Biophysics Instrumentation Facility

DETECTION LEVEL OF DROPLET DIGITAL PCR

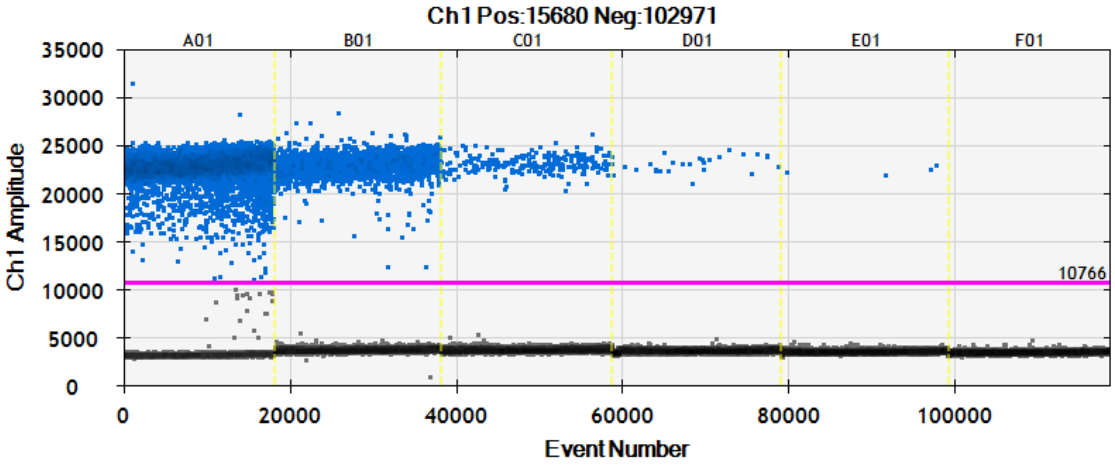
INTRODUCTION:

The QX-200 Droplet Digital PCR system is a new technology capable of detection and absolute quantitation of DNA targets. The system is capable of analyzing DNA samples using probe-based or generic DNA-binding based analysis. A typical sample is mixed with mastermix containing dye, primers, template and droplet generation oil. Following the addition of samples, droplets are generated, PCR is run and the droplets are read individually. Each droplet is about 1 µL in volume and approximately 20,000 droplets are analyzed per sample. As a result, the system is very sensitive and has the theoretical detection limit of a single copy of DNA. To test this detection limit, a standard sample was used with a dilution series with a lower limit of a single copy per well.

RESULTS:

To determine if the detection limits of the QX200 are truly a single copy of DNA, a dilution series of control template DNA was made. These samples were mixed with mastermix containing primers, dye and droplet generation oil. Droplets were made using the droplet generator and the sample was run on the C1000 Touch Thermal Cycler. Following PCR, the samples were read on the droplet reader.

As is shown in the figure below, the analyzed dilution series showed decreasing amounts of target DNA present. Each blue dot on the histogram represents a single positive droplet, while the black dots correspond to droplets without any dye fluorescence. Following quantiation by the software, the concentration of each sample was reported (see table.)



Sample	*Copies of DNA per analysis*	*DNA concentration (copies/µL)*	*Reported DNA concentration (copies/µL)*
1	40,000	2000	1485
2	4,000	200	146.4
3	400	20	14.1
4	40	2	1.51
5	4	0.2	0.23
6	1	0.05	0

The results show that the reported DNA concentrations in the sample were lower than expected. The samples showed a linear relationship following the dilution. However, the detection of a single molecule of DNA was not recorded.

CONCLUSIONS:

Using droplet digital PCR to determine very low levels of DNA is an effective method. However, it appears, using this sample, that detection of a single copy of DNA might not be easily attainable. Additionally, the reported concentrations of DNA are lower than calculated concentrations. This could be a result of the sample, the droplet production process or other factors.

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METHOD FOR DNA DETECTION BY DROPLET DIGITAL PCR:

-Prepare your samples.

-Mix up an appropriate amount of mastermix for all of your samples. (Each sample requires 20 µL of supermix and sample together – it is best to prepare enough for at least two samples)

Mastermix for EvaGreen (makes enough for 1 replicate):

12.5 µL QX200 EvaGreen ddPCR supermix (2X)

1.25 µL primers (20x)

Scale for the number of samples you want to run (for example, if you had 10 samples, mix 125 µL supermix and 12.5 µL primers)

-Using a 96-well plate, aliquot out 11 µL into wells (one well per sample).

-Add DNA samples and water to balance (11.25 µL total) to each well and slowly pipet up and down to mix. Droplet oil is viscous and requires thorough mixing. Avoid the creation of any bubbles as they will prevent the creation of proper droplets.

-Place a new cartridge in a cartridge holder and snap into place.

-Using a multi-channel pipet aliquot 70 µL of droplet generation oil into the oil wells of the cartridge.

-Add 20 µL of sample into the sample wells.

-Place a rubber gasket over top and put into droplet generator.

-Seat properly and push button to close and begin sealing – sealing takes about 2 minutes.

-Once finished, remove from generator and remove gasket.

-Pipet 40 µL into a twin-tec PCR plate.

-Continue this process until all samples are done.

-Place a foil seal on top and seal the plate.

-Put into C-1000 Touch Thermal Cycler and Run according to desired specifications.

-Following PCR run, load into droplet reader and follow directions for droplet reader operation.