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

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ABSTRACT

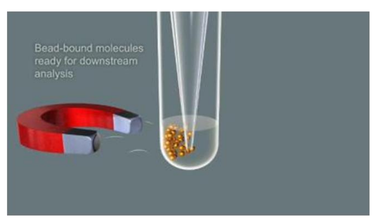
Residual host cell DNA is a common process-related impurity that is typically co-monitored with host cell protein. We have previously reported on a quantitative PCR (qPCR) based assay for monitoring host cell proteins (A customizable, qPCR-based immunoassay platform for accurate quantitation of protein 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 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 pg/mL to 300,000 pg/mL of CHO residual DNA..

INTRODUCTION

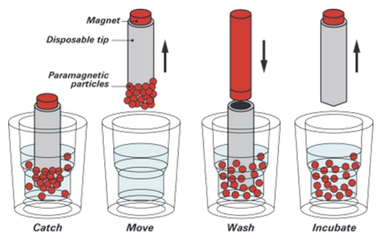
Ensuring the safety of biologic drugs requires rapid and accurate screening methods for in-process testing during manufacturing. High-throughput, automated approaches offer the advantage of processing many samples quickly and reliably. The use of functionalized magnetic bead technology to capture analytes of interest and purify them allows for sensitive and selective detection of target compounds. This technology has been incorporated into industry standard Applied Biosystems™ PrepSEQ™, resDNASEQ™ kits and ProteinSEQ™ kits and is easily adaptable to a 96-well plate format. For automated sample analysis, the Thermo Scientific™ KingFisher Flex™ Magnetic Particle Processor offers a fully integrated platform with real-time PCR to quantify a broad spectrum of bioproduction 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 covered with a specially 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 of isolation. The effectiveness of bead collection and transfer leads to superior washing, efficient elution, and rapid processing.



Traditional: Transfer liquids, not beads

- Loss of beads, and hence product, with liquid transfer
- Current step contaminated by leftover liquid from previous steps
- Higher elution volumes necessary



KingFisher: Move magnetic beads instead of liquids

- Superior purification
- Contaminants left behind
- High quality, concentrated end product

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 8 96-deep well plates. The instrument functions 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.

PrepSEQ Kit

ResDNA CHO Kit

MATERIALS AND METHODS

The KingFisher Flex instrument was used to purify CHO DNA from various samples in a high throughput mode using PrepSEQ kit chemistries 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 Kit. Applied Biosystems™ AccuSEQ™ software was used to process the data.

Samples were prepared in the following matrices.

- M4: 3% Mannitol, 2% Sucrose, 10mM L-Arginine, 0.01% Tween 20 and 100 mg/ml human IgG (simulation of conditions in cell culture)
- M5: same as M4 but with 50mg/ml human IgG
- PBS: Phosphate buffered saline

For the M4 and M5 matrixes, triplicate samples were prepared for each plate and analyzed in quadruplicate. Spiked sample levels were: 100 pg, 10 pg and 1 pg per well. For the PBS buffer, two plates were prepared with eight spikes of 10 pg/well.

Standards were prepared from genomic control DNA in the CHO resDNASEQ kit at five levels ranging from 3000 to 0.03pg per well using serial dilution.

The plate layout for the M4 and M5 buffer samples is shown below

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	1	2	3	4	5	6	7	8	9	10	11	12
A	M4	M4	M4	M4	M4	M4	M4	M4	M4	3000	3000	3000
B	M5	M5	M5	M5	M5	M5	M5	M5	M5	300	64	64
C										30	30	30
D										3	3	3
E	M4	M4	M4	M4	M4	M4	M4	M4	M4	0.3	0.3	0.3
F	M5	M5	M5	M5	M5	M5	M5	M5	M5	0.03	0.03	0.03
G												
H										NTC	NTC	NTC
	100pg			10pg			1pg			Standard Curve		

At the start of the run four 96 deep well plates are placed on the KingFisher Flex instrument. One plate is for the lysis step, two contain wash solutions and the last one contains the elution solution. A 200 uL standard plate is also added with the tip combs on top. The instrument will automatically pick up the tip comb during the run and deposit it when finished. After the run, the elution plate is removed and aliquots are added to a PCR reaction plate for quantitation.

To prepare the experiment, 100 µL of test sample solution was added to the wells of a 96 deep well plate. 10 µL of diluted CHO DNA was added to get final DNA amounts of 100 pg, 10 pg, and 1 pg. 60 µL of proteinase K buffer and 10 µL of proteinase K were then added to the wells. To adjust the salt concentration of the samples to ~0.5 M NaCl, 10 µL of 5 M NaCl was added to each sample. The plate was then placed into the instrument for 30 minutes at 56˚C to allow the proteinase K reaction to proceed. After proteinase K treatment, the plate was removed from the instrument. Lysis Solution (containing yeast tRNA and glycogen), magnetic particles, and binding solution were added to the wells. The plate was placed back into the instrument where DNA capture, wash, and elution were performed automatically. DNA was eluted with 200 µL of Elution Buffer.

Real-time PCR reactions were carried out on the extracted DNA and standard samples. A real-time PCR standard curve was generated by the amplification of 10 µL of each of the CHO DNA standard curve samples. In addition, 10 uL of each sample eluate was also analyzed with real time PCR and the resulting amplification values were used to calculate the amount of DNA recovered using the standard curve.

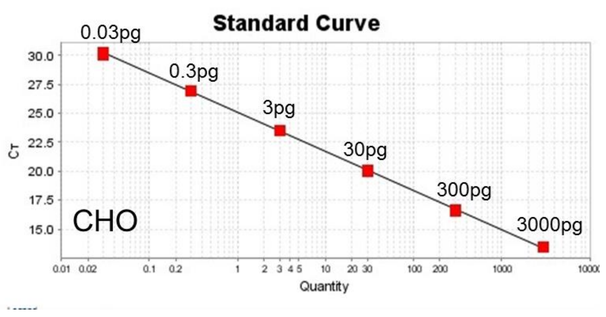
Processing the magnetic beads

The KingFisher Flex is designed for automated transfer and processing of magnetic particles in a micro plate format. It has a number of parameters that can be optimized for maximum processing efficiency of a specific application such as residual DNA extraction. The KingFisher Flex has a feature that allows the magnetic head to pause during mixing or the bead collection step for maximum sample collection. Other features include variable bead release time and speed, variable mixing times and speeds, bead collection counts and count collection times as well as heating of the well pads. The optimized protocol controlling the KingFisher Flex is shown below and allows for samples to be extracted in 1 hour and 45 minutes.

	Bead release time	Bead release speed	Mix time	Mix speed	Bead collection counts	Count Collection Time	Heating
PK lysis	--	--	30 min	Slow	--	--	56 C
Pause (add lysis solution, beads, binding agent)	--	--	--	--	--	--	--
DNA Binding	--	--	15 min	medium	5	10 sec	--
3x bead collection	--	--	--	--	5	10 sec	--
Wash 1	10 sec	Fast	1 min	slow	5	10 sec	--
Wash 2	--	--	1 min	slow	5	10 sec	--
Dry(15 seconds)	--	--	--	--	--	--	--
Resuspend beads	10 sec	Fast	10 min	Medium	--	--	--
DNA Elution	10 sec	Fast	10 min	Medium	5	10	70 C
3x bead collection	--	--	--	--	5	10	--

RESULTS

The figure below shows the standard curve of amplified CHO DNA generated for the five different non-extracted samples. 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 0.999, showing excellent linearity over the range of 0.03 pg CHO DNA per well to 3000 pg CHO DNA per well. This 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/resDNASEQ CHO kits.



The table below shows the recovery efficiency of CHO residual DNA from CHO DNA spikes of 100 pg, 10 pg, and 1 pg of in buffers containing 50 or 100 mg of IgG 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 6% to 11% demonstrating excellent reproducibility across the samples analyzed.

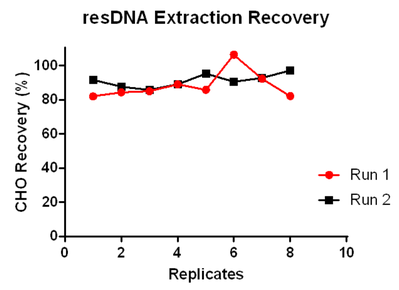
M4 Matrix:

	100pg		10pg		1pg	
CHO	Recovery%	CV%	Recovery%	CV%	Recovery%	CV%
1	90.9	8.3	78.5	2.3	71.9	3.8
2	91.3	3.7	86.2	5.1	76.7	7.2
3	103.9	4.5	88.8	3.8	90.8	9.7
4	102.0	3.4	91.6	1.5	86.9	5.5
Avg	97.0		86.3		81.6	
SD	6.9		5.6		8.8	
CV%	7.1		6.5		10.7	

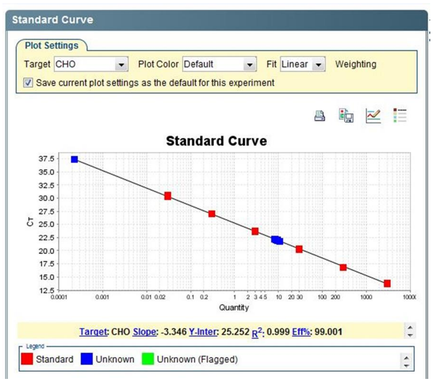
M5 Matrix:

	100pg		10pg		1pg	
CHO	Recovery%	CV%	Recovery%	CV%	Recovery%	CV%
1	97.9	5.9	91.0	2.8	82.1	7.9
2	95.4	4.5	89.8	2.2	80.1	4.9
3	107.5	3.7	93.6	3.3	100.3	6.4
4	109.6	5.2	94.6	4.8	97.1	3.8
Avg	102.6		92.3		89.9	
SD	7.0		2.2		10.3	
CV%	6.8		2.4		11.4	

In another experiment, eight 10 pg/well samples spiked in PBS were extracted on two different KingFisher Flex instruments. In the figure below, the recoveries of each eight samples on the the two instruments are shown. Recoveries for instrument 1 averaged 88.4% with CV(%) = 9.2 while on instrument 2, the recoveries averaged 91.3% with a CV(%) of 4.2. The results demonstrate high extraction efficiency and also good reproducibility between different instruments.



In addition the standard curve is comparable to the previous experiment. The figure below shows the standard curve generated for this study as shown in the AccuSEQ software window. The standard curve gave a slope of –3.346, demonstrating the high efficiency of the PCR reaction, and an R2 value of 0.999, showing excellent linearity.



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 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. The high recovery rate is consistent for spiked DNA amounts ranging from 1 pg to100 pg/well and is also consistent among different instruments. Additional experiments have produced similar performance with other cell line sample types.

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