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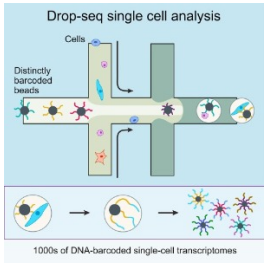
Measuring Droplet Volume in Home-Made Microfluidic Devices



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Human Cell Atlas Metho...

Max Brown

OPEN ACCESS

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External link: http://mccarrolllab.com/dropseq/

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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.

Attachments

PDF

Measuring-Droplet-Vo...

114KB

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).

1

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

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.

3

Load the syringe pump intended for cells with regular PBS.

Note

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

4

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

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.

6

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

7 Here is how to calculate droplet volume:

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