UF | ICBR Gene Expression & Genotyping

QIAcuity Digital PCR Frequently Asked Questions

Q: How much DNA is needed per reaction?

A: The amount of gDNA that can be loaded depends on the desired plate type and the species of your sample. For human samples, the ideal range is 56-330ng per well for 8.5K plates and 73-430ng per well for 26K plates. Rat and mouse samples are similar to human, while bacterial samples require significantly lower inputs. For example, E. coli gDNA inputs range from 85-500pg per well for 8.5K plate and 110-650pg for 26K plates.

If your DNA is >30kb, it is recommended to use a restriction enzyme to ensure even partitioning. It is the customer's responsibility to determine which enzymes are used.

cDNA inputs will need to be determined on a case-to-case basis. It is recommended to submit a range of serial dilutions to ensure the ideal input is 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 QIAcuity Digital PCR platform supports both EvaGreen and Probe-based chemistries. Primers will need to be provided for EvaGreen based chemistries, while both primers AND probes will need to be provided for probe based chemistries. Probes can be FAM, HEX, VIC, TAMRA, Atto 550, ROX, Texas Red, Cy5, Quasar 680, Atto 680, Cy5.5, LSS G/Y, LSS Y/O, LSS O/R, LSS R/C, and LSS C/Fr. Probes can be multiplexed between the Green, Yellow, Orange, Red, and Crimson ranges. Ensure the specificity of the probes/primers, that the amplification products are of similar lengths, and that the annealing temperatures are similar if multiplexing. 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 $25.93 per 10 samples.

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

A: For a full service dPCR request the core will prepare the plate, set up and run the QIAcuity, and finally organize and deliver the data. For the drop-off/walk-up service, the customer drops off a fully prepared plate, upon which the core will run it on the QIAcuity instrument. The data will then be organized and delivered.

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: The data is provided in an organized excel table format with the final results reported in copies/uL and copies/ng. We also include copies of the 1D plots as a reference for data quality. The raw data files from the QIAcuity software can be delivered on request.

Q: What steps are involved in dPCR?

A: An individual master mix is prepared for each target. The reaction mixes are aliquoted into a well plate and the samples are added at the desired input. The reaction mix+sample is them transferred to a QIAcuity plate and sealed. The QIAcuity instrument primes the plate by evenly distributing the reaction mixes into either 8,500 or 26,000 individual partitions depending on the plate type. Discrete PCR reactions then take place in each individual partition, after which the plate is imaged.

Q: What is the purpose of dPCR?

A: Digital PCR offers high precision and sensitivity which allows researchers to quantify template molecules that may not be detected by traditional techniques. Specific applications include absolute quantification without the need of a standard curve, mutation detection, genome edit detection, copy number variation, and gene expression quantification.

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 performed well with a similar assay in the past. We also suggest including a negative template control or an NRT control when applicable. We do not provide positive controls. We will always run a technical negative control prepared with our nuclease-free water.

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

A: If you have not tested your primers/probes, we recommend that you run a gradient plate to determine the ideal annealing temperature. If you do not have the ability to do so, we can perform the test with the QIAuity. The core will run your sample in several wells ranging in temperature from 52-64C. After imaging, we will determine which temperature produced the clearest separation between the positive and negative partitions. To perform this test you will need to provide extra volume of your positive control.