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| **The Francis Crick Institute**  **Advanced Sequencing Facility**  **Project Proposal Form** |
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| **Date** | **23/05/2022** |
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| **Project name** | Investigating the interaction between mSWI/SNF chromatin remodelling complex and ASCL1 in human neurogenesis |
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| **Investigator name** | **Oana Paun** |
| **Investigator email** | **oana.paun@crick.ac.uk** |
| **PI / Lab name** | **Francois Guillemot**  **francois.guillemot@crick.ac.uk** |
| **Budget Code for sequencing work** | **10309** |
| **Is this from a grant or Core funded?** | **Core funded** |
| **No. of samples planned for the project** | **ChIP-Seq: 12 libraries** |
| **Expected Date for Sample Submission** | **25th of May 2022** |
| **Material to be submitted**  **Please provide as much detail as possible** | **Libraries from DNA following ChIP for ASCL1 and mSWI/SNF subunit using wild-type iPSC-derived neural progenitors at day 20 and day 24, respectively.** |
| **Risk Assessment and Category Level Containment Information**  **For all projects please state the containment level these samples need to be handled at. Please also confirm an appropriate risk assessment has been carried out for this work** | **Containment Level 2** |
| **Type of Libraries (e.g mRNAseq, ChIPseq, Exome)** | **ChIP-Seq** |
| **Sequencing Read Length (eg SR100, PE100)** | **SR100** |
| **Organism** | **Human, GRCh38** |
| **Any special run requirements/machine type** |  |
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| **Number of reads per sample**  **Please be aware that this is expected to vary within a margin of +/- 20 % per sample** | **50 million** |
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| **Project Summary**  The generation of the mammalian brain involves rapid and time-specific changes in gene expression. The mammalian SWI/SNF chromatin remodelling complex plays critical roles in the regulation of transcription. Proneural transcription factors drive the proliferation of neural progenitors and their differentiation into neurons. We hypothesise that the proneural factor ASCL1, which has been shown to act as a pioneer factor, recruits mSWI/SNF complexes to the DNA, resulting in opening of the chromatin that becomes accessible to other transcription factors. Supporting this model, we have established by co-IP that ASCL1 interacts physically with the SMARCC1/BAF155 subunit of the BAF complex. We chose SMARCC1 as it is a core subunit of the complex, presumably present in all variants of the complex. Furthermore, preliminary data from ChIP-Seq that we performed in wild-type human iPSCs-derived neural cultures shows that ASCL1 and SMARCB1 (another core subunit of the complex) have largely overlapping DNA-binding landscapes (>60%). However, when we overlapped the ASCL1 binding sites with publicly available ChIP-seq datasets for core subunits of the mSWI/SNF complex, we noticed the overlap being even higher (up to 85%). We suspect this difference is coming from the high variability between the replicates that were sent for sequencing (Figure 1). Therefore, we would like to sequence three more replicates for the SMARCB1 ChIP. By combining the six replicates, we will be able to generate a higher quality consensus set of peaks for SMARCB1.  Chart, bar chart  Description automatically generated  Figure 1. SMARCB1 ChIP-Seq shows a high degree of variability between biological replicates of the same genotype based on the number of MACS called peaks.  The experiments described so far have been performed in human iPSC-derived neuronal cells at day 24, when ASCL1 is highest expressed, or day 12, when ASCL1 is not expressed at all (Figure 2). At day 24, all genomic sites where ASCL1 binds and regulates accessibility are already found in open chromatin, making impossible to address whether ASCL1 acts as a true pioneer transcription factor that is able to bind heterochromatin. On the other hand, at day 12, all these genomic sites are found in closed chromatin. In order to investigate the dynamics of ASCL1 binding, we would like to determine the set of ASCL1 binding sites at an intermediate stage between the two timepoints, but when the protein is detectable. Therefore, we decided to perform ASCL1 ChIP-seq at day 20, and correlate the binding sites with already generated ATAC-seq data at day 20. This way, we will be able to describe the DNA binding profile during ASCL1 expression time period, as well as determining whether it acts as a true pioneer transcription factor.  Chart, line chart  Description automatically generated  A picture containing text  Description automatically generated  Figure 2. ASCL1 expression pattern (RNA – left hand side, protein – right hand side) during *in vitro* human cortical neuronal differentiation. |
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| **Experimental Approach:**  Please include details such as the number of biological and / or technical replicates:  Neural progenitors and neurons are derived from human iPSCs by using the dual SMAD inhibition protocol.  For this experiment, 3 independent neural inductions will be performed from wild-type iPSCs. Cells will be collected at day 20 (one of the first days ASCL1 protein can be detected in the system) and day 24 (when ASCL1 expression is at its peak).  ChIP-Seq for ASCL1 and mSWI/SNF subunit is performed using established antibodies and protocols prior to library preparation. As controls, input chromatin samples will be taken from each samples prior to performing the ChIP. |

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| **Bioinformatics Analysis Requirements – please complete this section in as much detail as possible** | |
| **Bioinformatics support required** | *Yes* |
| **Requested Bioinformatician** | *If any* |
| **Budget code for analysis work** | **10309** |
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| **Analysis goals** | **Please provide an outline of the goals of the analysis**:  *-Identify sites with ASCL1 binding at day 20 (narrow peaks), as well as mSWI/SNF binding (broad peaks) in human iPSC-derived neural progenitors at day 20 and day 24, respectively.*  *(ChIP-Seq: peak calling)* |
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| **Analysis details** | **Please provide details of the data analysis required.**  -Three replicates will be provided for the ASCL1 ChIP-seq experiment:  ASCL1 ChIP wild-type day 20 neural progenitors: ASCL1 ChIP WT R1, ASCL1 ChIP WT R2, ASCL1 ChIP WT R3  By performing MACS peak calling for each of them, we aim to generate a consensus set of ASCL1 binding sites at day 20.  - Three replicates will be provided for the mSWI/SNF subunit ChIP-seq experiment:  mSWI/SNF complex ChIP wild-type day 24 neurons:  mSWI/SNF ChIP WT R1, mSWI/SNF ChIP WT R2, mSWI/SNF  ChIP WT R3  We would like to combine the data generated from this experiment with the previously generated SMARCB1 ChIP-seq replicates (found on run 190924\_K00102\_0399\_BHCFW3BBXY, ASF Lims ID DN19154; the three ChIP-seq samples and their inputs can be found in the attached Excel spreadsheet, highlighted in yellow). By applying “"--min\_reps\_consensus 2" on the six SMARCB1 replicates, we aim to generate a consensus set of SMARCB1 binding sites at day 24. |

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| **BABS time estimate:** |  |
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| **ASF Cost Estimate:** |  |
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Additional information from BABS:

**By submitting this form, you are confirming that your PI has agreed on the project and that the cost-code can be used for this purpose.**

Please be aware that the number of hours listed to do the analysis is an estimate and numbers may change. If the project takes longer than estimated you will be informed, and a new estimation will require approval before continuing. Likewise, if a project takes less time than the initial estimate you will be charged accordingly.

This charge does not affect the [Crick's authorship policy](https://intranet.crick.ac.uk/our-crick/research-integrity/pages/publication-authorship): regardless of whether it is Core or Grant funded, we generally expect our significant contribution to be recognised in papers - if this needs discussion, please do so at the project proposal meeting.