Detecting Residual CHO Host Cell DNA Using the Agilent Mx3005P QPCR System

Application Note

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Abstract

Chinese Hamster Ovary (CHO) cells are commonly used as a host system for the production of human therapeutic proteins. An important part of the purification process is the removal of host cell material such as DNA, which poses a safety concern if administered along with the therapeutic compound. Quantitative real-time polymerase chain reaction (qPCR) is an affordable, sensitive, and rapid method for the detection of residual CHO host cell DNA using sequence-specific primers and probe. In this application note, we demonstrate the use of a commercially available kit for CHO DNA detection, the resDNASEQ Quantitative CHO DNA Kit (Life Technologies), on Agilent's Mx3005P QPCR System. This method produces more specific, more sensitive, and faster results than using the traditional methods such as blot hybridization.

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Introduction

Recombinant protein production in mammalian cells is a widely established industry, of which CHO-based therapeutics account for a large proportion. This is because CHO cells have been well-characterized and successfully used to manufacture an array of approved biologics. One of the regulations that govern the use of mammalian host systems is the requirement to completely remove host cell DNA. While the effects of residual DNA contamination are still unclear, the transfer of host cell genetic material can be potentially harmful to the patient. According to the Food and Drug Administration (FDA) guidelines, the DNA content in the final product should be as low as possible as determined by the most sensitive method, with the maximum acceptable amount of residual host DNA being 100 pg/therapeutic dose[1].

Traditional methods for the detection of species-specific DNA such as blot hybridization are laborious and lack sensitivity[2]. In contrast, qPCR-based assays are very sensitive, specific, and produce results in less than three hours. Such assays have thus become the method of choice for detection of residual host cell DNA. The Mx3005P qPCR instrument provides superior uniformity, thermal accuracy, and sensitivity, making it an ideal system for quantitating small amounts of residual CHO DNA.

Methods

The CHO DNA standards were prepared and qPCR assay performed according to the manufacturer's instructions provided with the resDNASEQ Quantitative CHO DNA Kit. Briefly, six 10-fold serial dilutions of control CHO DNA were prepared using DNA dilution buffer such that the final amount of CHO DNA in each reaction ranged from 3000 pg–0.03 pg. Forward and reverse primers and a FAM-labeled probe that were designed to amplify a hamster-specific region of a multi-copy gene were provided. Each 30-µl PCR reaction mix contained 2 µl of negative control, 3 µl of 10x primer/probe mix, 15 µl of 2x Environmental Mastermix, and 10 µl of diluted CHO DNA. All reactions were performed in triplicate on the Agilent Mx3005P QPCR System and the ABI 7500 Fast Real-Time PCR System using the following cycling conditions: 10 minutes at 95 °C to activate the enzyme, followed by 40 cycles of 15 seconds at 95 °C and then 1 minute at 60 °C. A replicate experiment was performed on the Mx3005P system separately.

Results and Discussion

Biopharmaceuticals contain variable amounts of residual CHO DNA at different stages of manufacturing. Therefore, it is necessary for the assay and the instrument to have the dynamic range and sensitivity to detect both large and small amounts of contaminating host DNA. Figures 1A and 1B demonstrate the performance of the resDNASEQ assay on the Mx3005P instrument, where CHO DNA standards were diluted 10-fold from 0.3 ng–3 fg. The R squared (Rsq) value, which denotes linearity, was 1.00, indicating that the efficiency of amplification was consistent across a range of concentrations. The efficiency of the amplification was calculated to be 99.5 percent.

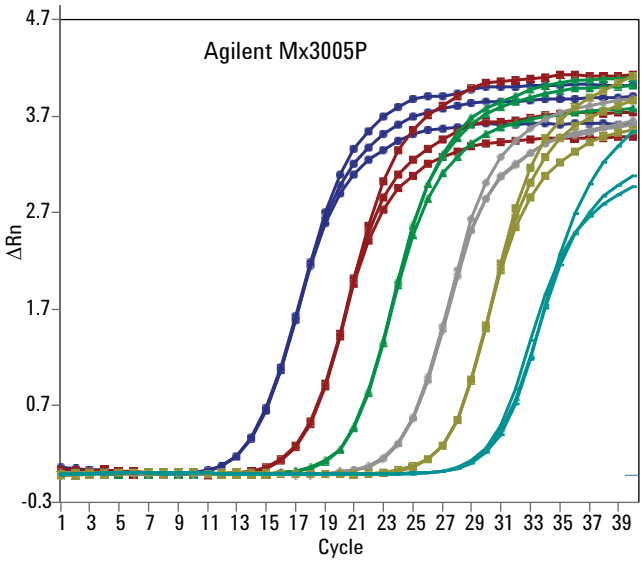

Figure 1A. *Amplification plots generated from running the resDNASEQ assay with 10-fold serial dilutions from 3000 pg–0.03 pg. The plots show the dynamic range and sensitivity of the assay when run on the Agilent Mx3005P QPCR System.*

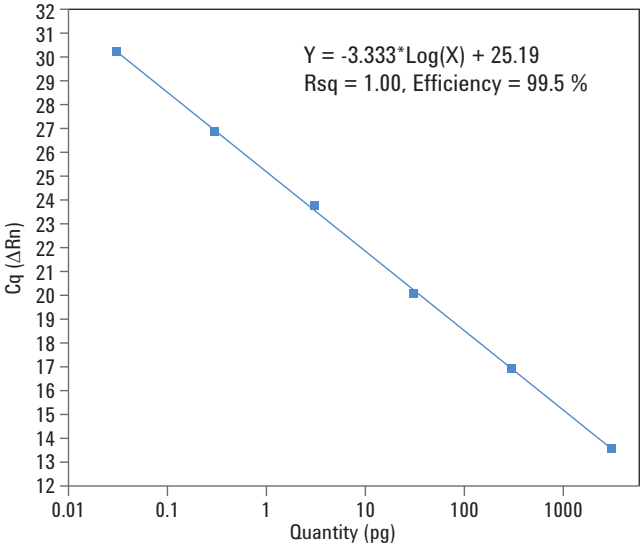

Figure1B. *Standard curve generated from data in Figure 1A with Rsq and efficiency values indicated.*

The same standard curve was quantitated on the ABI 7500 Fast instrument and results are shown in Figures 2A and 2B. The assay performed similarly as on the Mx3005P, with Rsq being 1.00 and efficiency of amplification being 99.66 percent.

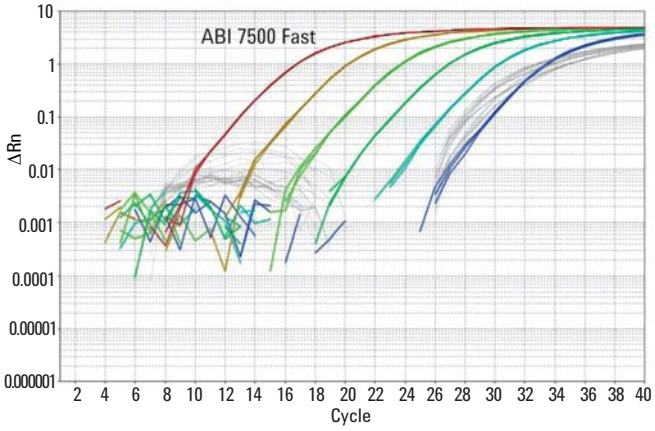

Figure 2A. *Amplification plots generated from running the resDNASEQ assay with 10-fold serial dilutions from 3000 pg–0.03pg. The plots show the dynamic range and sensitivity of the assay when run on the ABI 7500 Fast Real-Time PCR System.*

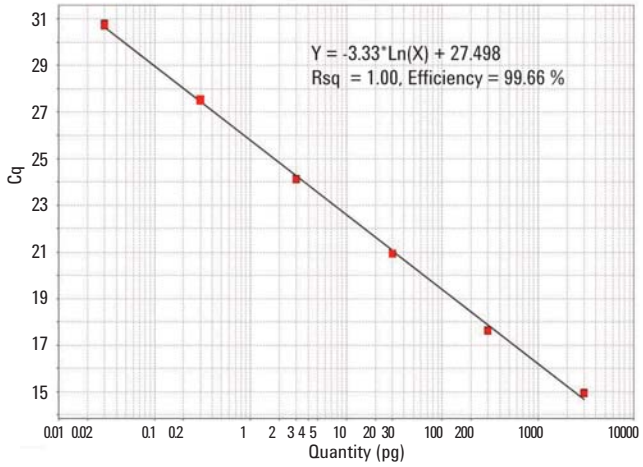

Figure 2B. *Standard curve generated from data in Figure 2A with Rsq and efficiency values indicated.*

To determine reproducibility, the CHO DNA standard dilutions were prepared fresh and quantitated using the Mx3005P on a separate occasion. Figure 3 shows both curves, with Standard 1 having a Rsq value of 1.00 and efficiency of 99.5 percent and Standard 2 having a Rsq value of 1.00 and efficiency of 101.8 percent. Hence, we show that residual host CHO DNA is quantitated reproducibly using the Agilent Mx3005P QPCR System.

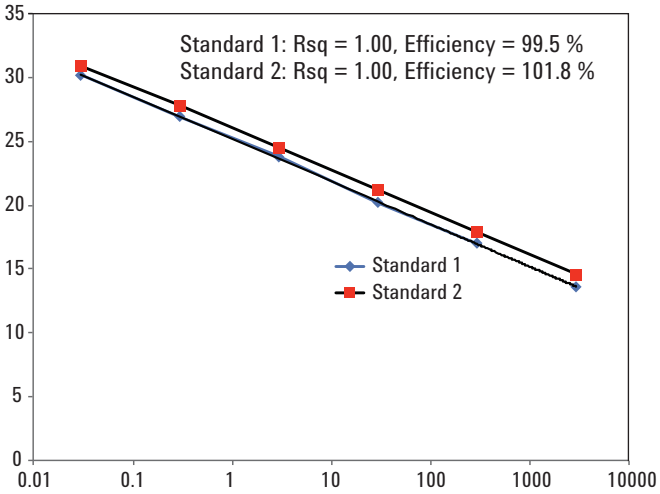

Figure 3. *Two standard curves were generated from 10-fold serial dilutions of CHO DNA from 0.3 ng–3 fg using the Mx3005P on separate occasions. The Rsq and efficiency values are indicated for both standards, showing reproducibility of the assay when run on the Mx3005P system.*

Conclusion

This study validates the performance of the resDNASEQ Quantitative CHO DNA Kit using the Agilent Mx3005P QPCR System and shows that residual CHO DNA in recombinant protein therapeutics can be accurately and reproducibly quantitated by the Mx3005P, even when present in low amounts. While the manufacturers of this assay recommend the use of the ABI 7500 Fast, it has been demonstrated here that linearity and efficiency of amplification are similar for both the Mx3005P and the ABI 7500 Fast. Compared to traditional methods, quantitating residual CHO DNA using the Agilent Mx3005P QPCR System produces more specific, more sensitive, and faster results. This study shows that residual CHO DNA in recombinant protein therapeutics can be accurately and reproducibly quantitated by the Mx3005P, even when present in trace amounts. While the manufacturers of this assay recommend the use of the ABI 7500 Fast platform, it has been demonstrated here that linearity and efficiency of amplification are similar for both the ABI 7500 Fast and the Mx3005P. This study validates the performance of the ResDNASEQ Quantitative CHO DNA Kit using the Agilent Mx3005P QPCR System, which compared to traditional methods, produces more specific, more sensitive, and faster results.

References

1. US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research, Feb 28, 1997.
2. Lee, D.H., Jung, E.B., Jung, H.L., Jeong, S.S., In, S.K. (2010) **Quantitative detection of E.coli host cell DNA by real-time PCR.** *J. Microbiol. Biotechnol.* 20(10): 1463–1470.

Ordering Information

Agilent Mx3005P QPCR System	
Description	**Part Number**
Mx3005P QPCR System (110v) with notebook computer	401449
Mx3005P QPCR System (110v) with desktop computer	401456
Mx3005P QPCR System (230v) with notebook computer	401457
Mx3005P QPCR System (230v) with desktop computer	401458

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