**Single cell RNA sequencing**

Methods:

1. Sample collection
2. Cell isolation
3. Single cell capture
4. Whole transcriptomic amplification
5. Library preparation
6. Loading on NextSeq 2000

**1. Sample Collection:**

Type of sample used: Nasopharyngeal sample

Nasopharyngeal swab will be collected in a cryovial containing 90% FBS + 10% DMSO and frozen using a slow cooling device (Mr. Frosty) before keeping it a -70C.

**2. Cell isolation**

Purposes: to isolate single cells from frozen nasopharyngeal swab

**Procedure**: https://www.protocols.io/view/human-nasopharyngeal-swab-processing-for-viable-si-5jyl8myz9g2w/v1/materials

* Step-1: Place the swab **(tube A)** in RPMI (**tube B**) and then move the nasal swab to digestion media in **tube C** (RPMI + DTT).
* Step-2: Centrifuge **tube B** and remove the supernatant. Resuspend the pellet in RPMI and DTT. Incubate the tube for 15min. centrifuge and discard the supernatant. Resuspend the pellet in accutase (the pellet has our cells). Incubate the resuspended pellet in **tube B** for 30min. *This is the first digestion for cell isolation.*
* Step-3: Take **tube C** (from step 1) that still contains the nasal swab along with RPMI. Incubate the tube. Then place the nasal swab into another tube (**tube D)** that contains accutase. Now centrifuge **tube C** until a cell pellet is formed. Resuspend the cell pellet in accutase. Incubate the resuspended pellet in **tube C** for 30min. *This is the second digestion for cell isolation.*
* Step-4: incubate **tube D** for 30 min that contains accutase and nasal swab. (from step 3). *This is the third digestion for cell isolation.*
* Step-5: the following steps wash away any residual DMSO, debri and digestion buffer.
  + Place a 70um filter in a conical tube and wet it with quenching buffer (RPMI + 10% FBS + EDTA)
  + Add the contents from **tube B, C, and D** onto the filter.
  + Centrifuge the conical tube and remove the supernatant
  + Resuspend the cell pellet in RPMI and FBS
* Step-6: Count the number of cells using a haemocytometer. The number of cells should be around 50,000-100,000 cells/sample