Master 2 project 2024-2025: **Comparison of methods for introducing cell-type genetic tracers into porcine in vitro systems.**

Key words: genetic tracer, genome editing, ESC, organoids.

Laboratory location: INRAE, GABI unit, GeMS team, Jouy-en-Josas.

Supervisor: Giorgia Egidy PhD.

https://eng-gabi.jouy.hub.inrae.fr/the-teams/gems

INRAE, the French public research organisation dedicated to agricultural, food, and environmental research, is one of the world's leading research institutes in these fields. GABI (Génétique Animale et Biologie Intégrative) is a research unit dedicated to animal genetics and integrative biology (https://gabi.jouy.hub.inrae.fr).

Pigs need a transition to sustainable agriculture and are an increasingly important biomedical model. Embryonic stem cells (ESCs) are of particular interest because they have the potential to differentiate into all cell lineages *in vitro.* Furthermore, their genome can be edited to study many biological aspects while addressing ethical concerns related to the 3Rs principle of animal experimentation. Genetic tracers are essential tools for studying cell behaviour, lineage, and fate. They involve labelling specific genes or genomic loci with fluorescent proteins or other markers. Cas9-based systems have emerged as powerful tools for generating genetic tracers. Our laboratory has recently isolated and characterised porcine ESCs (1) and employed the CRISPR/Cas9 and Cas9D10A strategy to knock out and knock in genes in pigs. However, non-homologous end joining methods such as CRISPaint (2) or MAGIK (3) have also proved effective for site-specific insertion into genomes. Our laboratory is also an active member of the "*in vitro* models hub" of the European EuroFAANG research infrastructure project (https://eurofaang.eu).

In this project, you will have the opportunity to work at the forefront of genome editing technology to study the function of genes involved in cell differentiation using porcine in vitro systems. The aim of this project is to compare 3 different strategies for the generation of porcine reporter lines and to efficiently perform loss and gain of function for genes of interest.

You will gain hands-on experience with advanced genome editing tools and learn to culture and analyse stem cells and organoids. As a member of our research team you will benefit from mentorship from experienced researchers, access to state-of-the-art facilities and a supportive and collaborative environment.

We are looking for a highly motivated and enthusiastic student with a strong interest in genome editing and organoid research. Please send your CV, a covering letter detailing your research interests and qualifications, and your transcripts to Giorgia Egidy: Giorgia.egidy-maskos@inrae.fr by November 14th 2024.

References:

1. Dufour *et al* Cell specification and functional interactions in the pig blastocyst inferred from single-cell transcriptomics and uterine fluids proteomics. Genomics 2024 *doi: 10.1016/j.ygeno.2023.110780*

2. *Schmid-Burgk et al.* CRISPaint allows modular base-specific gene tagging using a ligase-4-dependent mechanism *Nat Commun. 2016 doi: 10.1038/ncomms12338*

3. *Haideri et al.* MAGIK: A rapid and efficient method to create lineage-specific reporters in human pluripotent stem cells *Stem Cell Rep. 2024 doi: 10.1016/j.stemcr.2024.03.005*