Flow cytometry analysis and sorting of DE20 droplets

This document should be treated as a guide. Adaptation may be needed depending on the fluorochromes and encapsulated cell types. This protocol is for Xdrop double-emulsion droplets with a diameter of 20 µm (DE20 droplets) that contain DNA or cells.

Considerations for flow cytometry of DE droplets

Droplet weight: DE20 droplets are stable and relatively heavy. Start with a high sample pressure to draw the droplets into the channels of the flow cytometer. Note that it can take 5 to 10 minutes for the DE20 droplets to reach the point of interrogation and appear on the plot.

Droplet density: Due to the density of the DE20 droplets, they will quickly sediment at the bottom of the sample tube and will not remain in suspension during sorting. This is generally not a problem provided the sample probe is positioned just above the bottom of the sample tube.

Required assay chemistry: The assay chemistry within the DE20 droplets should emit fluorescent light compatible with your flow cytometer's detection spectrum. The DE droplet dye 🟠 emits green fluorescence upon binding to nucleic acids. The oil shell of the DE20 droplets is transparent.

Threshold trigger channel and doublet discrimination: Use forward scatter (FSC) as the threshold channel to exclude contaminating events such as particles in the buffer. Doublet discrimination can be performed but is not required as DE20 droplets do not stick together.

Materials

- DE20 droplets containing DNA or cells
- A flow cytometer or cell sorter with a 100 µm nozzle or sorting chip
- For DE20 droplets containing DNA: DE PCR buffer 🔵 and DE droplet dye 🟠
- For DE20 droplets containing cells: phosphate-buffered saline (PBS)

(For DE20 droplets containing DNA only) Staining DE20 droplets

1. Spin down DE droplet dye 🟠 at 1,700 g (equivalent to 5,000 rpm in a minifuge) for 2 minutes.
2. Add **1 ml** 1x DE PCR buffer 🔵 then **10 µl** DE droplet dye 🟠 to a flow cytometry sample tube or 1.5 ml tube (tubes depend on the instrument). Mix gently to dissolve the dye in the buffer.
3. Remove the supernatant from the tubes containing your DE20 droplets, leaving the droplets undisturbed at the bottom.
4. Add **200 µl** buffer from the flow cytometry sample tube to the tubes containing the DE20 droplets, then transfer all the DE20 droplets from their tubes to the flow cytometry sample tube.
5. Leave at room temperature protected from light for 5 minutes for staining to occur.

Main workflow

1. Load the sample tube containing the DE20 droplets in their buffer and start the analysis. Display at least 100,000 events on the live plot to ensure that rare events are visible on the plot.
2. Identify the DE20 droplet population on an FSC-H vs. SSC-H plot (Fig. 1). DE20 droplets have a higher SSC than oil droplets.
3. To sort or analyze the droplets based on their fluorescent signal, plot the identified DE20 droplet population in a fluorescence vs. SSC plot. See Fig. 2 for an example.
4. Sort selected population into a collection tube containing 15 µl water, buffer, or medium.

5. After sorting, keep the tube with the sorted droplets at 4°C, but do not store **sorted** DE20 droplets longer than a few hours before proceeding with your workflow.

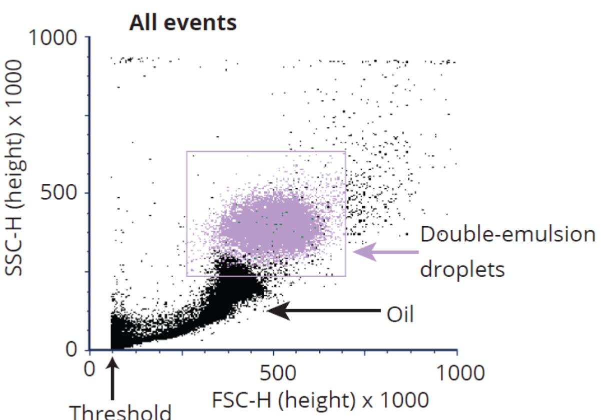

Fig. 1. Identification of DE20 droplets. Gate DE20 droplets on a plot of FSC-H vs. SSC-H. The **height** setting more clearly displays the population of DE20 droplets. Sorted on a Sony® SH800S Cell Sorter.

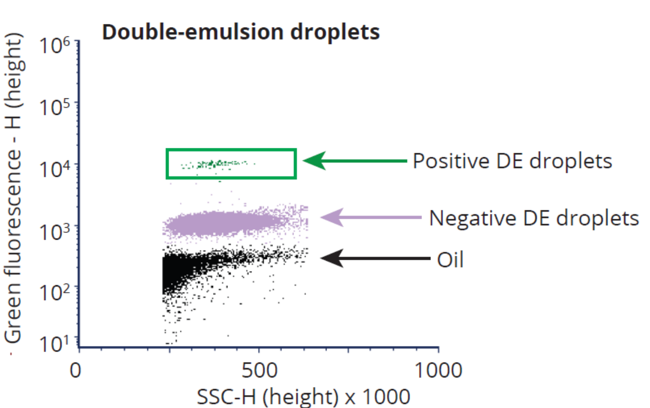

Fig. 2. Identify high and low fluorescent DE droplets. The DE droplet dye ● is excited by the 488 nm laser and is detected in the green channel. Sorted on a Sony SH800S Cell Sorter.

Please refer to the Xdrop Manual or Xdrop Sort Manual for more detailed information on droplet production.

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