illumina®

Eco™ Real-Time PCR System User Guide

FOR RESEARCH USE ONLY

ILLUMINA PROPRIETARY
Catalog # EC-900-1001
Part # 15017157 Rev B
Current as of August 2010

INTENDED USE: The Eco Real-Time PCR System is intended to support the Real-Time polymerase chain reaction (PCR) application needs of life science researchers. This includes gene expression quantification and analysis as well as genotyping by allelic discrimination or high-resolution melting. The system is able to support other applications and protocols as well. Eco features high-quality optical and thermal modules to provide optimal performance and data quality. The system includes data analysis software that is preloaded on a netbook computer and provided on a separate USB drive for installation on additional computers as needed. Additional accessories and consumables are provided or available for purchase to ensure the best user experience.

Use of the Eco for specific intended uses, such as polymerase chain reaction (PCR), Real-Time qPCR, or high-resolution melting (HRM) may require the user to obtain rights from third parties. It is solely the user's responsibility to obtain all rights necessary for the intended use of Eco.

The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product(s).

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY.

ILLUMINA DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE) OR ANY USE OF SUCH PRODUCT(S) OUTSIDE THE SCOPE OF THE EXPRESS WRITTEN LICENSES OR PERMISSIONS GRANTED BY ILLUMINA IN CONNECTION WITH CUSTOMER'S ACQUISITION OF SUCH PRODUCT(S).

FOR RESEARCH USE ONLY

Table of Contents

Table of Contents

Part # 15017157 Rev B

Overview

Chapter 1

Introduction

The Eco Real-Time PCR System offers life science researchers a full-featured real-time PCR system at an attractive price. Its features include:

- Four-color multiplexing
- High Resolution Melt (HRM)
- Fast PCR cycling: 40-cycle PCR in 40 minutes
- User-friendly, MIQE-compliant software

Eco's proprietary technologies provide excellent optical performance along with unmatched temperature control and thermal uniformity for a plate-based format (± 0.1°C).

Its robust optical system contains two sets of 48 LEDs for a broad range of fluorophores, along with four emission filters and a CCD camera for detection, enabling multiplexing of up to four targets. It is factory-calibrated for SYBR Green I dye, FAM, HEX, VIC, ROX, and Cy5.

Eco supports multiple applications, including gene expression quantification and analysis, and genotyping by allele discrimination or high-resolution melt (HRM). The system includes easy-to-use data analysis software preloaded on a netbook computer along with other accessories and consumables to provide the best user experience. The software is also provided on a USB drive so that it can be installed on additional computers for convenient access.

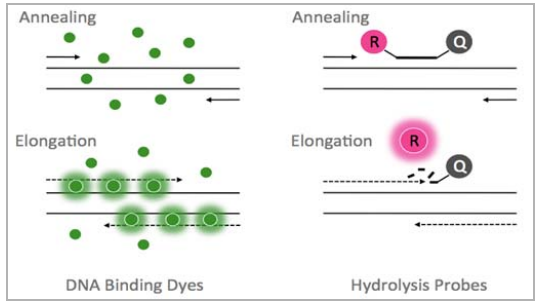
To order Eco materials and accessories, go to **https://icom.illumina.com**. If you do not have an account yet, click **Create New User**.

Go to **http://www.illumina.com/ecoqpcr** for Eco resources, including tutorials, customer stories, and information about the many possible applications of Eco technology.

Real-Time PCR

Polymerase Chain Reaction (PCR) denotes the amplification of DNA templates catalyzed by DNA polymerase in the presence of primers, dNTPs, divalent cations (like Mg+2), and a buffer solution.

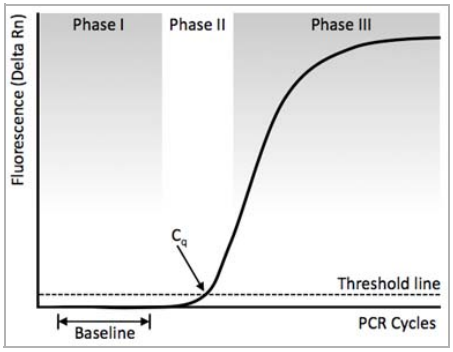
Figure 1 Main Real-Time PCR Chemistries



The ability to visualize and quantify the amplification of DNA as it occurs during PCR is called Real-Time qPCR or Quantitative PCR. This is made possible by the use of fluorescent chemistries, an optical system that can capture the emitted fluorescence at every PCR cycle, and software that can quantify the amplification.

The two most commonly used qPCR chemistries are DNA binding dyes and hydrolysis probes (Figure 1). DNA binding dyes fluoresce when bound to double-stranded DNA. Hydrolysis probes fluoresce when the reporter molecule is removed from its quencher molecule by the 5′ endonuclease activity of DNA polymerase.

Figure 2 The Three Phases of qPCR



Little fluorescence is generated during initial PCR cycles (Figure 2). Data from these early cycles define the baseline for the assay (Phase I). As fluorescence approaches the level of optical detection, the reaction reaches the exponential phase (Phase II), which is the region where the Cq is determined. Cq is the PCR cycle at which the fluorescent signal crosses the detection threshold level and is used for quantification. Finally, as reaction components are consumed and amplicons become abundant, the generation of additional fluorescent signal slows down and eventually reaches a reaction plateau (Phase III).

Resources

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Science 230: 1350–1354

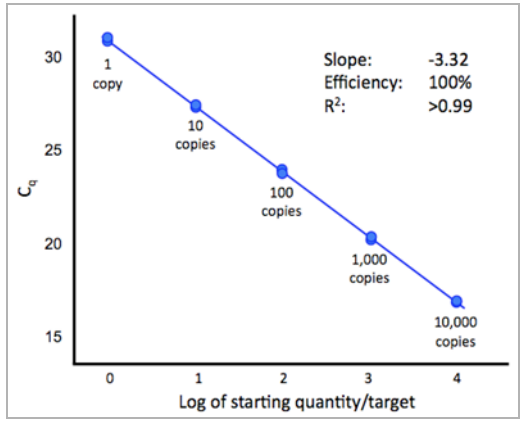
Higuchi R, Fockler G, Dollinger G, and Watson R (1993) Biotechnology (N.Y.) 11: 1026–1030

Absolute and Relative Quantification

The two primary methods used to quantify nucleic acids by qPCR are the absolute and relative quantification methods.

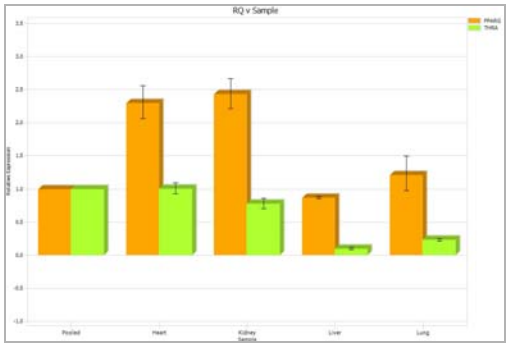
The absolute quantification method is based on a standard curve generated from serial dilution of a DNA template of known concentration (Figure 3). Quantification of unknown samples is determined by interpolating the sample Cq from the standard curve.

Figure 3 Five-Point (10-Fold) Standard Curve



A standard curve is useful for assay validation. The slope of the standard curve measures the efficiency of the assay (E = 10-1/slope). A slope outside the acceptable range (slope -3.1 to -3.6 and E value between 90 and 110%) typically indicates a problem with the template or assay design. The R^2 value, a measure of assay performance, should be > 0.99 for the assay to accurately quantify unknown samples.

Figure 4 Relative Quantification Experiment



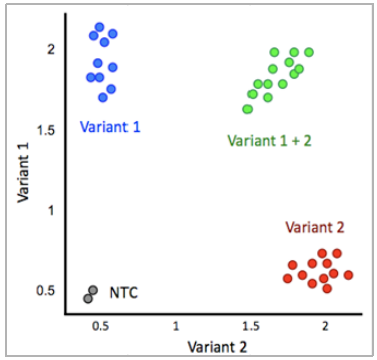
The relative quantification method measures the level of gene expression in a sample relative to level of expression of the same gene in a reference sample. In addition, the level of expression of every gene in the assay is normalized to the expression of a reference gene. As a best practice, use multiple reference genes when quantifying gene expression.

The results obtained are expressed as relative levels in gene expression compared to the reference sample (R) (Figure 4).

Allelic Discrimination and High Resolution Melt

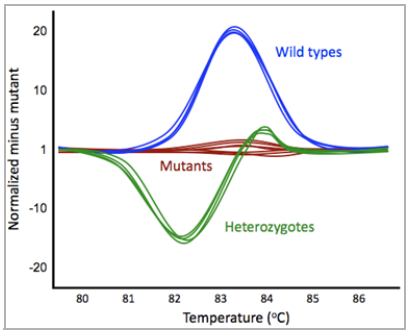
Allelic discrimination assays using hydrolysis probes provide a rapid and sensitive method to genotype samples. Two variants/alleles are interrogated at the same time (multiplex qPCR). One probe is typically labeled with a FAM dye and the other with a VIC dye. Samples with FAM signal and no VIC signal are homozygous for variant 1; samples with VIC signal and no FAM signal are homozygous for variant 2; and samples with both FAM and VIC signal are heterozygous (Figure 5).

Figure 5 Allelic Discrimination Scatter Plot



High Resolution Melt (HRM) enables the detection of almost any genetic variation (SNPs, mutations, and methylation patterns). Because HRM assays only require primers and a dye (no probes or DNA sequencing), the method is simpler and cheaper than traditional approaches. After the amplification phase, the amplicon is slowly heated until it melts. The melting temperature and profile are directly linked to the amplicon sequence.

Figure 6 HRM Difference Plot



HRM's power comes from the resolution of the sample's melt profile. It requires a high quality optical system and precise thermal uniformity. HRM PCR amplicons below 300 bp provide the best resolution. The shape of the resulting melting curves, which is sensitive to almost any genetic change, determines sample identity. To easily cluster equivalent samples, a reference curve (e.g. mutant) is subtracted from the other curves to generate a difference plot (Figure 6).

Resources

Livak KJ (1999) Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal Biomol Eng 14: 143–149.

POLAND server (http://www.biophys.uni-duesseldorf.de/local/POLAND/poland.html)

Wojdacz TK, Dobrovic A, Hansen LL (2008) Methylation-sensitive high-resolution melting. Nature Protocols 3(12): 1903–1908.

Multiplexing Real-Time PCR

The simultaneous detection of multiple targets in a single reaction is called multiplexing. An advantage of multiplexing is that it permits the inclusion of an internal control assay for normalization purposes, significantly increasing data accuracy. Another advantage is that multiplexing conserves sample, allowing more data to be obtained from the same amount of material.

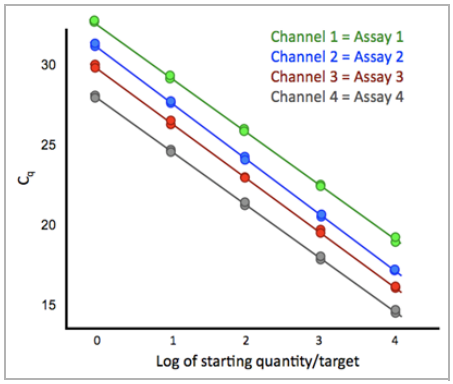
Validating a multiplex qPCR assay can be challenging. The advent of more advanced qPCR master mixes has significantly reduced the amount of optimization typically required, making multiplex qPCR a much more attractive alternative. Validation of assays using a standard curve is a must to ensure data accuracy.

Figure 7 Examples of Eco-Compatible Dyes

Channel 1 (λ = 505–545 nm)
SYBR Green[a], FAM[1]
Channel 2 (λ = 604–644 nm)
ROX[1], Texas Red, TAMRA
Channel 3 (λ = 562–596 nm)
HEX[1], JOE, TET, VIC[1]
Channel 4 (λ = 665–705 nm)
Cy5[1], Quasar 670

a. Factory-Calibrated Dyes

Figure 8 Standard Curves for Four Multiplexed Assays



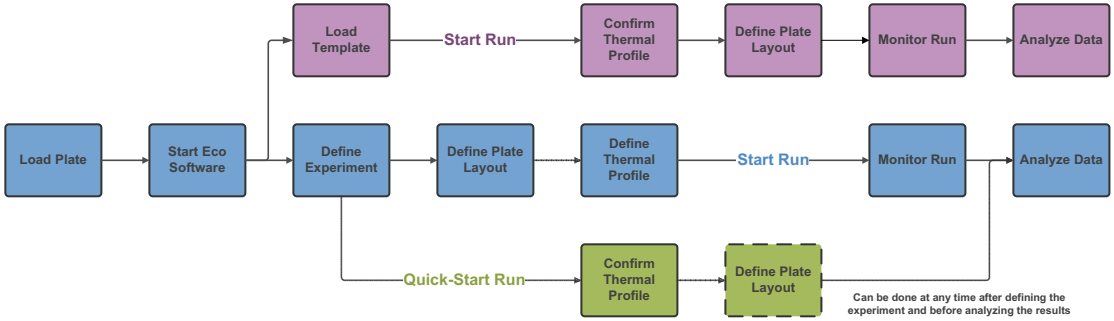
The Eco Real-Time PCR System includes four filter channels (Figure 7), which enable detection of up to four separate targets in a single reaction (Figure 8).

Eco is factory-calibrated for certain dyes within each channel (marked in Figure 7), but also supports additional dyes that are excited and detected within the instrument specifications.

Workflow

Chapter 2

Eco System Workflow



1 Prepare the sample plate, load it into the Eco, and close the lid (page 9).

2 Launch the Eco software on the netbook PC.

3 Define the experiment by selecting the application, detection chemistry, and starting material (page 10).

TIP

If you wish, click **Start Run** to begin the quick-start run now. You will be prompted to confirm the thermal profile and name the run. Set up the plate layout during the run or any time before analyzing the results.

4 Set up the plate layout by defining assays, samples, and standards and assigning them to wells (page 12).

5 Review the thermal profile and adapt it if needed (page 18).

6 Start the run. The software has a tab for monitoring the run in real time (page 20).

CAUTION

Do not open the lid while a run is in progress. This allows extraneous light into the system and can corrupt the data.

7 When the run is complete, open the Eco lid. Press the plate release lever and remove the plate from the block. Dispose of any hazardous materials in biohazard, caustic material, or other appropriate containers, according to your local safety regulations.

Load the Plate

1. Thaw all necessary reagents (templates, primers, probes, and master mix).
2. Turn on Eco and the netbook PC, and wait until the Eco **Ready** light is solid blue.
3. Confirm that the block and optical path are clear of visible contaminants and there is no physical damage to the system, such as dents, frayed cords, or damaged levers.
4. Place a 48-well plate into the Eco sample loading dock, aligning the notch with the matching indentation on the adapter.
5. Turn on the dock light and incline the dock to a comfortable angle for pipetting.
6. Pipette samples and qPCR reagents into the plate according to your protocol.

WARNING

Wear protective gloves and eyewear when handling any material that might be considered caustic or hazardous.

7. Seal the plate with an Eco optical seal. Holding the plate in place on the Eco sample loading dock, drag the squeegee firmly across the surface to ensure the seal is secure.
8. Place the plate adapter with your loaded and sealed plate in the centrifuge carrier along with the second supplied plate adapter for balance. Centrifuge the plate at 250 g for 30 seconds. Do not spin more than 500 g. Verify that there are no air bubbles at the bottom of the wells.
9. Open the Eco lid and place the plate on the block, aligning the notch against the top-left corner.

WARNING

Forcing the plate into any other orientation could damage the instrument.

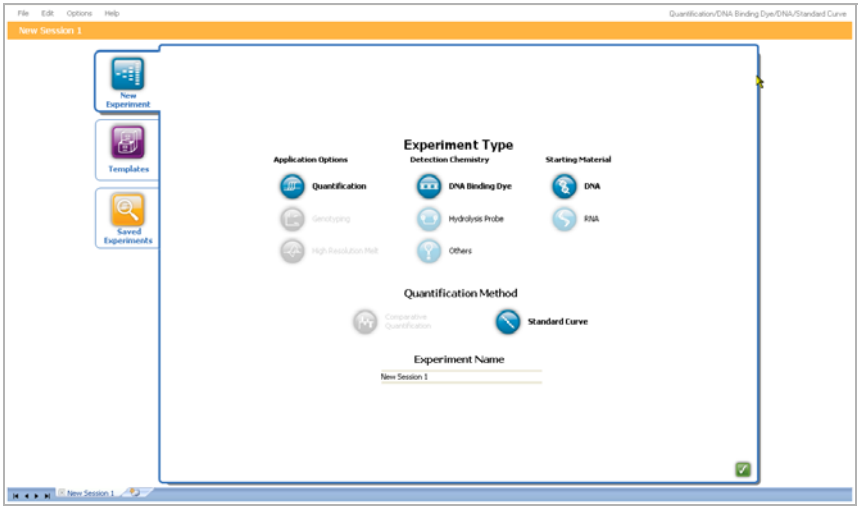
WARNING

Be careful not to touch the thermal bonnet above the plate. It heats to 105°C (221°F) when the instrument is turned on and could result in burns.

10. Close the Eco lid. The heated cover automatically creates a seal around and on top of the plate to prevent evaporation. Proceed to define a new experiment.

Define a New Experiment

Figure 9 New Experiment Tab



1 Double-click the Eco icon on the desktop to open the software. The New Experiment tab opens.

> TIP
> If you wish, you can re-open recent experiments or example runs from the Saved Experiments tab on this screen.

2 Select an **Application Option**, **Detection Chemistry**, and **Starting Material**.
When you select the application, the software automatically configures options for downstream setup and analysis. For example, High Resolution Melt (HRM) is associated with DNA Binding dyes.

3 If this will be a quantification experiment, select **Comparative Quantification** or **Standard Curve**.

4 Enter an experiment name of up to 20 characters.

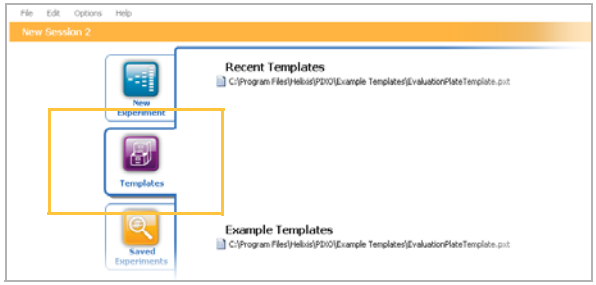
5 [Optional] If you want to save the experiment as a template, select **File** > **Save As** and save in the *.ecot file format.

6 Click . The Setup window opens, with the Plate Layout tab visible.

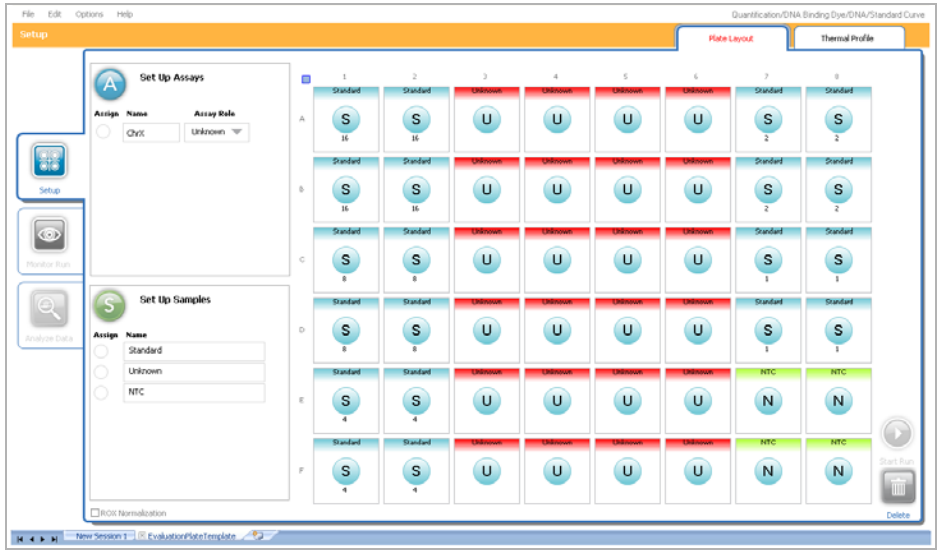
Load a Template (Optional)

After designing an experiment, you can save the experiment as a template using the **File** | **Save As** menu option. The template retains the experiment setup, plate layout, and thermal profile.

1 Start a new session in the Eco software and click the **Templates** tab. Recent and saved templates appear.



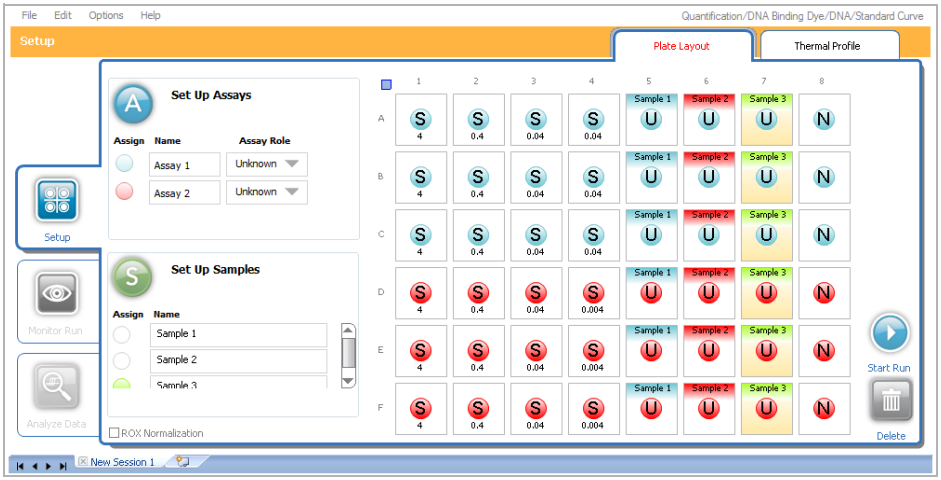
2 Click a template to select it. The **Plate Layout** tab opens.



3 If you wish, you can change the assay and sample designations on the plate, or adjust the thermal profile. You cannot redefine the application, detection chemistry, starting material, or quantification method.

Set Up the Plate Layout

Figure 10 Plate Layout Tab



The Plate Layout tab lets you assign assays and samples to wells. An image of an actual plate layout appears at the right. Plate layout involves four steps:

1 Set up assays (page 13)

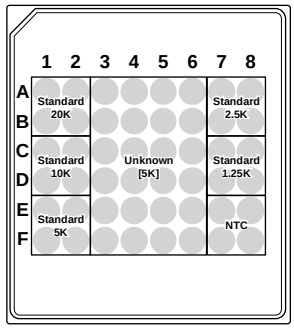
2 Set up samples (page 14)

3 Assign assays and samples to wells (page 15)

4 Define standards (page 16)

You can lay out the plate any time between defining the experiment and analyzing the data. The Analyze Data window (page 20) will not become active at the end of the run until the plate layout is complete.

Figure 11 Image of Eco Evaluation Plate



Set Up Assays

An assay is the set of primers or primers/probe used to quantify a gene. Assays can have different roles, such as Unknown, Standard, Negative, Positive, or NTC (Non-Template Controls).

Each assay is associated with a reporter dye which identifies the assay during analysis. Reporter dyes can belong to one of four "channels," each of which is defined by a specific excitation and emission range.

You can assign up to four assays per well. Within each well, assays cannot use reporter dyes from the same channel (see following table). If they did, the data from the assays would be indistinguishable during analysis. A red outline around a well indicates that it contains more than one reporter dye from the same channel and requires correction before you can start the run.

Channel	Excitation (nm)	Emission (nm)	Fluorophores Calibrated on the Eco (Reporter)
1	452–486	505–545	SYBR Green I, FAM
2	542–582	604–644	ROX[a]
3	452–486	562–596	HEX, VIC
4	542–582	665–705	Cy5

a. If you use ROX as a passive reference for normalization, you must use the other three channels for multiplexing.

1 On the Plate Layout tab, click Assays to open the Assays dialog box.

2 Select the number of assays. For each one:

- [Optional] Define a name and color.
- Select a **Reporter** dye, thereby setting the channel. If your dye is not listed, select a reporter with the same excitation and emission range as your dye (see the Channel table above).
- Select a **Quencher**. Quencher molecules absorb fluorescent emissions of reporter dyes when in close proximity.

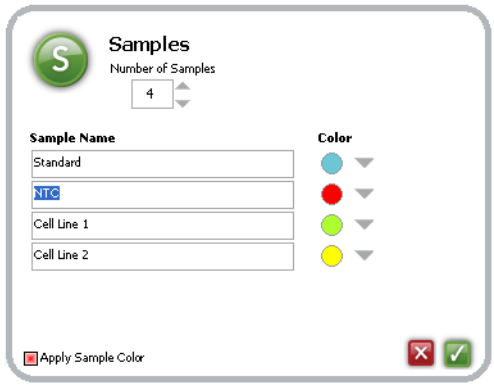
By default, the quencher is set to **None** for DNA binding dye chemistry and **Non-fluorescent** for Hydrolysis probes. Note that BHQ and MGB are considered non-fluorescent quenchers.

3 Click to close the Assays dialog box and return to Plate Layout. [Optional] Proceed to set up samples.

Set Up Samples

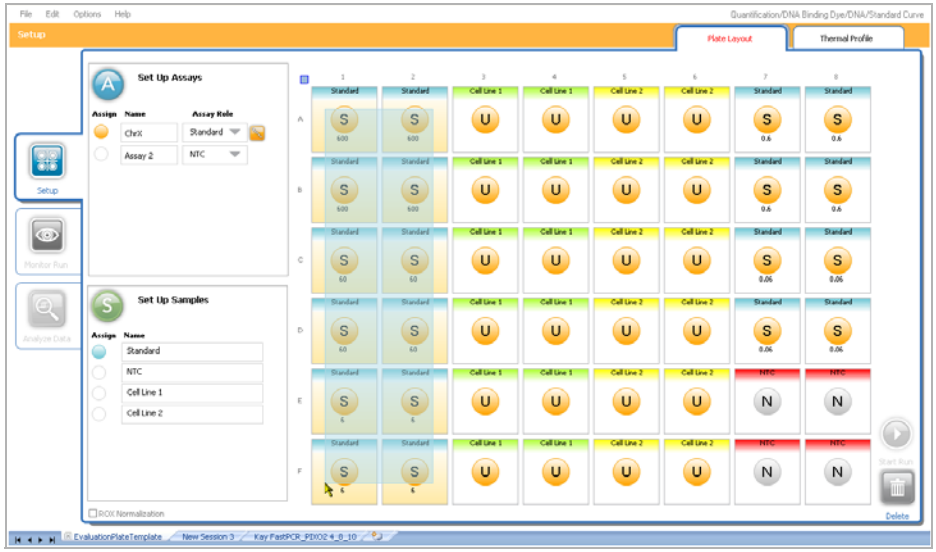
1. On the Plate Layout tab, click Samples to open the Samples dialog box.
2. Select the number of samples. Define a name and color for each sample.
3. Click to close the Samples dialog box and return to Plate Layout. Proceed to assign assays and samples to wells.

Figure 12 Samples Dialog Box

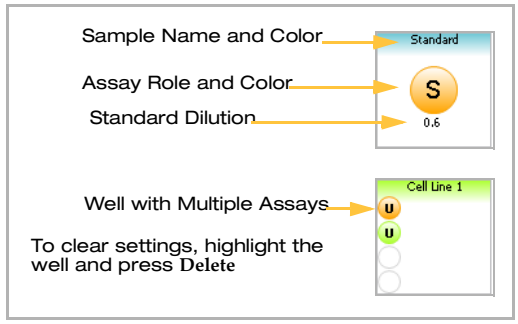


Assign Assays and Samples to Wells

Figure 13 Plate Layout Tab, Assigning Assays and Samples



1 For each type of assay and sample, left-click and drag the mouse to highlight the corresponding wells, as shown in Columns 1 and 2 of Figure 13. Wells turn gold when they are highlighted.



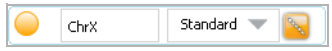
2 Click the **Assign** button beside any assay or sample name on the left side of the window. You can assign up to four assays to a well.

To remove the assay or sample designation, highlight the well and click **Assign** again to toggle the setting off.

3 To change the role of a given assay, select the corresponding **Assay Role** from the drop-down list. Proceed to define standards.

Define Standards

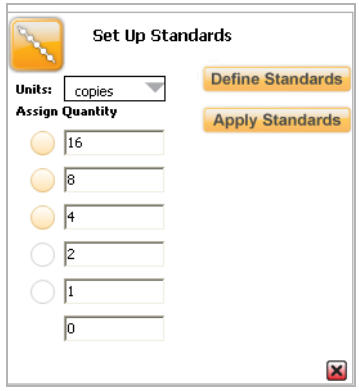
When you set an Assay Role to Standard, a small orange Standards button appears to the right of the assay role.



1 Click Standards to open the Set Up Standards pane in the lower left of the window.

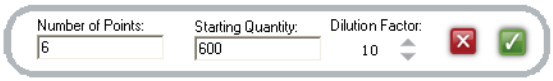
2 Select the units that are used in your standards, and then enter the dilution information.

Figure 14 Set Up Standards Pane



Auto-Calculate Serial Dilutions

1 To auto-calculate serial dilutions, click Define Standards. The Dilutions dialog box opens.



2 Enter the number of points in the standard curve, the quantity of the most concentrated standard, and the desired dilution factor, and then click .

Manually Enter Dilutions

1 Enter the value of the first standard into the first **Quantity** field below Units.

2 Press **Enter** to commit the value and open the next **Quantity** field.

Assign Standard Dilutions to Wells

You can assign standard dilutions to wells manually or automatically.

To assign dilutions automatically (Recommended)

1 Left-click and drag the mouse over a group of Standard Assay wells.

 # Vertical Wells = # Points on Standard Curve

 # Horizontal Wells = # Replicates

The **Apply Standards** button becomes active when you have selected a suitable group of wells.

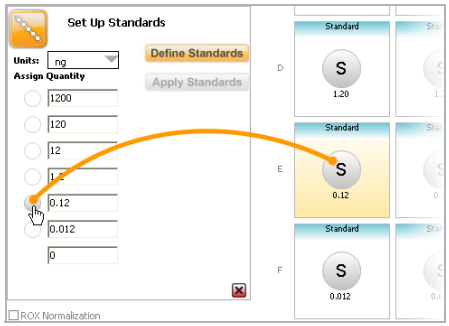
2 Click **Apply Standards**. The dilutions and replicates are automatically added in the highlighted group of wells.

Figure 15 Selecting Standard Assay Wells

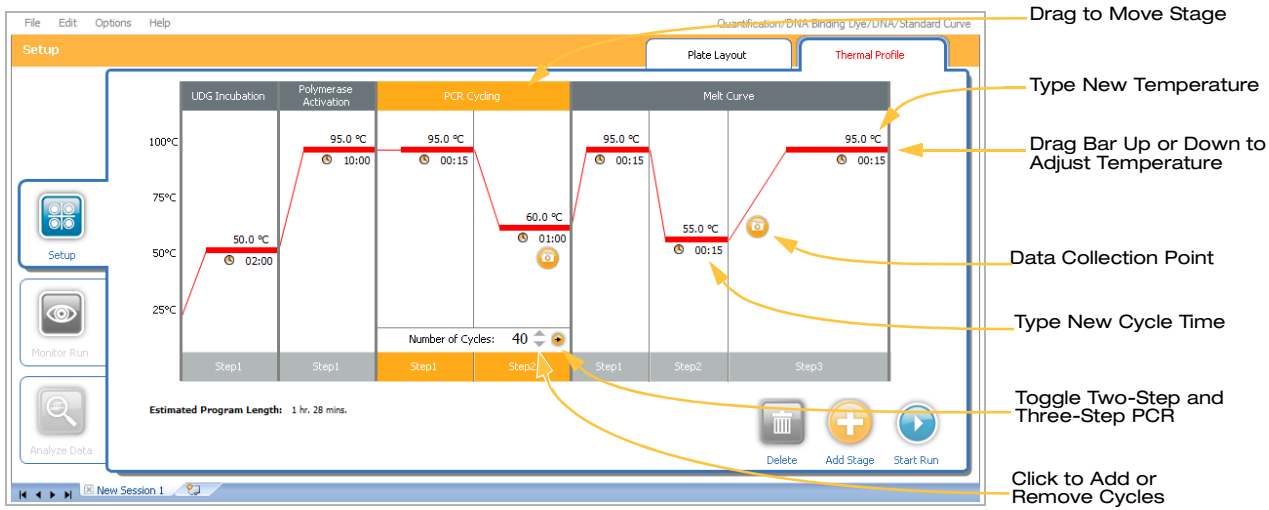
To assign dilutions manually

Highlight a Standard Assay well and click the **Assign** button beside the appropriate dilution quantity (Figure 16).

Figure 16 Assigning Dilutions



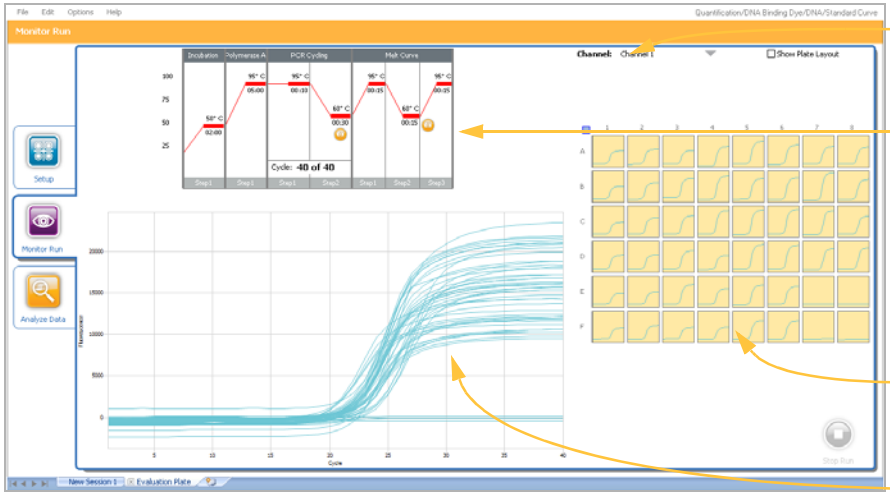
Set Up the Thermal Profile



When you define the experiment a corresponding default thermal profile is selected automatically. You can use this or modify it based on your reagent recommended protocol. You can set up cycle parameters in the Thermal Profile at any time after defining the experiment, but before starting the run.

- Click  to add a new stage, such as a reverse transcription incubation at the beginning. The stage will appear at the end of the cycle, and you can drag it to the desired location. Alternatively, you can drag the  icon to the location within the profile where you would like the new stage to be added.
- The camera icon  indicates when the Eco collects image data. In three-step PCR, you can select whether to collect data at the annealing or extension step. Extension is the default. To move it to annealing, mouse over the annealing step and click the dim camera icon that appears, and then drag it to the annealing step.
- To remove a stage, drag it to the  trash can or highlight it and press **Delete**.

Monitor Run



Channel:
Select channels to view in Amplification Plot

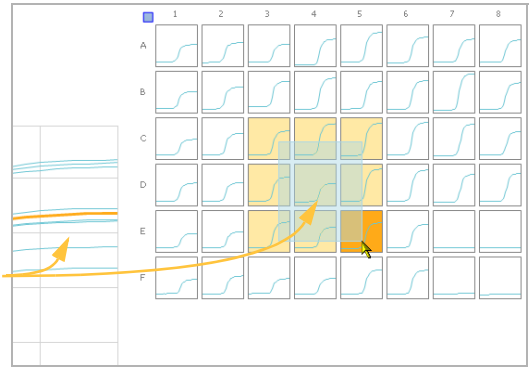
Thermal Profile:
Shows current stage highlighted in orange

Amplification View:
Shows amplification as it occurs in each well

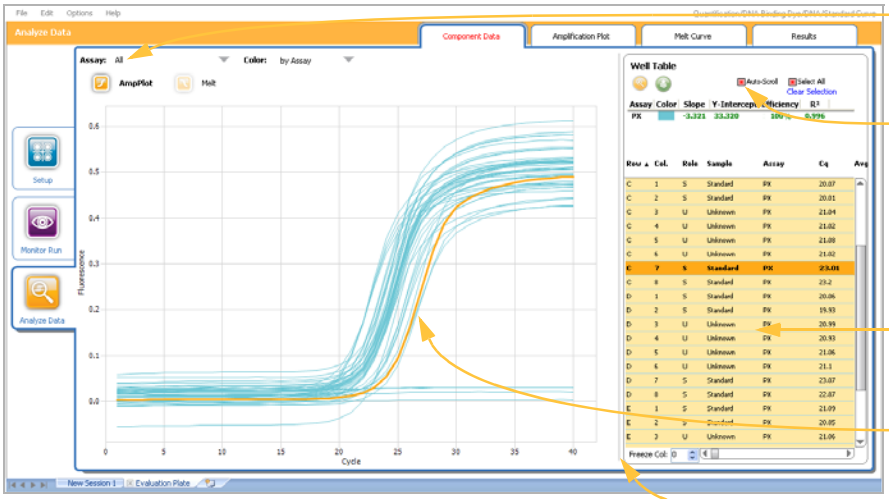
Amplification Plot:
Shows PCR signal in real time

Plate Layout View:
Shows sample type, sample identity, dilution, and assays

Amplification Plot:
Highlight wells to display a subset of data



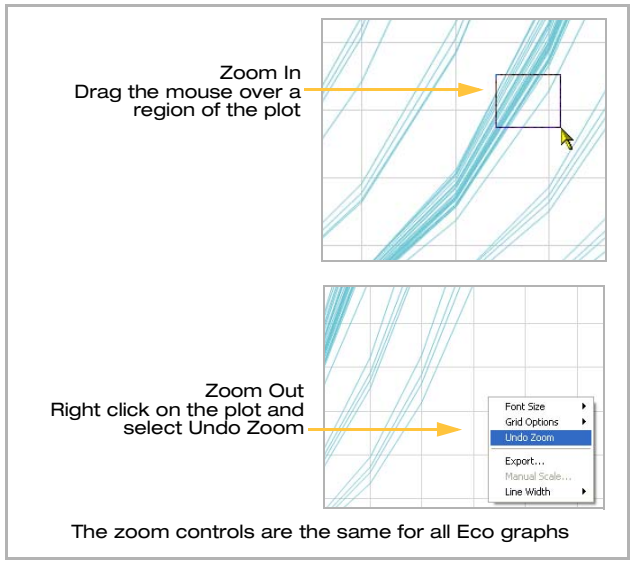
Analyze Data



There are four tabs:

- Component Data
- Amplification Plot
- Melt Curve (when applicable)
- Results

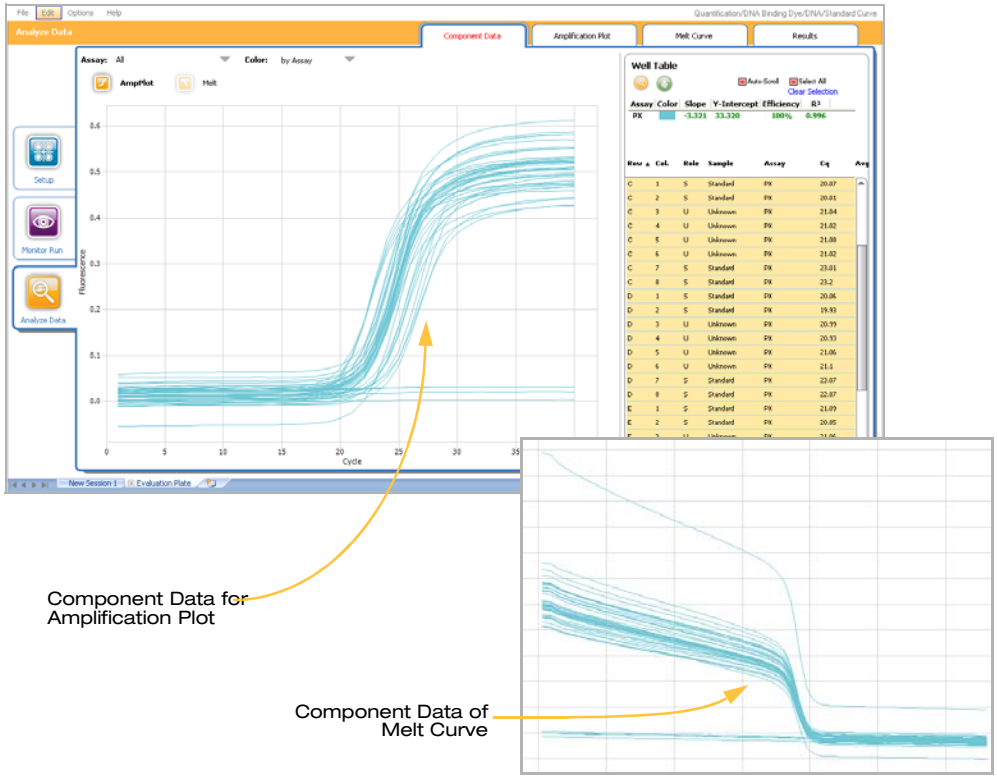
The window controls are the same for each tab.



Component Data Tab

The Component Data tab displays RFU (Relative Fluorescent Unit) versus cycle number. The data displayed are dye deconvoluted to remove dye cross-talk when multiplexing.

Figure 17 Component Data Tab

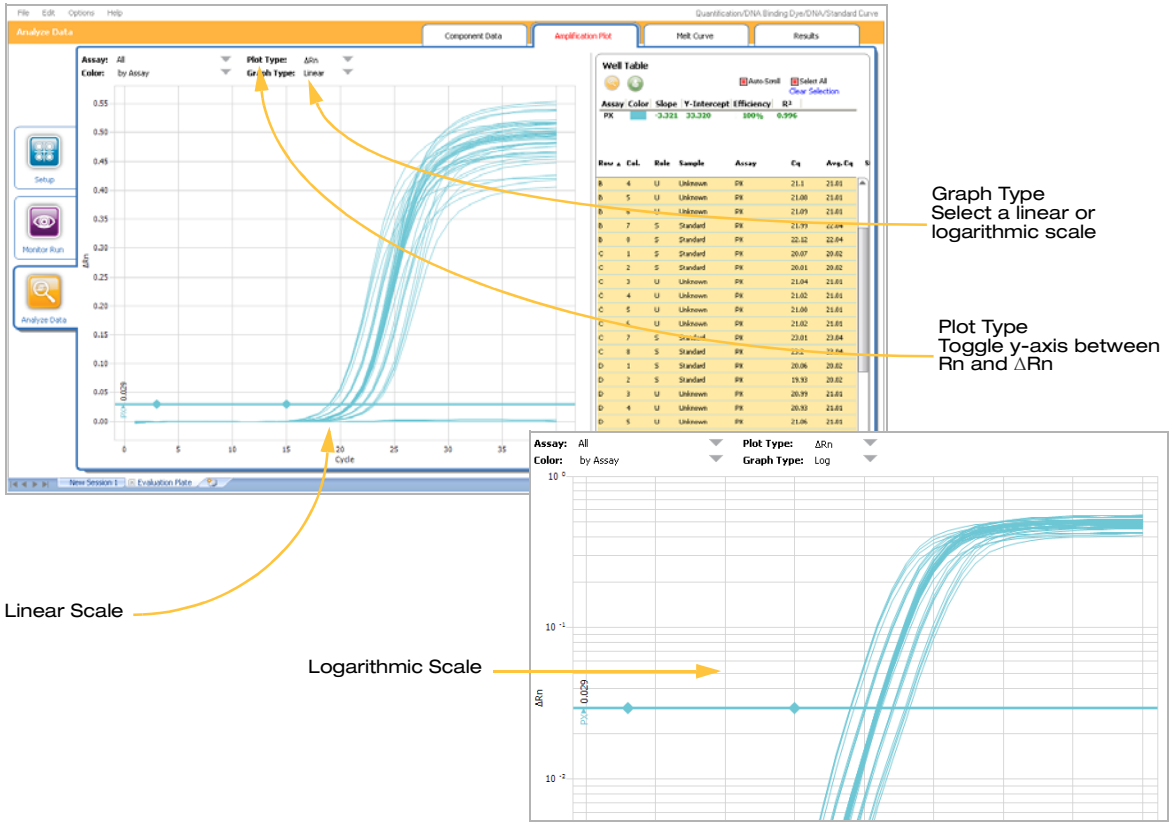


Above the graph, click  to view the Amplification Plot or  to view the Melt Curve.

Amplification Plot Tab

The Amplification Plot tab displays RFU normalized (Rn) to the ROX fluorescence level (if used) versus the cycle number. ΔRn is the Rn normalized to the background.

Figure 18 Amplification Plot Tab



Baseline Correction

Baseline Subtraction is based on background fluorescence detected between cycles 3 and 15 by default. You can adjust the baseline graphically, numerically, or by using a built-in algorithm that sets the best baseline for each well, based on the unique profile of their amplification plot.

To set the baseline automatically (Recommended)

1 Click **Analysis Settings** in the right panel. The Analysis Settings dialog box opens.

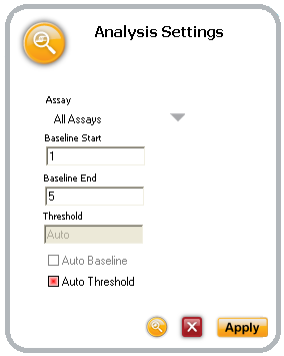
2 Select the **Auto Baseline** check box, then click Apply.

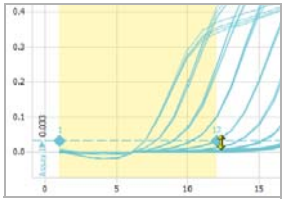
To adjust baseline numerically

1 Click **Analysis Settings** in the right panel. The Analysis Settings dialog box opens.

2 Enter the desired **Baseline Start** and **Baseline End** cycles in the Analysis Settings window, then click Apply.

Figure 19 Analysis Settings Dialog Box





To adjust baseline graphically

Drag ◆ diamonds sideways to indicate the interval boundaries.

Threshold Adjustment

By default, Eco sets a threshold approximately in the exponential growth phase of the amplification curves. This threshold can be adjusted manually or automatically to improve the quality of the data.

Eco performs an algorithm on all amplification curves to calculate the initial threshold for determining Cqs. The region of the amplification curve that corresponds to exponential growth changes depending on whether you select linear or log view.

To set the threshold automatically

1 Click **Analysis Settings** in the right panel. The Analysis Settings dialog box opens.

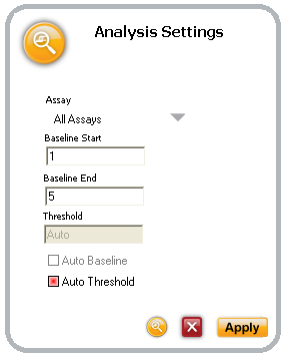
2 Select the **Auto Threshold** check box, then click **Apply**.

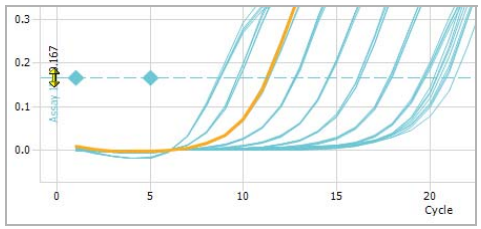
To adjust threshold numerically

1 Click **Analysis Settings** in the right panel. The Analysis Settings dialog box opens.

2 Enter the desired Threshold value, then click **Apply**.

Figure 20 Analysis Settings Dialog Box





To adjust threshold graphically

Drag the horizontal bar up or down into the exponential growth phase of the curve.

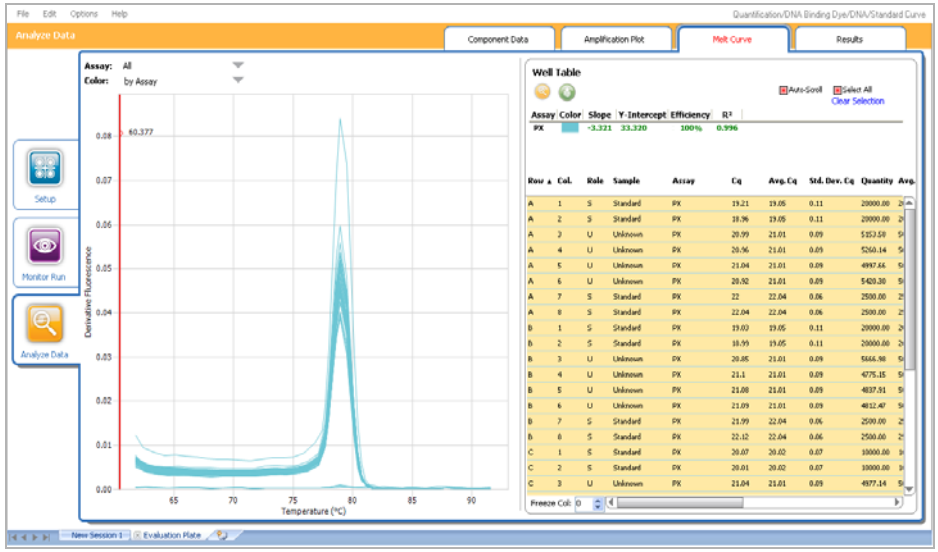
- In a linear scale view, this needs to be set close to the inflexion point of the amplification plots.
- In a log scale view, it should be set in the middle of the exponential phase.

Melt Curve Tab

This tab is active if you ran a melting curve in your thermal profile while using DNA binding dyes such as SYBR green. The Melt Curve tab displays the negative derivative of RFU versus temperature (-dRFU/dT) and calculates melting temperatures (Tm) based upon peak calls.

Tm calls (up to three per well) are listed in the Well Table on the right side of the tab and are ranked based upon maximum amplitude.

Figure 21 Melt Curve Tab



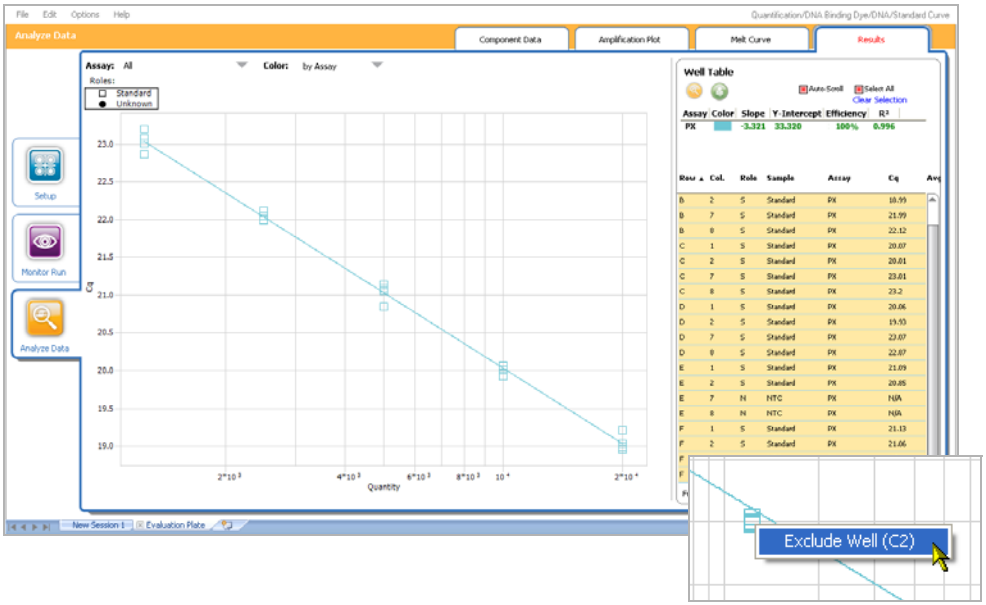
Results Tab

Eco software automatically analyzes the data and generates a plot based upon the experiment type along with any baseline or threshold adjustments.

- **Absolute quantification** experiments generate a standard curve.

  The slope, PCR efficiency, and R^2 appear in a table in the upper left.
- **Relative quantification** experiments generate a bar graph that includes error bars.
- **Allelic discrimination** experiments generate a scatter plot.
- **HRM** experiments generate a melting curve graph, which can be displayed as either a normalized melting profile or a difference plot to maximize cluster resolution.

Figure 22 Results Tab, Example of a Standard Curve display



In some cases you might want to exclude outlier data from the analysis. Right-click the data point in the graph or the well location in the table and select **Exclude Well** from the context menu (see inset). The Well Table will list the well as **Excluded**.

System Information

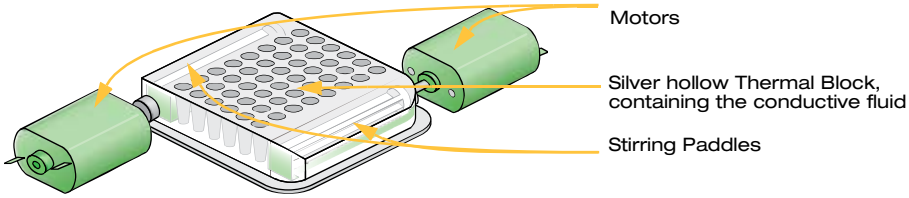
Chapter 3

Components

Thermal System

- Proprietary hollow silver thermal block filled with circulating conductive fluid provides superior temperature control and thermal uniformity across the sample plate
- Standard Fast protocol performs 40 PCR cycles in approximately 40 minutes

Figure 23 Eco Thermal System

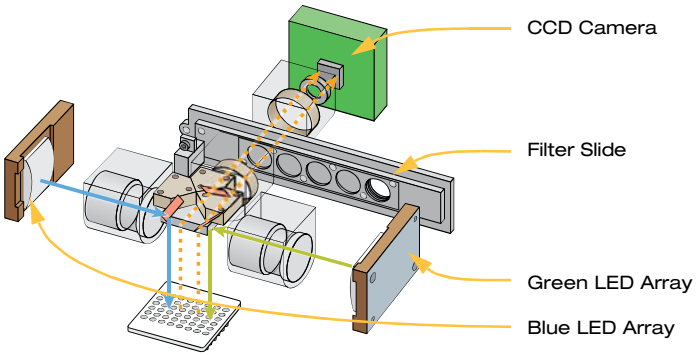


Optical System

- Two LED arrays provide individual sample well excitation
- Four detection filters support almost all PCR chemistries and multiplex detection (ROX is optional)
- CCD camera acquires high-quality data in all wells and filters at each PCR cycle

Factory-calibrated optics support SYBR Green/FAM, HEX, VIC, ROX, and Cy5 dyes. You can also use other dyes that are compatible with the excitation and emission wavelengths.

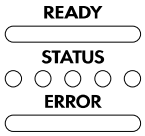
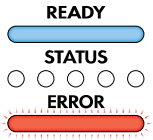
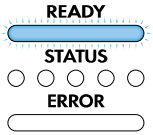
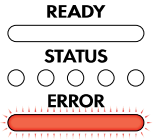
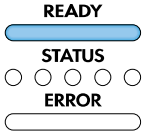
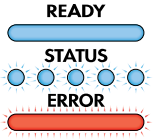
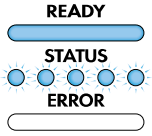
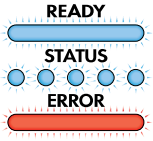
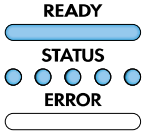
Figure 24 Optical System



Lights

The Eco System has three indicator lights on the front: **Ready**, **Status**, and **Error**. The following table shows the meaning of each combination of off, on, and flashing lights.

NOTE
Flashing lights are indicated in the table by dashed lines around the outside of the light.

Lights	Description	Lights	Description
	Power Off		Non-Fatal Error Decide whether you want to terminate the run
	Initializing (conducting self tests and heating the thermal bonnet)		Fatal Error: Run Terminated Instrument might have overheated or encountered a hardware failure
	Ready/Idle		Software Updating
	Run In Progress Do not switch off or open the door while a run is in progress		Communicating with Netbook
	Run Complete		

Specifications and Environmental Requirements

Optical	Light Source	Two sets of 48 LEDs (452-486 nm and 542-582 nm)
	Detector	CCD camera (4 filters)
Thermal	Thermal Cycling	Proprietary hollow silver block with Peltier-based system
	Thermal Uniformity	± 0.1°C
Operational	Sample Format	48-well plate
	Reaction Volume	5–20 µl
	Warmup Time	< 5 minutes
	Typical PCR Run Time	Less than 40 minutes for 40 cycles
	Sensitivity of Detection	1 copy
	High Resolution Melt	Supported resolution to 0.1°C
	Multiplexing	Detection of up to four targets simultaneously (four-plex)
	Passive Reference	Optional (ROX)
Physical	Dimensions	34.5 cm W x 31 cm D x 32 cm H (13.6 in. W x 12.2 in. D x 12.6 in. H)
	Weight	13.6 kg (30 lb) including power supply
Environmental	Electrical	120–240 VAC, 50/60 Hz, 8A
	Temperature Range	Operating: 15°C to 30°C (59° F to 86° F) Storage: 10°C to 38°C (50° F to 100° F)
	Humidity Range	Operating: 15–90% Relative Humidity Storage: 5–95% Relative Humidity

Symbols

	CAUTION: Hot Surface
	Do Not Throw in Trash: At end of useful life, recycle the system or device
EC REP	European Representative
	Fuse: replacement fuses must meet the stated rating
	Humidity Range (on packaging: indicates acceptable shipping and storage limits)
	Manufactured By
CE	Mark of European Conformity: device complies with the EMC Directive (2004/108/EC) and the Low Voltage Directive (2006/95/EC)
REF	Model Number
○	Off
I	On
SN	Serial Number
	Temperature Range (on packaging: indicates acceptable shipping and storage limits)

Electromagnetic Compatibility

This equipment complies with the emission and immunity requirements described in IEC 61326-1:2005 and IEC 61326-2-6:2005. To confirm proper operation:

- The electromagnetic environment should be evaluated prior to operation of the system.
- Do not use this system in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources), as these may interfere with proper operation.
- If you notice any interference, discontinue using the system until all issues are resolved. Resolution may include moving cords from other equipment away from the system, plugging the system into an outlet on a different circuit from other equipment, or moving the system away from the other equipment. If you continue to have difficulties, contact Illumina.

Cleaning And Maintenance

Clean the block and housing as needed, following these directions.

CAUTION

If hazardous or biohazardous material is spilled onto or into the equipment, clean it immediately.

1 Turn the system off and allow the block to cool completely.

2 Using a lint-free cloth slightly dampened with clean water, gently wipe the surfaces of the equipment. If a stronger cleaning agent is needed, use a lint-free cloth slightly dampened with 95% isopropyl alcohol.

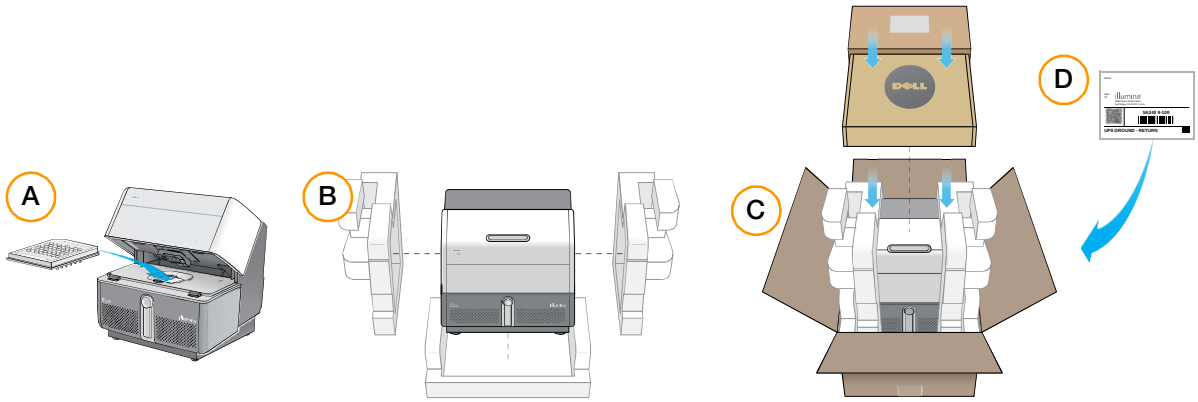
Follow these practices for proper maintenance of your Eco system.

- Every time you use the system, visually check it to confirm there is no obvious physical damage such as dents, frayed cords, or damaged levers. If you see any damage, discontinue use and contact Illumina Technical Support.
- Sign up for Illumina's monthly Illuminotes newsletter, which tells you about new versions of systems or software, informational resources, and product discontinuations.
- Once a year, run a known test sample to confirm accurate analysis.

CAUTION

The Eco system contains materials that may be hazardous to the environment if not disposed of properly. Be sure to dispose of materials according to all local, state/provincial, and national regulations.

Return Process



Follow these directions if you need to return the Eco instrument to Illumina for any reason (for repairs, for example.)

1. Follow the instructions in *Cleaning And Maintenance* on page 33 to clean the instrument.
2. Obtain the original shipping box and packaging materials used to ship Eco to you. If you do not have them, go to **http://www.illumina.com/ecoqpcr** for instructions on ordering return shipping materials.
3. Put an empty plate into the instrument for safe shipping, and then close the lid (A).
4. Place the instrument into the white foam packaging, ensuring that it is properly positioned for complete protection in shipping (B).
5. Put the packaged instrument into the shipping box (C).
6. Package the Dell netbook, dock, and squeegee into their original boxes and place the boxes on top of the Eco in the shipping box (C). You do not need to return the Evaluation Plate, buffers, extra plates, seals, or USB drive.
7. Tape the box securely for shipment and attach a shipping return label (D).

Concepts

Chapter 4

The weight of one genome (g) = (size of genome in bp) x (618 g/mol/bp) x Avogadro's number
One human genome (g) = (3 x 109 bp) x (618 g/mol/bp) x (6.02 x 1023) = 3.08 x 1012 g
One haploid cell (sperm/egg) = 3.08 pg of DNA
One diploid cell = 6.16 pg of DNA

There is approximately one copy of every non-repeated sequence per 3.08 pg of human DNA.
The average cell contains 10–20 pg of total RNA.
About 90–95% of total RNA is rRNA (18S, 5.8S and 28S). 1–3% is mRNA.

RNA concentration (µg/µl) = (A_{260} * 40 * D)/1000, where D = dilution factor and A_{260} = absorbence at 260 nm.
DNA concentration (µg/µl) = (A_{260} * 50 * D)/1000, where D = dilution factor and A_{260} = absorbence at 260 nm

The exponential amplification of PCR (Xn) is described by the following equation:

$Xn = Xo * (1+E_x)n$

where X_n = number of target molecules at cycle n; X_o = initial number of target molecules; E_x = efficiency of target amplification; and n = number of cycles

Amplification efficiency (E_x) is described by the following equation:

$E_x = 10^{(-1/slope)} - 1$

The acceptable range of assay efficiency = 90% to 110%, or a slope between -3.1 and -3.6
A slope of -3.32 indicates 100% efficiency, meaning that the number of template molecules doubled in each PCR cycle.

Common reference genes:

High expression: 18S ribosomal RNA (18S), Beta actin (ACTB), Beta-2-microglobulin (B2M), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and phosphoglycerokinase (PGK)

Medium expression: Transferrin receptor (TfR)

Low expression: Transcription factor IID TATA binding protein (TBP) and glucuronidase (GUS)

Glossary

Absolute Quantification—An assay that quantifies unknown samples by interpolating their quantities from a standard curve based on a serial dilution of a sample containing known concentration.

Allelic Discrimination—An assay that discriminates between two alleles (gene variants).

Amplicon—A fragment of DNA synthesized by a pair of primers during PCR.

Assay—The set of primers or primers/probe used to quantify an amplicon.

Baseline—The initial PCR cycles when little fluorescence signal is generated. This will be used to subtract the background.

Channel—The combination of excitation and emission spectra used to monitor amplification for a given assay.

Ct—Threshold Cycle. See Cq.

Cq—Quantification Cycle. The cycle number at which the fluorescent signal crosses the threshold. It is inversely correlated to the logarithm of the initial copy number.

Dark Quencher—A quencher without any native fluorescence. Black Hole Quencher (BHQ) dyes are an example.

Delta Rn (ΔRn)—The normalized Fluorescence of an amplification plot with background and ROX normalization dye correction.

Derivate Melt Curve—A plot of temperature (x axis) versus the derivate of fluorescence with respect to temperature (-dF/dT) (y axis). Used to analyze the Tm of an amplicon.

DNA Binding Dye—A dye that increases its fluorescence in the presence of double-stranded DNA.

dsDNA—Double-stranded DNA.

Dual-Labeled Hydrolysis Probe—See hydrolysis probe.

Dynamic Range—The range of template concentration over which accurate Cq values can be determined. Extrapolation is not recommended.

Efficiency—See Slope.

Endogenous Control—An RNA or DNA template that is naturally present in each sample.

End-Point Analysis—Qualitative analysis of PCR data at the end of PCR. Allelic discrimination assays (genotyping) are an example.

Exogenous Control—A RNA or DNA template that is spiked into each sample at a known concentration.

FAM (6-carboxy fluorescein)—The most commonly used reporter dye at the 5′ end of a Taqman probe.

Filter—Components used to limit the bandwidth or the excitation or emission energy to the next component of the optical path.

Fluorophore—The functional group of a molecule that absorbs energy at a specific wavelength and emits it back at a different wavelength.

Fluorescence—The immediate release of energy (a photon of light) as a result of an increase in the electronic state of a photon-containing molecule.

HEX—Carboxy-2′,4,4′,5′,7,7′-hexachlorofluorescein.

High Resolution Melt (HRM)—An enhancement of the traditional melt curve analysis which increases the detail and information captured.

Hybridization Probe—A probe that is not hydrolyzed by Taq polymerase. Hybridization probes can be used for melt curve analysis. Examples include Roche FRET and Molecular Beacons.

Hydrolysis Probe—A probe that is hydrolyzed by the 5′ endonuclease activity of Taq polymerase. Taqman probes are hydrolysis probes.

Internal Positive Control (IPC)—An exogenous control added to a multiplex qPCR assay to monitor the presence of inhibitors in the template.

JOE—Carboxy-4′,5′-dichloro-2′,7′ dimethoxyfluorescein.

LED—Light Emitting Diode. A light that is illuminated by the movement of electrons in a semiconductor material. LED lights do not have filaments that burn out and do not get very hot.

Linear View—A view of an amplification plot using linear dRn values (y-axis) versus PCR cycles (x-axis).

Log view—A view of an amplification plot using log dRn values (y-axis) versus PCR cycles (x-axis).

LUX Primer Set—A self-quenched fluorogenic primer and a corresponding unlabeled primer. When the primer is incorporated into DNA during PCR the fluorophore is de-quenched, leading to an increase in fluorescent signal.

Melt Curve—See Derivative Melt Curve.

Minor Groove Binders (MGBs)—dsDNA-binding agents typically attached to the 3′ end of Taqman probes. MGBs increase the Tm value of probes, thus leading to smaller probes.

Molecular Beacons—Hairpin probes containing a sequence-specific loop region flanked by two inverted repeats. A quencher dye at one end of the molecule quenches the reported dye at the other end. Sequence-specific binding leads to hairpin unraveling and fluorescent signal generation.

Multiplexing—Simultaneous analysis of more than one template in the same reaction.

No Template Control (NTC)—An assay with all necessary components except the template.

Normalization—The use of control genes with a constant expression level to normalize the expression of other genes in templates of variable concentration and quality.

Passive Reference—A fluorescence dye such as ROX that the software uses as an internal reference to normalize the reporter signal during data analysis.

Peltier—Element used for heating and cooling in a qPCR machine.

Quencher—Molecule that absorbs fluorescence emission of a reporter dye when in close proximity. TAMRA and BHQ are quenchers.

R^2 (Coefficient of Correlation)—The coefficient of correlation between measured Cq values and the DNA concentrations. It is a measure of how closely the plotted data points fit the standard curve. The closer to 1 the value, the better the fit. R^2 is ideally > 0.99.

Reference—A passive dye or active signal used to normalize experimental results.

Reference Genes—Genes with a wide and constant level of expression. Typically used to normalize the expression of other genes. Examples of commonly used reference genes: 16S/18S, GAPDH, and b-actin.

Relative Quantification—An assay used to measure the expression of a target gene in one sample relative to another sample and normalized to a reference gene.

Reporter Dye—Fluorescent dye used to monitor amplicon accumulation. This can be a dsDNA binding dye or a dye attached to a probe. Each dye is associated with a certain channel.

Rn (Normalized Reporter Signal)—Reporter fluorescent signal divided by fluorescence of the passive reference dye.

ROX (carboxy-X-rhodamine)—The most commonly used passive reference dye.

Slope—The slope of a standard curve. It is a measure of assay efficiency. $E = 10^{(-1/slope)}-1$, where a slope of -3.32 is equal to 100% efficiency (E) or an exact doubling of template molecules in each PCR cycle. Acceptable efficiencies range from -3.6 (90%) to -3.1 (110%). Overly high efficiencies indicate qPCR inhibition, usually due to contaminants in the sample. Overly low efficiencies typically indicate problems with the reaction mix concentration.

Standard—A serial dilution of a target of known concentration used as template to generate a standard curve.

Standard Curve—A plot of Cq values against the log of target amount. Used to determine an assay's dynamic range, efficiency (slope), R^2, and sensitivity (y-intercept).

Standard Deviation (SD)—The SD of replicate Cq measurements is a measure of the precision of the assay.

TaqMan Probe—See Hydrolysis Probe.

TAMRA—Tetramethyl-6-carboxyrhodamine. Commonly used as a quencher.

Target—The DNA or RNA sequence to be amplified.

Template—See Target. Template can also refer to a saved experiment that can be used as a model for new experiments in the software.

Threshold—A level set above the background signal generated during the early cycles of qPCR. When adjusted manually, it should be set in the middle of the exponential stage of qPCR.

TET—Carboxy-2',4,7,7'-tetrachlorofluorescein.

Tm—The temperature at which 50% of dsDNA is single-stranded (melted).

Unknown—A sample containing an unknown amount of template.

Y-Intercept—In a standard curve, the value that crosses the y-axis at x = 1 (single copy target).

Part # 15017157 Rev B

Technical Assistance

For technical assistance, go to **http://www.illumina.com/ecoqpcr**.

MSDS

Material safety data sheets (MSDSs) are available on the Illumina website at **http://www.illumina.com/msds**.

Product Documentation

If you require additional product documentation, you can obtain PDFs from the Illumina website. Go to **http://www.illumina.com/support/documentation.ilmn**.

When you click on a link, you will be asked to log in to iCom. After you log in, you can view or save the PDF. To register for an iCom account, please visit **https://icom.illumina.com/Account/Register**.

Illumina, Inc.
9885 Towne Centre Drive
San Diego, CA 92121-1975
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com