# Setting up transfection protocoles on porcine gut organoids

Celia Carbonne, Jean-Luc Coville, Giorgia Egidy, Elisabetta Giuffra

# Setting up transfection protocols on porcine gut organoids

Célia Carbonne, Jean-Luc Coville, Giorgia Egidy Maskos, Elisabetta Giuffra
**Université Paris-Saclay, INRAE, AgroParisTech, UMR 1313 Génétique Animale et Biologie Intégrative (GABI), Jouy-en-Josas**

**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 (Figure 1). A vast majority of expressed genes was shared between organoids and their tissue counterparts at low TPM thresholds. At higher TPM, 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 (Figure 2).

**Aim:** We aim to optimize cutting-edge genome-editing tools and protocols on pig intestinal organoids for our host:virus interaction studies. As a proof of concept, we will generate knock-out organoids for the *ANPEP* gene, a well-known receptor of a porcine virus (TGEV). We report here preliminary results on the transfection efficiency of porcine gut organoids by electroporation of a fluorescent reporter plasmid, adapting the method described for human organoids (Fuji et al, 2015).

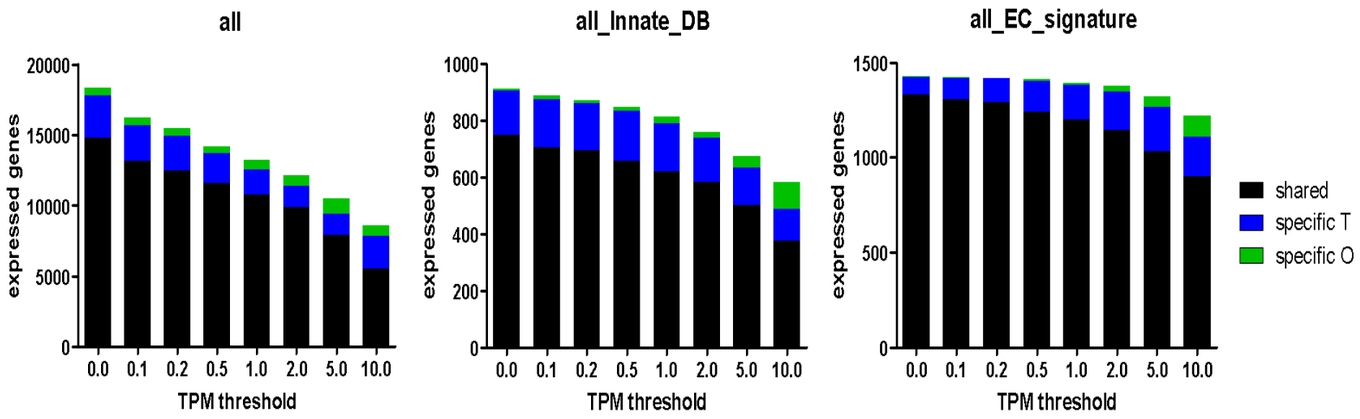



**Figure 1:** Number of genes whose expression is shared between tissues and organoids or specific to tissues (T) or organoids (O) depending on a TPM threshold varying from 0 to 10. A gene was considered as expressed in one condition if TPM was > threshold in ¾ of samples. From Blanc et al. (unpublished).

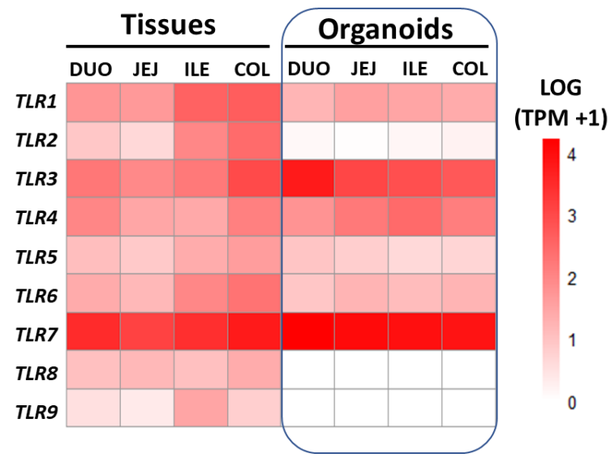
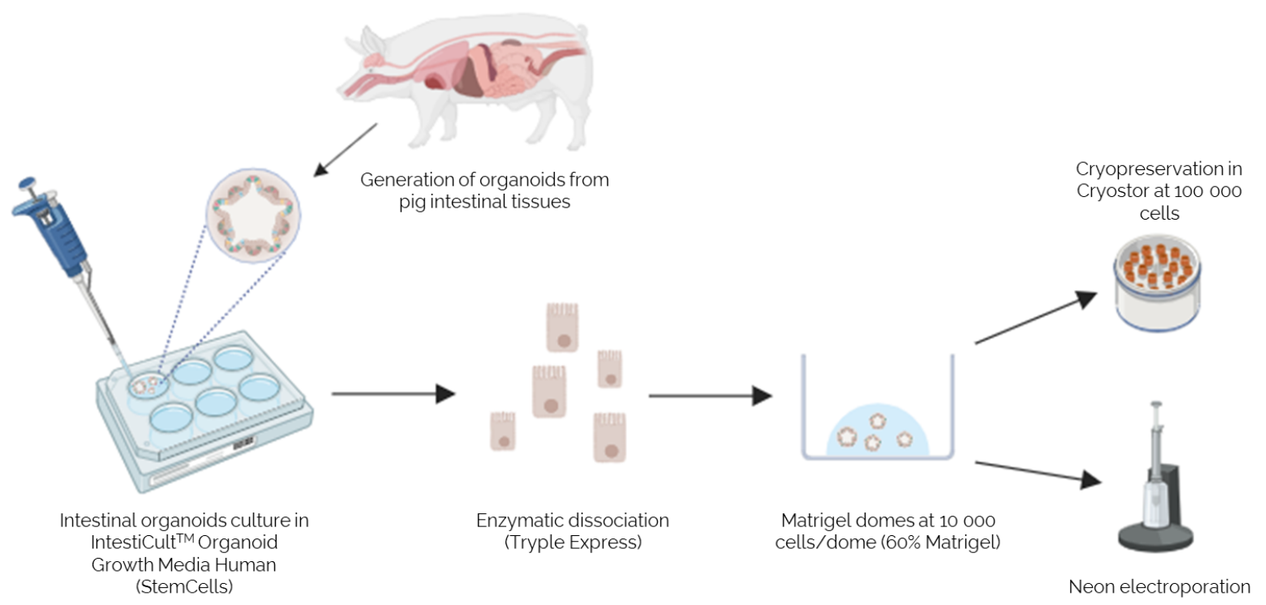



**Figure 2:** TLR expression in pig tissues and organoids cultures derived from gut segments, expressed in log of TPM

## Materials and methods:

**Evaluation of 3D organoid growth** by phase-contrast microscopy was performed with an Olympus IX83 microscope. Intestinal pig organoids (colon and duodenum) were grown from our biobank of porcine organoids. The protocol was optimized from existing protocols for human tissue, using IntestiCult™ medium (Beaumont et al, 2021).

**Transfection of organoids with Neon electroporation.** Organoids transfection was performed with the Neon™ electroporation system (Invitrogen) at 1 x $10^5$ cells for 0,5 µg of a 10,4 kb plasmid DNA harboring *mcherry* gene under the CMV promoter. Transfection efficiency was monitored by counting fluorescent organoids over their total number on phase-contrast, with UPLFLN 4X and PLCN 10X objectives.




## Results:

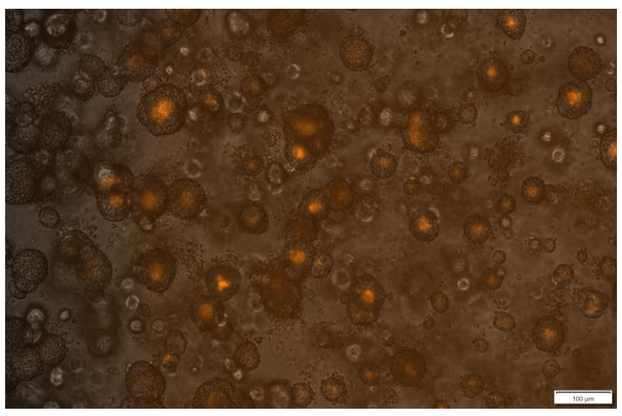


**Figure 3:** Transfection of duodenum organoids with pCMV::mcherry plasmid at day 5. Overlay of phase-contrast and mcherry filter channels. Estimated transfection efficiency : 63%

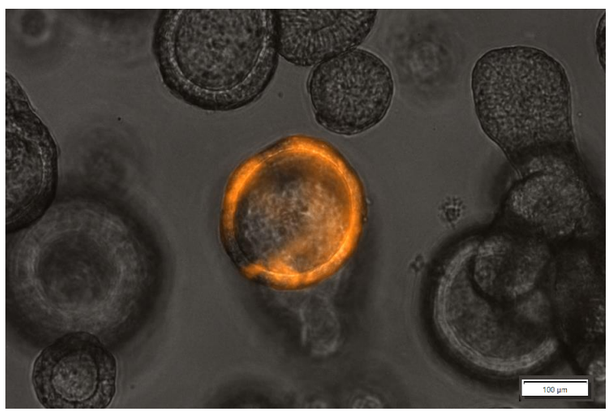


**Figure 4:** Transfection of colon organoids with pCMV::mcherry plasmid at day 5 after one passage of transfected organoids (x10). Overlay of phase-contrast and mcherry filter channels. Estimated transfection efficiency : 31%

### Intestinal pig organoids can be efficiently transfected by electroporation

Neon electroporation of the fluorescent reporter plasmid produced successful porcine organoids transfection. The expression of *mcherry* was detected upon transfection in electroporated duodenum organoids (Figure 3). Fluorescence in the lumen of organoids was not maintained through passaging, suggesting the plasmid had not been integrated into the porcine genome.
In colon organoids, cytoplasmic fluorescence was detected after one passage suggesting the genomic integration of the reporter plasmid (Figure 4). Moreover, the numbers of organoids was not affected by electroporation (no differences in number of dissociated cells obtained after passaging).

**Conclusion:** Our preliminary results indicate that transfection of intestinal pig organoids is efficient with the Neon™ Electroporation system. Ongoing assays are in progress to produce *ANPEP* knock-out organoids by CRISPR-Cas9 strategies, to be validated with TGEV infection, followed by transcriptional characterization. Additionally, protocols of genome editing applied on swine intestinal organoids and results produced will enable us to support the standardization effort made in the frame of EuroFAANG infrastructure project.

### References
Beaumont M., Blanc F., Cherbuy C., Egidy G., Giuffra E. et al. (2021) Vet. Res. 52(1):1-15. https://doi.org/10.1186/s13567-021-00909-x
Blanc F., Chalabi S., Rau A., Mongellaz M., Pepke F., Bevilacqua C., Riviere J., Vilotte M., Egidy G. and Giuffra E. Unpublished data.
Fujii M., Matano M., Nanki K. and Sato T. (2015) Nature Protocols volume 10, pages 1474–1485. doi:10.1038/nprot.2015.088

### Acknowledgments
Stéphanie Pons, NISC unit Institut Pasteur Paris, for providing the pCMV::mcherry WRPE plasmid

celia.carbonne@inrae.fr