康龙化成 PHARMARON

Application of CGE for Purity Analysis in Cell and Gene Therapy

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CASSS CE Pharm

16th September 2024

Laboratory Services

Chemistry, Manufacturing and Control

Clinical Development

Biologics & CGT

SCIEX

Overview

- Introduction to Pharmaron
- Overview of Plasmids and AAV
- CE for Plasmid Analysis - Kit Comparison
- CE for AAV Purity and Genome Integrity Analysis
- Summary

Pharmaron Overview

PHARMARON is a leading fully integrated pharmaceutical R&D services platform with global operations and has a well-established team of over 20,000 employees working in 21 different sites located in China, the United States and the United Kingdom.

Mission

To support our partners' success in discovery, development and commercialization of innovative medicines

Vision

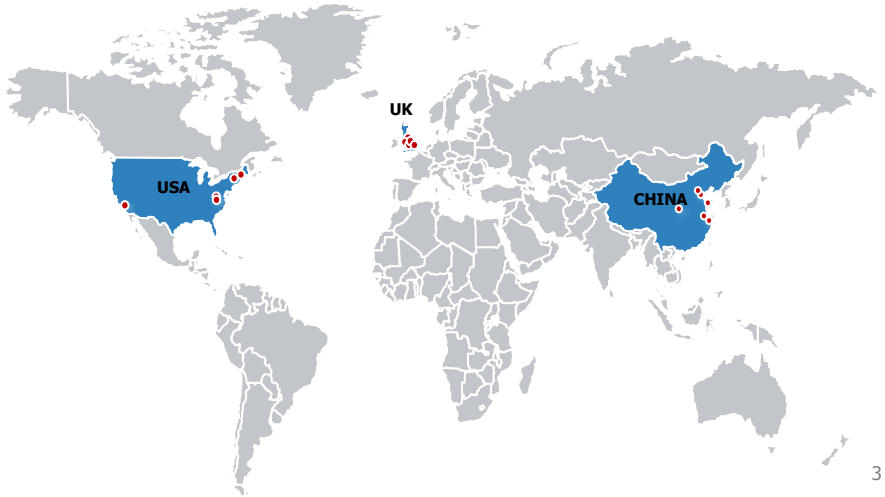
To become the world-leading life science R&D service company

Global Footprint

6 US Sites

5 UK Sites

10 China Sites



Gene Therapy Capability at Pharmaron, Liverpool, UK

World Class Centre for Gene Therapies

cGMP
cGMP multi-product bio-manufacturing facility with the latest automation

Viral Vector
Specific site focus of viral vector development and clinical manufacture

Gene Therapy
Key site focus since 2018

c.200 FTE
Highly talented employees focused on supporting the development of innovative biologics

Analytics
State of the art equipment and enhanced through collaborations with centres of excellence

MHRA MA (IMP)
Facility authorised to manufacture Human Investigational Medicinal Products for Clinical Trials

Pharmaron Plasmids
Pharmaron design including GOI backbone

Pharmaron HEK293 Cell Line
Research and GMP Master Cell Banks

Plasmid Manufacturing
Research Grade, GMP-like and GMP grades

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Overview of Plasmids and Adeno-Associated Virus

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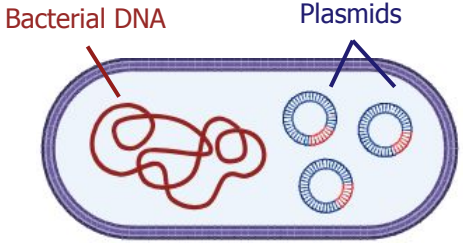
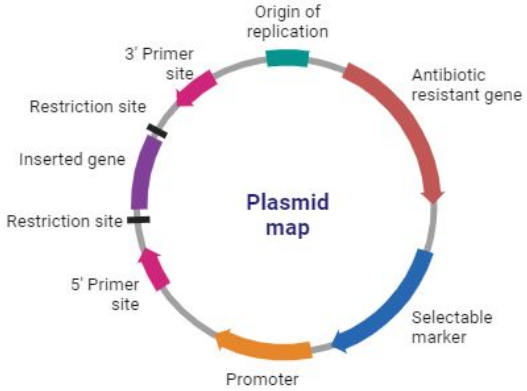
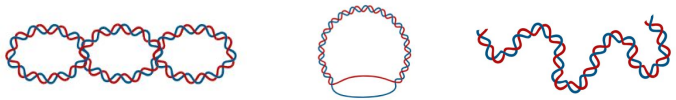
What Are Plasmids?

- **Plasmids** (pDNA) are small, circular, double-stranded DNA molecules, that exist as separate entities to the chromosomal DNA of bacterial cells.
- Genetic engineering allows for the manipulation of DNA sequences, enabling the expression of genes of interest.
- *In* vivo, pDNA typically exists in a **supercoiled** form, however other isoforms may form as breaks in the DNA strands occur.



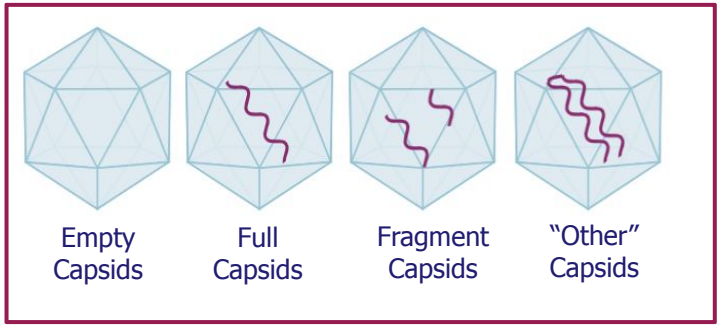




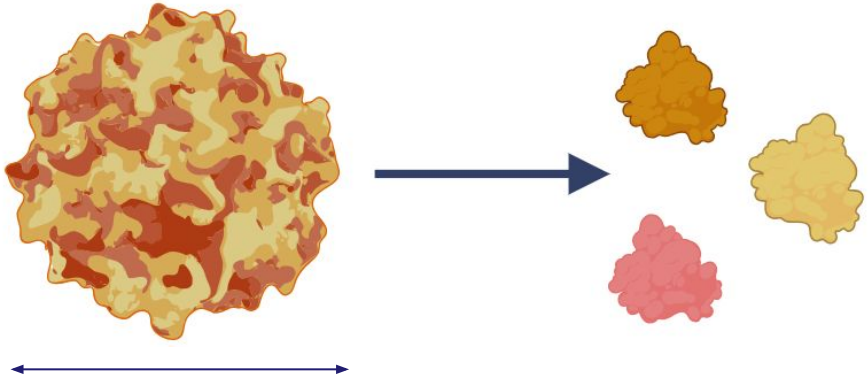
Diagrams created in BioRender.com

Overview of Adeno-Associated Virus (AAV)

- Top choices to deliver DNA to target cells.
- Safest strategies for GT; mild immune response and its non-pathogenic nature.
- Lack genes for viral integration and replication.
- Target tissue serotype specific.



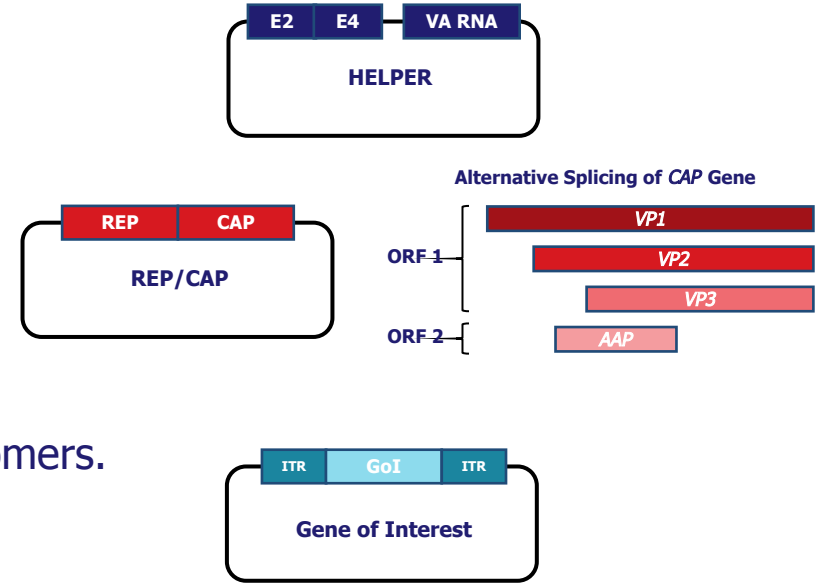
Modes of production can influence heterogeneity with respect to the packaging of the DNA.



3 repeating viral proteins in ~10:1:1 ratio (VP3:VP2:VP1)

Multiple Plasmids are Required to Support AAV Production

- 3 Plasmids typically used for Transient Transfection-based production of AAV
  - HELPER (enables AAV production)
  - REP/CAP (Replication and capsid formation)
  - Gene of Interest
- Pharmaron have created a **catalogue of AAV plasmid constructs** to support our customers.



High quality plasmid as a starting material produces higher quality AAV

Pharmaron Approach to Plasmid Analysis

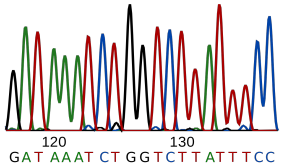
- Addressing **key challenges** in purified plasmid characterisation:
  - Extensive analysis of Plasmid CQA's.
  - Portfolio of assays **READY** to support plasmid manufacture for use as **starting material** and **batch release**.

Impurities

- Residual Antibiotic ELISA
- Residual hcDNA by qPCR
- Residual hcRNA by HPLC
- Residual HCP ELISA

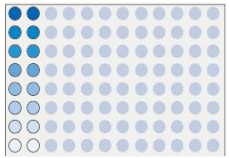
Identity

- Sanger Sequencing
- NGS
- Restriction Digest and Gel Migration



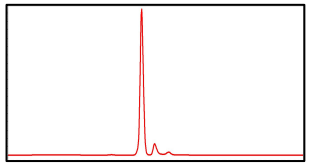
Titre

- UV by A260
- Anion Exchange HPLC



Purity

- A260/280 ratio by UV
- DNA Homogeneity by CE-LIF



Compendial /Safety

- Endotoxin
- Bioburden
- pH
- Osmolality
- Appearance

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Capillary Electrophoresis (CE) for Plasmid Purity Analysis

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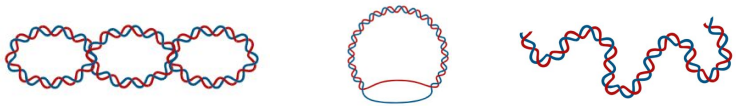
Improving Plasmid Isoform Determination

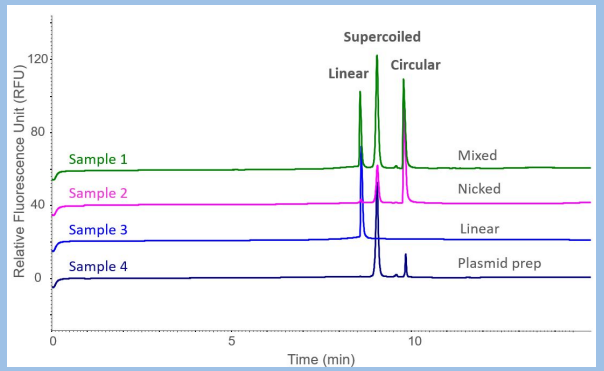
- **Purity** of plasmid is determined by **percentage (%) supercoiled isoform**.
- Requirement for high % supercoiled to **meet release specifications**.
- Traditionally gel-based methods are utilized, however limitations around **quantification** and **resolution** of impurities.
- We investigated **CE-LIF** for a more **accurate purity evaluation**.



Supercoiled (SC) Open Circular (OC) Linear (L)

dsDNA 1000 Kit from SCIEX

Assay designed for 1 kb plasmid, but can be optimised for larger plasmids (7-15 kb)

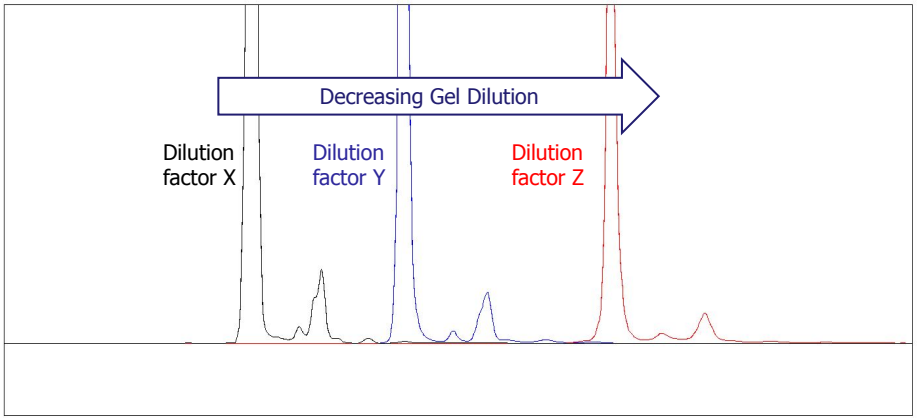


SCIEX Application Note: 'Simple, Fast and Robust Method for Plasmid Purity Testing and Degradation Monitoring'

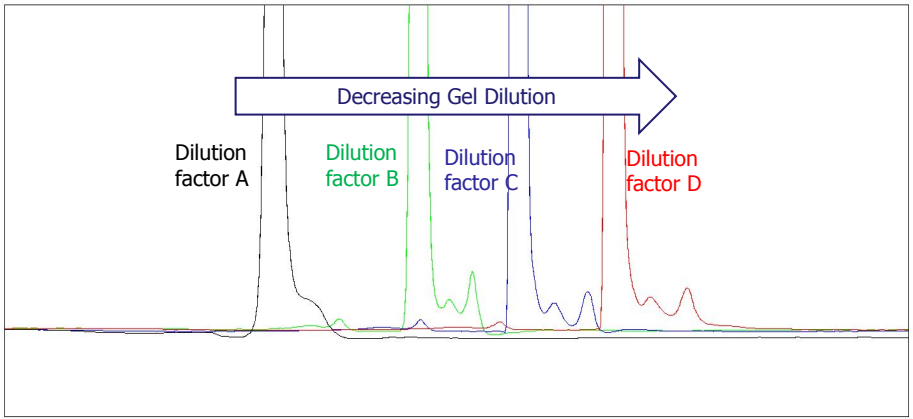
Optimising CE-LIF for Plasmid Purity Analysis

- dsDNA 1000 kit gel acts as sieving matrix – plasmid size specific.
- Optimum gel dilution for each plasmid size for separation of SC and impurities.
- Separation of isoforms is more challenging with larger plasmids.

7 kb plasmid



11 kb plasmid

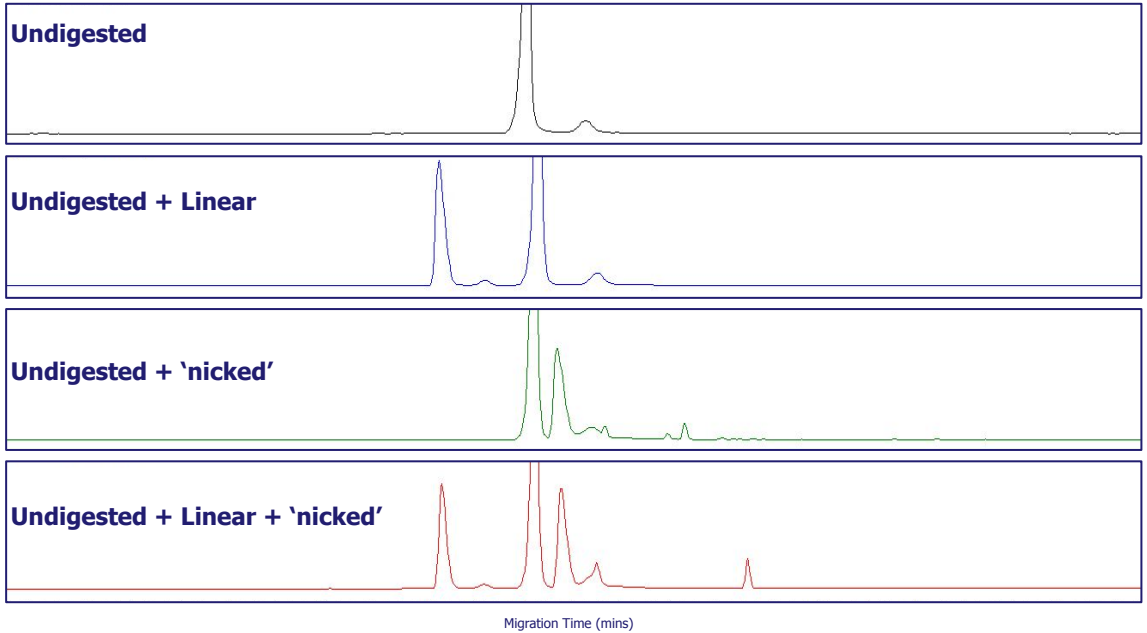


Dilution of the separation gel can be optimised for accurate purity determination of different plasmid sizes

Characterising Plasmid Isoforms

- Capillary Gel Electrophoresis is inherently challenging to characterise impurities.
- Restriction digestion can be used to generate specific isoforms.
- Digested samples can be spiked into undigested sample to help identify impurity peaks.

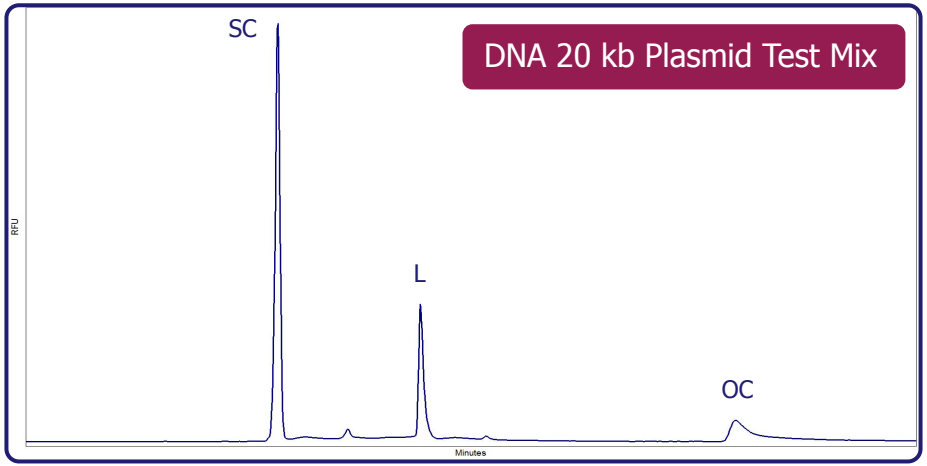
Restriction digest of a 7 kb plasmid



Restriction digest can be used to characterise various plasmid isoforms

DNA 20 kb Plasmid and Linear kit from SCIEX

- Streamlined plasmid purity determination with great resolution of plasmid isoforms.
- Separation of linear dsDNA between 100 and 20,000 bp allows for size estimation and fragment analysis.
- No optimisation of gel dilution required; neat gel used for analysis of broad size range of dsDNA.



Streamlined analysis of a range of Plasmid dsDNA with the new DNA 20 kb Plasmid and Linear Kit

Comparison of dsDNA kits

Kits from SCIEX	dsDNA 1000 kit 	DNA 20 kb Plasmid and Linear kit 
Size range	Up to 1 kb, optimised for analysis of larger dsDNA.	2-15 kb (up to 20 kb)
Gel state	Dehydrated solid gel, requires reconstitution in water prior to use	Liquid, no reconstitution required
Fluorescent dye	LIFluor EnhanCE or SYBR™ Gold Nucleic Acid gel stain[1]	SYBR™ Gold Nucleic Acid gel stain[1], potential use with LIFluor EnhanCE
Capillary	DNA Coated Capillary, 40.2 cm length	Bare Fused-Silica Capillary, 30.2 cm length Dynamic coating of capillary during conditioning, required every 50 injections
System compatibility	CESI 8000 Plus system, PA 800 Plus system DNA capillary not available for the BioPhase 8800 system	CESI 8000 Plus system, PA 800 Plus system and BioPhase 8800 system (multi-capillary)
Optimisation required	Yes, optimisation of gel dilution required for each new plasmid and plasmid size	No, gel is universal up to 20 kb plasmid size

Simplified plasmid purity analysis with the new DNA 20 kb Plasmid and Linear kit

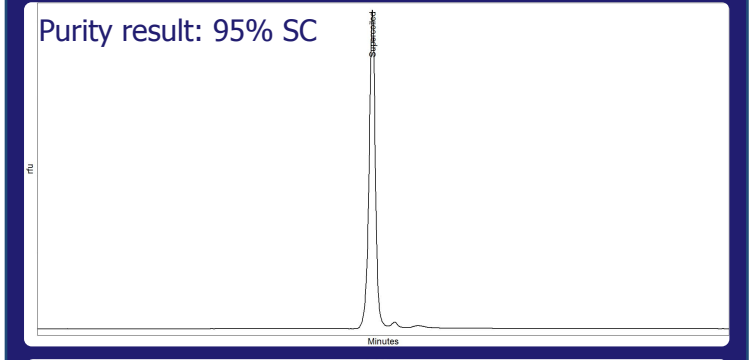
[1]SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Gold Nucleic Acid gel stain is not available for resale.

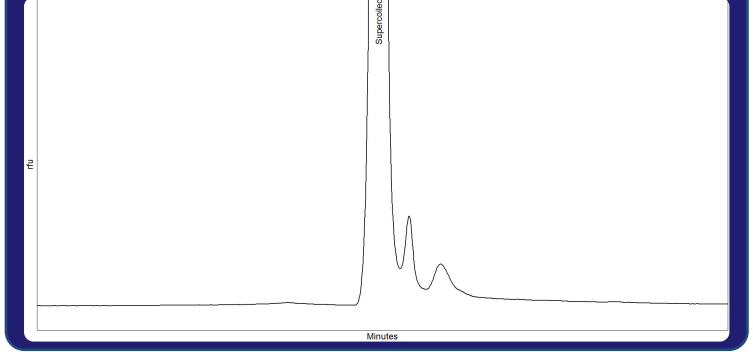
Kit comparison: 7 kb Plasmid

[1]SYBR™ is a trademark of the Life Technologies Corporation.
SYBR™ Gold Nucleic Acid gel stain is not available for resale.

dsDNA 1000 kit (LIFluor EnhanCE)

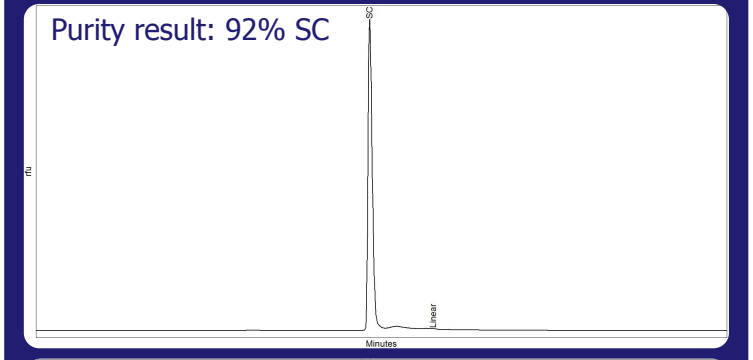
Purity result: 95% SC

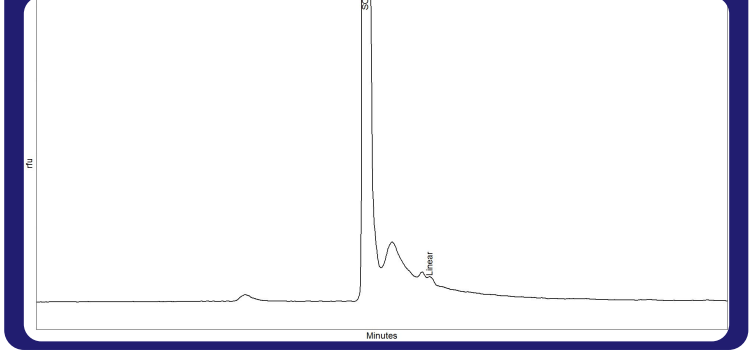




DNA 20 kb Plasmid and Linear kit (SYBR™ Gold Nucleic Acid gel stain[1])

Purity result: 92% SC





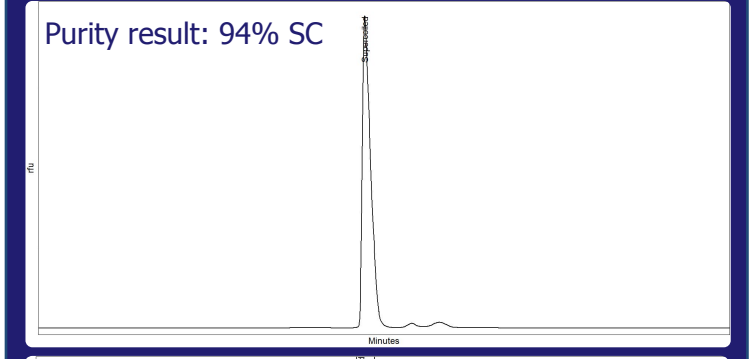
Comparable plasmid purity profile of 7 kb plasmid with both dsDNA kits

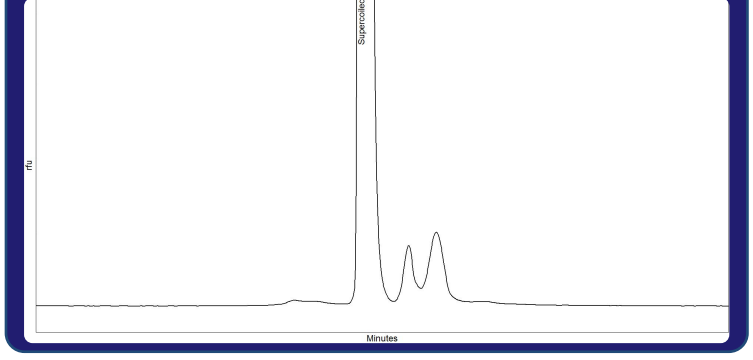
Kit comparison: 12 kb Plasmid

[1]SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Gold Nucleic Acid gel stain is not available for resale.

dsDNA 1000 kit (LIFluor EnhanCE)

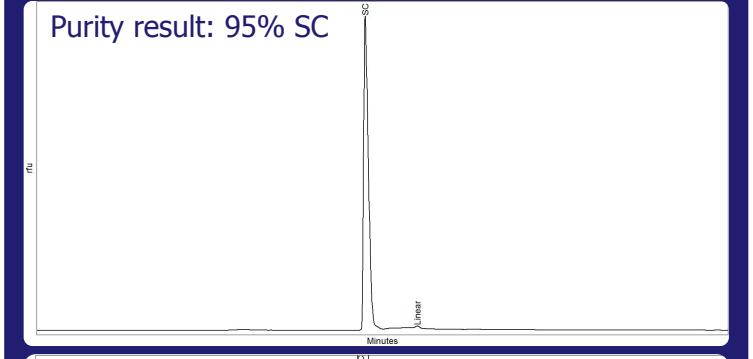
Purity result: 94% SC

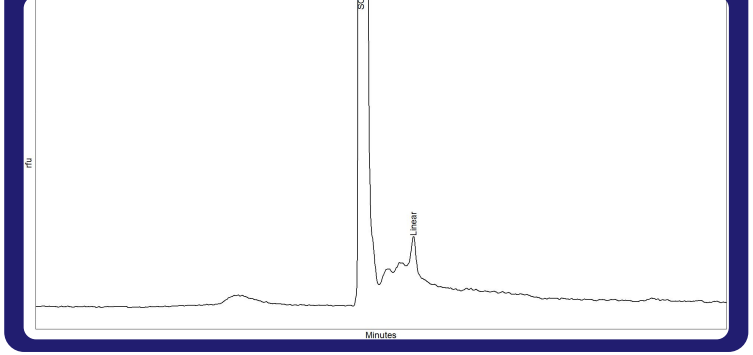




DNA 20 kb Plasmid and Linear kit (SYBR™ Gold Nucleic Acid gel stain[1])

Purity result: 95% SC



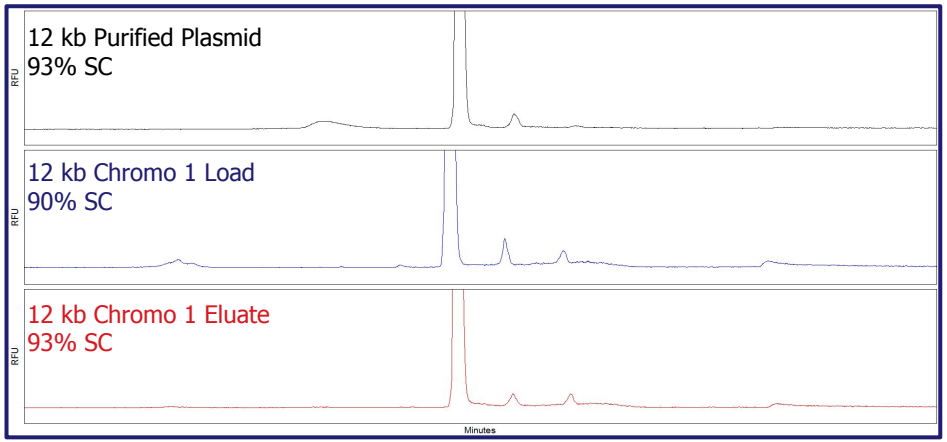


Comparable plasmid purity profile of 12 kb plasmid with both dsDNA kits

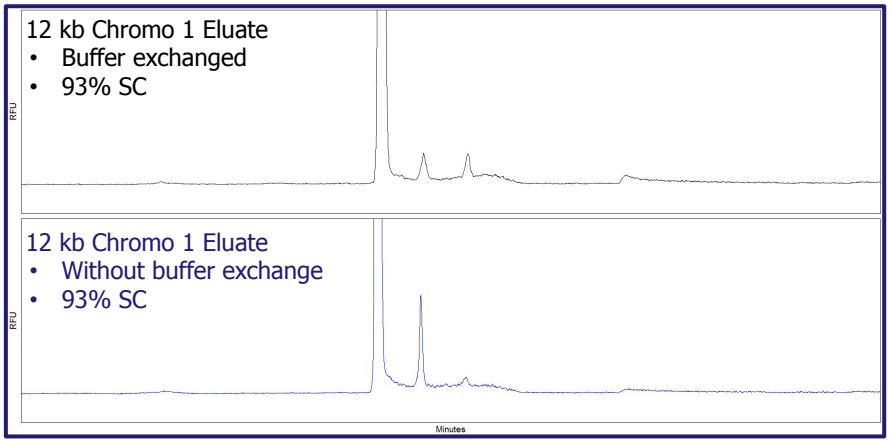
In-process Sample Analysis with the DNA 20 kb Plasmid and Linear kit

- Both SCIEX kits for dsDNA analysis allow for plasmid purity analysis throughout the production process including cell paste, in-process and bulk purified plasmid material.
- Buffer exchange typically required to minimise affect of high salt concentration.

Buffer exchanged in-process samples



Comparison with and without buffer exchange, 0.3M salt

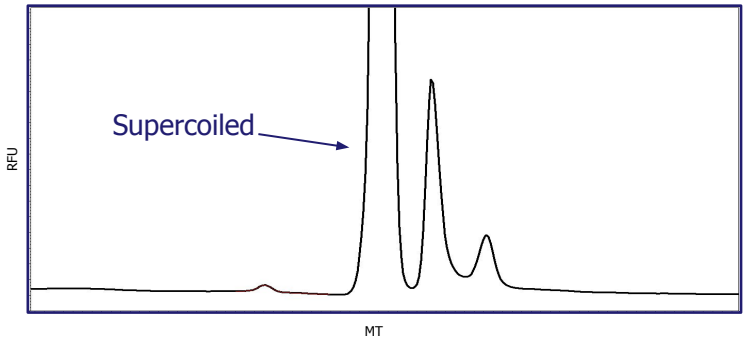


Successful plasmid purity monitoring throughout the production process

CE-LIF for Plasmid Purity - Summary

- **Purity** of plasmid is determined by **percentage (%) supercoiled isoform**.
- **CE-LIF** method developed for **accurate purity evaluation**.
- **Successfully validated platform method** for ~6-15 kb plasmid size range.
- The new **SCIEX DNA 20 kb Plasmid and Linear kit** evaluated for plasmid purity analysis across two different plasmid sizes throughout the manufacturing process.
- Comparable purity data was achieved between the dsDNA 1000 kit and the DNA 20 kb Plasmid and Linear kit

Electropherogram for Plasmid Purity



Benefits of the DNA 20 kb Plasmid and Linear kit:

- Compatible across single and multi-capillary CE systems
- Reduced analyst intervention
- Improved robustness and reduced assay variability

Reduced optimisation for different plasmid sizes using the new DNA 20 kb Plasmid and Linear kit

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AAV Purity Determination

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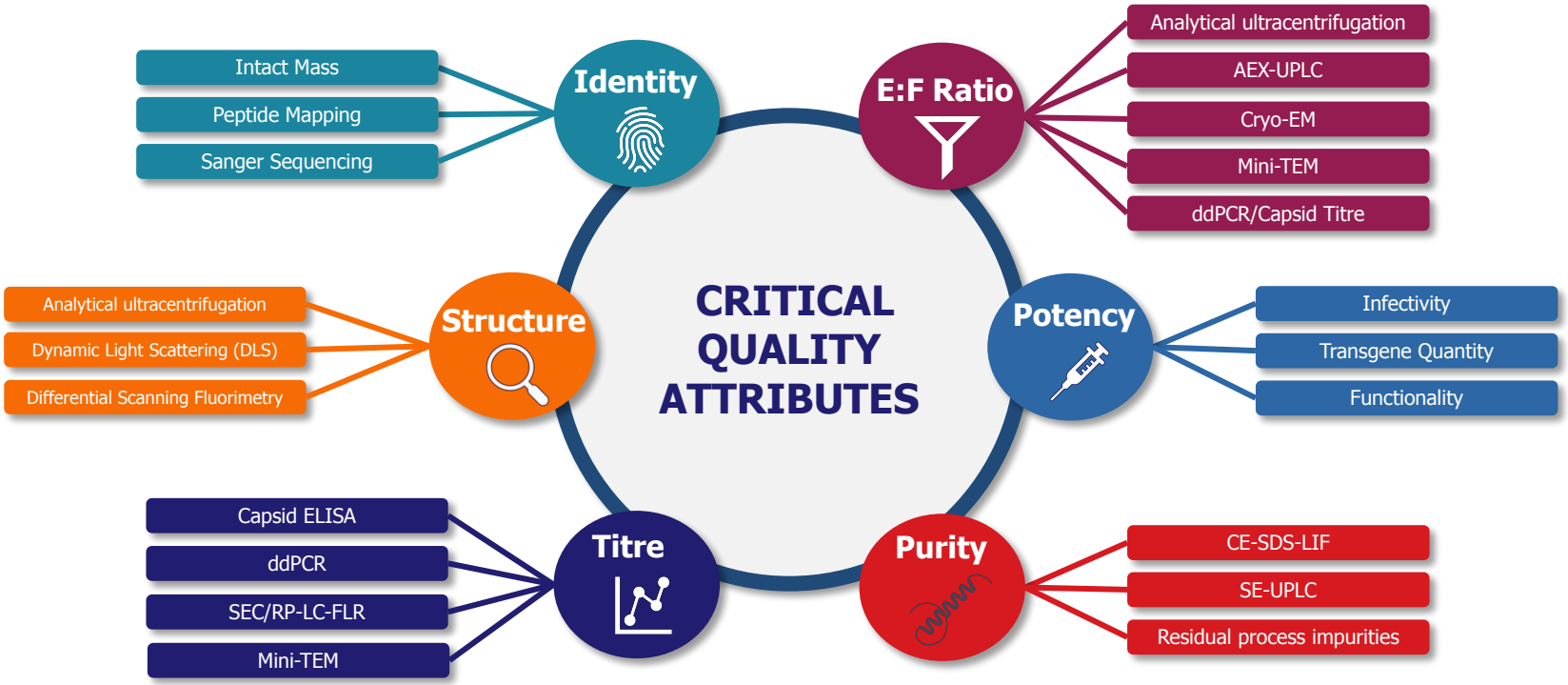
Pharmaron Approach to Viral Vector Critical Quality Attribute Analysis



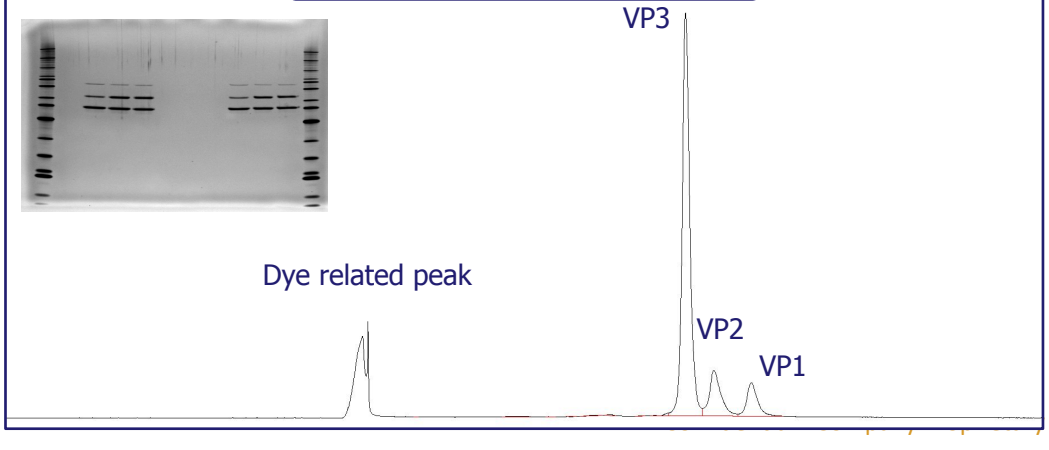
Full analytical toolkit to support product CQA analysis

CE-SDS-LIF for AAV Purity

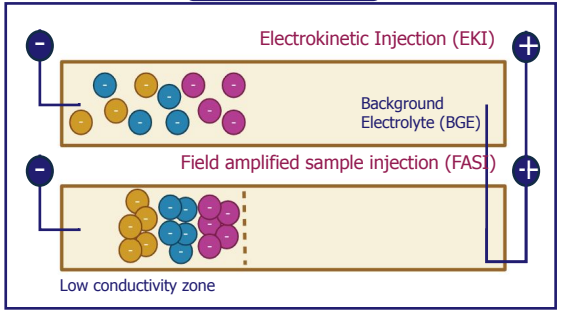
- Protein-SDS complexes separated based on electrophoretic mobility through a gel filled capillary when voltage is applied.
- Can be used to monitor viral proteins (VP1, VP2, VP3) once capsid is denatured – allows purity assessment of sample (% VPs).
- Ratio of viral proteins can also be estimated.

Sample prep overview → **Denature and reduce capsid** → **Incubate to label with dye** → **Dilute** → **Inject samples**

AAV Capsid Protein Separation



Water plug



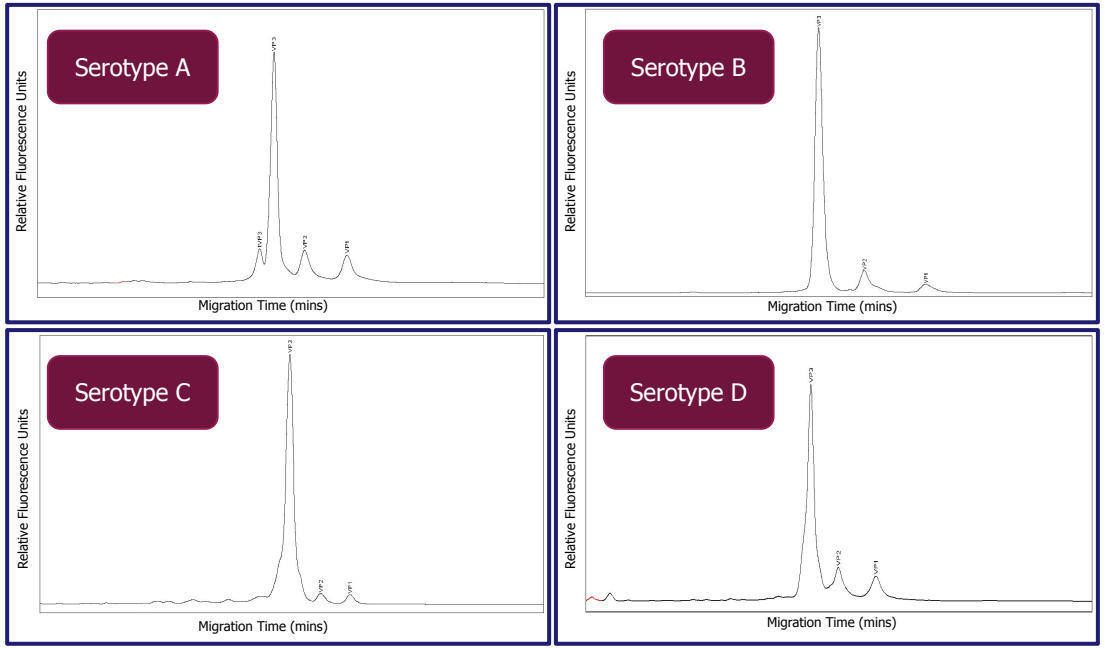
SDS-MW Analysis Kit from SCIEX

CE-SDS-LIF: Multiple AAV Serotypes

Electropherograms showing various AAV serotype purity profiles

Assay performance:
<5% RSD Total area
<2% RSD for % Purity result

- Minor modifications to the method required for each serotype based on physical properties.



CE-SDS-LIF assay has been optimised to allow analysis of multiple AAV serotypes

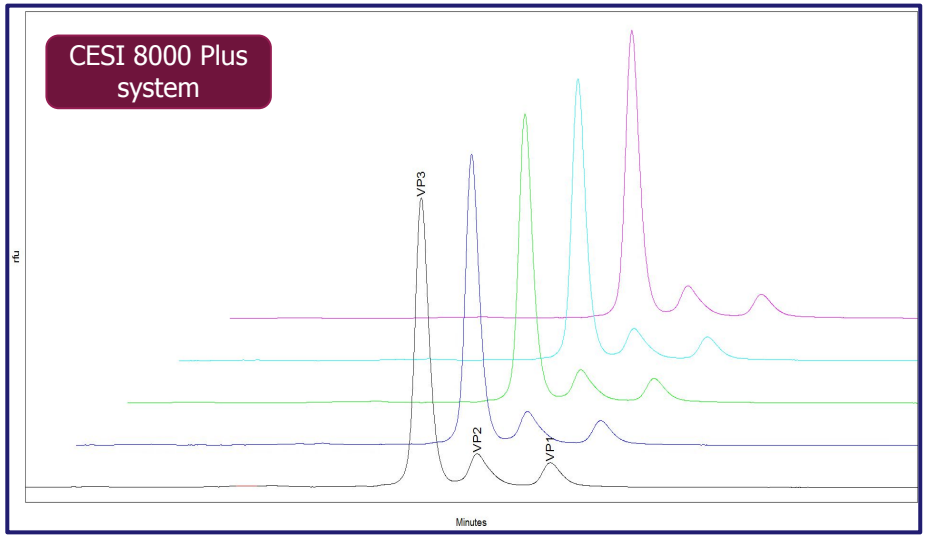
CE-SDS-LIF: BioPhase 8800 system

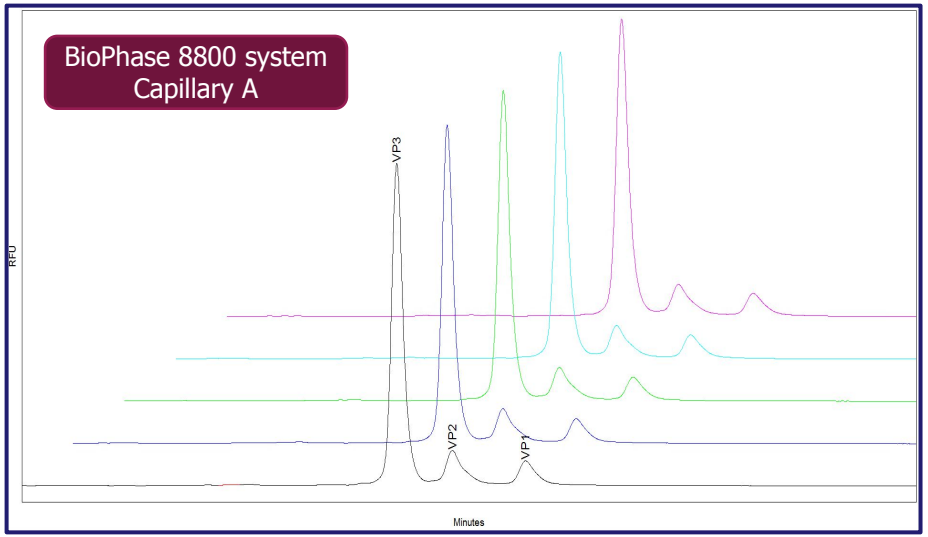
- The BioPhase 8800 system is a high throughput, **multi-capillary** CE system which allows for analysis of **8 injections** at once.
- **Reduced** method development and sample analysis time.
- Compatible with the Waters **Empower™** software for ease of use.
- **Simple method transfer** between standard one capillary systems, CESI 8000 Plus system/PA 800 Plus system and the BioPhase 8800 system.

Increased analytical capabilities with the new BioPhase 8800 system at Pharmaron

AAV Purity by CE-SDS-LIF

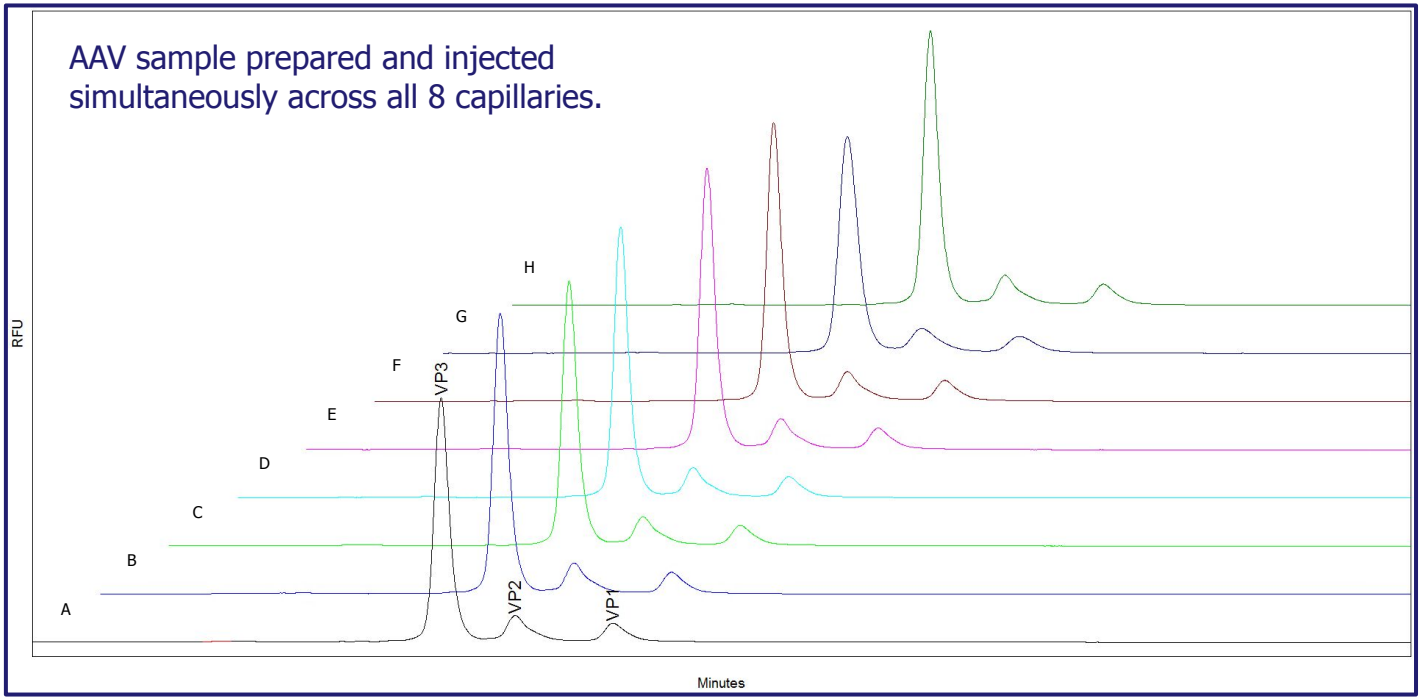
- Transfer of Pharmaron's AAV purity assessment by CE-SDS-LIF method from the CESI 8000 Plus system/PA 800 Plus system to the BioPhase 8800 system.





Method successfully transferred to HTP multi-capillary system with minimal optimisation

AAV Purity with the BioPhase 8800 system



Comparable AAV viral protein profile achieved across all 8 BioPhase 8800 system capillaries

AAV Purity with the BioPhase 8800 system: Peak Area Comparison

- AAV sample prepared and injected 5 times across all 8 capillaries (total of 40 injections).
- Peak areas were compared and % RSD calculated.

Sample Injection	Cap A	Cap B	Cap C	Cap D	Cap E	Cap F	Cap G	Cap H	% RSD Across Capillaries
1	90	104	99	99	104	104	101	101	**4.6**
2	90	103	96	98	104	102	102	98	**4.6**
3	89	100	96	98	103	101	101	98	**4.4**
4	89	102	94	96	102	102	101	98	**4.6**
5	89	101	94	96	101	101	98	99	**4.1**
% RSD	**0.6**	**1.5**	**2.0**	**1.4**	**1.5**	**1.3**	**1.4**	**1.1**	

Comparable peak areas observed across different capillaries and within the same capillary

AAV Percentage Purity Comparison: BioPhase 8800 system vs CESI 8000 Plus system

BioPhase 8800 system

Sample Injection	Cap A	Cap B	Cap C	Cap D	Cap E	Cap F	Cap G	Cap H	Mean	% RSD Across Capillaries
1	99.02	98.77	98.86	98.90	98.71	98.74	99.49	98.96	98.89	**0.2**
2	99.33	98.95	98.98	98.98	98.87	98.72	99.20	98.98	98.99	**0.1**
3	99.47	99.02	98.95	99.02	99.03	99.03	99.68	98.97	99.08	**0.2**
4	99.43	98.93	99.33	98.91	98.47	98.97	99.34	98.34	99.05	**0.2**
5	98.49	98.97	99.02	98.79	98.09	98.74	99.19	98.73	98.95	**0.1**
Mean	99.15	98.93	99.03	98.92	98.63	98.84	99.38	98.80		
% RSD	**0.4**	**0.1**	**0.2**	**0.1**	**0.4**	**0.2**	**0.2**	**0.3**		

CESI 8000 Plus system

Sample Injection	% Purity
1	98.82
2	98.68
3	98.64
4	98.65
5	98.59
Mean	98.68
% RSD	**0.1**

High precision AAV purity results achieved increasing sample throughput **8x** over single capillary system

康龙化成
PHARMARON

AAV Genome Integrity

Laboratory Services

Chemistry, Manufacturing and Control

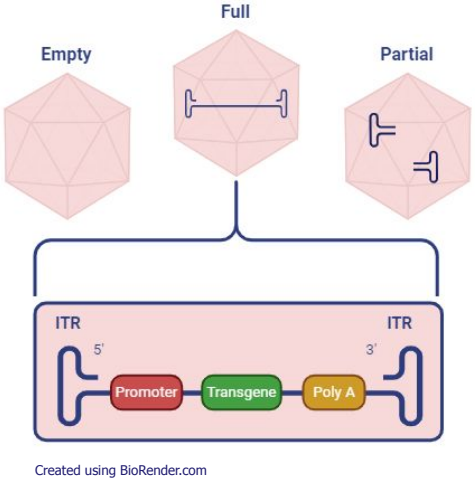
Clinical Development

Biologics & CGT

AAV Genome Integrity by CE-LIF

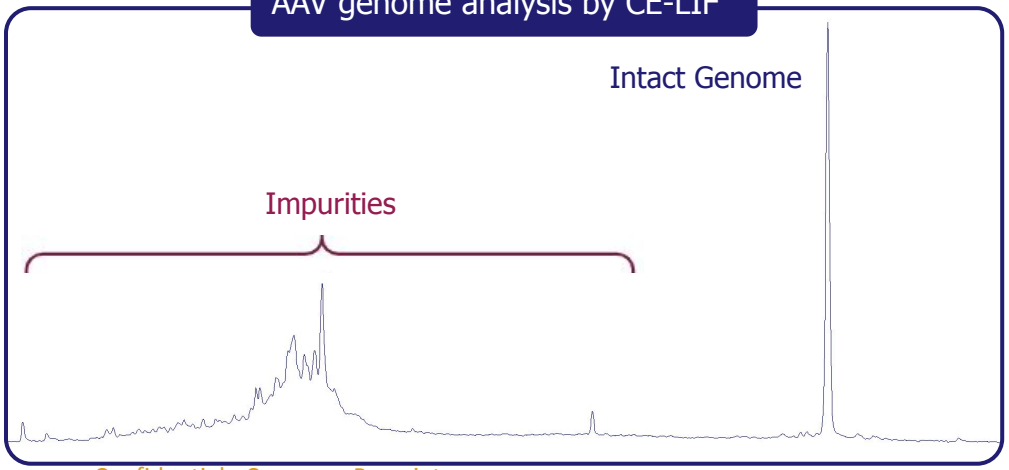
- The AAV genome is composed of two inverted terminal repeats (ITR), a promoter, transgene and a poly-A-tail.
- Errors during encapsidation of the intact genome can result in AAV capsids containing a partial/truncated genome which can impact transgene expression, therapeutic efficacy and safety.
- It is therefore critical to assess the quality and size of the encapsidated genome.
- The sample preparation involves genome release, PCR purification and sample incubation prior to analysis.

RNA 9000 Purity & Integrity kit from SCIEX



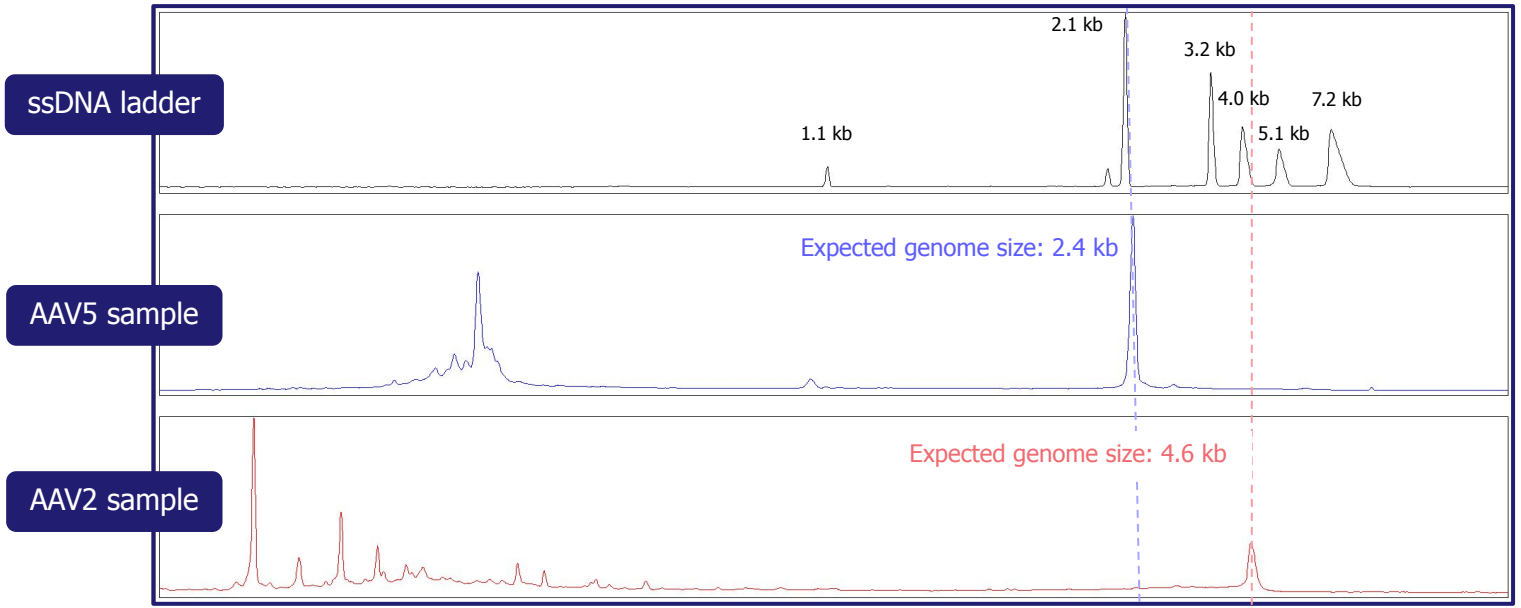
Created using BioRender.com

AAV genome analysis by CE-LIF



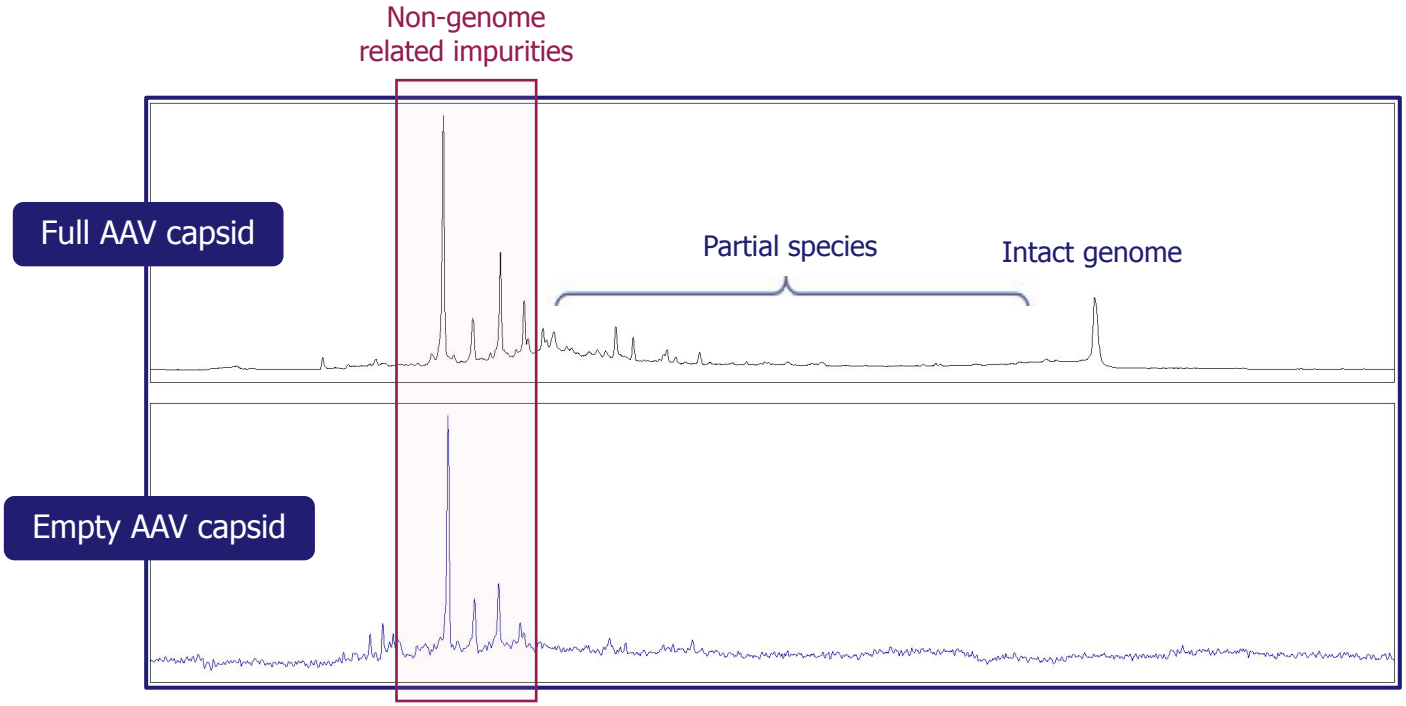
AAV Genome Analysis

Range of AAV serotypes and genome sizes analysed against ssDNA ladder for intact genome size assessment.



Successful confirmation of intact genome size with ssDNA ladder

Empty:Full Capsid AAV Analysis



Empty:full AAV analysis can help determine encapsidated non-genome related species

Summary

- Given the complexity of viral vector manufacture, **Pharmaron's analytics** are critical for successful **production and release** of GT products.
- **CE-LIF** is a powerful technique to obtain accurate information on **plasmid and AAV purity** for production of GT products with minimal sample volume.
- We demonstrated comparable plasmid purity analysis across a broad size range with the new **DNA 20 kb Plasmid and Linear kit** and the dsDNA 1000 kit from SCIEX and observed less optimisation was required for the new kit.
- We transferred the AAV purity assay onto The **SCIEX BioPhase 8800 system** and achieved an **increased throughput** (8x) and **reduced method development** time.
- **Pharmaron** successfully **validated platform CE assays** for plasmid and AAV purity determination which are **readily available** for analysis of a **range of plasmid sizes** and **AAV serotypes**.

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If you are interested in learning more about our advances in Gene Therapy product development watch **Pharmaron's CGT On-Demand Webinar Series here**: