

Real-time quantitative PCR

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Instrumentation. A Bio-Rad iQ5 is available to users for real-time PCR experiments. It enables to monitor PCR product accumulation on line during the PCR reaction in presence of a fluorescent dye (SYBR Green) or of fluorescently labeled oligonucleotide probes (Hybridization probes). The Bio-Rad iQ5 uses 96-well reaction format and up to five targets can be multiplexed in one well. For more information on the iQ5 capabilities and consumables visit: <http://www.bio-rad.com> under Life Science Research, and <http://www.biocompare.com/gonl.asp?id=114482> for multiplexing kits.

Procedure for using the iQ5

- Reserve the instrument by signing on the calendar or calling 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 iQ5/USERS folder; you will keep all your data and protocols in this folder.
- After the run, before turning the mercury lamp off, check with the MCIC staff, if there are other users scheduled after you.
- 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

- Our instrument is calibrated for Bio-Rad microtiter plates and optical tape (Bio-Rad, PCR Plastic Consumables, Cat#2239441, with optical tape Cat# 2239444), therefore you need to use these plates and tape. If not, the instrument would need to be re-calibrated.
- Use PCR or Rt-PCR kits that contain fluorescein as internal standard for well factor correction. Fluorescent dye Rox containing kit are now available on the market, but his dye can not be read by our instrument. You can use a Rox dye containing kits, which does not interfere with data collection, but you can not perform dynamic well factor correction, and you will need to set up an external well factor plate or use persistent well factors for data normalization.
- 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 (there is one in the drawer near the iQ5 machine).
- Do not keep your plates in direct contact with ice, because ice or droplets of water may be transferred into the iQ5 block. This will cause wrong fluorescence readings (think of lens effect). Your plate has to be completely dry before you put it in the iQ5 machine. Deposit the plate into the iQ5 block gently, and don't press it down into the block wells.
- If you do not have a full 96-well microtiter plate, you can cut it, and run strips. You have two choices: (1) if the strip does not have the side skirt, place it in the middle of the block so that the heated lid presses it down straightly, or (2) place it at one end of the block filling the empty spaces with the empty strips available to you near the machine. You need to do this second option, if your strip has the side skirt.
- In the plate set up window, choose the reaction volume of 20 ul, because the wellfactor correction module is for a 20 ul reaction. You can still run a slightly different volume, for example 15 ul or 25 ul, but make sure that samples that will be compared have the same volume.
- In the Run set-up window, choose the dynamic mode for well-factor corrections, by selecting "Collect well factor from experimental plate", if you are using a kit that contain fluorescein as internal well standard.

- For data analysis, chose the “PCR baseline subtraction” as Analysis Mode.
- During the run, do not use other programs on the iQ5 computer as this may interfere with the data collection.

Some references

For a tutorial on realtime PCR: <http://pathmicro.med.sc.edu/pcr/realtime-home.htm>

For a tutorial on cDNA synthesis go to: <http://www.dur.ac.uk/biological.sciences/Staff/Croy/cDNAfigs.htm>

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.

If you are ordering Rio-Rad supplies for your PCR work, use the Bio-Rad quotation *07-Q29825V2* to receive the University discout. The quote lists the discounted item, and it can be used by anyone on the OARDC campus using the Bio-Rad OARDC account number. *Download Bio-Rad 07-Q29825V2 quote*