DNA Recovery From Droplets

QX100 System

Modified 11.02.12

Purpose: This protocol explains how to recover DNA from droplets for downstream analysis and applications. Droplets read and analyzed by the Droplet Reader cannot be recovered. However, droplets thermocycled in parallel can be recovered.

Materials:

- 1X TE
- Chloroform (e.g. Sigma Cat# 288306)
- 1.5 mL tubes

A. Generate droplets by following the ddPCR standard workflow and protocols

1. If the goal is to read droplets as well as recover material from droplets in parallel, make desired number of wells to be read on the Droplet Reader (non-recoverable), and also make replicates to be broken open (not to be read on the Droplet Reader).
2. For example, a column of 8 wells could be generated, 4 of which are read after thermal cycling, and 4 of which are not read. In QuantaSoft, set up the template such that only 4 of the 8 wells are read. After the Droplet Reader has finished the run, remove the plate and pierce the foil of the 4 remaining unread wells and proceed with breaking the droplets from those wells.
3. Alternatively, if readout on the Droplet Reader is not required, DNA extraction from droplets can proceed directly following thermocycling.

B. DNA Recovery from Droplets

1. Pipet and discard the bottom oil phase of the well
2. Add 10µL of TE.
3. In a fume hood, add 40µL of chloroform.
4. Pipet up and down 5 times at a setting of 25µL volume.
5. Pipet out the entire volume into 1.5mL tube and cap the tube.
6. Vortex at maximum speed for 1 minute.
7. Centrifuge at 15,500g for 10 minutes.
8. Remove the upper phase by pipetting, avoiding the chloroform phase, and transfer to another 1.5mL tube. Dispose of the chloroform phase appropriately.
9. The aqueous phase contains PCR product(s), as well as dNTPs, primers, and probe.

C. Downstream Cleanup and Analysis

- The aqueous phase recovered from droplets contains PCR product(s) as well as dNTPs, primers, and probe. PCR products above a certain length can be further purified by using an appropriate filtration unit (column clean-up).
- Concentration and purity can be assessed by using UV-VIS spectrophotometry.
- Products can be visualized by gel analysis or microfluidic electrophoresis
    - E.g. Experion 1K DNA chip & reagents
- ddPCR can be used to re-quantify the product. For this, make a 10-fold dilution series.