CHAI


# Open qPCR User Manual

**Research Use Only**

Open qPCR User Manual
Revision B

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For product warranty information, please refer to the documentation that came with the Open qPCR instrument.

**Research Use Only. Not for use in diagnostic procedures.**

**DISCLAIMER**
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# PREFACE

## Intended Use

The Open qPCR User Manual serves to provide a comprehensive understanding of the setup, operation and maintenance of the Open qPCR system.

The Open qPCR instrument is intended for Research Use Only (RUO). Results produced from the instrument are not for use in diagnostic procedures.

The Open qPCR instrument is intended for indoor use.

## Safety Conventions

The following alert icons appear in this user manual at points where you need to be aware of relevant hazards and/or important information regarding operation of the instrument.

| Icon | Definition |
|---|---|
|  | CAUTION. Indicates that you should consult the manual for further information and proceed with appropriate caution. Failure to do so may lead to physical injury or could cause damage to the instrument. |
|  | ELECTRIC SHOCK HAZARD. Indicates the presence of an electric shock hazard and to proceed with appropriate caution. |
|  | HOT SURFACE. Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution |
|  | TECHNICAL NOTE. Important information regarding procedures relating to the current section of the manual or the use of the Open qPCR system. |

# Safety Warnings and Precautions

Warnings and precautions must always be adhered to avoid risk of personal injury and/or damage to the instrument. If the instrument is used in a manner not specified by the manufacturer, the protection provided by the instrument may be compromised. The information in this manual is intended to supplement the normal safety requirements established in the user's country.

The Open qPCR instrument complies with safety standards IEC 61010-1, level of pollution 2, and overvoltage category II.

**General Safety**

| | |
|---|---|
|  | The Open qPCR Instrument must be connected to a 3-wire grounded outlet with the correct voltage rating. A break in the electrical ground path may create a hazardous condition. |
|  | Do not touch the power switches or power cord with wet hands. |
|  | Always power down the instrument and disconnect the power cord before performing cleaning or maintenance procedures. |
|  | The heat block and lid temperature rises above 95 °C. To avoid injury, do not touch the heat block, lid, or tubes immediately after a run. Let the surface cool for a few minutes before removing the tubes. If samples must be removed immediately, avoid contact with surfaces by extracting tubes with tweezers. |
|  | Do not deliberately modify safety features of the instrument. |
|  | Do not block ventilation at the front and back of the instrument. Leave at least six inches of space on all sides of the instrument when powered on. |
|  | Wear protective eyewear, clothing, and gloves when handling reagents or operating the instrument. |

| | |
|---|---|
|  | Follow all local, state/provincial, and/or national regulations for proper disposal of the instrument calibrators. |

## Marks of Conformity

| Icon | Definition |
|---|---|
|  | This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.<br><br>This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense. |
|  | This device complies with the Electromagnetic Compatibility Directive (2014/30/EU) and Low Voltage Directive (2014/35/EU) of the European Union. |
|  | Waste Electrical and Electronic Equipment Directive 2012/19/EU. Do not dispose of the instrument with general waste. |

# Technical Support

Customer support is provided for all technical and service issues related to the Open qPCR instrument.

**Support Site**

Please visit https://support.chaibio.com for support-related inquiries. You will find resources and tips for getting started as well as troubleshooting help on this site.

**Community Forum Site**

Please visit https://community.chaibio.com to connect with the global community regarding Chai products, application uses, and general tips on PCR.

For instrument purchases made through a distributor, please contact your local support service representative for assistance.

For instrument purchases made directly through Chai, please contact the Chai support staff for assistance.

Phone: +1 (800) 642-4002
+1 (650) 779-5577

Website: www.chaibio.com

Welcome! Please read through this manual to become acquainted with your new real-time PCR thermocycler.

# CHAPTER 1 – The Open qPCR System

## 1.1 Introduction

The Open qPCR is a portable, real-time PCR instrument for amplifying and detecting DNA and RNA targets of interest. Real-time PCR provides fast and sensitive quantification of nucleic acids in various sample types. Diverse applications include, but are not limited to, cancer phenotyping, bacteria and pathogen detection, genetically modified organism detection in food, and gene expression analysis.

Based on a photodiode detection and dual-Peltier thermal system, the instrument contains 16 wells and supports sample volumes between 10 – 50 μL per reaction. With a maximum ramp rate of 5 °C/s, the system's heating and cooling efficacy results in rapid cycling and leads to assay completion in as little as 20 minutes. Multiple Open qPCR units may be connected to one computer via Wi-Fi or Ethernet, allowing simultaneous analysis for as many samples as desired.

Chai offers two models of the Open qPCR system: single channel and dual channel. The two instruments differ primarily in the number of fluorophores each can detect. The single channel detects either SYBR Green, Chai Green, or FAM fluorophores through channel 1 whereas the dual channel allows for additional detection of HEX, VIC, or JOE fluorophores through channel 2. Multiplexing by optical channel is available only on the dual-channel instrument.

The system supports high resolution melt (HRM), touchdown PCR, controlled ramp rates, and unlimited cycling stages. Analysis features include baseline subtraction, color compensation, and melt curve analysis. Open qPCR software is browser-based and is compatible with Mac, PC, Linux, and tablets. The instrument may be run via Ethernet, Wi-Fi or a connected USB cable.

## 1.2 Instrument Technical Specifications

### Connectivity

Networking: Ethernet, 802.11n Wi-Fi, USB
Touchscreen: 5.0 inch, capacitive
Software: HTML5/JavaScript web app
Device supported: PC, Mac, iPad, Android Tablet
Integration: REST API

### Operating Environment

Operating temperature: 10 – 35 °C

Relative humidity: 20 – 80% non-condensing
Max altitude: 2,000 m

### Power

Voltage: 100 – 240 V*
Frequency: 50 – 60 Hz
Average Power: 75 W**
Peak Power: 350 W

### System Specifications

Samples: 16 wells, 100 µL tubes
Sample volume: 10 – 50 µL
Sensitivity: 1 copy
Dynamic range: $10^9$
Dimensions: 11 x 9.5 x 7.5 inches (28 x 24 x 19 cm)
Weight: 10 lbs. (5 kg)

### Thermal Performance

Temperature range: 4 – 100 °C
Max ramp rate: 5.0 °C/s
Min ramp rate: 0.00001 °C/s
Design: dual Peltiers
Temperature accuracy: ±0.2 °C at 65 °C/s
Temperature uniformity: ±0.4 °C at 65 °C/s
Lid temperature: Ambient to 120 °C

Additional Notes:
*Allowed supply voltage fluctuations up to +/-10% of the nominal voltage
**Average power rating tested at room temperature with default protocol

## 1.3 General Features

1. Adjustable lid knob
2. Lid lever
3. Lid latch
4. LCD touchscreen
5. Front air inlet
6. Ethernet connection port
7. USB cable inlet for connection to a PC or Mac
8. Reset button
9. Wi-Fi adapter port
10. Power inlet
11. Power switch
12. Vent


Figure 1.1


Figure 1.2

## 1.4 Heating & Cooling

The Open qPCR's dual-Peltier design and heated lid provide for precise temperature control of the individual reactions. With one side of the Peltier modules attached to a heat sink, the opposite side can then be cooled and heated with respect to the heat sink (Figure 1.3).

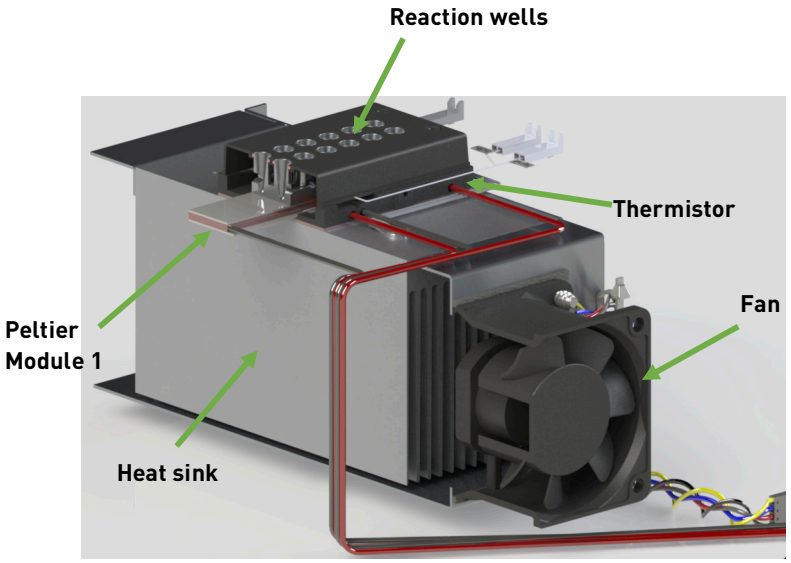


Figure 1.3

A copper heat spreader beneath the Peltiers ensures temperature uniformity and rapid heat transfer during cycling. Two thermistors, attached directly to the heat block, monitors well temperature. Cooling is achieved with a high-efficiency fan that dissipates air across the heat sink.

Open qPCR's fast ramp rate of up to 5 °C/s allows for rapid thermal cycling, resulting in quick turnaround times from start to finish. The instrument's firmware controls the thermal cycling temperature by using a control loop to ensure rapid heating and to control temperature overshoot around the desired point. A heated lid minimizes tube condensation throughout the cycling process.

## 1.5 Optics

The optical system of the Open qPCR detects one spectral band for the single channel model and two spectral bands for the dual channel model. Each of the 16 wells has its own LED and photodiode detector(s). Figure 1.4 depicts all components involved in the optical detection.

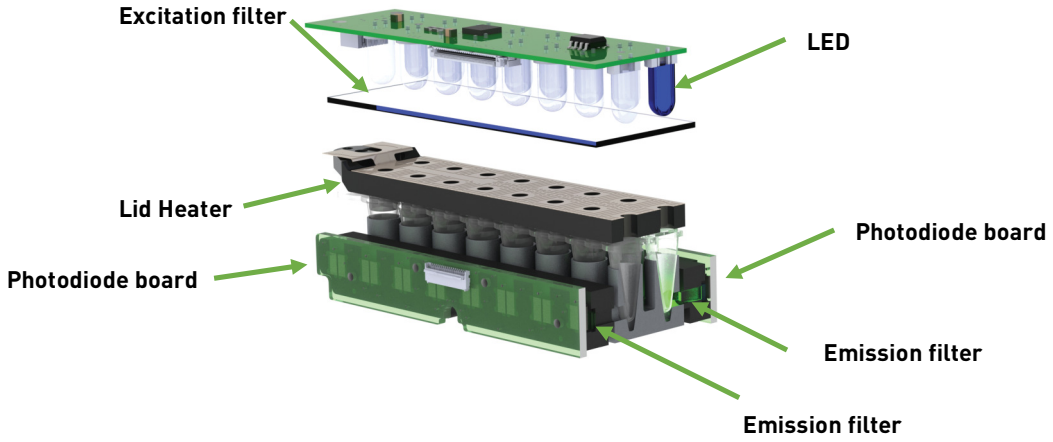


Figure 1.4

During a run, the LED emits light through an excitation filter embedded within the heating block on the instrument lid. The incident light strikes the top of each reaction tube and excites the fluorophores in the sample. The emerging light then passes through the emission filters before being detected by the photodiode board.

Detection wavelengths for the dual channel and single channel models are illustrated in Table 1.5 below:

Table 1.5

| Model | Channel | Fluorophores/Dyes | Detection Wavelength |
|---|---|---|---|
| Single Channel | 1 | FAM, Chai Green, SYBR Green | 513 - 555 nm |
| Dual Channel | 1 | FAM, Chai Green, SYBR Green | 508 - 532 nm |
| | 2 | HEX, VIC, JOE | 573 - 597 nm |

Fluorophores supported by the Open qPCR instrument are indicated in Table 1.6:

Table 1.6

| Fluorophore/Dye | Absorption wavelength (max) | Emission wavelength (max) |
|---|---|---|
| Chai Green | 488 nm | 510 nm |
| SYBR Green | 497 nm | 520 nm |
| FAM | 494 nm | 518 nm |
| HEX | 535 nm | 556 nm |
| JOE | 520 nm | 548 nm |
| VIC | 538 nm | 554 nm |

While the Open qPCR system does not require ROX dye for normalization purposes, including ROX in your master mix will not impact instrument performance.

## 1.6 Supported Consumables

The instrument utilizes 100 µL low-profile PCR tube strips. Due to Open qPCR's unique architecture, both cap and tube sides must be optically clear and they must not auto-fluoresce. For optimal results, please use Chai's validated DNAse and RNAse-free tubes.

Table 1.7

| Product | Catalog # |
|---|---|
| PCR 8-Cap Strips, Optically Clear Flat Caps, 100 µL, qty 250 | S02112 |
| PCR Tube & Cap Strips, 8-Well Strips, Optically Clear, 100 µL, qty 125 | S02132 |
| PCR 8-Cap Strips, Optically Clear Flat Caps, 100 µL, qty 250 | S02122 |

# CHAPTER 2 – Installation

## 2.1 Getting Started

The following items are packaged within the Open qPCR shipping box:

- Open qPCR user manual
- Open qPCR instrument
- Wi-Fi adapter
- Power cord
- USB cable (USB Type A to Mini B)
- Calibrators:
    - FAM/HEX calibrators, 3 cals (dual channel instruments) OR
    - Fluorescein calibrator, 3 cals (single channel instruments)

**Setting up the instrument**: Once the instrument is unpacked, set it on a stable, flat, and non-vibrating surface near an appropriate power source. Make sure to leave at least 6 inches of space on all sides of the instrument when powered on and operating.

Do not block ventilation in front of or behind the instrument. Route cables in an appropriate manner to prevent mechanical force from breaking connectors. Remove plastic covering from the LCD screen before use.

**Powering the instrument:** Connect the power cable from the power source to the back of the instrument before turning on the power switch. After the switch is turned on, the LCD screen will display "Booting" on the bottom right corner during initialization. Give the instrument approximately five minutes to boot up. Ensure that the instrument's software version and serial number are displayed on the LCD screen before accessing the Open qPCR software.

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**The instrument must be connected to a 3-wire grounded outlet with the correct voltage rating. Do not touch the power switch or power cord with wet hands.**

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When the Open qPCR system is not in use, ensure that the instrument lid is closed and secured in place. This avoids any dust or debris, which may interfere with the system performance.

## 2.2 Connectivity Options: USB, Ethernet, Wi-Fi

You can connect to the instrument from any computer using USB, Ethernet, or Wi-Fi. Tablets are limited to either Ethernet or Wi-Fi connection. **Make sure the Open qPCR LCD screen**

**displays the software version and serial number (bottom right corner) before the USB cable, Ethernet cable, or Wi-Fi dongle is plugged into the respective ports on the device**.

If you are connecting by Ethernet or Wi-Fi, please note the following:

Some networks require the Media Access Control (MAC) address to be registered with the network administrator first. If you see *No Network Connection* displayed on the LCD screen after Ethernet or Wi-Fi adapter connection, use the USB to connect to the device first. Go to **Settings > Network Settings**. The MAC address is displayed for Ethernet and/or Wi-Fi (if adapter is connected). Relay the appropriate MAC address to your network administrator to grant access permission.

Ethernet and Wi-Fi options: You are connecting directly to your Open qPCR via the local network and generally will not be able to access this IP address over the Internet.

Please follow the instructions below for each option.

## Option A - Connecting to Open qPCR via USB

The following operating systems are required for connections made by USB:

- Windows 7 or later
- macOS Sierra (10.12) or later
- Linux (Debian or Ubuntu)

1) **a. For Windows Users***:* Please ensure that the computer has internet connectivity before proceeding to allow required drivers to be automatically downloaded and installed.

   Use the included USB cable to connect the instrument to the designated computer.

   A message stating that a device is being set up/installed may display automatically. The setup should take no more than a few minutes. Once the setup is complete, a message may pop up, displaying that the USB/Ethernet/RNDIS Gadget is ready to use.

   **b. For Mac Users***:*

   Use the included USB cable to connect the instrument to the designated computer.

   No pop-up message will display to state that a device is being set up/installed. Proceed directly to step 2.

2) Open an internet browser and type into the address bar **192.168.7.2**.

## Option B - Connecting to Open qPCR via Ethernet

1) Connect an Ethernet cable to the Ethernet port on the back of the Open qPCR.

2) Once the Ethernet connection is established, the instrument will take a few minutes before displaying the IP address on the LCD screen.

3) Open an internet browser on a computer connected to the same network, and type in the instrument IP in the address bar.

## Option C - Connecting to Open qPCR via Wi-Fi

Please note that in order to connect via Wi-Fi, the instrument must first be set up using either USB or Ethernet connection.

1) Make sure the Open qPCR is powered off before plugging the Wi-Fi adapter into the Wi-Fi port on the back of the instrument.

2) Power on the instrument and let the system initialize. Allow the "booting" message to clear from the LCD screen before continuing.

3) Follow the procedures previously indicated in section **Option A - Connecting to Open qPCR via USB** or in section **Option B - Connecting to Open qPCR via Ethernet**.

4) Enter the appropriate IP address (192.168.7.2 for USB connection, or the IP address displayed on the LCD screen for Ethernet connection). You will be brought to the login page. Create a new account and username if this is the first time setting up the instrument. Otherwise, log into the software with your existing username and password.

5) On the Open qPCR home page, select **Settings > Network Settings**.

6) Ensure that the Wi-Fi toggle is green before selecting the appropriate network to connect the instrument.

7) Type in the password for the network and note the IP address stated under **Current Settings** (Figure 2.1). Make sure this IP address is also displayed on the bottom left of the instrument's LCD screen. If the Wi-Fi IP address is not displayed, turn off the instrument, wait for 30 seconds, and power the device back on.

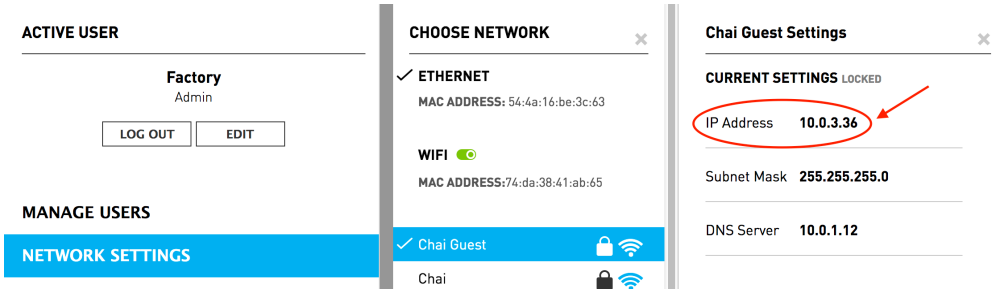



Figure 2.1

8) Unplug the USB cable and close out of the current browser. Open a new browser and type in the instrument IP address to access the software.

## 2.3 Setting a Static IP Address

The Open qPCR system must be connected by Ethernet in order to set a static IP address. If applicable, consult with your IT department before proceeding.

1) On the home page, navigate to **Settings > Network Settings**.

2) Select **Ethernet**. Ensure that the toggle next to **Set Automatically** is in the off position (Figure 2.2).

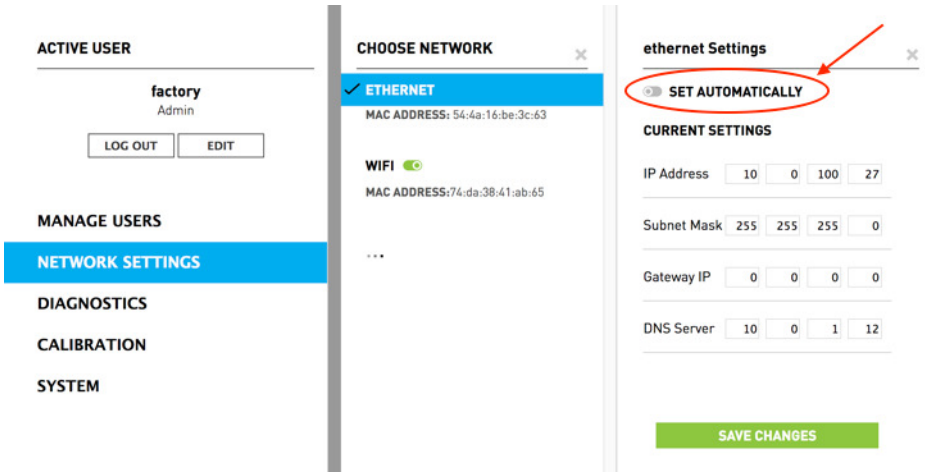



Figure 2.2

3) Proceed to set the appropriate static IP address and select **Save Changes** once complete.

4) Once the changes are saved, the screen will automatically refresh to the home page. The new IP address will display on both the current web browser and the instrument's LCD screen.

## 2.4 Logging in and Creating an Account

Navigate to the appropriate address in your web browser to view the following screen.

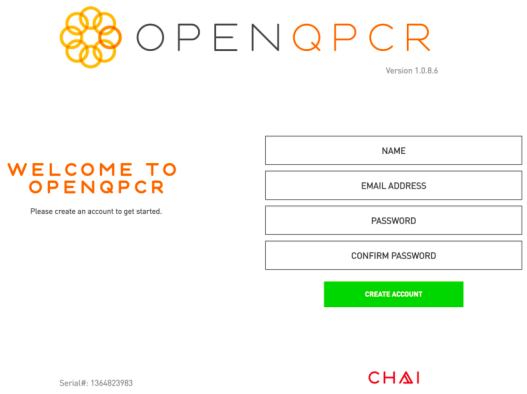



Figure 2.3

Proceed to create a new user account for logging into the software.

If the instrument is connected by Ethernet or Wi-Fi, different users may log into the software simultaneously to view previous test results and export data.

# CHAPTER 3 – Calibration

## 3.1 When to Calibrate the Open qPCR

The Open qPCR needs to be calibrated before its initial use. Calibration must also be run each time any of the following events occur:

- The lid height is adjusted
- The tube type is changed

## 3.2 Adjusting the Open qPCR Lid Height

When using the instrument for the first time, set the lid height for the 100 µL PCR tubes that will be used in your qPCR runs. Calibrate the instrument for that specific lid height.

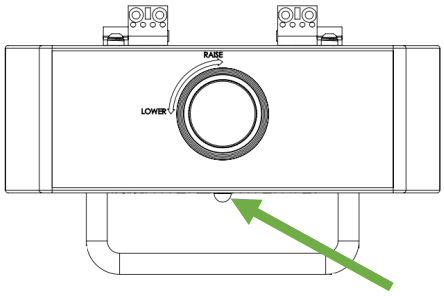



Figure 3.1

Open the lid by pressing the small silver button near the lid bottom. You should feel the latch mechanism release.

There are 2 rows of 8 wells, accommodating either 8-strip 100 µL PCR tubes or individual 100 µL PCR tubes. Use gloves while handling the tubes as prints on the tubes may affect the fluorescence measurements. Ensure there are 16 capped tubes in the heat block during the lid calibration process.

Raise the lid height by turning the knob on the lid clockwise. Place the PCR tubes in the instrument and then close the lid until it latches. Proceed to turn the knob counter-clockwise to lower the lid. Set the knob so that it just touches the top of the tubes and you feel a light resistance.

Test the lid height by opening the lid and making sure you can close it again easily with the tubes in place. If it does not close easily and you need to adjust the height for the next set of tubes, a recalibration of the instrument will be required.

Calibrate using the built-in procedure. If calibration fails, re-adjust the lid height and repeat the calibration process.

**Always make sure that your tubes are dry externally and capped tightly before inserting them into the Open qPCR. Failure to do so may cause liquids to vaporize and create deposits on the internal components of the instrument.**

## 3.3 Open qPCR Single Channel System Calibration

The Open qPCR single channel system requires calibration with the Fluorescein Calibrator kit. The calibration process, including solution preparation, will take approximately 20 minutes to complete.

Please note that the instrument lid height must not be adjusted at any point during calibration. If the lid height is adjusted, a re-calibration is required.

PCR tubes used for the calibration process must be the same tubes that will be used for subsequent test runs.

### Fluorescein Calibrator Kit

Table 3.2

| Fluorescein Calibrator | # of Calibrations | Quantity | Catalog # |
|---|---|---|---|
| Shipped with instrument | 3 | 1 x 1.3 mL/vial | - |
| Available for order | 6 | 2 x 1.3 mL/vial | S0110M |

### Storage Temperature

Store at 4 °C or -20 °C (long term), protected from light. The product is stable for two years from date of receipt when stored at the recommended temperature.

### Materials Required for Calibration

- Fluorescein calibrator solution
- Distilled or deionized water
- 200 µL pipette
- 200 µL pipette filter tips
- Chai PCR Tube & Cap Strips, 8-Well Strips, Optically Clear, 100 µL
- PCR tube rack
- Mini centrifuge

## Preparing for Calibration

The Fluorescein solution is light sensitive. Keep aliquoted solution protected from light. If you are using calibrators that have previously been stored at 4 °C, you do not need to equilibrate the solution to room temperature before running the calibration process. You may use the calibrator solution directly from the fridge.

1. Obtain 4 new 8-well 100 µL PCR tube strips. Check that there are no cracks on the tubes.

2. Vortex for 10 seconds or invert the fluorescein vial 10 times to ensure homogenous mixing of the solution.

3. Spin down the vial before aliquoting 25 µL/tube of the solution into 2 new 8-well PCR tube strips. Cap tubes tightly and spin down.

4. Prepare the water strips by aliquoting 25 µL/tube of water into 2 new PCR strip tubes. Cap strip tubes tightly and spin down.

## Calibration Procedures

Please note that the calibration process must be completed in one sitting.

**The heat block and lid temperature rises above 95 °C. To avoid injury, do not make direct contact with the heat block, lid, or tubes immediately after a run. Let the surface cool for a few minutes before removing the tubes. If samples must be removed immediately, avoid contact with surfaces by extracting tubes with tweezers.**

**Always wear gloves and protective eye wear when handling reagents or operating the instrument.**

1. Log into your account through a web browser. On the software home screen, navigate to **Settings → Calibration**.

2. Under Optical Calibration, select **Run Now**.

3. The calibration time and materials required will be shown on the next screen (Figure 3.3). Select **Begin.**


Figure 3.3

4. The screen will prompt you to prepare 4 new 8-well PCR tubes (Figure 3.4). Gather the previously prepared strip solutions and select **Begin.**

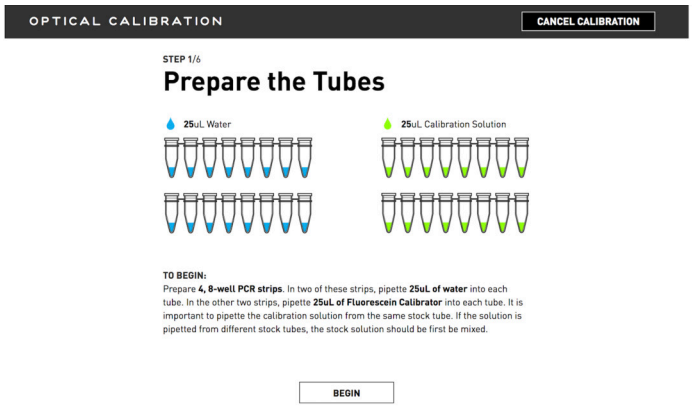



Figure 3.4

5. Insert two water strips into the instrument. Press down on the tubes gently once they are inserted. Adjust the lid height per the instructions in section **Adjusting the Open qPCR Lid Height**.

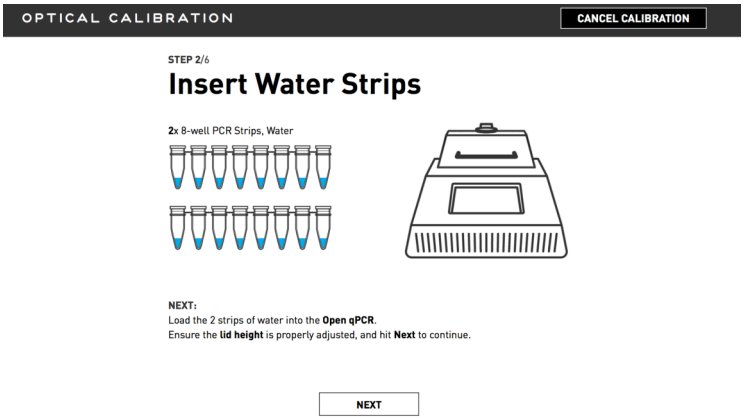



Figure 3.5

6. Close the lid, making sure that the latch clicks and select **Next**. You will see the screen below during the lid heating and data collection process.

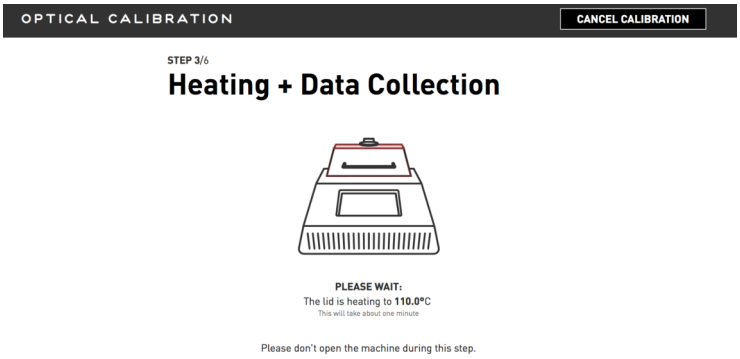



Figure 3.6

7. Allow the instrument to read fluorescence for 2-3 minutes before proceeding to the next step.

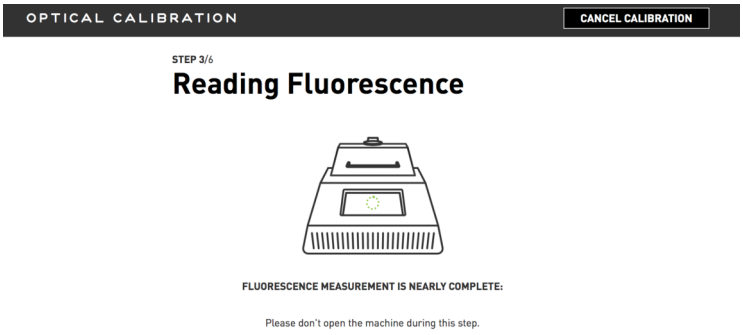



Figure 3.7

8. Once the reading completes, insert the Fluorescein solution strips (Figure 3.8). Press down on the tubes gently once they are inserted. Click **Next**.

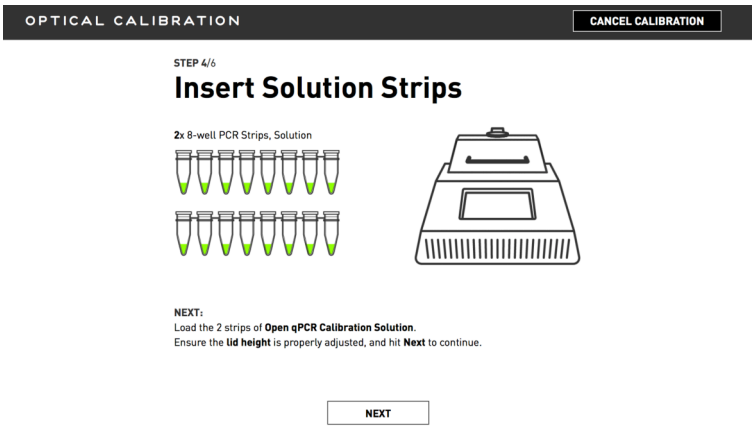



Figure 3.8

9. Following the fluorescein reading, **Calibration Complete** will be displayed on the screen (Figure 3.9). You may now proceed to your experiment setup.

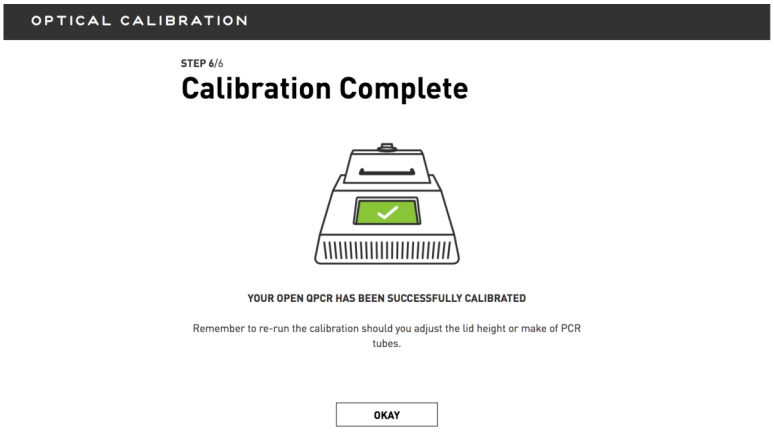



Figure 3.9

10. If calibration fails, **Calibration Failed** will be displayed on the screen (Figure 3.10). Repeat the calibration process in such instances. If failure persists on repeat, contact Technical Support.

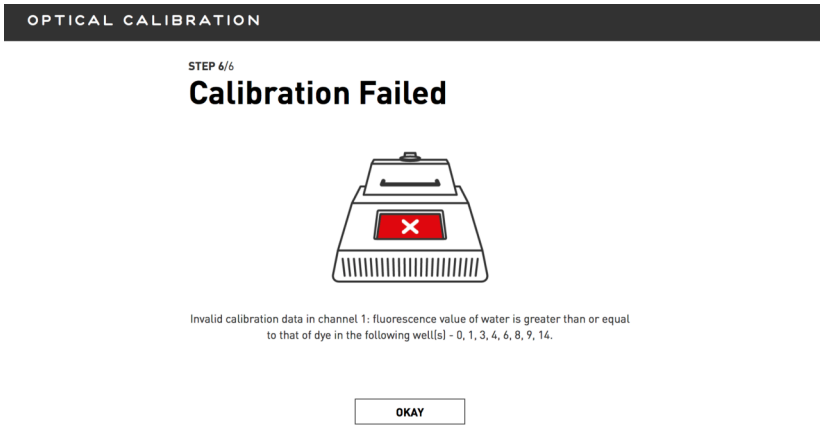



Figure 3.10

It is not recommended to reuse the calibration dyes because photo-bleaching occurs at different levels from well to well. If you would still like to reuse the dyes, pool the solution first and aliquot again as before.

## 3.4 Open qPCR Dual Channel System Calibration

The Open qPCR dual channel system requires calibration with the FAM + HEX Calibrator Set. The calibrators are provided in lyophilized format. Prior to calibrating the Open qPCR, ensure that the lyophilized calibrators are reconstituted thoroughly (~ 45 minutes). The calibration process, including calibrator reconstitution, will take approximately 75 minutes to complete.

Please note that the instrument lid height must not be adjusted at any point during calibration. If the lid height is adjusted, a re-calibration is required.

PCR tubes used for the calibration process must be the same tubes that will be used for subsequent test runs.

## FAM + HEX Calibrator Set

Table 3.11

| FAM + HEX Calibrator Set | FAM Calibrator | HEX Calibrator | Reconstitution Buffer | # of Calibrations | Catalog # |
|---|---|---|---|---|---|
| Shipped with instrument | 1x 0.66 nmol/vial | 1x 1.32 nmol/vial | 2x 1.34 mL/vial | 3 | - |
| Available for order | 2x 0.66 nmol/vial | 2x 1.32 nmol/vial | 1x 5.5 mL/bottle | 6 | S0140S |

The final concentrations of the reconstituted FAM and HEX calibrators are at 500 nM and 1000 nM, respectively.

### Storage Temperature

Store the lyophilized calibrators and the reconstitution buffer between 4 – 20 °C. The calibrators are light sensitive. Ensure that the vials are placed in a dark location. Storage temperature of the reconstituted calibrators should be at 4 °C and protected from light.

When stored at the recommended temperature and condition, the lyophilized calibrator and reconstitution buffer are stable for two years from the date of manufacture. The reconstituted calibrator is stable for one year when stored at 4 °C and protected from light.

Do not freeze the calibrator once reconstituted.

## Materials Required for Calibration

- FAM/HEX calibrator solution
- Distilled or deionized water
- 200 µL pipette
- 200 µL pipette filter tips
- Chai PCR Tube & Cap Strips, 8-Well Strips, Optically Clear, 100 µL
- PCR tube rack
- Mini centrifuge

## Reconstitution Procedures

Please note that the lyophilized calibrators may be difficult to see.

1. Before opening the calibrator tubes, use a mini centrifuge to spin down the lyophilized HEX and FAM vials for 10 seconds. It is important to gather all materials at the bottom of the tube.

2. Using a new tip, pipette 1.32 mL of the provided reconstitution buffer into each of the calibrator vials. Mix well by pipetting the solution up and down 10 times or by vortexing the vials for 10 seconds. Make sure to spin down the vials.

3. Incubate the vials at room temperature for at least 30 minutes. This is necessary to ensure that the product has completely dissolved. During the 30-minute incubation period, protect the calibrator vials from light by placing them in an opaque drawer or covering them with aluminum foil.

4. After the 30-minute incubation, open the vial caps and use a new tip to pipette up and down ten times or vortex for 10 seconds.

5. Spin down the vials using a mini centrifuge to collect all liquid contents at the bottom of the tube. The reconstituted calibration vials are now ready for use.

## Preparing for Calibration

The FAM and HEX solutions are light sensitive. Keep aliquoted solutions protected from light. If you are using calibrators that have previously been stored at 4 °C, you do not need to equilibrate the solution to room temperature before running the calibration process. You may use the calibrator solution directly from the fridge.

1. Obtain 6 new 8-well 100 µL PCR tube strips. Check that there are no cracks on the tubes.

2. Vortex both the FAM and HEX vials for 10 seconds or invert the vials 10 times to ensure homogenous mixing of the solution. Spin down the vials.

3. Aliquot 25 µL/tube of the FAM solution into 2 new PCR tube strips. Cap tubes tightly and spin down.
4. Aliquot 25 µL/tube of the HEX solution into 2 new PCR tube strips. Cap tubes tightly and spin down.
5. Prepare the water strips by aliquoting 25 µL/tube of water into 2 new PCR tube strips. Cap strip tubes tightly and spin down.

## Calibration Procedures

Please note that the calibration process must be completed in one sitting.

**The heat block and lid temperature rises above 95 °C. To avoid injury, do not make direct contact with the heat block, lid, or tubes immediately after a run. Let the surface cool for a few minutes before removing the tubes. If samples must be removed immediately, avoid contact with surfaces by extracting tubes with tweezers.**

**Always wear gloves and protective eye wear when handling reagents or operating the instrument.**

1. Log into your account through a web browser. On the software home screen, navigate to **Settings → Calibration**.

2. Under Optical Calibration, select **Run Now**.

3. The calibration time and materials required will be shown on the next screen (Figure 3.12). Select **Begin.**

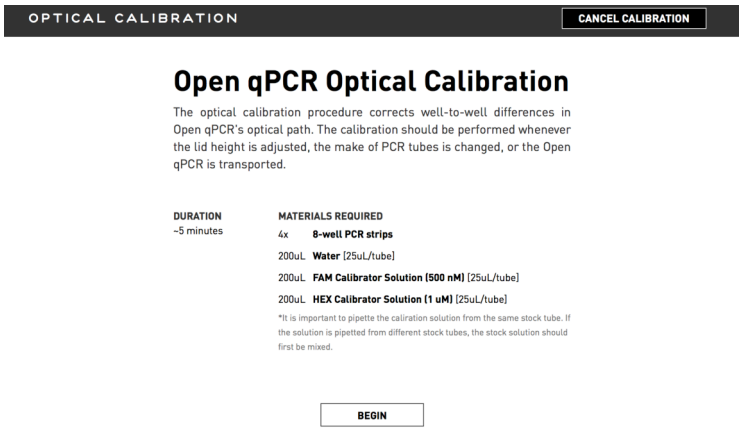



Figure 3.12

4. The screen will prompt you to prepare 6 new 8-well PCR tube strips (Figure 3.13). Gather the previously prepared strip solutions and select **Next.**

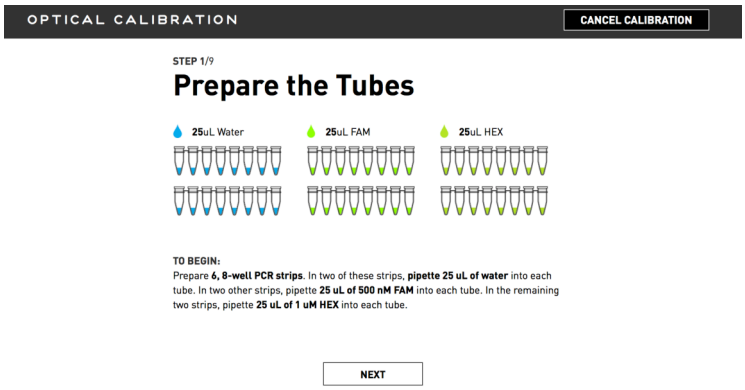



Figure 3.13

5. Insert two water strips into the instrument. Press down on the tubes gently once they are inserted. Adjust the lid height per the instructions in section **Adjusting the Open qPCR Lid Height**.

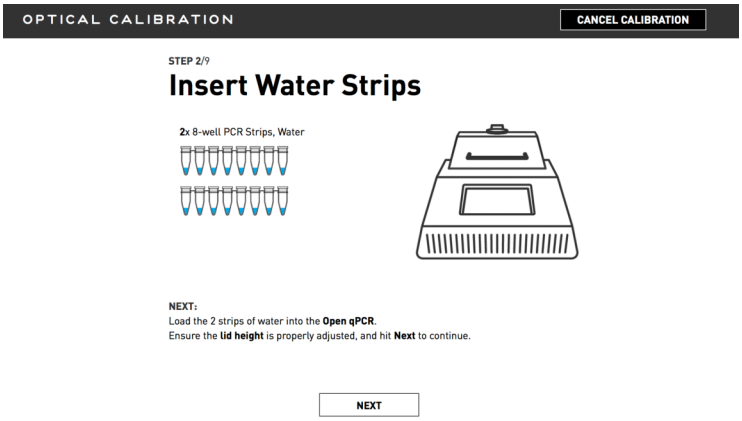



Figure 3.14

6. Close the lid, making sure that the latch clicks and select **Next**. You will see the screen below during the lid heating process.

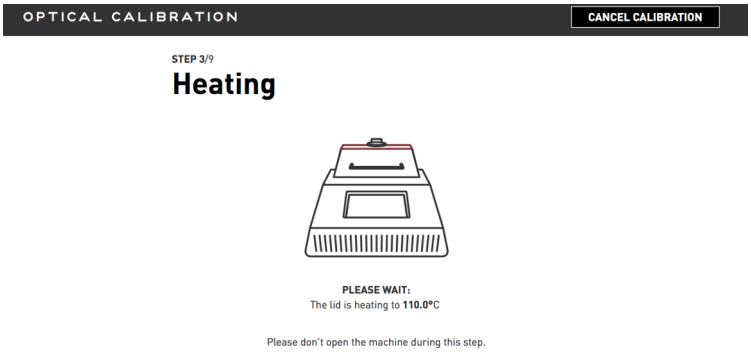



Figure 3.15

7. Allow the instrument to read fluorescence for 2-3 minutes before proceeding to the next step.

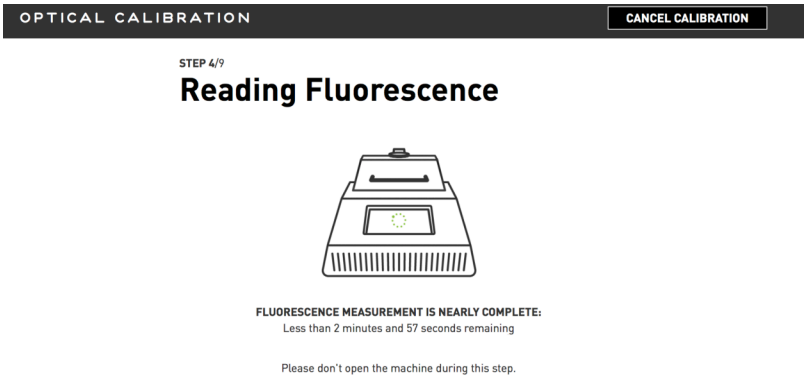



Figure 3.16

8. Once the reading completes, insert the FAM strips (Figure 3.17).

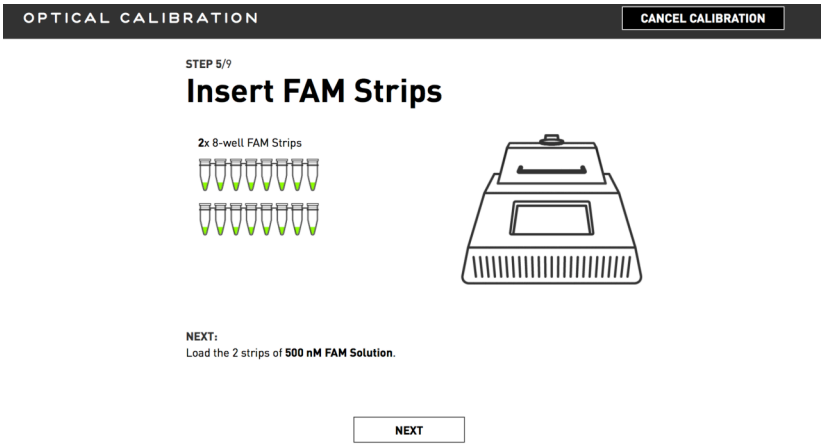



Figure 3.17

9. Repeat steps 6 - 7 for both the FAM and HEX strips.

10. Following the subsequent fluorescence reading, you will receive a status displaying **Calibration Complete** (Figure 3.18). You may now proceed to your experiment setup.

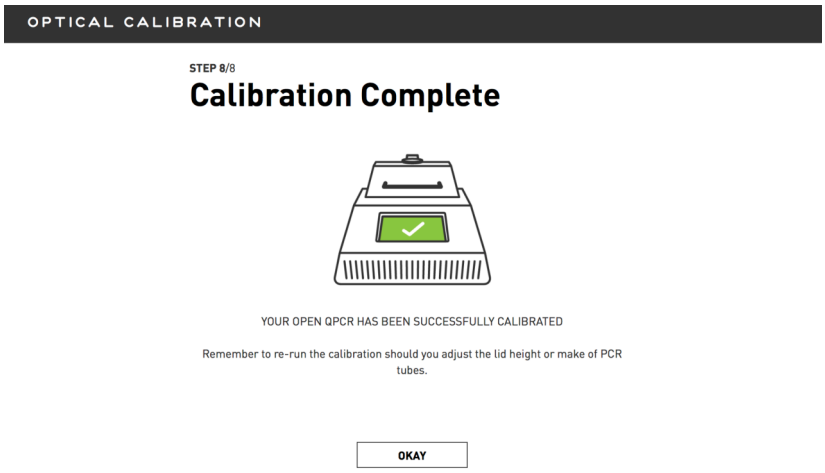



Figure 3.18

11. If calibration fails, you will see a screen displaying **Calibration Failed** (Figure 3.19). Repeat the calibration process in such instances. If failure persists on repeat, contact Technical Support.

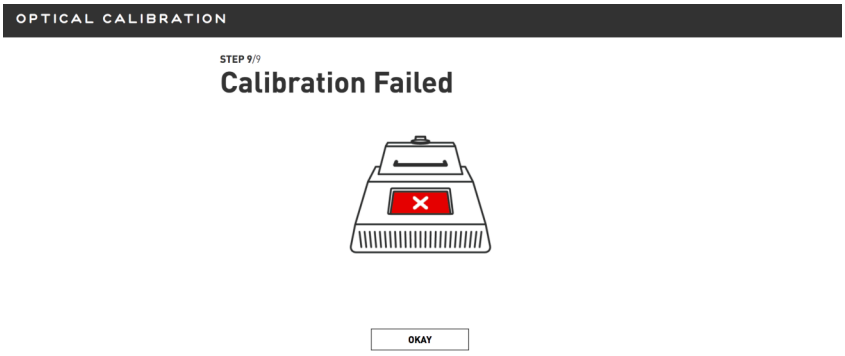



Figure 3.19

It is not recommended to reuse the calibration dyes because photo-bleaching occurs at different levels from well to well. If you would still like to reuse the dyes, pool the solution first and aliquot again as before.

# CHAPTER 4 – Assay Setup

## 4.1 General PCR Considerations

It is important to minimize sample contamination and PCR product carryover when setting up a new experiment. Please take note of the following precautions:

- Wear a clean lab coat, clean gloves, and eye protection when preparing samples for PCR amplification. Change gloves whenever you think there may be a contamination carryover.
- Ensure there are separate areas for PCR setup and PCR amplification. Avoid bringing amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Avoid splash-back of the PCR samples.
- Use aerosol resistant pipette tips and change tips after each use.
- Ensure that reaction and reagent components are always capped when not in use.
- Clean lab benches and equipment periodically with 70% ethanol or 10% bleach followed by 70% ethanol.

## 4.2 Create a New Experiment

To set up a new assay procedure, log into your account through the appropriate web address. On the home screen, click on the green icon titled **Create A New Experiment**. You will then see a box asking for the experiment name. Input the information and click on **Create Experiment**.

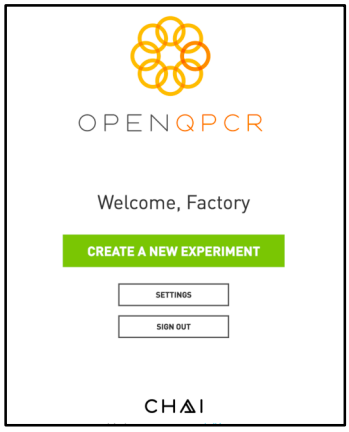



Figure 4.1


Figure 4.2

You will be brought to the assay setup screen. The protocol parameters must be defined on this page prior to running the experiment. The default cycling conditions are shown in Figure 4.3. In the default protocol, data is collected during the annealing step after completion of each cycling stage.

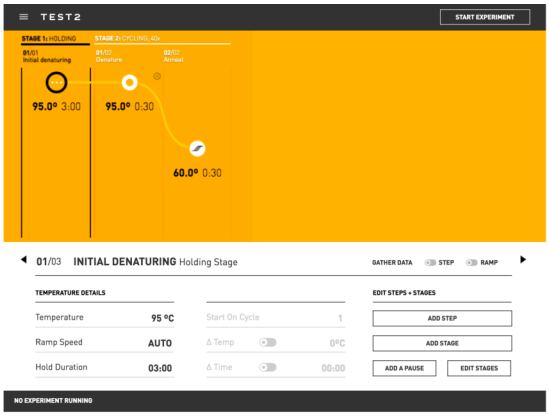



Figure 4.3

## TEMPERATURE DETAILS

Temperature details are displayed on the bottom left of the page. The **Temperature**, **Ramp Speed**, and **Hold Duration** can be manually adjusted by clicking directly on the values. Ramp speeds can be set between a range of 0.00001 and 5 °C/s. If no ramp speed is designated, the system defaults to *Auto*. *Auto* equates to the maximum ramp speed on the instrument.

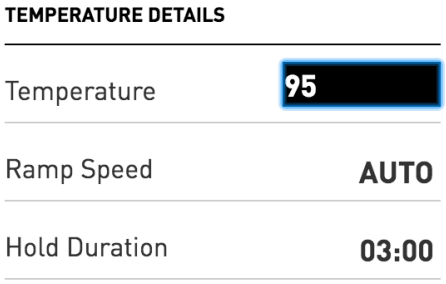



Figure 4.4

## INFINITE HOLD

This feature is available only during the last step with **Gather Data** turned off. To implement an infinite hold, set the **Hold Duration** to 00:00 and press *Enter* on your keyboard. The infinity symbol will appear next to the temperature (Figure 4.5).

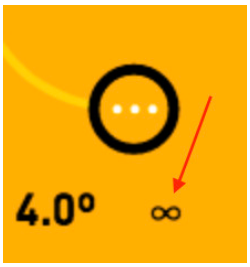



Figure 4.5

## ADD STEP

On the right side of the page, there is an option to **Add Step** (Figure 4.6). Designate the respective sections to add the additional step(s). To do so, click on the arrows at either side of the screen or click directly on the appropriate stage in the orange section; the editable step will be bordered in black (Figure 4.7).

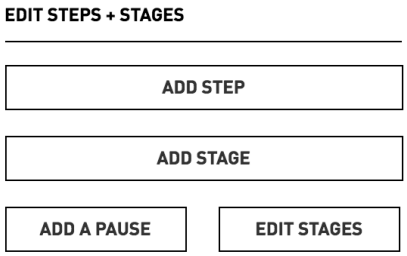



Figure 4.6

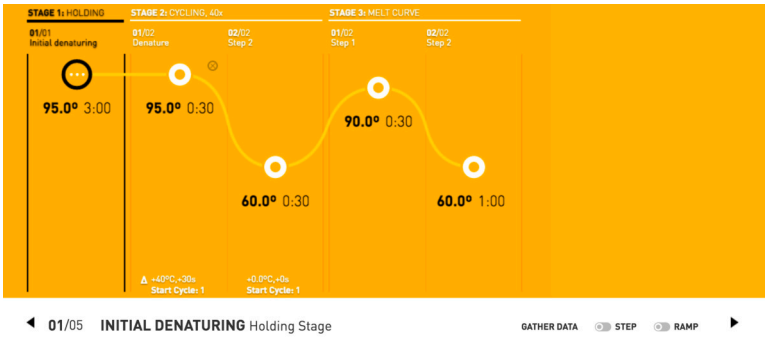



Figure 4.7

## ADD STAGE

Under the "Add Stage" icon, there are three different stage options to select. Each stage may consist of one or more steps.

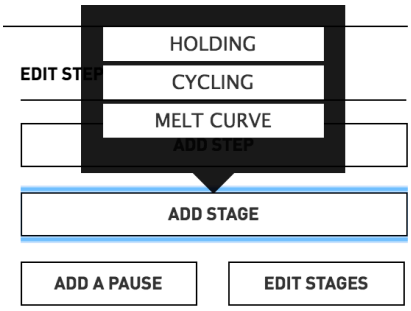



Figure 4.8

**Holding Stage**

The default protocol comes with a holding stage that contains the initial denaturation step. Additional steps may be added to a holding stage if needed. Generally, in both two-step and three-step PCR protocols, a holding stage exists at the very beginning since it houses the initial denaturation step.

In some cases, depending on whether you plan to run downstream applications such as a post-PCR agarose gel, an additional holding stage may be added at the very end of the amplification cycle for the final extension step.

**Cycling Stage**

The cycling stage is akin to a holding stage, except that it repeats for a specified number of times. Denaturation, annealing and extension occurs during this stage so the number of DNA copies doubles with each completed cycle.

**Melt Curve Stage**

A melt curve is typically performed immediately after the amplification protocol in one continuous sitting. The purpose is to determine specificity of the PCR reaction and to check whether nonspecific amplification products, such as primer dimers, were formed. Refer to Section 1.4 Melt Curve Analysis for more details.

## ADD PAUSE

You may also select **Add Pause** to a certain step. When a pause is implemented, the reaction will be held for an indefinite amount of time at a set temperature (figure 5.10). Once an experiment reaches the pause stage, the *In Progress* time will display *00:00:00.* Select the **Resume** icon on the bottom of the page layout's palette to continue the experiment (Figure 4.9).

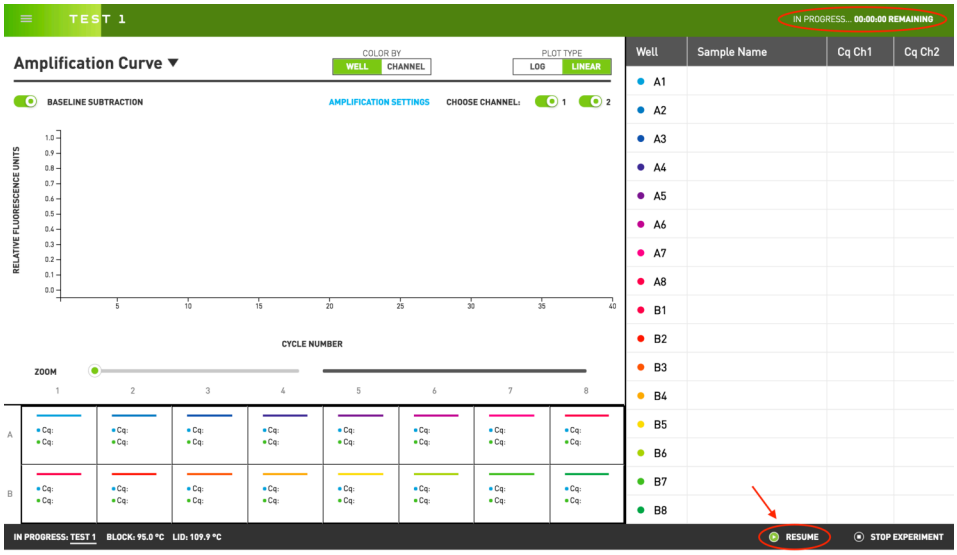



Figure 4.9

## AUTODELTA

The *Autodelta* option is used for Touchdown PCR (TD-PCR) and can be applied only to the cycling stages on the protocol setup page. Touchdown PCR can be incorporated as part of any standard PCR to increase the specificity, sensitivity and yield of the PCR reaction. It increases the likelihood that your target of interest is amplified in cases where there may be significant mis-priming. It is usually applied if your primers tend to bind non-specifically at temperatures close (but lower) to the annealing temperature of the correct positions.

TD-PCR involves a cycling program that gradually decreases the annealing temperature over the course of successive cycles until the calculated melting temperature ($T_m$) of the primers is reached. Typically, the initial annealing temperature is set approximately 10 °C higher than the expected Tm of the primers.

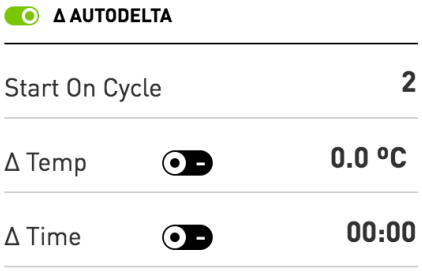



Figure 4.10

DEFINE CYCLE NUMBER

To set the number of cycles for a certain stage, click directly on the underlined default ***40x*** *Cycles* shown in Figure 4.11 and type in the value.

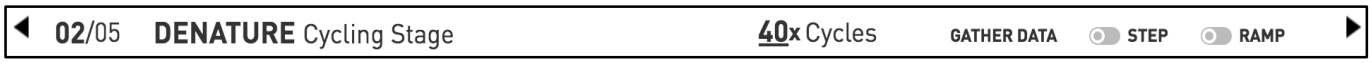



Figure 4.11

EDIT STAGES

To delete or reposition a step, click on **Edit Stages** located on the right-hand side. To delete a step, select the ⊗ icon. To reposition a step in the protocol, click and drag the dotted icon at the bottom of the orange section.

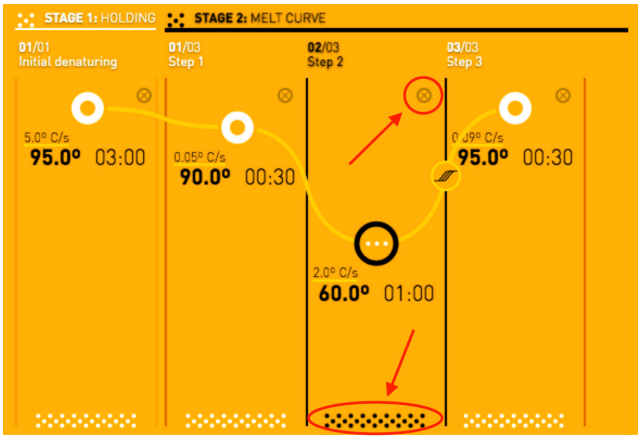



Figure 4.12

ENDPOINT REACTIONS

For end-point reactions with no fluorescence detection (conventional PCR), make sure to turn off **Gather Data** for Step and/or Ramp in all stages of your protocol. No amplification curves will be displayed on the results screen with endpoint reaction settings.


Figure 4.13

ESTIMATED TIME TO COMPLETE

To view the estimated time until test completion before starting a run, click anywhere on the right panel of the protocol. Once clicked, the **Est. Time to Complete** will be displayed on the page layout's palette on the bottom portion.

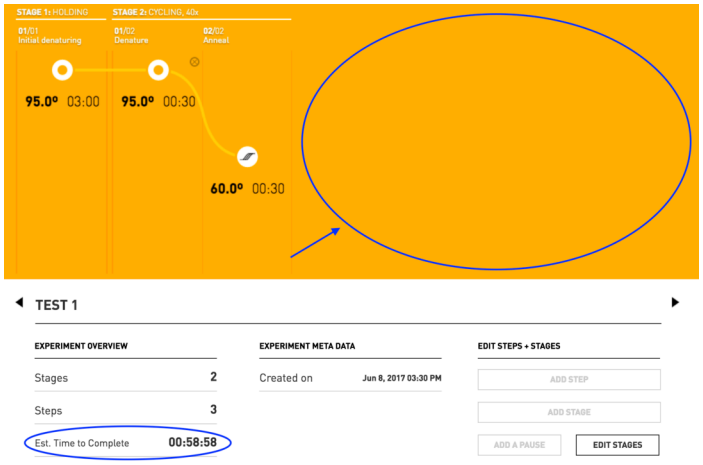



Figure 4.14

## 4.3 Starting & Cancelling Runs

1. When you are ready to begin the experiment, select the START EXPERIMENT icon on the top right corner of the screen. You will be prompted to "Confirm Start" before proceeding. As the PCR runs, the amplification curves will display in real time for your viewing.

2. The estimated time remaining on the experiment is shown on the top right corner. To abort the current run, click on the STOP EXPERIMENT icon on the bottom right corner.

3. While the experiment is running, you may access other screens without aborting the test. To do so, click on the icon located at the top left near the experiment name. You will have options to go back to the home page or view the experimental details.

   Under the **Experiments** column on the home screen, the current run will display as *In progress*.

---

**Never open the instrument lid or adjust the lid height while an experiment is running. The experiment will abort if the lid is opened. Adjustment of the lid height will lead to inaccurate result reporting. Recalibrate machine if lid height adjusted. Repeat the run if lid is opened.**

---

# CHAPTER 5 – Result Analysis

## 5.1 Amplification Curve

Depending on the protocol specifications, run results may be viewed as either an amplification curve, melt curve, or thermal profile. The default setting displays the amplification curve on the result screen (Figure 5.1). The plot may be viewed in either log or linear format.

During a run, amplification curves are generated and can be monitored in real-time. The curves are created when the fluorescence signal from each sample is plotted against the cycle number. The analyzed data is expressed as a numerical representation (Cq values) that is displayed on the right side of the page palette. These Cq values are inverse to the amount of target nucleic acid present in a given reaction. A low Cq value indicates high quantities of target nucleic acid whereas a high Cq value indicates minimal target quantities.


Figure 5.1

AMPLIFICATION SETTINGS

Assay parameters may be adjusted by selecting the Amplification Settings located at the top right of the graph (Figure 5.2).


Figure 5.2

Once selected, you will see the following screen with options to adjust for the Cq Calling Method, Parameters, and Baseline Cycles (Figure 5.3).

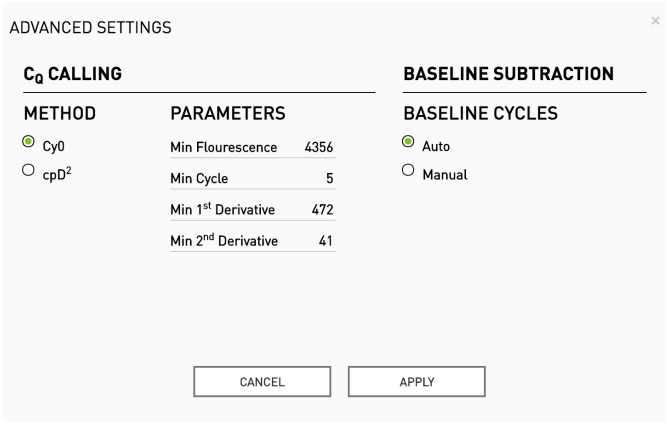



Figure 5.3

**Cq Calling:** The software defaults to the Cy0 method for Cq calling as it is recommended. You may choose to select cpD2 for data analysis as the characteristics of the data itself can affect how Cq methods perform.

**Parameters:** The parameters section is set to default at the values shown in Figure 5.3. You may customize as needed per each experiment.

*Min. Fluorescence* – The minimum fluorescence threshold for Cq calling. Cq values will not be called if fluorescence is below this threshold
*Min. Cycle* – The earliest cycle to use in Cq calling and baseline subtraction. Data for earlier cycles will be ignored

*Min. 1st Derivative* – The threshold which the first derivative of the curve must exceed for a Cq to be called

*Min. 2nd Derivative* – The threshold which the second derivative of the curve must exceed for a Cq to be called

**Baseline Cycles**: Baseline refers to the background signal level prior to any significant product amplification. The baseline should be wide enough to eliminate any background found in early cycles of amplification.

*Auto* – Allows the software to automatically detect the best baseline for each well

*Manual* – Allows for manual adjustment for baseline cycles as appropriate. The baseline will be the median fluorescence of the cycles you select.

BASELINE SUBTRACTION

The software's baseline subtraction aligns the baseline of each curve. Baseline subtraction is performed to reduce well-to-well variation so that there are similar levels of fluorescence during the initial cycling stages.

The algorithm performs baseline subtraction on background subtracted values. Background subtracted values are obtained after the algorithm adjusts for well-to-well variations and deconvolution (for dual channels only) on the amplification data.

Baseline subtraction is turned on by default. This can be turned off by toggling the Baseline Subtraction option on the top left of the amplification plot. Please note that Cq values are calculated from baseline-subtracted values. This means that the Cq values will remain the same regardless of whether baseline is turned on or off.

Figure 5.4 displays the results screen of a completed sample run in a dual channel cycler. To view the exact Cq and Relative Fluorescence Units (RFU) for a specific cycle, click on one of the curves (Figure 5.5).

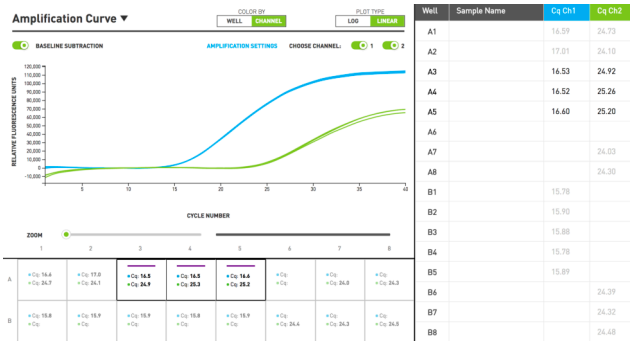



Figure 5.4

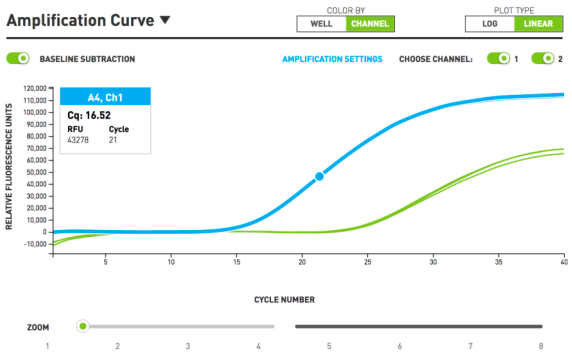



Figure 5.5

## SAMPLE NAME INPUT

Sample names may be entered in the software post-run for tracking purposes. Once the run has completed, select each box under the **Sample Name** column and type in the sample name as appropriate. The names will be automatically saved once entered into each row (Figure 5.6).

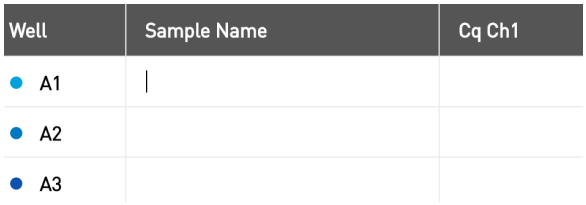



Figure 5.6

## GRAPH DISPLAY FUNCTIONS

**Select/Deselect samples**

Drag to select all adjacent wells simultaneously or select noncontiguous wells to view curves from specific samples. To view data from noncontiguous wells, click on each well while holding down the Command key (for Mac users) or the Control key (for PC users).

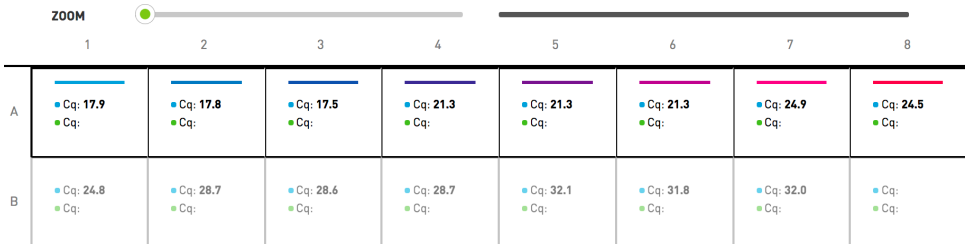



Figure 5.7

**Zoom**

To zoom in and out of the amplification plot, toggle the cursor over the "Zoom" bar.

DUAL CHANNEL & SINGLE CHANNEL DISPLAY DIFFERENCES

**Dual Channel Model**

For the dual channel Open qPCR, amplification curves may be viewed by well or channel. Channels may also be turned on or off by toggling between the options (Figure 5.8).


Figure 5.8

**Single Channel Model**

For the single channel Open qPCR, amplification curves may only be viewed in either Log or Linear format. There is no option for differentiating between channels since there is only one channel.

## 5.2 Melt Curve

MELT CURVE ANALYSIS

The Melt Curve feature may be used as a quality control for cycling analysis. The purpose of a melt curve analysis is to determine the peak dissociation temperature of the resulting amplicons. This aids in measuring an assay's analytical specificity, such as formation of non-specific products (primer-dimers). This information is useful for confirming product specificity and for assay optimization. Chemistries compatible with melt curve analysis include dual hybridization probes, molecular beacons, and intercalating dyes.

Melt curve analysis may also be used for genotyping purposes based on the differences in melt temperature between alleles.

There are two types of curves used in the melt analysis application: normalized and derivative.

**Normalized Plot**: This displays the various temperature increments on the x-axis and the normalized fluorescence signal intensity on the y-axis. Melt curve analysis function by incremental temperature changes over time, thereby resulting in DNA strand dissociation. Since the overall fluorescence intensity is directly proportional to the amount of double-stranded target DNA, strand dissociation results in decreased fluorescence intensity.

The inflection point seen in the sigmoidal shaped curve below equates to the melting temperature ($T_m$) of the amplified product.

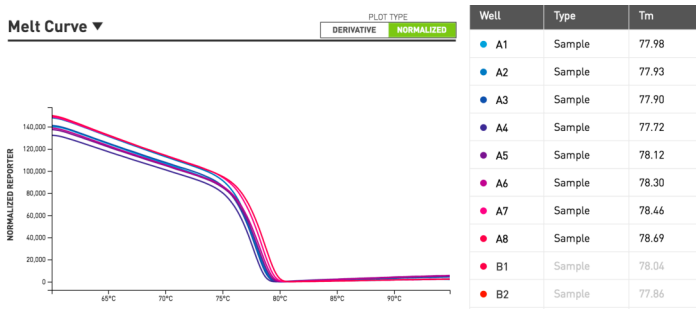



Figure 5.9

**Derivative Plot**: This is a plot of the negative first derivative of the normalized curve. Each peak is characteristic for a specific amplicon. The $T_m$ is easily identified here since it is the maximum point of the peak. The $T_m$ is defined as the temperature at which 50% of the product has "melted" from double-stranded DNA to single-stranded DNA.

In Figure 5.10 below, temperature is displayed on the x-axis and the negative first derivative of the normalized curve is displayed on the y-axis (-dF/dT).

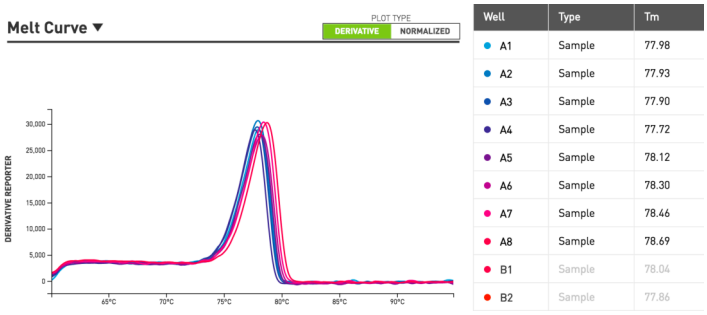



Figure 5.10

## 5.3 Thermal Profile

To access the thermal profile, click on the arrow next to **Amplification Curve** at the top left of the page for a specific experiment. Select Thermal Profile (Figure 5.11).

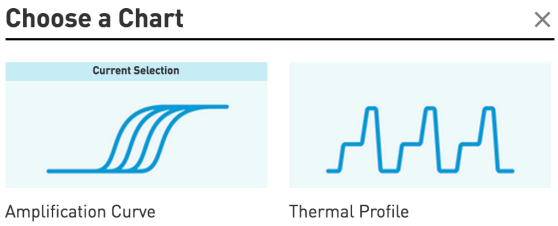



Figure 5.11

The thermal profile graph displays time on the x-axis and heat block temperature on the y-axis. You can manually hover over the graph to view the heat block and lid temperature at each time point.

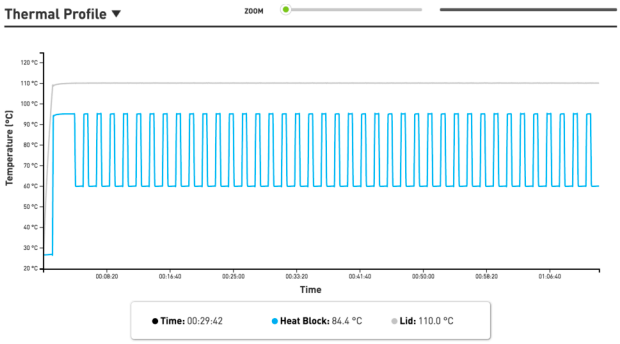



Figure 5.12

## 5.4 Navigation Icon on Results Screen

To navigate out of the results screen, select the icon at the top left of the page. You will then see options as shown in Figure 5.13.

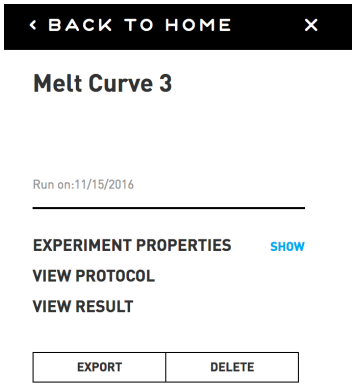



Figure 5.13

**Back to Home**: Returns to the home screen

**Experimental Properties**: Displays the lid temperature throughout the experiment

**View Protocol**: Displays the initial protocol setup page for the experiment

**View Result**: Displays the results screen

**Export**: Downloads the experiment file for further data analysis.

**Delete**: Permanently deletes the experiment run from the database

## 5.5 Data Export

Data can be exported via a .csv file. To export the run file, click on the  icon at the top left of the page. You will then see the following window on the left. Select **Export** to download the run file.

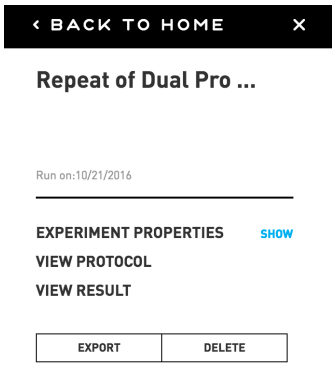



Figure 5.14

Amplification runs contain the following files: amplification, cq, and temperature_log.
To determine the absolute copy number from the starting material, use the cq.csv file to generate the standard curve.

- **amplification.csv** contains the baseline subtracted, background subtracted, and sample fluorescence values for each corresponding channel, well, and cycle number.
- **cq.csv** provides the Cq values for each channel and well in the instrument.
- **temperature_log.csv** contains data for the lid and heat block temperatures per cycling period.

Melt curve analysis contains the following files: melt_curve_analysis, melt_curve_data, and temperature_log

- **melt_curve_data.csv** contains the normalized and derivative values for each incremental temperature point
- **melt_curve_analysis.csv** contains the Tm(s) for each sample

- **temperature_log.csv** contains data for the lid and heat block temperatures per cycling period.

## 5.6 Changing an Experiment Name

To change the name of your experiment post-run, select the icon on the top left of the results screen. Proceed to click on the experiment name (Figure 5.15) to delete before typing in the new name.

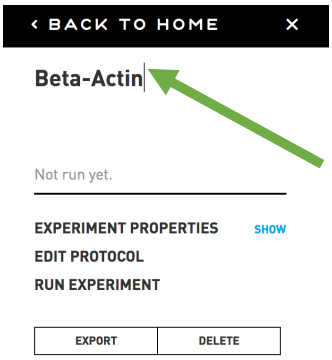



Figure 5.15

## 5.7 Deleting Experiments

To delete previous test runs, click on the **Edit** tab in the "Experiments" column on the home screen. Once you select **Edit**, you will see the icon next to each test. Click on it to delete the respective run. Select **Edit** to return to the original screen.

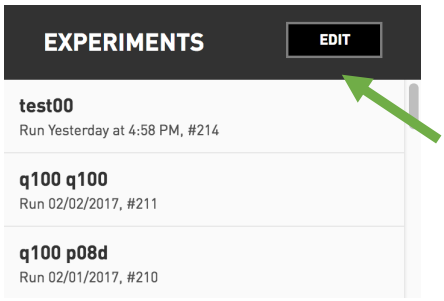



Figure 5.16

# CHAPTER 6 – System Settings

## 6.1 Manage Users

Once you are logged in, you can add additional users under the **Settings** tab on the home screen. Click on **Manage Users** and proceed to **Add User.**

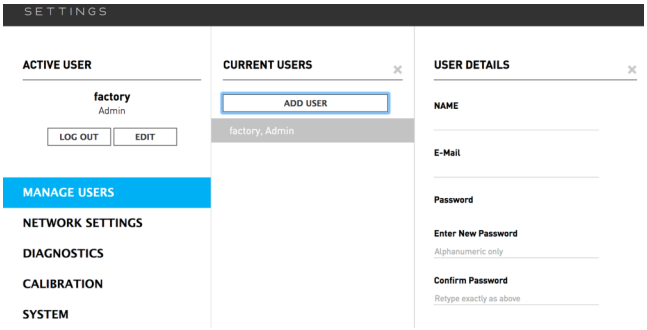



Figure 6.1

## 6.2 Software Update

It is highly recommended to keep your software updated to the latest version as this ensures optimal performance of your experimental runs. If your instrument is not on the most current version, the software will automatically prompt you to update once logged in. This prompt occurs only when the instrument is connected by Ethernet or Wi-Fi. The update process will take approximately 30 - 60 minutes.

**Ethernet or Wi-Fi**: If the Open qPCR is connected to the local network, the software update image will be displayed as follows (Figure 6.3).

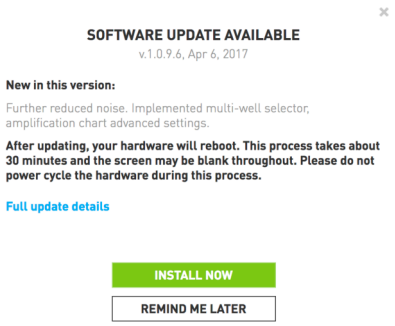



Figure 6.3

**USB**: If the Open qPCR is connected via USB, select **Download Image**. This will download as a zip file. Do not open the zip file once downloaded. Select **Browse & Install** to upload the zip file.


Figure 6.4

To update the software at a later time, access it through **Settings > System > Software Update Status.**

Either the instrument or the computer needs to be connected to the internet for the software to update. If you are unable to successfully update the software or do not have internet access via an instrument or computer, please contact Technical Support.

## 6.3 Network Settings

You may connect your Open qPCR instrument to any available network via Ethernet or Wi-Fi. Ensure that your computer is connected to the same network as the instrument. You have the option to adjust your Ethernet settings as necessary under the **Network Settings** tab.

Refer to **Section 2.2 Connectivity Options** for procedures on Ethernet and Wi-Fi setup of the device.

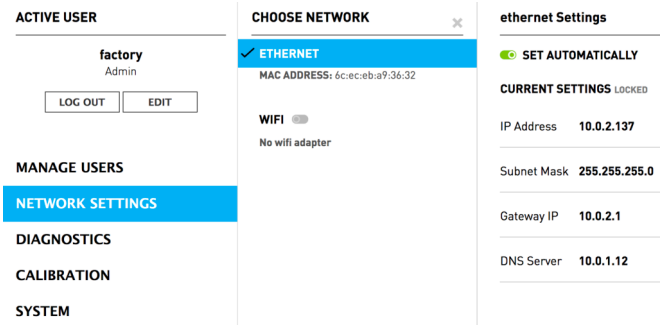



Figure 6.5

## 6.4 Diagnostics

The diagnostics section checks the thermal and optical integrity of the system. To perform either test, click on the respective **Run Now** tab. Instructions will be displayed on the screen once you begin. Run times for each diagnostic is approximately 5 minutes.

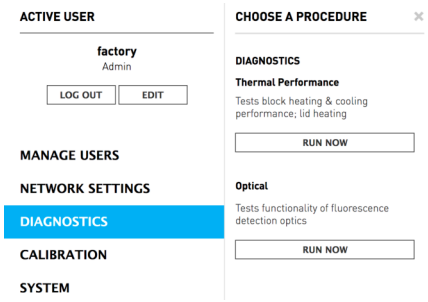



Figure 6.6

### Thermal Performance

The thermal performance diagnostic tests the lid heating as well as the heating and cooling efficiency on the processing block. Once completed, you will see the following screen (Figure 6.7). A pass is indicated by a green check mark for the specific tested component.

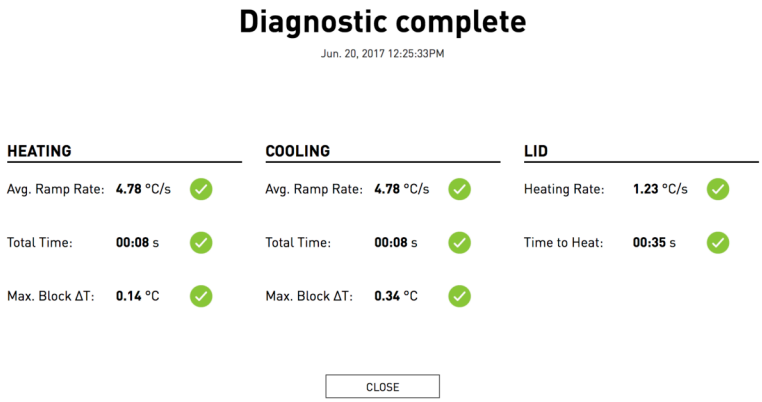
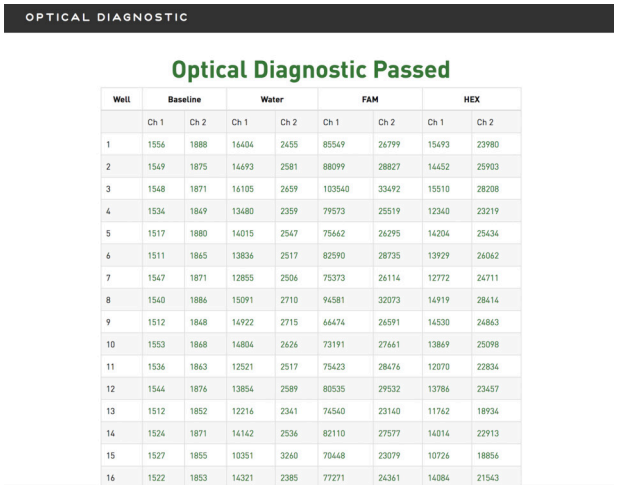



Figure 6.7

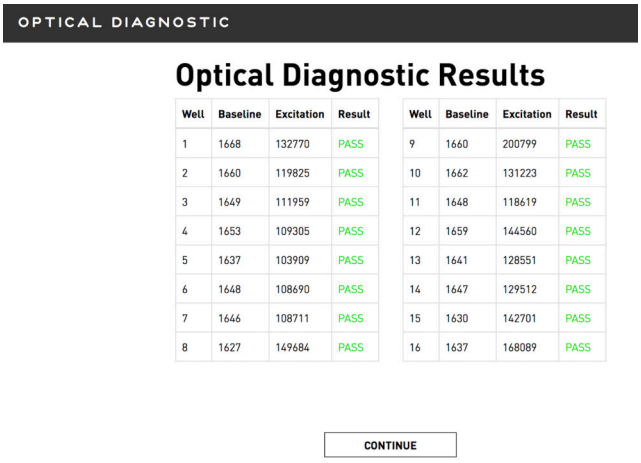
### Optical Diagnostic

The optical diagnostic checks for functionality of the fluorescence detection optics. The single channel model does not require any materials for the diagnostic performance. The dual channel model requires the same materials necessary to perform a calibration.




| Well | Baseline | | Water | | FAM | | HEX | |
|---|---|---|---|---|---|---|---|---|
| | Ch 1 | Ch 2 | Ch 1 | Ch 2 | Ch 1 | Ch 2 | Ch 1 | Ch 2 |
| 1 | 1556 | 1888 | 16404 | 2455 | 85549 | 26799 | 15493 | 23980 |
| 2 | 1549 | 1875 | 14693 | 2581 | 88099 | 28827 | 14452 | 25903 |
| 3 | 1548 | 1871 | 16105 | 2659 | 103540 | 33492 | 15510 | 28208 |
| 4 | 1534 | 1849 | 13480 | 2359 | 79573 | 25519 | 12340 | 23219 |
| 5 | 1517 | 1880 | 14015 | 2547 | 75662 | 26295 | 14204 | 25434 |
| 6 | 1511 | 1865 | 13836 | 2517 | 82590 | 28735 | 13929 | 26062 |
| 7 | 1547 | 1871 | 12855 | 2506 | 75373 | 26114 | 12772 | 24711 |
| 8 | 1540 | 1886 | 15091 | 2710 | 94581 | 32073 | 14919 | 28414 |
| 9 | 1512 | 1848 | 14922 | 2715 | 66474 | 26591 | 14530 | 24863 |
| 10 | 1553 | 1868 | 14804 | 2626 | 73191 | 27661 | 13869 | 25098 |
| 11 | 1536 | 1863 | 12521 | 2517 | 75423 | 28476 | 12070 | 22834 |
| 12 | 1544 | 1876 | 13854 | 2589 | 80535 | 29532 | 13786 | 23457 |
| 13 | 1512 | 1852 | 12216 | 2341 | 74540 | 23140 | 11762 | 18934 |
| 14 | 1524 | 1871 | 14142 | 2536 | 82110 | 27577 | 14014 | 22913 |
| 15 | 1527 | 1855 | 10351 | 3260 | 70448 | 23079 | 10726 | 18856 |
| 16 | 1522 | 1853 | 14321 | 2385 | 77271 | 24361 | 14084 | 21543 |

Figure 6.8 (Dual Channel)




| Well | Baseline | Excitation | Result |
|---|---|---|---|
| 1 | 1668 | 132770 | PASS |
| 2 | 1660 | 119825 | PASS |
| 3 | 1649 | 111959 | PASS |
| 4 | 1653 | 109305 | PASS |
| 5 | 1637 | 103909 | PASS |
| 6 | 1648 | 108690 | PASS |
| 7 | 1646 | 108711 | PASS |
| 8 | 1627 | 149684 | PASS |

| Well | Baseline | Excitation | Result |
|---|---|---|---|
| 9 | 1660 | 200799 | PASS |
| 10 | 1662 | 131223 | PASS |
| 11 | 1648 | 118619 | PASS |
| 12 | 1659 | 144560 | PASS |
| 13 | 1641 | 128551 | PASS |
| 14 | 1647 | 129512 | PASS |
| 15 | 1630 | 142701 | PASS |
| 16 | 1637 | 168089 | PASS |

Figure 6.9 (Single Channel)

# CHAPTER 7 – Maintenance

## 7.1 Cleaning and Disinfecting

**Biological Agents and Hazardous Chemicals**
For most biological agents, use a 10% bleach solution (dilute with water) followed immediately by a 70% ethanol solution (dilute with water) to disinfect the surface or the wells. Complete with a water cleanse.

**Bleach is corrosive to the Open qPCR body. It is important that you follow the 10% bleach solution immediately (no longer than 30 seconds) with a 70% ethanol solution.**

**Always power down the instrument and disconnect the power cord before performing cleaning or maintenance procedures.**

**Important Cleaning Procedure Notes:**

- Do not flood the instrument with the cleansing solution
- Do not spray the cleaning agent directly onto the instrument as this could damage internal components
- Spray the cleaning agent on low-lint cloth or paper towel and gently wipe down all surfaces
- To clean the wells of the PCR instrument, use a low-lint cotton swab dipped in 70% ethanol

Avoid using bleach if possible. This is corrosive and may damage the instrument. If bleach is necessary, dilute the stock 1:10 for a 10% working bleach solution. Follow the bleach cleanse immediately with a post-water wipe down. Do not let the bleach solution dry or sit on the instrument for more than 30 seconds.

**Do not allow ethanol, bleach, or water to seep into any openings on the machine as this will damage the optics and may contribute to adverse run performance.**

## 7.2 Open qPCR Return and Repair

If the instrument needs to be returned for service, it must be disinfected prior to shipment.

For direct customers of Chai, please contact Chai Technical Support for a copy of the Decontamination Certificate to initiate the return process.

For international customers, please contact your local supplier for the instrument repair process.

# CHAPTER 8 – Troubleshooting

## 8.1 Amplification Curves

| Amplification Curves | | | |
|---|---|---|---|
| | **Problem** | **Cause** | **Solution** |
| 1 | Inconsistent data where replicate samples do not display similar results | a. Low enzyme concentration | Increase enzyme concentration or use a different enzyme |
| | | b. Low target copy number below the LOD for the assay | Increase sample concentration |
| | | c. Pipetting errors | Repeat assay |
| 2 | No amplification | a. Lack of target in sample | Confirm by testing a positive control sample |
| | | b. Assay design failure | Try a different assay |
| | | c. Sample degradation | For RNA samples, denature via formamide and then run an agarose gel. For DNA samples, run an agarose gel or use a capillary electrophoresis system. A smeared gel reflects sample degradation. If there is a smear, prepare a fresh sample |

| Amplification Curves | | | |
|---|---|---|---|
| | **Problem** | **Cause** | **Solution** |
| 2 | No amplification | d. Instrument not calibrated | Calibrate the instrument |
| | | e. Dye detector assigned to incorrect channel | Ensure that the channel used to detect the fluorophore matches correctly |
| 3 | No real-time curve and no visible bands on gel | a. Master mix preparation error | Confirm master mix calculations |
| | | b. Protocol requires optimization | Test different annealing temperatures and times |
| | | c. Sample component inhibiting PCR | Dilute sample or change extraction procedure |
| | | d. Excess fluorescent dye inhibiting PCR | Decrease fluorescent dye concentration |
| | | e. Degraded PCR reagent | Rerun protocol with fresh reagents |

| Amplification Curves | | | |
|---|---|---|---|
| | **Problem** | **Cause** | **Solution** |
| 4 | Delayed Cq values | a. Decreased PCR efficiency | Design specificity: Confirm there are no mismatches between target and primer/probe sequences by performing a BLAST search to confirm specificity or target and assay sequences. Also confirm that the primer/probe does not span a SNP site |
| | | | Reaction setup: Confirm whether reaction efficiency has decreased over time by re-running a previously working assay. If not assay specific, confirm proper storage of master mix and/or template |
| | | | Instrument: Confirm that the cycling temperatures and time parameters are correctly set |
| | | b. Master mix differences | Test different master mixes with the same protocol, assay design, and reaction components. Confirm assay reproducibility |
| | | c. Sample component inhibiting PCR | Dilute sample or change extraction procedure |
| 5 | Cq values are very early (<15) | a. Template concentration too high | Dilute sample as necessary to obtain Cq values greater than 15 |

| Amplification Curves | | | |
|---|---|---|---|
| | **Problem** | **Cause** | **Solution** |
| 6 | Jagged curves | a. Very low fluorescence signal | Optimize fluorescent dye or probe concentrations |
| | | b. Air bubbles present or droplets adhered to tube walls | Spin down tubes for 10 seconds using a microcentrifuge |
| | | c. Incorrect tube type used | Use Chai's validated PCR-strip tubes. The instrument requires 100 µL optically clear, non-auto fluorescent tubes |
| 7 | Very low fluorescence signal | a. Low copy number of template | Increase concentration or add more volume of sample |
| | | b. High background signal | Decrease fluorescent dye or probe concentration. Check probe purity |
| | | c. Sample component inhibiting PCR | Dilute sample or change extraction procedure |
| | | d. Degraded PCR reagent | Rerun protocol with fresh reagents |

| Amplification Curves | | | |
|---|---|---|---|
| | **Problem** | **Cause** | **Solution** |
| 8 | Amplification in No Template Control (NTC) | a. Reagent or genomic contamination | Repeat protocol with fresh reagents |
| | | b. Primer-dimer formation | Use a master mix with good hot start activity |
| 9 | Looping Data Points during early cycles; noisy curve in early cycles | a. Baseline adjustment contains too many cycles | Reset baseline to 3 cycles before indication of amplification |
| | | b. Too much starting material | Use less starting material |
| 10 | Slope of curve is greater than or less than -3.34 and R^2 is less than 0.98 | a. Inaccurate dilutions | Recalculate standard concentration or gene copy number using a spectrophotometer; make new stock solutions of the control standards; eliminate extreme concentrations |
| | | b. Standard curve exceeds the linear range of detection | |
| 11 | Plateau has lower than expected fluorescence units | a. Limiting reagents | Confirm master mix calculations |
| | | b. Degraded reagents (i.e. master mix or dNTPs) | Repeat protocol with fresh reagents |

## 8.2 Melt Curves

| MELT CURVES | | | |
|---|---|---|---|
| | **Problem** | **Cause** | **Solution** |
| 1 | Large quantity of non-specific product amplified | a. Excess primer | Decrease primer concentration |
| | | b. Polymerase activity begins before thermal cycling starts | Use hot start polymerase and ensure that reaction tubes are kept chilled before placing into the thermal cycler |
| | | c. Primer design not optimal for the assay | Run the sequence through Primer-BLAST to check for homology |
| | | d. Excessive hold times | Reduce the number of hold times |

## 8.3 Factory Reset

A factory reset will restore your machine to factory condition. Remember to back up your data before performing a reset as this procedure will erase all data.

To perform the factory image reset:

1. Turn off your Open qPCR. Disconnect any USB cables.

2. Using a toothpick, press the reset button through the small hole in the rear of the machine. See attached photo for exact location. Press the button and continue to hold it down.


Figure 8.1

3. While holding down the reset button, power on the Open qPCR. Continue to hold the reset button down for 30 seconds.

4. Release the reset button. Keep the machine powered on for about 30 minutes. After 30 minutes, the device will reboot, and the Open qPCR logo will be displayed on the LCD.

5. The device is now configured in the manner it was when you first received it. You may now reconnect USB cables, and create a new account. Additionally, you should update your software to the latest version, as it will be downgraded by the factory reset procedure.

# APPENDIX A – Glossary

**Amplicon** – A DNA segment that is amplified during PCR

**Amplification Plot** – Displays a plot of the amplification cycles (X axis) versus fluorescence units (Y axis)

**Assay** – Pertaining to the Open qPCR platform, this is a test that contains a PCR master mix and specific primers/probes to amplify a target sequence

**Background Fluorescence** – This represents the baseline of a real-time PCR amplification curve. This is the fluorescence emitted from free dye, unbound probe, non-specific cleavage of probe or sample auto-fluorescence

**Baseline** – Defined as the PCR cycles in which a reporter fluorescent signal accumulates but is beneath the instrument's limit of detection (LOD)

**Cq** – This is the quantification cycle used to calculate starting quantity of the DNA template. A low Cq value equates to a high quantity of the target sequence in the sample. A high Cq value equates to a low quantity of the target sequence in the sample.

**Cycling Stage** – Also termed as the amplification stage, this refers to a stage that is repeated in the thermal profile setup.

**FAM** – A carboxyfluorescein fluorescent dye with $\lambda_{max}$(absorption) at 494 nm and $\lambda_{max}$(emission) at 518 nm

**HEX** – A hexachlorofluorescein fluorescent dye with $\lambda_{max}$(absorption) at 535 nm and $\lambda_{max}$(emission) at 556 nm

**JOE** – A dechlorinated, dimethoxylated version of the fluorescent dye fluorescein with $\lambda_{max}$(absorption) at 520 nm and $\lambda_{max}$(emission) at 548 nm

**Melt Curve** – A plot of data that is collected during the melt curve stage. The peaks generated from a melt curve are indicative of the target melting temperature ($T_m$) and can identify nonspecific PCR amplification

**Primer/probe mix** - A component of the PCR reaction containing primers designed for target amplification and a specific probe designed to detect target amplification

**Ramp speed** – This pertains to the speed at which the block temperature changes between steps during a PCR run

**Singleplex PCR** – This type of PCR contains a single primer set in the tube/well where only target or endogenous control can be amplified at a time.

**VIC** – A fluorescent dye with $\lambda_{max}$(absorption) at 538 nm and $\lambda_{max}$(emission) at 554 nm

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