Transcriptional profiling
Modified for zebrafish by A.Quillien and M. Shin from Hrvatin et al, PLOS One, 2014

1) **Deyolk embryos:** pipette embryos up and down with p1000 in calcium free ringers buffer
2) **Disperse cells:** disperse to a single cell suspension using TrypLE Express –Aurelie's fridge - (Invitrogen).
    a. Prewarm buffer - 4mL TrypLE Express in 14mL tube (Falcon 352059)
    b. Incubate fish at 28C
    c. Pipette up and down with p1000 filtered tip every 5min for 15min
    d. (Maximum incubation 1.5-2hrs)
3) Everything **ON ICE** after this step
4) Cells were passed through a 70μm filter (BD Falcon 352340; filters are on the lab shelf with tissue culture supplies) into a 50mL conical tube. Transfer cells from 50mL to 15 mL tube.
5) Fill tube with 1xPBS and spin down (@4C) at 3000g for 3min.
6) Add 1mL to pelleted cells, transfer to a 2mL tube, (take 1μl for cell count) and spin again.
7) **4% PFA fix and permeabilize**: Cells were fixed and permeabilized for 30min rotating at 4C in the 2mL tube with 1.4mL of 4% PFA/PBS supplemented with 0.1% saponin (Sigma- Aldrich 47036; chemical shelf).
    a. 4%PFA + 0.1% saponin stored at 4C
8) **Permeabilize:** Cells were pelleted by centrifugation at 3000g for 3min at 4C and washed in Wash Buffer: PBS containing 0.25% BSA/0.1% saponin (chemical room; Sigma-Aldrich), 1:100 RNasin Plus RNase Inhibitor. Before adding RNasin Plus, filter wash buffer with 0.22um filter and 20mL syringe every time to prevent from clogging during FACS.
9) **Blocking:** Cells were blocked in 500μl of PBS containing 1%BSA/0.1% saponin/1:100 RNasin Plus fro 15min at 4C that spin at 3000g for 3min at 4C.
10) **Primary Antibody staining (optional):** Add 500μl of Primary antibody with appropriate concentration in the blocking solution was carried out while 3D rocking for 2.5hr at 4C (note that certain antibodies may require longer incubation times). Cells were pelleted and washed 2x in 1mL of Wash Buffer 5min at 4C.
11) **Secondary Antibody staining**: Stain with 1/1000 secondary antibody in the blocking solution. Following secondary antibody staining cells were washed 1-2x in Wash buffer
12) **Resuspension**: Resuspend 30M of cells with 3mL of wash buffer (also for sample collection) in 14mL Falcon tube.
13) **FACS sort:** Cells were sorted on the FACS Aria (BD Biosciences) using FACSDiva software. Gates were set with reference to negative controls. The sorting speed was adjusted to ensure sorting efficiency above 90%. Cells were collected in tubes that were coated with a small amount of Sort buffer.
14) **RNA isolation:** After sorting, cells were pelleted by centrifugation at Max speed for 5min at 4C. The supernatant was discarded. Total RNA was isolated from the pellet using the RecoverAll Total Nucleic Acid Isolation kit (Ambion), starting at the protease digestion stage of manufacturer-recommended protocol. Incubate the cells in 100μL of digestion buffer with 4μL proteinase for **1hr at 50C (critical!)**.
15) **RNA analysis :** BioAnalyzer and qPCR