Adenovirus Type B Detection Using the Primerdesign genesig® Kit and xxpress® qPCR Thermal Cycler

The Primerdesign genesig® Adenovirus Type B (AdVB) Kit is designed for the *in vitro* quantification of AdVB genomes. The kit is designed to remain specific to this genome whilst offering the broadest detection profile possible. This paper investigates the performance of this kit as well as its compatibility with the ultrafast xxpress® qPCR thermal cycler through performing standard dilution series and multiplex qPCR experiments.

1. Introduction

Human Adenovirus (HAdV) is caused by the *Adenoviridae* family of viruses. This group of viruses are medium-sized and known for having a double-stranded DNA genome and a nonenveloped icosahedral nucleocapsid (Wieczorek *et al*., 2015). They are classified into 7 subgroups (A-G), each causing various infectious symptoms. Type B (AdVB) is the second most abundant and known for manifesting respiratory and urinary symptoms (Bibby and Peccia, 2013). The prevalence of AdVB, along with the severity of the symptoms it causes, makes it of major public health importance. Consequently, fast and accurate detection of this virus is necessary (Laconelli *et al*., 2017).

Primerdesign offers an ideal solution, see Figure 1. Their genesig® Adenovirus Type B Kit targets the hexon gene, shown in previous real-time PCR-based studies to be a good genetic marker (Hernroth *et al*., 2002). The primer and probe sequences were chosen based on the results of a comprehensive bioinformatics analysis and have 100% homology with an extensive range of clinically relevant reference sequences.

Figure 1: The genesig® Adenovirus Type B Kit by Primerdesign.

The xxpress® is the perfect qPCR thermal cycler to perform these detection studies on. This open platform performs ultrafast thermal cycling with multiplexing capability in up to 5 channels. This paper investigates the performance of the Primerdesign genesig® AdVB Kit as well as its compatibility with the xxpress® qPCR thermal cycler.

2. Method

Standard Dilution Series

First, a standard dilution series was performed as outlined in the kit instructions, using the thermal protocol detailed in Table 1. The reaction mixture used was as follows: 15µL precision multiplex 1-step RT-qPCR master mix, 6µL nuclease free water, 1.5µL AdVB primer/probe mix, 7.5µL AdVB positive control template dilution.

The positive control template provided in the kit was used to create 10-fold template dilutions ranging from $2x10^5$ copies/µL to 2 copies/µL. Each dilution was performed in triplicate alongside 3 no template control reactions. A final reaction volume of 10µL per well was run on a 54 well xxplate™ SBS.

Step	Temperature	Dwell	Repeats
Enzyme Activation	95 °C	120 seconds	x1
Denaturation	95 °C	10 seconds	x40
Annealing, Extension	60 °C	60 seconds	

Table 1. Table detailing the thermal protocol used with the genesig AdVB Kit on the xxpress® qPCR thermal cycler.

Multiplex

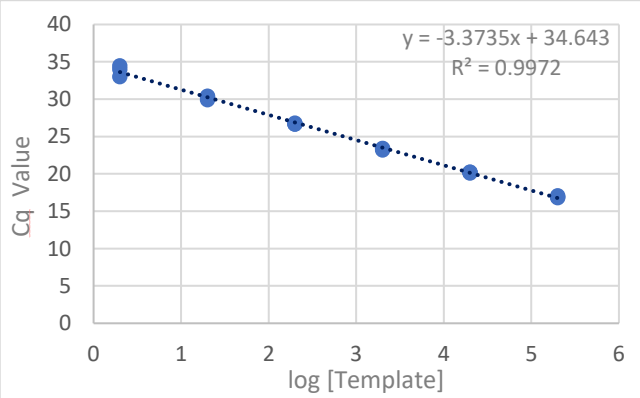
Secondly, the simultaneous detection of the AdVB hexon gene and an internal extraction control gene was performed using the positive samples provided in the kit and the thermal protocol outlined in Table 1. The reaction mixture was as follows: 15 µL precision multiplex 1-step RT-qPCR master mix, 4.5µL nuclease free water, 1.5µL AdVB primer/probe mix, 1.5µL internal extraction control primer/probe mix, 3.75µL AdVB positive control template, 3.75µL internal extraction control DNA.

The reaction was performed in triplicate alongside 3 no template control reactions. A final reaction volume of 10µL per well was run on a 54 well xxplate™ SBS. The hexon gene was detected in the FAM channel (channel 1 with the xxpress® qPCR thermal cycler) and the internal extraction control gene was detected in the VIC channel (channel 2 with the xxpress® qPCR thermal cycler).

3. Results

Standard Dilution Series

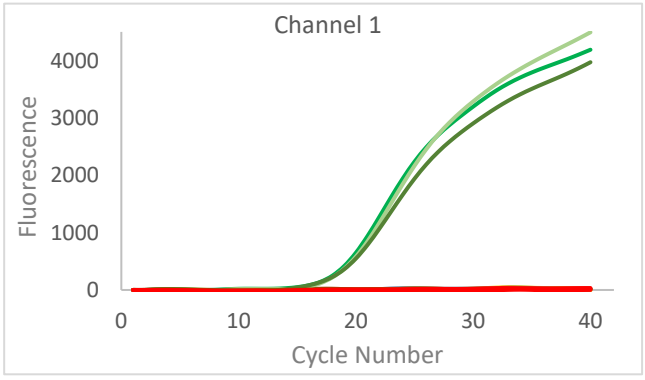
The performance of the kit with the xxpress® was very good. The Cq values for each reaction were plotted against the log of the template concentration to produce a standard curve. This can be seen in Graph 1. The efficiency of the reaction was 98% and the R^2 was 0.9972. The results show that the detection limit of the kit when used with the xxpress® is also very good; as little as 2 copies/µL can be detected.



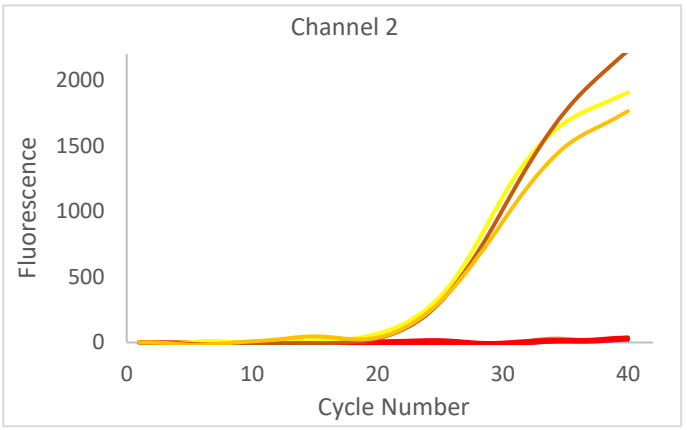
Graph 1: Standard curve graph produced using the genesig® AdVB kit and the xxpress® qPCR thermal cycler.

Multiplex

The multiplex reaction was also successful and both the hexon gene and internal extraction control gene were detected. The run took 57 minutes to complete using the thermal protocol recommended in the Primerdesign kit manual. A faster protocol was also successful and reduced the total run time by >20%.



Graph 2: Amplification curve in channel 1 for the AdVB hexon gene when PCR was performed using the Primerdesign genesig® AdVB Kit and the xxpress® qPCR thermal cycler. Red = no template control replicates.



Graph 3: Amplification curve in channel 2 for the internal extraction control gene when PCR was performed using the Primerdesign genesig® AdVB Kit and the xxpress® qPCR thermal cycler. Red = no template control replicates.

4. Conclusions

The results of this investigation indicate that the Primerdesign genesig® AdVB Kit is very compatible with the xxpress® qPCR thermal cycler, shown in figure 2. This is demonstrated by the high efficiency and repeatability of the qPCR reaction as well as the low limit of detection.

The multiplexing ability of the xxpress® makes the simultaneous detection of the AdVB hexon gene and internal extraction control gene possible too.

Figure 2: The ultrafast xxpress® qPCR thermal cycler by BJS Biotechnologies.

5. References

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