UF | ICBR
Gene Expression & Genotyping

Droplet Digital PCR - Frequently Asked Questions:

Q: How much DNA is needed per reaction?

A: We can load up to 330ng of genomic DNA, and can load between 1-9 ul of DNA. If you want replicates (recommended), please supply enough volume for two reactions per sample. It is suggest that reactions with >66 ng of genomic DNA input should be digested with restriction enzymes. It is the customer's responsibility to determine which enzymes are used.

Q: What kind of DNA can be submitted?

A: Genomic DNA, cDNA, plasmid, or viral DNA.

Q: Do I need to supply primers and probes?

A: Yes. The Droplet Digital PCR platform supports both EvaGreen and Probe-based chemistries. For EvaGreen chemistry we need primers; for Probe-based chemistries we need primers and probes. Probes can be either FAM, HEX, or VIC. Multiplexing can only be FAM/HEX or FAM/VIC. If you need advice on probe design, you can contact Core Director at yanp@ufl.edu.

Q: How precise should the sample concentrations be?

A: Accurate concentrations are important when you wish to have a certain input in nanograms. You can either tell us how many nanograms you need loaded, or tell us what volume in microliters you want us to load. If you do not have a way to quantify samples, our core offers the Qubit service which precisely measures concentration, for an additional charge of $20 per 10 samples.

Q: What is the difference between full service and drop-off/walk-up?

A: Walk-up means you prepare the reactions before bringing them to us, and we perform droplet generation, PCR, and droplet reading. You would need the BioRad supermix and Bio Rad ddPCR plates to do this. Full service means we prepare the reactions using your primers/probes and DNA, as well as provide the supermix and ddPCR plates.

Q: How long is turnaround time?

A: We can generally deliver data 3 to 5 days after sample drop off.

Q: What format will the data be delivered in?

A: We provide the raw data (in excel format) and the QuantaSoft run file. The QuantaSoft run file requires the QuantaSoft Software to open and edit. If you need further analysis, you can contact Yanping to discuss.

Q: What steps are involved in ddPCR?

A: First we prepare the reaction mixtures for PCR, then use the BioRad Automated Droplet Generator to separate the mixture into approximately 20,000 tiny (nanoliter) and uniform droplets. After droplet generation, PCR is performed. After PCR the reactions are read by the Bio Rad QX200 Droplet Reader

Q: What is the purpose of ddPCR?

A: Digital PCR offers high precision and sensitivity which allows researchers to quantify template molecules that may not be detected by traditional techniques. Some applications include genomic alteration, rare sequence detection, copy number variation (CNV), and absolute quantification without a standard curve.

Q: Do I need to supply a positive and negative control?

A: It is recommended to run a positive control if you have a sample that has performed well with a similar assay in the past. We also suggest to include a negative template control such as RT reagent control without RNA template added. Please provide us those controls as a sample. We do not provide positive controls. We will run a technical negative control with our nuclease-free water.

Q: How should I choose an optimal annealing temperature for PCR?

A: If you have never tested the probes before, we recommend having us perform a gradient test to determine which annealing temperature is best. Based on your probe/primer temperatures listed, we can set up PCR with the same sample in 8 reactions so that each of the 8 wells is run at a different temperature in a gradient from approximately 50-60 degrees Celsius. After droplet reading, we look at which temperature produced the clearest separation between positive and negative droplets. To perform this test you will need to provide extra volume of your positive control.