Protocol Sheet

Applied Biosystems® StepOnePlus (for software version 2.0) instrument setup instructions for qBiomarker Copy Number PCR Arrays

Important points before starting

- Please read the handbook supplied with the qBiomarker Copy Number PCR Array, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

Procedure

1. **Open the ABI StepOnePlus software on the desktop of the computer that is connected to the ABI StepOnePlus system.**
2. **Select "New Experiment" on the upper toolbar.**
3. **"Define: Experiment Properties"**
    - **Label experiment.**
        - **Type in "Experiment Name."**
        - **Type in "Barcode," "User Name," "Comments" (optional).**
    - **Select instrument.**
        - **"StepOnePlus Instrument (96 wells)"**
    - **Select experiment type.**
        - **"Quantitation"**
    - **Click "Next" on bottom of the screen.**
4. **"Define: Methods & Materials"**
    - **"Quantitation Method"**
        - **"Standard Curve"**
    - **"Reagents to Detect Target Sequence"**
        - **"SYBR® Green Reagents"**
        - **Keep "Melt Curve" checked.**
    - **"Ramp Speed"**
        - **"Standard (~2 hours to complete a run)"**
    - **"Template Type"**
        - **"cDNA" (complementary DNA)**
    - **Click "Next" on bottom of the screen.**

5. **"Set Up: Targets"**
    - **"How Many Targets Do You Want to Quantify?"**
        - **"1"**
    - **Uncheck "SetUp Standards"**
        - **"Target Name":**
            - **"Target 1"**
        - **"Reporter"**
            - **"SYBR"**
        - **"Quencher"**
            - **"None"**
    - **Click "Next" on bottom of the screen.**
    - **Ignore the warning — click "OK."**
6. **"Set Up: Standards"**
    - **"How Many Points?"**
        - **"2"**
    - **"How Many Replicates?"**
        - **"1"**
    - **Click "Next" on bottom of the screen.**
7. **"Set Up: Samples"**
    - **"How Many Samples?"**
        - **"96"**

Note: If the instrument is not recognizing all 96 wells, please see additional instructions on the last page.

- **"How Many Replicates?"**
    - **"1"**
- **"How Many Negative Controls?"**
    - **"0"**
- **"Which Sample/Target Reactions Do You Want To Set Up?"**
    - **Select: "ALL Sample/Target Reactions"**
- **Verify all wells in "Plate Layout" view have the "U" symbol ("U" = unknown).**
- **Click "Next" on bottom of the screen.**

8. **"Set Up: Run Method"**
    - **This setting should default to the run protocol with melting curve.**
        - **"Verify Data Capture" icon is present at:**
            - **"Cycling Stage": 60°C (1 minute step)**

- **"Melting Curve Stage": During ramp from 60°C to 95°C**

- **Set "Reaction Volume" to 25 µl.**
- **Verify "Number of Cycles" is set to 40.**

9. **Click "Finish Designing Experiment."**
10. **Ignore warning.**
11. **Click "OK" when prompted "You did not set up standards on the plate."**
12. **Load your plate into the instrument.**
13. **Start the run for this experiment.**
14. **Save your experiment before starting the run.**

Note: For those customers whose instruments do not recognize all 96 wells of the PCR arrays, please use the following instructions.

ABI StepOnePlus — modified setup

1. **Open the ABI StepOnePlus software on the desktop of the computer that is connected to the ABI StepOnePlus system.**
2. **Select "Advanced Setup."**
3. **"Define: Experiment Properties"**
    - **Label the experiment.**
        - **Type in "Experiment Name."**
        - **Type in "Barcode", "User Name", "Comments" (optional).**
    - **Select instrument.**
        - **"StepOnePlus Instrument (96 wells)"**
    - **Select experiment type.**
        - **"Quantitation — Standard Curve"**
    - **Select reagents.**
        - **"SYBR Green"**
    - **Select ramp speed.**
        - **"Standard (~2 hours to complete)"**
4. **Click "Plate Setup" (on left).**
    - **Click "Assign Targets and Samples" tab.**
        - **Highlight the entire plate.**
        - **Check the box next to "Target 1" under "Assign Targets to the Selected Wells."**
        - **Verify all wells in "Plate Layout" view have the "U" symbol ("U" = unknown).**
5. **Click "Run Method" (this setting should default to run protocol with melting curve.)**

- **Verify "Data Capture" icon is present at:**
  - **"Cycling Stage": 60°C (1 minute step)**
  - **"Melting Curve Stage": during ramp from 60°C to 95°C**
- **Set "Reaction Volume" to 25 µl.**
- **Verify "Number of Cycles" is set to 40.**

6. Click "Start Run."

The qBiomarker Copy Number PCR Arrays are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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