**M2 Internship description**

**Chromatin Dynamics Team** (team leader: Daan NOORDERMEER)

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 <https://www.i2bc.paris-saclay.fr/equipe-chromatin-dynamics/>

*Determination of 3D genome organization in mouse embryonic stem cells using* in-vitro *transcription based Hi-C*

The three-dimensional (3D) organization of chromosomes is an essential component of gene regulation in mammalian cells. The promoters of many genes form DNA loops with enhancer elements that are located far away on the chromosome, thereby regulating their activity. This process is aided by the presence of TADs, which are insulated 3D chromatin domains that promote the formation of contacts within the same domain and prevent contacts between neighboring domains. Perturbation of TADs leads to a range of diseases and embryonic defects, confirming the importance of these domains for gene regulation (see e.g. Lupianez et al, 2015).

The most popular sequencing-based approach to determine the 3D organization of mammalian genomes is Hi-C, which is based on the Chromosome Conformation Capture (3C) assay. In a Hi-C experiment, DNA fragments that are interacting in a cell are ligated together, followed by PCR amplification and Illumina high-throughput sequencing.

PCR is the most common methodology to amplify DNA fragments. PCR can only be used in a test tube though, which complicates its use for single-cell and “in-situ” genomics applications. These powerful new techniques allow insights into the variation of biological processes, either within isolated populations of cells or even within intact biological samples. One such example is Spatial-CUT&Tag in mouse embryos, which helps to precisely position different cell types within the developing embryo (Deng et al, 2022). For now, the use of PCR in the Hi-C protocol prevents similar developments. Therefore, our insights into the variation in 3D genome organization between cells remains limited.

The aim of this M2 project in the Noordermeer team at the I2BC is to modify the Hi-C protocol for future spatial genomics applications. Instead of PCR, the project aims to optimize an approach that uses *in-vitro* transcription for amplification (following up on our recent study: Chang et al, 2021). Depending on the progress of the project, we expect to obtain the following outcomes:

1. A direct comparison between Hi-C protocols using *in-vitro* transcription and PCR. This analysis will allow the identification of biases introduced by PCR in the Hi-C protocol.

2. Determination of cell-to-cell variation of 3D genome organization in mouse stem cells by combining *in-vitro* transcription Hi-C with single-cell genomics (10X Genomics).

This M2 project, with a focus on technology development, will incorporate:

1. Optimization of random integration of T7 promoters in 3C libraries using a Tn5-transposase strategy (see e.g. Deng et al, 2022).

2. Optimization of *in-vitro* transcription for the preparation of Illumina sequencing libraries

3. Preparation of Hi-C libraries from *in-vitro* transcribed DNA fragments, both from pooled cells and within cells.

The student will be supervised on a daily basis by a research engineer in the team (supervisor 1), in close collaboration with the team leader (supervisor 2).

**Techniques used during the internship**

Embryonic stem cell culture

3C and Hi-C

Tn5 transposase assays

*In-vitro* transcription

Illumina sequencing library preparation

Bioinformatics analysis of Hi-C data (under direct supervision of a bioinformatician in the team)

**Bibliography**

* D. G. Lupianez, K. Kraft, V. Heinrich, P. Krawitz, F. Brancati, E. Klopocki, et al. **Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions (2015)** Cell 161, Pages 1012-1025.
* Y. Deng, M. Bartosovic, P. Kukanja, D. Zhang, Y. Liu, G. Su, et al. **Spatial-CUT&Tag: Spatially resolved chromatin modification profiling at the cellular level (2022)** Science 375, pages 681-686.
* L. H. Chang, S. Ghosh, A. Papale, M. Miranda, V. Piras, J. Degrouard, et al. **A complex CTCF binding code defines TAD boundary structure and function (2021)** bioRxiv. <http://biorxiv.org/content/early/2021/04/15/2021.04.15.440007.abstract>

**The team**

The Chromatin Dynamics team studies the function of 3D genome organization in biological processes, with a focus on gene regulation. They primarily focus on mammalian cells, including models for human disease (cancer and imprinting disorders). The team consists of 3 permanent scientists, 2 PhD students a postdoc and a research engineer.

See website for more detail and recent publications:

<https://www.i2bc.paris-saclay.fr/equipe-chromatin-dynamics/>

The team is part of the I2BC and will move to new rooms in September 2023.

**How to apply**

Send an email, including your CV and your reasons to apply for the project, directly to the main supervisor: joanne.edouard@i2bc.paris-saclay