Generate cDNA Libraries with the Polaris Single-Cell Dosing mRNA Seq IFC

Before You Begin

Before using the maps and for detailed instructions on preparing the mRNA Seq chemistry, see the Polaris mRNA Seq Protocol (PN 101-0082).

Best Practices

- Use good laboratory practices to minimize contamination of samples. Use a new pipette tip for every new sample. Whenever possible, separate pre- and post-PCR activities. Dedicate laboratory materials to designated areas.
- Unless otherwise specified, thaw reagents on ice before use.
- When pipetting into the IFC, pipet only to the first stop to avoid creating bubbles.
- Ensure that the surface of the IFC is kept clean. If necessary, gently dab debris or liquid away with a lint-free cloth or transparent tape (such as Scotch™ tape).
- Before returning the IFC to the instrument, check the Environmental Control interface plate (EC IP) and tray for liquid residue and wipe down as needed with a delicate, lint-free cloth that contains IPA.
- When prompted to run a **Clean** step, remove the IFC from the Polaris instrument, place the EC IP into the instrument tray, and tap **Clean**.

Safety

Use standard laboratory safety protocols. Read and understand the SDSs before handling chemicals. To obtain SDSs, go to fluidigm.com/sds and search for the SDS using either the product name or the part number.

Workflow

Perform the following steps with the Fluidigm Polaris™ system and Polaris Single-Cell Dosing mRNA Seq IFC (integrated fluidic circuit) to capture individual cells, perform single-cell functional studies, and amplify cDNA for mRNA sequencing.

1 Prime | 2 Select cells | 3 Dose/culture | 4 Post-stain/wash | 5 Run chemistry

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Prime the IFC

☐ **1** Bring the Polaris Bead Suspension Reagent and Polaris Cell Capture Beads to room temperature 1 hour before use.

☐ **2** Dilute 1 µL of ROX™ Reference Dye stock solution in 60 µL of Cell Wash Buffer and protect from light until used.

☐ **3** (A) Pipet 300 µL Actuation Fluid into each of the two accumulators.

☐ **4** (18)(20) Pipet 25 µL/inlet Cell Wash Buffer into inlets 18 and 20.

☐ **5** (1)(2)(3)(4)(5) Pipet 25 µL/inlet Polaris Blocking Reagent into inlets 1 to 5.

☐ **6** H1 Pipet 200 µL Polaris Blocking Reagent into the large reservoir on the top left.

☐ **7** (v) Pipet 20 µL/inlet Valve Priming Reagent into each of the 32 inlets.

☐ **8** (v) Pipet 25 µL/inlet Valve Priming Reagent into each of the 2 additional inlets.

☐ **9** (13) (Optional) For culturing adherent cells, you need to additionally pipet 25 µL of user-provided extracellular matrix (ECM) solution into inlet 13.

☐ **10** In a DNA-free hood, prepare 1,200 µL Cell Capture Bead Mix, as follows:

a Combine the following two reagents into a 1.5 mL tube:

☐ Polaris Bead Suspension Reagent	Ensure at room temperature before use.	1,154 µL
☐ Polaris Cell Capture Beads	Ensure at room temperature before use. Vortex vigorously for 30 sec immediately before pipetting. **Do not centrifuge.**	10 µL

b Vortex the 1.5 mL tube for 10 seconds.

c Add the following reagent to the 1.5 mL tube:

☐ Polaris Bead Linking Reagent	Keep on ice until use. Centrifuge for 3 sec. Mix gently by pipetting up and down. **Do not vortex.**	36 µL

d Mix the total volume of 1,200 µL in the 1.5 mL tube by gently and slowly pipetting up and down 7 times with a pipet set to 1,000 µL.

☐ **11** (b) Immediately transfer 142 µL of Cell Capture Bead Mix to each tube of an 8-tube strip.

☐ **12** Use a 20 µL, 8-channel pipette set at 20 µL to deliver 20 µL/inlet into all 48 inlets of Zone 2, avoiding all bubbles:

a Slowly draw up 20 µL of Cell Capture Bead Mix.

b Slowly eject to the first stop into the same strip tube containing the Cell Capture Bead Mix.

c Draw up 20 µL of the Cell Capture Bead Mix again.

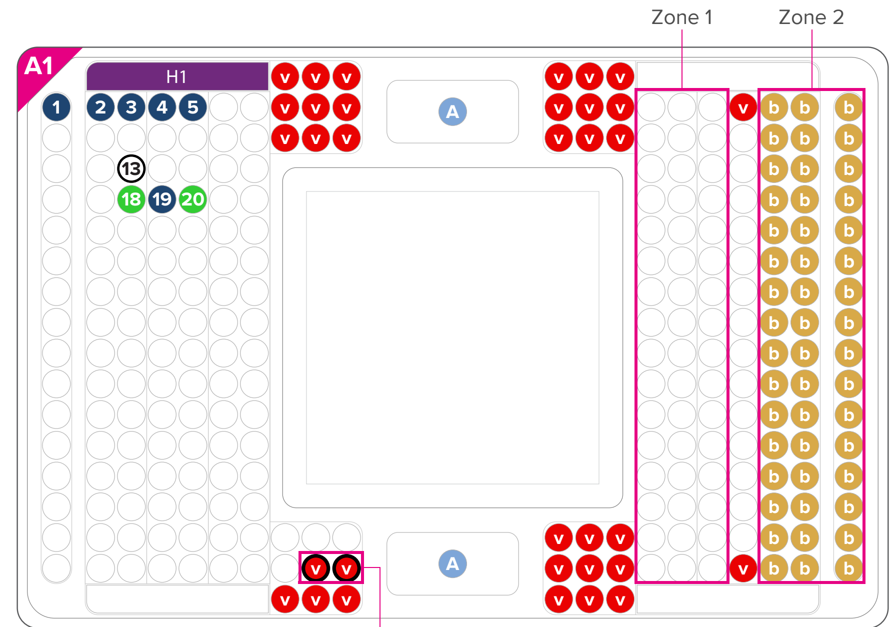
d Keep the pipette tips upright as you place them at the bottoms of each inlet of the Polaris IFC with gentle but firm pressure.

e With the same pressure at the bottoms of each inlet, slowly eject to the first stop. (Never pipet to the second stop.)

f Withdraw the pipette tips slowly repeat with remaining inlets (a total of 6 times).

☐ **13** (19) Pipet 20 µL of diluted ROX into inlet 19. (Store the remaining volume of diluted ROX for cell selection.)

☐ **14** Place the IFC into the Polaris instrument with the Environmental Control interface plate (EC IP).

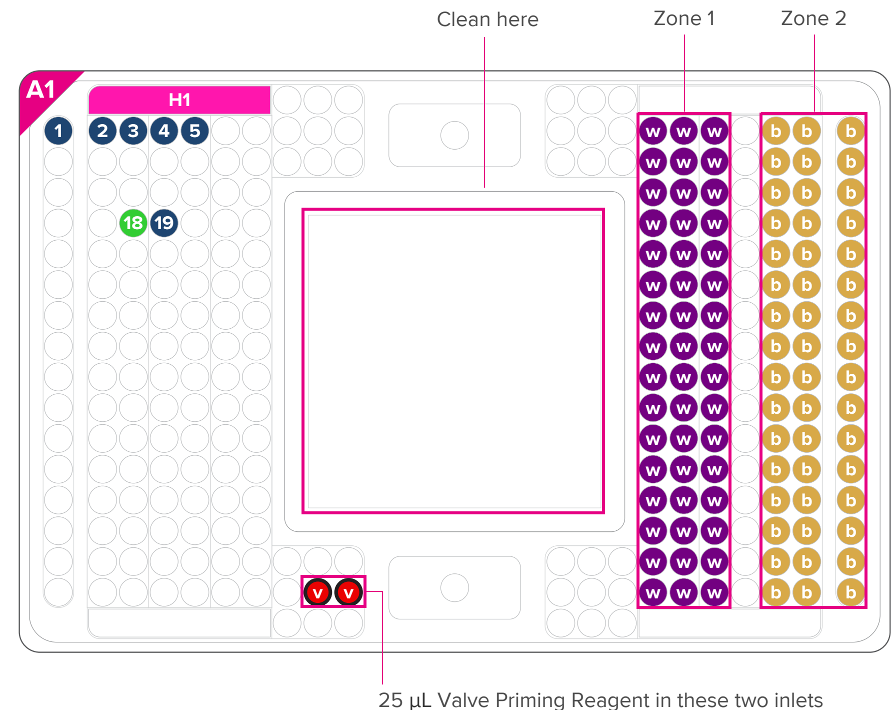


Select the Cells on Polaris

In this procedure, you prepare the IFC and load the cells on it for the Cell Selection step on Polaris.

- ☐ **1** (w) (b) Using an 8-channel pipette, remove the contents of all inlets in Zone 1 and Zone 2.
- ☐ **2** **H1** Remove any remaining Polaris Blocking Reagent from the upper-left reservoir.
- ☐ **3** (1)(2)(3)(4)(5) Remove any remaining Polaris Blocking Reagent from inlets 1 to 5.
- ☐ **4** (18) Replace the contents of inlet 18 with 25 µL Cell Wash Buffer.
- ☐ **5** (v) Replace the contents of the 2 lower inlets with 25 µL/inlet Valve Priming Reagent.
- ☐ **6** **H1** Pipet 200 µL of the cell selection medium of your choice into the upper-left reservoir.
- ☐ **7** (w) Pipet 5 µL/inlet PCR-Grade water into all 48 inlets of Zone 1.
- ☐ **8** (1)(2)(3)(4)(5) Load the cell suspension mixed with Cell Suspension Reagent (cell mix) that was optimized for your experiment (as described in the procedure Load the Cells into the IFC in the Polaris mRNA Seq Protocol) into inlets 1–5.

  NOTE Be certain to fill all inlets with the input volume that you specified during your experimental setup, where the input volume is the total volume of cell mix that will be loaded on the IFC. Each of the 5 cell-loading inlets holds 25 µL.
- ☐ **9** (19) Replace the contents of inlet 19 with 20 µL of diluted ROX.
- ☐ **10** Gently clean the surface of the IFC where indicated with clear adhesive tape (such as Scotch tape).
- ☐ **11** Place the IFC into the Polaris instrument with the Environmental Control interface plate (EC IP).



Dose/Culture on Polaris

Polaris can perform either of the following two tasks according to the experiment profile that you set up: Dose Response or Culture Cells.

☐ **1** Using an 8-channel pipette, remove the waste from all inlets of Zone 1 and Zone 2.

☐ **2** H1 Remove the cell selection medium from the upper-left reservoir.

☐ **3** Waste Remove the waste from the lower-left and upper-right reservoirs.

☐ **4** Remove any remaining reagents from Zone 1 and Zone 2.

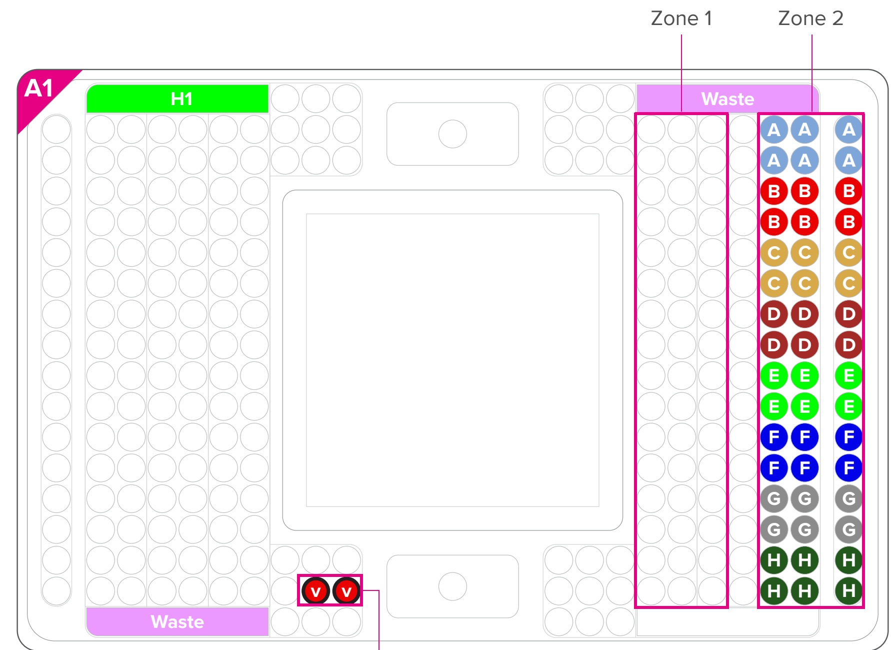
☐ **5** V Replace the contents of the 2 lower inlets with 25 µL/inlet Valve Priming Reagent.

☐ **6** H1 Pipet 200 µL cell culture medium into the upper-left reservoir.

☐ **7** A B C D E F G H If you are running a Dose Response experiment, pipet 27 µL/inlet of your choice of dosing reagents into the 48 inlets in Zone 2.
If you are running a Culture Cells experiment, leave the 48 inlets in Zone 2 empty.

☐ **8** Pipet 3.0 mL of PCR-Grade water along the top and sides of the hydration sponge, clip the hydrated sponge into the EC IP, and wipe off excess liquid with a lint-free wiper.

☐ **9** Place the IFC into the Polaris instrument with the Environmental Control interface plate (EC IP).

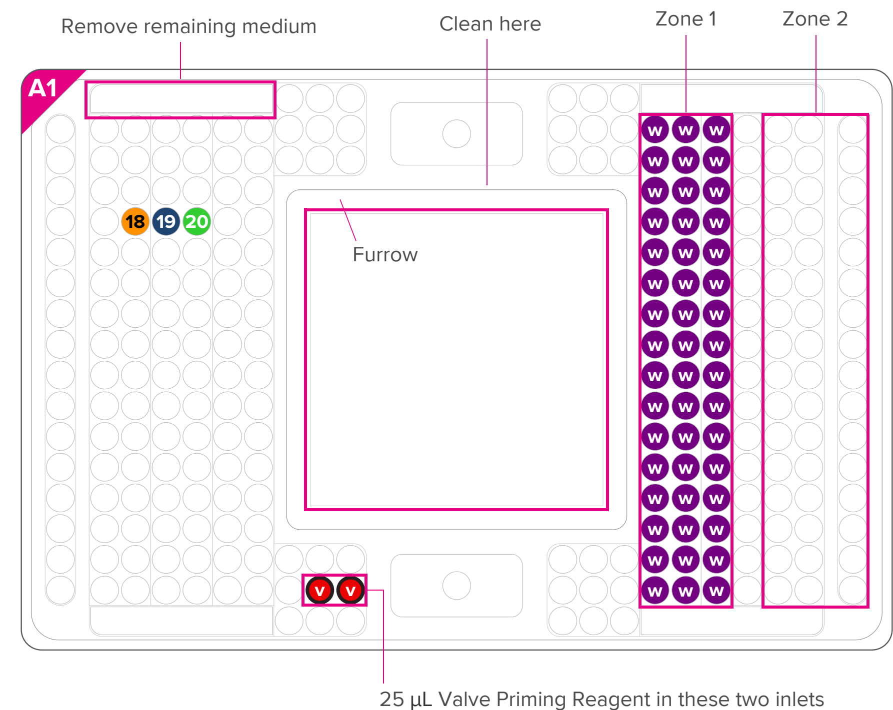


Post Stain/Wash Cells on Polaris

The Post Stain/Wash setting in your experimental profile allows you to set your preference for performing a final staining and washing immediately prior to running the chemistry step on Polaris.

- ☐ **1** Freshly dilute 1 µL of ROX Reference Dye stock solution in 60 µL of Cell Wash Buffer and protect from light until used.
- ☐ **2** Remove the sponge clip from EC IP and wipe off any excess liquid from the EC IP with a lint-free wiper.
- ☐ **3** Pipet off any excess water from the IFC that accumulated during the Dose/Culture step, including water that accumulated in the furrow.
- ☐ **4** Remove any remaining cell selection medium from the upper-left reservoir.
- ☐ **5** 18 19 20 Remove any remaining reagents from inlets 18, 19, and 20.
- ☐ **6** Remove any remaining reagents from Zone 1 and Zone 2.
- ☐ **7** V Replace the contents of the 2 lower inlets with 25 µL/inlet Valve Priming Reagent.
- ☐ **8** W Pipet 20 µL/inlet Harvest Reagent into the 48 inlets of Zone 1.

  NOTE If you are only washing, go directly to step 13 in this procedure.
- ☐ **9** If you are washing and staining, prepare your stain.
- ☐ **10** 18 Add 20 µL of the prepared stain solution to inlet 18.
- ☐ **11** 20 Add 20 µL of Cell Wash Buffer to inlet 20.
- ☐ **12** 19 Add 20 µL of diluted ROX Reference Dye into inlet 19.
- ☐ **13** Gently clean the surface of the IFC where indicated with clear adhesive tape (such as Scotch tape).
- ☐ **14** Place the IFC into the Polaris instrument with the Environmental Control interface plate (EC IP).



Run mRNA Seq Chemistry on Polaris

☐ **1** Remove the black tape beneath the IFC.

IMPORTANT

- With the black tape removed, you may also image your cells on a microscope compatible with the Polaris Single-Cell Dosing mRNA Seq IFC. Guidelines for the selection of a microscope are outlined in Minimum Specifications for Imaging Cells in Fluidigm Integrated Fluidic Circuits, PN 100-5004. Contact technical support for this document.
- Be certain that you removed the sponge clip from EC IP and wipe off any excess liquid from the EC IP with a lint-free wiper.

☐ **2** h Remove any remaining reagents from Zones 1 and 2 of the IFC.

☐ **3** w W Remove any remaining reagents from inlets 18 (inlet "w"), and 20 (inlet "W").

☐ **4** w Pipet 25 µL of Cell Wash Buffer into inlet 18.

☐ **5** W Pipet 25 µL of Preloading Reagent into inlet 20.

☐ **6** v Replace the contents of the 2 lower inlets with 25 µL/inlet Valve Priming Reagent.

☐ **7** (Optional) Use two pieces of C1 Single-Cell Array Barrier Tape to cover all the inlets of Zone 1.

☐ **8** Rinse the upper-left reservoir with Cell Wash Buffer to remove any remaining residual cell selection or cell culture medium.

☐ **9** H H Pipet 220 µL/reservoir of Harvest Reagent into the upper-left and lower-right reservoirs.

☐ **10** P P Pipet 20 µL/inlet of **P**CR Mix to inlets 14 and 15.

☐ **11** R Pipet 10 µL of **R**everse Transcription (RT) Reaction Mix to inlet 16.

☐ **12** L Pipet 10 µL of Cell **L**ysis Mix to inlet 17.

☐ **13** Place the IFC into the Polaris instrument with the Environmental Control interface plate (EC IP).