



Application of a BHK-21 gDNA control in the detection of residual host cell DNA in biopharmaceuticals

Abstract

Residual host cell genomic DNA (gDNA) poses a significant safety risk in biopharmaceutical manufacturing. The precise detection and quantification of this impurity is, therefore, essential. In this study, we demonstrate the application of our high-quality quantitative gDNA control material in validating quantitative PCR (qPCR) assays designed to detect residual host gDNA impurities in biologics.

Introduction

When manufacturing biopharmaceuticals such as vaccines and other biologics, cell substrates are often used to produce the desired product. One of the primary concerns when using these complex expression systems is the potential presence of residual host cell gDNA in the final product, even after downstream purification processes. The effective removal of this impurity is essential as host gDNA may harbor oncogenic or viral sequences that can put patient safety at risk. In response to this concern, regulatory agencies such as the U.S. Food & Drug Administration (FDA),¹ European Medicines Agency (EMA),² and World Health Organization (WHO),³ have set criteria about the maximum amount of residual host gDNA allowed in such products (< 10 ng/dose, ≤ 200 bp in length).

A widely accepted methodology for the precise quantification of residual host cell gDNA is quantitative PCR (qPCR). This approach is a highly sensitive and specific high-throughput technology that can detect femtogram amounts of residual host gDNA in samples.⁴⁻⁶ While qPCR offers many advantages, biases associated with different protocols or reagents can affect the resulting data. Therefore, it is essential to thoroughly validate assay performance using high-quality, authenticated reference materials to ensure reliable, reproducible, and comparable results.

To support the need for highly qualified reference materials, USP and ATCC have collaborated to develop gDNA control materials derived from host cell lines commonly used to manufacture vaccines and other biologics. These commercially available controls are manufactured, tested, and quantitated using robust processes, providing high-quality controls for evaluating qPCR detection methods.

This report demonstrates the utility and performance of our baby hamster kidney-21 (BHK-21) gDNA control material against a highly sensitive PCR assay developed to detect residual host cell gDNA.⁴ This assay targets intracisternal A-particle (IAP), which is a transposable element class with about 1000 copies in the rodent genome.⁷

Materials and Methods

In this study, we used gDNA (catalog # 1592100) extracted from the BHK-21 cell line (ATCC® CCL-10™). This gDNA product was evaluated for critical attributes such as concentration, total amount, purity, and integrity, as well as, most importantly, its utility as a reliable qPCR control material.

Development and quality control of the reference material

Product integrity and purity were evaluated via agarose gel electrophoresis and the Agilent TapeStation system (Agilent Technologies, Inc.). Restriction digestion using *Bam*HI (New England Biolabs, catalog number R0136S) was conducted according to the manufacturer's recommendations. Undigested gDNA was incubated under the same conditions as the treated sample (i.e., temperature, duration, buffer) but without the restriction enzyme.

qPCR assays

Table 1 summarizes the dynamic range features of the assay we used in this study. The Wang *et al.* assay has been shown to have a wide dynamic range and high sensitivity for detecting traces of baby hamster kidney (BHK) gDNA.

Table 1: Linear range assessment of the assay used in this study to detect residual BHK gDNA

Publication	Target Seq.	Detection range (pg/ reaction) in the publication	Linear range (pg/reaction) in the publication	Testing range (pg/ reaction) in this study
Wang <i>et al.</i> 2018 ⁴	IAP	0.025 - 25	0.025 - 25	0.00125 - 2.5

qPCR experiments were performed in a 20 µL reaction containing 10 µL of iTaq™ Universal Probes Supermix (Bio-Rad, catalog number 1725131). The primer and probe sequences used were those from the published Wang *et al.* assay (**Table 2**),⁴ and the Taqman probes were tagged with a FAM dye and quenched by a combined ZEN/Iowa Black fluorescent quencher from Integrated DNA Technologies (IDT). The cycling parameters and the master mix formula are summarized in **Table 3** and **Table 4**, respectively. All samples were tested in triplicate.

Table 2: Primers and probes used in the study

Assay Publication	Oligo names	Oligonucleotide sequences	Amplicon Size (bp)
Wang <i>et al.</i> , 2018 ⁴	BHK_IAP_F	TTTGCCTTCACTATCCCTTCTC	103
	BHK_IAP_R	GCTGACATATTGTTGGGCTATTG	
	BHK_IAP_P	ATTTCAGTGAAGGTACTGCCGCA	

Table 3: Cycling parameters

Publication	Initial Denature [Cycles x Target °C/Hold Time (min:sec) / Ramp Rate in °C/sec]	PCR Cycling [Cycles x Target °C/Hold Time (min:sec) / Ramp Rate in °C/sec]	Instrument
Wang <i>et al.</i> , 2018 ⁴	1 × 95°C/15:00/3.3	40 × (95°C/00:30/3.3 - 60°C/01:00/3.3)	CFX96™ (Bio-Rad)
	1 × 95°C/15:00/1.6	40 × (95°C/00:30/1.6 - 60°C/01:00/1.6)	QuantStudio™ 3 (Thermo Fisher Scientific)

Table 4: Master mix formulation

Component	Final Concentration
qPCR Reaction Mix	1×
10 µM Forward Primer	1000 nM
10 µM Reverse Primer	1000 nM
10 µM Probe	250 nM
PCR-grade water	n/a
Template	variable

To ensure data quality and reproducibility throughout the assay's dynamic range, we paid particular attention to the diluent. Despite using low-bind or siliconized tubes, some amounts of gDNA still bind to the inner wall of these vessels. This phenomenon confounds PCR results, especially at dilutions containing very low gDNA concentrations. We were able to solve this problem by using Poly(A) buffer as a diluent; Poly(A) aids DNA recovery by coating the inner walls of the storage tubes, and it protects nucleic acids from degradation by serving as a substrate for contaminating nucleases. In our study, we used 0.25 mg/mL Poly(A) (Millipore Sigma/Roche, catalog number 10108626001) solution as a diluent for serial dilutions. Serial dilutions were made in low-bind tubes (Thomas Scientific, catalog number 1149X75), and PCR-grade water (QIAGEN, catalog number 17000-10, or Amerigo Scientific, catalog number PER1136265AMP) was used throughout all experiments.

PCR inhibition assessment

We used FBS (Sigma-Aldrich, catalog number F0926) and molecular-grade BSA (Thermo Fisher Scientific, catalog number B14) for spike-in PCR experiments.

Results

We conducted a series of tests to assess the quality and applicability of the BHK-21 gDNA product.

Quality attributes of the gDNA control

We assessed gDNA integrity via the Agilent TapeStation system (Agilent Technologies, Inc) (**Figure 1**). As expected, the uncut gDNA was a single band >48 kb. In contrast, the product appeared as a <48 kb smear following treatment with the *Bam*HI restriction enzyme. These findings indicate that the product has no contaminating material residues from extraction. We found similar results when analyzing the gDNA via agarose gel electrophoresis (data not shown). No traces of residual RNA were observed. We also assessed product purity via spectroscopy. The A_{260}/A_{280} values were within 1.7-2.0, which are industry-acceptable purity parameters and within specifications outlined in the product Certificate of Analysis (data not shown).

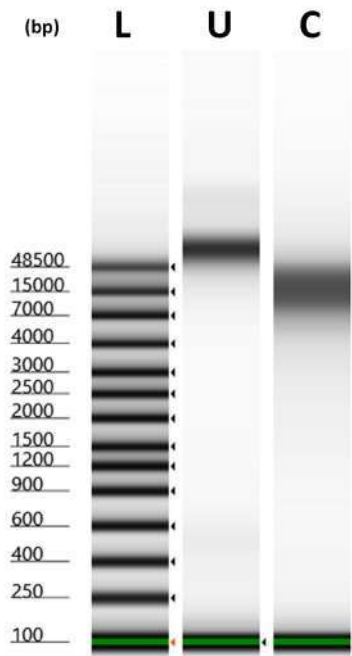


Figure 1: Evaluation of the integrity and purity of the BHK-21 gDNA. Analysis of cut (C) and uncut (U) gDNA via the Agilent TapeStation system. The sizes of resultant products were compared against the reference ladder (L).

Assessment of the qPCR assay sensitivity and linearity with the gDNA control material

We assessed the utility of the product as a qPCR control in a highly sensitive assay.⁴ Using the BHK-21 gDNA, we successfully confirmed the linearity, repeatability, intermediate precision, and published lower limit of quantitation (LLOQ). We focused our work on the lower end of the dynamic range to better assess the quality and utility of the gDNA as a reference material. The assay maintained linearity at concentrations up to 20× below the published LLOQ. These qPCR experiments were executed repeatedly by two users and using two separate instruments on separate days (**Figure 2**). Results are consistent within each instrument. Minor variations observed between devices are due to inherent thermocycling conditions, hence inherent instrument design.⁸⁻⁹ Variations in thermocycling patterns among PCR instruments could affect assay performance; therefore, users might be able to avoid potentially unexpected outcomes of sensitive PCR-based assays by designing their PCR protocols and establishing reliable workflows with the understanding that thermocycling conditions could vary among instruments.

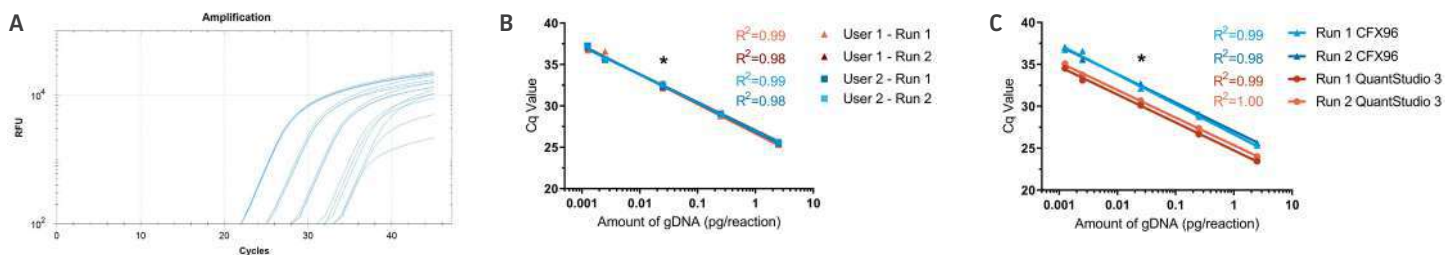


Figure 2: Comparative qPCR results summary regarding linearity and sensitivity of the Wang *et al.*⁴ assay using BHK-21 gDNA. (A) Amplification plots were generated by running the assay with serial dilutions ranging from 2.5 pg gDNA/reaction to 1.25 fg gDNA/reaction. (B) qPCR data between two users using CFX96™ thermocyclers (Bio-Rad). (C) qPCR data executed by one user using CFX96™ (Bio-Rad) or QuantStudio™ 3 (Thermo Fisher Scientific) thermocycler instruments. All samples were tested in triplicate. * = Lower Limit of Quantitation claimed by the original assay developers in their publication.⁴

Evaluating the gDNA product as a control for assessing PCR inhibition during the bioproduction process

To evaluate the utility of the gDNA control in assessing the extent of qPCR inhibition during the bioproduction process, we diluted the BHK-21 gDNA in materials that mimic in-process samples or the final product. Then we compared the overall performance of the Wang *et al.* assay under each condition (**Figure 3**). As a cell lysate surrogate (i.e., in-process sample), we used fetal bovine serum (FBS) due to its rich protein content and high amounts of salts, lipids, carbohydrates, and other constituents; FBS also has a pH that is not a critical PCR inhibition factor. As a highly purified protein material (i.e., final product), we used molecular-grade bovine serum albumin (BSA). Poly(A) was used as a control.

FBS appeared to inhibit PCR significantly—not only undiluted but also at 1:20 or 1:50 dilutions. Even in instances when there was amplification, the reaction's fluorescence was reduced by at least 50% as compared to those containing the Poly(A) control and BSA diluents. These results were similar to those reported in the literature, showing that cell lysates inhibit PCR.⁴ In contrast, the PCR containing BSA had a minimal but not negligible impact on assay performance. At higher gDNA concentrations, the estimated gDNA in the BSA mixtures was on average up to 20% of the pure gDNA, which is within the acceptable range for the qPCR. At lower gDNA concentrations close to the LLOQ, the amount of gDNA in the BSA mixtures was overestimated by 1.1-2.5× (relatively lower Cq/Ct qPCR values). Overall, these results demonstrate the BHK-21 gDNA product can be used as a reliable control material for assessing the extent of PCR inhibition during the development and optimization of bioproduction processes.

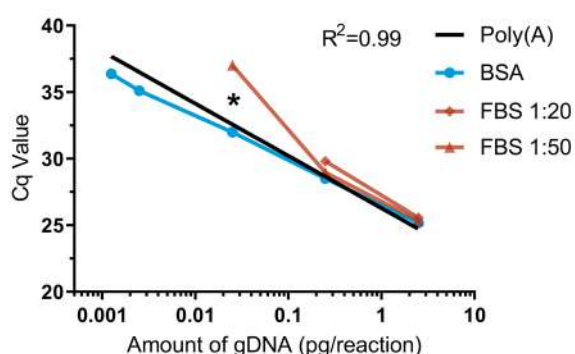


Figure 3: Using BHK-21 gDNA to assess PCR inhibition. Comparative performance of the Wang *et al.*⁴ PCR assay using the BHK-21 gDNA diluted in Poly(A) (control), FBS as a cell lysate surrogate, and BSA as a highly purified protein product. All samples were tested in triplicate. * = Lower Limit of Quantitation claimed by the original assay developers in their publication.⁴

Conclusion

In this study, we demonstrate the quality of the BHK-21 gDNA product and its applicability as a reliable control material for qPCR assays designed to detect residual host gDNA in biologics. The gDNA is free of impurities, has high integrity, and is quantifiable and amplifiable. Furthermore, it is compatible with sensitive qPCR assays developed for host residual gDNA detection. We evaluated one qPCR assay using the BHK-21 gDNA and successfully confirmed its linearity, sensitivity, repeatability, intermediate precision, range, and LLOQ. The PCR assay yielded consistently positive results with very low gDNA concentrations, even below the originally claimed LLOQ, indicating that the BHK-21 gDNA has good quality and is a reliable PCR control material.

Product Utility Recommendations

- Test the drug substance and process intermediates to assess clearance of residual gDNA using fully validated PCR assays with analytical reference materials derived from authenticated cell lines.
- According to ICH¹⁰ and USP¹¹ guidelines, it is essential to develop and thoroughly validate assay performance using high-quality, authenticated reference materials to ensure reliable, reproducible, robust, and comparable results.
- Users might be able to avoid potentially unexpected outcomes of sensitive PCR-based assays by designing their PCR protocols and establishing reliable workflows with the understanding that thermocycling conditions could vary among instruments.

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