DE20 droplet sorting with Xdrop® Sort

Allow all reagents to equilibrate to room temperature for at least 30 minutes before use.

Sample preparation

For DNA enrichment workflows

1. After DE20 droplet production and PCR, collect your DE droplets in a 1.5 ml tube
2. Remove the aqueous phase without disturbing the droplets at the bottom of the tube.
3. Add 1 ml of DE Staining buffer ● to the droplets and gently mix it by rotating the tube.
4. Incubate for 5 min at room temperature in the dark.

For cell workflows

1. After DE20 droplet production, pipette 200 µl of droplets and buffer into a 1.5 ml tube.
2. Add 100 µl of DE Sorting Buffer ●.
   If the DE droplet volume is below 200 µl, add enough of the DE Sorting Buffer ● to reach a total volume of 300 µl to be loaded on the cartridge.

Load the Xdrop DE20 Sort Cartridge

1. Unpack the Xdrop DE20 Sort Cartridge in an LAF hood or similar dust-free environment.
2. Seal the entire cartridge with Foil for sorting (#FI00200), ensuring a tight seal on all the wells.
3. Use the Xdrop Sort Lane Opener (#ACXSCHOP100) to punch open the wells in your chosen lanes.
   Note: If not running all 8 lanes, use only even or odd lanes, i.e., 1, 3, 5, and 7.
4. In the LAF hood, load the cartridge in this order:

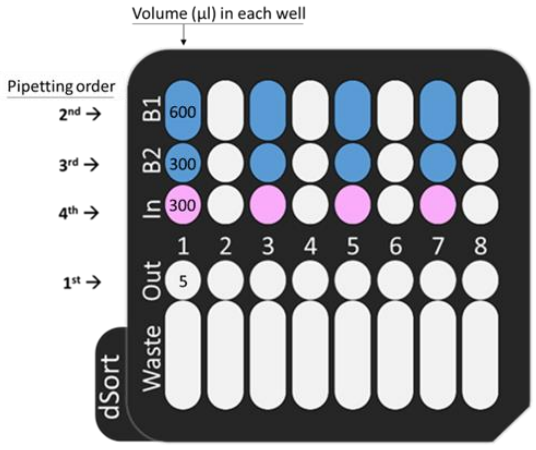
   #Out: 5 µl of Xdrop Blank Droplets ○

   #B1: 600 µl DE Sorting Buffer ●

   #B2: 300 µl DE Sorting Buffer ●

   #In: 300 µl of sample and buffer

   Note: If you are loading with stained droplets containing DNA, pipet from the bottom of the sample tube. Do not resuspend the sedimented droplets.



5. Ensure there are no air bubbles in any of the wells and cover the cartridge with the Xdrop DE Sort Gasket (#GADES100).

Sort the DE20 droplets

1. Press Open on the instrument, place the cartridge in the drawer securely, and press Close.
   Leave the cartridge to sit in the instrument for 5 minutes.
2. Press Next and select Sort for the Xdrop Sort Cartridge.
3. Select the lanes you wish to run, and press Run.
4. Once the run has started, droplets are detected in two zones: Detection and Sorting zone. Two signals are seen: ● Red, for detection zone background and ● Blue, for sorting zone background.
5. Adjust the thresholds for each lane pair (e.g., 1 and 2, 3 and 4) by sliding the white and green threshold lines between the upper and lower droplet populations.
6. Once the thresholds are set, select Start Sorting to initiate sorting on all lanes.
   Two new signals are seen: ○ White for detection zone positive and ● Green for sorting zone positive.
7. After sorting, carefully remove the cartridge from the instrument and gently pipette the positive sorted droplets from the **#Out** well into a clean 1.5 ml tube.
8. Let the droplets settle for 5 min in the collection tube and carefully remove the aqueous phase.

Prepare the Xdrop DE20 Sort Cartridge for storage

1. Wash the **#Out** well of the used lanes with Sort Buffer from well **#B1**.
2. Empty and discard any remaining liquid from the other wells (**#Out**, **#B1**, **#B2**, **#In** and **#Waste**) of the used lanes, taking care not to contaminate any unused lanes.
3. If all lanes have been used or this was the second use of the cartridge, discard the cartridge. If any lanes remain unused and this was the first use of the cartridge, seal it with Foil for sorting as described in the manual and mark the used lanes.
4. The cartridge can be stored for up to 4 weeks at room temperature.
   Note: Do not reuse lanes to prevent contamination.

Please refer to the Xdrop Sort Manual for more detailed information.