

High-Performance Optical System

Real-time PCR uses fluorescent reporters to detect amplification of nucleic acid targets after each PCR cycle. The fluorescence signal is captured in real time to enable quantitative analysis at the appropriate phase of the reaction. The Eco system facilitates four-color multiplex applications and is calibrated for use with SYBR, FAM, HEX, VIC, ROX, and Cy5 dyes, but can be used with any real-time PCR chemistry. Its optical system uses light emitting diodes (LEDs), which are very stable over their lifetime, contributing to accurate data generation and increased instrument longevity (Figure 2). Two panels—48 fixed LEDs each—provide fluorescent dye excitation over a broad spectrum. Each of the 48 wells is individually illuminated, optimizing the signal per well and minimizing cross-talk between wells.

The high-performance Eco optical system enables real-time detection of up to four targets in a single reaction. Four emission filters in a linear filter slide and a high-performance CCD camera detect the fluorescence from all wells, preventing any data loss and allowing changes to the plate setup and data analysis even after the run is completed. Standard melt curve and HRM analysis protocols support continuous data acquisition in a single dye channel during the melt for increased data collection and reduced run times.

Table 1: Reaction Mix for SYBR Assay and DNA Sample Specifications

Reagent	Amount (1 rxn)
2x SYBR Master Mix	5.0 µl
20x Human F5 Assay Mix	0.5 µl
Human Genomic DNA (2500 c/µl)	2.0 µl
Water	8.0 µl
Total Volume	10 µl

Table 2: Reaction Mix for FAM, HEX, and ROX Assays and DNA Sample Specifications

Reagent	Amount (1 rxn)
2x PCR Master Mix	5.0 µl
20x Human B2M Assay Mix (FAM, HEX or ROX labeled)	0.5 µl
Human Reference cDNA (5 ng/µl or 2.5 ng/µl)	2.0 µl
Water	8.0 µl
Total Volume	10.0 µl

Table 3: PCR Thermal Protocol

Stage	Temperature (°C)	Time (min)	Acquisition
Activation	95	0:10:00	
PCR (x40 cycles)	95	0:00:15	
	60	0:01:00	x
	95	0:00:15	
Melt	55	0:00:15	
	95	0:00:15	on ramp

Gage R&R Study Parameters

Gage R&R studies are valuable Six Sigma tools for evaluating and identifying variables in equipment and technician performance that could impact analyses. A Gage R&R study was performed to determine the uniformity of results generated by the Eco system. The study was conducted by two different operators using four randomly selected Eco systems, with the same experiment performed in triplicate (three plates) using four different real-time fluorescent chemistries (SYBR, FAM, HEX, and ROX).

The SYBR assay was performed with 5,000 copies of human genomic DNA template in a 10 µl reaction volume, while the FAM, HEX and ROX assays were performed with 5 ng and 10 ng of Human Reference cDNA templates in 10 µl reaction volumes (Tables 1 and 2). Each plate was run with the same PCR thermal profile as recommended by the master mix manufacturer (Table 3).

Results

The Eco system demonstrated high precision within and between all four instruments (Figure 3). The mean Cq* (cycle of quantification) value for the SYBR assays was 21.17, with the standard deviation (SD) for each instrument ranging from 0.06 to 0.08. Such precision enables a statistically significant call to be made between samples with as little as 20% difference in target expression levels when run in duplicate in any position on the three plates. A Cq standard deviation value of 0.167 measured across all well positions indicates sufficient precision to discern a two-fold difference in starting target copy number with 99.6% confidence, and is a good measure of real-time performance. In our study, the precision between instruments is also high, with a 0.12 SD Cq for all four instruments. This value was also significantly below the 0.167 threshold and would enable detection of ~40% differences in starting target quantity among samples run in duplicate on any of the twelve plates run on any of the four instruments tested.

Equally solid precision data can be seen when the samples were run with FAM, HEX, and ROX chemistries. In these analyses, two different sample quantities (5 ng and 10 ng) were assayed. Once again, the instrument reproducibility was high with SD Cq values varying by < 0.03 between the 5 ng and 10 ng assays on the same instrument, and < 0.02 between instruments.

*Cq (also known as C_i) value is the cycle number when the amplification plot of the fluorescent signal crosses the threshold fluorescence value. Cq is the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) recommended unit for this point.

