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APPLICATION NOTE

Quantification of RNA Transcripts using One-Step RT-dPCR on Clarity™ Digital PCR System

I. Overview

Information is transcribed from genes through the synthesis of RNA transcripts, either mRNA or other classes of RNA, in the process of gene expression. Gene expression analysis by absolute measurement of RNA transcripts are widely employed in molecular biology and is an important method in diagnostic fields. Several approaches have been developed to quantify RNA transcripts, which include digital PCR-based technologies. In this study, we demonstrate the absolute quantification of RNA transcripts using the Clarity™ digital PCR system with high accuracy and precision.

II. Quantitation of RNase-P transcripts with one-step Reverse Transcriptase Digital PCR (RT-dPCR) using Clarity™ digital PCR system

In this study, one-step RT-dPCR using the Clarity™ system was validated with RNase-P transcripts as the target. RNase-P is a type of ribonuclease that is responsible for generating mature 5′-end of tRNAs by a single endonucleolytic cleavage of their precursors[1,2]. RT-dPCR was performed on a serial dilution of RNA samples obtained by extracting total RNA from HEK293 cells. Using a hydrolysis probe-based SensiFast One-step RT-qPCR mastermix, RNase-P transcripts were amplified with thermal cycling condition as detailed in Table 1. Results showed that one-Step RT-dPCR can be performed in the Clarity™ digital PCR system. The measured RNA transcripts were close to the expected values and relative uncertainties (RU) was ⩽ 10% on each RNA dilution, except for 0.25 copies/µl. Furthermore, the results on Table 2 showed that the measured concentrations were of RNA origins as no-RT controls did not produce any positive signals. Additionally, commercially available mastermixes from 3 other vendors were also tested for their compatibilities with the Clarity™ digital PCR system. Results showed that mastermixes from different vendors are compatible with Clarity™ digital PCR system for performing RT-dPCR.

Table 1. Thermal cycling condition for one-step RT-dPCR using Clarity™ digital PCR system.

Steps	Cycle (s)	Temperature (°C)	Time (s)
Reverse Transcription	1	45*	1200
Polymerase Activation	1	95	300
Denaturation	40	95	50
Annealing and Extension		58	90
Final Hold	1	70	300

*Temperature used can be adjusted to manufacturer's recommendation

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Table 2. Detection of RNase-P transcripts using Clarity™ digital PCR system. Three independent experiments were performed in duplicates with no-template-controls (NTCs) and no-RT-controls using SensiFast One-Step RT-qPCR mastermix. RNA eluent from HEK293 cells was serially diluted from 2500 copies/µl down to 0.25 copies/µl. Measured concentrations are provided in copies/µl reaction mix.

Expected Concentration (copies/µl)	RT-dPCR		No-RT Control	
	Measured Concentration (copies/µl)	RU (%)	Measured Concentration (copies/µl)	RU (%)
2500	2417.51	3.67	0	N.A.
250	272.8	0.63	0	N.A.
25	28.63	1.67	0	N.A.
2.5	2.39	0.87	0	N.A.
0.25	0.21	13.33	0	N.A.

Figure 1. Measured RNA concentrations against its expected concentrations. Results showed high accuracy and excellent linearity for quantitation of RNA from 2500 copies/µl down to 0.25 copies/µl.

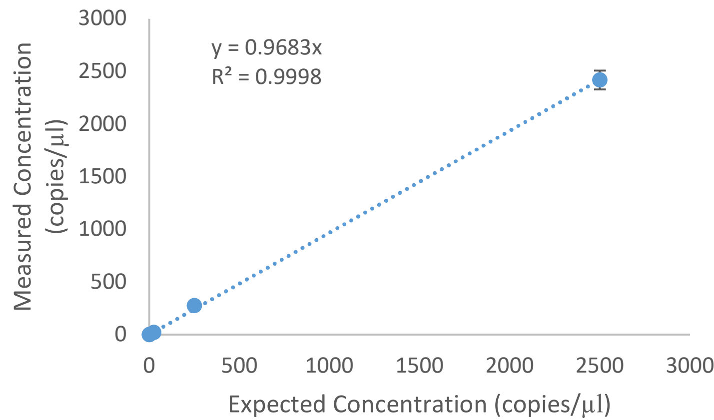

Table 3. Detection of RNase-P transcripts using four different commercial mastermixes. Serially-diluted RNA was quantified using four commercial mastermixes. Measured concentrations are provided in copies/µl reaction mix.

Expected Concentration (copies/µl)	Bioline SensiFast Probe No-ROX One-Step Kit (copies/µl)	RU (%)	Promega AccessQuick RT-PCR System (copies/µl)	RU (%)	Thermo Fisher TaqMan Fast Virus 1-Step kit (copies/µl)	RU (%)	NEB Luna Universal Probe One-Step RT-qPCR kit (copies/µl)	RU (%)
250	272.80	0.63%	291.80	2.73%	297.47	0.26%	282.40	3.67%
25	28.63	1.67%	26.07	8.09%	27.54	9.49%	27.35	1.69%
2.5	2.39	0.87%	1.25	18.48%	2.48	13.63%	2.42	1.75%
0.25	0.21	13.33%	0.14	22.24%	0.23	41.72%	0.25	19.21%
0.25 (No RT)	0	N.A.	0	N.A.	0	N.A.	0	N.A.
NTC	0	N.A.	0	N.A.	0	N.A.	0	N.A.

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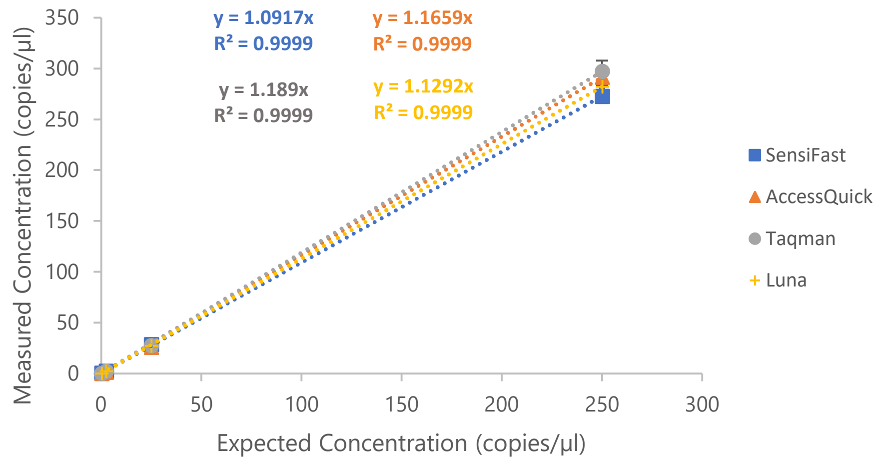
Figure 2. Measured RNA concentrations against its expected concentrations of RNase-P transcripts using four different commercial mastermixes. Results showed that different mastermixes provide high accuracy and excellent linearity for quantitation of RNA from 250 copies/µl down to 0.25 copies/µl.



III. Conclusion

In this study, we demonstrated that the Clarity™ digital PCR system offered a sensitive and accurate way for the quantitation of RNA transcripts using one-Step RT-dPCR, where trace amount of RNA transcripts of as low as 0.25 copies/µl could be detected. Additionally, four different commercial mastermixes have been tested to work with comparable performances (p-value = 0.999, $p > 0.05$) in Clarity™ Digital PCR system, which opens up the possibility for flexible assay design and clinical applications, such as gene expression analysis and mutation detection.

References

1. Guerrier-Takada C, Gardiner K, Marsh T, Pace N, Altman S (1983). The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell. 35 (3 Pt 2): 849–57.
2. Frank DN, Pace NR (1998). Ribonuclease P: unity and diversity in a tRNA processing ribozyme. Annu. Rev. Biochem. 67: 153–80.

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