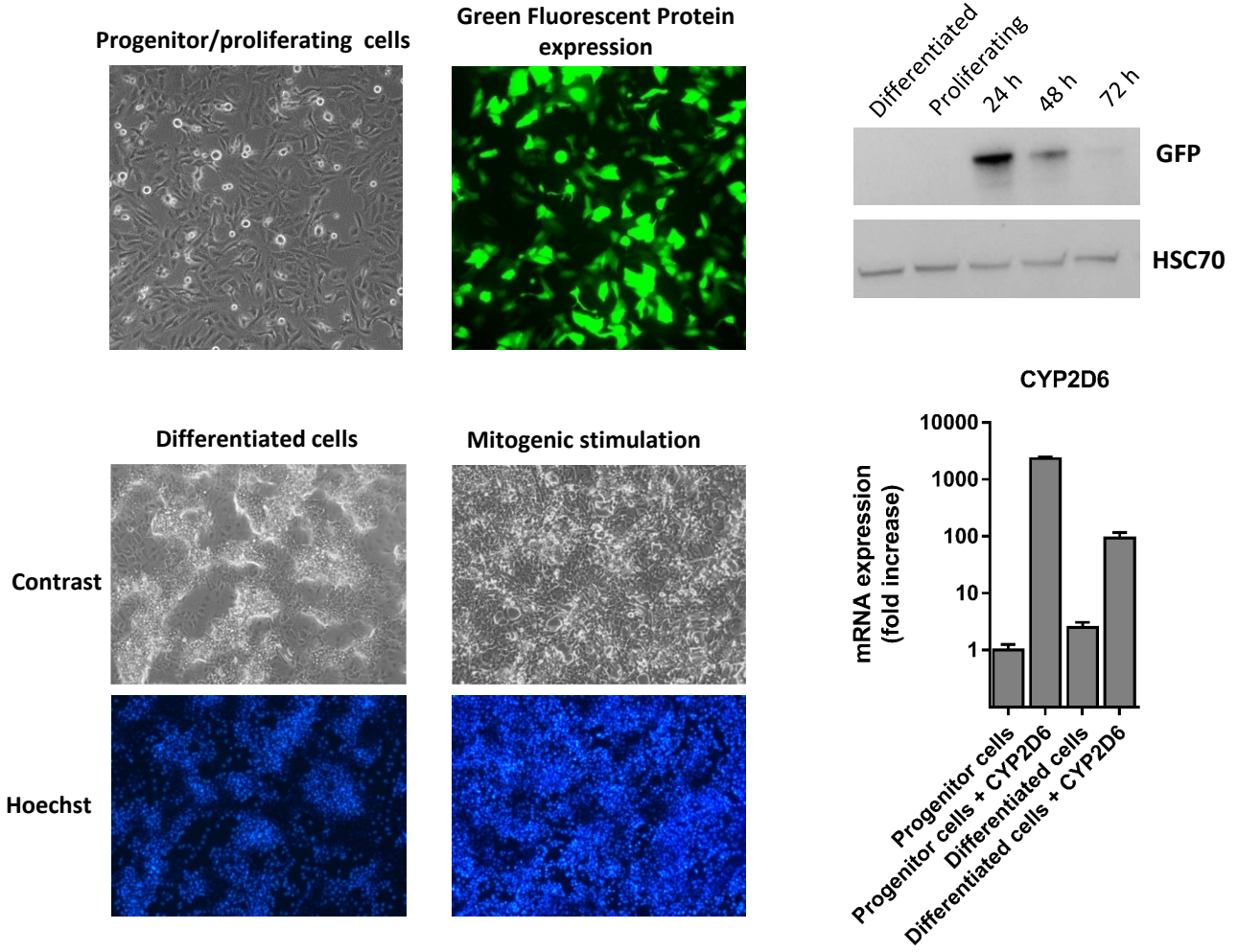
- NanoJuice® Core Transfection reagent (1mL) : 460€
  Merck Millipore **Ref: 71900-3**

Different technologies to achieve enforced expression :

➢ Lipofection (synthetic nanovectors for transient transfection)



- Lipofectamine: R2
- NanoJuice: R0.75
- Viafect: R1.8



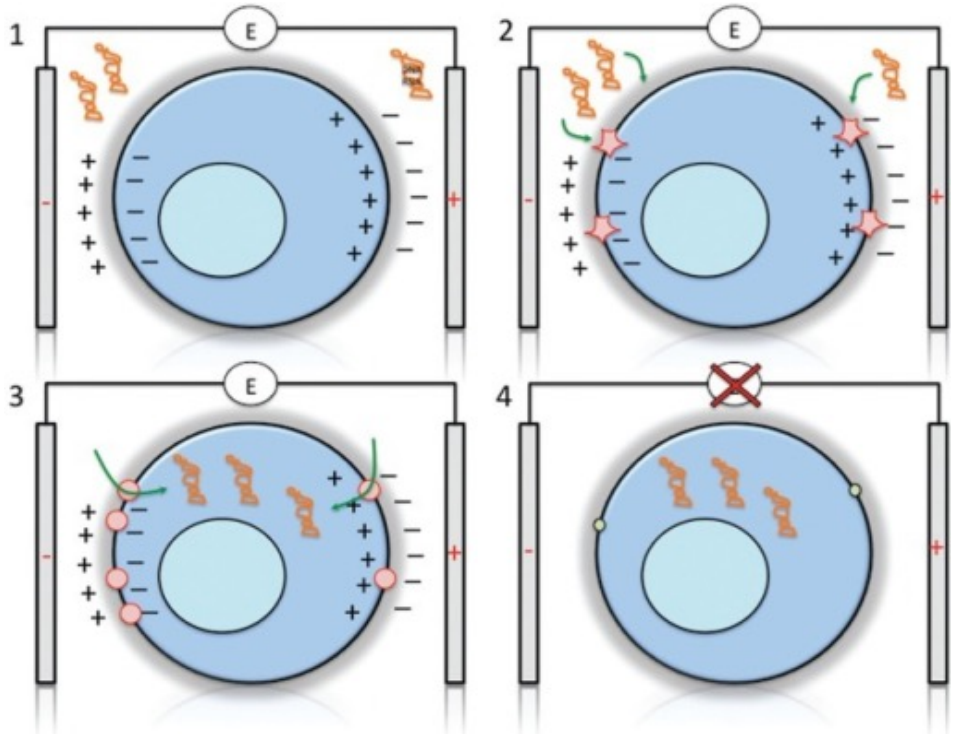
V. Laurent et al., *Biotechnol J*, **2010** ; Laurent V et al., , *Methods Mol Biol* **2013** ; Demazeau et al., *Int J Pharm* **2017** ; Vlach et al., in preparation

Different technologies to achieve enforced expression or gene repression :

- **Electroporation : efficient gene transfer in both progenitor and differentiated cells**

Invitrogen : Neon™

Price : 7500€



Luft C et al., J Biomol Screen, 2015

Technical features :

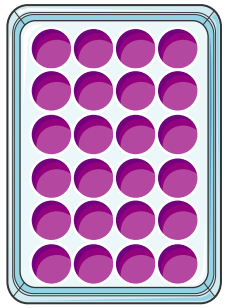
- 2 different tips: 10 μl for 100 000 cells
  100 μl for 1 000 000 cells

- 2 different resuspension buffer : R for volatge up to 1900V
  T for higher voltage

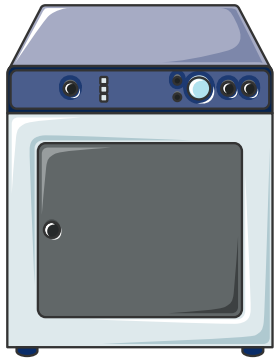
- 2 different electrolytic buffer : E for 10 μl tips
  E2 for 100 μl tips

Protocol for Electroporation of HepaRG cells :

Preparation of the Electroporator:

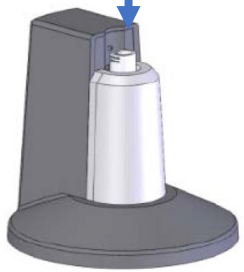


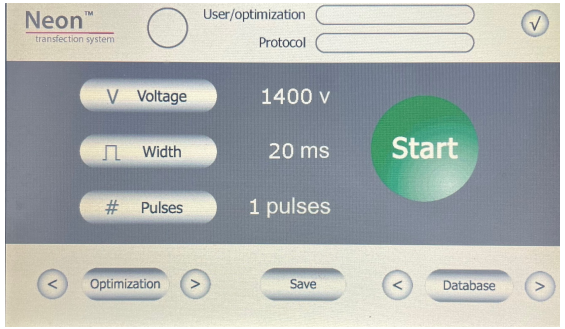
Prepare medium to recieve the cells



Incubate at 37° - 5% CO2

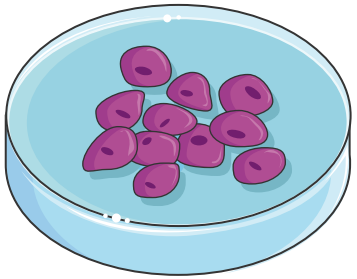
3mL of E (10 μL) or E2 buffer (100 μL)



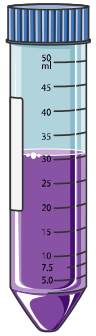


For HepaRG, 1 pulse of 20ms at 1500V for DNA plasmid (or 1700V for CRIPSR-Cas9)

Preparation of the cells:

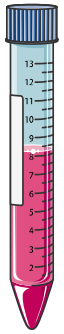


Harvest the cells



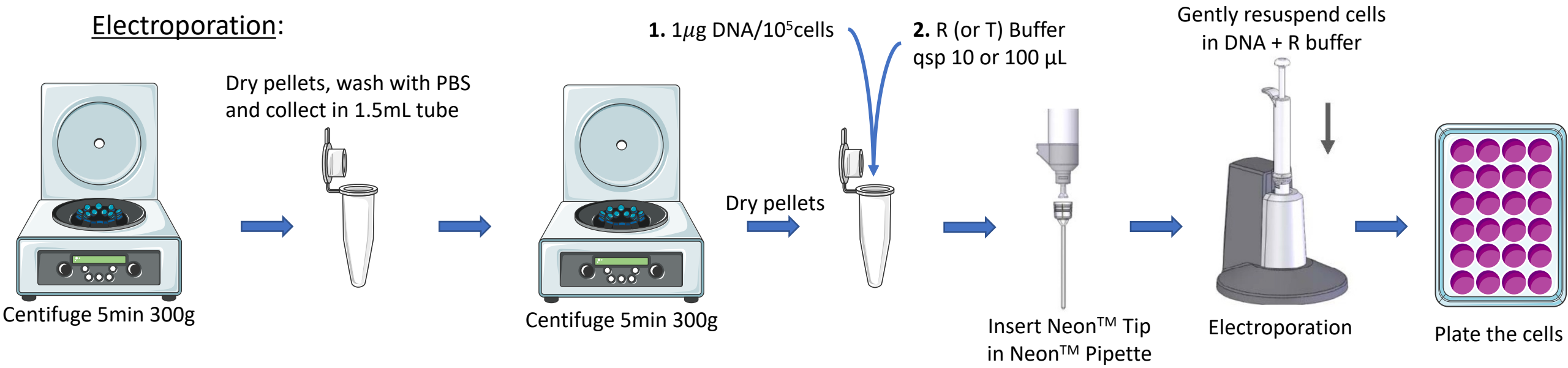
Count the cells

Retrive the desired number of cells



Protocol for Electroporation of HepaRG cells :

<u>Electroporation</u>:



⚠ Critical point: Max. 15-30 min in Resuspension Buffer at RT to avoid toxicity and a reduce transfection efficacy
Avoid air bubble to avoid arcing during electroporation
Neon™ Tip can be used 2-3 times in a row but must be changed between different DNA conditions
The cells can be centrifuged after electroporation and before plating to eliminate R buffer (toxicity)

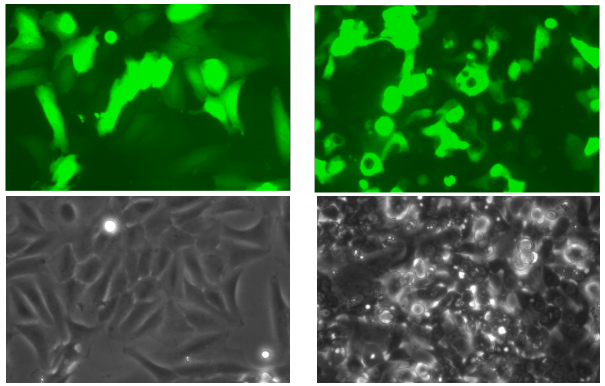
2 Invitrogen kit (tips and buffer) :

- Neon™ Transfection System 100µL Kit **Ref:** MPK10096 with 25 tips : 592€ or 96 tips : 1712€
- Neon™ Transfection System 10µl **Ref:** MPK1025 with 25 tips : 596€ or 96 tips : 1678€

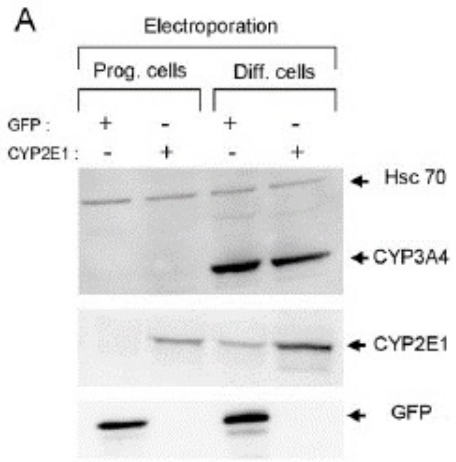
Different technologies to achieve enforced expression or gene repression :

- **Electroporation : efficient gene transfer in both progenitor and differentiated cells**

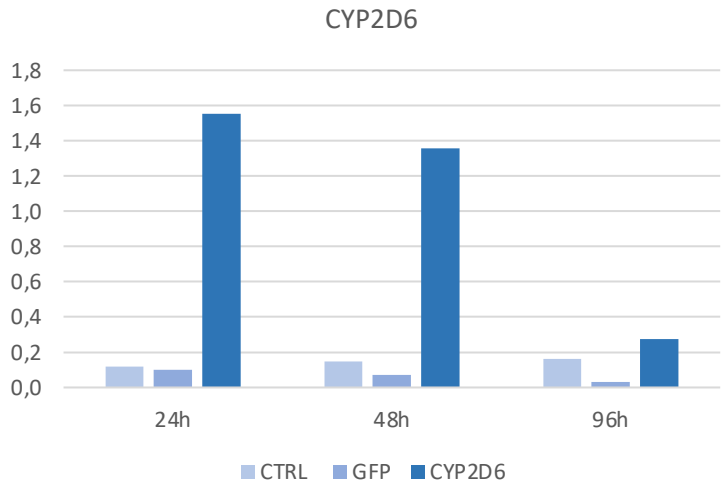
GFP expression

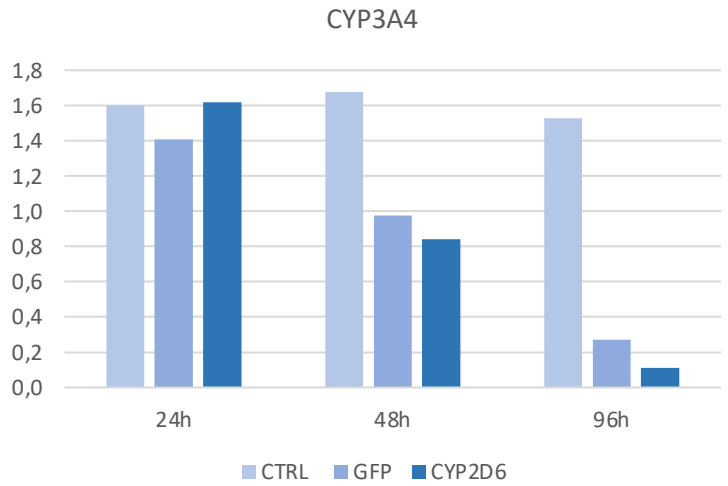


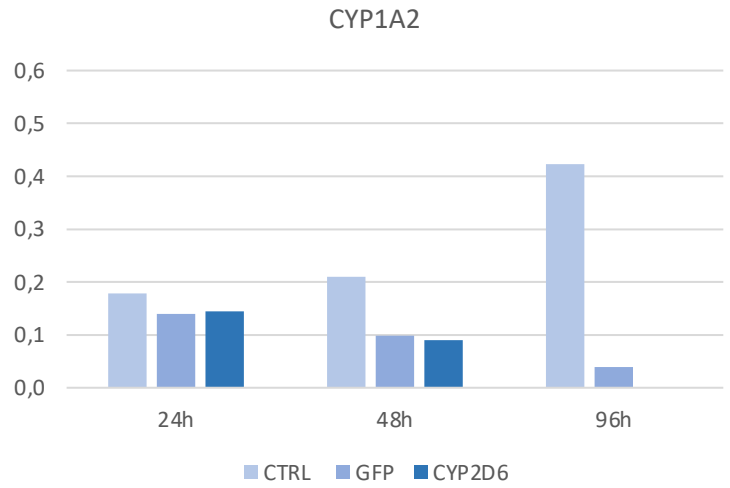
Progenitor cells | Differentiated cells

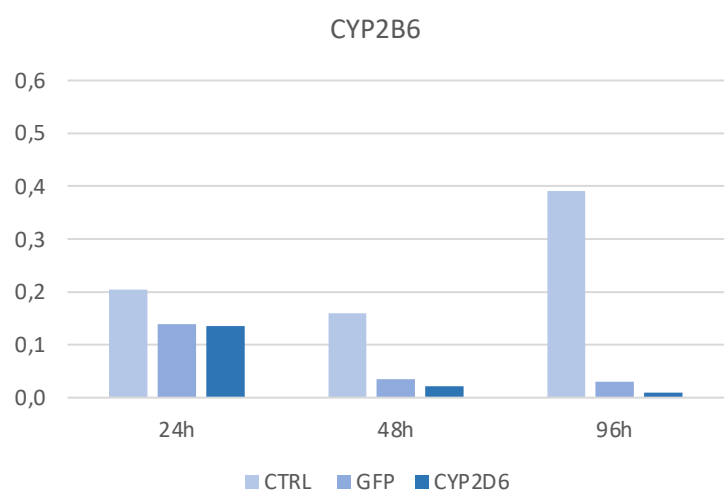


Enzymatic activities after Electroporation of Differentiated HepaRG with CYP2D6 (nmol/h/mg prot)





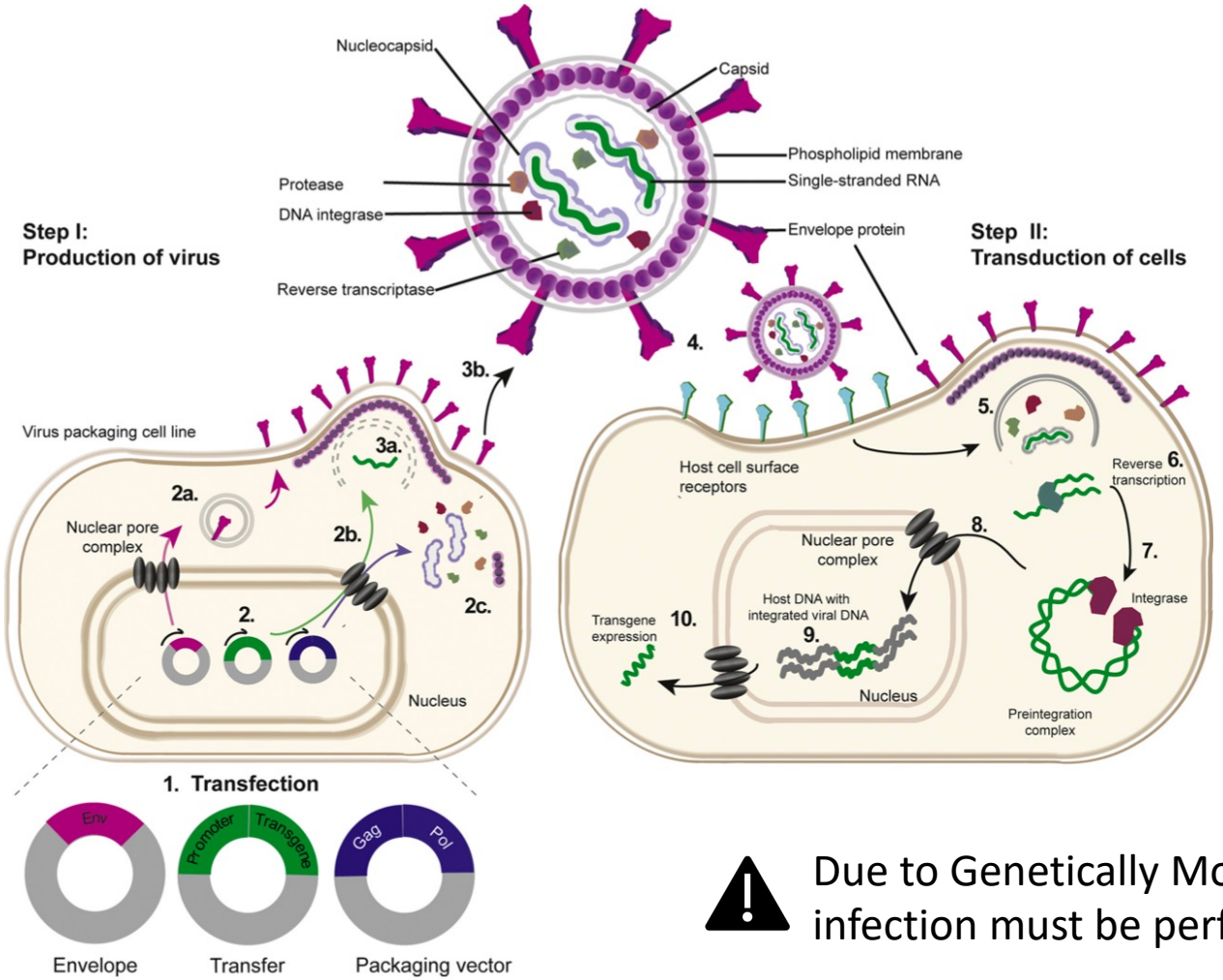




V. Laurent et al., *Biotechnol J*, 2010 ; Dumont et al., *Toxicol Appl Pharmacol* 2010 ; Laurent V et al., , *Methods Mol Biol* 2013

Different technologies to achieve enforced expression or gene repression :

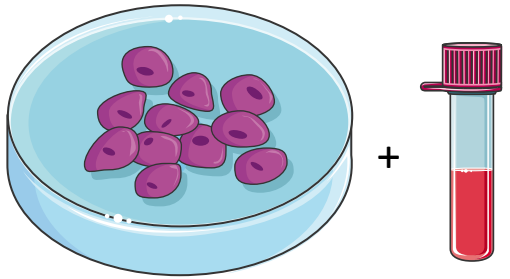
- **Lentiviral transduction : efficient gene transfer in progenitor (stable expression)**



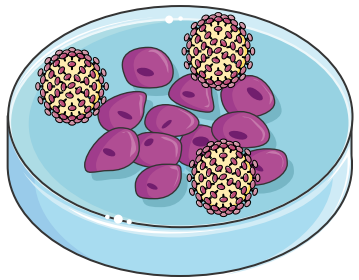
⚠ Due to Genetically Modified Organism (GMO) manipulation, infection must be performed in C4 culture area

Meaes et al., Neurosciences Letters, 2019

Protocol for Lentiviral infection of HepaRG cells :

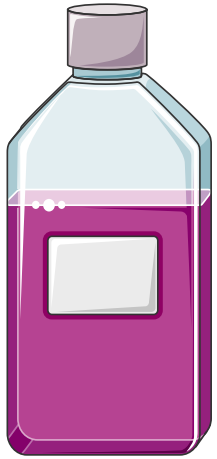


Thaw proliferative HepaRG cells and lentiviral solution



Infect at different Multiplicity of Infection (MOI) the cells depending of the Plaque Forming Unit (PFU)

J+1



Renew with fresh medium the next morning

⚠ Inactivate every material in contact with lentiviral solution in chlorine bleach

For example:

PFU = 1.10^7 viral particle/mL

For an infection at MOI = 1 infect 1M HepaRG cells with 0.1mL of lentivirus solution

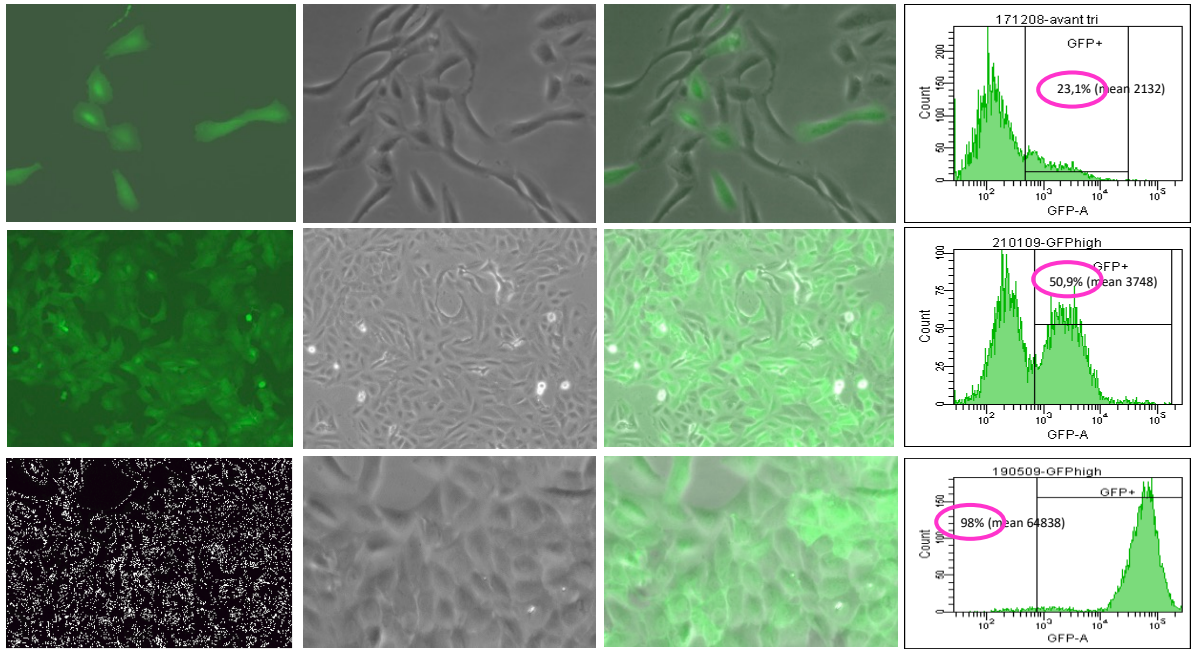
For an infection at MOI = 2 infect 1M HeapRG cells with 0.2mL of lentivirus solution

For an infection at MOI = 3 infect 1M HeapRG cells with 0.3mL of lentivirus solution

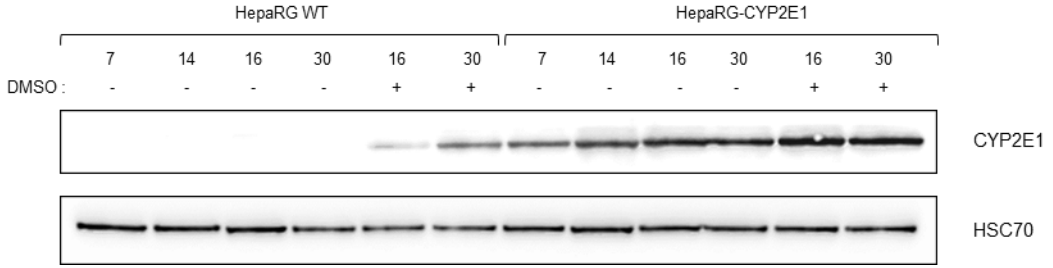
Different technologies to achieve enforced expression or gene repression :

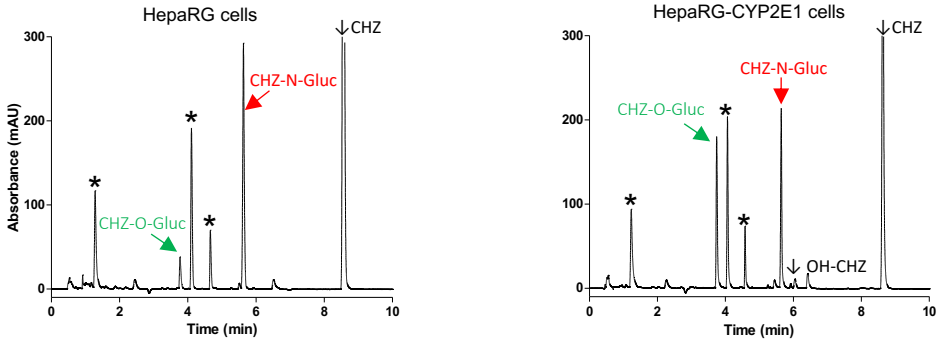
➢ Lentiviral transduction : efficient gene transfer in progenitor (stable expression)

Transduction at low viral titer (MOI < 1 pfu/cell)
GFP expression



Enforced expression of CYP2E1





Chlorzoxazone (CHZ) metabolism

Peyta et al., *BBA-Bioenergetics* **2015** ; Quesnot et al., *Arch Tox* **2018**.

Conclusions and R&D

Different technologies to achieve enforced expression or gene repression in HepaRG cells :

- **Lipofection/polyfection**
- **Electroporation**
- **Lentiviral transduction**

Knowledge and expertise in the culture procedure of HepaRG cells

- ✓ **Select the suitable technology for each application : transient vs stable expression**
- ✓ **Maintain the ability of HepaRG cells to proliferate and to differentiate after gene transfer**

R&D:

- ❑ **Production on large scale of recombinant HepaRG cells with enforced expression of selected genes**
- ❑ **Development of KO cells (CRISP/CAS9 invalidation)**

Thank you for your attention !

Questions ?

NPhDA

NuMeCan PhD Association