

**THESIS TOPIC**

Subject N° (to be completed by the ED):	<b>FUNDING:</b> <input checked="" type="checkbox"/> Requested <input checked="" type="checkbox"/> Acquired	Funding origin: Association Gaétan Saleun
Thesis title: <b>Study of <i>CFTR</i> cis-regulatory elements</b>		3 keywords: transcriptional regulation, chromatin organisation, <i>CFTR</i> related disorders.
Unit / team: <b>INSERM UMR1078 / Genetics, Functional Genomics and Biotechnology</b>		
Supervisor's name: <b>Pr Claude FEREC</b>		Phone number: 0298017281 Email address: claud.ferec@gmail.com
<p><u>Socio-economic and scientific context (approximately 10 lines):</u>  <b>In our Genetics, Functional Genomics and Biotechnology– Inserm UMR 1078 unity, directed by Emmanuelle GENIN, we have access to many facilities and platforms that allow us to achieve all experiences of our project on site (<a href="https://www.univ-brest.fr/umr1078/menu/Technologies/equipements#">https://www.univ-brest.fr/umr1078/menu/Technologies/equipements#</a> / plateforme Epigen Brest (CPER) <a href="https://www.univ-brest.fr/drive/menu/Valorisation/Financeurs/EpiGen">https://www.univ-brest.fr/drive/menu/Valorisation/Financeurs/EpiGen</a>).</b>  <b>Our hospital laboratory is widely recognized in genetic, in particular as reference lab for molecular diagnosis of several genetic diseases.</b>  <b>Our team CRED (Cis-Regulatory Elements &amp; Diseases) is now well known in France for our research in the study of chromatin conformation field and the implication on gene regulation. Chromosome conformation studies are mastered in our lab and different projects on analysis of long-range regulation of genes implicated in several diseases are already conducted. We developed a recognized expertise in the analysis of cis-regulatory regions allowing the functional impact exploration of certain genomic rearrangements or variants in the chromatin organization (<i>CFTR</i> project - Moisan et al. 2016, <i>Nucleic Acids Res</i> / Collobert et al. 2021, <i>Int. J. Mol. Sci</i> ; <i>PKD2</i> project – Moisan et al. 2018, <i>BMC Genomics</i>; <i>DFNB1</i> deafness project - Moisan et al. 2019 <i>Human Genetics</i>).</b></p>		
<p><u>Working hypothesis and aims (approximately 8 lines):</u>  <b>About 8% of the human genome is covered with candidate cis-regulatory elements (cCREs). Disruptions of CREs, described as ‘disruptions’ have been identified as being involved in various genetic diseases. Thanks to the development of chromatin conformation study techniques, several long-range cystic fibrosis transmembrane conductance regulator (<i>CFTR</i>) regulatory elements were identified, but the regulatory mechanisms of the <i>CFTR</i> gene have yet to be fully elucidated.</b>  <b>The aim of this work is to improve our knowledge of the <i>CFTR</i> gene regulation, and to identify factors that could impact the <i>CFTR</i> gene expression, and potentially account for the variability of the clinical presentation of cystic fibrosis as well as <i>CFTR</i>-Related Disorders. We are interested in identifying molecular mechanisms that control transcription through changes in 3D chromatin organization. Once these CREs are well documented, we further study the potential involvement of cis-regulatory variants in unresolved patients of diseases studied in our laboratory.</b></p>		
<p><u>Main milestones of the thesis (approximately 12 lines):</u>  <b>We will perform 3D genomics approaches (3C-based methods) to map chromatin folding and topologically associating domains (TADs). We also will apply classical molecular biology techniques such as cloning, real-time quantitative PCR and protein immunoprecipitation to identify CREs and study their disruptions by CRISPR/Cas9 technologies:</b>  <b>- Gene reporter activity tests: Identification of CREs and validation of potential regulatory variants with functional assays.</b>  <b>- Conformational analyses with 4C-Seq: Mapping of chromatin folding and topologically associating domains (TADs).</b>  <b>- Chromatin Immunoprecipitation (ChIP): Analyze of transcription factors binding within CREs.</b>  <b>- CRISPRi : An RNA-based method, CRISPR interference (CRISPRi), will be used for targeted silencing of transcription.</b>  <b>- CRISPR/Cas9 editing of variants to determine their impact on gene expression and chromatin architecture.</b></p>		
<p><u>Scientific and technical skills required by the candidate (2 lines):</u>  <b>The candidate must have knowledge of molecular biology, genetic and epigenetic.</b>   <b>Scientific formation student with a Master of Genetics.</b></p>		
<p><u>3 publications from the team related to the topic (last 5 years):</u>   <b>Collobert M, Bocher O, Le Nabec A, Génin E, Férec C, Moisan S. <i>CFTR</i> cooperative cis-regulatory elements in intestinal cells. <i>Int. J. Mol. Sci</i>, 2021, 22, 2599.</b>   <b>Moisan S, Le Nabec A, Quillévéré A, Le Maréchal C, Férec C. Characterization of <i>GJB2</i> cis-regulatory elements in the <i>DFNB1</i> locus. <i>Hum. Genet</i>, 2019, 138, 1275-1286.</b>   <b>Moisan S, Berlivet S, Ka C, Le Gac G, Dostie J, Férec C. Analysis of long-range interactions in primary human cells identifies cooperative <i>CFTR</i> regulatory elements. <i>Nucleic Acids Res</i>. 2016 Apr 7;44(6):2564-76.</b></p>		
<p><u>National and international collaborations:</u></p>		

This project will be in continuity with numerous national and international collaborations nationales mais aussi internationales :

- Partnerships with groups specialized in the study of chromatin conformation field and the implication on gene regulation:

- Dostie Lab - McGill University – Montreal,
- Harris Laboratory - Northwestern University - Ann & Robert H. Lurie Children's Hospital of Chicago.

- Partnerships with groups specialized in cystic fibrosis and *CFTR*-related disorders :

- Pr Antoine Valeri, urologue au CHRU de Brest,
- Laboratoire Génopathies – Mucoviscidose de Lille (Adrien Pagin),
- Laboratoire Mucoviscidose: physiopathologie et phénogénomique de Paris (Harriet Corvol),
- Laboratoire Génétique méd, mal rares et médecine personnalisée de Montpellier (Caroline Raynal),
- Registre et Mucodoméos - Vaincre la Mucoviscidose de Paris (Lydie Lemonnier),
- Harris Laboratory - Northwestern University - Ann & Robert H. Lurie Children's Hospital of Chicago.