

FemINDICATOR™ Data Analysis

qPCR Plant Gender Detection Assay on the BIO-RAD CFX96 Touch™
Real-Time PCR Detection System

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Please refer to <http://www.medicinalgenomics.com/product-literature/> for updated protocols and Material Safety Data Sheets (MSDS). Consult MSDS before using any new product.

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FemINDICATOR™ Analysis Quick Table

FemINDICATOR™ Assay	Cq Value	Fluor	Negative Control (Cq)	Cq threshold
Male	< 35	FAM	> 35	Presence/Absence
Female	> 35	FAM	> 35	Presence/Absence
Internal Control*	<35	HEX	*Internal control verifies the presence or absence of plant DNA	
Assay Positive Controls	<35	FAM		

Detailed Assay Data Analysis

1. Presence / Absence Assays

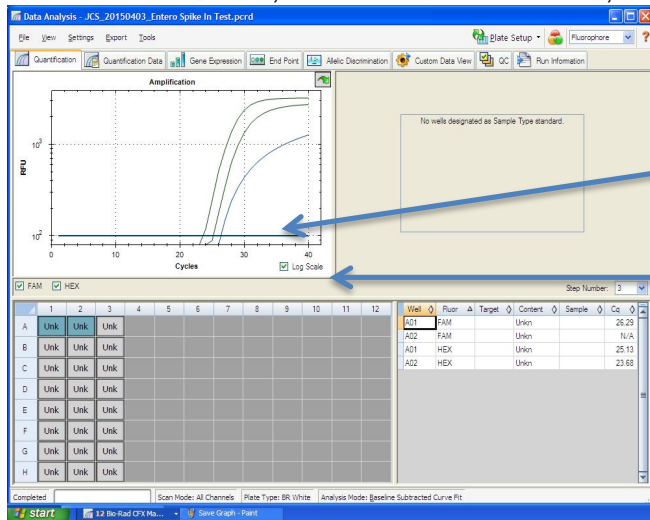
1.1. The Data Analysis window will open automatically when the run is complete.

1.2. Highlight the well of interest.

- The graph will appear above.
- The Cq values will appear to the right.

1.3. To analyze the results

- Start by turning the graph to Log Scale and manually moving the threshold to 10^2 for the FAM flourophore. Also manually move the threshold for the HEX flourophore half way between 10^2 and 10^3 .
 - To turn the graph to Log Scale, click on the box at the bottom right of the graph.
 - To move the threshold, click on the horizontal lines, and move them to 10^2 on the y-axis.



Threshold Line

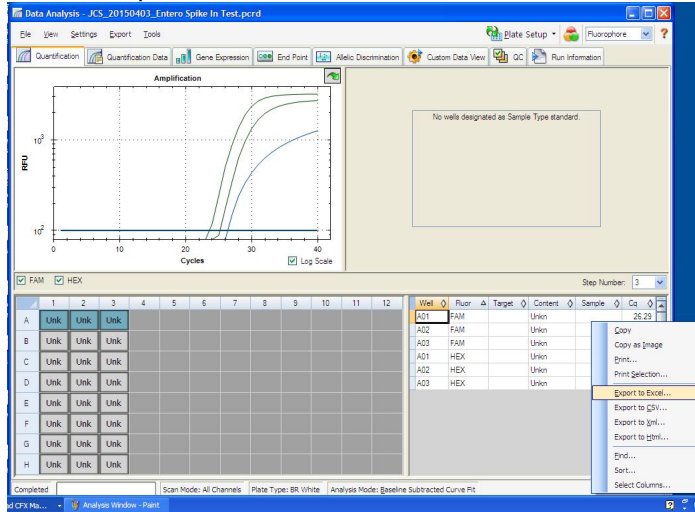
Log Scale

- Controls
 - Male specific Positive Control, on the FAM flourophore, has a Cq value < 35.
 - Visually confirm with the curve on the graph.
 - Male specific Negative Control, on the FAM flourophore, has a Cq value > 35 or no Cq value.
 - Visually confirm with the curve on the graph.
- Unknown Samples
 - Internal Control, on the HEX flourophore, has a Cq value < 35.
 - Visually confirm with the curve on the graph
 - A “presence or male” result for the unknown sample.
 - Any Cq value for the FAM flourophore < 35.
 - Visually confirm with the curve on the graph. (It is very important to confirm with the amplification curve when a presence result occurred. Sometimes the background amplification will give a false positive reading, especially when Cq reading is less than 15. See troubleshooting guide below.)
 - An “absence or female” result for the unknown sample.
 - No Cq value for the FAM flourophore or a Cq value >35.
 - Visually confirm no curve on the graph.

2. Export the Data

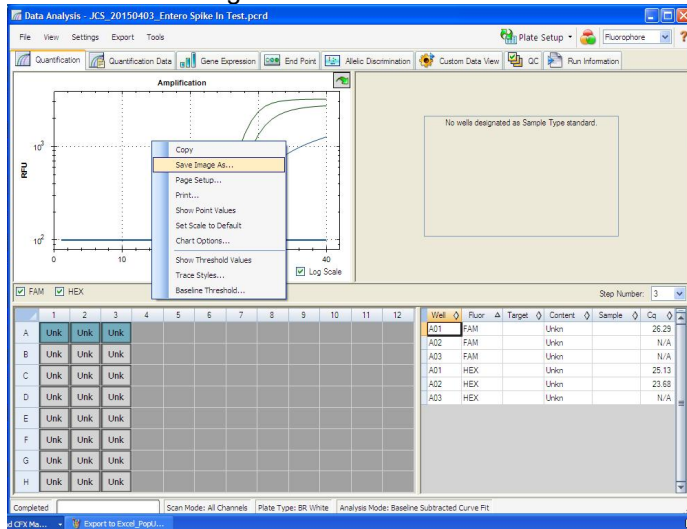
2.1. Exporting the Cq values into an Excel spreadsheet.

- To export the Cq values to an Excel spreadsheet, right-click on the chart on the bottom right of the screen.
- Choose Export To Excel...

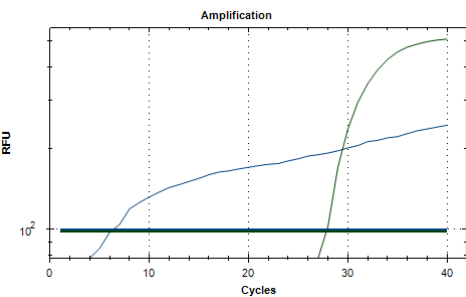


2.2. Saving a visual of the graph

- To save a picture of the graph, right click on the graph on the top left of the screen.
- Choose Save Image As



Troubleshooting Guide

Symptom	Reason	Solution
Internal control (SCCG Primer) failure	Extraction Failure	Repeat SenSATIVAx™ and FemINDICator™ by following the protocol.
	Residual ethanol in elution	Ethanol is an inhibitor to PCR. Return to the SenSATIVAx™ protocol and repeat all steps.
	Mix up in Reaction Setup	Repeat the Decontamination and qPCR by following the protocol.
	Missing Fluorophore on plate set up	In the Data Analysis window click on View/Edit Plate Setup from the Settings drop down. All wells should have both FAM and HEX. Once completed and window is closed the analysis should automatically update.
Positive Negative Control	Small Cq value <15	Visually confirm that there is an amplification curve. If not, this is low level background and is to be expected.
	Carry over	Repeat the Decontamination and qPCR by following the protocol.
	Insufficient pre-setup bleaching	Wipe down the lab workspace and all equipment with 10% Bleach. Repeat Decontamination and qPCR Protocols.
Negative Positive Control	Mix up in Reaction Setup	Repeat the Decontamination and qPCR by following the protocol.
Total run failure	Excessive vortex of the qPCR Master Mix	Repeat the Decontamination and qPCR by following the protocol.
<p>Background Amplification</p> 	Unclear	This is usually seen with a very low Cq reading (<15), the curve is usually missing the exponential growth phase, but rather a gradual increase of florescent signal. This is usually a negative result, but should be repeated.

Glossary and Definitions

Deoxyribonucleic acid (DNA) is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms.

Polymerase Chain Reaction (PCR) is a technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

A **fluorophore** is a fluorescent chemical compound that can re-emit light upon light excitation.

The **Negative Controls** are the samples where no or a very high Cq is expected. It helps to ensure that all Assay specific reactions are clean of contaminants.

The assay specific **Positive Controls** are the samples where a Cq is expected. It helps ensure that all Assay specific reactions are working correctly. The Assay specific Positive Control is targeting the pathogen using the FAM fluorophore.

The **Internal Control** is added to every sample where a Cq is expected. It ensures the effectiveness and efficiency of each reaction. The internal control is targeting a Single Copy Control Gene or SCCG, using the HEX fluorophore.

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