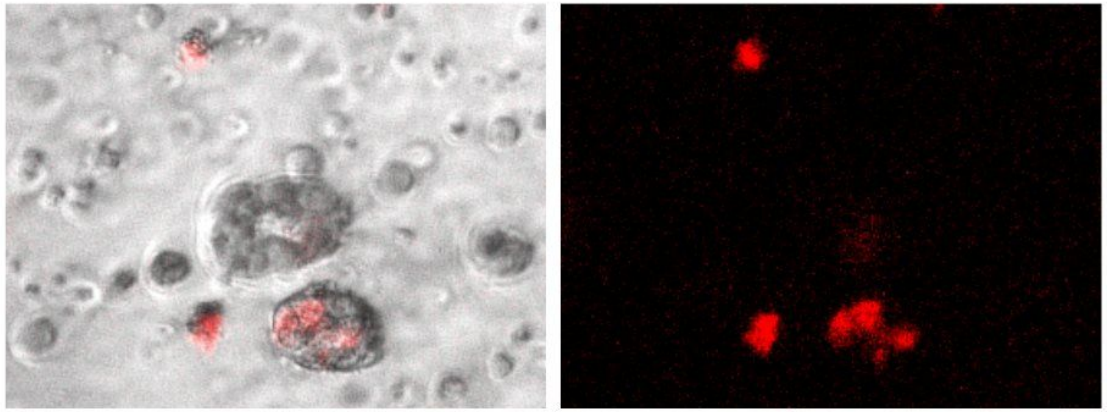
Transfection ADN ou ARNm d'organoïdes intestinaux de porc pour une application en édition de l'épigénome

Katia Feve, Tanguy Bourrec, Guillaume Devailly



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Guillaume Devailly

Séminaire Organoïde

2022-06-29

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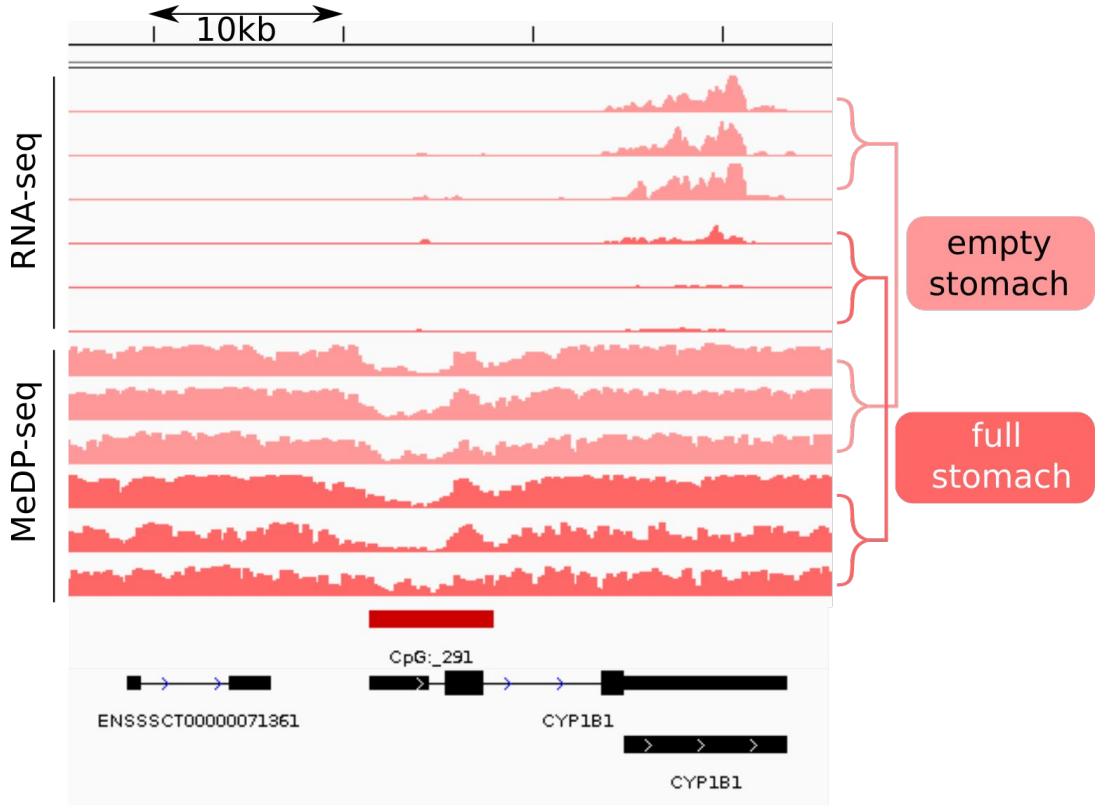
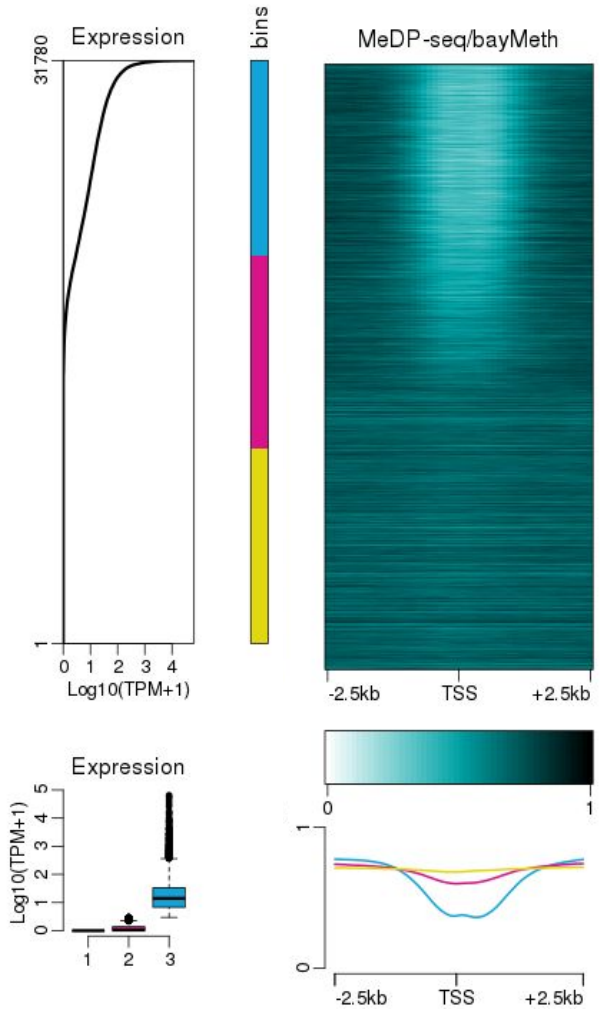
DNA methylation of the intestine

The intestine is a key organ of interest in the pigs:

- feed efficiency
- co-product feeds
- post-weaning diarrhea
- hunger/satiety regulation

DNA methylation and other epigenetic marks are involved in gene expression regulation



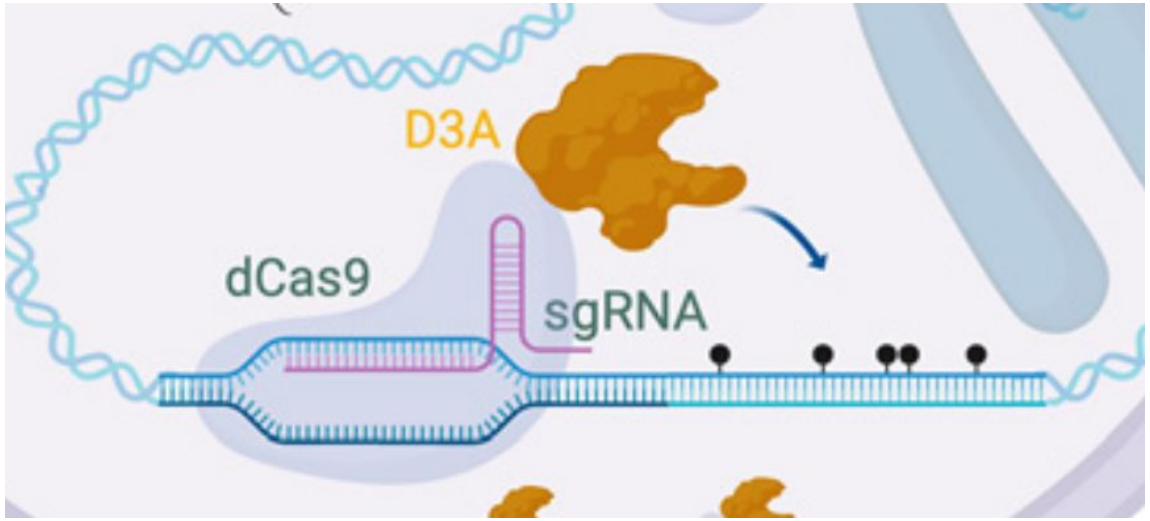


Epigenetic editing allows targeted gene repression

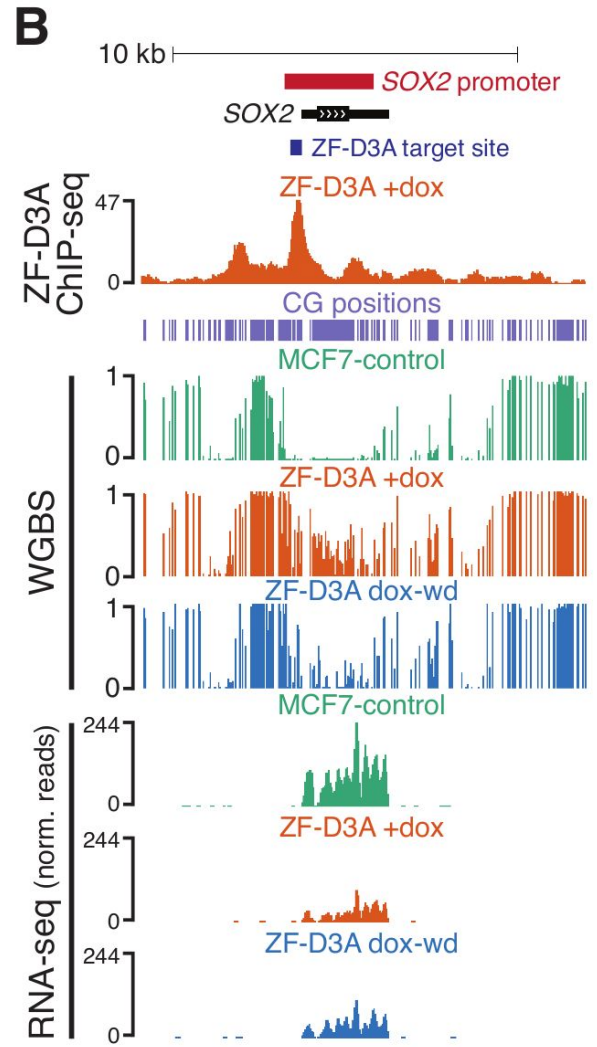
Coupling of an epigenetic writer (DNMT3A) with a targeting system:

- ZF array
- dCas9

Long term (?) targeted gene repression without altering the DNA sequence



Pflueger et al. 2019, 10.1042/EBC20190029

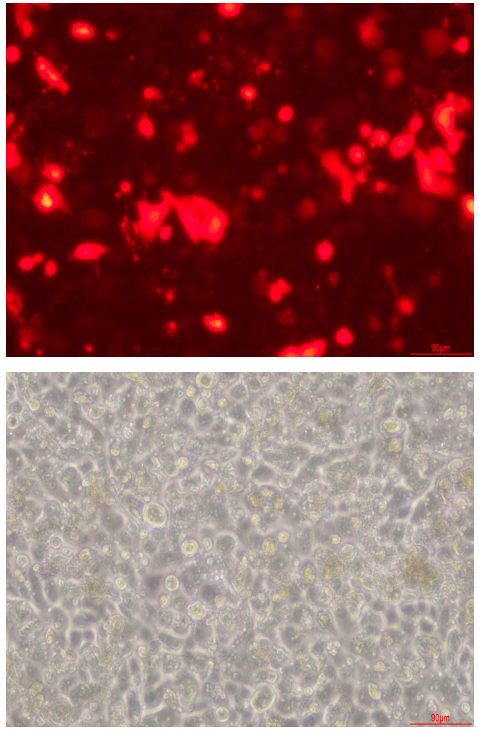


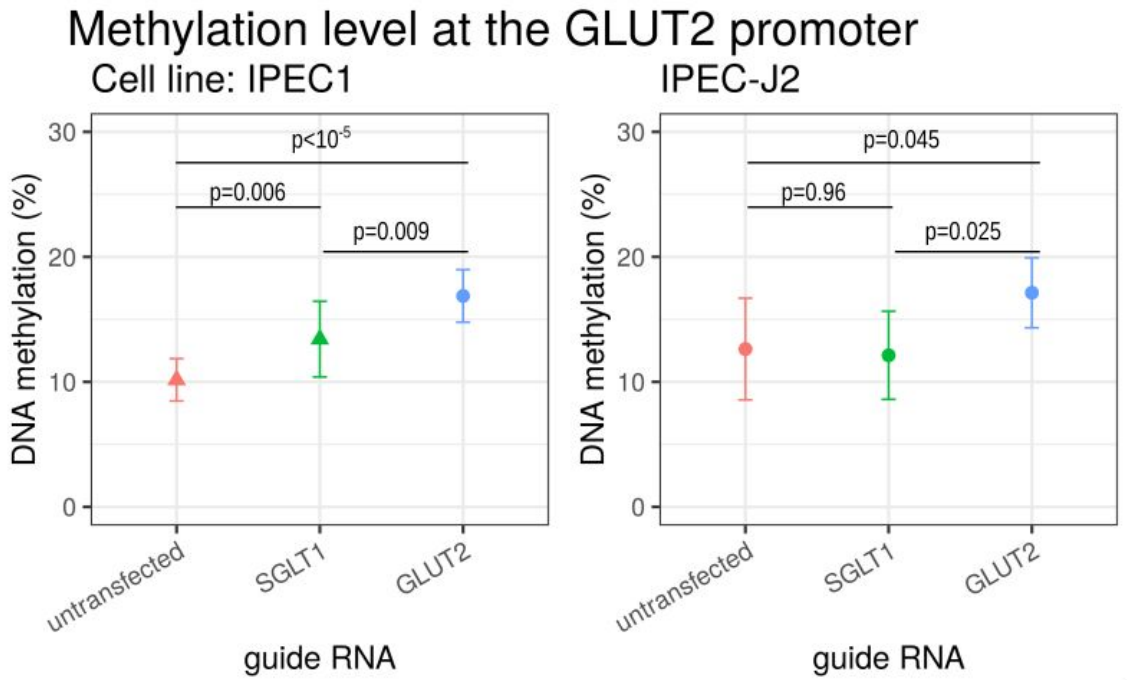
Ford et al. (2017) doi: 10.1101/170506

Epigenetic editing in Intestinal Porcine Pig Epithelium Cell lines

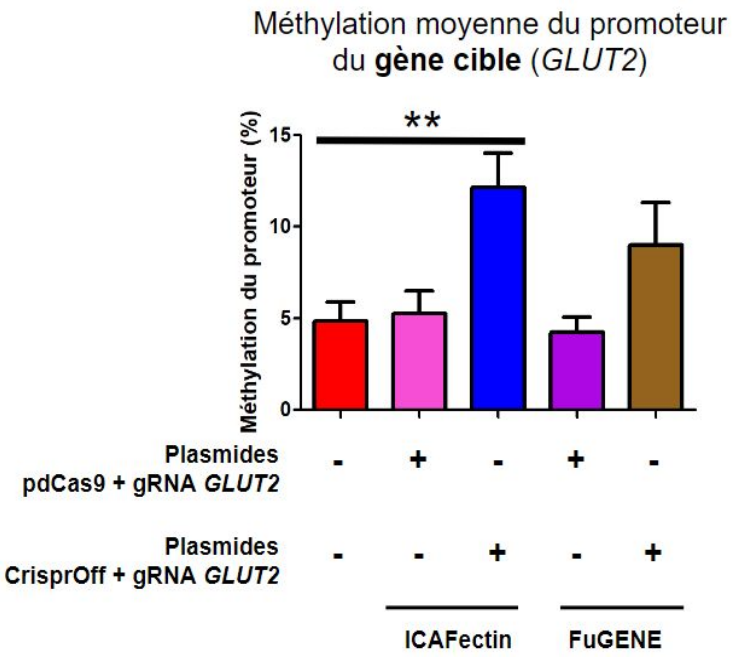
Lipofection of plasmids with **Fugene 6**

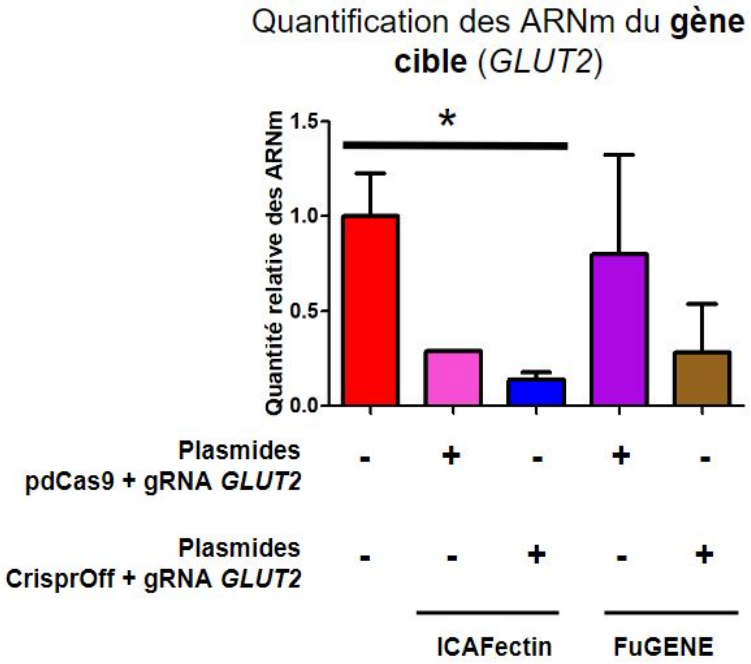
DNA methylation levels measured by pyro-sequencing after bisulfite conversion **48h** post transfection.

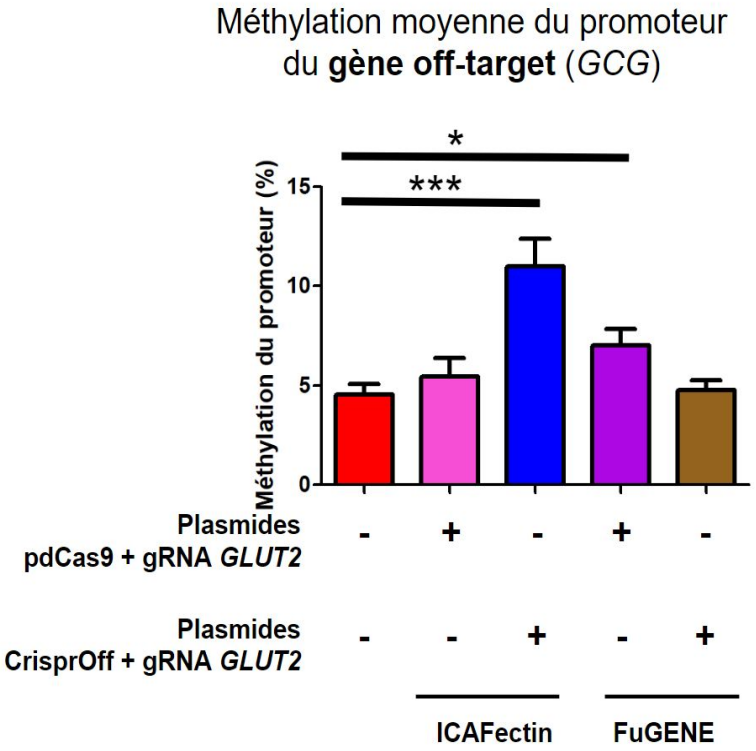




Epigenetic editing in Intestinal Porcine Pig Epithelium Cell lines

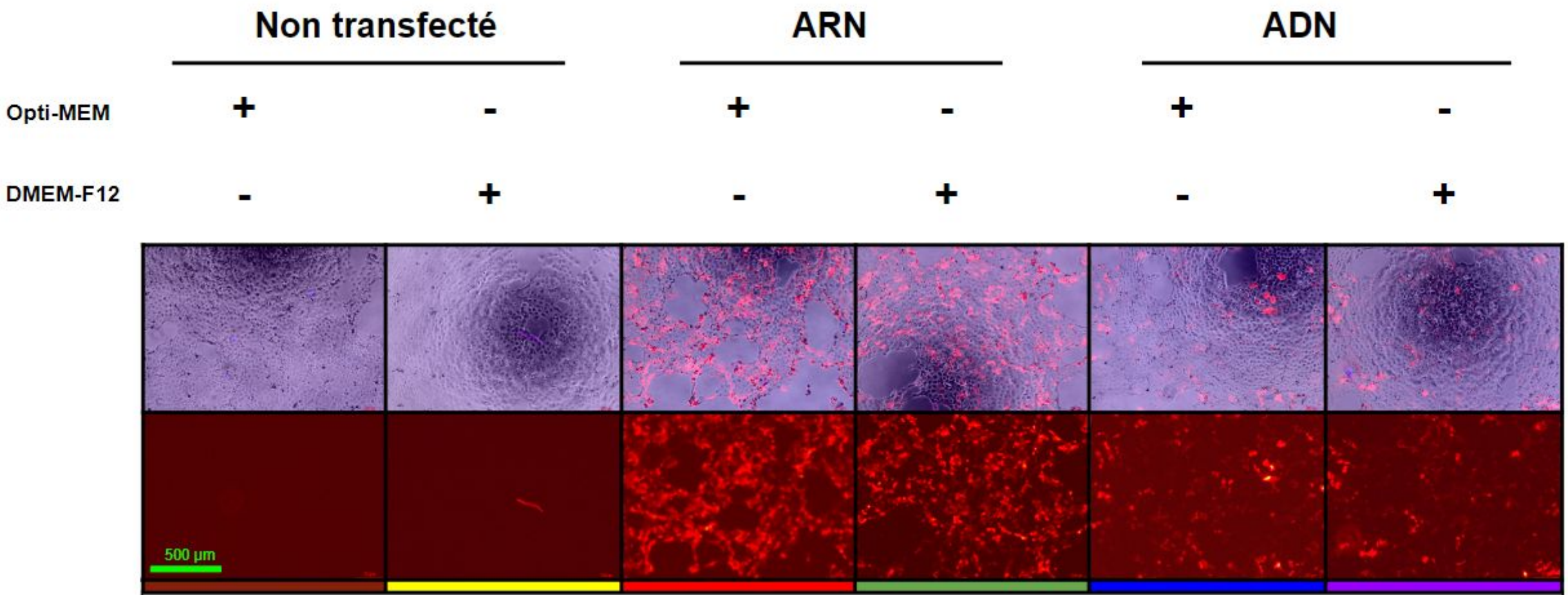




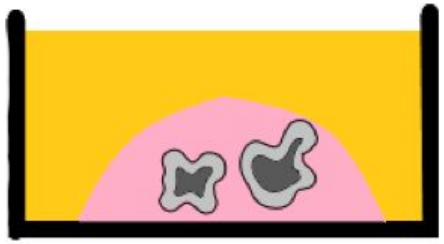


DNA vs mRNA transfection efficiency in IPEC cell lines

Commercial mRNA coding for mCherry (ozbiosciences) transfected using ICAfectin-mRNA

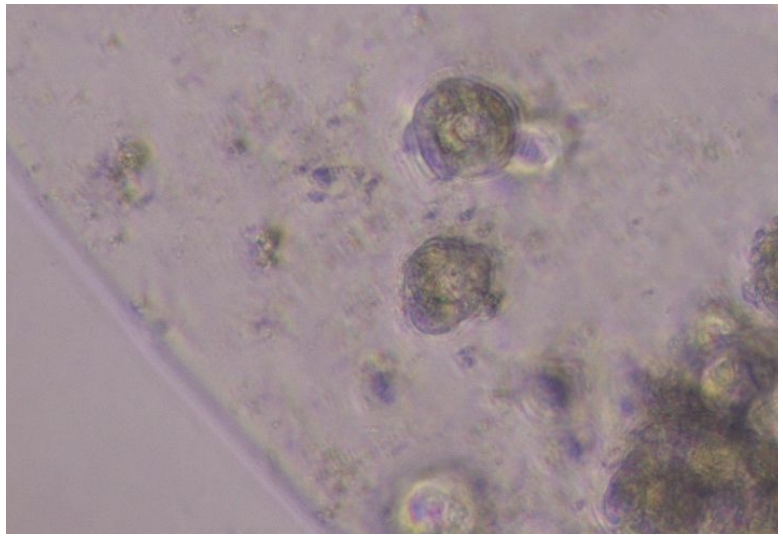


Intestinal organoid transfection



The matrigel is likely to block the transfection vesicles

Intact organoids have the basal side exposed. Apical side transfection might be more efficient?

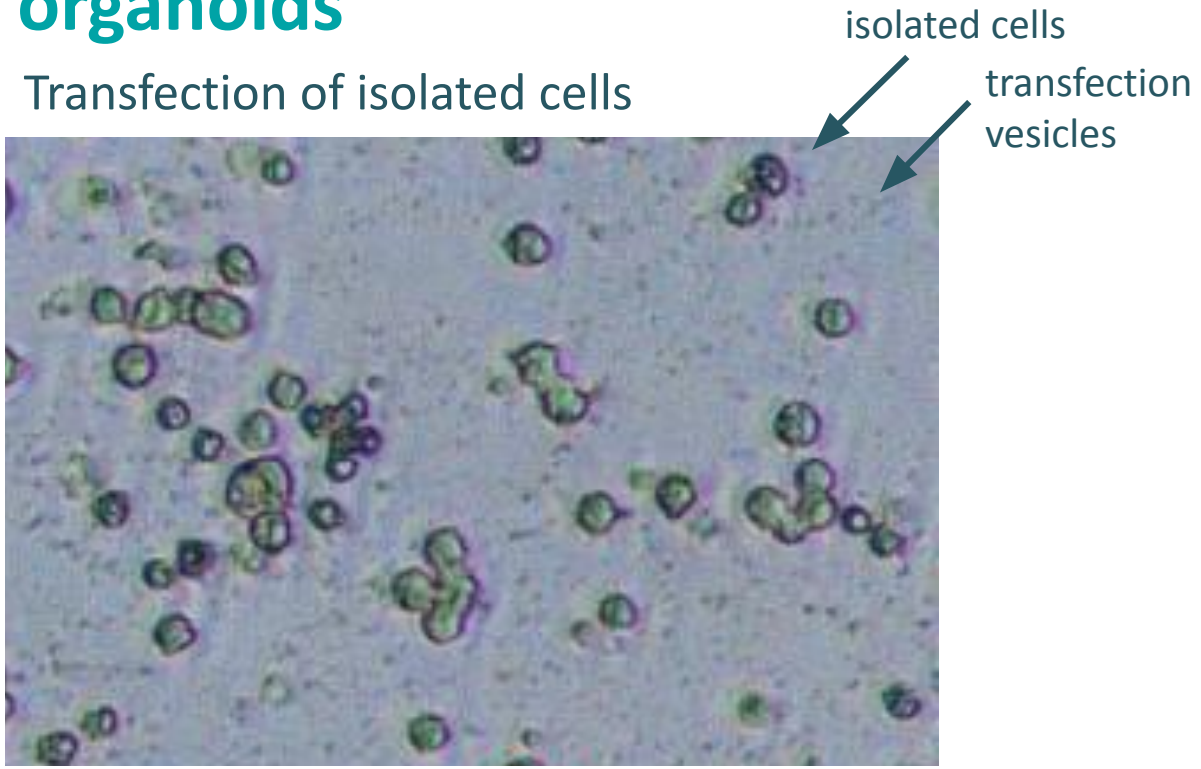


Transfection of intestinal porcine organoids

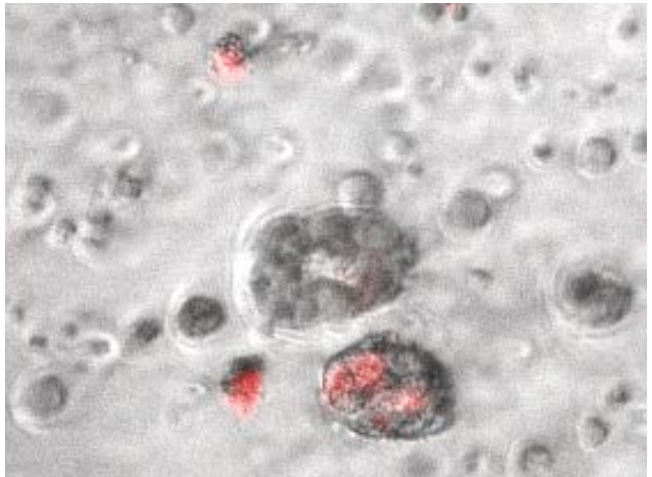
Protocol 1: suspension cells

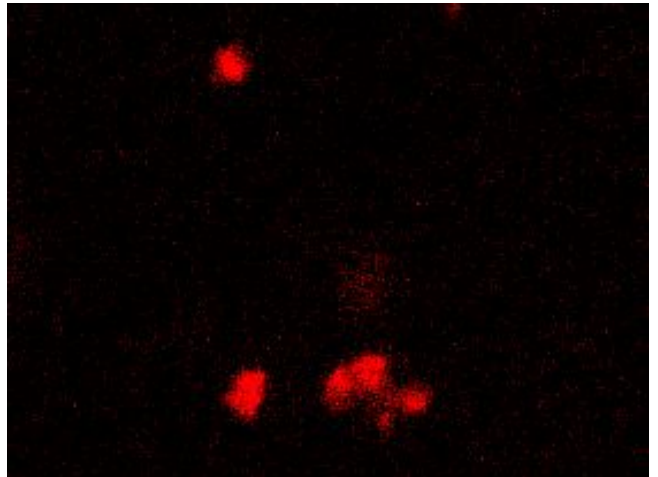
- Thorough mechanical and enzymatic dissociation
- Addition of transfection vesicles in suspension
- Centrifugation (10 minutes, 1h)
- Incubation (4h, ON)
- Back in matrigel dome

Transfection of isolated cells



72h post transfection





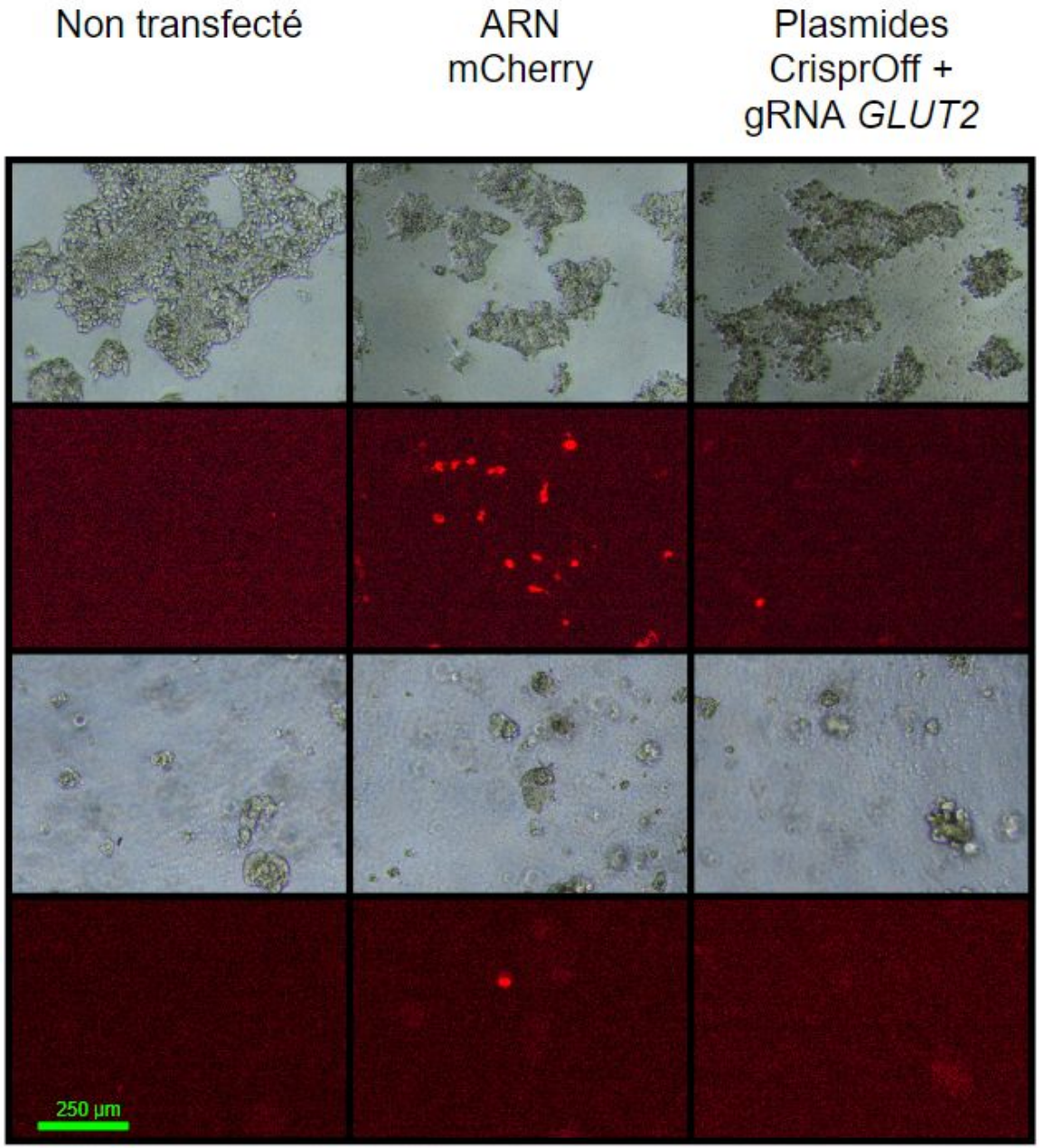
Transfection of intestinal porcine organoids

Protocol 2: monolayer

- Gentle mechanical and enzymatic dissociation
- Seeding in mono-layer in wells coated with matrigel
- ON adhesion of the cells
- Transfection as for adherent cell lines
- Back in matrigel dome 48h post transfection

2D culture (48h)

3D culture (5d)



Intestinal organoids transfection: conclusion and path forward

mRNA >>> DNA transfection efficiency

'Monolayer' protocol probably better than the 'suspension' protocol

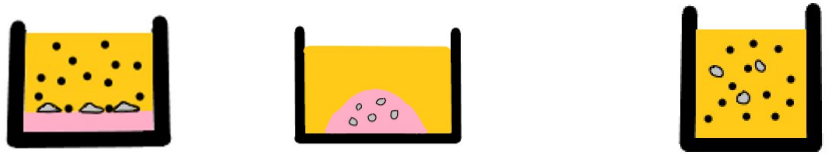
Monolayer protocol easier to work with (fluorescent microscopy)

Cellular density is a key factor, and we have not yet mastered it.

Other mRNA transfectant?

One secret weapon left: cell sorting

	Transfection sur matrice 2D	Après remise en dômes	Transfection en suspension 4H	Transfection en suspension 12H
Densité	50%	15%	20%	15%
Mortalité	5%	5%	5%	20%
Taux de transfection	15%	15%	10%	10%



Oral-introduced mRNA transfection of the gut epithelium in vivo?

Acknowledgments

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Le département GA

GenPhySE

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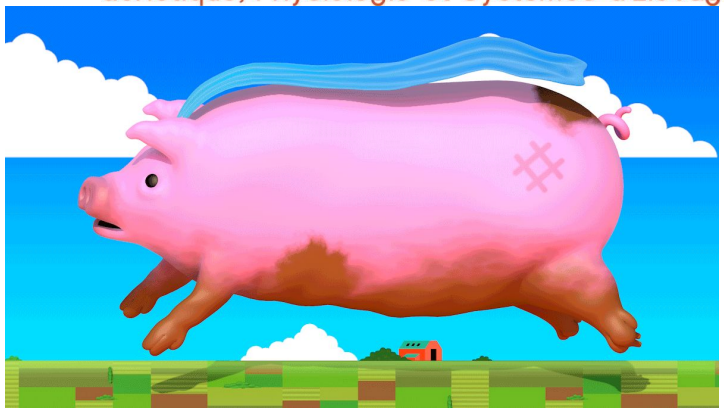

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