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| **The Francis Crick Institute**  **Advanced Sequencing Facility**  **Project Proposal Form** |
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| **Date** | **21/01/2022** |
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| **Project name** | Single-cell multiome ATAC and RNA sequencing of adult hippocampal neural stem cells. |
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| **Investigator name** | Sara Ahmed de Prado |
| **Investigator email** | saraahmeddeprado@crick.ac.uk |
| **PI / Lab name** | **Francois Guillemot** |
| **Budget Code for sequencing work** | **10309** |
| **Is this from a grant or Core funded?** | **Wellcome Trust grant** |
| **No. of samples planned for the project** | 9 |
| **Expected Date for Sample Submission** | 6 weeks-old mice on 3rd February 2022 (Replicate 1)  12 months-old mice on 14th September 2022 (Replicate 1)  18 months-old mice on 5th September 2022 (Replicate 1) |
| **Material to be submitted**  **Please provide as much detail as possible** | mCherry+ nuclei suspension resuspended in Diluted Nuclei Buffer comprising 1X Nuclei buffer (10x Genomics), 1mM Dithiothreitol (DTT) and 1 U/ μL RNase inhibitor in nuclease-free water. |
| **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** | **CL1** |
| **Type of Libraries (e.g mRNAseq, ChIPseq, Exome)** | Chromium Next GEM single cell multiome ATAC and RNA sequencing |
| **Sequencing Read Length (eg SR100, PE100)** | Paired-end, dual indexing |
| **Organism** | *Mus musculus* |
| **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** |  |
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| **Project Summary**  **Introduction**  In mice, the subgranular zone of the hippocampal dentate gyrus has lifelong neurogenic capabilities due to the presence of adult hippocampal neural stem cells (NSCs or AHNSCs). Activation of NSCs proliferation gives rise to neural progenitors, which will form the dentate granule neurons upon differentiation, and/or new NSCs. In absence of mitogenic signals, NSCs enter a non-dividing reversible state or quiescence. As NSCs remain quiescent for longer duration, they move progressively into deeper quiescence and display a reduced sensitivity to growth signals. In juvenile mice, all proliferating hippocampal NSCs differentiate into neuronal progenitors, whereas in adult mice (6 months of age) more than half of the dividing NSCs self-renew and return to quiescence.  Previous results from the lab demonstrate that the decision of adult hippocampal NSC to re-enter the cell cycle or remain quiescent depends on ASCL1 protein levels (Harris L. et al 2021). ASCL1 is a pioneer transcription factor and thus it has the capacity to associate with condensed chromatin to facilitate the binding of additional transcription factors and it can directly modulate chromatin accessibility, recruit other transcription factors and chromatin modification enzymes. Other results from the lab also suggest that significant transcriptional and chromatin accessibility changes occur between activated and quiescent NSCs, based on previous snRNA-Seq (Fig. 1) analysis and preliminary snATAC-Seq analysis.    Figure 1. single cell RNA-Seq analysis of adult hippocampal NSCs. After two iterations of subsetting and re-clustering, a dataset of 2,947 NSCs was ordered using Slingshot, revealing a pseudotime trajectory from the most quiescent NSCs (blue) to proliferating NSCs (red) (Harris L. et al 2021).  **Hypothesis**  We propose that adult hippocampal NSC behavior is defined by their epigenetic status, and thus the decision of adult hippocampal NSCs to proliferate or remain quiescent is controlled cell intrinsically at the chromatin level. In this model, quiescent NSCs from young mice would present a partially closed chromatin configuration at loci involved in NSC activity, and the chromatin configuration in these loci would be closed more tightly as NSCs enter a deeper quiescence state in old mice.  **Purpose**  Profiling of genome-wide chromatin accessibility and RNA expression in the same individual adult hippocampal NSCs.  To explore the gene regulatory network that controls the maintenance of quiescent AHNSCs and the progression of NSCs from quiescence to activation, we plan to combine discovery of regulatory elements with gene expression by simultaneous detection of mRNA and chromatin accessibility from the same cell. The analysis of associations between variation in gene expression and chromatin accessibility within the same single cell will allow relationships to be identified unambiguously.  We will perform single cell multiome ATAC and RNA sequencing *in vivo.* For this we will use a mouse strain generated by Dr. Kaja Maczulska in which cells transcribing *Ascl1* express a histone-tagged mCherry. To select the hippocampal NSCs we will use FACS.  The scRNA-Seq data analysis will allow us to identify quiescent and active NSCs and associate opened chromatin regions with active genes, whereas the scATAC-Seq analysis will allow us to determine the difference between quiescent and active NSCs at the chromatin level. We propose that the chromatin accessibility level will correlate with the position of cells along the quiescent to active axis.  Moreover, we plan to examine the role of TFs including ASCL1 and MYCN in driving chromatin accessibility changes in NSCs by 1) examining transcription factor binding sites in differential ATAC-Seq regions between quiescent and active NSCs and 2) comparing the ATAC-Seq signal with the CUT&RUN signals for ASCL1 and MYCN generated in a parallel project (PM22021). This will determine the role of TFs including Ascl1 and MycN in driving chromatin changes between quiescent and active NSCs, and address the hypothesis that Ascl1 has distinct activities in quiescent and active NSCs which we proposed in the above-mentioned CUT&RUN project proposal (PM22021). |
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| **Experimental Approach:**  Please include details such as the number of biological and / or technical replicates:  **10x Genomics single cell multiome ATAC + gene expression sequencing**  The experiments will be carried out with a mouse strain in which cells transcribing *Ascl1* express a histone-tagged mCherry (Dr. Kaja Maczulska). Multiomics (snRNA-Seq and snATAC-Seq) experiments will be performed at three time points (6 weeks-old, 12 and 18 months-old mice) with 4 mice of both males and females per age to obtain sufficient NSCs. Three replicates will be generated per time point.   |  |  |  | | --- | --- | --- | | **Age group** | **Technical Replicates** | **Biological Replicates** | | 6 weeks-old | Replicate 1 | 4 mice (males & females) | | Replicate 2 | 4 mice (males & females) | | Replicate 3 | 4 mice (males & females) | | 12 months-old | Replicate 1 | 4 mice (males & females) | | Replicate 2 | 4 mice (males & females) | | Replicate 3 | 4 mice (males & females) | | 18 months-old | Replicate 1 | 4 mice (males & females) | | Replicate 2 | 4 mice (males & females) | | Replicate 3 | 4 mice (males & females) |   **Sample preparation**  All animal-related experimental procedures will be carried out under ethical guidelines.  Mice will be killed by cervical dislocation and their brains removed. Mouse dentate gyrus will be dissected, cut into small pieces and transferred to a tube containing 300 μL ice cold NP40 nuclei lysis buffer comprising 10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% Nonidet P40, 1mM DTT and 1 U/ μL RNase inhibitor in nuclease-free water. The following procedure will be followed to extract the nuclei from the dentate gyrus tissue: homogenization 15 times on ice cold nuclei lysis buffer using a Pellet Pestle, incubation with 1 mL of ice-cold NP40 nuclei lysis buffer on ice for 5 min. Nuclei suspensions were passed through a 70 μm Flowmi filter (VWR Bel-Art SP Scienceware). Then, nuclei will be pelleted by centrifugation at 500rcf for 5 min at 4ºC leaving 50 μL of supernatant and adding 1mL of PBS 1% BSA 1 U/ μL RNase inhibitor without mixing. After incubation on ice for 5 min, nuclei pellet willbe resuspended and the nuclei suspension centrifugated at 500 rcf for 5 min at 4ºC. Finally, nuclei pellet will be resuspended with 1mL of PBS 1% BSA 1 U/ μL RNase inhibitor and 10 μL of 7-amino-actinomycin D (7AAD) ready-made solution will be added to analyse viable cells in flow cytometry.  To select only the nuclei of the cells that express *Ascl1*, mCherry+ 7AAD+ nuclei will be sorted using a 100 μm nozzle and a flow rate of 3 on a FACS Fusion A flow cytometer into a 5 mL FACS tube containing 500 μL BSA with 5000U RNase inhibitor. After sorting, nuclei concentration will be assessed using a hemocytometer/ the Countess II FL Automated Cell Counter. The sorted nuclei will be pellet by centrifugation at 500 rcf for 5 min at 4ºC, resuspended in 100 μL of 0.1X Lysis Buffer comprising 10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% Tween-20, 0.1% Nonidet P40, 0.01% Digitonin, 1% BSA, 1mM DTT and 1 U/ μL RNase inhibitor in nuclease-free water and incubated on ice for 2 min. Nuclei suspension will be mixed with 1 mL of Wash buffer containing 10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% Tween-20, 1% BSA, 1mM DTT and 1 U/ μL RNase inhibitor in nuclease-free water. Nuclei will be pelleted by centrifugation at 500 rcf for 5 min at 4ºC and resuspended in ice cold Diluted Nuclei buffer comprising 1X Nuclei buffer (10x Genomics), 1mM DTT and 1 U/ μL RNase inhibitor in nuclease-free water. Finally, nuclei concentration will be assessed using a hemocytometer/ the Countess II FL Automated Cell Counter. |
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| **Bioinformatics Analysis Requirements – please complete this section in as much detail as possible** | |
| **Bioinformatics support required** | *Yes* |
| **Budget code for analysis work** | **Specifically, the code for BABS to charge against.** |
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| **Analysis goals** | **Please provide an outline of the goals of the analysis**:  *Please provide a bullet point list of the analysis objectives and questions that these data should address.*   * Identification of cell types and assign cell-type identities to snATAC-Seq clusters. * Identification of quiescent and activated states of NSCs. * Compare chromatin accessibility in quiescent NSCs and active NSCs within each aged group to determine the difference between quiescent and active NSCs at the chromatin level. * Correlation between ATAC peak accessibility and gene expression levels. * Compare snATAC-Seq and snRNA-Seq data to ASCL1 and MYCN binding sites from CUT&RUN project (PM22021) to identify binding sites potentially involved in gene expression and chromatin opening.   *These objectives should be specific deliverables that constitute the end-points for this project* |
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| **Analysis details** | **Please provide details of the data analysis required.**  *Please list all experimental factors for consideration, e.g. genotype, phenotype, cell-line, treatment, batch, replicate etc.*  Experimental factors for the analysis:   * Replicates: 3 replicates per age group. * Age: young adults, middle-age adults and old adult mice (6 weeks-old, 12 and 18 months).   *Please provide all sample information in a separate Excel sheet to be submitted to ASF and BABS in the sample sheet. Any factor names and labels defined above should match those in the sample sheet.*  *Please use these factors to list group comparisons to run e.g treatment vs control, tumour vs normal, ChIP vs input etc. and the definitions of any controls and how they are applied. In the case of scRNA-Seq the characterization of specific cell types with marker genes should be provided along with any trajectory analysis requirements.*  *Any anticipated follow-up analyses including the integration of other datasets such as previously analysed or public datasets should also be defined here.*  *If a specific analysis technical is being applied beyond the standard analysis workflows please provide details of the approach, a link to software and any relevant publications/documentation.* |

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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.