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| **The Francis Crick Institute**  **Advanced Sequencing Facility**  **Project Proposal Form** |
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| **Date** | **06/07/21** |
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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** |
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| **Investigator email** | [**oana.paun@crick.ac.uk**](mailto:oana.paun@crick.ac.uk) |
| **PI Details**  **Please ensure they have checked the proposal** | **Francois Guillemot**  **francois.guillemot@crick.ac.uk** |
| **BUDGET CODE to charge to** | **10089** |
| **Is this from a grant or Core funded?** | **Core funded** |
| **No. of samples planned for the project** | **ChIP-Seq: 30 libraries**  **ATAC-Seq: 15 libraries**  **RNA-Seq: 15 libraries** |
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| **Expected Date for Sample Submission** | **15th of July 2021** |
| **Material to be submitted**  **Please provide as much detail as possible** | **DNA from ChIP for ASCL1 on human iPSC-derived neural cultures, including both wild-type and mSWI/SNF-mutant cells**  **Tagmented DNA for both wild-type and mSWI/SNF-mutant cells prepared using the Illumina Tagment DNA TDE1 Enzyme and Buffer Kit**  **RNA for both wild-type and mSWI/SNF-mutant cells** |
| **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, ATAC-Seq, mRNA-Seq** |
| **Sequencing Read Length**  **For example, SR50, SR100, PE50, PE100)** | **SR100** |
| **Organism** | **Human, GRCh38** |
| **Any special run requirements/machine type** |  |
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| **Number of reads per sample** | **50 million** |
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| **60Project 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%) (Figure 1).  A picture containing chart  Description automatically generated  **Figure 1. Overlap between SMARCB1 and ASCL1 binding landscapes.**  Previous ChIP-Seq experiments we performed in human wild-type iPSC-derived neurons showed that SMARCB1-containing BAF complexes share more than 6% of their binding sites with the binding landscape of the proneural transcription factor ASCL1.  To further characterise the ASCL1-mSWI/SNF interaction, we performed ATAC-seq in wild-type, ASCL1 and mSWI/SNF knockout neurons. Analysis of these datasets revealed 3000 genomic sites where ASCL1 binds and regulates chromatin accessibility. Moreover, one third of these sites are also bound by the mSWI/SNF complex and also dependent on these complexes for chromatin accessibility. This suggests the ASCL1-mSWI/SNF complex interaction is required to regulate chromatin accessibility in human neuronal cultures.  The experiments performed so far involved disassembly of the mSWI/SNF complex by generating an acute double mutant for SMARCC1 and SMARCC2 core subunits of the complex, previously shown to regulate its assembly. Generation of these mSWI/SNF deficient cells require at least three days, timeframe which could also affect the neural identity of the cells. In addition, mSWI/SNF complex also binds ASCL1 promoter. As a result, ASCL1 expression and binding is significantly reduced within the three days and makes difficult to address questions regarding the recruitment mechanisms between mSWI/SNF complex and ASCL1. In order to address this question, we found an alternative method to abolish the activity of the mSWI/SNF chromatin remodeler: a small molecule called BRM014 that blocks the activity of the two ATPases that can incorporate in mSWI/SNF assemblies. This results in the remodeling activity of the complex to be rapidly reduced  (24 – 48 hours) without physically removing mSWI/SNF from the DNA. Analysing both chromatin accessibility and ASCL1 binding after BRM014 treatment will enable us to investigate the recruitment mechanisms between ASCL1 and the mSWI/SNF complex in a dynamic fashion. Finally, the RNA-Seq experiments will allow us to investigate how the expression of different downstream targets is affected when impeding the ASCL1-mSWI/SNF complex interaction. Since BRM014 changes chromatin accessibility quicker than the acute mutation approach used before, we expect the mutant cells to retain their neural identity.  **Experimental Approach:**  Please include details such as the number of biological and / or technical replicates:  Neurons are derived from human iPSCs by using the dual SMAD inhibition protocol to generate neural progenitors followed by Notch inhibition to generate neurons (Figure 3A).  For this experiment, 3 independent neural inductions will be performed from wild-type iPSCs. 48 hours before collection, cells will be treated with either DMSO or BRM014. Wild-type, DMSO and BRM014 samples will be collected at day 24 & 48 (in cells expressing high levels of ASCL1) for ATAC-Seq and RNA-Seq. = 18 samples each  For ChIP-Seq- one time point will be selected from the ATAC data, with 18 samples for each IP (36 samples)  ChIP-Seq for ASCL1 and SMARCB1 is performed using established antibodies and protocols prior to delivery for library preparation. As controls, input chromatin samples will be taken from each sample prior to performing the ChIP.  DNA for the ATAC-Seq experiment is prepared using the Illumina Tagment DNA TDE1 Enzyme and Buffer Kit.  RNA is isolated using the RNeasy Micro Kit.  I will first submit the ATAC-Seq samples to confirm mSWI/SNF complex inactivation. Once the ATAC-Seq data is analysed, I will proceed with submitting samples for RNA-Seq and ChIP-Seq. |  | **0** |
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| **Who will perform the Bioinformatics?:** If you are not using a member of the core Bioinformatics team at Crick, please provide details of how you will perform the analyses. If BABS are to analyse the data, please be sure to discuss the intent to publish and authorship with the analyst.  **We request Harshil Patel from the core Bioinformatics team to perform the analysis.** |  |  |
| ***Indicative Costing (ASF Use):***  ***RNA***   |  |  |  |  | | --- | --- | --- | --- | | **Type** | **Units** | **Prep** | **Crick Cost** | | **QC** | 18 | QC (includes Premade Libraries) and Project Management | £256.03 | | **Lib. Prep** | 18 | KAPA mRNA polyA HyperPrep | £749.39 | | **Sequencing** | 1 | Single End High Output Sequencing | £701.11 | |  |  | **Total** | **£1,706.53** |   ***ChIP***   |  |  |  |  | | --- | --- | --- | --- | | **Type** | **Units** | **Prep** | **Crick Cost** | | **QC** | 36 | QC (includes Premade Libraries) and Project Management | £512.06 | | **Lib. Prep** | 36 | NEB Ultra II DNA | £1,421.61 | | **Sequencing** | 3 | Single End High Output Sequencing | £2,103.34 | |  |  | **Total** | **£4,037.01** |   ***ATAC***   |  |  |  |  | | --- | --- | --- | --- | | **Type** | **Units** | **Prep** | **Crick Cost** | | **QC** | 18 | QC (includes Premade Libraries) and Project Management | £256.03 | | **Lib. Prep** | 0 | NEB Ultra II DNA | £0.00 | | **Sequencing** | 2 | Paired End High Output Sequencing | £2,600.77 | |  |  | **Total** | **£2,856.80** |   ***Total £7,970.34*** |  |  |