ROY J. CARVER CENTER FOR GENOMICS

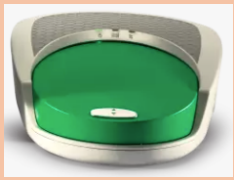
"ACCELERATING GENOMICS RESEARCH"

Newsletter: Volume 2- April 2024

RECENT NEWS:

The BD-FACS Melody Cell sorter is now available to schedule time on the system. Sort days will be limited to Monday, Wednesday, and Friday mornings. Please see the services page of the CCG website for procedures for sample prep and scheduling time.

https://biology.uiowa.edu/research/carver-center-genomics-ccg/services



Bio-Rad QX200 ddPCR system

CCG UPDATES

- **EVENT:** The CCG will be hosting an Applications Seminar and Training Session on Wednesday, April 17. The seminar will focus on this newsletter's highlighted equipment: the Bio-Rad QX200 Droplet Digital PCR (ddPCR) System. The seminar will be presented by the Bio-Rad Applications Specialist at 12:30pm with a free lunch in Room 401, Biology Building East (BBE). Please register via email (ccg@uiowa.edu) by 3pm on Friday, April 12 if you plan to attend, so we can plan accordingly.
- **TRAINING:** In addition to the Seminar, the Bio-Rad Specialist will be doing a training session for the ddPCR system. The training session is limited to 4 people and will be held in the CCG (232 BB) starting at 9am on Wednesday, April 17. This is an all-day training session with breaks for the seminar. Please sign up for the session by emailing the CCG (ccg@uiowa.edu) by 3pm on Friday, April 12. The number of lab members is limited to 1 person per lab to allow availability for as many labs as possible. Future training will be done as needed by the CCG staff.
- **REMINDER:** A sign-in log sheet is next to the Cytation 5 machine. Please continue to log your runs and number of plates in addition to scheduling time online. Also, please note on the sign-in sheet if you log into the machine to just download data (for example: Date, Name, Lab, "data download"). The machine logs are cross-checked, and this will enable accurate counting of the number of runs done for billing purposes.
- **LOCATION CHANGE:** The ddPCR system will be moved from the 3rd floor of BB to the CCG (232 BB) and the Classic LI-COR System will be placed in the current location of the ddPCR system on the 3rd floor of BB (in the Common Equipment Room in 343 BB). This change will happen during the first week of April.
- If you have any questions or would like to be added to our listserv for future newsletters, please email the CCG (ccg@uiowa.edu).

Equipment Highlighted:

Bio-Rad QX200 Droplet Digital PCR System is a multiplex digital PCR system utilizing a water-oil emulsion droplet technology with the droplets acting like individual wells in a plate. Samples (20ul) are fractionated into 20,000 uniform nanoliter-sized droplets. Each droplet contains a separate template DNA or RNA molecules that are individually amplified using standard TaqMan probe-based assays (FAM, HEX or VIC) and EvaGreen fluorescence detection. Following PCR, each droplet is analyzed to determine the number of PCR-positive droplets (at least 1 copy of the target molecule per droplet) in the original sample. The data is further analyzed using Poisson statistics to determine the target template concentration in the original sample.

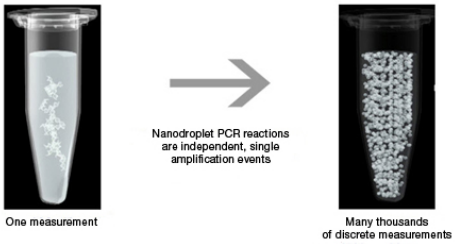


Image of Sample Fractionation

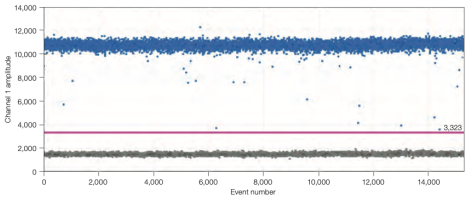
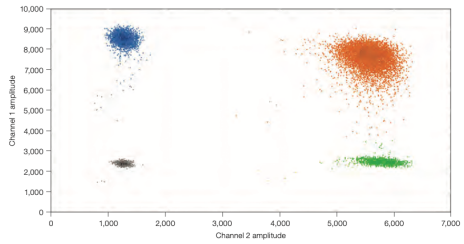


Fig. 1.9. Each droplet from a sample is plotted on the graph of fluorescence intensity vs. droplet number.

1-D plot of droplet data graph of fluorescence intensity vs droplet number. Positive droplets are blue and above red threshold line. Negative droplets are grey and below threshold.



2-D plot of FAM vs HEX fluorescence for each droplet. Black: double neg, Blue: FAM only positive, Green: HEX only positive, Orange: double positive

Additional info (select documents in link): https://www.bio-rad.com/en-us/life-science/digital-pcr/qx200-droplet-digital-pcr-system?ID=MPOQQE4VY

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The QX200 system consists of two components - a droplet generator, and a droplet reader. The system is designed to process 8 samples simultaneously and can be scaled up to run a 96-sample experiment in 5 hours. The droplet generator partitions each sample in a random fashion into 8 x 20,000 droplets which are transferred to a 96-well plate for amplification. After PCR, the plate is placed in the droplet reader which analyzes the droplets for fluorescence in a single file manner recording negative and positive readings for each droplet. Thus, a single sample can generate tens of thousands of data points providing an absolute count of target copies per input sample. This eliminates the need for running standard curves or calibration standards. High-copy targets and background are diluted allowing for detection of rare targets as low as 0.0001% of total copies. Applications of ddPCR:

- Absolute quantification of target DNA measurements, viral load analysis, and microbial quantification.
- Genomic alterations such as a) gene copy number variation (CNV): measuring as much as 1.2x differences in copy number, and b) detection of rare mutations (tumor mutants, SNPs or small indels) or rare sequences (viral, invasive species, rare gene transcripts, or indels)
- Gene expression and microRNA analysis: small changes in amounts of mRNA & miRNA
- NGS: quantification of sample library preps and validate sequence results (single nucleotide polymorphisms or CNV) without use of a standard curve.
- Genome edit detection: homology directed repair and NHEJ generated by CRISPR or other methods.
- Single cell analysis: cell-cell variation (10- to 100-fold) in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations

Applications guide link: https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6407.pdf

Important Use Information:

Please reserve a time to run your experiments using the online scheduling system: https://bioweb.biology.uiowa.edu/servicecenters/login.php. You will also need to log the number of samples you run on the sign-in sheet next to the machine. Please email the CCG (ccg@uiowa.edu) to remove your reservation if you need to cancel. This will allow other labs to sign-up in your canceled spot.

Billing Information:

Billing is done monthly, and you are charged for the number of runs and the number of samples run as follows:

ddPCR Droplet Reader: $45 per run
Total number of channels run that month: $8 per channel
Please note that each channel does 8 samples, and the machine can run a total of 12 channels per run. Channels are billed per run and totaled at the end of the month.