Workshop on single-cell sequencing techniques

Aim: Understand the molecular biotechnology of single-cell sequencing to gain a better understanding of the data.

Each group is assigned one sequencing technique and will compile the information related to it. During a final presentation on Friday each group will communicate their results to the whole CSAMA course.

Workshop: Thursday afternoon, read, discuss and prepare your presentations

Presentations: Friday 1.30-2 pm, 5 min each group

Groups

- **SMART-seq2 with FACS sorting of target cells**
- **Drop-seq** and **10x Genomics Chromium 3' sequencing (V3)** (please also explain the differences between both)
- **10x Genomics Chromium 5' including VDJ sequencing**
- **SPLiT-seq (V3)**

Include in the presentation:

Library preparation

- What biological questions can you answer using this technique?
- Advantages/disadvantages compared to other techniques?
- Design a detailed scheme of the library preparation, starting from a single cell suspension of target cells and ending in the final sequencing read.
- Does the method use cell barcodes and UMIs? During which steps are they added?
- Does the method include other control reagents and what are they used for? (spike in, ...)
- Which parts of the mRNA are covered by the sequencing reads?
- For each of the steps in your scheme, which biases do you suspect that could affect correct assessment of transcript abundance? (transcript length, sequence composition, transcript abundance, alternative isoforms)
- How many cells can you measure in one experiment?
- How many genes per cell can you detect on average?
- How long does one experiment take approximately?
- Advantages/disadvantages compared to other techniques?

General experiment design

You have 2 blood samples from 5 donors:

- timepoint 1: 1 day before influenza vaccination
- timepoint 2: 7 days after influenza vaccination.

You want to investigate which activated lymphocyte populations appear in the peripheral blood after the vaccination and how they differ from one donor to the other. To this end, you will sequence B cells and T cells.

How many cells of each type would you sequence and how would you design the experiment (using your assigned method)? Where do you expect batch effects? Which reagents are in your opinion most affected by batch variation?

Try to estimate how much time and money do you will need.

List of available material

You can of course check for more material online - but due to internet connection problems we also put together some information:

- **General material of single-cell sequencing:**
  - Chen et al. 2018: Review on scRNA-seq in *Annual Review of Biomedical Data Science*
  - Svensson et al. 2018: Perspective on scRNA-seq in *Nature Protocols*

  - Presentation “Introduction to scRNA-seq technologies” provided by Lior Pachter
- **SMART-seq2 with FACS sorting of target cells**
  - SMART-seq2 protocol by Picelli et al. 2014
  - SMART-seq2 original publication by Picelli et al. 2013
  - SMART-seq original publication by Ramsklöd et al. 2012
  - SMART-seq2 entry from Teichmann lab github page https://teichlab.github.io/scg_lib_structs/
- **Drop-seq**
  - Website on Drop-seq from McCarroll Lab
  - Online protocol from McCarroll Lab
  - Original publication of Drop-seq from Macosko et al. 2015
  - Drop-seq entry from Teichmann lab github page
- **10x Genomics Chromium 3’ sequencing (V3)**
  - assay scheme for 3’ protocol
  - 10x Genomics protocol from the corresponding library prep kit
  - 10x Chromium 3’ sequencing entry from Teichmann lab github page
- **10x Genomics Chromium 5’ including immune-profiling**
  - assay scheme for 5’ VDJ protocol
  - 10x Genomics protocol from the corresponding library prep kit
  - 10x Chromium 5’ sequencing entry from Teichmann lab github page
- **SPLiT-seq (V3)**
  - Original publication of SPLiT-seq from Rosenberg et al. 2018
  - Supplementary information for Rosenberg et al.
  - SPLiT-seq protocol V3
  - SPLiT-seq entry from Teichmann lab github page