Streamlined High Performance Extraction and Quantitation of Host Cell Residual DNA in Bioproduction

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ABSTRACT

Residual host cell DNA is a common process-related impurity that is typically co-monomed with host cell protein. We have previously reported on a quantitative PCR (qPCR) based assay for monitoring host cell proteins (A customizable, qPCR-based immunoassay platform for accurate quantitation of protein impurities and contaminants). Here we report on high performance Host Cell Residual DNA extraction and quantitation using a streamlined and high throughput semi-automated workflow. Sample preparation and extraction is achieved in under two hours using a sample preparation kit based on antibody coated magnetic beads and a magnetic processing instrument. Sample quantitation is carried out on a real-time PCR instrument. Recoveries of greater than 85% are obtained from a standard spiked solution with CV% less than 10% for Chinese Hamster Ovary (CHO) host cell DNA. Excellent linearity is obtained for concentration ranges 3 pg/mL to 300,000 pg/mL of CHO residual DNA.

INTRODUCTION

Ensuring the safety of biologic drugs requires rapid and accurate screening methods for in-process testing during manufacturing. High-throughput, automated approaches offer the advantage of processing many samples quickly and reliably. The use of functionalized magnetic bead technology to capture analytes of interest and purify them for sensitive and selective detection of target compounds. This technology has been incorporated into industry standard Applied Biosystems™ PrepSEQ™, resDNASEQ™ kits and ProSEQ™ kits and is expanded/labeled to a 96-well plate format. For automated sample analysis, the Thermo Scientific™ KingFisher Flex™ Magnetic Particle Processor is a processor platform with real-time quantitation of magnetic bead based DNA purification impurities and contaminants using these specialized sample preparation and quantitation kits. The KingFisher Flex instrument is ideal for high throughput processing of functionalized magnetic particles. The patented technology of the KingFisher Flex employs 96-well plates and magnetic rods containing uniquely designed disposable tip combs. Samples mixed with functionalized magnetic beads and reagents are dispensed into plate wells, and the tip combs are automatically loaded when the run begins. The instrument collects magnetic beads from solution and then releases the beads into plate wells containing reagents for the next step or reaction. The elution, wash, and elution steps are dispensed and collected to transfer beads without washing, effluent elimination, and rapid processing.

MATERIALS AND METHODS

The KingFisher Flex instrument was used to prepare CHO DNA from various samples in a high throughput mode using PrepSEQ kit chemistry and magnetic beads. Host cell DNA quantitation was carried out using real-time PCR technology on the Applied Biosystems™ 7500 PCR system along with components of the resDNASEQ Quantitative CHO DNA Residual DNA Sequencing™ Assay software was used to process the data. Samples were prepared in the following matrices.

- CHO: 3 pg/mL, 10 pg/mL, 100 pg/mL, 10 pg/mL (CHO DNA per well).
- M4 Matrix: 10 pg/mL, 10 pg/mL, 10 pg/mL (CHO DNA per well).
- M5 Matrix: 10 pg/mL, 10 pg/mL, 10 pg/mL (CHO DNA per well).

RESULTS

The figure below shows the standard curve of amplified CHO DNA generated for the five dilutions. Each point is an average of triplicate experiments. The standard curve had a slope of -3.37, demonstrating the high efficiency of the PCR reaction. The standard curve also gave an R2 value of 0.998, which confirmed linearity over the range of 0.05 pg CHO DNA per well to 1000 pg CHO DNA per well. The standard curve can be used to determine recovery levels from the spiked samples that were extracted from matrix using the KingFisher Flex instrument and PrepSEQ kit DNA Residual DNA Sequencing™ Assay.

The table below shows the recovery efficiency of CHO residual DNA from CHO DNA spiked at 100 pg, 10 pg, and 1 pg in buffers containing 50 or 100 mg of IgG protein. Triplicate samples were analyzed in quadruplicate and some of the data shown in the above table. The data show that the DNA was recovered with very high efficiency using the automated procedure, with sample replicates ranging from 90% to 100% recovery. To demonstrate that only one PCR reaction was performed for each extracted DNA sample, the CV% values ranged from 0% to 7% demonstrating excellent reproducibility across the samples analyzed.

SUMMARY

The KingFisher Flex magnetic particle processor can be used with PrepSEQ kits to extract small amounts of residual host cell DNA from complex mixtures in a high-throughput mode. Samples can be processed in a 96-well plate format in under 2 hours and quantitated using resDNASEQ CHO Residual DNA kits and real-time PCR. The sample preparation procedure can effectively extract DNA from a variety of matrices including a solution containing 100 mg/mL IgG. This high recovery rate is consistent for spiked DNA amounts ranging from 1 pg to 100 pg/mL and is also consistent among different instruments. Additional experiments have produced similar performance with other cell line sample types.

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