Projects list

Block 1

Project 1: Cell-type-specific epigenetic profiling using Fluorescence-Activated Nuclear Sorting (FANS) from brain tissue
Instructor: Dr. Rafael Alcalá Vida (Instituto de Neurociencias, UMH-CSIC, San Juan Alicante Spain)

Background and aims: The CNS is composed of a highly complex set of cellular types, differently contributing to both physiological and pathological processes. The urgent need to characterize specific mechanisms occurring in particular brain cell types triggers the development of new approaches to improve the cell-specific resolution of epigenomic and transcriptomic techniques. A gold standard for generating cell-type-specific epigenomic data is based on the separation of nuclear populations using fluorescent markers and flow cytometry, so-called Fluorescence-Activated Nuclear Sorting (FANS). In this project, we will learn about the pros and cons of these particular approaches and the compatibility with downstream applications for epigenetic profiling of histone post-translational modifications using Chromatin Immunoprecipitation coupled to sequencing (ChIP-seq) and the recently developed Cleavage Under Target and Tagmentation (CUT&TAG), and using as starting material frozen brain samples. We will perform nuclear purification and immunostaining protocols from frozen mouse brain using native and cross-linked conditions, and purify specific populations using flow cytometry. The quality of purification will be verified by different approaches. In addition, a set of purified nuclear preparations will be processed for epigenetic profiling, targeting specific histone modification (e.g. H3K27ac and H3K27me3) using CUT&TAG and ChIP-seq. Several validations steps will be performed to assess the efficacy of enrichment and sample quality.

Techniques:
- Nuclear preparations from frozen tissue (native and cross-linked)
- Nuclear immunostaining
- Flow cytometry
- Western blot
- CUT&TAG
- ChIP-seq
- qPCR

Project 2: Astrocyte epigenome of mouse hippocampus
Instructor: Dr. Isabel Paiva de Castro (Laboratory of Cognitive and Adaptive Neurosciences, LNCA, Strasbourg, France)

Background and aims: Astrocytes play a key role in brain homeostasis and functions that are compromised in aged brains, such as memory. These mechanisms are sustained by the bidirectional communication between astrocytes and neurons that regulates neuronal excitability and synaptic plasticity. Therefore, understanding how astrocytes are altered in aging is crucial to determine the mechanisms occurring in brain aging and contributing to aging-related diseases. In recent years, development of next generation sequencing techniques led to a broader characterization of transcriptional changes occurring in astrocytes in the aging brain. However, the astrocytic epigenetic alterations related to histone

modifications have not been elucidated. Thus, this project aims to assess the epigenetic landscape of astrocytes in aging, using the recently developed Cleavage Under Target and Tagmentation (CUT&Tag). For that we will assess histone marks of active (H3K27ac) and repressive (H3K27me3) transcription in astrocytes from young and aged mice hippocampi, a critical brain region that regulates memory and learning. Moreover, in order to assess genome-wide chromatin accessibility, the Assay for Transposase-Accessible Chromatin (ATAC)-sequencing will be performed.

Techniques:

- Isolation of astrocytes from mouse hippocampus using ACSA2 magnetic --beads (MACS):
- CUT&Tag
- ATAC-seq

Project 3: Transcriptomic profiling of adult mouse brain tissue using 10X Genomics single-nuclei RNA-sequencing
Instructor: Lalit Kaurani (DZNE-Göttingen)

Background and aims: Single cell RNA sequencing (scRNA-Seq) has emerged as a revolutionary technology over the past few years, and as the methodologies continue to be refined, it is being implemented more frequently. Understanding the role of transcriptional regulation in learning and memory, development of the nervous system, and the etiology of a variety of neurodegenerative and psychiatric disorders has progressed significantly over the past decade thanks to bulk RNA sequencing (and prior to that, DNA microarrays). Nonetheless, bulk RNA-Seq disregards the cellular heterogeneity within the same piece of tissue, both in terms of basal gene expression and, more critically, transcriptional response to a stimulus. scRNA-Seq is an alternative method for studying the cellular heterogeneity of the brain, as it profiles tens of thousands of individual cells/nuclei as an effort to characterize the intricate cellular changes in healthy brain physiology. During the course, we will generate the single nuclei data by profiling droplet-based single-nucleus cortical/hippocampal transcriptomes from young healthy mouse brain. The resulting experiment will show cell type-specific and common gene expression markers, specific cellular subpopulations based on unique gene expression profiling, and provides a unique cellular-level perspective of transcriptional profiling of healthy mouse brain. To achieve this objective, we will use 10X Genomics Single Cell 3' (v3) RNA sequencing, a microdroplet-based method for capturing and sequencing mRNA and pre-mRNA molecules from individual nuclei. As we know, RNA molecules are transcribed and processed within the nucleus prior to export to the ER for translation into proteins. Therefore, nuclear RNA is a mixture of nascent transcripts, partially or completely processed mRNA, and other noncoding RNA molecules. Nuclei can be quickly extracted from frozen tissues using a combination of chemical and physical techniques that effectively avoid the non-uniform or incomplete dissociation of solid tissues into single cells, as well as RNA degradation or artifacts (such as the stress response) during dissociation.

Techniques:

- Mouse brain dissection (CA1/PFC)
- Nuclei isolation
- Nuclei barcoding and GEM preparation
- cDNA and Library preparation
- Qubit library concentration measurement
- Bioanalyzer for library size selection

Project 4: Cell type-specific detection of lncRNAs in the mouse brain using RNAscope and immunofluorescence
Instructor: Sophie Schroeder (DZNE-Göttingen)

Background and aims: Long non-coding RNAs (lncRNAs) are emerging as important regulators of neuronal plasticity and have recently been linked to the onset and progression of neurodegenerative diseases. However, our knowledge about the role of CNS-specific lncRNAs in neuronal and non-neuronal cells is limited. It has been found that many lncRNAs show a high tissue- and cell type-specificity, but as single-cell sequencing of non-coding RNAs is still challenging, their expression pattern in the different cell types in the brain remains elusive. Therefore, the aim of this project is to identify the expression pattern of selected lncRNAs in the brain. We will use RNAscope to detect the lncRNAs of interest in brain tissue sections. Using the software CellProfiler, we will also learn how to quantify the expression levels of these lncRNAs. In the second part, we will combine the RNAscope method with immunofluorescence staining for cell type markers (neurons and astrocytes as an example) to investigate the cell type-specificity of the selected lncRNAs.

Techniques:
- Brain isolation
- Tissue sectioning using a cryostat
- RNAscope (+ immunofluorescence staining)
- Image acquisition using fluorescence and confocal microscopy
- Image analysis with CellProfiler

Project 5: Investigation of single cell DNA methylome landscape of cortical brain cells
Instructor: Mykhailo Batiuk (Neuroepigenetics lab, EPFL, Lausanne, CH)

Background and aims: Last decade of single cell genomics advancements was crucial for deciphering brain cell complexity and perturbations during brain diseases. Plethora of commercial, high throughput, and user friendly assays have appeared allowing assessment of millions of single cells on the level of mRNA expression and chromatin accessibility. However, molecular complexity of cell goes beyond this. And single cell assessment of different modalities of epigenetic gene expression regulation is more challenging for a regular investigator, while being crucial for understanding brain physiology and pathology. The project will focus on assessment of single cell DNA methylation of brain pre-frontal cortex – area involved in memory, cognition, and brain pathology. Students will learn to microdissect pre-frontal cortex from mouse brain and isolate high-quality nuclei using optimized gradient centrifugation protocol and FACS. Further, students will learn to produce single nuclei DNA methylome sequencing libraries using snmC-seq2 protocol, and assess methylation of selected loci using qPCR. By the end of the course students will get practical knowledge of single cell DNA methylation assessment in neural tissue. This will help to familiarize investigators with one of the more technical and non-commercial epigenome profiling technologies.

Techniques:
- Brain subregion microdissection
- Isolation of cell nuclei using optimized protocol
- FACS of nuclei
- Single nuclei DNA methylome library preparation (snmC-seq2)
- qPCR

Project 6: Cell-type and brain-region specific epigenetic manipulation in Alzheimer's disease models
Instructor: Dr. Sanchez-Mut (Instituto de Neurociencias, UMH-CSIC, San Juan Alicante, Spain) and Dr. Marta Kulis (IDIBABS, Barcelona, Spain)

Background and aims: There is increasing evidence of cell- and brain-region specific effects of epigenetic manipulations on brain function. Using a combination of stereotaxic and genetic tools, we will manipulate the DNA methylation profiles of selected cell types in specific brain regions in a mouse model of Alzheimer's disease. We will perform a series of quality controls and prepare the samples for high-throughput techniques such as Whole genome bisulfite sequencing (WGBS) and/or single-nuclei ATAC-RNA multiome analysis.

Techniques:
- Stereotaxic surgeries: e.g., viral delivery of Camk2a driven DNMT3A expression in Hippocampus of APP/PS1 mice (or other available models, if not, wt)
- IHC (or ink) for testing the target-efficiency. If AD model, abeta plaques, etc..
- Fresh brain dissections: CA1, CA3 and DG (other regions such as frontal cortex, striatum, thalamus, hypothalamus, entorhinal cortex, amygdala, etc.. are also possible).
- RT-PCR with brain specific markers for testing the efficiency of the dissection
- Fresh/frozen samples for simultaneous ELISA (abeta), RNAseq, WGBSseq and/or singe-nuclei_ATAC-RNAseq (multiome)
- Pyrosequencing, Bisulfite cloning, RT-PCR, bioanalyzer, … for quality control and marker detection.

Project 7: CRISPR-dCas9 systems for targeted epigenetic repression
Instructor: Dr. Davide Martino Coda (Neuroepigenetics lab, EPFL, Lausanne, CH)

Background and aims: By being at the same time dynamic and stable, epigenetic modifications have long been proposed as the molecular substrates for memory and learning, but the underlying mechanisms remain to be elucidated. Since its recent introduction, CRISPR-based epigenetic editing tools became an essential resource for researchers in the neuroepigenetics field, enabling to alter the epigenetic landscape at specific sites in the genome and thus shed light on the importance of the epigenetic code for cognition and behavior in both health and disease. This hands-on project will focus on CRISPR-dCas9 systems for epigenetic repression, also referred to as CRISPRoff. The goal of the project is to target genes crucial for memory and learning (e.g. Arc, Homer, Npas4, Fos) in N2a cells, and to compare the efficiency of different CRISPRoff systems in repressing gene expression. The participants will also learn principles of sgRNA design for enhancers/promoters targeting and sgRNA multiplexing. Finally, changes in epigenetic modifications at the targeted loci will be explored by performing ChIP q-PCR for different histone markers.

Techniques:
- Selection of sgRNAs for CRISPR-based epigenetic repression,
- Design of vectors for sgRNA multiplexing
- Transfection of N2a cells with different CRISPRoff systems (dCas9KRAB-MeCP2, dCas9HDAC2/8)
- Analysis of transcriptional and epigenetic changes by RT-PCR and ChIP q-PCR

Project 8: Epigenome editing technology based on the CRISPR/dCas9 system.
Instructor: Dr. Beatriz del Blanco Pablos (Instituto de Neurociencias, UMH-CSIC, San Juan Alicante Spain) and Marta Alaiz Noya (Instituto de Neurociencias, UMH-CSIC, San Juan Alicante Spain)

Background and aims: For years, attempts have been made to clarify the relationship between chromatin state, genome regulation and cellular phenotype. However, most of the studies aimed at understanding the role of epigenetic marks in gene transcription and more currently in 3D chromatin structure only provide correlative data. In addition, the use of epi-drugs capable to modify chromatin has an unexpected genome-wide impact, giving rise to search for alternative gene therapies with higher target specificity. The recent emergence of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system as a robust and easy epigenome-engineering platform has the potential to allow site-specific rewriting of epigenetic information. Within the "Hands-on project" of the *Cajal Training Program*, we offer training in the design, development and application of epigenome editing tools based on the nuclease-deficient Cas9 (dCas9) fused to specific catalytic domains to precisely modulate transcriptional activity in hippocampal neurons. Within this project, we will integrate the use of viral vectors as a technology for in vivo integration of the epi-editing system. The objective of the project is to acquire skills and abilities in neuronal genome epi- editing, including strategies for target gene selection, design of guide RNAs and use of viral vectors.

Techniques:

- Design of guide RNAs and target selection.
- Production of viral particles expressing different components of the CRISPR system in HEK293 cells.
- Primary cultures of hippocampal neurons.
- Epi-editing of specific loci in the hippocampal neuron genome using viral vectors.
- Analysis of transcriptional changes in the targeted gene after epi-editing.

Block 2 - Computational projects

In the second part of the course, all students will learn state-of-the-art approaches for computational analysis and interpretation of the next generation sequencing (NGS) data obtained in the epigenomic experiments covered in the first block. This 10-day bioinformatics part will be divided into a short NGS-data analysis introduction, where the instructors will explain the foundations of the NGS data analyses (including quality-control, read mapping, normalization, basic RNA-Seq quantification and comparative analyses) and guide all the participants through the hands-on examples of NGS data analysis tasks. In a few days, every participant will have a chance to gain the necessary knowledge to do the basic processing of their own data from a typical NGS experiment under guidance of experienced instructors. In the second part of the bioinformatics block, the participants will be divided into small teams focusing on a particular type of NGS data, relevant to their interests. There will be several units available, each tailored to the needs of a particular type of NGS-based experiment:

- ChIP-Seq and Cut&Tag data analysis
- Advanced RNA-Seq data analysis
- ATAC-Seq and DNAse-Seq data analysis

- DNA Methylation from bisulfite-seq data analysis
- Chromatin structure through HiC-4C-seq data analysis
- Single cell data analysis

In each of the units, we will be using pre-processed data from model organisms and experiments relevant to a typical setup for an epigenomic experiment in the neuroscience field. All of the course topics will have a representative dataset prepared, but there will be time to also discuss questions on multi-modal data integration and best approaches to data analyses of the data generated by the students. The sample datasets will also include typical noise patterns and issues in NGS-data analyses, so that the course attendees will be prepared for typical scenarios they might encounter in their own research.

Instructors:
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