scWGS+scRNAseq
Sample Submission Guidelines

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The table below shows a quick summary for the sample requirements. Please refer to the whole document for more details.

scWGS+scRNAseq Sample Requirement	Sample Submission Format
Single intact and viable cells (e.g. dissociated from fresh tissues; from cell lines)	Submission in **Lobind 96-well or 384-well** plate

Section 1. Sample staining

All cells should be stained for viability check using a 2-stain system.

- **DAPI** staining **should NOT be used**.
- Passive stain (propidium iodide) will be used to visualize non-intact cells.
- Active stain (Calcein-AM) is only fluorescent upon uptake and enzymatic cleavage by the cell, thus ensuring that the cell is not only intact, but also actively viable.
- **Propidium iodide NEGATIVE and Calcein-AM POSITIVE cells** should be sorted into wells.
- Further stains may be utilized to identify cells of interest.
- You may refer to detailed information in **Appendix A**.

Section 2. Cell sorting and dispensing into 96-well or 384-well plate

- Primary method for cell deposition is FACS sorting, and plate alignment is critical to downstream success.
- If cells will be dispensed by a method other than sorting, ensure the cells are deposited in the bottom of the well.
- You may refer to detailed information in **Appendix A**, but specifications may vary depending on instrument used.

Section 2.1 Notes for Pre-sorting into 96-well plate format

- The 96-well plate used must be **LoBind.**
- We recommend using Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/clear (Cat no.: HSP9601), which has been tested at Mirxes Genomics Lab.
- We recommend adding **3µL of BioSkryb Genomic's proprietary cell buffer** to each target well (where a single cell is to be added into) prior to the start of sorting.
- Plates containing cell buffer should be sealed and spun briefly to collect the contents at the bottom of the well.
- Please leave wells **A1, B1, C1, D1, E1, F1, G1 and H1 empty** during dispensing, for Mirxes to use for control samples.

Section 2.1 Notes for Pre-sorting into 384-well plate format

- The 384-well plate used must be **LoBind** and **Optical**.
- We recommend using MicroAmp™ Endura Plate™ Optical 384-well Clear Reaction Plates (Cat no.: 4483285), which has been tested at Mirxes Genomics Lab.
- We recommend adding **1µL of BioSkryb Genomic's proprietary cell buffer** to each target well (where a single cell is to be added into) prior to the start of sorting.
- Plates containing cell buffer should be sealed and spun briefly to collect the contents at the bottom of the well.
- Although not optimal, it is also possible to 'dry-sort' the samples without adding cell buffer to the target wells prior to sorting.
- Please leave wells **A1, B1, C1, D1, E1, F1, G1 and H1 empty** during dispensing, for Mirxes to use for control samples.

Section 2.1 Notes for Post-sorting (96-well or 384-well plate format)

- After sorting, all plates should be spun down aggressively to ensure that any deposited cell comes into contact with the cell buffer.
- **PLATES SHOULD NOT BE SUBJECT TO VORTEX AT ANY TIME.**
- After spinning down, all plates should immediately be placed as flatly as possible directly onto dry ice.
- Plates should not be stacked on top of each other.
- Dry ice should not be placed on top of the plates. After all sorting is completed, plates should be stored at -80°C until use.

Section 3. Shipping instruction (sealed 96-well or 384-well plates for scWGS+scRNAseq)

- Plates will need to be well sealed, spun down, and immediately stored on dry ice or -80°C prior to being shipped. Please ensure that the **sealing film remains adhesive at -80°C and in dry ice.**
- Shipping must be on dry ice, in secondary containment to prevent damage.
- Eg. Sealed plates will be placed into individual baggies, plates in bags will be placed into a cardboard box (eg. Tube box) which will be in direct contact with dry ice.
- Dry ice shipping container will need at LEAST a 2" (5.1cm) layer of dry ice on the bottom, and 2" (5.1cm) on the sides of the cardboard box containing the plates.
- Dry ice should fully cover the top of the cardboard box.

Appendix A: FACS Plate Alignment Considerations (Materials are adapted from BioSkryb)

Rationale

FACS sorting is the primary method of cell deposition for the ResolveDNA and ResolveOME workflows. Plate alignment is critical to downstream success, as it ensures that cells are accurately deposited into wells with minimal negative impact on the cell itself. This is not a fast process and must be performed every 5-10 plates. In many facilities, where core-personnel are not actively performing the sorting, this is a source of poor assay performance. The purpose of this document is to describe plate alignment procedures that are performed at BioSkryb Genomics on a Sony SH-800 instrument, and to allow customers insight into our process so that they may achieve success on their own instruments. Where applicable, this document will indicate Sony SH-800 specific software and describe the goal of the step so that customers may apply the principles.

General Method

Prior to sorting plates, the FACS instrument is calibrated using a 130µm chip. Use of a 70µm chip is NOT recommended. Cells that are dual-stained (active and passive) for viability are diluted (1 million cells in 1200µL final staining buffer) and maintained on ice during this alignment step.

Accessing Plate Alignment

On the Sony SH-800, after designing experiment parameters that allow for dual stain viability sorting, as well as any desired secondary gating strategies, navigate to ‘Sort Settings’ (boxed in red in figure 1). Place the target plate, with sealing film over all wells into a PCR cooler that has thawed for 10 minutes.

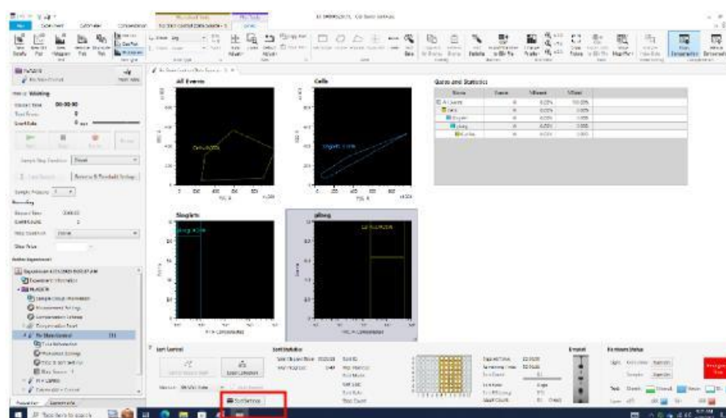

Figure 1 Navigation to Sort Settings

After opening sort settings, ensure that the sorter is set to single cell dispense by entering ‘1’ in the stop count (red box in figure 2), and navigate to ‘Plate Adjustment’ (boxed in green in figure 2).

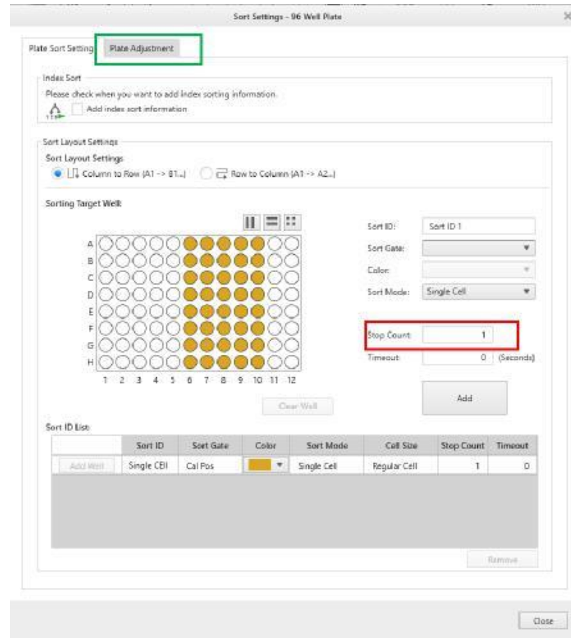

Figure 2 Plate Adjustment Navigation & Confirmation of Single-Cell Selection

This will open the plate adjustment settings. Start by performing a ‘4 corners and center well’ check by selecting the appropriate circle and clicking ‘Start’ (figure 3). The system will automatically bring the plate to the front when the test is complete. Visually observe the plate to see if the droplet appears in the middle of each well.

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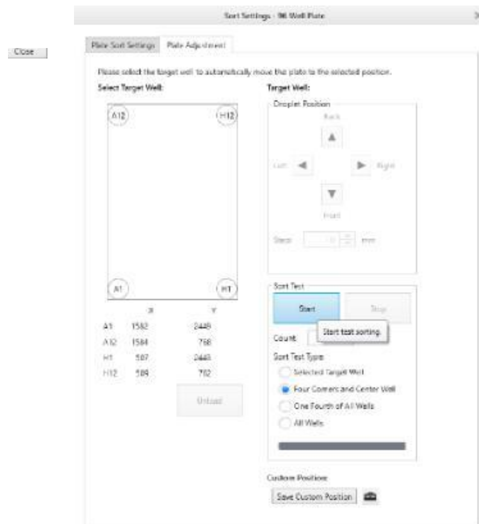

Figure 3 Initial assessment of plate adjustment

Adjust each well individually by clicking on the well (highlighting it in red) and moving in the appropriate direction in 0.5mm increments. Test **each** well by selecting ‘Selected Target Well’ and clicking ‘Start’. At the end of each test, the user must click ‘Unload’.

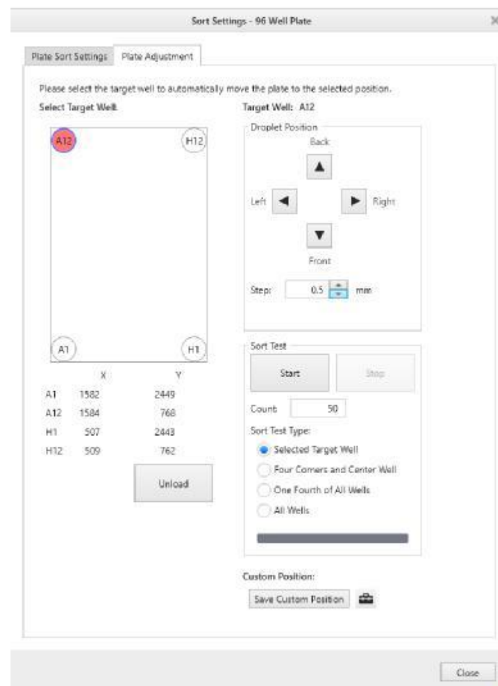

Figure 4 Adjusting the alignment at each corner well

After adjustment, perform a brief 4 corners and center well assessment again to confirm wells are well aligned (droplet in the dead center of each well). When wells are aligned appropriately, perform a final test by selecting ‘All wells’ and clicking start. The plate will automatically unload when completed.

Defining Success

Well-adjusted plates will have a droplet in the very center of the vast majority of each of the wells. As all sorting is dependent on the mechanisms of given instruments, it is **expected** that up to 10% of wells will not have well aligned droplets. An example of a well aligned plate (with a few poorly aligning wells) can be found in figure 5 at the top of the next page. **Note wells D and E in column 2. These individual wells are NOT well aligned.** Well D has the droplet too far to the left, while well E has a droplet too low and slightly to the right. This is **OK.** Looking at the remainder of the plate, we can clearly see that **the majority of the wells are well centered and that the droplet will not impact the side of the well upon being sorted**. These results are **TYPICAL.**

Figure 5 Example of a typical well aligned plate.

Considerations for 384 Well Plates

Sorting into 384 well plates is minimally more challenging than sorting into a standard 96 well plate. The steps above are the same, with the difference being that the wells that will be aligned to are A01, A24, P01, and P24. **Note: When using a Sony SH800 instrument, please use the metal 384 well plate holder provided with the instrument.**

When aligning these plates, there may be less ability to accurately define a droplet in the very center of each well. For this reason, the dropout rate may be slightly higher than in 96 well sorting. An image of a well aligned plate can be seen in figure 6 at the top of the next page. **Note that when aligning a 384 well plate, the emphasis is on getting a droplet above the well.** As much as is possible, the droplet should be centered. Machine limitations do exist, and it will not be possible to completely align each well. A typical dropout rate for 384 well plates is ~20%, when the plate has been optimally aligned.

Figure 6 Well aligned 384 well plate

Other Considerations for FACS Success Beyond Alignment

There are many considerations for a successful FACS deposition of cells into a given plate. While we at BioSkryb Genomics feel that plate alignment is among the most important, please see the few additional considerations below that are likely to improve data quality.

Viability Staining

All cells should be stained for viability using a 2-stain system. In this system, one stain is passive (propidium iodide) and will be used to visualize non-intact cells. The other stain is an active stain (Calcein-AM) and is only fluorescent upon uptake **and** enzymatic cleavage by the cell, thus ensuring that the cell is not only intact, but actively viable. **We aim to sort**

propidium iodide NEGATIVE and Calcein-AM POSITIVE cells into wells. Further stains can be utilized by the flow cytometry core facility to identify cells of interest.

When sorting nuclei, please note that **ONLY** a passive nuclei stain should be used, and that unlike in cells, the **propidium iodide POSITIVE nuclei should be ACTIVELY SORTED.**

Please note that DAPI staining should NOT be utilized for either cells or nuclei.

Plate Handling Pre- and Post- Sort

All 96 well plates should have 3µL of BioSkryb Genomic's proprietary cell buffer added to each target well (where a single cell is to be added) **prior to the start of sorting.** Plates containing cell buffer should be sealed and spun briefly to collect the contents at the bottom of the well. Plates that have 384 wells may be 'dry-sorted' where no cell buffer is added to the wells prior to sorting beginning.

After sorting, all plates should be spun down aggressively to ensure that any deposited cell comes into contact with the cell buffer. **AT NO TIME SHOULD PLATES BE SUBJECT TO VORTEX.** After spinning down, all plates should be placed as flatly as possible directly onto dry ice. Plates should not be stacked on top of each other. Dry ice should not be placed on top of the plates.
After all sorting is completed, plates should be stored at -80°C until use.