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

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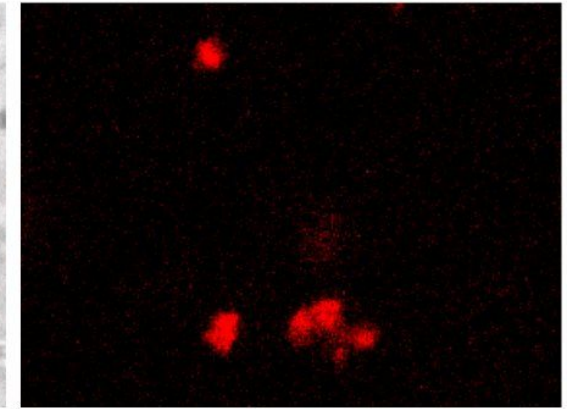
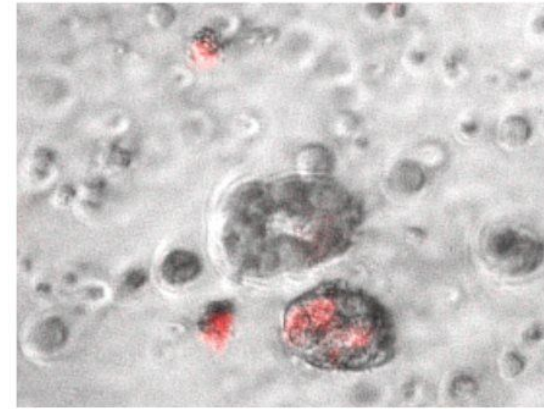
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➤ Transfection ADN ou ARNm d'organoïdes intestinaux de porc pour une application en édition de l'épigénome

Guillaume Devailly

Séminaire Organoïde

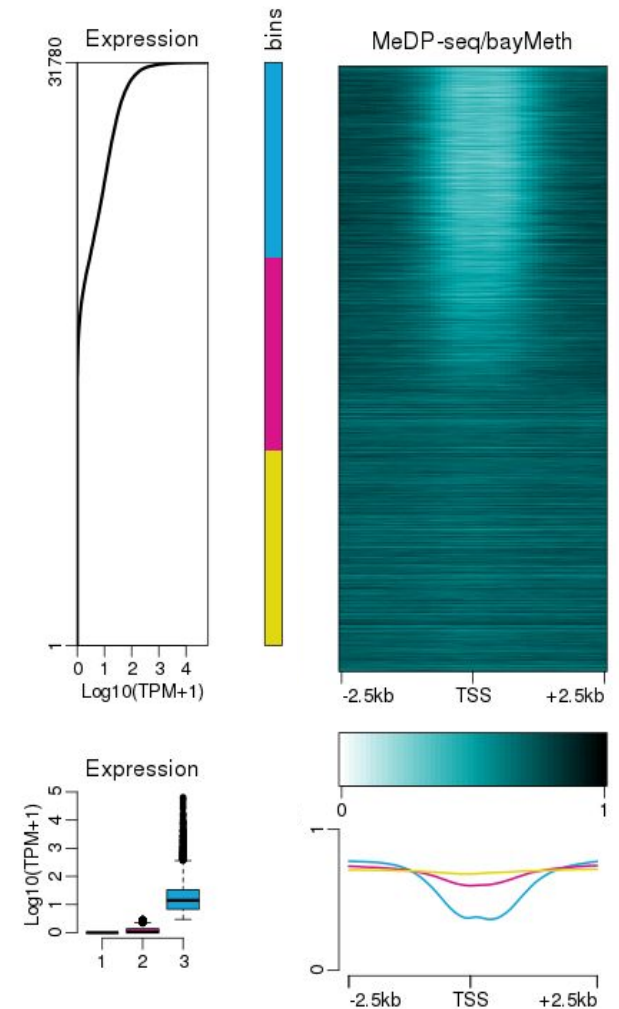
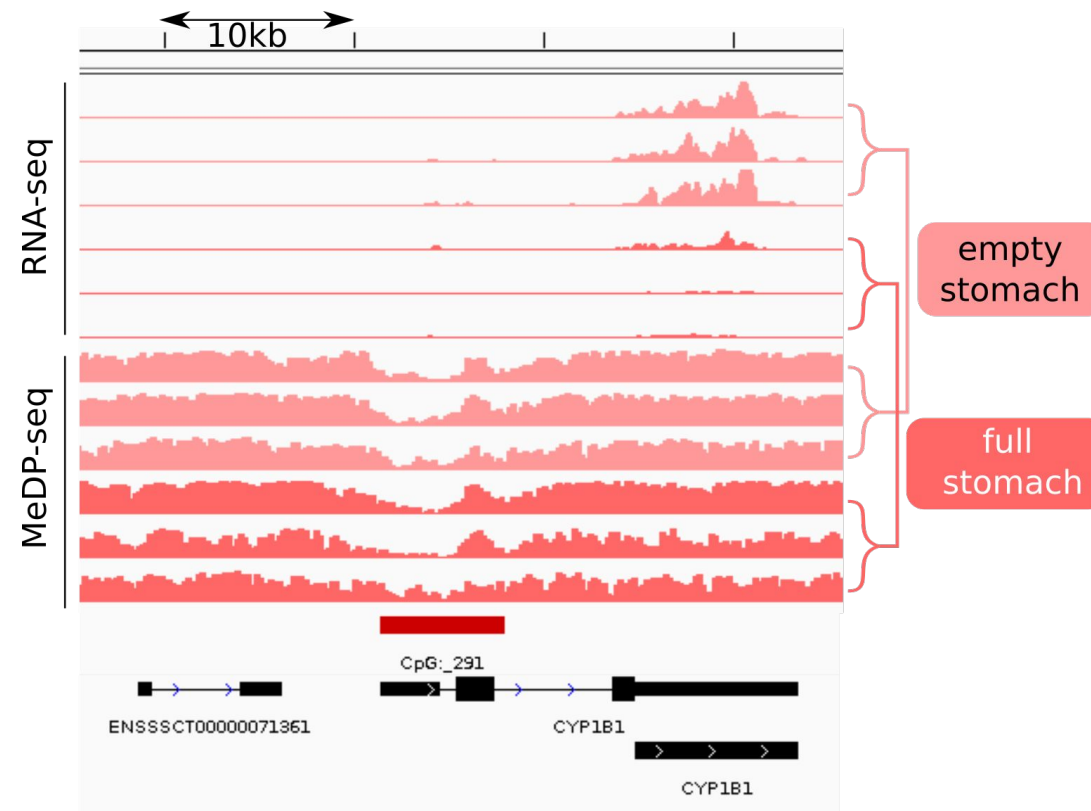
2022-06-29

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

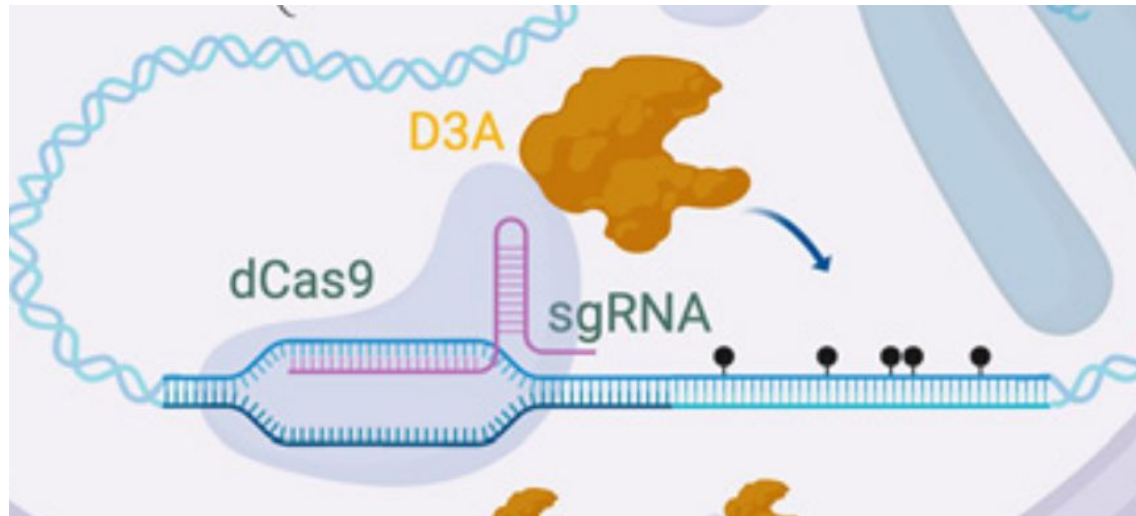


Epigenetic editing allows targeted gene repression

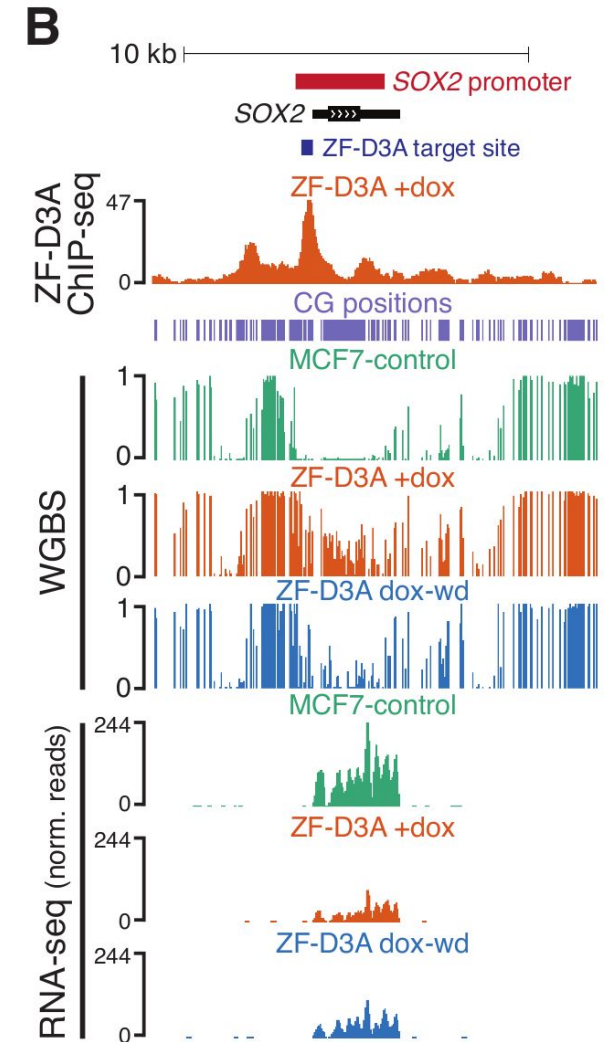
Coupling of an epigenetic writer (DNMT3A) with a targeting system:

- ZF array
- dCas9

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



Pflueger et al. 2019, 10.1042/EBC20190029

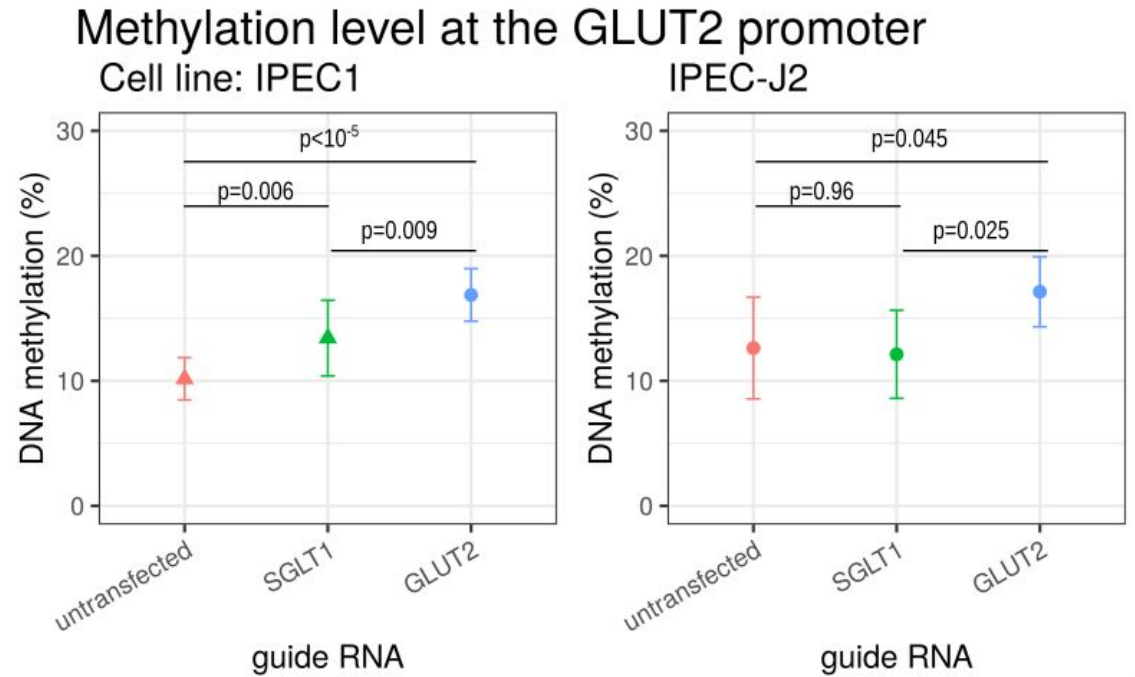
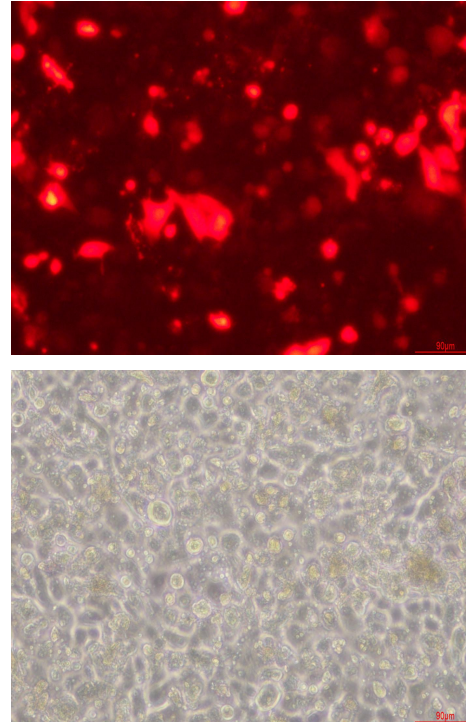


Ford et al. (2017) doi: [10.1101/170506](https://doi.org/10.1101/170506)

Epigenetic editing in Intestinal Porcine Pig Epithelium Cell lines

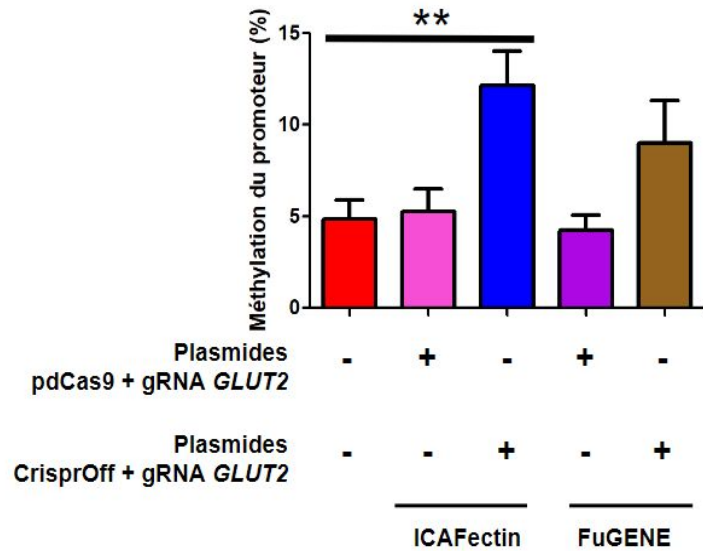
Lipofection of plasmids with **Fugene 6**

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

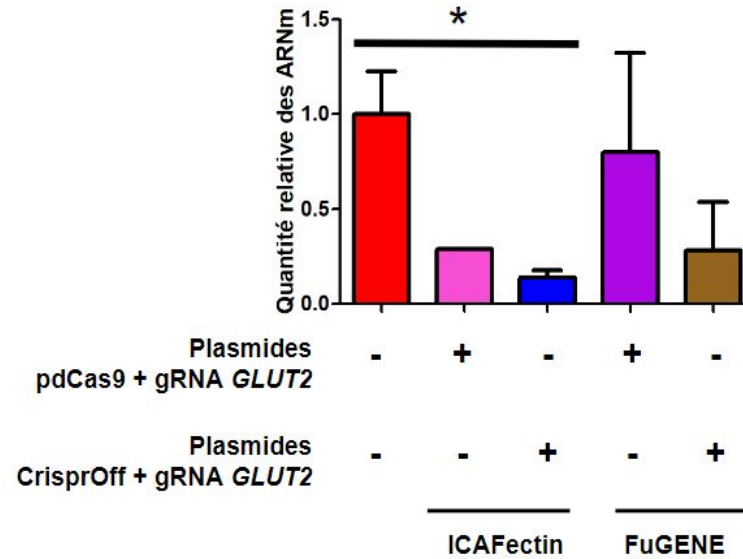


Epigenetic editing in Intestinal Porcine Pig Epithelium Cell lines

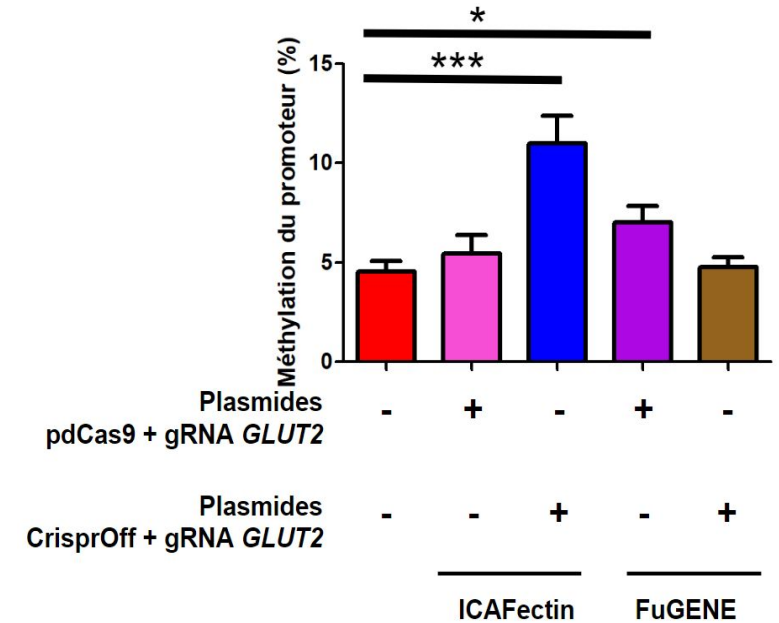
Méthylation moyenne du promoteur du gène cible (*GLUT2*)



Quantification des ARNm du gène cible (*GLUT2*)



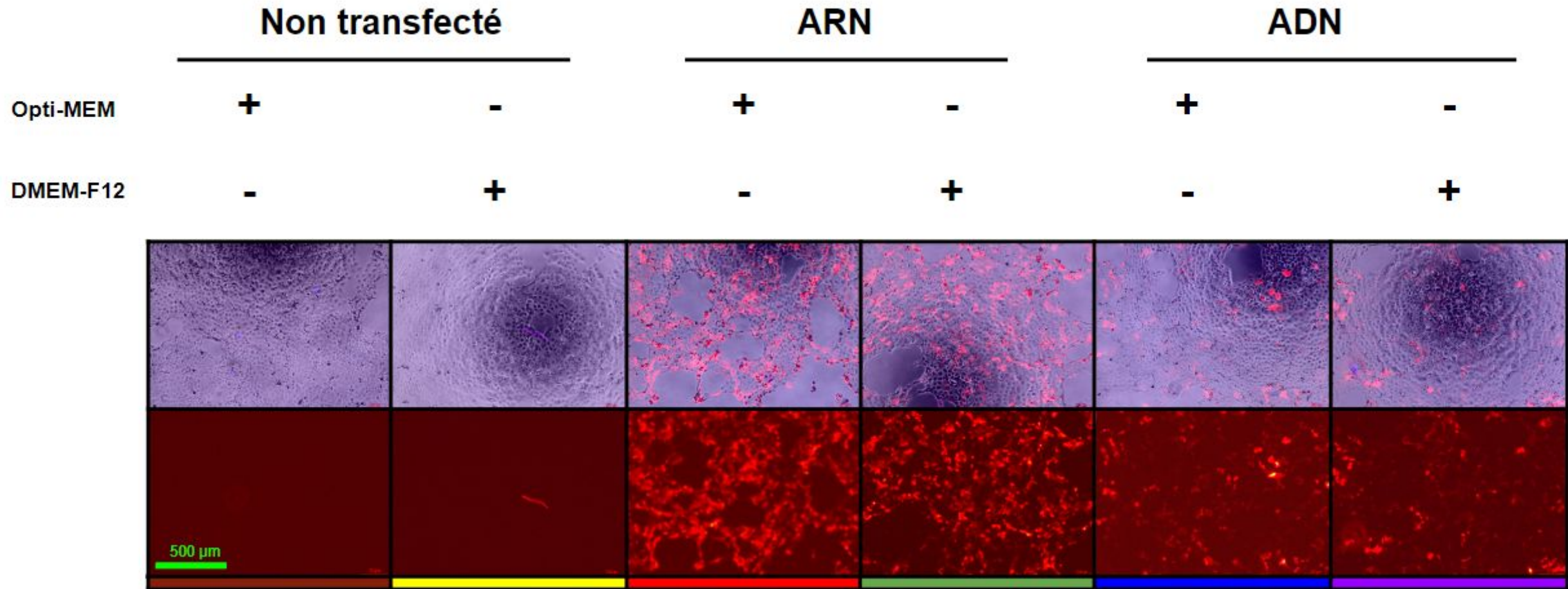
Méthylation moyenne du promoteur du gène off-target (*GCG*)



DNA vs mRNA transfection efficiency in IPEC cell lines



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



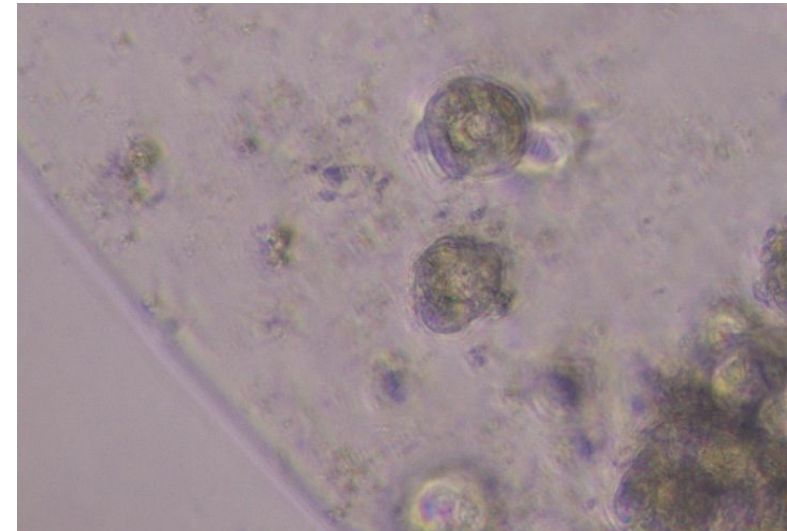
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Intestinal organoid transfection
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Intestinal organoid transfection

The matrigel is likely to block the transfection vesicles

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

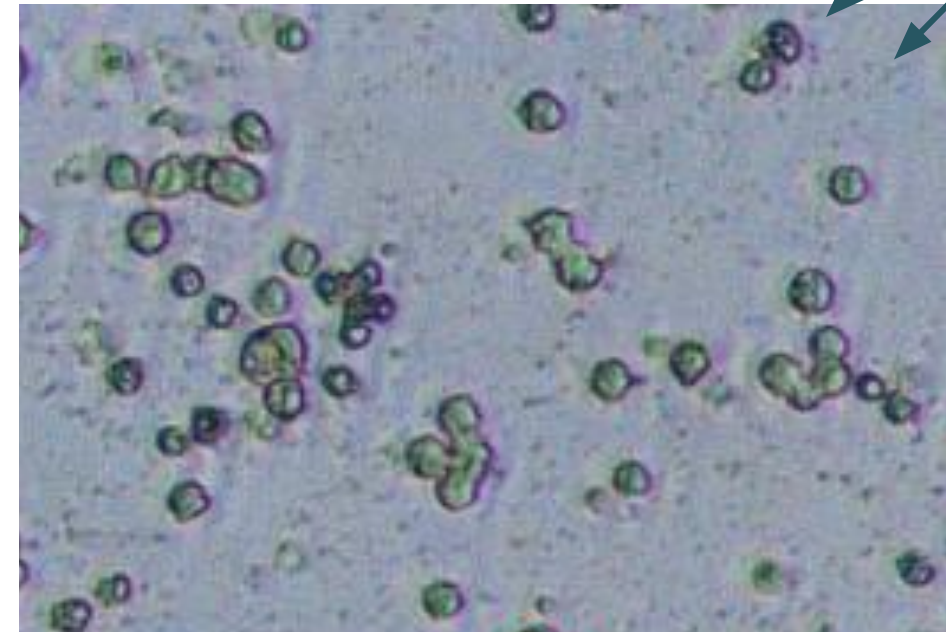


Transfection of intestinal porcine organoids

Protocol 1: suspension cells

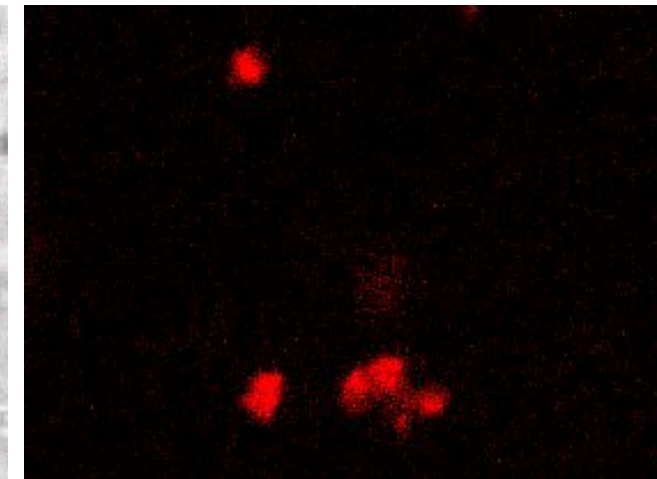
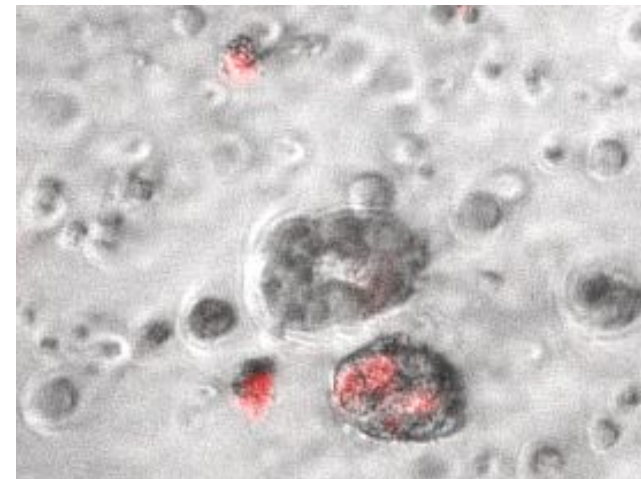
- Thorough mechanical and enzymatic dissociation
- Addition of transfection vesicles in suspension
- Centrifugation (10 minutes, 1h)
- Incubation (4h, ON)
- Back in matrigel dome

Transfection of isolated cells



isolated cells
transfection vesicles

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

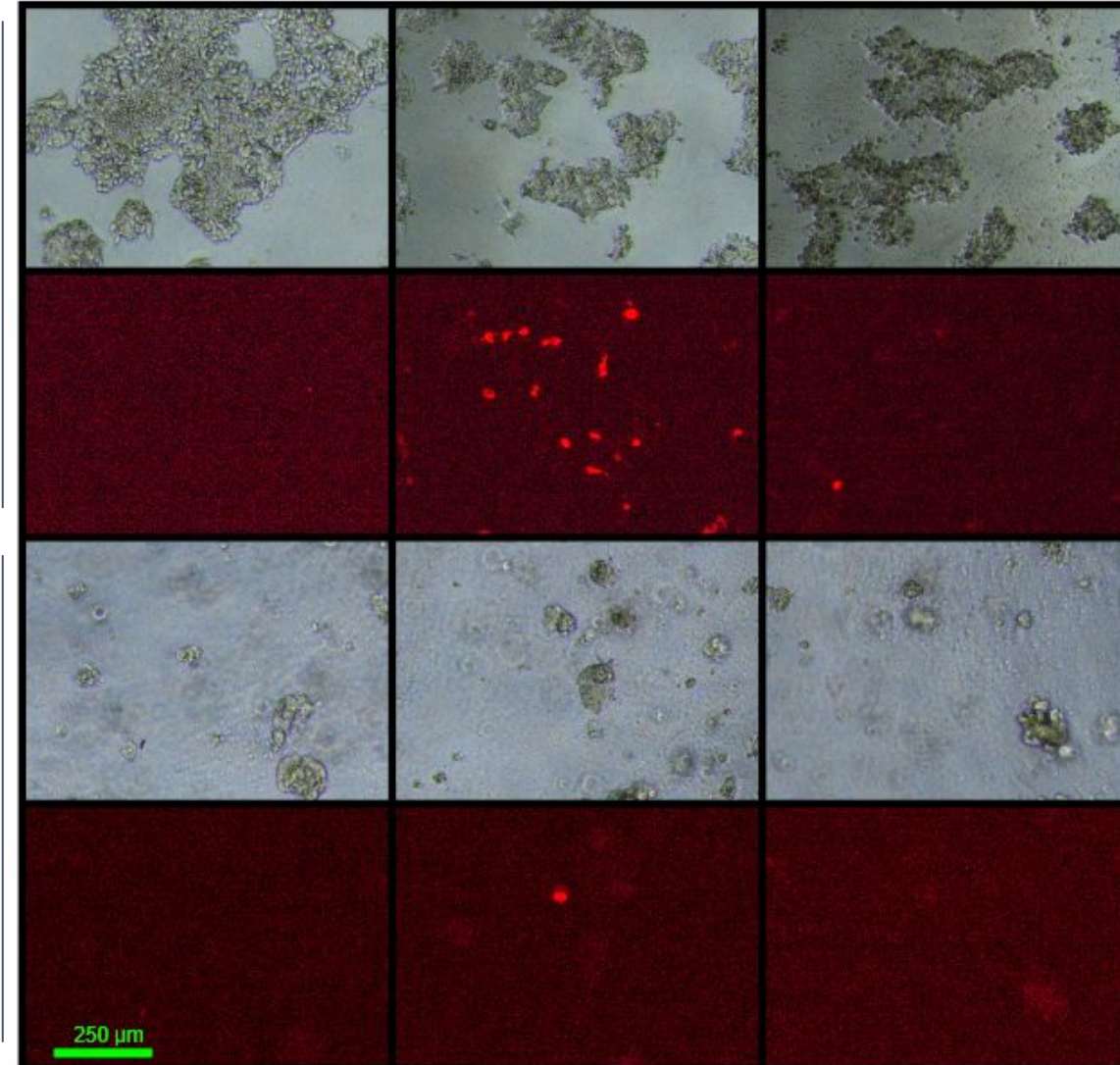
2D culture (48h)

3D culture (5d)

Non transfecté

ARN
mCherry

Plasmides
CrisprOff +
gRNA *GLUT2*



Intestinal organoids transfection: conclusion and path forward

mRNA >>> DNA transfection efficiency

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

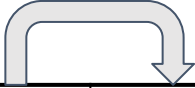
Monolayer protocol easier to work with (fluorescent microscopy)

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

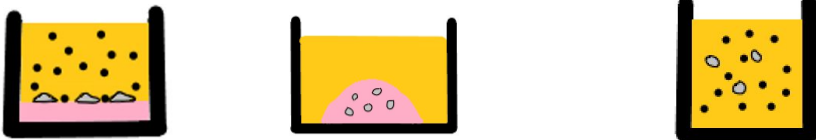
Other mRNA transfectant?

One secret weapon left: cell sorting

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



	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%



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Intestinal organoid transfection
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