SOP: Cell sorting into Xpress-seq lysis plate

This SOP describes how to sort cells into Xpress-seq lysis plates, and how to store and ship sorted plates. Familiarize yourself with this procedure before proceeding with the cell sorting.

Materials and reagents

- Dry-ice
- PBS (eg. Thermo Fisher Catalog #14190094)
- Seal for -80 °C Storage: provided by Xpress Genomics
  Alternative: Adhesive PCR Plate Foils (Thermo Fisher Catalog #AB0626) or Axygen Aluminium Sealing Film (Corning Catalog #PCR-AS-200)
- Xpress-seq plates. Plates can be stored at -20 °C until sort.

Instruments

- Fluorescence activated cell sorter (FACS), or a cell sorter capable of dispensing in <50 nl of volume. Our service is not compatible with manual cell-picking, mouth pipetting or similar for capturing and dispensing single cells in high volumes. The following instruments have been successfully used for Xpress-seq:
  - BD FACSMelody
  - BD FACSAria / BD FACSAria Fusion
  - BD Influx
  - BD FACSDiscover S8
  - Sony SH800S
  - Sony MA900
  - Beckman CytoFLEX SRT
  - Cellenion CellenONE
  - Cytena F.SIGHT Omics
- Centrifuge with buckets for PCR plates
  (capable of spinning to 1000xg)

Considerations

- Xpress-seq operates in nanoliter volumes and therefore depositing cells in nanoliter volumes is required, typically via FACS. We have successfully sorted cells into Xpress-seq lysis plates using a range

of FACS instruments and cell printers that all typically dispense the cell in ~5-10nl or less. If your instrument dispenses cells in higher volumes (> 50nl), the risk for failure increases.

- The buffer used for the cell suspension while sorting is important. Since the relative difference between sorted cell volume and lysis volume is lower in Xpress-seq, common additives like FBS, BSA, EDTA can interfere and negatively affect downstream reaction if present in higher amounts. We recommend to sort cells in a solution of **PBS + 0.04% BSA**. Avoid using buffers with Mg2+, Ca2+ or other cations for sorting. If EDTA is required for the cell suspension, aim to minimize the amount used. Completely avoid other additives or preservatives like DNAse I or Sodium Azide.
- Not all RNase Inhibitors are compatible when added into the cell suspension, and some can have severe negative impact on the downstream reaction and ultimately library quality. If required, it is highly recommended to use the following RNase Inhibitor: Recombinant RNase Inhibitor (Takara Bio Inc. Catalog #2313A). Alternative RNase Inhibitors, that have been tested and are compatible: NXGen RNase Inhibitor (Lucigen Catalog #30281-2), RNaseOUT Recombinant Ribonuclease (Thermo Fisher Scientific Catalog #10777019), Protector RNase Inhibitor (Sigma Aldrich Catalog #3335399001). Do NOT use SUPERaseIn RNase Inhibitor (Thermo Fisher Scientific, AM2694).

Procedure

Step 1. Prepare cells for sorting according to your working protocol, taking the above considerations into account.
Step 2. Thaw Xpress-seq lysis plates on ice and quick spin them before proceeding with the sort.
Step 3. Sort single cells into each well of a 384-well plate provided by Xpress Genomics. The plates are ready to use and contain lysis reaction mix overlayed by an evaporation barrier.
Step 4. If possible, keep both the cell sample and lysis plates cooled (~4°C) throughout sorting. Our plates have been used for extended sort durations and the cell material is effectively stabilized in our lysis buffer.
Step 5. Seal plates with a provided cold storage foil seal.

Step 6. Quick pulse centrifugation to 1000 x g to make sure the droplet with the cell reaches the bottom of the plates and merges with the lysis buffer.
Step 7. Immediately store at -80 °C or on dry ice.

Caution. *Do not put the sorted plates on dry-ice before centrifugation as this will introduce a detrimental freeze-thaw cycles that could compromise the sample integrity and affect the experiment outcome.*

For plate submissions to Xpress Genomics, please fill the Customer Information Sheet for Xpress-seq indicating:

- Number of plates submitted
- Sequencing Depth
- Read Length
- Species
- Any transgenes used
- Sample type (single cells/single nuclei)
- Sorted tissue/cell type

For questions, please contact us at service@xpress-genomics.com.

FACS Sorting Tips & Tricks

Often, final single cell library success comes down to the initial health or state of the sorted cells, as well as the sorting itself. This section includes some tips and things to consider when FACS sorting your cells, based on the cumulative experience at XG sorting cells and using different FACS machines. These tips are not to be considered guarantees but can hopefully increase the overall success-rate of sorted libraries.

• Your dissociation protocol matters. Choose carefully and perform pilot experiments, if possible. Finding the best protocol that both best represents the composition of your tissue, whilst maintaining cell health is key to a good sorting and library outcome.

• After dissociation, keep your samples cooled, on ice, at all times if possible. FACS sort with both sample and lysis plate cooled if possible.

• Always include a live/dead marker when sorting cells or a nuclei stain of preference when sorting on nuclei, to make sure you are sorting on viable material.

• If possible, try and remove as much debris from the samples prior sorting. This can usually be done with a gradient centrifugation step, or some manufacturers have kits available for specific removal of for example myelin.

• **SORT SLOW(ER).** Frequently, facilities and machines boast that they can single cell sort fast and efficient. However most often in our experience it helps to slow down a little, and sort slower. **We always recommend to sort on the slowest speed possible.**

• Dilute your samples. If you are concerned with cells sticking and clumping, dilute your sample with PBS and keep them on ice. This will also help you with sorting slower. Typically, we recommend an event rate between 50-500 events/s, but preferentially 100-250 events/s.

• If you have read and/or followed the above tips and you are now concerned about sorting times, then fear not. Apart from being a logistical issue, in terms of FACS machine booking times etc, we have successfully generated great data from plates that took ~2hours to complete sort. If both plate and sample are properly cooled, your transcriptomes are suspended and "stable", meaning we do not see significant shifts in transcriptome profiles between first and last cells sorted even on long plate sorts.