Direct Quantification of Residual Host Cell DNA

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Introduction

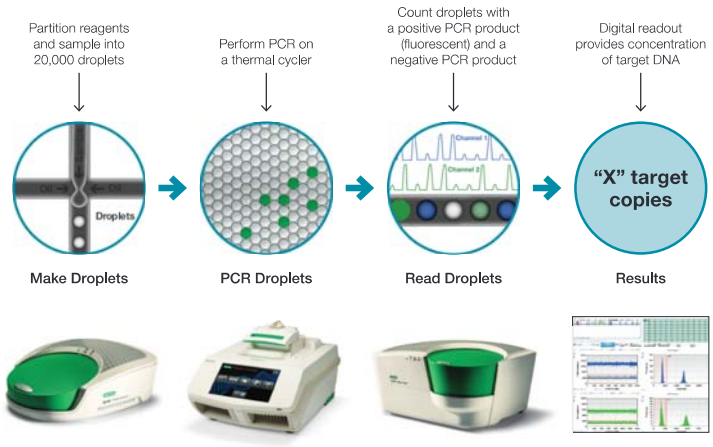
Many therapeutic proteins and vaccines are manufactured using bacterial and mammalian host cells. Manufacturing processes are prone to leaving biological impurities derived from these cells, such as host cell DNA (HCD). The presence of HCD in drug substances poses safety concerns and HCD must be removed to ensure product quality and safety. Regulatory agencies, such as the U.S. Food and Drug Administration (FDA) and the World Health Organization (WHO), have provided guidelines defining acceptable levels of HCD allowed in final drug products; the upper limit for residual HCD is 100 pg/dose and 10 ng/dose as stated by the FDA and WHO, respectively. Therefore, the method to quantify residual HCD and monitor DNA clearance should be highly sensitive to meet these regulatory requirements.

Real-time quantitative PCR (qPCR) is currently the most commonly used technique to monitor residual HCD, yet this methodology requires purifying nucleic acids from inhibitor-rich sample matrices, which adds time and expense to the process. In addition, this type of sample preparation can be inefficient, resulting in an inaccurate assessment of the true amount of HCD in the samples.

Here, we introduce a highly precise and sensitive method for residual HCD quantification, without the need for DNA extraction, using Bio-Rad's QX200™ Droplet Digital™ PCR (ddPCR™) System. We created a panel of test matrices to simulate various process intermediates and analyzed these samples using ddPCR. More than 80% of spiked-in DNA was recovered for five out of the six samples tested. The poorly recovered sample was inhibited by high amounts of IgG, but pretreatment of the sample with proteinase K (PK) alleviated the inhibition and allowed for full recovery of the DNA from the sample. Data from these samples also showed high precision and femtogram (fg)-level sensitivity in complex matrices. The results from our study clearly show the effectiveness of ddPCR in detecting residual HCD by a direct method and without the need for DNA extraction.

Materials and Methods

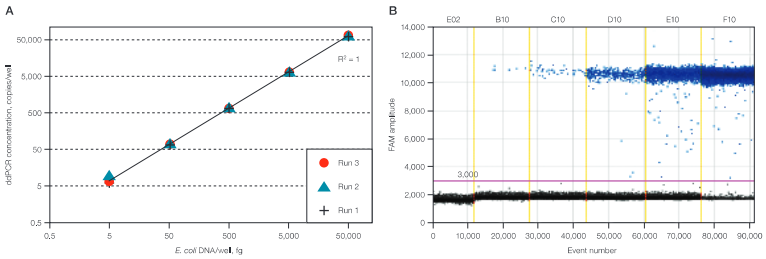
Droplet Digital PCR Workflow



- Chinese hamster ovary (CHO) DNA was purchased from Cygnus Technologies, and *Escherichia coli* (*E. coli*) DNA was purchased from American Type Culture Collection
- ddPCR reagents for residual DNA detection were made at Bio-Rad
- Primers and probes were designed targeting short interspersed element (SINE) sequences for CHO genomic DNA (gDNA) and ribosomal RNA (rRNA) repetitive sequences for *E. coli*. All oligonucleotides were ordered from Integrated DNA Technologies, Inc
- ddPCR was performed on a QX200 Droplet Generator and QX200 Droplet Reader
- Mock samples were assembled in-house using chemicals purchased from Sigma-Aldrich Corporation
- qPCR reagents were obtained from a commercially available source

ddPCR Sensitivity and Precision

Dynamic Range and Precision of ddPCR Results Using *E. coli* DNA

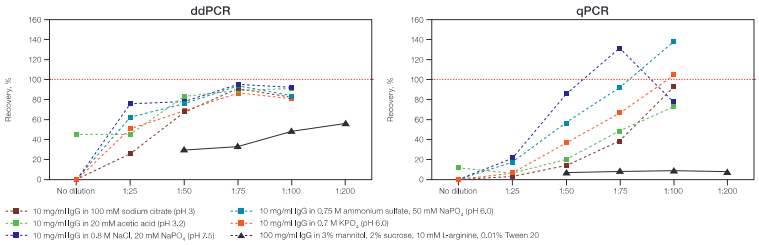


E. coli DNA was serially diluted tenfold from 50 pg down to 5 fg and analyzed using ddPCR. **A**, ddPCR reproducibility and precision. The experiment was repeated three times on different days. **B**, the 1-D fluorescence amplitude plot for Run 3. Demonstrates consistent performance across different concentrations.

- 4 logs linearity
- <5% coefficient of variation (CV) between runs
- Low femtogram-level detection and quantification

Inhibitor Tolerance in ddPCR

Percentage of DNA Recovery Across Various Sample Matrices and Dilutions

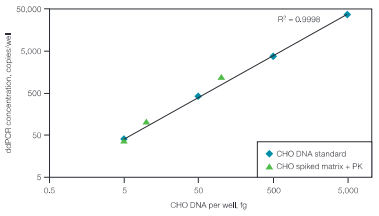


CHO DNA (100 fg) was spiked into various dilutions of different sample matrices and analyzed using ddPCR and qPCR. Data were normalized to a control, CHO DNA in water, and reported as percentage recovery. No data were generated at 0 and 1:25 dilutions for the highest IgG sample, indicated by the solid black lines, because PCR inhibition is assumed at these IgG concentrations.

- Direct ddPCR of samples in complex matrices can be achieved with sufficient dilutions
- Good recovery of CHO DNA samples in ddPCR requires less dilution than in qPCR
- qPCR data were highly variable due to PCR efficiency bias; the results change significantly with small shifts in quantification cycle (Cq)
- Recovery of CHO DNA with high amounts of protein (100 mg/ml IgG) was poor using both ddPCR and qPCR; however, PK pretreatment can alleviate inhibition and allow for full recovery of the DNA with ddPCR (see below)

Improved IgG Tolerance with Proteinase K Pretreatment

DNA Detection in Samples with High IgG Levels Using Proteinase K Pretreatment



CHO DNA was spiked into a sample matrix composed of 100 mg/ml IgG, 3% mannitol, 2% sucrose, 10 mM L-arginine, and 0.01% Tween 20. The sample was then treated with PK and diluted to 100, 10, and 5 fg of CHO DNA prior to ddPCR analysis. CHO standards were also included as controls to determine recovery of the test sample. Full recovery of the CHO DNA was obtained at the three concentrations tested. The data show good linearity, sensitivity, and IgG tolerance. The amount of IgG equivalent in the 100 fg CHO DNA sample is 5 mg/ml.

Conclusions

Here, we propose ddPCR as an alternative method for routine testing of residual host cell DNA. Our data demonstrate that direct quantification of residual HCD using ddPCR is highly sensitive and precise. More than 80% recovery of spiked-in CHO DNA was obtained with all samples at various dilutions, except for one sample containing high levels of IgG (100 mg/ml). However, pretreatment of this sample with PK prior to ddPCR analysis allowed for full recovery of the DNA sample. ddPCR performance remains the same, even in complex samples without DNA. By eliminating the need for DNA extraction, ddPCR can save time, cost, and labor without compromising performance.

References

U.S. Food and Drug Administration (2012). FDA Briefing Document: Vaccines and Related Biological Products Advisory Committee Meeting, September 19, 2012. Cell Lines Derived from Human Tumors for Vaccine Manufacture, pp. 17–25.

World Health Organization (1997). WHO Expert Committee on Biological Standardization: Highlights of the 46th Meeting, October 1996. WHO Weekly Epidemiological Record 72, 141–145.

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