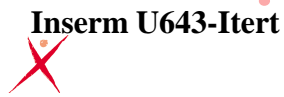




Institut national
de la santé et de la recherche médicale



La Transgénèse lentivirale



Application à la génération
de rats transgéniques

Séverine Remy

Historique

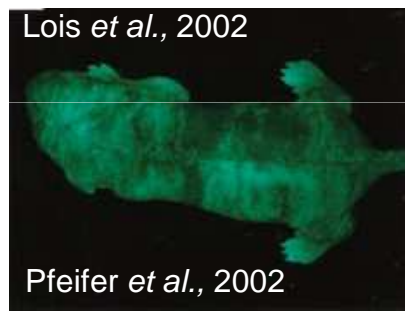
📖 1976: 1^{ère} Utilisation des Oncoretrovirus en Transgénèse *Jaenisch et al., 1976*

«silencing» au cours du développement (méthylation)

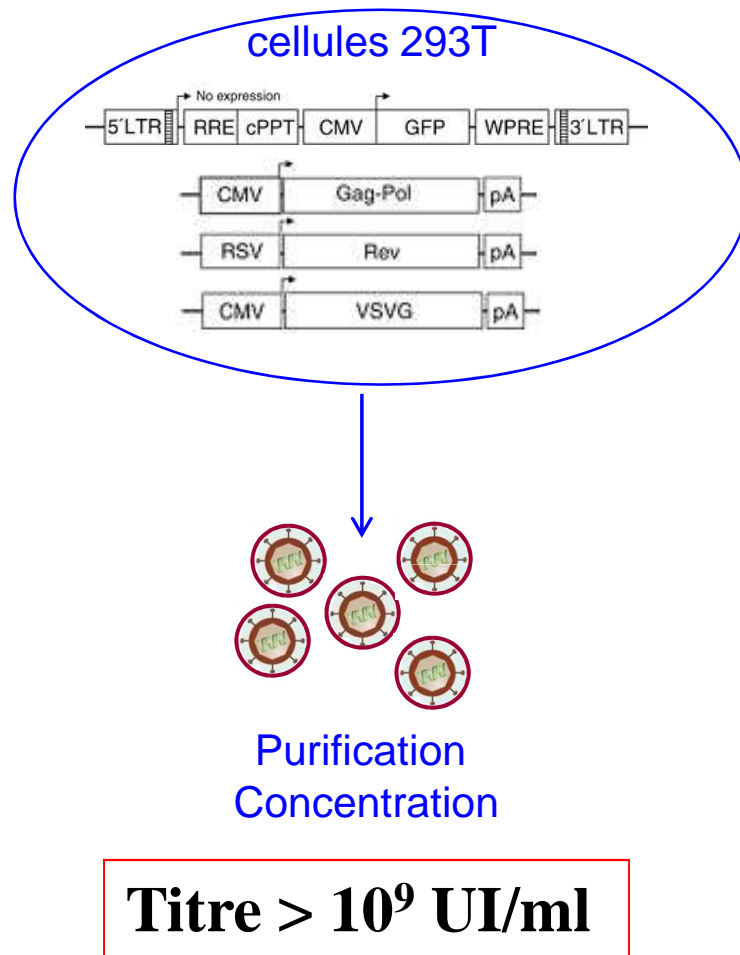
→ peu ou pas d'expression du transgène (souris, bovins)

📖 1996: 1^{ère} Utilisation des vecteurs lentiviraux en thérapie génique *Naldini et al., 1996*

📖 2002: 1^{ère} Utilisation des vecteurs lentiviraux en Transgénèse



Design des vecteurs lentiviraux



Promoteur

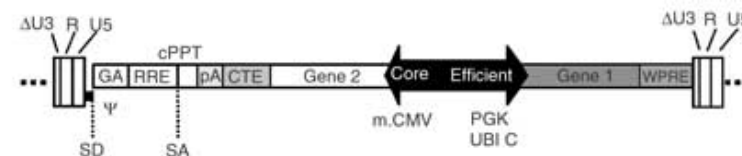
- ubiquitaire
- tissu-spécifique
- inductible

Multiple transgènes

- Monocistronique

IRES, 2A

- Promoteurs bidirectionnels



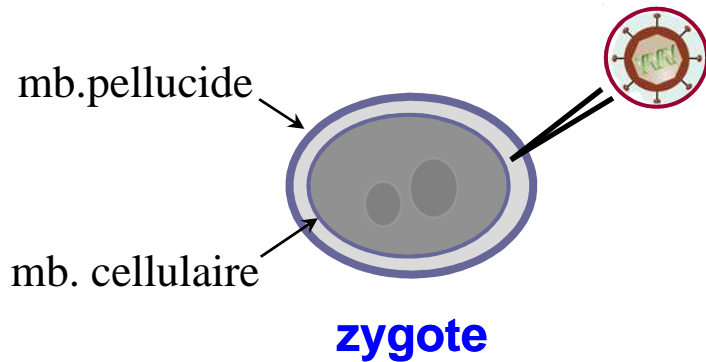
Capacité d'empaquetage

~5kb (+ ~2.2 kb backbone/WPRE)

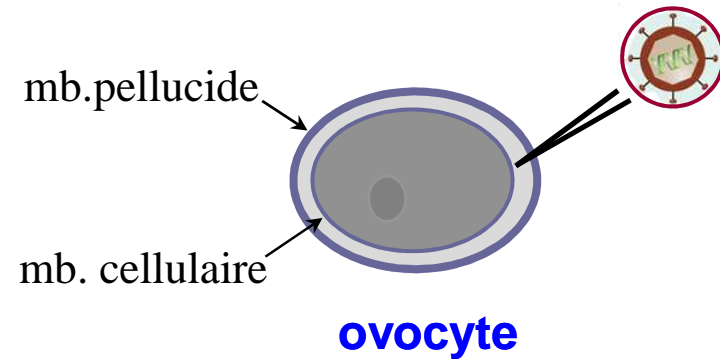
G. Tiscornia, Nat. Protocols 2006

S. Remy, TH. NGuyen et al. Methods Mol Biol. 2010 in press

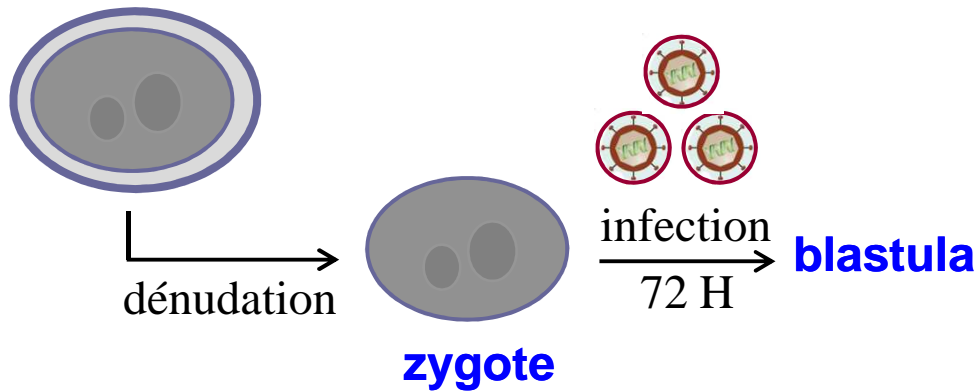
Méthodes



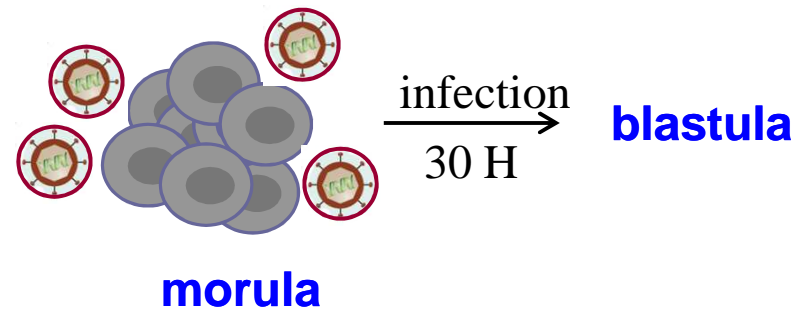
- Souris** Lois *et al.*, 2002
- Rat** Lois *et al.*, 2002; van den Brandt *et al.*, 2004
- Porc** Hofmann *et al.*, 2003; Whitelaw *et al.*, 2004
- Ovins** Ritchie *et al.*, 2009
- Macaques** Kubisch *et al.*, 2008



- Bovins** Hoffman *et al.*, 2004
- Ovins** Ritchie *et al.*, 2009
- Macaques** Kubisch *et al.*, 2008



Souris Lois *et al.*, 2002



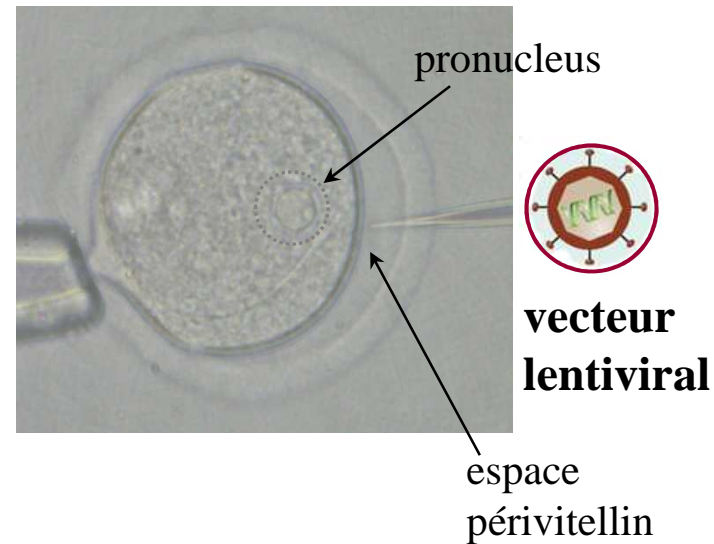
Souris Pfeifer *et al.*, 2002

Male germ-line stem cells

- Souris** Nagano *et al.*, 2002
- Rat** Hamra *et al.*, 2002

Atouts

- ☺ techniquement + facile
- ☺ moins invasif, survie +++
Rat -Souris 60-95%
- ☺ taux élevé de transgéniques
Rats 20-60% (DNA -MI : 1-5%)
Souris 70-80% (DNA -MI : 5-20%)
Porc 70-90% (DNA -MI : 10%)



| | Espèce | Souche | Taux de Tg | Références |
|-----------------------------|--------|--------|------------|---------------------------------|
| ADN | Rat | Lewis | 6.7 % | Popova et al., Transg.Res. 2005 |
| Vecteurs lentiviraux | Rat | Lewis | 33 % | Van den Brandt, Genesis 2004 |

- ☺ taux d'expression du transgène dans F0 élevé > 60%
- ☺ 1 à plusieurs sites d'insertion → augmente efficacité

Limites

☹ accès

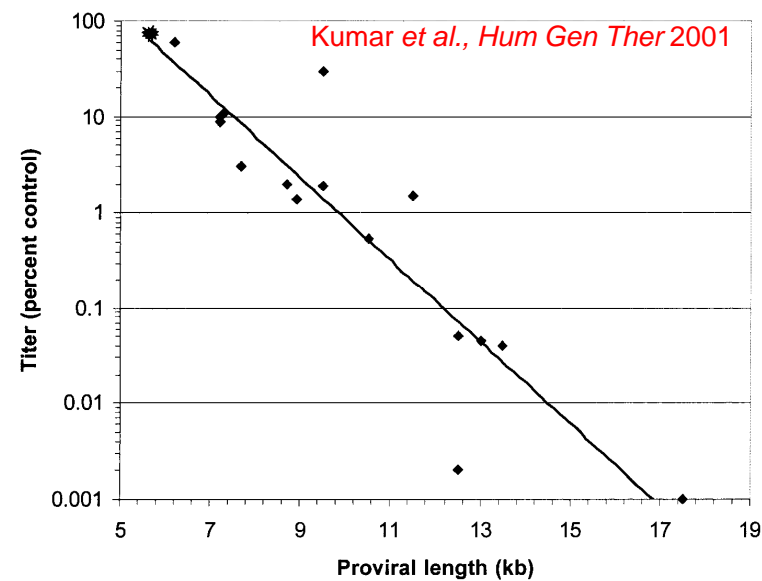
☹ pureté et titre

| | Titre (UI/ml) | Taux de survie à la microinjection | Taux de Naissances | Taux de Tg |
|--------|----------------------|------------------------------------|--------------------|------------|
| LV 1* | 1.8×10^{10} | 87% | 11% | 60% |
| LV 2* | 1.98×10^9 | 70% | 35% | 18% |
| LV 3* | 1.2×10^9 | 71% | 27% | 12.5% |
| LV 4** | 2.5×10^8 | 85% | 25% | 86% |

*ultracentrifugation

**chromatographie

☹ capacité d'empaquetage limitée



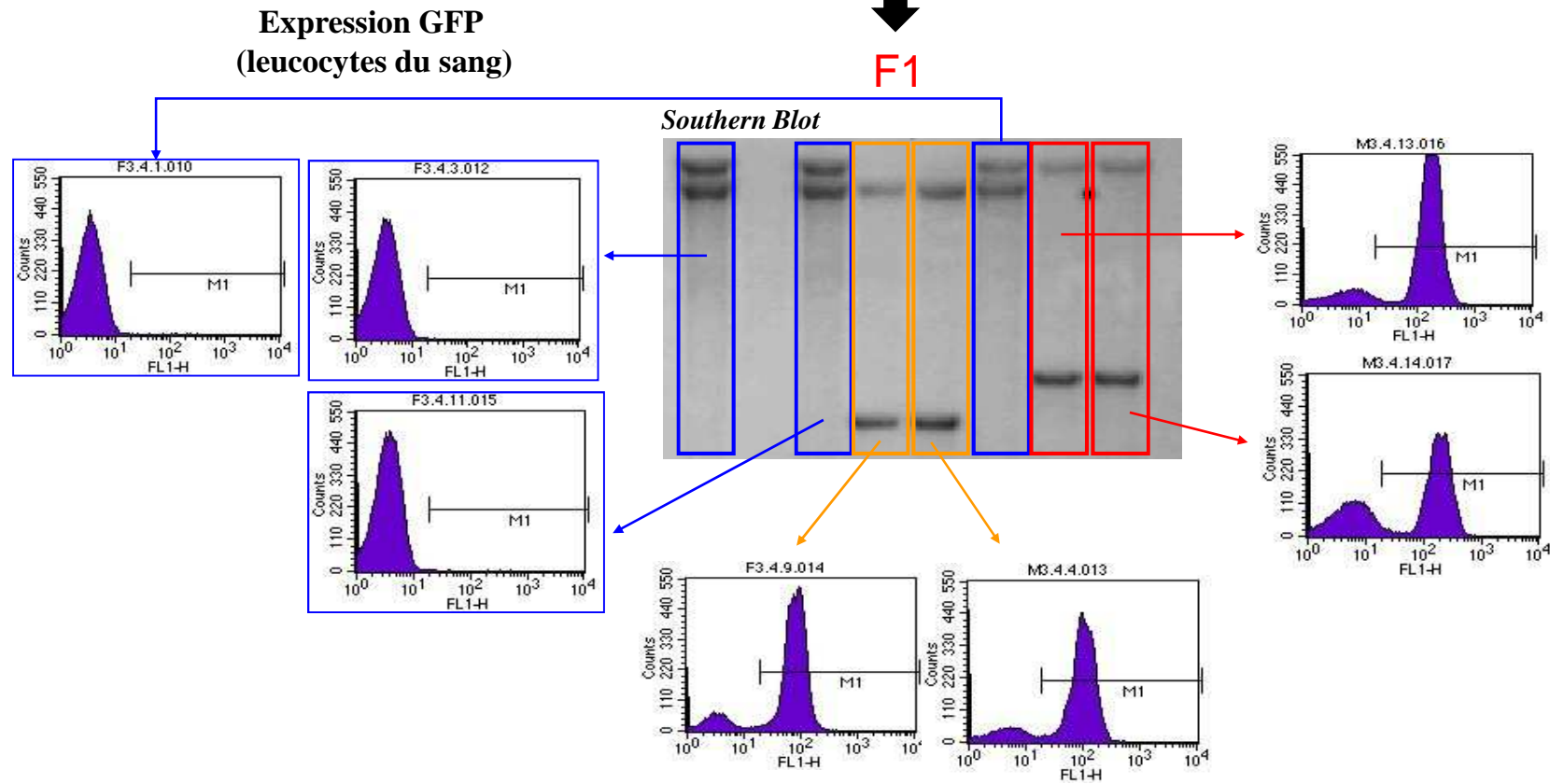
Limites

☺ multiples sites d'insertion → ségrégation au hasard dans la descendance

F0: 3 sites d'insertions



F1



☺ risque biologique modéré

Points à éclaircir

⊕ degré de mosaïcisme

- minimal chez la souris,
intégration dans le génome ~4-16h après transduction MO. Sauvain *et al.*, J. Virol. 2008
- plus élevé chez le rat J. van den Brandt *et al.*, Genesis 2004

⊕ régulation épigénétique

- hyperméthylation (30% sites) A. Hofmann *et al.*, Mol. Ther. 2006

Lentiviral vectors containing an enhancer-less ubiquitously acting chromatin opening element (UCOE) provide highly reproducible and stable transgene expression in hematopoietic cells

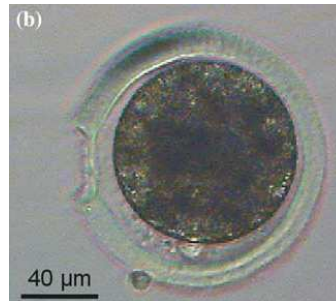
Fang Zhang,¹ Susannah I. Thornhill,¹ Steven J. Howe,¹ Meera Ulaganathan,¹ Axel Schambach,² Joanna Sinclair,¹ Christine Kinnon,¹ H. Bobby Gaspar,^{1,3} Michael Antoniou,⁴ and Adrian J. Thrasher^{1,3}

Blood, 2008

Innovations en Transgénèse lentivirale

↳ Alternatives à la microinjection sous la pellucide:

- ⊕ utilisation du laser + infection



Bovins

Ewerling *et al.*, 2006

- ⊕ microinjection dans le cytoplasme (10^9 UI/ml)

Efficacité ~75% (46% microinjection périvitelline) **Souris** Yang *et al.*, 2007

↳ Knock-down: Vecteurs lentiviraux exprimant shRNA

Souris Tiscornia *et al.*, 2003

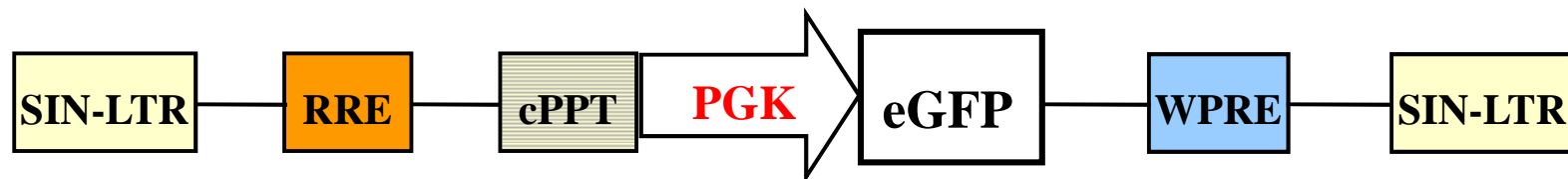
Rat stabilité sur plusieurs générations Dann *et al.*, 2006

↳ Vecteurs lentiviraux non-intégratifs pour expression transitoire

Rats transgéniques GFP

Applications

- Analyse de lignages cellulaires, ontogénèse et différenciation
- Transplantation cellules/organes
- Médecine régénérative
- Mécanismes de Tolérance immunitaire



Sprague-Dawley

Nb.embryons
injectés

109

Nb.embryos
transférés

95

Nb.petits

10

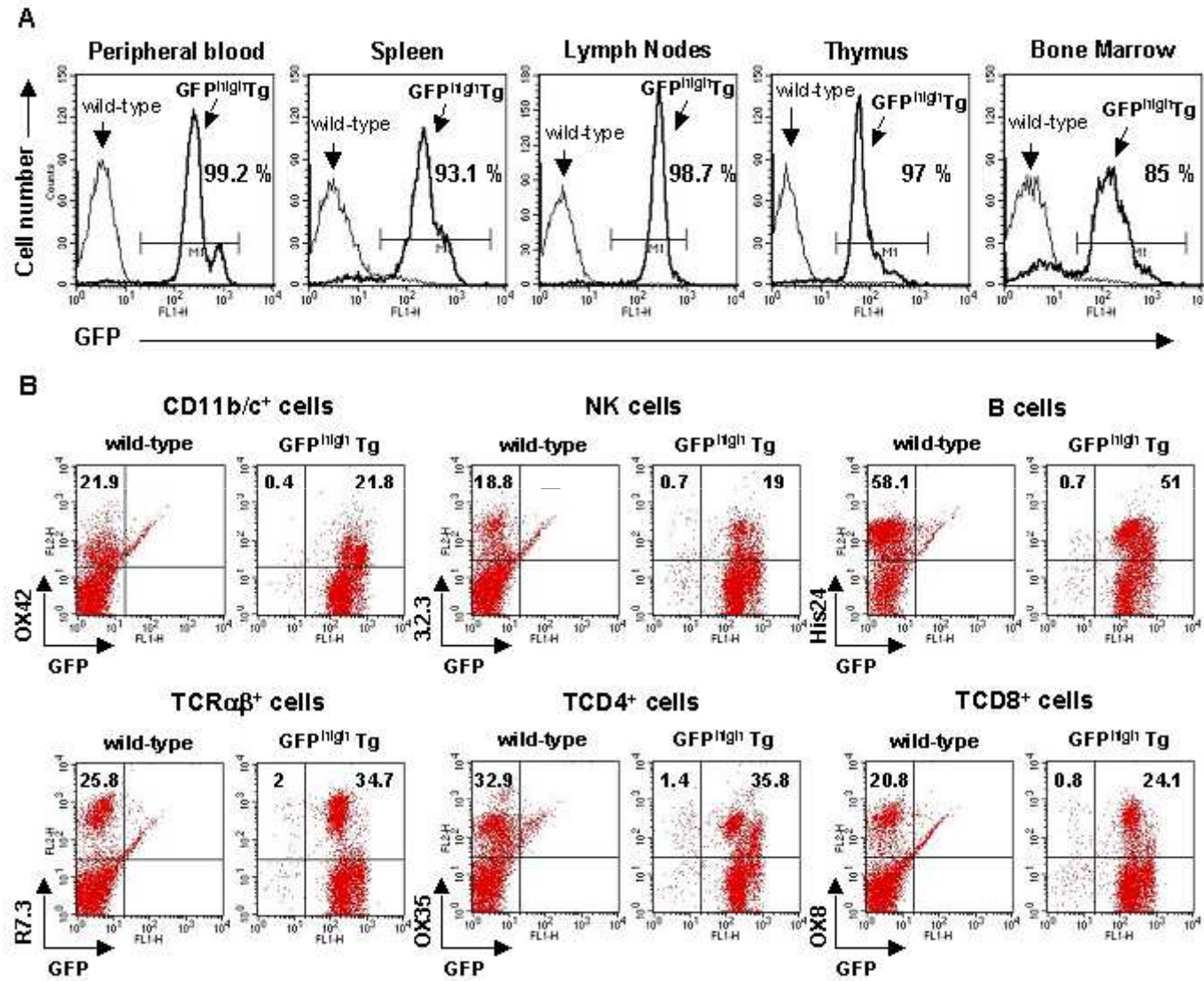
Nb.Tg

6 (60 %)

Nb.Tg/
injectés

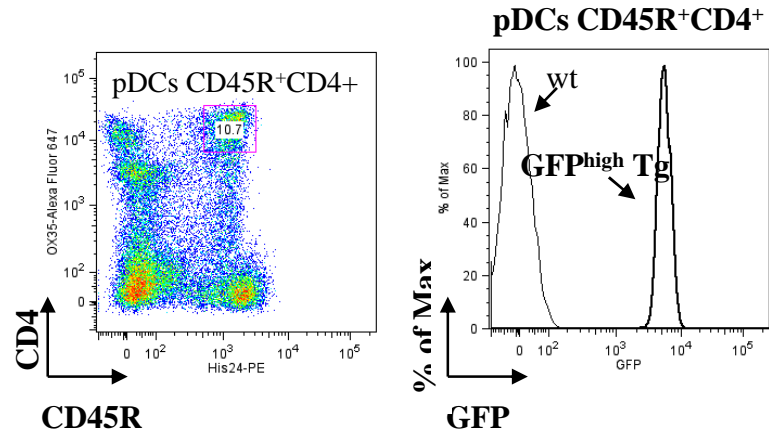
6.3 %

Rats Tg GFP "high expressers"

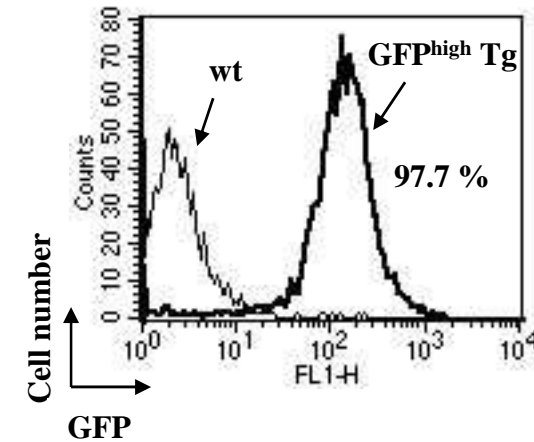


Rats Tg GFP "high expressers"

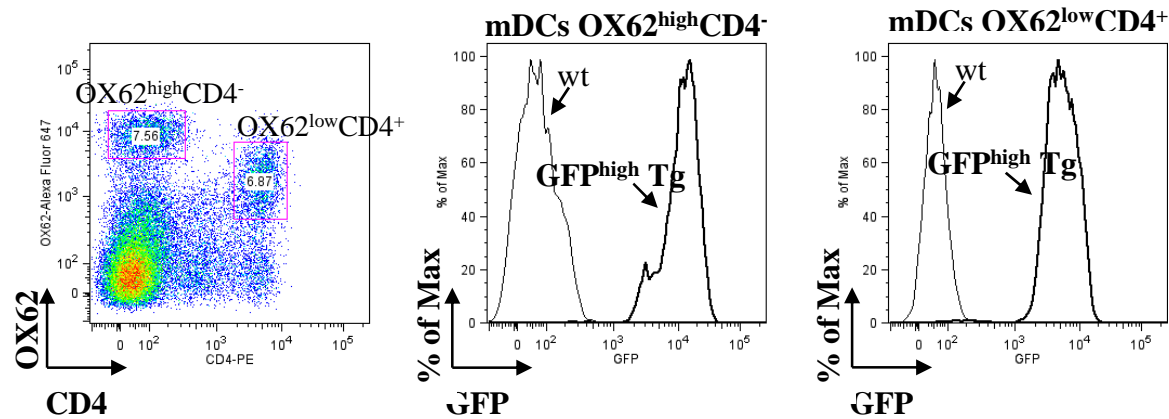
Plasmacytoid Dendritic Cells



Bone-marrow-derived dendritic cells

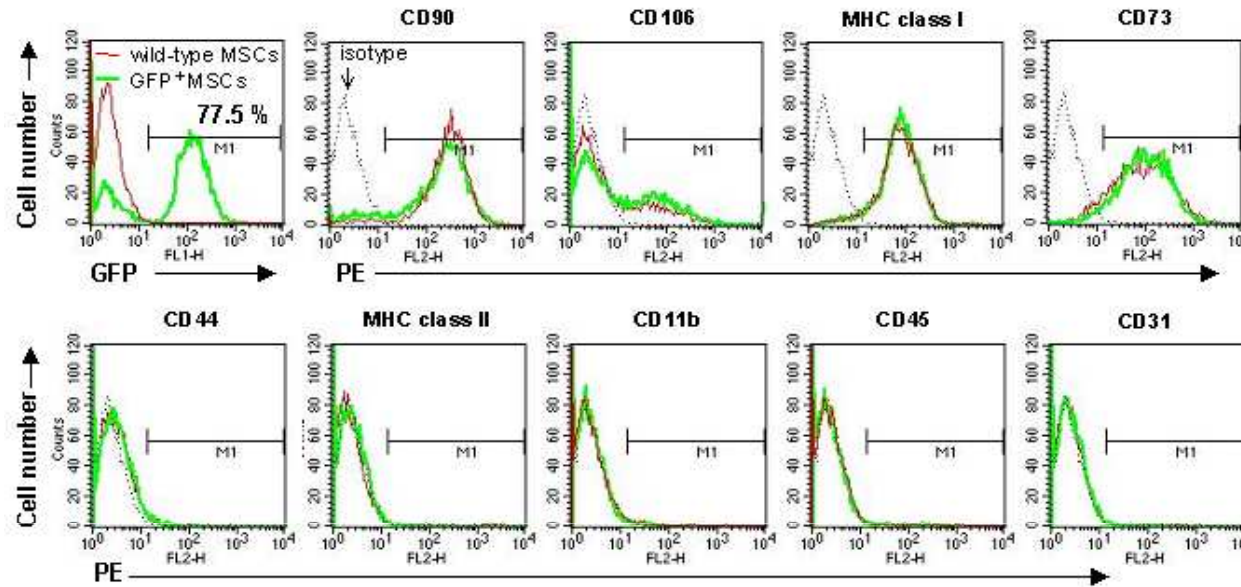


Myeloid dendritic cells

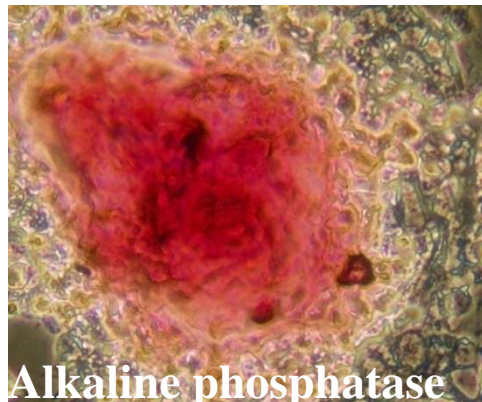


Rats Tg GFP "high expressers"

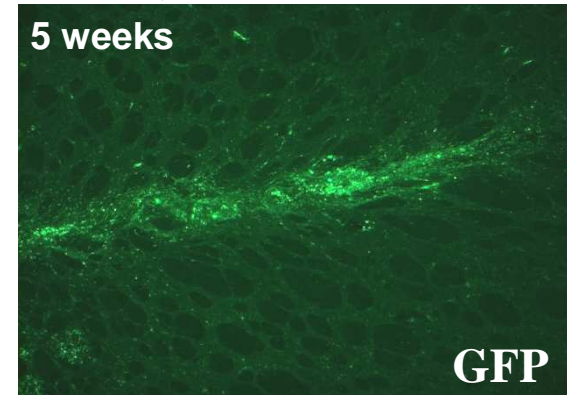
Mesenchymal stem cells



Differentiation en osteoblastes

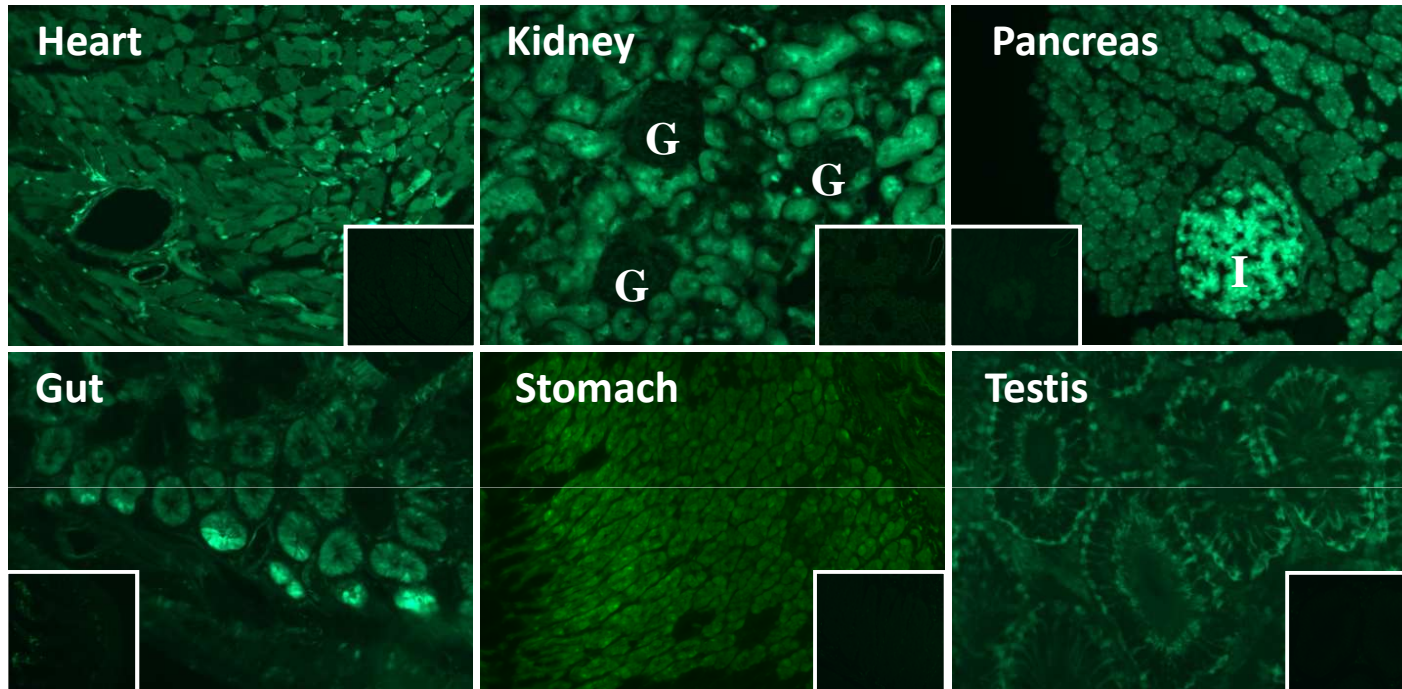


Greffe dans le striatum de rat



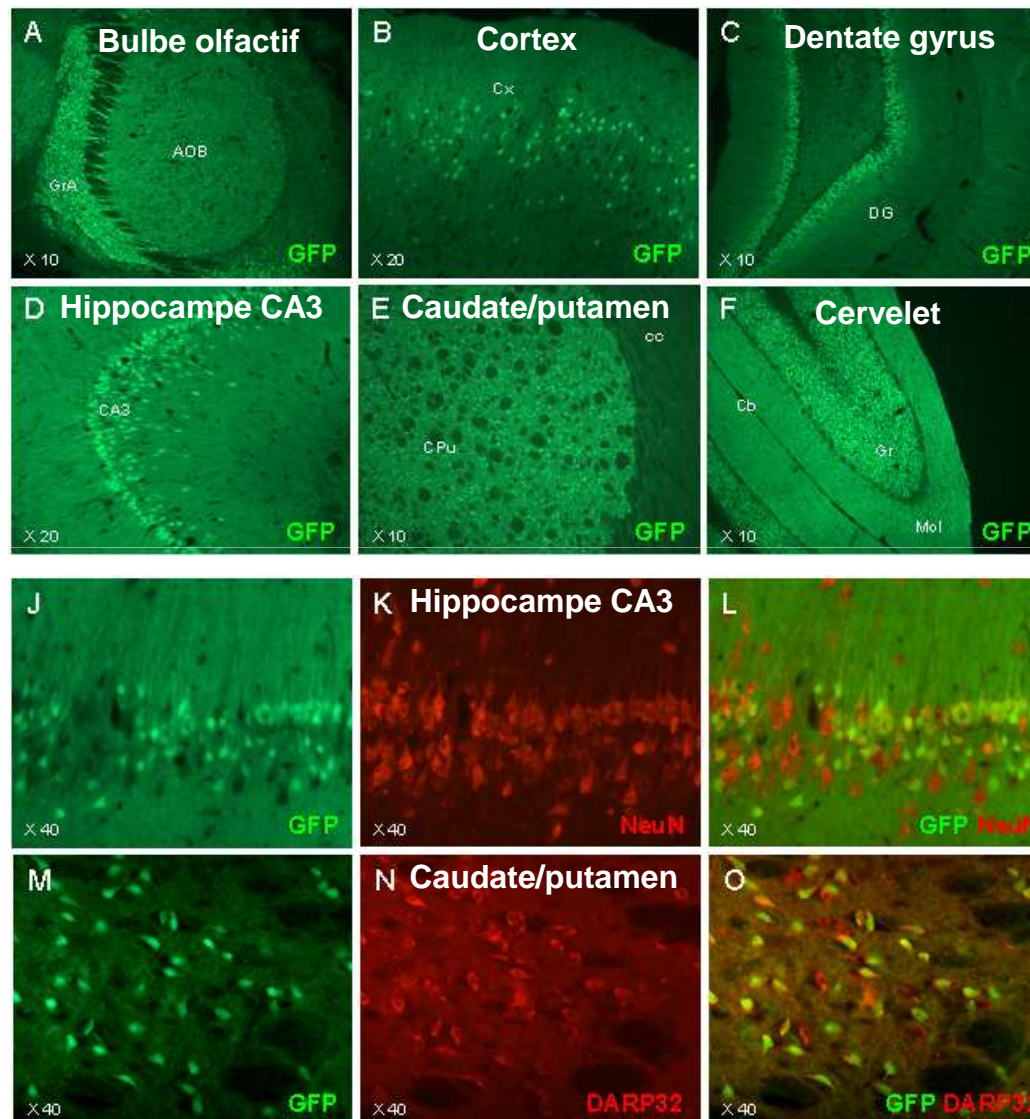
S. Remy et al. Trang. Res. 2010 in press

Rats Tg GFP “high expressers”



| | |
|------------------------|-----------|
| Ovary | ++ |
| Liver | + |
| Skin | + |
| Skeletal muscle | - |

Rats Tg GFP "high expressers"

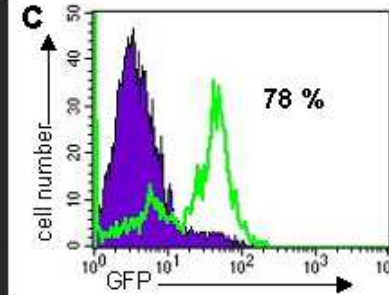
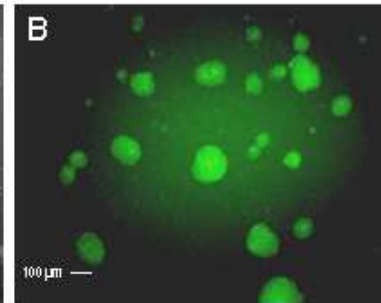
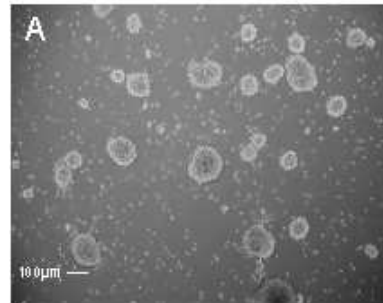
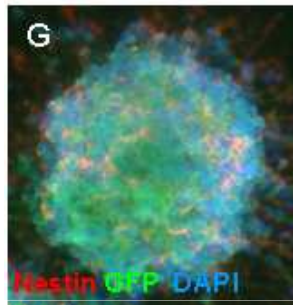


Rats Tg GFP "high expressers"

Neural Stem Cells

Neurospheres from E15 brains

5 days *in vitro* with FGF

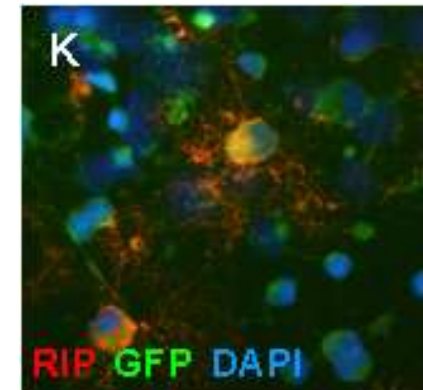
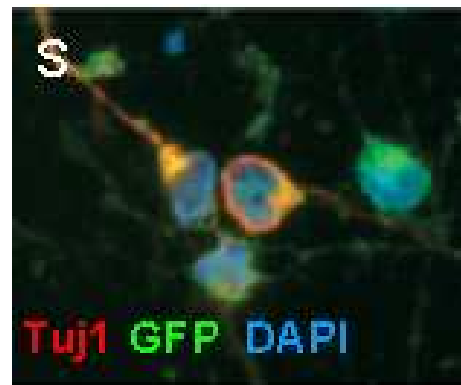
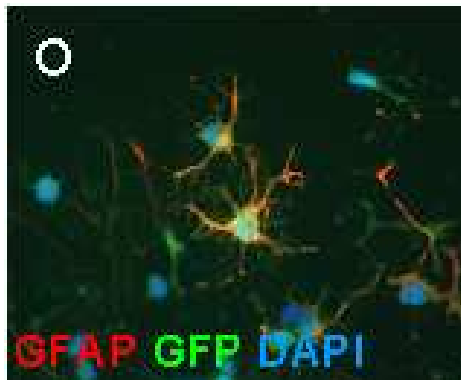


Differentiation

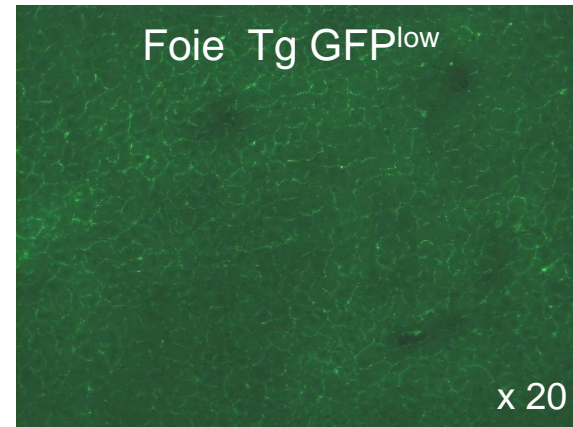
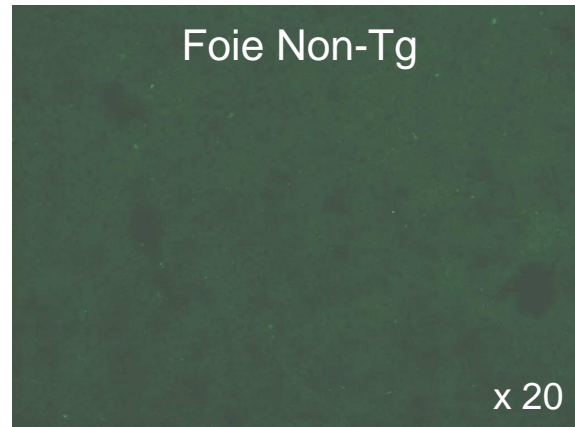
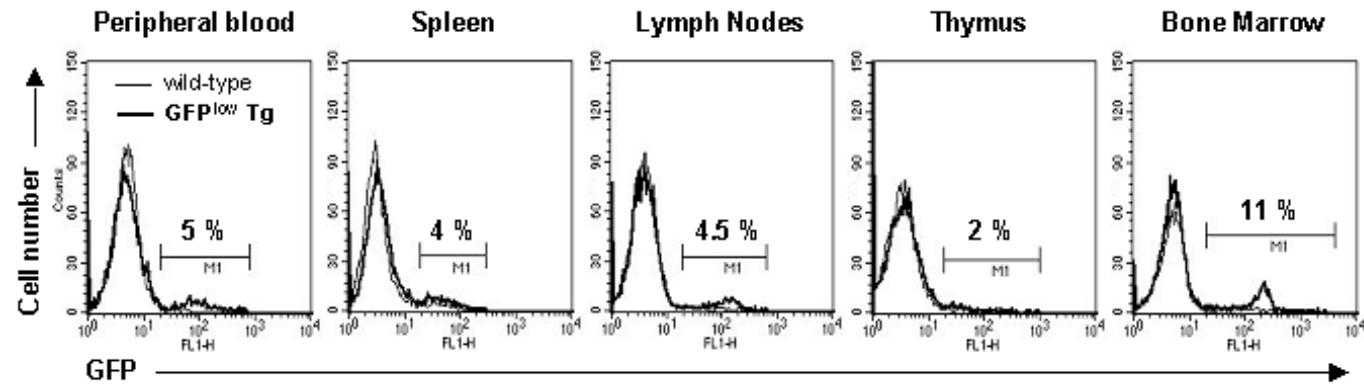
astrocyte

neurone

oligodendrocyte



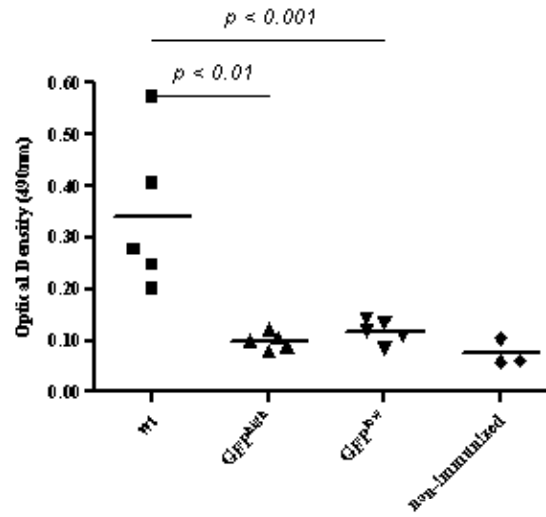
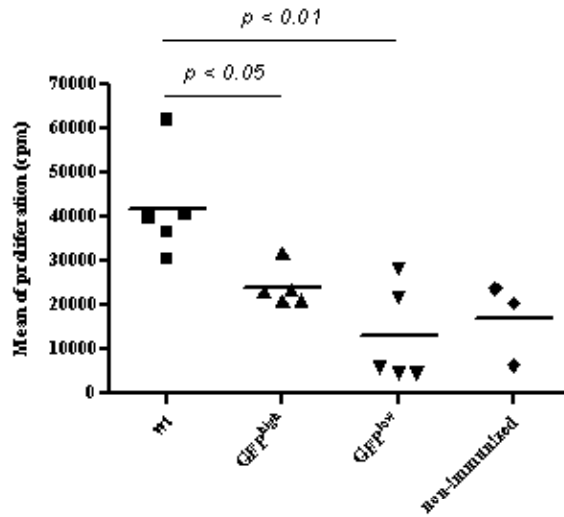
Rats Tg GFP "low expressers"



Rats Tg GFP "low expressers"

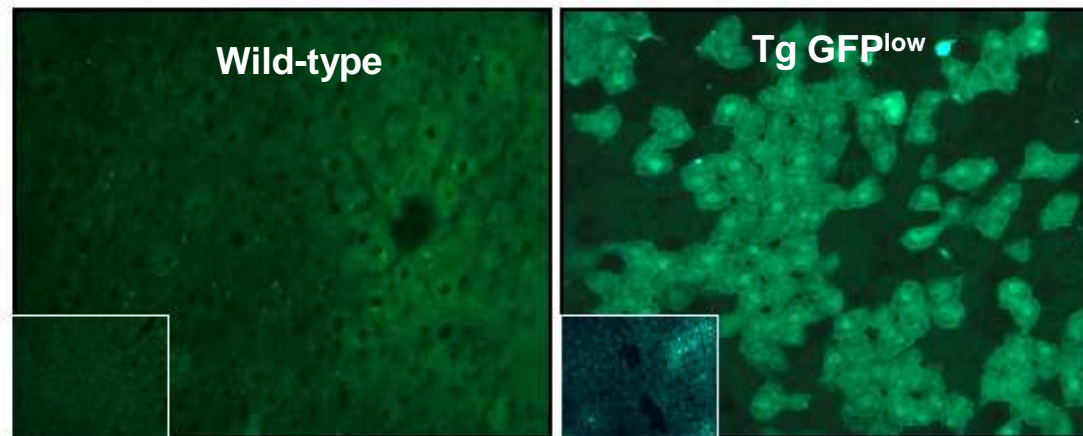
Transfert adoptif de splenocytes GFP+.

14 days



Injection intraportale de 0.5×10^7 UI/g

65 days



Conclusions

- **lentiviral vectors well established method for transgenesis**
- **species where high efficiency is needed (livestock and primates)**
- **DNA microinjection with low efficiency (rat, cattle, rabbit)**
- **or mouse strains difficult to genetically modified (NOD/scid, C57Bl/6)**
- **for knock-down/shRNA in species with no ES cells**



PF Transgénèse Rat



Inserm U643-Itert



Ignacio Aregon

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[http:// www.ifr26.nantes.inserm.fr/ITERT/transgenese-rat/](http://www.ifr26.nantes.inserm.fr/ITERT/transgenese-rat/)