Real-time quantitative PCR

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Instrumentation. A Bio-Rad CFX96 (c1000 Touch) is available to users for real-time PCR experiments. It enables to monitor amplification traces in real time in presence of a fluorescent dye (SYBR/BRYT Green) or of fluorescently labeled oligonucleotide probes (Hybridization probes). The Bio-Rad CFX96 uses 96-well reaction format and up to five targets can be multiplexes in one well. User can utilize the included CFX Maestro software to effortless and intuitively design your experiment and analyze results. For more information on the CFX96 capabilities and consumables visit: http://www.bio-rad.com under Life Science Research, or https://www.bio-rad.com/en-us/product/cfx-maestro-software-for-cfx-real-time-pcr-instruments?ID=OKZP7E15.

Students or new user must have training on the Real-Time PCR machine prior to use. To set up a training session on how to operate the CFX96 touch, assistance, or troubleshooting, please contact MCIC staff at 330-263-3828.

Procedure for using the CFX96 Touch

- Reserve the instrument by signing on the sign-up sheet or calling/email the MCIC staff.
- When you come to use the instrument, sign-up with your name, PI name, time, etc. on the sign-up sheet near the instrument.
- Set up a folder with your name in the Bio-Rad/CFX/Users/admin folder; you will keep all your data and protocols in this folder.
- As our instrument is calibrated for BioRad consumables, you will need to use them for your experiments. Read the "Instruction and tips for running real-time PCR" below.

Instructions and tip for running real-time PCR on the iQ5

- For reliable performance in real-time PCR, we recommend the CFX96 instrument compatible plate (e.g. Cat#HSP9601 or HSP9655 etc.) and optical clear micro seal “B” (Cat# MSB1001).
- When you cover your plate with the optical tape, make sure that you do not touch the tape with your hands, or gloves: grease from your hands or powder from the gloves may deposit on the optical film and cause wrong fluorescence readings. To completely seal the plate, press the optical tape down using the protective paper strip that you peeled off the tape, or use a roller.
- Do not keep your plates in direct contact with ice, because ice or droplets of water may be transferred into the CFX96 block. This will cause wrong fluorescence readings (think of lens effect). Your plate has to be completely dry before you put it in the CFX96 machine. Deposit the plate into the CFX96 block gently, and don’t press it down into the block wells.
- For protocol setup, plate setup, please use CFX real-time PCR detection system (https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10000068706.pdf).
- Data analysis can be done with CFX Maestro ver2.0, for more information see https://www.bio-rad.com/en-us/sku/12013758-cfx-maestro-software-2-0-for-windows-pc?ID=12013758 and CFX Maestro user guides (https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10000068703.pdf).

Some references

- A good reference for beginners is: Fraga, D., Meulia, T. and Fenster, S. Real-time PCR. 2007. Chapter 32 in ‘Current Protocols in Essential Laboratory Techniques.’ John Wiley & Sons Publishing.
- For ultimate qPCR assay design guide: https://www.biorad.com/webroot/web/pdf/lsr/literature/Bulletin_6894.pdf