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Setting up transfection protocols on porcine gut organoids

Génétique, Microbiote, Santé
UMR 1313 Génétique Animale et
Biologie Intégrative (GABI)
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Introduction: Organoids are *in vitro* models derived from stem cells that recapitulate many aspects of the complex structure and function of the corresponding *in vivo* tissue. We previously characterized organoid cultures from different segments of the porcine gut vs. the original tissue by RNA-seq (data not shown). A vast majority of expressed genes was shared between organoids and their tissue counterparts. Innate immune and epithelial gene lists maintained a higher proportion of shared genes vs. the full gene catalogue, confirming that organoids are a good *in vitro* “3Rs” proxy to evaluate genotype-to-phenotype relationships involving innate immunity responses of the gut epithelia. These systems could be more appropriate than regular cell lines for host-virus interaction studies.

Aim: We aim to optimize cutting-edge genome-editing tools and protocols on pig intestinal organoids. As a proof of concept, we generate knock-out organoids for the aminopeptidase N (*ANPEP*) gene, a well-known receptor of the transmissible gastroenteritis coronavirus (TGEV). We report here preliminary results on the transfection efficiency of porcine gut organoids by electroporation, adapting the method described for human organoids (Fujii et al, 2015) and by a GFP-lentiviral vector transduction.

Materials and methods:

Culture of duodenum organoids

Organoids were generated from duodenum or colon segments of Large white pigs. Organoids are grown in 20 µl Matrigel® domes with Intestinal Organoid Growth Media Human (StemCells). Each week, they are dissociated into single cells with Tryple Express and seeded into new Matrigel domes.

Plasmid preparation

Guide RNA for exon 2 of the porcine *ANPEP* gene was designed using CRISPOR and cloned behind the hU6 promoter into pX458 plasmid (Addgene #48138) coding for *SpCasg* and *GFP*. Plasmid preparation was performed with NucleoSpin Plasmid transfection grade (Macherey-Nagel).

Transfection of organoids with Neon electroporation. Duodenum or colon organoids transfection was performed with the Neon™ electroporation system (Invitrogen) with 5×10^4 dissociated cells for 0,5-1 µg of either a pCMV::mcherry plasmid or pX458 plasmid with guides RNA against the *ANPEP* gene. Electroporation conditions tested were 1200V/20ms/2 pulses or 1700V/20ms/1 pulse with Buffer R (Neon).

Transduction of organoids with lentiviral vector. Colon organoids were transfected with a lentiviral vector expression GFP, at different MOI for 2×10^4 dissociated cells (incubation 4 hours at 37°C).

Transduction and transfection efficiency were monitored by counting fluorescent organoids over their total number on phase-contrast, with PLAPON 2x, UPLFLN 4X and PLCN 10X objectives.

Results:

Intestinal pig organoids can be transfected by electroporation with plasmids

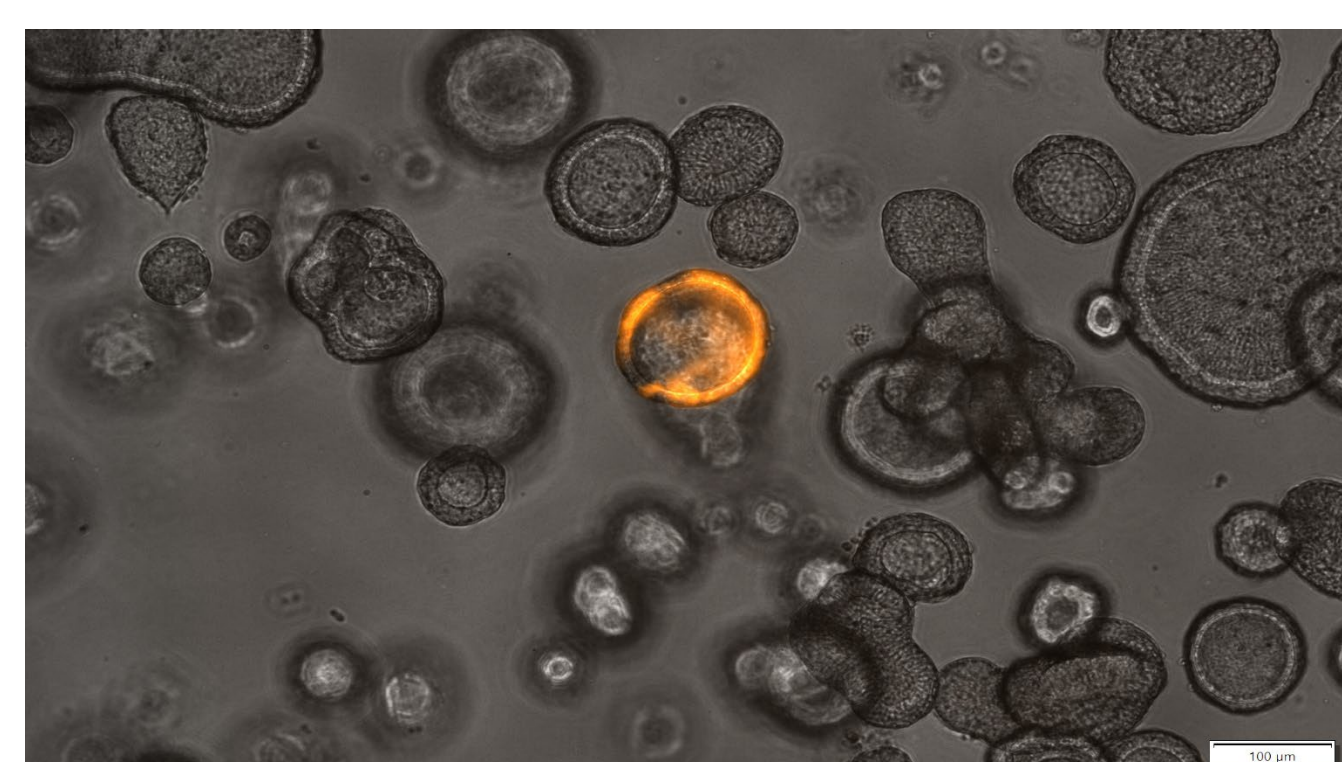


Figure 1: Transfection of porcine colon organoids with pCMV::mcherry plasmid at day 5, one passage after electroporation. Overlay of phase-contrast and mcherry filter channels (PLCN 10x objective)

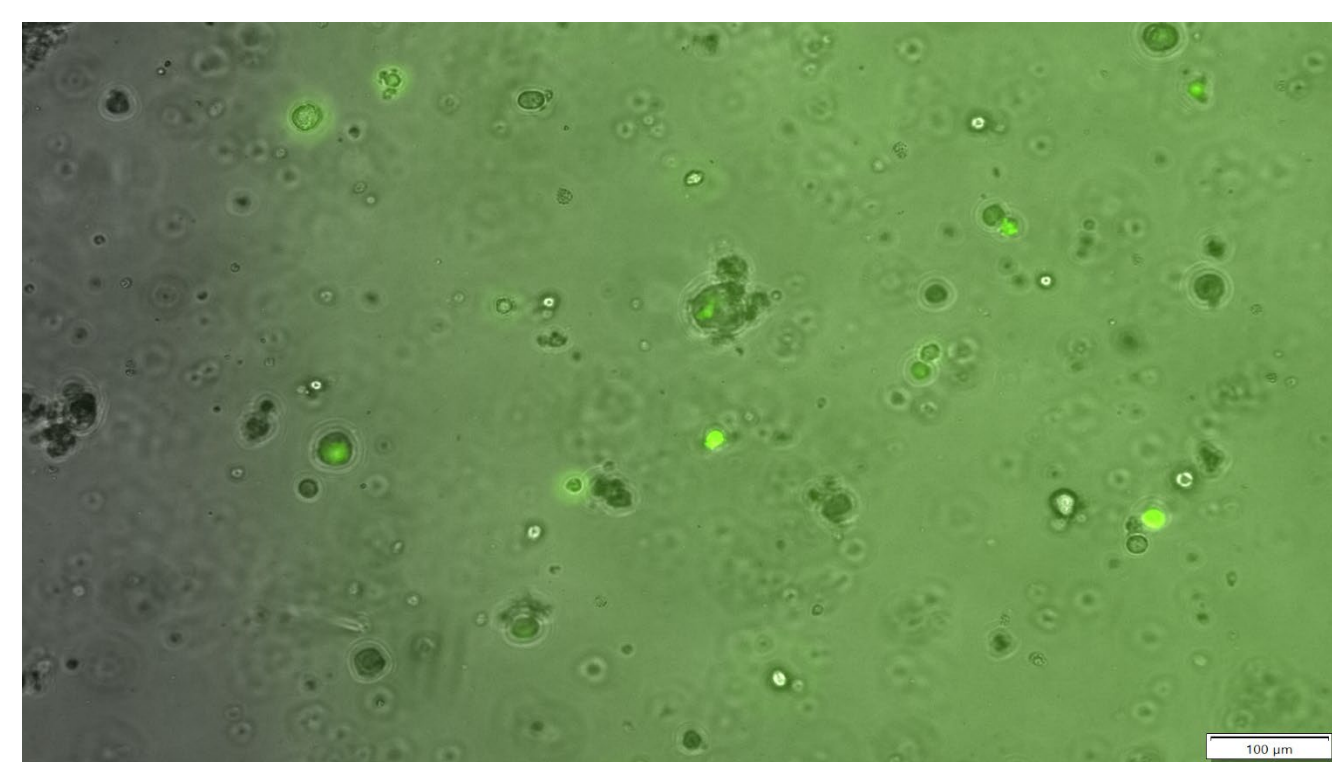


Figure 2: Transfection of duodenum organoids with plasmid pX458 with guide RNA against *ANPEP* exon 2, at day 2. Overlay of phase-contrast and GFP filter channels (UPLFN 4x objective). Estimated transfection efficiency : 31%

Neon electroporation of two plasmids carrying fluorescent reporter produced successful porcine organoids transfection. The expression of *mcherry* and *GFP* was detected upon transfection in electroporated duodenum organoids (Figures 1 & 2) with about 30% of transfection efficiency, either for 1200V/20ms/2 pulses or 1700V/20ms/1 pulse. However, selection of fluorescent dissociated organoids by FACS needs further improvement in terms of cells recovery.

Intestinal pig organoids transduction by lentivirus leads to a higher transfection efficiency

Lentiviral transfection on colon organoids at MOI 1 produced fluorescent organoids with 75% efficiency. This method with lentiviral vector seems to be the most efficient comparing to plasmid electroporation. However, this system is less flexible than plasmids as it is necessary to produce a lentiviral vector for each gene which is aimed to be inactivated.

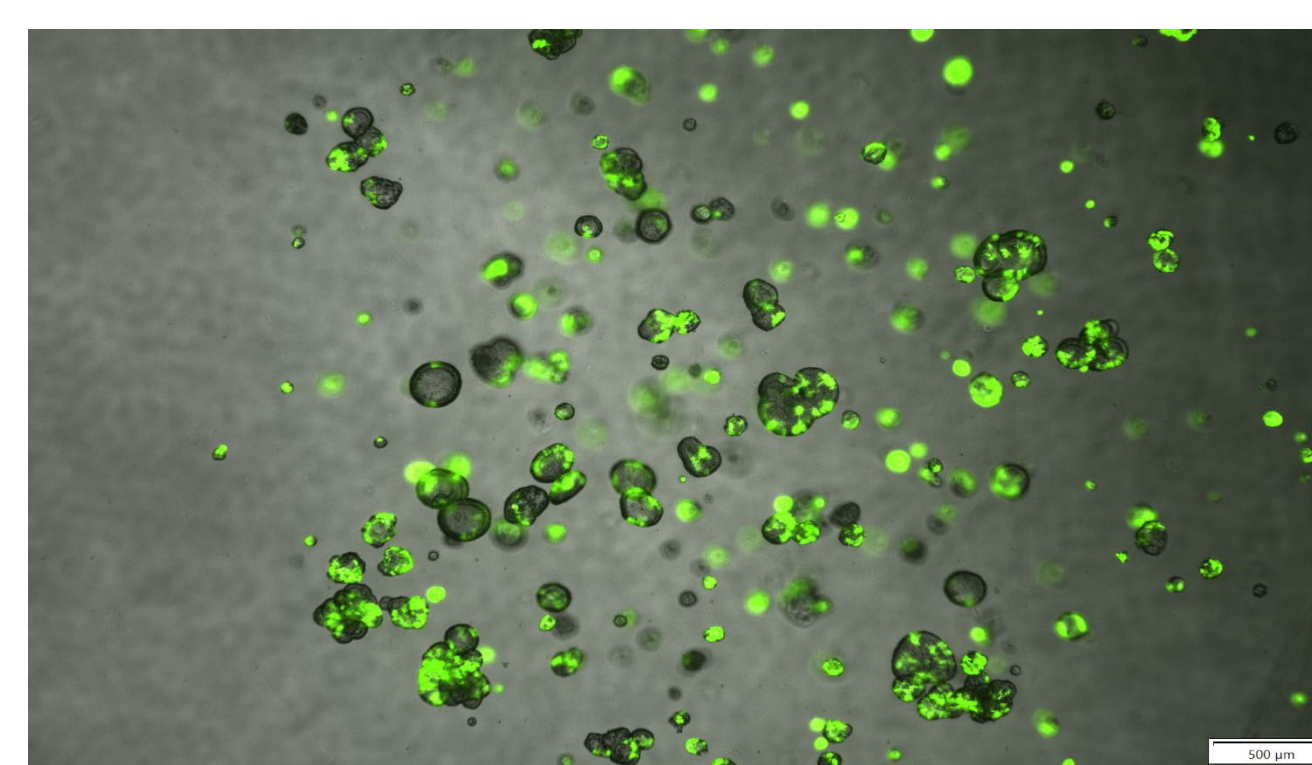


Figure 3: Transfection of colon organoids with a lentiviral vector expressing GFP at MOI 1. Overlay of phase-contrast and GFP filter channels (PLAPON 2x objective). Estimated transfection efficiency : 75%

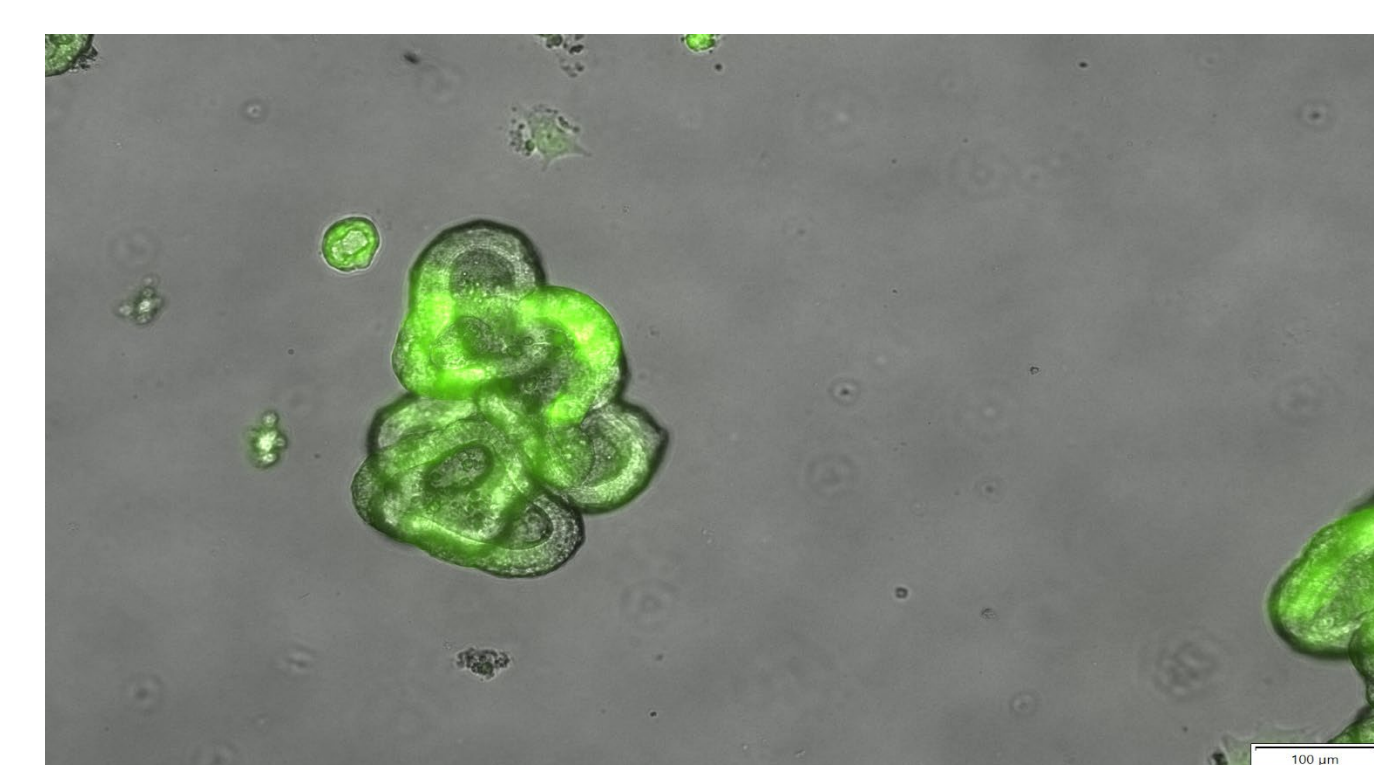


Figure 4: Transfection of colon organoids with a lentiviral vector expressing GFP, passage 7. Overlay of phase-contrast and GFP filter channels (PLCN 10X objective).

Conclusion: Our preliminary results indicate that transfection of intestinal pig organoids is efficient with the Neon™ Electroporation system and with lentiviral transduction, with a higher efficiency for the latter. Our project relies on plasmids electroporation as it is more flexible to test different candidates. Ongoing assays are in progress to isolate and produce organoids knock-out for *ANPEP* gene, either with plasmids or with RNP proteins, to be tested soon. These knock-out organoids will then be validated by a TGEV infection, followed by immunofluorescence and transcriptional characterization.



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