Polaris Training with K562 Cells

Contents

Purpose

This training document describes the procedure for performing a cell-based test for the Polaris™ system using software version 1.2.1. It is intended to be used in conjunction with the detailed protocol Generate cDNA Libraries with the Polaris Single-Cell Dosing mRNA Seq IFC (PN 101-0082), referred to as Polaris mRNA Seq Protocol (PN 101-0082) for brevity. Use this training document to test all systems of the Polaris instrument to ensure proper function prior to beginning experiments on the instrument.

IMPORTANT Before using this procedure, read and understand the detailed instructions and safety guidelines in Polaris mRNA Seq Protocol (PN 101-0082).

Materials

IMPORTANT Store reagents according to manufacturer's recommendations as soon as they are received.

Required IFC, Reagent Kits, and Reagents

Polaris Single-Cell Dosing mRNA Seq IFC (integrated fluidic circuit; Fluidigm, PN 101-0317)

Polaris Training Kit (Fluidigm, PN 101-0583), which contains 3 modules: 4 °C, −20 °C, and −80 °C

SMARTer® Ultra™ Low RNA Kit for the Fluidigm C1™ System, 10 IFCs (includes Boxes 1 of 2 and 2 of 2 and Advantage® 2 PCR Kit; Clontech, PN 634833)

CellTracker™ Orange CMRA Dye (CTO, Thermo Fisher Scientific, PN C34551)

CellTracker Green CMFDA (CTG, Thermo Fisher Scientific, PN C2925 or C7025)

Anhydrous Dimethyl sulfoxide (DMSO, Thermo Fisher Scientific, PN D12345)

Required Equipment Supplied by Fluidigm

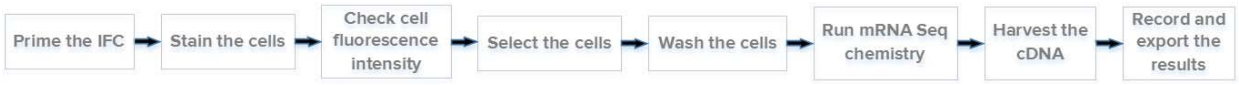
☑	Product Name	Company	Part Number
☐	Polaris system (software version v1.2.1)	Fluidigm	100-9009

Required Equipment from Other Suppliers

☑	Product Name	Company	Part Number
☐	Two freezers and one refrigerator: one freezer at −20 °C, one freezer at −80 °C, and one refrigerator at 4° C	Major laboratory supplier (MLS)	—
☐	PCR thermal cycler	MLS	—
☐	Water bath set to 37 °C	—	—
☐	Two centrifuges: one for Eppendorf® microcentrifuge tubes and one for 8-well strips for 96-well plates	MLS	—
☐	Pipettes (0.2–2 µL; 2–20 µL; 20–200 µL; 100–1,000 µL; and L8–10 8-channel pipettes 2–20 µL)	MLS	
☐	Filtered pipette tips	MLS	
☐	Sterile, Eppendorf DNA LoBind tubes for sample prep: 0.5 mL (PN 0030 108.035) and 1.5 mL (PN 0030 108.051)	MLS	—
☐	8-tube TempAssure™ strip with attached caps	USA Scientific	1402-4700
☐	96-well TempPlate™ PCR plate, semi-skirted	USA Scientific	1402-9700
☐	Clear adhesive film for 96-well MicroAmp™ plate	Thermo Scientific	4306311
☐	Two-chamber INCYTO™ C-Chip Disposable Hemocytometer (or other cell-counting device)	Neubauer Improved	DHC-N01
☐	Vortexer	MLS	—
☐	For DNA quantitation, use one of these systems and reagents: • 2100 Bioanalyzer® (Agilent® Technologies) • 384-well fluorometer for PicoGreen® assay (MLS) • Microsoft Excel worksheet Single-Cell mRNA Seq PicoGreen Template with Qubit® fluorometer (Thermo Fisher Scientific)	—	—
☐	Ice bucket	MLS	—
☐	Glass bottle	MLS	—
☐	Blood gas pressure regulator	MLS	—
☐	Input fitting to two-stage blood gas pressure regulator*	MLS	—
☐	Cylinder of gas with clean, dry, pre-mixed gas (standard grade) containing either 5% CO2, 5% O2, and 90% N2; or 5% CO2, 20% O2, and 75% N2	MLS	—
☐	USB key with at least 2 GB storage	Any supplier	—

* Consult gas supplier.

Workflow



Portions of this workflow refer you to these Polaris documents for detailed instructions:

- Protocol: Generate cDNA Libraries with the Polaris Single-Cell Dosing mRNA Seq IFC (PN 101-0082); also referred to in this document (for brevity) as Polaris mRNA Seq Protocol (PN 101-0082).
- Quick Reference: Generate cDNA Libraries with the Polaris Single-Cell Dosing mRNA Seq IFC (101-0075); also referred to in this document (for brevity) as Pipetting Maps for the Polaris Single-Cell mRNA Seq Protocol (PN 101-0075).

See the complete list of related documentation in Appendix B on page 10.

Procedure

Set up an experimental profile on Polaris that contains the steps shown in Table 1:

Table 1. Experimental profile of the training test on Polaris

Step	Parameter	Setting
Select an experiment to run	Experiment Type	No Treatment
Prime the IFC	Suspension/Adherent	Suspension
Cell Selection	Number of Populations	1 Population
	Selection Pressure	Standard Pressure
	Number of Inlets	2 Inlets
	Number of Selection Channels	1 Selection Channels (Green, Yellow)
	Selection Thresholds	6000 to 65000 (for both Green and Yellow)
Post Stain	Stain & Wash or Wash Only	Wash Only

Before you begin the Prime the IFC step, select **No Treatment** as the experiment type.

Prime the IFC

The Prime the IFC step for priming the IFC prepares the Polaris Single-Cell Dosing mRNA Seq IFC prior to loading the cells.

Run the Prime the IFC step according to the instructions in the Polaris mRNA Seq Protocol (PN 101-0082) and the Pipetting Maps for the Polaris Single-Cell mRNA Seq Protocol (PN 101-0075).

Select the parameter listed in Table 1.

Stain the Cells

While the second part of Prime is running on the Polaris system (after adding the capture beads), prepare the staining solutions, thaw the K562 cells, and then stain the cells as described in the following instructions:

- Prepare the Cell-Staining Solutions on page 5
- Thaw the Frozen K562 Cells on page 6
- Stain the Thawed K562 Cells on page 6

Prepare the Cell-Staining Solutions

1. Allow the lyophilized CellTracker Orange CMRA Dye (CTO), CellTracker Green CMFDA (CTG), and DMSO (−80 °C) to thaw at room temperature.

   IMPORTANT Protect the CellTracker dyes and all solutions containing the dyes from light at all times.

2. Prepare 10 mM stock CTO and CTG solutions:
   a. Label the lyophilized stock CTO and CTG tubes as “10 mM CTO” and “10 mM CTG.”
   b. Add 9.1 µL DMSO to the stock CTO tube and add 10.8 µL DMSO to the stock CTG tube.
   c. Vortex the tubes well and centrifuge them to collect the 10 mM solution in the bottom of each tube.
3. Prepare 1 mM CTO and CTG solutions from the 10 mM stock solutions:
   a. Label 2 new tubes as “1 mM CTO” and “1 mM CTG.”
   b. To each tube, add 9 µL DMSO and 1 µL of either 10mM stock CTO or CTG solution.
   c. Vortex the tubes well and centrifuge them to collect the 1 mM solution in the bottom of each tube.
4. Prepare 0.1 mM solutions from the 1 mM stock solutions:
   a. Label 2 new tubes as “0.1 mM CTO” and “0.1 mM CTG.”
   b. To each tube, add 18 µL Cell Wash Buffer and 2 µL of either 1 mM CTO or CTG solution.
   c. Vortex the tubes well and centrifuge them to collect the 0.1 mM solution in the bottom of each tube.
5. Prepare 0.8 µM final cell-staining solutions from the 0.1 mM stock solutions:
   a. Label 2 new tubes as “CTO Final” and “CTO + CTG Final.”
   b. Assemble the final cell-staining solutions according to the following table:

Table 2. Final cell-staining solutions

Reagent	CTO and CTG Final (µL)	CTO Final (µL)
Cell Wash Buffer	1,000	1,000
0.1 mM CTO	8	8
0.1 mM CTG	8	—

Thaw the Frozen K562 Cells

1. Warm the K562 media to 37 °C in a water bath.
2. Thaw the K562 cells (100-5340, −80 °C) in a 37 °C water bath.
3. Transfer the cells to a 15 mL conical tube (preferred) or a 1.5 mL microcentrifuge tube.
4. Slowly add (dropwise) 0.8 mL of warm media to the cells, swirling the tube gently to mix between drops.
5. Mix by gently inverting the tube.
6. Pellet the cells for 5 min at 300 x *g*.
7. Remove supernatant, leaving only ~100 µL medium in the tube with the pelleted cells.
8. Gently flick the tube to resuspend the pelleted cells in the remaining ~100 µL medium.
9. Slowly add (dropwise) 1 mL of warm media to the resuspended cells.
10. Mix by gently inverting the tube.

Stain the Thawed K562 Cells

1. Pipet 500 µL of the resuspended, thawed K562 cells into 2 new 1.5 mL microcentrifuge tubes.
2. Centrifuge each tube of resuspended cells for 5 min at 300 x *g* to pellet them.
3. Resuspend 1 tube of pelleted cells in 1 mL “CTO + CTG Final” solution, and resuspend the other tube of cells in 1 mL of “CTG Final” solution.
4. Incubate both tubes of resuspended cells in stain solution for 30 min at room temperature.

   IMPORTANT Protect the incubating cells from light at all times.
5. Centrifuge the cells for 5 min at 300 x *g*.
6. Wash the cells 3 times:
   a. Resuspend the cells in 1 mL Cell Wash Buffer.
   b. Centrifuge the cells for 5 min at 300 x *g*.
   c. Repeat Steps 6(a) and 6(b) 2 more times for a total of 3 washes.
7. Gently resuspend each pellet in 300 µL Cell Wash Buffer.

8 Determine the cell concentration of each cell suspension using a hemocytometer or automated cell counter, and adjust the cell concentration to 300 cells/µL in Cell Wash Buffer.

9 Prepare a 50:50 mix of each cell type by combining 100 µL of each cell suspension in a new 1.5 mL microcentrifuge tube. Keep the prepared cells at room temperature.

IMPORTANT Protect the stained cells from light.

Check Cell Fluorescence Intensity

When the Prime the IFC step is complete, check the fluorescence intensity of the stained cells.

You can use the hemocytometer in Stain the Thawed K562 Cells on page 6 or a new one with the 50:50 cell mix from that procedure.

Guidelines: The intensity should be >7000 in both channels for most cells. If the intensity is under 7000, repeat the staining process with an additional 20 min of incubation time, wash and count the cells, and check the final intensity again on a hemocytometer.

Select the Cells (and Begin Tube Controls)

1 After you check the fluorescence intensity of the stained cells, prepare the final cell mix by combining 40 µL of Cell Suspension Reagent with 60 µL of the 50:50 cell mix and mixing by pipetting gently.

IMPORTANT Do not vortex the cells.

2 Save the remaining cells for tube controls.

3 Load the cells according to the instructions in the Polaris mRNA Seq Protocol (PN 101-0082) and the Pipetting Maps for the Polaris Single-Cell mRNA Seq Protocol (PN 101-0075).

Use 220 µL Cell Wash Buffer as the selection medium in the R1 reservoir and select the parameters listed in Table 1.

4 Start the **Cell Selection** step.

5 Record the cell selection results:

Table 3. Cell selection results

Cell Selection Results	
Number of empty:	
Number of single:	
Number of multiple:	
Total time (min):	

6 While the Cell Selection step is running, prepare reagent mixes for the mRNA Seq Chemistry step and begin running the tube controls according to the instructions in Prepare the Reagent Mixes for mRNA Seq Chemistry on Polaris (PN 101-2819).

Wash the Cells

When the Cell Selection step is complete, run the Post Stain step according to the instructions in the Polaris mRNA Seq Protocol (PN 101-0082) and the Pipetting Maps for the Polaris Single-Cell mRNA Seq Protocol (PN 101-0075).

Select the parameter listed in Table 1.

Run the mRNA Seq Chemistry

When the Post Stain step is complete, run the mRNA Seq Chemistry step according to the instructions in the Polaris mRNA Seq Protocol (PN 101-0082) and the Pipetting Maps for the Polaris Single-Cell mRNA Seq Protocol (PN 101-0075).

Harvest the cDNA

When the mRNA Seq Chemistry step is complete, harvest the cDNA according to the instructions in the Polaris mRNA Seq Protocol (PN 101-0082) and the Pipetting Maps for the Polaris Single-Cell mRNA Seq Protocol (PN 101-0075).

Record and Export the Results

1 When harvest is complete, do the following using the Results Tables on page 9:

- Record the harvest volumes.
- Analyze at least 9 randomly selected samples and 2 tube controls using a Bioanalyzer high-sensitivity chip, Qubit, or PicoGreen assay.

2 Export the results and save the standard Export file and the Debug file from the View Runs menu.

Appendix A: Results Tables

Harvest Volumes (µL)

Well	1	2	3	4	5	6
A						
B						
C						
D						
E						
F						
G						
H						

cDNA Yield for Selected Samples (ng/µL) via Bioanalyzer, Qubit, or PicoGreen

Sample	Plate Position	cDNA Concentration (ng/µL)
Sample 1		
Sample 2		
Sample 3		
Sample 4		
Sample 5		
Sample 6		
Sample 7		
Sample 8		
Sample 9		
Positive Tube Control		
Negative Tube Control		

cDNA Yield for Full Harvest Plate (ng/uL) via Qubit or PicoGreen)

Well	1	2	3	4	5	6
A						
B						
C						
D						
E						
F						
G						
H						
Positive Tube Control						
Negative Tube Control						

Appendix B: Related Documentation

Document Title	Part Number
Generate cDNA Libraries with the Polaris Single-Cell Dosing mRNA Seq IFC This is a protocol. For brevity, referred to in this document as: Polaris mRNA Seq Protocol	101-0082
Generate cDNA Libraries with the Polaris Single-Cell Dosing mRNA Seq IFC This is a Quick Reference. For brevity, referred to in this document as: Pipetting Maps for the Polaris Single-Cell mRNA Seq Protocol	101-0075
Prepare the Reagent Mixes for mRNA Seq Chemistry on Polaris This is a Quick Reference.	101-2819
Polaris User Guide	100-9580

For technical support visit techsupport.fluidigm.com. For general support visit fluidigm.com/support.

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