



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L214

QX200 Droplet Digital PCR System: Instrument Familiarization and Standard Operation

Standard Operating Procedure

SOP Number	SOP-L214
Revision	0.1
Author(s)	Marc Los Huertos
Date	2026-06-26
Organization	EA Program, Pomona College

FOR INTERNAL USE ONLY

This document is the property of the Environmental Analysis Program at Pomona College.
Unauthorized distribution is not permitted.

Table of contents

1 Purpose	2
2 Scope	2
3 Responsibilities	2
4 Definitions	3
5 Safety and Hazards	3
6 Equipment, Consumables, and Reagents	4
6.1 Instruments	4
6.2 Consumables and reagents	4
7 Procedure	5
7.1 System Overview	5
7.2 Daily Startup	5
7.3 Consumables Orientation	5
7.4 Droplet Generation	5
7.5 Plate Sealing (PX1)	6
7.6 PCR Amplification	6
7.7 Droplet Reading	6
7.8 QuantaSoft Orientation (Reading the Output)	6
7.9 Shutdown	7
7.10 Maintenance and Care	7
7.11 Troubleshooting	7
8 Quality Control and Acceptance Criteria	8
9 Waste Disposal	8
10 References	8
11 Training Record	8
12 Appendix A: Pre-Run Checklist	9

13 Appendix B: Generic Thermal Cycling Template

9

1. Purpose

This procedure orients new personnel to the Bio-Rad QX200 Droplet Digital PCR (ddPCR) system and describes its safe, standard operation: the components of the system, the consumables and reagents, the four-stage workflow (droplet generation, plate sealing, thermal cycling, and droplet reading), basic use of QuantaSoft software, routine maintenance, and common troubleshooting.

It is a familiarization and operation SOP. It does not cover the science of assay or primer design (see [SOP-L701](#)) or the analysis and interpretation of results (see [SOP-L702 Applications Guide](#)). The workflow described here follows Bio-Rad's published guidance for the platform ([Bio-Rad Laboratories, 2018, 2024](#)), which itself implements the droplet-counting principle of absolute quantification ([Hindson et al., 2011](#)).

2. Scope

- **Applies to:** Operation of the QX200 ddPCR system in the lab, comprising the QX200 Droplet Generator, the PX1 PCR Plate Sealer, the C1000 Touch Thermal Cycler with 96-deep-well reaction module, and the QX200 Droplet Reader, together with QuantaSoft software.
- **Does not apply to:** Assay/primer design ([SOP-L701](#)), data analysis and interpretation ([SOP-L702](#)), or DNA extraction ([SOP-L700](#)).
- **Prerequisite:** A prepared, validated reaction mix and plate plan from [SOP-L701](#).

SOP numbering

This instrument SOP is numbered L214, placing it ahead of the L700-series analysis SOPs in the lab scheme. Conceptually it precedes analysis: complete this familiarization before running samples for [SOP-L702](#).

3. Responsibilities

Role	Responsibility
Lab PI	Approve instrument access; oversee major maintenance
Lab Manager	Maintain consumable stock, oil, and service records
Trained Personnel	Operate the system per this SOP; log each run
New Students	Complete familiarization and supervised runs before independent use

4. Definitions

- **Amplitude:** The fluorescence signal intensity of a droplet; positive droplets (containing target DNA) have high amplitude; negative droplets have low amplitude; a clear separation between positive and negative populations is required for accurate quantification.
- **Droplet digital PCR (ddPCR):** A method of absolute quantification of nucleic acids in which the sample is partitioned into ~20,000 nanoliter-sized water-in-oil droplets, each containing zero or one target molecule; after PCR amplification, the fraction of fluorescent positive droplets is used to calculate target concentration using Poisson statistics.
- **FAM:** A green fluorescent dye (fluorescein amidite) used to label one probe or primer in a duplex ddPCR assay; detected in the FAM channel (channel 1) by the QX200.
- **HEX:** A yellow-orange fluorescent dye used to label a second probe in a duplex ddPCR assay, or as an alternative to VIC; detected in channel 2 by the QX200.
- **Poisson correction:** A statistical adjustment applied by the DropletQuant software to account for the probability that some droplets contain more than one target molecule; necessary when target concentrations are high.
- **Copies per microliter (copies/ μ L):** The absolute concentration of the target DNA in the final ddPCR reaction, as reported by DropletQuant; must be back-calculated to the original sample concentration using the appropriate dilution factor.
- **Threshold:** The fluorescence amplitude boundary separating positive from negative droplets; set manually or automatically in the analysis software; incorrect threshold setting is the most common source of ddPCR quantification error.

5. Safety and Hazards

Hazards and precautions

- **Droplet generation oil and reader oil.** Use the oil specified for the chemistry (probe vs. EvaGreen). Avoid skin and eye contact; wear gloves and eye protection; consult the SDS for each oil. Clean spills promptly.
- **Reagents.** ddPCR supermixes and any sample DNA are handled with gloves. Environmental soil DNA is low biohazard risk, but treat all extracts with standard lab precautions.
- **Moving parts.** The droplet reader autosampler moves during a run. Keep hands clear and the lid closed while running.
- **Electrical and thermal.** Standard bench-instrument electrical safety. The thermal cycler block reaches high temperatures; do not touch the block during or immediately after a run.
- **Sharps / glass.** None in normal operation; cartridges and plates are single-use plastics.

Consumables are not interchangeable

ddPCR requires Bio-Rad's proprietary droplet-generation reagents, cartridges, and gaskets. Substituting non-specified consumables will produce unusable droplets ([Bio-Rad Laboratories, 2018](#)). Match the oil to the supermix chemistry.

6. Equipment, Consumables, and Reagents

6.1. Instruments

Item	Role in workflow
QX200 Droplet Generator	Partitions each 20 uL reaction into ~20,000 droplets
DG8 cartridge holder	Holds the DG8 cartridge during generation
PX1 PCR Plate Sealer	Heat-seals droplets into the 96-well plate with pierceable foil
C1000 Touch Thermal Cycler (96-deep-well module)	Endpoint PCR amplification within droplets
QX200 Droplet Reader	Streams droplets single-file and reads two-color fluorescence
Instrument computer (QuantaSoft)	Plate setup, run control, thresholding, export

6.2. Consumables and reagents

Item	Notes
DG8 cartridges and gaskets	Single-use; one cartridge = 8 samples
Droplet generation oil (probes) or QX200	Match to supermix chemistry
EvaGreen droplet oil	
Droplet reader oil	Loaded in the reader
ddPCR Supermix for Probes or QX200 ddPCR	Per assay (SOP-L701)
EvaGreen Supermix	
ddPCR 96-well plates and pierceable foil heat seals	For PX1 sealing and reading
Filtered pipette tips, low-bind tubes	Standard molecular-biology consumables

Tip

Keep a stock log for cartridges, gaskets, oils, plates, and foil. The droplet generator and reader each draw oil from onboard bottles; check levels before every session.

Ordering supermixes, oils, and assays

Order supermixes, droplet oils, cartridges, plates, and validated PrimePCR ddPCR assays from the Bio-Rad digital assay catalog: <https://www.bio-rad.com/digital-assays>. Confirm chemistry (probe vs EvaGreen) before ordering so the supermix and matching oil are consistent.

7. Procedure

7.1. System Overview

The QX200 system performs absolute quantification by partitioning, amplifying, and counting (Bio-Rad Laboratories, 2018; Hindson et al., 2011). A single 20 uL reaction is split into roughly 20,000 uniform water-in-oil droplets. Each droplet is a tiny independent PCR. After endpoint amplification, the reader passes droplets one at a time through a two-color optical detector (channel 1 for FAM, channel 2 for HEX or VIC), scoring each droplet as positive or negative. QuantaSoft fits the positive fraction to a Poisson model to return target concentration in copies per microliter.

The work proceeds through four instruments in sequence:

1. **Droplet Generator** (Section 6.4) makes the droplets.
2. **PX1 Plate Sealer** (Section 6.5) seals them into a plate.
3. **C1000 Touch Thermal Cycler** (Section 6.6) amplifies.
4. **Droplet Reader** (Section 6.7) counts.

7.2. Daily Startup

1. Power on the instrument computer and the instruments to be used.
2. Launch QuantaSoft and confirm it connects to the droplet generator and/or reader.
3. Check oil levels in the droplet generator and the reader; top up or replace bottles per the manual (Bio-Rad Laboratories, 2024).
4. Empty the reader's waste bottle if near full.
5. Confirm consumable stock for the planned run (cartridges, gaskets, oil, plate, foil).

7.3. Consumables Orientation

Before the first run, identify each consumable and where it loads:

- **DG8 cartridge** seats in the cartridge holder. The middle row of wells receives sample; the bottom row receives generation oil; the top row collects the generated droplets.
- **Gasket** snaps over the loaded cartridge to seal it for generation.
- **Generation oil** is chemistry-specific. Using probe oil with EvaGreen supermix (or vice versa) will fail.
- **Pierceable foil** is the only seal type compatible with the reader's autosampler; do not use hard-seal films.

7.4. Droplet Generation

1. Prepare reactions per SOP-L701 and load into the cartridge holder with a DG8 cartridge.
2. Pipette each sample into the sample wells (avoid bubbles; pipette to the first stop).
3. Pipette the matched generation oil into the oil wells.
4. Place the gasket over the cartridge.

5. Load the holder into the QX200 Droplet Generator and close the lid; the run takes about two minutes per cartridge.
6. After generation, slowly transfer the droplets from the top row of the cartridge to the corresponding wells of a ddPCR 96-well plate using a multichannel pipette. Pipette gently and at an angle; droplets are fragile and shearing them lowers the usable count.

 Warning

Damaged or sheared droplets reduce accepted-droplet counts and can fail the QC threshold (see SOP-L702, Section 6.2). Pipette droplets slowly and avoid introducing air.

7.5. Plate Sealing (PX1)

1. Place the plate and a sheet of pierceable foil in the PX1 Plate Sealer.
2. Seal at the manufacturer-specified temperature and time (commonly 180 C for 5 s; confirm against the current manual ([Bio-Rad Laboratories, 2024](#))).
3. Inspect the seal: the foil should be uniformly bonded with no lifted edges.

7.6. PCR Amplification

1. Transfer the sealed plate to the C1000 Touch with the 96-deep-well module.
2. Run the validated cycling program for the assay (from SOP-L701).
3. **Set the ramp rate to about 2 C/s.** This slower ramp is important for even amplification across the droplet population on this platform ([Bio-Rad Laboratories, 2018](#)).
4. Include a final hold and store at 4 C if not reading immediately; read within the window specified for the chemistry.

A generic template program is given in Appendix B; the assay-specific anneal temperature and cycle number come from SOP-L701.

7.7. Droplet Reading

1. Place the amplified, sealed plate in the QX200 Droplet Reader plate holder with the retaining clamp.
2. In QuantaSoft, set up the plate layout: sample names, assay/target names, fluorophores (FAM, HEX/VIC), experiment type (e.g., absolute quantification), and supermix/chemistry.
3. Start the run. The autosampler aspirates droplets well by well and the detector reads two channels per droplet.
4. Do not open the reader or disturb the plate while running.

7.8. QuantaSoft Orientation (Reading the Output)

This is orientation only; thresholding decisions and full analysis are covered in SOP-L702.

- **1-D plot:** fluorescence amplitude vs. droplet (event) number for one channel. Positive droplets sit above the threshold line; negatives below.
- **2-D plot:** channel 1 (FAM) vs. channel 2 (HEX/VIC) amplitude. In a duplex assay, droplets fall into four clusters: double-negative, FAM-only, HEX-only, and double-positive ([Bio-Rad Laboratories, 2018](#)).
- **Thresholding:** the boundary between positive and negative clusters. Review it for every well; adjust manually in QuantaSoft Analysis Pro where the automatic call is poor.
- **Well merging:** replicate wells of the same sample/target may be merged to increase the effective partition count before concentration is calculated.
- **Concentration:** QuantaSoft reports copies per microliter from the Poisson fit. Export as described in SOP-L702, Section 6.1.

7.9. Shutdown

1. Close the experiment and export/back up data (SOP-L702).
2. Remove and discard the plate and used cartridges/gaskets (Section 8).
3. Check and, if needed, empty the reader waste bottle.
4. Power down per the manual; leave the system in the state your lab specifies for the reader oil lines.

7.10. Maintenance and Care

- **Each session:** check oil levels and the waste bottle; inspect for leaks.
- **Routine:** keep the reader's fluidics maintained per the manual; do not let oil bottles run dry mid-run.
- **Do not:** force cartridges or gaskets, reuse single-use consumables, mix oil chemistries, or use non-pierceable seals.
- **Records:** log each run (date, operator, assay, plate, instrument notes) and any maintenance or service in the instrument logbook.

7.11. Troubleshooting

Symptom	Likely cause	Action
Low accepted-droplet count	Sheared droplets; pipetting too fast; bubbles	Pipette droplets slowly at an angle; avoid air; re-generate
Few or no droplets generated	Wrong/insufficient oil; cartridge/gasket seated poorly	Use matched oil; reseal cartridge and gasket
"Rain" (droplets between clusters)	Suboptimal anneal temperature; degraded sample	Optimize anneal (SOP-L701); check DNA quality (SOP-L700)
Reader error / clog	Low oil; debris; fluidics issue	Check oil and waste; run reader maintenance; see manual
High NTC positives	Contamination	Rerun with fresh reagents; review setup (SOP-L702, Section 6.2)

8. Quality Control and Acceptance Criteria

- Each well should yield at least 10,000 accepted droplets (target ~12,000 to 20,000); wells below 10,000 are excluded in analysis (SOP-L702).
- Run the controls defined in SOP-L702 (NTC, extraction blank, positive control) on every plate.
- Confirm uniform plate sealing and correct plate orientation before reading.
- Log instrument run metadata to support digital-PCR reporting standards (The dMIQE Group & Huggett, 2020).

9. Waste Disposal

- Used DG8 cartridges, gaskets, plates, foil, and tips: dispose as standard laboratory plastic/biohazard waste per the lab's waste plan.
- Generation and reader oil and oily waste: collect and dispose per the SDS and the lab's chemical-waste procedures; do not pour oil down the drain.
- Reagent containers: rinse and dispose per local guidance.

10. References

i Finding published methods

For peer-reviewed studies that used ddPCR, including environmental and microbial applications useful when validating an assay or interpreting low target concentrations, search Bio-Rad's ddPCR publication database: <https://www.bio-rad.com/ddPCR/publications>.

Bio-Rad Laboratories. (2018). *Droplet digital PCR applications guide*. Bio-Rad Laboratories, Inc.

Bio-Rad Laboratories. (2024). *QX200 droplet digital PCR system instruction manual*. <https://www.bio-rad.com/en-us/product/qx200-droplet-digital-pcr-system>

Hindson, B. J., Ness, K. D., Masquelier, D. A., Belgrader, P., Heredia, N. J., Makarewicz, A. J., Bright, I. J., Lucero, M. Y., Hiddessen, A. L., Legler, T. C., Kitano, T. K., Hodel, M. R., Petersen, J. F., Wyatt, P. W., Steenblock, E. R., Shah, P. H., Bousse, L. J., Troup, C. B., Mellen, J. C., ... Colston, B. W. (2011). High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Analytical Chemistry*, 83(22), 8604–8610. <https://doi.org/10.1021/ac202028g>

The dMIQE Group, & Huggett, J. F. (2020). The digital MIQE guidelines update: Minimum information for publication of quantitative digital PCR experiments for 2020. *Clinical Chemistry*, 66(8), 1012–1029. <https://doi.org/10.1093/clinchem/hvaa125>

11. Training Record

Before operating the QX200 system independently, personnel must:

1. Read this SOP and review the current Bio-Rad QX200 manual (Bio-Rad Laboratories, 2024) and Applications Guide (Bio-Rad Laboratories, 2018).
2. Read SOP-L701 Primer Design and SOP-L702 Applications Guide for context on what they are running and why.
3. Observe a full run (generation, sealing, cycling, reading) performed by trained personnel.
4. Complete at least one full run under supervision, including QuantaSoft plate setup and export.
5. Be signed off by the PI or lab manager.

Trainee	Date observed	Date supervised run	Signed off by	Date

12. Appendix A: Pre-Run Checklist

- ☐ Validated reaction mix and plate plan ready (SOP-L701)
- ☐ Instruments powered on; QuantaSoft connected
- ☐ Generation oil matches supermix chemistry; sufficient volume
- ☐ Reader oil level adequate; waste bottle emptied
- ☐ DG8 cartridges, gaskets, plate, and pierceable foil on hand
- ☐ Controls included on the plate (NTC, extraction blank, positive control)
- ☐ PX1 sealer at correct temperature/time
- ☐ Thermal cycler ramp rate set to ~2 C/s
- ☐ Run logged in the instrument logbook

13. Appendix B: Generic Thermal Cycling Template

Anneal/extension temperature and cycle number are assay-specific and come from SOP-L701. The structure below is a starting template for probe-based ddPCR; confirm against the supermix insert and current manual (Bio-Rad Laboratories, 2024).

Step	Temperature	Time	Cycles	Ramp
Enzyme activation	95 C	10 min	1	~2 C/s
Denaturation	94 C	30 s	39–40	~2 C/s
Anneal/extension	(assay)	1 min	39–40	~2 C/s
Enzyme deactivation	98 C	10 min	1	~2 C/s
Hold	4 C	hold	1	~2 C/s

i Note

EvaGreen chemistry uses a different supermix, oil, and cycling profile than probe chemistry. If the lab runs EvaGreen assays, add a separate template here once the standard profile is fixed in SOP-L701.