Internal quality controls to monitor EcoToxChip performance



BACKGROUND INFORMATION

EcoToxChips are based on QIAGEN RT2 Profiler PCR Arrays, which are a highly reliable platform for studying focused gene panels. Patented controls (GDC, RTC, PPC) (US patent #8, 597, 938) are an important feature of the EcoToxChip to ensure accurate results in any lab with regards to contamination, reverse transcription efficiency and inter-plate PCR efficiency.

APPROACH

GDC (Genomic DNA Control)

The GDC is a sensitive assay that detects the presence of genomic DNA. In our initial tests the one exception was the gDNA control well for the FHM v0.1 chip. This assay consistently yielded Cycle threshold values above the requisite cut-off due to unresolved primer design issues. Therefore, for the FHM EcoToxChip, independent no RT reactions are included as an alternate method to ensure the absence of genomic DNA contamination.

RTC (Reverse Transcription Control) & PPC (Positive PCR Control)

The RTC assay tests the efficiency of the reversetranscription reaction performed by the RT2 First Strand cDNA kit and detects an artificial RNA template control. The PPC tests the efficiency of the polymerase chain reaction and monitors samples for PCR inhibitors. During data analysis, ratios between the PPC and RTC are used to calculate the reverse transcription efficiency. The technical variability in a single sample and across multiple samples is monitored by analyzing the RTC and PPC assays.



Figure 1. EcoToxChip plate layout showing the location of the internal quality control wells

	Genomic DNA Contamination Control	Reverse Transcription Efficiency
V1 JQ	150/156 plates passed	156/156 plates passed
V1 FHM	0/113 plates passed	46/113 plates passed
V1 XL	8/8 plates passed	7/8 plates passed

Table 1. Results of the in-plate QC data for the initialV1 EcoToxChips tested for the three model species

TAKEAWAYS

Performance of EcoToxChips for three model species met most quality assurance/quality criteria guidelines established by Qiagen for the key internal control wells, though more work is needed for the FHM and an interim solution is available. These internal QC wells help provide confidence in EcoToxChip performance.

Notes

Reference: RT 2 Profiler PCR Array Handbook, 2019 (Qiagen).