ddPCR User Guide (FY23)

CCR Genomics Core

Location: Building 41, Room D310

Core e-mail: ncilecdnacore@mail.nih.gov

Core website: https://genomics.ccr.cancer.gov/

QX200 Droplet Digital PCR System (https://www.bio-rad.com/en-us/product/qx200-droplet-digital-pcr-system?ID=MPOQQE4VY)

1) Registration

   a) To request core services and to schedule equipment you must be registered under your PI as a member in our iLab website (https://nci.corefacilities.org/account/login). Log-in using your NIH your PIV card as an internal NCI user (even if you are from a different institute).

   b) If you are a new member, register as a member under your PI from the drop-down list. Please note that you will not be able to register unless your PI is already in system with a CAN number. Contact the Core manager (liz_conner@nih.gov) if your PI is not listed.

2) Experimental Design

   a) The primary focus of the Core is to process your samples in as timely a manner as possible. Usually within 24 hours and, often, in the same day.

   b) While we are capable of answering any questions you may have about processing your ddPCR samples, we will typically refer you to local Bio-Rad scientists who are familiar with our Core for experimental design and data analysis support. Those contacts are:

      i) Sarah Daniel, FAS
      sarah_daniel@bio-rad.com
      ii) Bo Song, Instrument Specialist
      Bo_song@bio-rad.com

3) Sample Submission

   a) Once you have settled on your experimental design and are ready to move forward with your samples, please contact the Core at ncilecdnacore@mail.nih.gov to set up an appointment with us.

b) We will email you a template for sample submission and instruct you how to fill it in. There are additional tabs at the bottom of this form with helpful information. Fill in this form and send it to us every time you submit samples.

c) Please also submit a service request for ddPCR on the iLab website for billing purposes.

   i) Select your service from the drop-down menu (ddPCR)
   ii) Enter sample names into the “Sample Grid.” Copy and paste sample names into the sample grid rather than uploading an excel file or typing in sample names.
   iii) Select Assay type from drop-down (Evagreen or probe)
   iv) Make sure to click “Confirm”.

d) The Core provides all reagents and consumables necessary to run your samples.

   i) The Bio-Rad ddPCR system uses specific plates and we provide them to our users as they are built into the cost of the run. It is ok to use any 96-well plate you have available to load your samples into the first time you submit samples but please pick up a couple plates from us for future runs.
   ii) Provide the Core with 11ul of template/primer(s)/probe(s) per sample well. The loading orientation of the plate should be vertical—A1 to H1, A2 to H2, etc. The Core will add 11ul of the appropriate supermix to your samples for a total reaction volume of 22ul.

4) Processing

a) Samples are processed on the Bio-Rad QX200 system.

   i) Plates loaded with 22ul of total sample mixture are placed in the ADG for droplet generation.
   ii) Plates are heat sealed with foil sheets.
   iii) Plates are transferred to a PCR instrument. Check tab5 of the submission form for PCR conditions. Let the Core know of any custom conditions required including gradients.
   iv) Plates are run on the Droplet Reader for analysis. Let the Core know of any special requirements for analysis setup on the reader. Check the assay type that applies under column “N” of the submission form.

5) Data

a) Data is currently sent as a .qlp file. The software, QuantaSoft Analysis Pro, is freely available through download from Bio-Rad’s website. You must register an account with Bio-Rad first. The software is PC-only.

b) A new software package from Bio-Rad, QX Manager, is in the works. Data run through this program will be saved as a .ddpcr file. The software will still recognize .qlp files.

c) Core staff may be able to assist with basic analysis questions but will again refer any questions we are not comfortable answering to the experts at Bio-Rad.

6) Billing

a) We do charge for this service. All the consumables for the instruments are furnished by us and we charge $7/sample.

7) Acknowledgement

The CCR Genomics Core would like to remind our customers that it is important to acknowledge the core in scientific publications, posters, and presentations that include data derived from the facility. Proper acknowledgment provides a visible measure of the impact of the core and is thus essential for our existence both for our continued funding and leadership support. It also helps tremendously in our future effort to secure additional instruments and services. Acknowledgment at the authorship-level would be strongly appreciated when extensive collaborative efforts are involved. Please send us a reprint of the paper, or an e-mail including the reference information for any publication in which the CCR Genomics Core is acknowledged.

An example of Acknowledgement: [Insert name of services(s) here] was conducted at the CCR Genomics Core at the National Cancer Institute, NIH, Bethesda, MD 20892.