INTERNSHIP PROPOSAL 2022-2023

Internship Title : Bovine fetal epigenome : effects of chromosomal sex, environment and their interaction.

Name of the laboratory : UMR 1198 BREED Biology of Reproduction, Environment, Epigenetics and Development. https://www6.jouy.inrae.fr/breed/

Place of the internship : Domaine de Vilvert. 78352 Jouy-en-Josas.

Supervisors :

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Name of the hosting team : MECP2 : Epigenetic Mechanisms of building/prediction of phenotypes Epigénétiques de construction/prédiction des phénotypes. Centre de Recherches Ile-de-France-Jouy-en-Josas-Antony.

Internship description :

Early mammalian embryo is very sensitive to environmental perturbations, which affect phenotypic traits in a sex-specific manner at adulthood. This assumes that sex-specific differences exist even before hormonal impregnation and that early environmental perturbations are somehow registered by the embryonic epigenome and later affect the gene expression and phenotype of the individual.

Such differences due to sexual chromosomes have been evidenced mainly in the mouse. Yet mouse is special among mammals due to its very early X-chromosome inactivation and high number of inactivated genes. This suggests that sex-related phenotypic differences are even more important in non murine species. The ANR project BoSeX-Dim ANR aims to (i) identify early sex-dimorphisms in bovine embryo (ii) analyze how these dimorphisms are modified in response to early embryo environment especially when the embryo is produced *in vitro*.

We generated transcriptomes and methylomes of male or female bovine fetuses developed to day 18 or day 40 from embryos produced either in vivo or in vitro during the first 7 days of their development. Transcriptome analyses have already been performed and lists of differentially expressed genes will be made available.

The student will be in charge of the analysis of methylomes produced by Reduced Representative Bisulfite Sequencing, a pan-genomic DNA methylation analysis, from the extra-embryonic tissue of 20 individuals at Day 18 and from the brain, liver, gonads and chorion of 20 fetuses at Day 40 (10 males and 10 females, half of which were produced in vivo and the other half in vitro i.e. a set of 100 methylomes), in order to identify regions differentially methylated according to the sex of the individuals or according to the early embryonic environment (in vivo vs. in vitro).

HE/She will compare these regions to genes differentially expressed according to sex or developmental conditions previously identified by analysis of the corresponding transcriptomes. The most interesting

candidate regions either for their methylation differential or for their correlation with expression differences will be confirmed by bisulfite pyrosequencing methylation analysis.
For genes with different methylation profiles, he/she will determine the gene ontology terms, functional category, biological pathway and integrated network using : EnrichR platform (http://amp.pharm.mssm.edu/Enrichr), The Database for Annotation, Visualization and Integrated Discovery (DAVID ; https://david.ncifcrf.gov/) andGene Set Enrichment Analysis (GSEA, www.broad.mit.edu/gsea).

Techniques used :
For RRBS analysis, our home made pipeline will be used. This pipeline is based on reference software solutions, such as trim_galore (reads cleaning and filtering), Bismark (mapping of bisulfite-converted DNA reads) as well as MethylKit (methylation level and differential analysis) and MethylSig (statistics for differentially methylated cytosines or regions). Differentially methylated cytosines (DMC) showing close genomic localization will be integrated in differentially methylated regions (DMR). Both lists of DMCs and DMRs positions will be annotated with genomic features: Few candidate regions will be validated by a new method based on bisulfite conversion and pyrosequening.

Keywords : DNA methylation, fetus, sexual dimorphism, in vitro development of embryo

References :
Okamoto I, Patrat C, Thépot D, Peynot N, Fauque P, Daniel N, Diabangouaya P, Wolf JP, Renard JP, Duranthon V, Heard E. Eutherian mammals use diverse strategies to initiate X-chromosome inactivation during development. Nature. 2011 Apr 21;472(7343):370-4.

Perrier JP, Sellem E, Prézelin A, Gasselin M, Jouneau L, Piumi F, Al Adhami H, Weber M, Fritz S, Boichard D, Le Danvic C, Schibler L, Jammes H, Kiefer H. A multi-scale analysis of bull sperm methylome revealed both species peculiarities and conserved tissue-specific features.
BMC Genomics. 2018 May 29;19(1):404.

Costes V, Chaulot-Talmon A, Sellem E, Perrier JP, Aubert-Frambourg A, Jouneau L, Pontlevoy C, Hozé C, Fritz S, Boussaha M, Le Danvic C, Sanchez MP, Boichard D, Schibler L, Jammes H, Jaffrézic F, Kiefer H. Predicting male fertility from the sperm methylome: application to 120 bulls with hundreds of artificial insemination records. Clin Epigenetics. 2022 Apr 27;14(1):54.

Duration of the internship : 5 to 6 months

Desired profile: Master 2 student or engineer end of study internship.
Interest in epigenetics and bioinformatics.

Gratification : About 600 euros / month.