Use of Oragene®•RNA samples in the development of ReaX™ Mastermix Beads for a two step RT-PCR

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RNA samples collected using the Oragene®•RNA kit were used in combination with Q Chip's two step RT-PCR ReaX™ mastermix beads and successfully employed in a qRT-PCR reaction to amplify the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase.

Introduction

Quantitative Reverse Transcription (RT) PCR is increasingly used as a method for the detection and quantification of RNA targets especially in the area of molecular diagnostics. However, despite its popularity, the process can be time consuming, prone to contamination and requires skilled technicians. As there is a clear requirement in a clinical environment for reliable and reproducible data, stream lining the workflow offers several advantages including reduced risk of contamination and increased throughput.

Collecting human RNA is usually a complicated and invasive technique, involving sourcing from blood or biopsy. These traditional supplies present difficulties in sample collection, storage and transport. The Oragene•RNA self-collection kit is designed to provide an easy non-invasive method for collection of high quality RNA from saliva. For sample collection, the donor delivers 2 mL of saliva into the Oragene•RNA collection vial. Once the kit is capped, the Oragene•RNA solution is released and the RNA is stabilized at room temperature for up to 8 weeks.

ReaX bead-based mastermixes are enhanced PCR formulations which simplify PCR assays and provide robust reproducible results. A new addition to the ReaX product line is a two step RT-PCR bead system. The ReaX RT bead supplies all the reagents required to reverse transcribe RNA to cDNA. Each encapsulated RT bead contains RT enzyme buffer, $MgCl_2$ and dNTPs along with a reverse transcriptase enzyme; at final reaction volumes. A second bead, the ReaX mastermix PCR bead, is then used to amplify the cDNA in an endpoint or real-time PCR.

This application note demonstrates that the combination of Oragene•RNA self-collection kits, ReaX RT and ReaX mastermix beads in a two step RT reaction, enables a simplified, streamlined workflow, delivering reliable and reproducible molecular diagnostic assays.

Materials and methods

The functionality of the Oragene•RNA ReaX system for RT-PCR was tested using a qPCR assay for the housekeeping gene GAPDH (glyceraldehyde 3-phosphate dehydrogenase). A primer set and a Taqman® probe for GAPDH were added to the ReaX mastermix bead in the second step of the two step RT-PCR. The composition of each of the beads in the ReaX RT-PCR system is summarized in Table 1.

RT Bead (20 µL reaction)	ReaX Mastermix bead (25 µL reaction)
1× RT buffer	800 µM dNTPs (200 µM of each)
6 mM $MgCl_2$	1× ReaX PCR buffer
500 µM dNTPs	1 unit of hot start Taq polymerase
200 U MULV reverse transcriptase	5 mM $MgCl_2$

***Table 1**: ReaX bead compositions*

Saliva samples were collected from donors and purification of RNA was carried out according to the protocol supplied with the Oragene•RNA kit. For each sample, the RNA yield obtained was assessed by measuring the optical density of the RNA at 260 nm wavelength using a spectrophotometer. For the reverse transcription step of the RT-PCR, 2 µg of total RNA, 2 µL of oligo (dT)15 primers and 4 µL of molecular biology grade water were combined, heated to 70°C for 5 minutes and cooled on ice for 5 minutes. 5 µL of this reaction

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was then added to a ReaX RT bead and molecular biology grade water was added to give a final volume of 20 µL. This was then placed in a Mastercycler Realplex2® qPCR thermal cycler (www.eppendorf.com) and subjected to the following program: 25°C for 5 minutes; 42°C for 60 minutes; 70°C for 15 minutes.

For the PCR step of the RT-PCR, 0.5 µL of the cDNA (made in the first step) was added to a ReaX Mastermix bead (5 mM $MgCl_2$), 0.4 pmol of each primer and 0.1 pmol of the GAPDH Taqman probe. Molecular biology grade water was added to give a final volume of 25 µL. Each sample was then placed in the Mastercycler Realplex2 qPCR thermal cycler and the following program was carried out: 94°C for 120 seconds; 95°C for 15 seconds; 58°C for 60 seconds. The last two steps were repeated 39 times. Liquid equivalent and no template controls were run alongside these reactions.

Results

The qPCR amplification curves for each Oragene•RNA ReaX RT-PCR reaction are given in Figure 1.

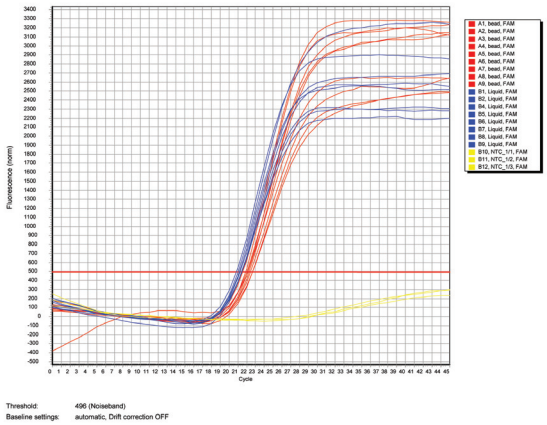

***Figure 1**: Oragene•RNA sample – ReaX RT-PCR amplification curves*

***Red** – amplification of GAPDH in a ReaX RT-PCR reaction*
***Blue** – amplification of GAPDH in a liquid RT-PCR reaction*
***Yellow** – no template controls*

Conclusions

The results demonstrate that a two step RT-PCR reaction can be simplified and streamlined using an Oragene•RNA/ReaX system for two step RT-PCR and that the results are equivalent to those obtained using a standard liquid two step RT-PCR reaction. Additionally, using an Oragene•RNA/ReaX system may reduce the need to repeat assays since all the assay components are pre-dispensed. The risk of contamination may also be reduced.