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| **The Francis Crick Institute**  **Advanced Sequencing Facility**  **Project Proposal Form** |
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| **Date** | **2022-05-07** |
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| **Project name** | ***Glossina* Slide-seq pilot** |
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| **Investigator name** | **Jason Somers** |
| **Investigator email** | [**Jason.somers@crick.ac.uk**](mailto:Jason.somers@crick.ac.uk) |
| **PI / Lab name** | **Lucia Prieto-Godino** |
| **Budget Code for sequencing work** | CC2067 |
| **Is this from a grant or Core funded?** | **To be transferred to Allen Investigator Award once cost centre is active** |
| **No. of samples planned for the project** | **6** |
| **Expected Date for Sample Submission** | **2022-05-14** |
| **Material to be submitted**  **Please provide as much detail as possible** | **cDNA libraries from cryosectioned *Glossina* antenna on Slide-seq pucks. Libraries will be prepared by ASFand QC’d with MiniSeq before submission.** |
| **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)** | **mRNAseq** |
| **Sequencing Read Length (eg SR100, PE100)** | **PE, 42 base pairs read 1 and as many as possible on read 2 (40-60)** |
| **Organism** | ***Glossina morsitans*** |
| **Any special run requirements/machine type** | **NovaSeq sequencing** |
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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** | **200M** |
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| **Project Summary**    Following on from Project SP22016 we now want to perform Slide-seq on a pest fly species *Glossina morsitans.* Again, as many pairs of dissected antenna as possible will be placed inside a 3mm diameter in a grid arrangement in order to get more replicates per puck and hopefully providing some landmark reference information when clustering libraries. Antennae of *Glossina morsitans* are significantly larger 700um \* 200um so we should have a greater chance of separating out individual pairs on antenna and hopefully see changes in spatial expression of different chemosensory genes with the ideal goal of being able to inform electrophysiological experiments.  If dataset generated looks promising I will also follow up with a single cell or nuclei sequencing experiment to get more accurate cell type information. |
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| **Experimental Approach:**  Please include details such as the number of biological and / or technical replicates:  As the tissue we want to test is small (700um \* 200um) we will arrange the tissues in a grid pattern and mount in OCT. We will then take several sections (10um) through the grid of tissues in order to fit as many individual antennal segments onto each puck.  Because it will be very difficult to follow the same individual antenna through each section we would consider each cryoblock to be a biological replicate and each section close to a technical replicate. Even though we will be sectioning different planes of the cryoblock between each section we will also be sectioning different inter-antennal planes.  This time I will try to take 3 sections from each of two separate pucks to see how repeatable expression patterns are across samples. I will image interleaving sections in order to have a visual representation of how many antenna should be present on each puck. |

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| **Bioinformatics Analysis Requirements – please complete this section in as much detail as possible** | |
| **Bioinformatics support required** | *Yes* |
| **Requested Bioinformatician** | Nourdine Bah |
| **Budget code for analysis work** | **PRJ\_11188** |
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| **Analysis goals** | **Please provide an outline of the goals of the analysis**:  QC and mapping of samples of transcriptome.  Spatial transcription profile of chemosensory genes of interest and cell type information. |
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| **Analysis details** | **Please provide details of the data analysis required.**  It will involve three pucks from two cryoblock. This will hopefully have multiple sections from multiple individual antenna.  Reads from each puck will need to be mapped to the Glossina morsitansreference sequence and demultiplexed to achieve spatial resolution. |

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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.