Size distribution analysis of residual host cell DNA fragments in lentivirus by CGE-LIF

PA 800 Plus

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Mammalian cell lines have become the most widely accepted host for the expression and preparation of biological products. Among biological products already commercially available and under clinical trials, 70% are derived from mammalian cell lines, such as the baby hamster kidney cell line (BHK), the Chinese hamster ovary cell (CHO), the mouse myeloma NS0 cell line and the human embryonic kidney cell line (HEK-293).[1] Residual host cell DNA (HCD) is a process-related impurity present in the finished product. Due to its pathogenicity, the quantification of HCD is a significant critical quality attribute. Existing studies have shown that the pathogenetic functional gene is at least 200 base pairs (bp), so the larger the residual DNA fragment, the higher the risk level. The US Food and Drug Administration (FDA) guidelines for producing new human genetic therapy products clearly state that HCD fragments should be less than 200 bp. The Division of Biological Products and Pharmaceuticals under the National Medical Products Administration (NMPA) in China also pointed out that fragments of HCD should be less than 200 bp in its draft of "Technical Guidelines for Pharmaceutical Research and Evaluation of Gene Therapy Products." Therefore, appropriate methods are required to measure the fragment size distribution of HCD in finished gene therapy products.

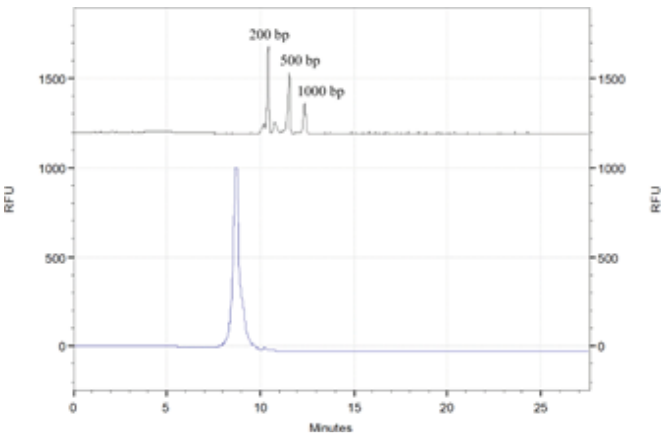

Figure 2. HCD fragment size analysis of lentiviruses by CGE-LIF. a: marker (200, 500, 1000 bp); b: DNA extract of lentiviruses.

Figure 1. PA 800 Plus(top) and dsDNA 1000 kit (bottom).

In this study, lentivirus samples were used as an example to showcase fragment distribution testing of HCD using the PA 800 Plus for drug analysis with a laser-induced fluorescence (LIF) detector and a dsDNA 1000 kit (Figure 1). Generally, LIF detection can detect as low as 10 pg/mL of total DNA, which is essential to fulfilling regulatory requirements for sensitivity.

Key features

- Simple sample preparation
- A quick assessment of HCD in lentivirus, ideal for quality control (QC) operations (15 min)

Methods

Instrumentation and software: A PA 800 Plus from SCIEX (Framingham, MA) was equipped with a laser-induced fluorescence (LIF) detector (with 488 nm excitation wavelength and 520 nm emission wavelength). 32 Karat software, version 10, from SCIEX was the data acquisition and analysis software. The DNA coated capillary ID was 100 µm with effective and total lengths of 30 and 40.2 cm, respectively. The injection conditions were 0.2 psi for 10 s; the separation voltage was -7.8 kV for 20 min; the capillary and sample temperatures were 20°C and 10°C, respectively. The capillary conditioning method consisted of a rinse with deionized water (5 min at 20 psi) followed by a gel buffer rinse (5 min at 20 psi). The capillary surface was voltage equilibrated for 10 min at -7.8 kV. Before each injection, the capillary was rinsed for 2 min at 20 psi with a gel buffer.

Reagents: The dsDNA 1000 kit from SCIEX included the DNA coated capillary. The SYBR Gold fluorescent dye was from Thermo Fisher (Carlsbad, CA). The 10 × Tris Borate-EDTA (TBE) buffer, DNA size ladder (200 bp, 500 bp and 1000 bp), was from Sigma-Aldrich (St. Louis, MO).

Gel buffer preparation: A total of 20 mL of deionized water was transferred into the lyophilized gel and stirred until the solid gel was completely dissolved. The solution was diluted 10-fold with 1 × TBE buffer. Then, 10 mL of gel buffer was mixed with 1 µL of SYBR Gold dye for the CGE-LIF analysis.

Sample preparation: The lentivirus samples were provided by a Chinese gene therapy company and purified with the HCD magnetic bead extraction kits manufactured by HZS Biotechnology Co., Ltd. The sample preparation workflow is shown in Figure 3.

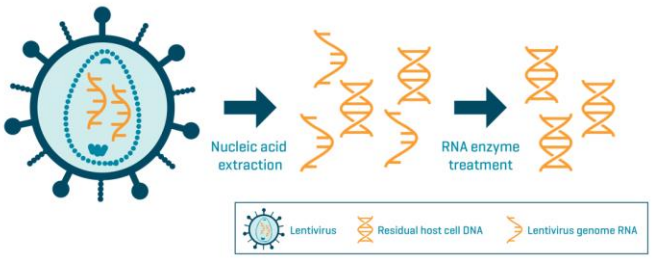

Figure 3. Sample pretreatment process flow of lentivirus HCD distribution analysis.

Extraction of residual DNA: The residual DNA extraction procedure followed the manufacturer's instructions. Briefly, 100 µL of the test sample was mixed with proteinase K solution and incubated at 55°C for 1 h to digest the membrane and DNA-binding proteins and to fully release the DNA. Then, 200 µL of isopropanol and 10 µL of magnetic beads were added to allow the DNA to bind to the magnetic beads. The magnetic beads were washed with the buffers provided to clean and isolate the DNA. The concentration of the supernatant containing the extracted and purified DNA was measured with a UV spectrophotometer.

Results and discussion

Figure 2 (front page) shows a representative electropherogram of the DNA size ladder (top) and the HCD from a lentivirus sample (bottom).

By comparing the migration time from the DNA extracted from the lentivirus sample with the migration time of the DNA size ladder, it is reasonable to conclude the residual DNA fragment is well below the threshold of 200 bp. This result demonstrates that the size of the HCD in this lentivirus sample can be determined quickly to help users optimize the processes for quality control.

Conclusions

- Different DNA can be isolated according to the fragment size in combination with DNA markers to evaluate the fragment size distribution of DNA
- Easy to operate: sample pretreatment kits and separation kits are commercially available

References

1. Distribution analysis of residual host cell DNA fragments by CE-LIF. SCIEX, RUO-MKT-02-9259-ZH-A

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