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-monitored with host cell protein. We have previously reported on a quantitative PCR (qPCR) based assay for monitoring host cell proteins (A customisable, 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 particle 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 observed for concentration ranges 3 pgM to 300,000 pgM 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 allows for sensitive and selective detection of target compounds. This technology has been incorporated into industry standard Applied Biosystems™ PrepSEQ™, realDNASEQ™ kits and KingFisher® platform and is extensively applied to 96-well plate format. For automated sample analysis, the Thermo Scientific™ KingFisher Flex™ Magnetic Particle Processor is available with real-time quantitation features that provide fast, high-throughput quantitation of residual DNA samples 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, each containing a precisely designed disposable tip comb. Samples mixed with functionalized magnetic beads and reagents are dispensed into plate wells, and the tip comb is 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 in the isolation. The final step in the process is an automatic wash and transfer beads to leders, efficient elution, and rapid processing.

The KingFisher Flex instrument is designed for automated transfer and processing of magnetic particles in microplate/deep well format. The patented technology of the KingFisher Flex is based on the use of magnetic rods covered with a disposable, specially designed tip comb. The KingFisher Flex instrument holds up to 96 deep well plates. The instrument function without any dispensing or aspiration parts or devices. Samples and reagents including magnetic particles are dispensed into the plates and run from a specially designed protocol that is selected via the keypad.

RESULTS
The figure below shows the standard curve of amplified CHO DNA generated for the five dilutions. The standard curve had a slope of -3.27, demonstrating the high efficiency of the PCR reaction. The standard curve also gave an R2 value of 0.989, which reflected linearity over the range of 0.02 pgM CHO DNA per well to 300 pgM CHO DNA per well. This standard curve can be used to determine recovery levels from the spiked samples that were extracted from media using the KingFisher Flex instrument and PrepSEQrealDNASEQ CHO kits.

In another experiment, eight 10 pgM spiked samples in PBS were extracted on two different KingFisher Flex instruments. In the figure below, the recoveries of each eight samples on the two instruments are shown. Recoveries for instrument 1 averaged 88.4% with CV% of 2.2 while on instrument 2, the recoveries averaged 91.3% with a CV% of 4.2. The result demonstrates high extraction efficiency and also good reproducibility between different instruments.

The table below shows the recovery efficiency of CHO residual DNA from CHO DNA spikes of 100 pg, 10 pg, and 1 pg of buffers containing 50 or 100 mg of Lg protein. Triplicate samples were analyzed in quadruplicate and are included in the table. These data show that the DNA was recovered with very high efficiency using the automated procedure, with sample replicates ranging from 80% to 100% recovery. Note that only one PCR reaction was performed for each extracted DNA. *CV% values ranged from 1% to 17%, demonstrating excellent reproducibility across the samples analyzed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Recovery%</th>
<th>CV%</th>
</tr>
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<tbody>
<tr>
<td>100pg</td>
<td>96.2</td>
<td>0.5</td>
</tr>
<tr>
<td>10pg</td>
<td>95.6</td>
<td>0.4</td>
</tr>
<tr>
<td>1pg</td>
<td>95.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

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 then quantitated using realDNASEQ 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. The high recovery rate is consistent for spiked DNA amounts ranging from 1 pg to 10 pgM and is also consistent among different instruments. Additional experiments have produced similar performance with other cell line samples.

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