Droplet Digital PCR System Instructions

These are basic instructions for the use of the Droplet Digital PCR system. For further details about setting up reactions, types of experiments, instrument usage and troubleshooting, consult the manuals and applications guide.

General tips for good ddPCR:

-The working range of the instrument is between 1-120,000 copies / 20 µL.
-Ideally, one should amplify a 60-200 bp product with 40-60% GC content.
-Wear gloves for all steps to avoid contamination (of your samples and future users' samples).
-Avoid the creation of bubbles as this will greatly affect the efficiency of droplet formation and droplet size consistency.

Step-by-step Directions:

1. Fill out logbook.
2. Retrieve proper supplies from the freezer and store on ice. Generally, these include your DNA/RNA samples, primers and specialized ddPCR supermix (Either for Probes or EvaGreen).
3. Prepare bulk supermix (everything except template) according to directions and aliquot out into striptubes or a 96-well plate (if you have samples that will use different primers, then it may not be beneficial to make bulk supermix).
4. Add template DNA to proper wells/tubes. For each sample, make 25 µL total volume (20 µL needed for each reaction).
5. Mix samples by vortexing or pipetting up and down. (The supermix with droplet formation oil is quite viscous so be sure to mix properly.) NOTE: Avoid bubbles at this point as they will disturb droplet formation.
6. Place a disposable cartridge into the cartridge holder cassette (figure 1) and snap shut (if cassette does not snap, then turn around the cartridge and retry).

Figure 1: Disposable cartridge properly snapped into droplet generation cassette.

7. Using a multi-channel pipet, add 20 µL of PCR reaction to the droplet generator cartridge (center wells, labeled Sample – See Figure 1). Avoid bubbles at this step. If using the BIF multi-channel pipet, use a speed of 4 to fill and dispense.
8. Using a multi-channel pipet, add 70 µL droplet generation oil to the front wells of the cartridge (Labeled Oil – See Figure 1). Use a speed of 4 if using BIF multi-channel pipets. NOTE: Different droplet generation oil is required for EvaGreen or Probe-based experiments; be sure to use the correct one.
9. Place a new rubber gasket over the top (securing with the white pegs – Figure 2). Plug in droplet generator (cord in the back) to turn on. Press green button to open droplet generator. Place prepared cassette into the droplet generator and press the button to close and begin droplet generation. (If the light on the generator flashes orange, open the lid, remove the cassette, reset in place and retry. If this still doesn't work, then remove cartridge from cassette and tap lightly on the countertop before retrying.)

Figure 2: Placement of gasket onto droplet generation cassette.

10. Following droplet generation (about 2 minutes), remove the cartridge and, using a multi-channel pipet, pipet the droplets into a PCR plate (twintec plate). Pipet 40 µL at speed 4 while holding the tips at a 45 degree angle (See Figure 3). This will likely not remove all of the droplets, so a second attempt will need to be done. NOTE: At this point, it is important to pipet the droplet mixture very gently. Fast pipetting or mixing can shear (damage) the droplets and ruin your run. Fortunately, air bubbles are no longer a problem.

Figure 3: Removal of newly formed droplets with proper pipet tip angle.

11. Continue making droplets until complete (maximally, 96 samples). Be sure to cover oil and PCR plate to minimize evaporation as components are volatile.
12. Once your PCR plate is ready, seal the top of the plate with a foil seal and the plate sealer (Use DDPCR program – 5.0 sec at 180°C). NOTE: leave plate sealer tray ejected during warmup time to avoid accidentally heating your samples – block will get hot and cook your samples!
13. Place sealed plate into Thermal Cycler and run PCR. NOTE: Once PCR is finished, the droplets are stable for ~24 h at 4-12°C and can be read the next day.
14. While waiting for PCR to run, turn on droplet reader and allow to warm up for ~30 minutes and check to make sure there is enough reader oil to complete your run. (If no one has used the instrument in more than a month, you should prime it before your run)
15. Following PCR run, remove plate and place into plate holder, securing the plate with black clamps (Figure 4).

Figure 4: Placement of sealed PCR plate into droplet reader plate holder.

16. Place plate holder in droplet reader, and set up run.

17. Log into the computer. (password is BIF)

Sample setup for ddPCR

For EvaGreen:

Table 1. ddPCR reaction components.

Component	Volume per Reaction	Final Concentration
2x QX200 EvaGreen ddPCR supermix	10 µl	1x
Forward primer	variable	100 nM
Reverse primer	variable	100 nM
DNA template* and RNase/DNase-free water	variable	50 fg to 100 ng
Total volume	**20 µl**	--

*Suggested amplicon length: 80–250 bp.

Table 2. Cycling protocol for C1000 Touch thermal cycler.*

Cycling Step	Temperature	Time	Ramp Rate	# Cycles
Enzyme activation	95°C	5 min	~2°C/sec	1
Denaturation	95°C	30 sec		40
Annealing/extension	60°C	1 min		
Signal stabilization	4°C	5 min		1
	90°C	5 min		1
Hold (optional)	4°C	infinite		1

*Use a heated lid set to 105°C and set the sample volume to 40 µl.

For Probes:

Table 1. ddPCR reaction components.

Component	Volume per Reaction	Final Concentration
2x ddPCR supermix for probes	10 µl	1x
20x target primer/probe mix (FAM)	1 µl	900 nM primers/250 nM probe
20x reference primer/probe mix (HEX)	1 µl	900 nM primers/250 nM probe
DNA template and RNase/DNase-free water	8 µl	50 fg to 100 ng
Total volume	**20 µl**	--

Table 3. Cycling conditions for Bio-Rad® C1000 Touch™, S1000™, and T100™ thermal cyclers.*

Cycling Step	Temperature	Time	# Cycles
Enzyme activation	95°C	10 min	1
Denaturation	94°C	30 sec	40
Annealing/extension	60°C	1 min*	
Hold	98°C	10 min	1
Hold (optional)	4°C	Forever	1

* Check/adjust ramp rate settings to ~2–3°C/sec. Use a heated lid set to 105°C and set the sample volume to 40 µl.