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INTERACTIONS

QuantStudio 6/7: CMI Getting Started Guide to qPCR

Sample Preparation Getting Started Data Collection Shutdown

Introduction

Quantitative PCR (qPCR), or Real-time PCR, allows PCR reactions to be monitored in real-time and is used for quantitation of DNA and RNA. Reactions are characterized by the time at which fluorescently-labeled PCR products are detected. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed.

Instrument Overview

The CMI has a modified QuantStudio 6/7 from Life Technologies used primarily for conventional Differential Scanning Fluorimetry (DSF). It is a hybrid between a QuantStudio 6 and 7, with decoupled excitation and emission filters.

Applications

- The SYBR Green I dye chemistry can be used for the following assay types:
    - One-step RT-PCR for RNA quantitation
    - Two-step RT-PCR for RNA quantitation
    - DNA/cDNA quantitation

Required Supplies

- 96-well FAST-block optical plate, such as LifeTechnologies MicroAmp FAST optical 96-well reaction plate, 0.1 mL, 4346907
- Optical adhesive film, such as LifeTechnologies MicroAmp Optical Adhesive Film, 4360954
- qPCR reagents including LifeTechnologies PowerUp SYBR Green Master Mix, A25742
- Primers and templates
- Pipettes (recommended)

Sample Preparation

Follow kit guidelines for sample preparation and experimental setup.

Getting Started

Resources

Additional resources are available at the instrument, including instrument manual.

Experimental Design Tips

- Target Ct values between 20-35:
    - If lower, reduce template concentration.
    - If higher, raise template concentration.

- Test primers and template:
    - Two fold dilution series.
    - One cycle difference in Ct ~ 2X difference in concentration.
- Repeat measurements (2-4 replicates).
- Calculate and plot 2^{-Ct}.

General Care and Maintenance

- Keep the instrument door (on the right side) free of obstruction.
- If the control software won't connect to the instrument, restart the software. If the control software still won't connect, cycle the power on both the instrument and the computer.

Startup

1. Book time on the PPMS calendar before you start.
2. Login to the computer using your PPMS credentials (eCommons ID and password).
3. Set up the qPCR reaction plate
    a. Following instructions from the kit protocol, mix samples and reagents.
    b. Load 96-well FAST block optical plate. Include replicates and No-Protein Controls.
    c. Cover the plate with optical film
4. Tap the touchscreen to wake the instrument from sleep by pressing the power button.
5. Open the instrument door.
6. Place the plate in the instrument and close the door.

Data Collection

1. Open the Quant Studio Real-Time PCR Software.
2. On the Setup tab, select Template or Experiment Setup.
    a. If you open from template, then you may use your own or open the file.
3. Setup Tab:
    a. Experiment Properties:
        i) Experiment name: provide a name
        ii) Instrument: **Quant Studio 7**
        iii) Block: FAST 96
        iv) Type: Standard Curve
        v) Reagents: SYBR Green reagents
        vi) Properties:
            (1) Speed: FAST (or standard, depending on template and kit)
            (2) Check the box to include melt curve.
    b. Define:
        i) Targets: Target 1.
            (1) Reporter: SYBR.
            (2) Quencher: NONE.
    c. Passive Reference: ROX.
    d. Assign:

i) Select wells to read (select entire plate to avoid losing data from human error in well selection).

e. Run Method:
   i) Set reaction volume: 10 µL (or 20 µL).
   ii) Graphical View is used to set ramp parameters (the example below highlights Fast ramp speed or according to kit instructions).
      (1) Hold Stage:
         (a) Fast ramp to 95 ˚C, hold 20 sec
      (2) PCR Stage:
         (a) 40 cycles
         (b) 95 ˚C, hold 1 sec
         (c) Fast ramp to 60 ˚C, hold 20 sec
      (3) Melt Stage:
         (a) Fast ramp to 95 ˚C, hold 15 sec
         (b) Fast ramp to 60 ˚C, hold 1 min
         (c) Slow ramp (0.05 ˚C/s) to 95 ˚C, hold 15 sec
   iii) Optical Filters: select x1-m1 (for SYBR green) and x4-m4 (for ROX).

4. Save the experiment in your folder.
5. Run Tab: click Start Run and select the instrument number from the pulldown.
6. When data collection is complete, you must press the **Analyze** button before proceeding to data processing.
7. Examine the melt curves to check for single melt (multiple peaks indicate non-specific amplification).
8. Export Results (Ct value).
9. Save experiment.

Shutdown

1. Remove your plate from the instrument.
2. Clean up in and around the instrument.
3. Close the control software.
4. Logoff from PPMS!

Book time and Report Problems through the PPMS system: https://ppms.us/hms-cmi

- rates are based on *booked and real-time usage.*

Contact cmi@hms.harvard.edu with questions.

last edited: 2021-07-09