Salt gradient separation of nucleic acid components in Adeno-Associated Virus using anion exchange chromatography

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Abstract

Purpose: To determine the viral genome content within the capsid then further analysis of genome integrity after digestion.

Methods: An anion exchange (AEX) method was developed for fast genome content analysis of AAV capsid before digestion. The same method was used for genome integrity assessment.

Results: AEX evaluation of AAV serotype 6 containing a DNA genome showed high genome integrity.

Introduction

Adeno-associated virus (AAV) has emerged as a promising transport system for gene therapy, allowing treatment for a wide range of diseases, including cancer, cystic fibrosis, heart disease, diabetes, hemophilia and AIDS. AAV is a small non-enveloped virus with the ability to encapsulate up to a 4.7 kb single-stranded DNA (ssDNA) genome within its capsid protein.[2] Characterizing the AAV vector requires identity, potency, and safety.[1] A key aspect of this characterization is determining the viral genome content genome integrity.

Materials and methods

Sample preparation

An AAV6 sample expressed in cells was used with a titer of 10^13 GC/mL.

Part 1: Perform serial dilutions using the provided buffer at ratios of 1:1, 1:4, 1:5 and 1:10.

Part 2: Use Proteinase K from Invitrogen™ PureLink™ Viral RNA/DNA Mini Kit to digest the capsid protein for 15 minutes, followed by extraction and isolation of the DNA genome.

Test method(s)

Columns

- ProPac ™ 3R SAX, 4.0 x 100 mm, 3.0 µm, (P/N 43203-104068)

Instrumentation

Thermo Scientific™ Vanquish™ Flex Binary system consisting of:

- Vanquish™ System Base (P/N VH-S01-A)
- Vanquish™ Quaternary Pump F (P/N VF-P20-A)
- Vanquish™ Sampler F (P/N VF-A10-A)
- Vanquish™ Column Compartment H (P/N VH-C10-A-02)
- Vanquish™ Diode Array Detector HL (P/N VH_D10_A) with light pipe standard flow cell (P/N 6083.0100B)
- Vanquish™ Fluorescence Detector C with Dual-PMT (P/N VC-D51-A) with standard bio flow cell (P/N 6079,4230)

Data Analysis

- The Thermo Scientific™ Chromeleon™ 7.3.1 Chromatography Data System was used for data acquisition and analysis.

Separation conditions

- Mobile phase A: 100mM Tris-HCl
- Mobile phase B: 0.8M NaClO4
- Mobile phase C: Water
- Flow rate: 0.3 mL/min
- Autosampler temperature: 10 °C
- Column temperature: 50 °C
- Injection volume: 5 µL
- Injection wash solvent: 10% Methanol

Fluorescence Detector settings:

- Excitation: 280nm; Emission: 330nm; Sensitivity 8
- Lamp mode: high power:

Diode Array Detector setting:

- Absorbance wavelength: 260nm and 280nm; 3D Field: 200-400 nm

Table 1. Gradient method

Time (min)	%A	%B	%C
0.0	20	0	80
3.0	20	0	80
5.0	20	30	50
10	20	40	40
15	20	45	35
16	20	80	0
19	20	80	0
19.1	20	0	80
25	20	0	80

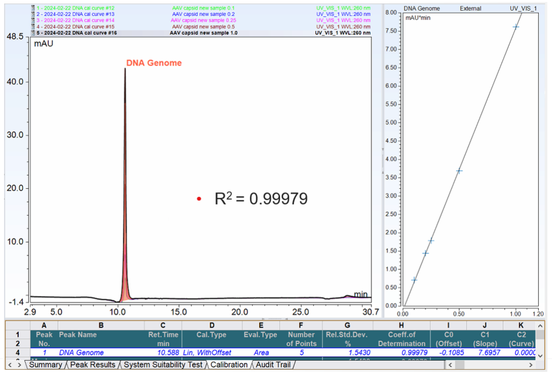
Results

Part 1: Quantification of encapsulated DNA genome using anion exchange chromatography

In this method, the DNA initially binds to the stationary phase at a lower salt concentration, as the specific interaction between the phosphate groups and the positively charged stationary phase is stronger. As the salt concentration increases, the competing ions in the mobile phase disrupt this interaction, causing the DNA to elute off the column. The elution order of DNA molecules in anion exchange chromatography is influenced by their size and the number of phosphate groups they possess. Longer DNA molecules contain more phosphate groups, resulting in a higher binding affinity toward the stationary phase and a slower elution rate. Conversely, shorter DNA molecules with fewer phosphate groups elute faster.

The overlay of all calibration samples showed excellent resolution and intensity for all peaks. The calibration curve demonstrated a high calibration coefficient of 0.99979. This calibration curve can be used to quantify DNA genome content in the capsid.

Figure 1. Calibration curve for AAV capsid with baseline subtraction



Part 2: Separation of released DNA genome using anion exchange chromatography

For further analysis of genome integrity, after sample digestion, the ssDNA, partial DNA and oversized DNA were released. The process involved less than one hour of sample preparation. The purity of the DNA genomes was assessed by calculating the absorbance ratio at 260 to 280 nm (Figure 2&3). [3] In addition, overlaid chromatograms allowed for visualizing the progress of DNA purification over the digest time (Figure 4 &5). Fluorescence data showed AAV capsid protein dramatically decreased with digestion time while UV data showed DNA dramatically increased with digestion time. Simultaneously, an alkaline agarose gel was employed to run the same DNA sample, enabling confirmation of DNA identification by comparing the DNA peaks in the chromatograms with the prominent bands in the agarose gel (Figure 3).

Figure 2. Overlay chromatogram at 260 m/280nm before digestion sample.

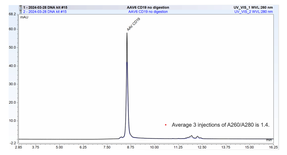


Figure 3. Overlay chromatogram at 260 m/280nm after digestion sample.

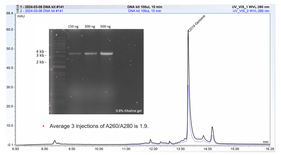


Figure 4. Digestion time with Proteinase K for 0, 5, 10 and 15 min using FLD detection

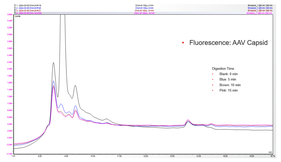
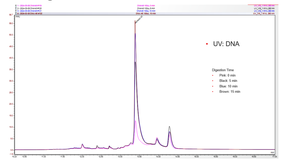
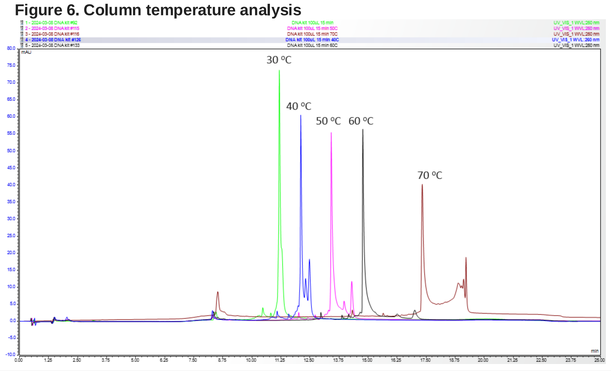


Figure 5. Digestion time with Proteinase K for 0, 5, 10 and 15 min using UV detection



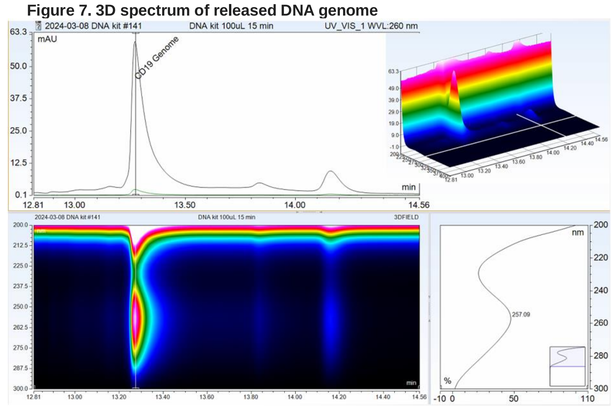
Salt effect analysis was performed using tetramethylammonium chloride (TMAC), NaCl and NaClO4. NaClO4 demonstrated the best separation (data not shown). Additionally, column temperature analysis indicated that 40 °C or 50 °C yielded the best separation (Figure 6). Since the conformation of the ssDNA is temperature-dependent, [4] an increase in temperature resulted in a corresponding increase in peak retention time.

Figure 6. Column temperature analysis



From the 3D spectrum (Figure 7). , The maximum absorbance at 260nm (257.09) observed for all three peaks at retention times 13.2, 13.8, and 14.2 minutes confirms the presence of released ssDNA, partial DNA, and oversized DNA. This suggests the successful detection of these DNA fragments in the analyzed sample

Figure 7. 3D spectrum of released DNA genome



Conclusions

- Superior peak intensity enabled confident quantification of DNA content.
- The separation method of released DNA genome demonstrates superior separation and repeatability with significantly reduced sample preparation time.
- The ProPac 3R SAX column is an appropriate column for the separation of long DNA molecules.

References

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[3] Genomic DNA Isolation Kits Technical resources. https://www.thermofisher.com/us/en/home/technical-resources/technical-reference-library/nucleic-acid-purification-analysis-support-center/genomic-dna-purification-support/genomic-dna-purification-support-getting-started.html?open=g1#g1

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