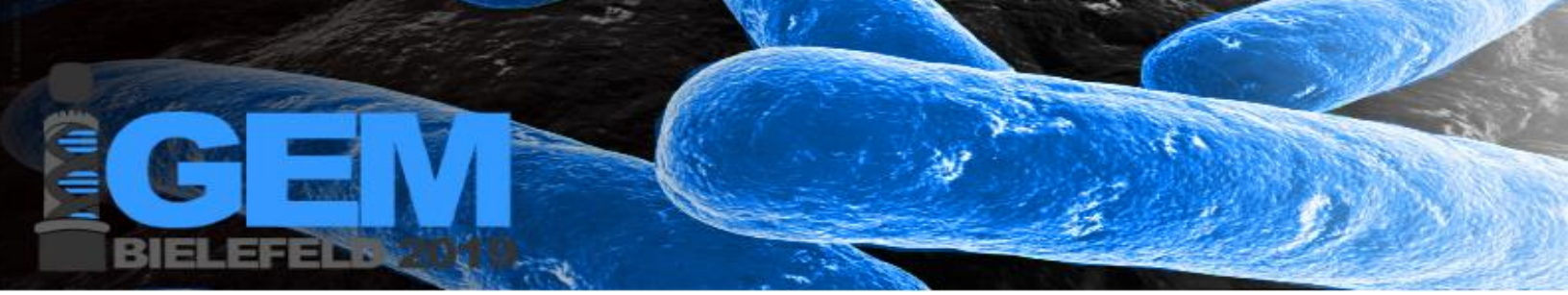


Droplet Digital™ PCR (ddPCR™) with QX200™ EvaGreen® Supermix

Required Equipment:

- QX200 Droplet Generator or Automated Droplet Generator (catalog #1864002 or 1864101)
- QX200 Droplet Reader (catalog #186403)
- C1000 Touch™ Thermal Cycler with 96-Deep Well Reaction Module (Catalog #1851197)
- PX1™ PCR Plate Sealer (catalog #1814000)

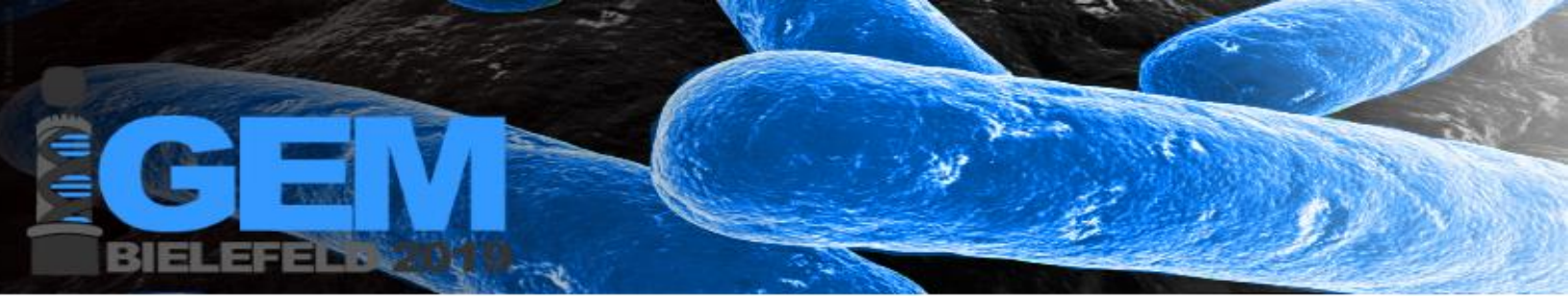
Reaction Setup:

1. Thaw all components to room temperature. Mix thoroughly by vortexing the tube to ensure homogeneity. Centrifuge briefly to collect contents at the bottom of the tube.
2. Prepare samples at the desired concentration before setting up the reaction mix.
3. Prepare the reaction mix for the number of reactions needed according to the guidelines (refer to the next table). Assemble all required components except the sample. Dispense equal aliquots into each reaction tube and add the sample to each reaction tube as the final step.

Table 1. Reaction Mix

Component	Volume per Reaction [µl]	Final Concentration
2x QX200™ddPCR™ EvaGreen® Supermix	10	1x
Forward Primer	Variable	100-250 nM
Reverse Primer	Variable	100-250 nM
Diluted restriction enzyme	1	Variable
DNA template and RNase-/DNase-free water	Variable	Up to 100 ng
Total volume	20	-

4. Mix thoroughly by vortexing the tube. Centrifuge briefly to ensure that all components are at the bottom of the reaction tube. Allow reaction tubes to equilibrate at room temperature for about 3 minutes.
5. Load 20 µl of each reaction mix into a sample well of a DG8™ Cartridge for QX200™/QX100™ Droplet Generator (catalog #1864008) followed by 70 µ of QX200 Droplet Generation Oil for EvaGreen® (catalog #1864005 or 1864006) into the oil wells, according to the QX200 Droplet Generator Instruction Manual (#10031907). For the Automated Droplet Generator, follow instructions in the Automated Droplet Generator Instruction Manual (#10043138)



Restriction Enzyme Digestion Prior to ddPCR:

1. Use 5 – 10 enzyme units per microgram DNA, and 10 – 20 enzyme units per microgram genomic DNA
2. Incubate the reaction for 1 hour at the temperature recommended for the restriction enzyme of choice.
3. For long-term storage, heat inactivate below 65°C.
4. Use a minimum 10-fold dilution of the digest to reduce the salt content of the sample in the ddPCR reaction.
5. Store digested DNA at -20°C or below.
6. Prepare the ddPCR reaction mix.

Thermal Cycling Conditions:

1. After droplet generation with the QX200 Droplet Generator, carefully transfer droplets into a clean 96-well plate or remove the droplet plate containing ddPCR droplets from the Automated Droplet Generator. Seal the plate with the PX1 PCR Plate Sealer.
2. Proceed to thermal cycling (Table 2) and subsequent reading of droplets in the QX200 Droplet Reader.

Table 2. Cycling conditions for Bio-Rad's C1000 Touch Thermal Cycler.

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

Data Acquisition and Analysis:

1. After thermal cycling, place the sealed 96-well plate in the QX200 Droplet Reader.
2. Open QuantaSoft™ Software to set up a new plate layout according to the experimental design. Refer to the QX200 Droplet Reader and QuantaSoft Software Instruction Manual (#10031906).
3. Under Setup, double click on a well in the plate layout to open the Well Editor dialog box.
4. Designate the sample name, experiment type, **QX200 ddPCR EvaGreen Supermix** as the supermix type, target name, and target type: **Ch1** for FAM.
5. Select apply to load the wells and when finished, select **OK.**
6. Once the plate layout is complete, select EvaGreen as the dye set used and run options when prompted.
7. After data acquisitiomm, select samples in the well selector under Analyze. Examine the automatic thresholding applied to the 1-D amplitude data and, if necessary, set thresholds or clusters manually.
8. The concentration reported is copies/µl of the final ddPCR reaction.

From: iGEM Bielefeld-CeBiTec

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BIELEFELD 2019