**Constellation Digital PCR FAQ**

[http://www.formµlatrix.com/demosite/pcr/index.html#tabbed-nav=tab4](http://www.formulatrix.com/demosite/pcr/index.html#tabbed-nav=tab4)

**1. What is Digital PCR?**

Digital PCR is an alternative to qPCR. It uses microfluidics to provide absolute quantification of the target DNA. In digital PCR, the sample, master mix and primers are mixed, then split into many individual partitions of equal volume. When the partitions are thermally cycled, only the partitions containing the target DNA will amplify. This results in a mixture of “positive” fluorescent partitions and “negative” dark partitions, hence the name “digital” PCR. By counting the number of positive and negative partitions, the original concentration of the target sequence can be determined.

**2. How Does dPCR Differ from Standard qPCR?**

qPCR and dPCR use similar reagents, and the amplification process is the same, but the readout is very different. In qPCR, the fluorescence of the reaction mixture is monitored every cycle, and the cycle that exceeds a threshold fluorescence value is recorded. This threshold cycle is compared to a standard curve, created with samples of known concentration, to calculate a target concentration. With digital PCR, the partitions are thermally cycled to completion (typically 40 cycles). The reactions do not need to be monitored during amplification. After cycling is complete, the number of positive and negative partitions are counted with a fluorescence imaging system. This gives the original concentration directly, without having to compare to a standard curve.

**3. Why is dPCR Better than qPCR?**

There are many cases where dPCR gives a more accurate and precise answer than qPCR. Because the quantification is absolute and binary, dPCR, unlike qPCR, is not dependent on small changes in the amplification efficiency. It is easy to directly compare multiple targets without having to create standard curves or match amplification efficiency. Please see this ["Advantages"](http://www.formulatrix.com/demosite/pcr/index.html#tabbed-nav=tab2) for the benefits of dPCR over qPCR.

**4. Is Amplification Efficiency Really Unimportant for dPCR?**

With digital PCR, each partition is cycled to completion. As long as the amplification efficiency is enough to produce a signal distinguishable from background fluorescence, efficiency will not affect the quantification.

**5. What If There are Mµltiple Copies of the Target in a Partition?**

dPCR can’t detect the number of copies of a template in a single partition, but this can be accounted for using Poisson statistics. The random distribution of a high concentration of templates in a known number of partitions follows the Poisson distribution. While CVs will increase at very high concentrations, concentrations of up to an average of four templates per partition can still obtain CVs under 10%.

### Constellation Digital PCR Questions

**1. How does the Constellation Digital PCR System work?**

The Constellation microplate has 96 sample input wells on the top surface, and microfluidic channels and chambers on the bottom surface. The microplates are primed and read on the Constellation instrument. See the [“How it works” and “Interactive Demo”](http://www.formulatrix.com/demosite/pcr/index.html#tabbed-nav=tab3) page for a more detailed explanation.

**2. How many partitions are there per well?**

Each of the 96 wells has 496 partitions. If a larger number of partitions or conditions are required, the plate can be arranged with replicates or different conditions and fewer samples.

**3. How much volume do I need per sample?**

The minimum per sample reaction volume is 10 µl. This includes master mix, primers and DNA samples.

**4. How long does it take to run a dPCR experiment on the Constellation?**

Once the reagents have been pipetted to the microplate, priming takes about 15 minutes. Then the microplate is thermally cycled for about 1-2 hours depending on the reagents and protocol. Once cycling is complete, reading the plate on the imager takes 5-10 minutes depending on the number of wavelengths read. The Constellation can prime one plate while simultaneously reading another plate, so one instrument can run up to four 96 well plates per hour.

**5. What detection chemistry does the Constellation support?**

The Constellation is designed to work with probe-based chemistries (e.g. Taqman or IDT probe assays). The Constellation is also compatible with EvaGreen.

**6. Can the Constellation do multiplexing?**

Yes, the Constellation can multiplex up to 3 probe wavelengths per sample. The default wavelengths are FAM and VIC/HEX. The third can be configured by the user.

**7. What range of sample concentrations can the Constellation detect?**

The linear dynamic range is from about 1-2 copies /µl to about 1000 copies/µl. If the samples are expected to have concentrations over a wider range, dilutions can be performed. Like any detection method, at very low concentrations, the variability will increase due to Poisson sampling errors.

**8. What quantification precision can I expect?**

Between 100 copies/µl and 1000 copies/µl each well will give a typical quantification CV of <8%. If higher precision is required, replicates can be used.

**Additional Questions?**  
Please [Contact Us](http://www.formulatrix.com/demosite/pcr/index.html#tabbed-nav=tab5) if you have any other questions. We will be happy to answer any additional questions or discuss whether your application would benefit from digital PCR.