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APPLICATION NOTE

Nucleic acid extraction

High-performance extraction and quantitation of host-cell residual DNA

Streamlined workflow in bioproduction

Abstract

In this study, we report high-performance extraction and quantitation of host-cell residual DNA using a streamlined, high-throughput, and semi-automated workflow. Preparation and extraction of sample are achieved in less than 2 hours using a sample preparation kit based on functionalized magnetic beads and a magnetic particle processing instrument. Real-time PCR yields quantitative recovery of Chinese hamster ovary (CHO) host-cell DNA, with excellent linearity for a concentration range of 3–300,000 pg/mL of CHO residual DNA.

Introduction

Ensuring the safety and quality of biologics requires rapid and accurate screening methods for in-process contaminant and impurity testing during manufacturing. High-throughput, automated approaches offer the advantage of quickly processing many samples, with a high degree of reproducibility. The use of functionalized magnetic bead–based technology to capture and purify analytes of interest allows for sensitive and selective detection of target compounds. This technology has been incorporated into industry-standard Applied Biosystems™ PrepSEQ™ Nucleic Acid Extraction Kit, resDNASEQ™ Quantitative DNA kits, and ProteinSEQ™ kits, and is easily adaptable to a 96-well plate format. These specialized sample preparation and quantitation kits are incorporated into an automated workflow by coupling the Thermo Scientific™ Pharma KingFisher™ Flex 96 Deep-Well Magnetic Particle Processor with real-time PCR instrumentation to quantify a broad spectrum of bioproduction contaminants and impurities.

Thermo Fisher Scientific™ KingFisher™ Flex purification system

The KingFisher Flex system is designed for automated transfer and processing of magnetic particles in microplate or deep-well format. The instrument functions without any dispensing or aspiration parts or devices. Samples and reagents including magnetic particles are dispensed into the plates and are run based on a specially designed protocol that has been preloaded into the onboard software, which is selected by the user via the keyboard and display.

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The KingFisher Flex system is ideal for high-throughput processing of functionalized magnetic particles. Patented technology of the KingFisher Flex system employs 96-well plates and magnetic rods covered with a specially designed disposable tip comb. The operating principle of the system is inverse magnetic particle processing (MPP), where the magnetic beads are moved from well to well between the required extraction reagents rather than traditional methods of transferring liquids while the beads remain stationary. Samples mixed with functionalized magnetic beads and reagents are dispensed into plate wells and loaded onto the instrument turntable. The tip comb is automatically loaded onto the magnetic rods at the beginning of the run. Magnetic beads are collected from the wells of one plate and released into the wells of another plate containing the appropriate reagent for the next sample preparation step.

The effectiveness of bead collection and transfer leads to superior washing, more efficient elution, and rapid processing compared to traditional methods (Figure 1).

This study evaluates the extraction efficiency and reproducibility of the PrepSEQ kit combined with the KingFisher Flex system via real-time PCR analysis of CHO cell DNA standard spiked at various concentrations into different simulated bioprocess sample matrices.

Materials and methods

CHO cell DNA standard, spiked into simulated cell culture sample matrices, was recovered and purified using the PrepSEQ kit with magnetic beads coupled with the high-throughput KingFisher Flex system. Quantitation of CHO cell DNA standard recovery was determined via real-time PCR using components of the resDNASEQ Quantitative CHO DNA Kit and performed on the Applied Biosystems™ 7500 Fast Real-Time PCR system, operated by Applied Biosystems™ AccuSEQ™ 2.0 Real-Time PCR software.

Samples and matrices

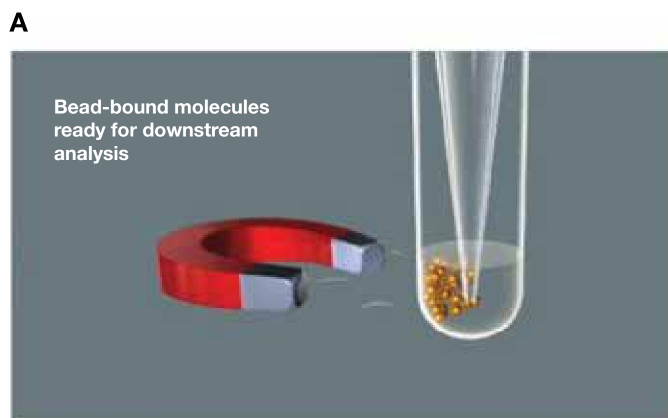
Test samples were prepared by spiking CHO cell DNA standard (included in the resDNASEQ kit) into one of the two matrices containing human IgG to simulate bioprocess matrices. Additionally, samples were extracted on a separate KingFisher Flex instrument to demonstrate reproducibility.

Simulated bioprocess matrices were prepared as follows:

- M4: 3% mannitol, 2% sucrose, 10 mM L-arginine, 0.01% Tween™ 20 buffer, and 100 mg/mL human IgG
- M5: same as M4 with 50 mg/mL human IgG

For each simulated bioprocess sample matrix (M4 and M5), quadruplicate samples were spiked with CHO cell DNA standard to obtain final PCR concentrations of 100, 10, and 1 pg/well. Extraction was performed on the KingFisher Flex system with a validated protocol for residual DNA.

A



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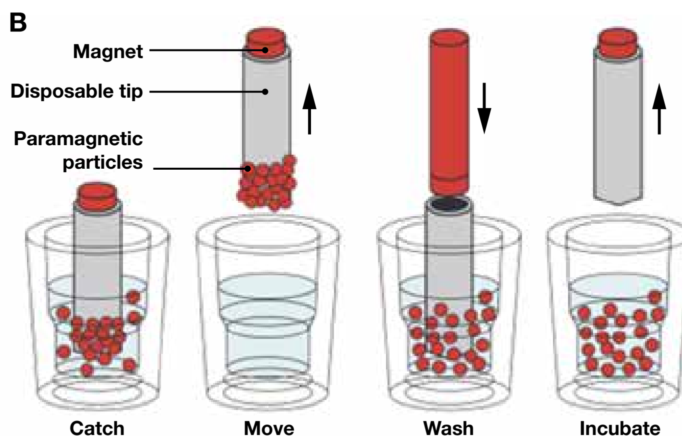

Figure 1. Comparison of traditional magnetic bead–based separation and inverse MPP. (A) Traditional techniques utilize a magnet to immobilize the magnetic beads to the tube or plate wall and the liquid is removed. The magnet is removed and the beads are resuspended in the next solution. This method is prone to loss of beads and product, and contamination by residual liquid, requiring greater elution volumes. **(B)** MPP, as employed by the KingFisher Flex system, utilizes a magnetic rod covered with a disposable cover. Beads are moved between the extraction solutions. This method eliminates contamination by residual liquid and results in superior purification, quality, and concentration of product.

In an additional experiment, triplicate samples of M4 and M5 matrices were spiked with CHO cell DNA standard to obtain final PCR concentrations of 100, 10, and 1 pg/well. The sample matrix was extracted utilizing a second KingFisher Flex system and evaluated via real-time PCR, as described below.

Real-time PCR

Results of the extraction process performed with the KingFisher Flex system were evaluated, in triplicate, using real-time PCR and the resDNASEQ kit. PCR experiments were performed on a 7500 Fast Real-Time PCR System supported by AccuSEQ software.

CHO cell DNA standard from the resDNASEQ kit was utilized to prepare dilutions for the standard curve. Tenfold serial dilutions of CHO cell DNA standard (3 ng/µL) in DNA Dilution Solution were prepared to obtain the following concentrations: 300 pg/µL, 30.0 pg/µL, 3.0 pg/µL, 0.3 pg/µL, and 0.03 pg/µL.

The standard curve was generated with 10 µL of each DNA dilution, plated in triplicate. Amplification occurred in parallel with the appropriate test samples.

Data analysis via AccuSEQ software was as follows:

1. Standard curve analysis with a manual Ct threshold value of 0.2, and either autobaseline or manual baseline from 3–12 for CHO cell standard. Analysis methods were chosen based on the best efficiency for the standard curve.

2. AccuSEQ software was used to label the standards and a linear fit was chosen for the standard curve.

3. AccuSEQ software was used to calculate spike recoveries and CV%.

Results

Extraction efficiency

The extraction efficiency of the PrepSEQ kit and the KingFisher Flex system was evaluated by spiking simulated cell culture sample matrices with CHO cell DNA standard and performing real-time PCR as described above.

CHO cell DNA standard recovered from the M4 and M5 matrices after extraction using the PrepSEQ kit and KingFisher Flex system was quantified using the standard curve in Figure 2. The slope of –3.37 and R^2 value of 0.999 demonstrate high PCR efficiency and excellent linearity over the range of 0.03–3,000 pg CHO DNA per well.

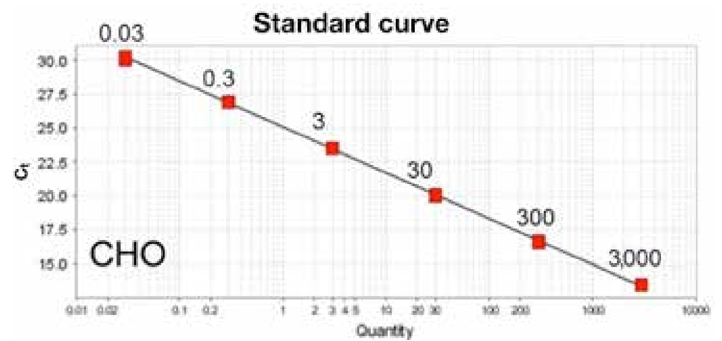

Figure 2. Standard curve used for quantitation of PCR.

Table 1. Percent recovery of residual DNA from CHO cells.

Matrix type	CHO DNA standard concentration (pg/well)	Extraction replicate	Recovery (%)			
			PCR replicate			Average
			1	2	3	
M4	100	1	82.90	97.81	92.01	90.91
		2	87.68	94.24	91.97	91.31
		3	98.81	104.81	107.95	103.86
		4	98.01	104.02	103.85	101.96
	10	1	80.60	77.10	77.90	78.53
		2	89.80	87.40	81.30	86.17
		3	92.50	87.80	86.00	88.77
		4	90.30	93.00	91.50	91.60
	1	1	72.62	68.94	74.26	71.94
		2	79.64	70.36	80.09	76.70
		3	89.87	82.52	100.00	90.80
		4	82.38	91.84	86.47	89.90
M5	100	1	92.00	103.56	98.03	97.86
		2	91.63.	100.12	94.38	95.38
		3	103.25	111.14	108.02	107.47
		4	111.35	114.19	103.29	109.61
	10	1	89.70	89.40	93.90	91.00
		2	91.70	90.00	87.70	89.80
		3	95.10	90.00	95.70	93.60
		4	94.90	99.00	89.90	94.60
	1	1	74.68	86.34	85.30	82.11
		2	77.21	84.56	78.56	80.11
		3	92.99	103.00	105.00	100.33
		4	99.15	92.78	99.21	97.05

Table 1 shows the percent recovery of CHO residual DNA from CHO DNA spikes of 100, 10, and 1 pg in buffers containing 50 or 100 mg of IgG protein. Spiked samples were extracted in quadruplicate and analyzed via real-time PCR, in triplicate.

Reproducibility

To demonstrate reproducibility, triplicate samples were prepared by spiking CHO cell DNA standard into M4 and M5 matrices to obtain a final concentration in PCR of 100, 10, and 1 pg/well. Samples were extracted on a second KingFisher Flex system and evaluated via real-time PCR as previously described. Table 2 shows the percent recovery results of extractions performed on a second KingFisher Flex system. There is excellent agreement between the CHO extraction recoveries on the two instruments.

Table 2. Reproducibility results from second KingFisher system.

Matrix type	CHO DNA standard concentration (pg/well)	Recovery (%)			
		PCR replicate			Average
		1	2	3	
M4	100	100.44	98.30	99.68	99.47
	10	93.60	79.50	92.70	88.60
	1	101.00	102.00	99.55	100.85
M5	100	104.50	104.14	98.42	102.35
	10	104.70	92.40	105.90	101.00
	1	99.71	112.00	104.00	105.24

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Summary and conclusion

The extraction efficiency and reproducibility of the PrepSEQ and resDNASEQ kits coupled with the KingFisher Flex system were tested and evaluated via real-time PCR. CHO cell DNA standard was spiked into different simulated sample matrices at various concentrations. Samples were extracted in a 96-well plate format in under 2 hours and PCR performed on a 7500 Fast Real-Time PCR instrument with AccuSEQ software.

The results show that the spiked CHO DNA standard was recovered with very high efficiency using the automated procedure. The average recovery from extraction ranged from 78% to 100% in the M4 matrix and from 86% to 107% in the M5 matrix.

Table 3 summarizes the average percent recovery, standard deviation, and CV% for each CHO DNA standard concentration in each simulated cell culture matrix type. The average percent recovery across all CHO DNA standard concentrations and matrix types was 82–103%. The CV values were all less than 5%, demonstrating excellent precision and reproducibility across the samples.

Table 3. Summary of CHO DNA recovery when spiked into M4 and M5 matrices at three concentrations.

Matrix type	CHO DNA standard concentration	Average recovery (%)	Standard deviation	CV (%)
M4	100	97.00	4.51	4.65
	10	86.27	2.06	2.39
	1	81.58	3.42	4.19
M5	100	102.58	4.10	4.00
	10	92.25	0.54	0.59
	1	89.90	3.37	3.75

Reproducibility was demonstrated by spiking CHO cell DNA standard into triplicate samples of M4 and M5 matrices, extracting with a second KingFisher Flex system and evaluated via real-time PCR as previously described. Figure 3 shows that the average percent recovery was greater than 80% across all concentrations, matrices, and separate instruments.

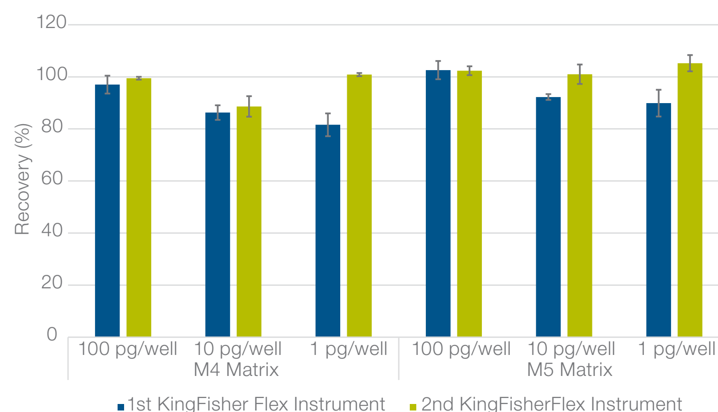

Figure 3: Percent recovery from two KingFisher Flex systems.

The sample preparation procedure provides excellent recovery of spiked DNA amounts ranging from 1 pg/well to 100 pg/well. It offers consistent reproducibility among replicates and across different KingFisher Flex systems. Additional experiments have produced similar performance with other residual DNA cell line sample types such as *E. coli* and Vero cells.

In conclusion, using the PrepSEQ kit coupled with the KingFisher Flex system and the resDNASEQ kit, we have demonstrated quantitative CHO residual DNA recovery from a variety of complex test sample matrices typical of those encountered during purification of biopharmaceutical products.

Find out more at **thermofisher.com/automate**

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