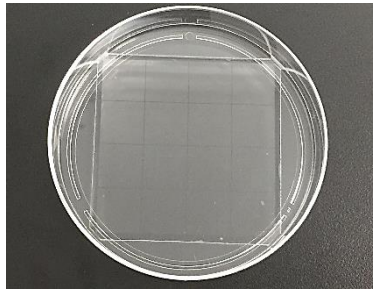




Protocol for Million Microwell Device (MMD)

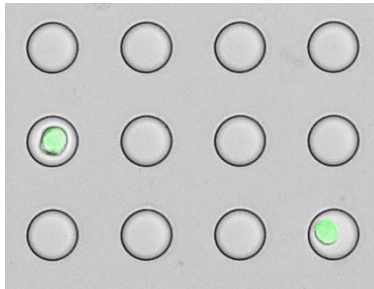
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Preparation



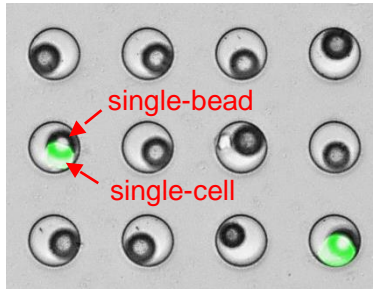
Transfer one MMD to a 100 mm diameter Petri Dish with the microwell side facing up (the microwell openings face down in the packaging). Treat the surface of MMD with plasma for 1-2 min for hydrophilicity

Single-Cell Capture



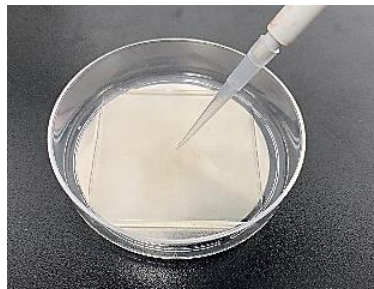
Pipette 2 mL of 30mg/mL BSA/PBS solution onto MMD; wash away unabsorbed BSA after 1 h of incubation. Add cell suspension (10^5 /mL); settle down for 10 min (~10% microwells will capture cells); wash away uncaptured cells

Bead Capture



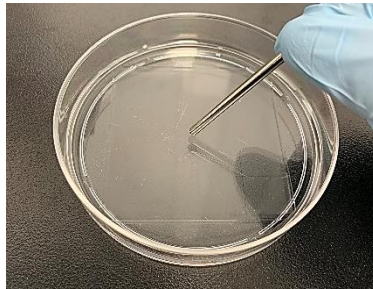
Place a magnet at the bottom of the MMD. Add the barcoded magnetic bead suspension ($\geq 10^6$ /mL); settle down for 10 min (most microwells will capture beads); wash away uncaptured beads

Single-Cell Lysis



Add cold cell lysis buffer onto the MMD surface; incubate for 10 min; add fresh lysis buffer (optional). The magnet at the bottom of MMD will increase the stability of magnetic beads within the microwells

Bead Collection



Remove the bottom magnet. Place a magnetic bar on the surface of MMD; move the magnetic bar across the entire surface of MMD to collect the magnetic beads with captured mRNA

Bead Transfer



Place a PCR tube containing PBS on top of a strong magnet. Insert the magnetic bar containing magnetic beads into the PBS; strongly stir up and down to transfer beads to the PCR tube

Applications

- Single-Cell Isolation
- Single-Cell RNA-Sequencing

