**Fabrication of microwell device**

The diameter and depth of the microwells were 28 µm and 35 µm, respectively. Firstly, a silicon plate with 100,000 microwells was manufactured by Suzhou Research Materials Microtech Co. Ltd. The silicon microwell plate was then used as a mold to make a PDMS plate that had the same number of micropillars. Prior to experiments, a disposable agarose microwell plate was made by pouring 5% agarose solution onto the surface of the PDMS plate. Both the silicon and PDMS plates are reusable. One silicon microwell plate allows for almost permanent use.

**Synthesis of barcoded beads**

Magnetic beads coated with carboxyl groups were provided by Zhiyi (苏州知益微球，diameter 20-25 µm). The barcoded oligonucleotides on the surface of the beads were synthesized by three rounds of split-pool. All the sequences used are listed in supplementary information Table S1.

For each batch of bead synthesis, 300-350 μl of carboxyl magnetic beads (50 mg/ml) were washed twice with 0.1 M MES (2-[N-morpholino]ethanesulfonic acid). The beads were then suspended in 0.1 M MES at a final volume of 635 μl. 3.08 mg of EDC (1-ethyl-3 (-3-dimethylaminopropyl) carbomiimide hydrochloride) were added to the beads. 6.2 μl of beads were then distributed into each well of a 96-well plate. 2.5 μl of amino modified oligonucleotide (50uM in 0.1 M MES) were then added into every well. After vortexing and incubation for 20 minutes at ambient temperature, 0.5 μl mix (Add 6 mg of EDC in 100 μl of 0.1 M MES) was distributed into every well. After another round of vortexing and incubation for 20 minutes at ambient temperature, 0.5 μl more mix (Add 6 mg of EDC in 100 μl of 0.1 M MES) was distributed into every well. After vortexing and incubate for 80 minutes at ambient temperature, the beads were collected in 1 ml of 0.1M PBS containing 0.02% Tween-20. After centrifugation, supernatant was removed carefully. The beads were then washed two times in 1 ml of TE (pH 8.0).

In the second split-pool, the beads were washed with water and split into each well of another 96 well plate containing the PCR mix: 1× Phanta Master Mix (Vazyme) and 5 µM oligonucleotides. The oligonucleotides in every tube encoded a sequence that was reverse complementary to linker 1, a unique barcode and a linker 2 sequence. PCR program was as follows: 94℃ 5 min; 5 cycles of 94℃ 15 sec, 48.8℃ 4 min, and 72℃ 4 min; 4℃ hold. The third split-pool procedure was the same as the second one. PCR program was as follows: 94℃ for 5 min, 48.8℃ for 20 min, 72℃ for 4 min and 4℃ hold. The oligonucleotides used in every tube encoded a linker 2 reverse complementary sequence, a unique barcode, a UMI sequence and a poly T tail. All the oligos were synthesized by Sangon Biotech Co. Ltd. with HPLC purification. Beads were resuspended in 1 ml of ddH2O. To remove complementary chains, put beads into 95℃ water bath for 6 min, separate beads with magnetic separator and remove the supernatant quickly for 2 times. Beads could be stored in TE-TW (10 mM Tris pH 8.0, 1 mM EDTA, 0.01% Tween20) for 4 weeks at 4℃.