



Application of an MDCK 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 very small amounts of residual host gDNA in samples.⁴⁻⁸ 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 Madin-Darby Canine Kidney (MDCK) gDNA control material against two highly sensitive PCR assays developed to detect residual host cell gDNA.⁴ These assays target the small subunit (SSU) ribosomal 18S RNA (18S rRNA), which is a highly repetitive and conserved sequence among mammalian species.⁹

Materials and Methods

In this study, we used gDNA (catalog # 1592111) extracted from the MDCK (NBL-2) cell line (ATCC® CCL-34™). 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 assays we used in this study. The André *et al.* assays have been shown to have a wide dynamic range and high sensitivity for detecting traces of MDCK gDNA.

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

Publication	Assay Variant (Amplicon size)	Target Seq.	Detection range (pg/reaction) in the publication	Linear range (pg/reaction) in the publication	Testing range (pg/reaction) in this study
André <i>et al.</i> , 2016 ⁴	123 bp 254 bp	18S rRNA	5–50000	5–50000	1–50000

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 André *et al.*, 2016 (**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)
André <i>et al.</i> , 2016 ⁴	HS_RIB_F_123	GCAATTATCCCCATGAACG	123
	HS_RIB_R	GGCCTCACTAAACCATCCAA	
	HS_RIB_P	AAGTCCCTGCCCTTTGTACACACCG	254
	HS_RIB_F_254	AACAGGTCTGTGATGCCCTT	
	HS_RIB_R	GGCCTCACTAAACCATCCAA	
	HS_RIB_P	AAGTCCCTGCCCTTTGTACACACCG	

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
André <i>et al.</i> , 2016 ⁴	1 × 95°C/10:00/3.3	40 × (95°C/00:10/3.3 – 62°C/00:30/3.3 – 72°C/00:10/3.3) + 1 × (40°C/00:30/3.3)	CFX96™ (Bio-Rad)
	1 × 95°C/10:00/1.6	40 × (95°C/00:10/1.6 – 62°C/00:30/1.6 – 72°C/00:10/1.6) + 1 × (40°C/00:30/1.6)	QuantStudio™ 3 (Thermo Fisher Scientific)

Table 4: Master mix formulation

Component	Final Concentration
qPCR Reaction Mix	1×
10 µM Forward Primer	500 nM
10 µM Reverse Primer	500 nM
10 µM Probe	200 nM
PCR-grade water	variable
Template	variable

We paid particular attention to the diluent to ensure data quality and reproducibility throughout the assay's dynamic range. 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 solved 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 fetal bovine serum (FBS, Sigma-Aldrich, catalog number F0926) and molecular-grade bovine serum albumin (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 MDCK 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).

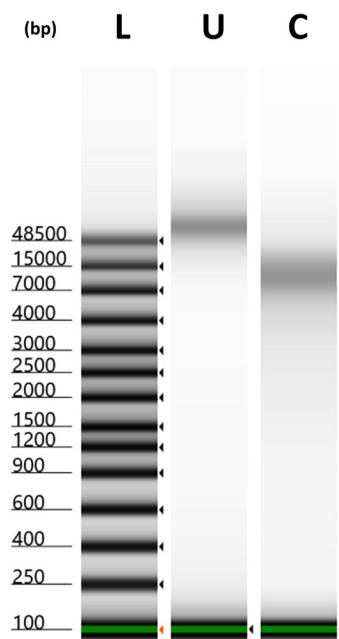


Figure 1: Evaluation of the integrity and purity of the MDCK gDNA. Analysis of cut (C) and uncut (U) gDNA via the Agilent TapeStation system. The sizes of resultant products were compared against the reference ladder (L).

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 MDCK gDNA, we successfully confirmed the dynamic range, 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 5× 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.¹⁰⁻¹¹ 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. The data obtained using the André *et al.*⁴ 254 bp amplicon assay were similar to those with the 123 bp amplicon and are shown in the supplementary information section.

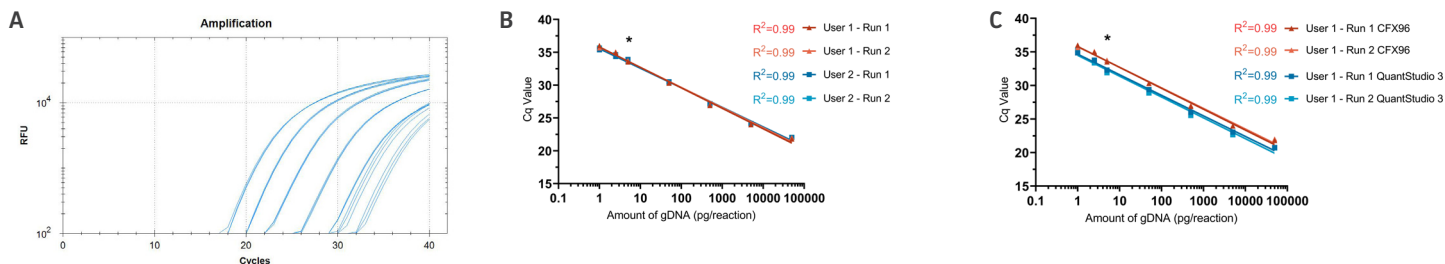


Figure 2: Comparative qPCR results summary regarding dynamic range and sensitivity of the André *et al.*⁴ 123 bp amplicon assay using MDCK gDNA. (A) Amplification plots were generated by running the assay with serial dilutions ranging from 50000 to 1 pg gDNA/reaction. **(B)** qPCR data between two users 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 MDCK gDNA in materials that mimic in-process samples or the final product. Then, we compared the overall performance of the André *et al.* assays 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 1:2 or 1:5 dilutions. These results were similar to those reported in the literature, showing that cell lysates inhibit PCR.⁸ In contrast, the PCR containing BSA had a minimal but not negligible impact on assay performance. Relative to the mixture containing Poly(A) along the linear range, the Cq/Ct qPCR values varied between 0.2-1.2 in those containing BSA, suggesting a 47% underestimate of the gDNA. Overall, these results demonstrate the MDCK 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.

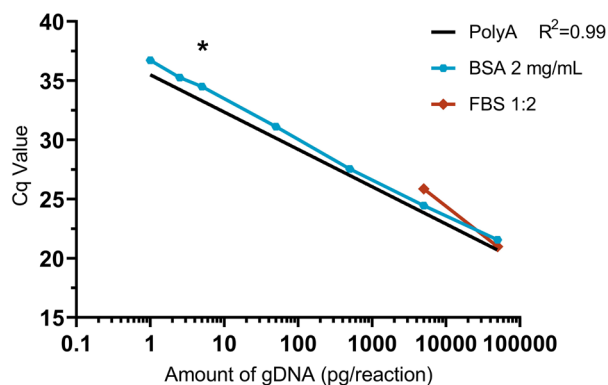


Figure 3: Using MDCK gDNA to assess PCR inhibition. Comparative performance of the André *et al.*⁴ 123 bp amplicon PCR assay using the MDCK gDNA diluted in Poly(A) (control), FBS as a cell lysate surrogate, and BSA as a highly purified protein product. All samples were tested in triplicate. * = Lower Limit of Quantitation claimed by the original assay developers in their publication.⁴

Conclusion

In this study, we demonstrate the quality of the MDCK 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 two qPCR assays using the MDCK gDNA and successfully confirmed their dynamic range, linearity, sensitivity, repeatability, intermediate precision, and LLOQ. The PCR assays yielded consistently positive results with very low gDNA concentrations, even below the originally claimed LLOQ, indicating that the MDCK 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 ICH¹² and USP¹³ 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.

References

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SUPPLEMENTARY INFORMATION

MDCK gDNA performance in the André *et al.*⁴ 254 bp amplicon assay.

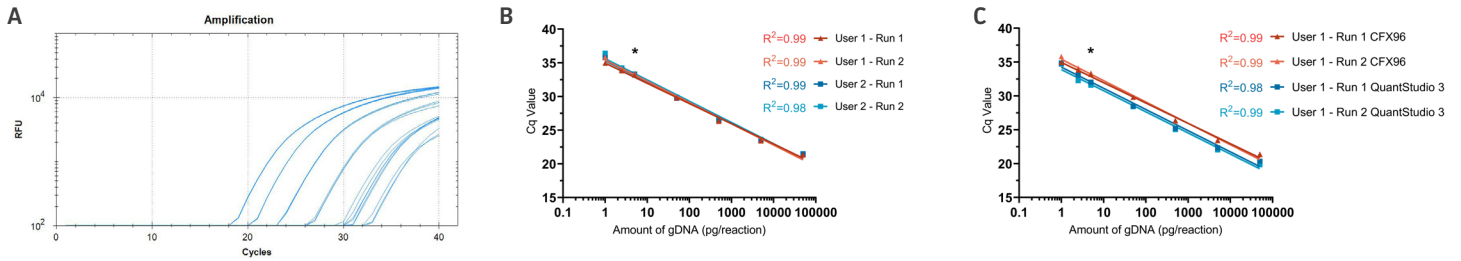


Figure S1: Comparative qPCR results summary regarding dynamic range and sensitivity of the André *et al.*⁴ 254 bp amplicon assay using MDCK gDNA. (A) Amplification plots were generated by running the assay with serial dilutions ranging from 50,000 to 1 pg gDNA/reaction. **(B)** qPCR data between two users using a CFX96™ thermocycler (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. * = assay developers' LLOQ.⁴

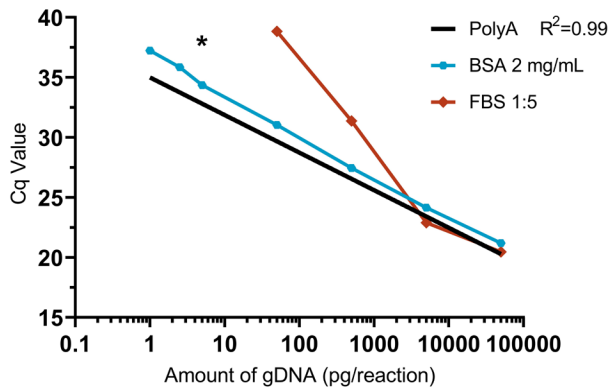


Figure S2: Using MDCK gDNA to assess PCR inhibition. Comparative performance of the André *et al.*⁴ 254 bp PCR assay using the MDCK gDNA diluted in Poly(A) (control), FBS as a cell lysate surrogate, and BSA as a highly purified protein product. All samples were tested in triplicate. * = assay developers' LLOQ.⁴



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