Evaluation of the IntelliQube® for Gene Expression Analysis using a One-Step RT-PCR Workflow

ABSTRACT

One-step reverse transcription-PCR (RT-PCR) is widely used for gene expression analysis, RNA virus detection, and basic RNA quantification experiments. In this study, we evaluated the IntelliQube for one-step RT-PCR performance using commercially available RNA, gene expression assays, and a one-step RT-PCR master mix. The IntelliQube combines liquid handling with real-time qPCR analysis in miniaturized reaction volumes, providing laboratories a flexible and economical platform to scale their screening workflows. In this experiment, RT-PCR data quality and reproducibility was consistent over nine consecutive arrays, highlighting the ability to process several arrays in a walk-away workflow. Combined with the automated process and economic benefits of Array Tape®, the IntelliQube can be a useful and powerful platform for one-step RT-PCR applications.

INTRODUCTION

One-step reverse transcription-PCR (RT-PCR) is a common technique used to directly amplify RNA samples. In a one-step workflow, the reverse transcription (RT) and quantitative PCR (qPCR) steps are performed in the same well, reducing the number of pipetting steps and decreasing processing time. However, unlike two-step strategies where cDNA is generated in a separate reaction, all of the cDNA is used up in the PCR process and unavailable for future testing. One-step RT-PCR workflows benefit users wanting to process many samples at a time against one or a few markers. The most popular research applications include virus detection and quantification, high throughput gene expression analysis, and basic RNA quantification experiments. While there are several PCR instruments on the market upon which these tests could be performed, there remains an unmet need for a fully-automated solution that provides laboratories the flexibility and scalability necessary to economically expand their screening workflows.

The IntelliQube from Douglas Scientific® is designed to address automation and throughput needs by producing accurate and reliable results with walk-away automation that substantially reduces reagent expenditures and labor needs. The IntelliQube is a fully integrated laboratory instrument that combines liquid handling with real-time qPCR analysis in miniaturized reaction volumes. The system utilizes Array Tape in a unique and innovative 384- or 768-well format in place of standard 384-well microplates. Array Tape is a thin and flexible polypropylene consumable that supports miniaturized reaction volumes. In this study, we evaluated the IntelliQube for one-step RT-PCR performance using commercially available RNA, gene expression assays, and a one-step RT-PCR master mix.

MATERIALS AND METHODS

Sample: Purified FirstChoice® Human Liver Total RNA was purchased from Thermo Fisher Scientific (Cat#: AM7960). The RNA was serially diluted in 10-fold increments with molecular grade water to cover a range of 500 ng – 0.5 fg of total RNA per reaction.

Assays and Reagents: TaqMan® Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, Cat#: 4444434) was used to perform the RT-PCR. The master mix was provided at 4X concentration and used according to the manufacturer's recommendations. ROX™ is incorporated into the 4X master mix for normalization. TaqMan probe assays (Thermo Fisher Scientific) targeting β-actin (Hs01060665_g1) and 18S (Hs03003631_g1) were used to assess gene expression. Assays were supplied at a 20X concentration and diluted in master mix to a 2X concentration before use. The final reactions consisted of 1X assay, 1X master mix, and RNA sample.

Instrumentation and Analysis: The IntelliQube, exhibited in Figure 1, was used for PCR reaction set-up, thermal cycling, and real-time fluorescence detection. DNA samples (800 nL) were dispensed into 768-well Array Tape with the multi-channel, pipette head from CyBio® Product Line. Master mix containing 2X assay (800 nL) was dispensed with the non-contact Dispense Jet to create a total reaction volume of 1.6 µL. A total of four replicates per dilution per assay were dispensed into each array. Nine consecutive arrays were run in-line on the IntelliQube to assess stability of the chemistry and consistency of the results over a multi-array protocol. Thermal cycling conditions followed the master mix manufacturer's recommendations. Real-time amplification curves, Cq values, and PCR efficiencies were generated using the IntelliScore® Software.

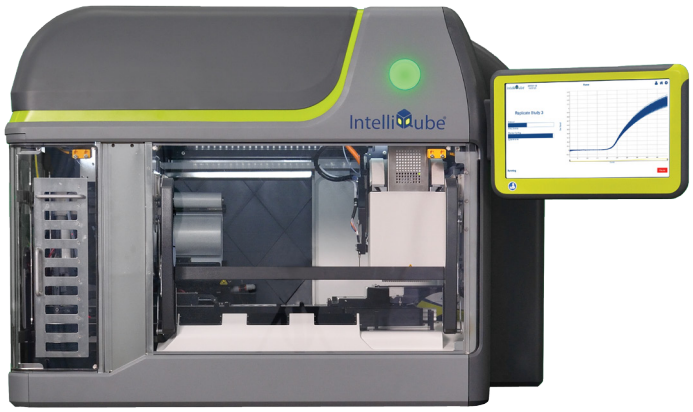

Figure 1: The IntelliQube is a fully integrated liquid handling and real-time quantitative PCR instrument optimized for use with miniaturized reactions in 384- or 768-well Array Tape.

RESULTS AND CONCLUSIONS

One-step RT-PCR was successfully performed on the IntelliQube using commercially available liver RNA and two gene expression assays. The two assays were tested over a concentration range of 500 ng to 0.5 fg of total RNA per reaction. The β-actin and 18S genes showed varying expression levels within the liver RNA sample, with 18S expressed at a higher level for this particular sample. The 18S assay demonstrated a linear dynamic range between RNA concentrations of 5 ng – 5 fg per reaction and an example of the associated amplification curves can be seen in Figure 2. The 18S assay appeared to be impacted by PCR inhibition with RNA concentrations ≥ 50 ng/reaction, likely due to the overabundance of the target template. In comparison to 18S, the β-actin gene was detected at a lower level in the RNA sample, as indicated by higher Cq values, and the linear dynamic range was observed between 50 ng – 5 pg of total RNA, as shown in Figure 2.

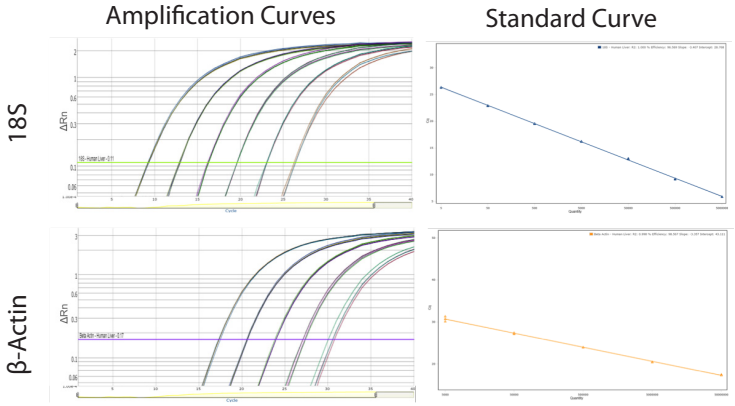

Figure 2: Comparison of the amplification curves and standard curves generated on the IntelliQube for 18S and β-actin assays. The amplification curves for both assays are in the logarithmic scale. The standard curves are generated with the IntelliScore Software. The concentration range shown for 18S is 5 ng – 5 fg of total RNA per reaction. The concentration range shown for β-actin is 50 ng – 5 pg of total RNA per reaction. The plots represent four replicates per dilution per assay.

PCR performance was monitored over the course of nine consecutive arrays processed in an inline, walk-away fashion. During the initial setup, an assay plate containing the required master mix for all arrays was loaded into the IntelliQube. Similarly, a plate containing the required RNA sample was loaded. After starting the protocol, the IntelliQube dispensed and thermal cycled all nine arrays without interaction from laboratory personnel. This nine array protocol was completed twice in independent experiments. The total run time for nine arrays was approximately 7 hours and 15 minutes, which includes time

for liquid handling and Array Tape movement within the instrument. Protocol time is partially dependent on the thermal cycling conditions used. For this particular master mix, the thermal cycling time for 40 cycles was 45 minutes per array. PCR efficiencies, R^2 values for the standard curves, and Cq standard deviations are shown in Table 1. The data highlights the run to run reproducibility and consistency of the instrument over nine consecutive arrays processed continuously in a walk-away protocol. Additionally, the variation of replicates was very low which can be attributed to accurate, consistent dispensing and overall uniformity of the thermal cycling and detection components. A common area of concern when working with RNA is stability of the sample over time. Our data suggests the RNA sample used in this experiment was stable over the course of the protocol. However, the purity of the RNA sample is an important factor and the stability of different RNA samples used in this system may vary.

This study demonstrates the ability of the IntelliQube and associated Array Tape, to process RNA samples using a one-step RT-PCR workflow. Although this study was limited to a few assays and a single sample source, it opens the door to a variety of RNA screening applications including gene expression or viral detection and quantification. In these experiments, data quality was not compromised over the course of nine consecutive arrays, highlighting the ability to process several arrays with limited hands-on time required. The integration of liquid handling, thermal cycling, and detection systems in the IntelliQube enables users to achieve efficient and economical high throughput RNA sample processing in Array Tape for one-step RT-PCR applications.

	Efficiency (%)				Standard Curve R^2				High Concentration Cq STDEV				Low Concentration Cq STDEV			
	18S		β-Actin		18S		β-Actin		18S (5 ng)		β-Actin (50 ng)		18S (5 fg)		β-Actin (5 pg)	
Array	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1	96.1	96.6	98.6	98.6	1.000	0.999	0.999	0.998	0.041	0.018	0.045	0.084	0.081	0.042	0.164	0.375
2	95.9	95.7	96.8	98.3	1.000	1.000	0.998	0.999	0.037	0.038	0.128	0.085	0.088	0.069	0.392	0.165
3	97.0	96.5	97.9	98.2	0.999	0.999	0.999	0.999	0.049	0.037	0.080	0.013	0.028	0.095	0.278	0.283
4	96.4	95.8	97.5	95.7	1.000	0.999	0.999	0.998	0.045	0.084	0.042	0.086	0.047	0.054	0.335	0.297
5	96.4	95.7	96.7	95.1	0.999	1.000	0.999	0.998	0.053	0.070	0.033	0.090	0.041	0.063	0.270	0.332
6	96.1	95.7	95.4	95.1	1.000	1.000	0.999	0.998	0.030	0.034	0.089	0.104	0.013	0.067	0.199	0.313
7	96.5	96.2	96.4	94.5	0.999	0.999	0.999	0.999	0.035	0.060	0.049	0.072	0.059	0.133	0.286	0.198
8	96.0	95.8	96.9	94.9	0.999	0.999	0.999	0.999	0.029	0.160	0.107	0.094	0.046	0.077	0.105	0.100
9	95.4	95.1	95.2	96.2	0.999	0.999	0.999	0.999	0.065	0.030	0.088	0.94	0.056	0.050	0.209	0.222
Average	96.2	95.9	96.8	96.3	0.999	0.999	0.999	0.999	0.043	0.059	0.073	0.080	0.051	0.072	0.249	0.254

Table 1: PCR efficiency, R^2, and Cq standard deviation determined for the highest and lowest concentration of total RNA within the linear range of the standards for 18S and β-actin tested on the IntelliQube. The results for all nine arrays over the two independent runs are shown.