Measuring Droplet Volume in Home-Made Microfluidic Devices

Steve McCarroll's lab

Abstract

Drop-seq is a technology we developed to enable biologists to analyze RNA expression genome-wide in thousands of individual cells at once. We first described this in a 2015 paper in *Cell*. Though commercial implementations of droplet-based single-cell RNA-seq also now exist, we have made Drop-seq open-source and want to make sure that any lab can build their own system. The materials for constructing a Drop-seq setup in one's own lab can be obtained for about $6,000. The reagents for performing Drop-seq cost about 6 cents per cell.

This is a supplemental protocol of our Drop-Seq Protocol for measuring droplet volume in home-made microfluidic devices.

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Guidelines

Videos and FAQs

These tutorials, images, and diagrams may be helpful in building your own Drop-seq setup and doing Drop-seq experiments in your lab.

We have also created a YouTube channel with a variety of videos to help scientists through the steps that most benefit from watching.

This FAQ provides also provides supplementary information.

Before start

To measure droplet volume, purchase some durable, monodisperse polystyrene beads with a hydrophilic coating (e.x. 10-micron carboxylated polystyrene beads from Bangs Labs, product #PC06N-11355. It can be helpful to use fluorescent beads to be sure you can identify them in droplets. Bangs cells these under product # FC06F-10163).

Protocol

Step 1.

Wash and resuspend beads in Drop-seq lysis buffer at a concentration of 1000 beads per microliter.

Step 2.

Draw the beads into a syringe with a magnetic mixer (as you would with the standard barcoded beads) and load into a syringe pump.

Step 3.

Load the syringe pump intended for cells with regular PBS.

NOTES
Anita Bröllochs 11 Jan 2018

Since we are co-flowing beads with PBS, we estimate that the concentration of beads in the droplet fluid will be 500 beads per microliter

Step 4.

Connect all tubing to the appropriate channels in the microfluidic device, and generate droplets.

Step 5.

For a given number of droplets, count the number of beads inside. You should count the beads inside several hundred droplets to make sure that you have a statistically sound estimate.

Step 6.

Divide the total number of beads counted inside droplets by the number of droplets you counted. This is your **droplet occupancy**.

Step 7.

Here is how to calculate droplet volume:

Droplet volume = (droplet occupancy) / (500 beads per microliter) = # microliters per droplet.