Highly Sensitive qPCR-based Method Development for Quantitation of Residual Host Cell DNA in Biopharmaceutical Samples

Poster#:

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What is Residual Host Cell DNA?

Residual Host Cell DNA (HCD) refers to trace amounts of DNA derived from host cells used in biopharmaceutical manufacturing. If not effectively removed during purification, HCD can persist in the final drug product, posing potential safety and immunogenicity risks, making its control a critical quality attribute.

Origin of Host Cell DNA & Analysis

HCD originates from the genome of the production cell line and may be released during cell lysis, cell culture, or harvest. These DNA fragments can enter process intermediates and require monitoring across purification steps to ensure effective removal.

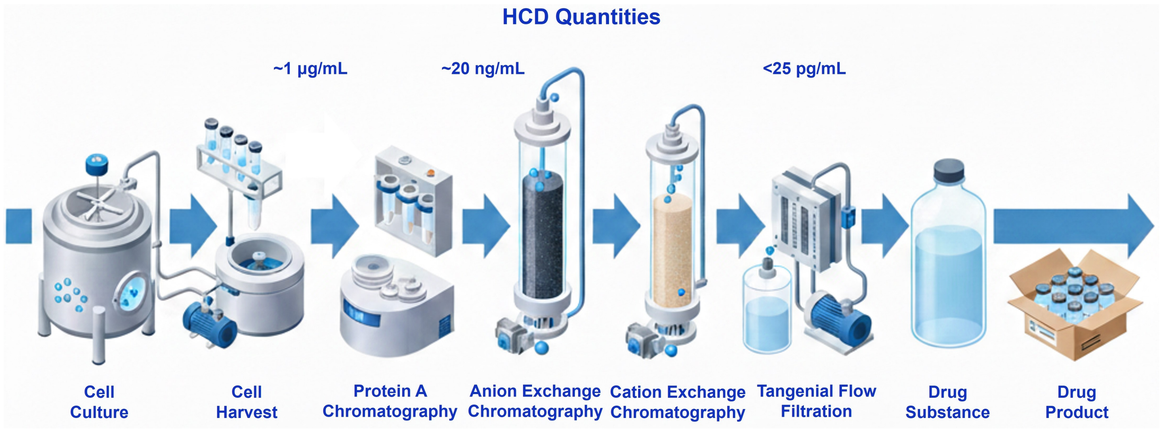

Figure 1: HCD Release and Progressive Reduction across Biopharmaceutical Manufacturing Steps

DNA quantification typically involves two critical steps: **isolation of DNA** from the sample matrix and subsequent **quantification**. Among available techniques, quantitative real-time PCR (qPCR) is the most widely used method for HCD quantification. However, many workflows rely on expensive, ready-to-use commercial qPCR kits. Here, we present Highly Sensitive in-house qPCR Method for Quantitation of Residual Host Cell DNA.

Principle

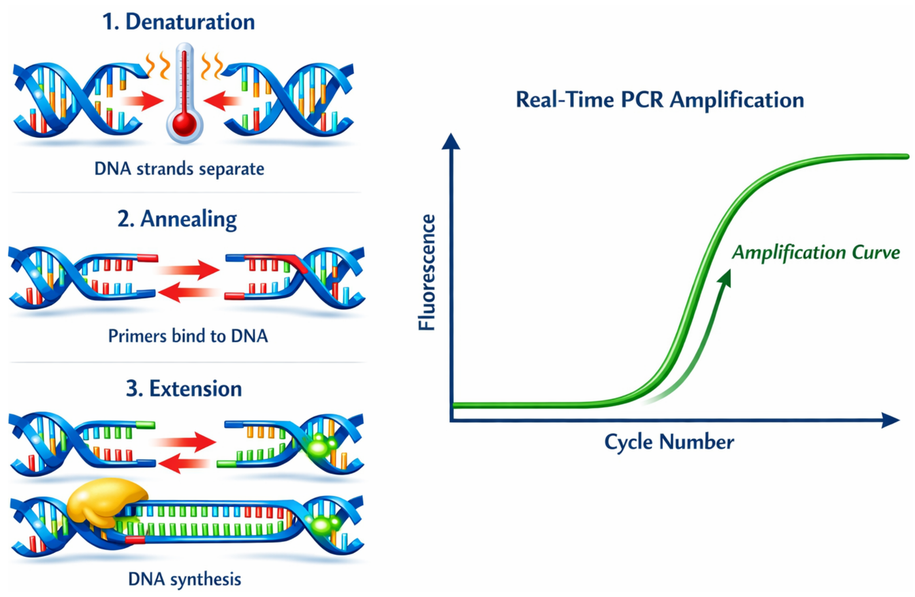

Figure 2: Schematic illustration of Real-Time PCR workflow

Real-time PCR (qPCR) is a powerful molecular technique that enables the **real-time monitoring** and **quantification** of nucleic acid amplification through fluorescence-based detection. During thermal cycling, amplified DNA is detected using sequence-specific probes. The 5→3 exonuclease activity of DNA polymerase cleaves the probe during extension, releasing a **fluorescent signal**.

Fluorescence intensity is measured at each cycle and used to determine the quantification cycle (Cq/Ct), which is inversely related to the initial target concentration. This real-time measurement allows accurate and sensitive quantification over a wide dynamic range, making qPCR a gold-standard method in **HCD Analysis**.

Performance: Commercial vs. In-house

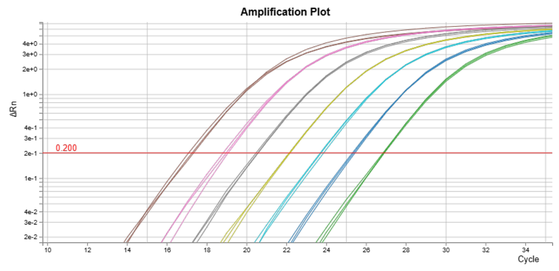

Figure 3: Amplification of Commercial qPCR Method

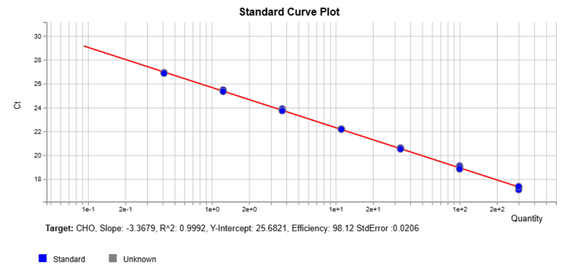

Figure 4: Standard Curve of Commercial qPCR Method

Commercial Kit provides standardized performance using a generic DNA reference material.
Fixed assay composition limits flexibility for process-specific optimization.

VS

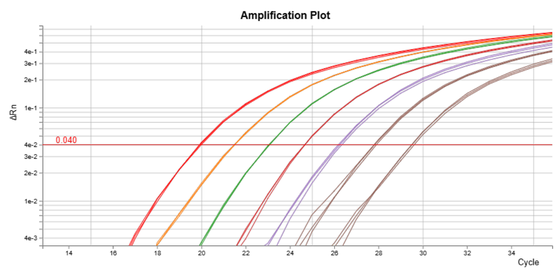

Figure 5: Amplification of In-House qPCR Method

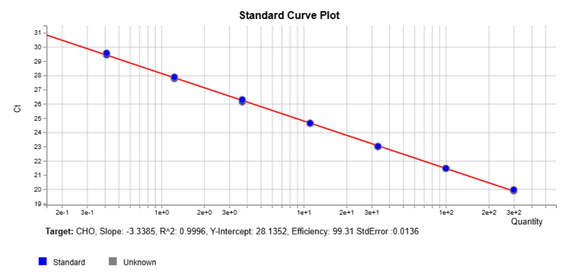

Figure 6: Standard Curve of In-House qPCR Method

Process-specific in-house DNA standard enables accurate quantitation across manufacturing intermediates.
Optimized primer and probe concentrations result in consistent amplification efficiency and high sensitivity.

- **High Sensitivity:** Limit of Quantification of 0.5 pg DNA/reaction corresponding to 2 ng per dose
- **Specificity:** No interference from cell culture media or process-related buffers
- **Precision:**
  - Repeatability: %CV 18%
  - Intermediate precision: %CV 19%
- **Accuracy:** Recoveries between 50% and 150%
- **Linearity:** Demonstrated across multiple process intermediates
- **Regulatory Impact:** Meets ICH Q2(R2) expectations for HCD control

	In-house Method	Commercial Method
Cost	Low cost (~150 $/plate)	High cost (~5000 $/plate)
Standard Origin	Process-specific standard	Unknown origin of standard
Performance	High sensitivity and accuracy	High sensitivity and accuracy
Flexibility	Assay customization	Fixed parameters

A **cost-effective, process-specific, and highly sensitive** in-house qPCR method for residual host cell DNA quantification, ICH Q2(R2)–compliant, was developed by the **AbdiBio R&D** team.

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[3] ICH Q2(R2) VALIDATION OF ANALYTICAL PROCEDURES.

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MORE INFORMATION

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