ReaX™ beads for convenient real-time PCR

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ReaX beads simplify PCR setup by containing all of the reagents necessary to perform PCR: PCR buffer, dNTPs, Taq polymerase, and Magnesium chloride. They are compatible with the Spartan DX™ desktop DNA analyzer, and may be used for end-point PCR, real-time PCR, multiplex PCR, and reverse transcription applications.

Introduction

Q Chip's ReaX™ mastermix beads are stable at room temperature and contain all of the reagents necessary for PCR and real-time PCR, except for primers, probes, and DNA template. Each reaction uses a single bead and the bead melts to become part of the reaction during the first few minutes of the thermal cycle program. Less user manipulation reduces pipetting errors, shortens reaction setup time, and standardizes reagent concentrations.

The purpose of this study was to determine the compatibility of ReaX beads with three real-time PCR assays on the Spartan DX: (1) *Neisseria gonorrhea* (NG), (2) Varicella zoster virus (VZV), and (3) human muscarinic acetylcholine receptor subtype M3 gene (*CHRM3*).

Materials and Methods

DNA extraction

Human DNA was extracted from whole blood, and NG DNA was extracted from clinical samples. For VZV, an artificial DNA template was generated by synthesizing a 78-bp single-stranded oligonucleotide, corresponding to a fragment from the ORF62 region of the VZV genome. Reverse transcription was used to convert the single-stranded oligonucleotide into a double-stranded template.

Real-time PCR

Oligonucleotide primers were designed against conserved regions of the human muscarinic acetylcholine receptor subtype M3 gene (*CHRM3*; Ref 1), the Varicella zoster virus *ORF62* gene (VZV; Ref 2), and *Neisseria gonorrhoeae porA* pseudogene (NG) using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Sequences can be located in Table 1.

Components of the PCR amplification mixtures are listed in Table 2, and cycling parameters are listed in Tables 3-5. Note that a two-temperature cycling program was performed by combining the annealing and extension steps. Reactions were performed in 0.2 ml thin-wall, flat-cap PCR tubes (VWR, Cat. No. 53550-106). Reactions were topped with 15 µl of mineral oil (Biotools, Cat. No. 20.032) to prevent evaporation, and real-time PCR was performed using the Spartan DX instrument. All PCR programs used an initial denaturation step of 5 min to activate the hotstart polymerase in the beads.

DNA analysis

Fluorescence results from the Spartan DX were downloaded to a computer and graphed using the Spartan Analyzer software. In addition, real-time PCR results were confirmed by agarose gel electrophoresis using 5 µl of the amplification

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
CHRM3 primers	TTg ggT CAT CTC CTT Tg	gCA CAg TTC TCT TTC CA	74
CHRM3 probe	6-FAM-TCC TTT ggg CTC CTg CCA TCT-BHQ1		
NG *PorA* primers	TTg gCg gCT CAg TTg gAT TT	ATC gAC ACC ggC gAT gAT TT	155
NG *PorA* probe	6-FAM-CCC gCg CAT CAg CTA TgC CCA-BHQ1		
VZV *ORF62* primers†	CCT Tgg AAA CCA CAT gAT CgT	AgC AgA AgC CTC CTC gAC AA	78
VZV *ORF62* probe†	6-FAM-TCg AAC CCg ggC gTC Cg-BHQ1		

6-FAM = 6-carboxy-fluorescein, BHQ1 = Black Hole Quencher 1

† Cohrs RJ, Gilden DH.(2006).Prevalence and abundance of latently transcribed varicella-zoster virus genes in human ganglia. *J. Virol.* 81(6): 2950-6.

Table 1. Primer/probe sequences and amplicon sizes.

Component	Final amount
1 ReaX master mix bead (1.5 mM $MgCl_2$) (Q Chip)	1 X
$MgCl_2$ (Invitrogen)	2.5-5.75 mM*
TaqMan probe (Biosearch Technologies)	0.1 µM
PCR primers (Biosearch Technologies)	0.5 µM each
Template DNA	2.5 µl
Sterile water	
Total reaction volume	**25 µl**

* 2.5 mM $MgCl_2$ was used for NG testing, 4 mM was used for VZV, and 5.75 mM was used for *CHRM3*.

Table 2. Components of PCR amplification mixture.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	300 s	1
Denaturation	95°C	28 s	50
Annealing/extension	44°C	20 s	50

Table 3. Cycling parameters for NG program.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	300 s	1
Denaturation	97°C	24 s	50
Annealing/extension	45°C	16 s	50

Table 4. Cycling parameters for VZV program.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	300 s	1
Denaturation	95°C	30 s	50
Annealing/extension	58°C	55 s	50

Table 5. Cycling parameters for *CHRM3* program.

products (data not shown).

Results

All three real-time PCR assays worked successfully with the ReaX beads (Fig 1-3). Agarose gel electrophoresis revealed no non-specific amplification products. Including the initial 5-min hotstart step, the total PCR run-times were 76 min, 41 min, and 35 min for the *CHRM3*, NG, and VZV assays, respectively.

Discussion and Conclusions

The ReaX beads yielded successful real-time PCR results for human, bacterial, and viral assays. For PCR setup, the beads significantly reduced the number of manual pipetting steps, and shortened the time to assemble the reagents. In summary, ReaX beads are a reliable and convenient way to set up

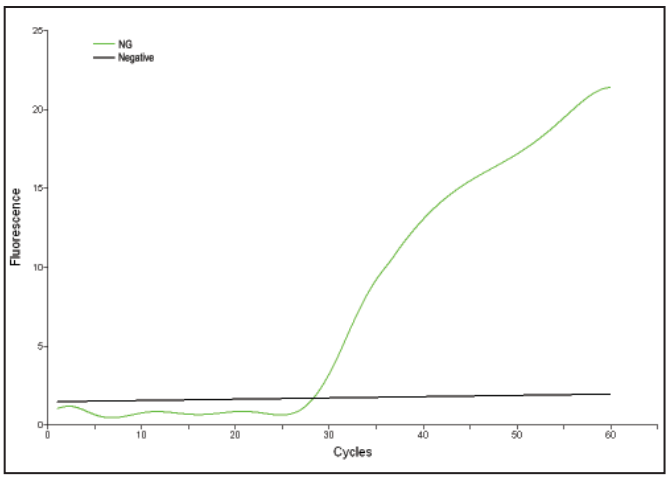

Figure 1. Real-time PCR results for *Neisseria gonorrhea* .

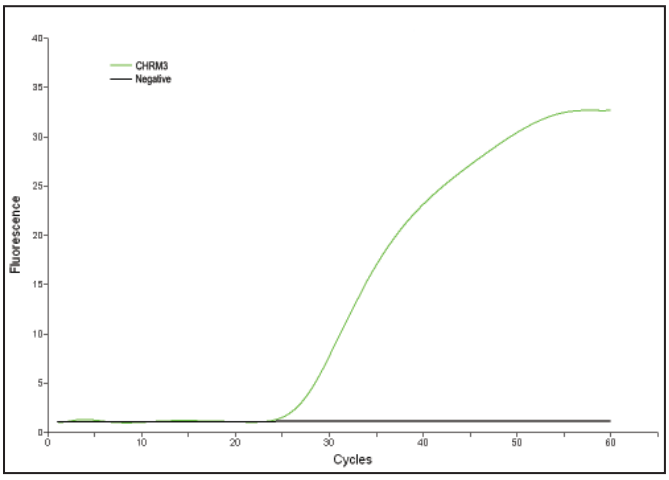

Figure 2. Real-time PCR results for *CHRM3*.

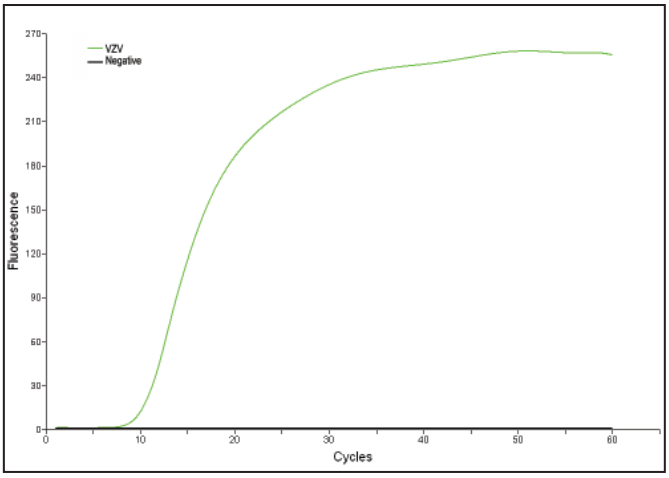

Figure 3. Real-time PCR results for VZV .

References

1. Prevost SL, Arbour NA, Sowers B, Harder CJ. (2007). Fluorophores compatible with the Spartan DX™. *Spartan Bioscience*. AN0021 Ver 1.2 October 2008.

2. Spartan Bioscience. *Protocol: Real-time PCR for Varicella Zoster Virus*. Online. Lit#P0012.