

Prepare Samples for Loading

- 1 Dilute 25–100 ng total RNA with water to 12.5 µl in a new plate. Pipette to mix.
- 2 Vortex RPB2.
- 3 Add 12.5 µl RPB2. Pipette to mix.
- 4 Place on the thermal cycler and run the mRNA Denaturation program.

Set Up Run and Load Library Card

- 1 Vortex the reagent plate for 3 seconds.
- 2 Centrifuge at 600 × g for 5 seconds.
- 3 Select **Prepare Libraries**.
- 4 Select a protocol or run. Select **Next**.
- 5 Configure the run. Select **Next**.
- 6 Review the run and sample information. Select **Next**.
- 7 Enter tracking information. Select **Next**.
- 8 Place the library card on the stage.
- 9 Close the door. Select **Verify Library Card**.
- 10 Place the guide on the library card.
- 11 Load the oil.
- 12 Insert pipette tips to the bottom of the wells of the prepared sample plate. Pipette up and down 1 time to mix.
- 13 Transfer 25 µl of samples 1–8 and 9–16.
- 14 Add 25 µl RSB to empty sample wells.
- 15 Transfer 125 µl of reagents i–iv and v–vii.
- 16 Vortex DMB.
- 17 Add 80 µl DMB to reagent well viii.
- 18 Transfer 15 µl of reagents 1–4, 5–8, 9–12, and 13–16.
- 19 Transfer 5 µl of reagents a–d and e–h.
- 20 Transfer 3 µl of adapters A–H and I–P.
- 21 Remove the guide.
- 22 Close the door. Select **Start Run**.
- 23 When the run is complete, select **Next**.

Unload Libraries

- 1 Add 10 µl RSB to a new plate.
- 2 Place the guide on the library card.
- 3 Transfer 20 µl from 1L–8L and 9L–16L to the plate. Pipette to mix.
- 4 Centrifuge briefly.
- 5 Transfer from plate wells 1–8 and 9–16 to the library separation tubes.
- 6 Let stand for 10 seconds.
- 7 Transfer from library separation tubes 1–8 and 9–16 to a new plate.
- 8 Remove the library card and guide.
- 9 Select **Home**.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 2 months.

Pool Libraries

- 1 Determine the number of samples to combine.
- 2 Transfer 5 μ l to a single well of a new plate. Pipette to mix.
- 3 Proceed to cluster generation.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 2 months.

Acronyms

Acronym	Definition
DMB	Digital Microfluidics Beads
RPB2	RNA Purification Beads 2
RSB	Resuspension Buffer