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Procedure

ISOFLUX™ PROTOCOL

Single Cell Isolation

Revision 2

For Research Use Only
Not intended to treat or diagnose any disease condition

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SUMMARY

This document is intended to guide the user through a method for selection and isolation of individual cells from samples collected on the IsoFlux System. Single cell selection is accomplished using fluorescence microscopy and a pipetting device (e.g. pipette, micromanipulator).

EQUIPMENT REQUIREMENTS

- IsoFlux Instrument (Catalog No. 950-0100)
- Fluorescence microscope
    - Equipped with bright field + DIC settings
    - Excitation/emission filters (FITC 495 nm/521 nm), Cy3 550 nm/570nm, and Hoechst33342 361 nm/497 nm).
    - Ensure that the microscope allows ample clearance and access to sample for picking. Inverted microscope works best.
    - Optional: micromanipulator, automated stage

CELL SELECTION CONSIDERATIONS

When selecting individual target cells from a sample, it is important to have a reliable means to identify the target cells. Three approaches are presented here for the user to consider, and other methods may be possible as well:

1. **Fluorescence illumination – Surface Expression (recommended)** Target cells can be labeled with a fluorescently-tagged surface-expressed marker. Common examples of this include EpCAM, Her2, EGFR, etc. This marker can be used to identify target cells under fluorescence microscope observation. Advantage: easier visual identification of target cells, better preservation of cell contents
   Disadvantage: may require optimization of antibody staining

2. **Fluorescence illumination – Internal Expression**
   Intracellular markers such as cytokeratin may be stained to identify target cells for selection. This generally requires the cells to be permeabilized. The IsoFlux CTC Enumeration Kit contains a CK marker as well as the permeabilization reagent. This approach can allow for other non-surface expressed markers to be used, but the permeabilization step might interfere with the cellular contents (e.g. DNA, RNA).
   Advantage: more options for markers, can use CTC Enumeration Kit
   Disadvantage: might interfere with downstream analysis

3. Fluorescence illumination – Negative Selection

If the target cells need to remain label free, the background cells can be fluorescently stained with a negative marker (e.g. CD45 for white blood cells).

Advantage: keeps target cells label-free
Disadvantage: may be more difficult to identify target cells

4. Brightfield illumination

Target cells in an IsoFlux sample have 1 or more magnetic beads attached to them. Additionally, the target cells may be, on average, larger than the background cells. It is possible to select single cells under brightfield illumination using these types of visual criteria.

Advantage: label-free, less processing time
Disadvantage: may be more difficult to identify cells

DOWNSTREAM ANALYSIS

Typical downstream analysis options for single cells include sequencing, mutational profiling, and gene expression analysis. Any downstream analysis methods and sample prep kits (i.e. DNA/RNA isolation, amplification) should be fully qualified to confirm an acceptable and appropriate level of sensitivity and reproducibility.

PRECAUTIONS

Before starting any experiment, it is advisable to consider all the criteria involved and select the most appropriate method. Care should be taken not to handle or treat the cells in any way the would preclude downstream analysis. Possible steps that could be problematic include: fixation/permeabilization, excessive agitation, buffer conditions, labware selection, and storage conditions.

This protocol is meant to serve as a starting point and guide for single cell selection. It may need to be modified to work with specific applications. Please contact Liquidbio's Application Support Team (support@fluionbio.com) for assistance in protocol development.

1. Microscope set-up: the user should have a fluorescence microscope equipped with brightfield/DIC settings, excitation/emission filters (FITC 495 nm/521 nm, Cy3 550 nm/570nm, and Hoechst33342 361 nm/497 nm). Ensure that the microscope allows ample clearance and access to sample for picking. An inverted microscope typically works best.

2. Prepare an appropriate number of PCR tubes for the amount of single cells to be picked. Depending on the application, add 10µL of PBS or appropriate buffer to each tube. Set aside on ice.

3. Resuspend the enriched sample in 0.2mL of binding buffer. Note: if working slow, resuspend in growth media to minimize cell lysis. Keep on ice.

4. Draw a circle of 0.5in (12mm) diameter at one end of a glass slide with a hydrophobic pen (PAP pen). On the distant end, draw several smaller circles. These are for placement of the picked cells. Allow to dry.

5. Coat the circled area on the slide with serum (human, fetal bovine, e.g.) for 10 minutes. Remove serum. Rinse each area once with PBS taking care not to spill over the drawn edge.

6. Place slide on the microscope. Add 0.2mL of Binding Buffer to the large circle and few drops into the smaller ones.

7. Gently resuspend the sample and transfer 10 to 20µL to the Binding Buffer in the large circle, gently move the pipette tip around to disperse the cells.

8. Open the shutter to allow for fluorescent visualization of cells. Use the filter that corresponds to the fluorescence marker being used to identify the cells. If relying on bright field for picking, use DIC and choose the cells that are covered with beads.

9. Identify the cell to be picked under 100x magnification (10x objective). Using the 10µL micropipette with extended tip set to maximum volume, place the tip next to the cell and gently aspirate it into the tip, taking in minimal volume.

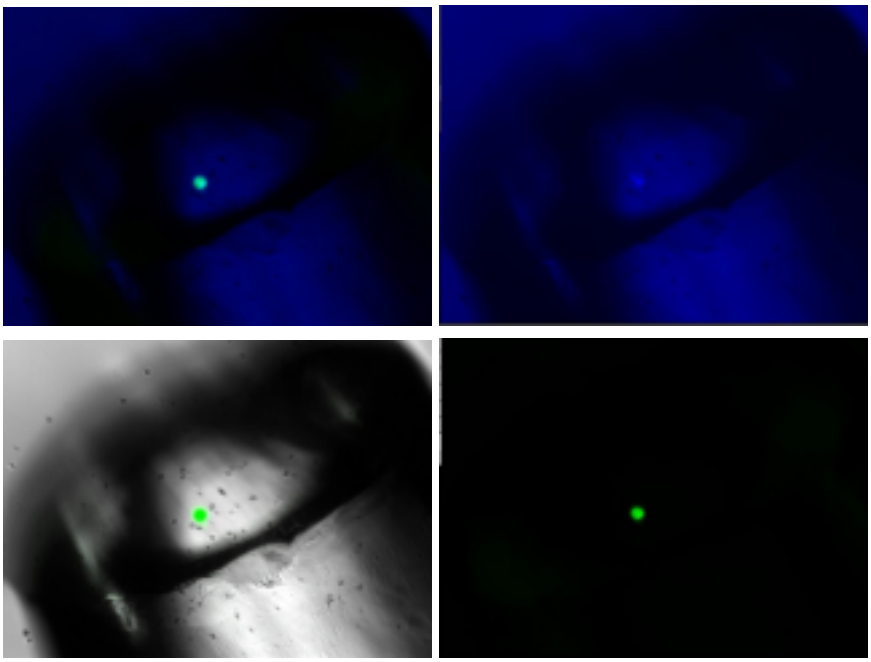
10. Place the picked cell in one of the small circles by dipping the tip into the droplet of buffer in the circle (i.e. liquid to liquid transfer). Examine to ensure that the picked cell is there and for other contaminants. Re-pick if necessary.

11. Pick and transfer the single cell by dipping the tip into the buffer in the PCR tube (liquid to liquid transfer). Snap freeze or place on ice.

12. Continue picking from the large circle, replenish with new sample as necessary.

13. Process the picked single cells immediately or snap freeze on dry ice and store at -20°C.

Examples of single picked cell
(note the false blue color was applied on bright field image)



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SUPPORT

For assistance with this protocol, please contact Liquidbio through one of these methods:

Email: support@liquidbio.co

WWW: www.liquidbio.co