



# Characterization of lentiviral vectors using capillary electrophoresis platform technology

Aaron Shafer, PhD

St. Jude Children's Research Hospital



# Agenda

- Lentiviral vector (LVV) background and analytical characteristics
- Platform methods for analyzing LVV based products

## LVV proteome analysis

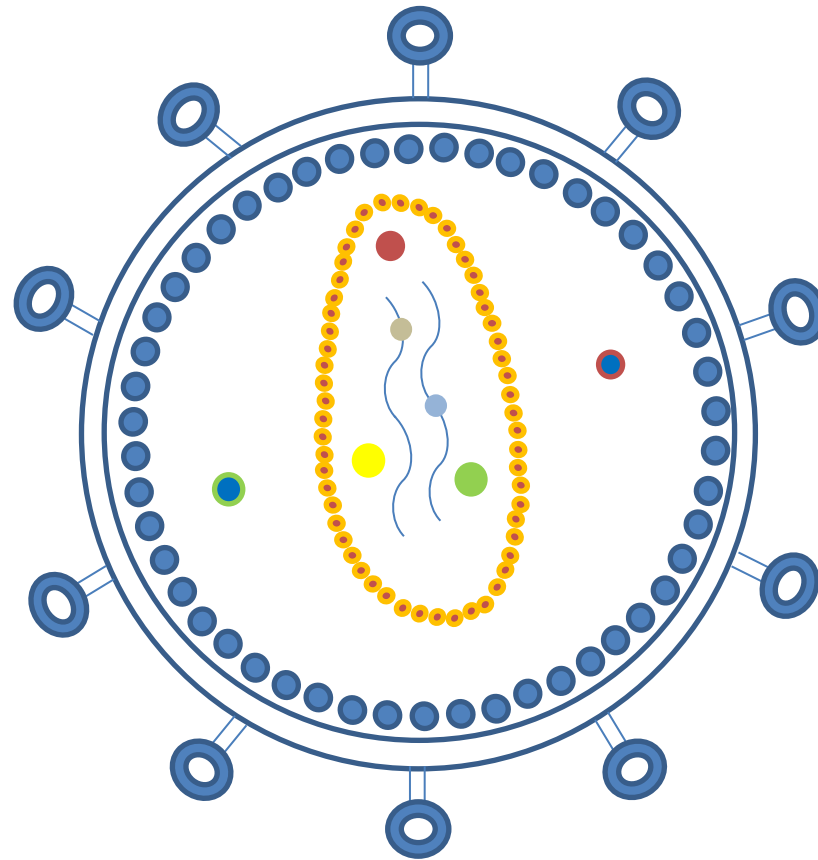
- Titer determination from p24 protein
- Lentiviral vector protein profiling

## LVV genome analysis

- Transgene sizing and impurity screening
- Summary



# Why is lentivirus proteome/genome analysis important?



Lentivirus

## Structural proteins:

- Nucleocapsid proteins: p7
- **Capsid proteins:** p24
- Matrix proteins: p17
- **Env. proteins:** GP120, GP41

## Non-structural proteins:

- Enzymes (4)
- Gene Regulatory Proteins(2)
- Accessory Proteins (5)
- HCPs
- etc.

## Proteome

- Envelope Proteins: The glycoprotein complexes enable the virus to attach to and fuse with target cells to initiate the infectious cycle
- P24 protein is typically used for lentiviral titer determination, a critical quality attribute (CQA) for lentivirus production from lab scale to GMP manufacturing
- Protein profiling of the lentiviral vector by CE can provide enhanced specificity for titer determination as well as the assessment of multiple structural and non-structural proteins

## Genome

- Product-related impurities can cause reduced transduction efficiency and efficacy:
  - Empty LVV particles
  - LVV containing a truncated transgene or incomplete 3' end
- Process-related impurities can be toxic to target cells and reduce transduction efficiency:
  - Residual host cell DNA and/or plasmid DNA
  - Increases the risk for innate immune response
- Lentiviral genome integrity analysis by CE offers high resolution insight into the quality of your therapeutic transgene



## Analysis of Lentiviral Proteins

- Titer determination from p24 protein
- Lentiviral vector protein profiling

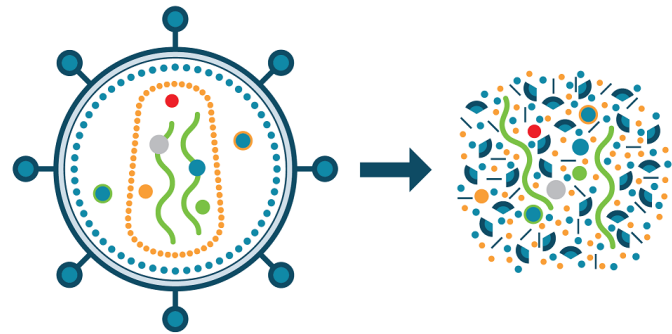
## Analysis of Lentiviral Genome

- Genome sizing and impurity screening



# Workflow for LVV protein profiling and p24 titer

## Easy sample preparation



Sample preparation in <15 min

## CE-SDS analysis



BioPhase 8800 system

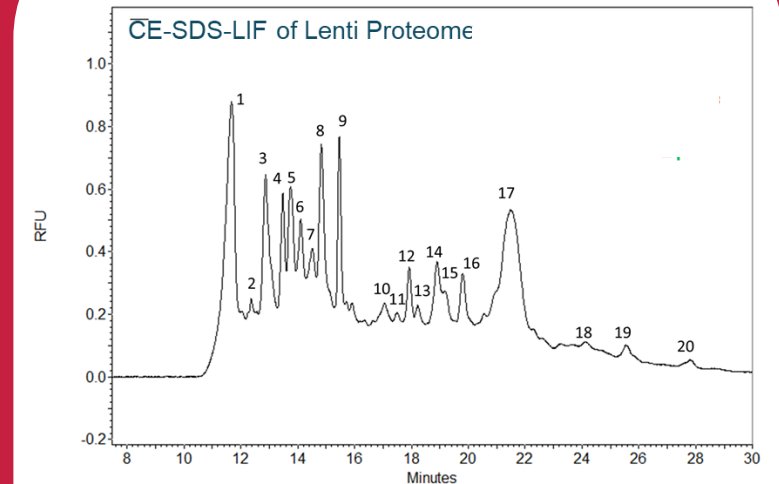


PA 800 Plus



CE-SDS workflow Kit and BFS cartridge

## Automated result generation

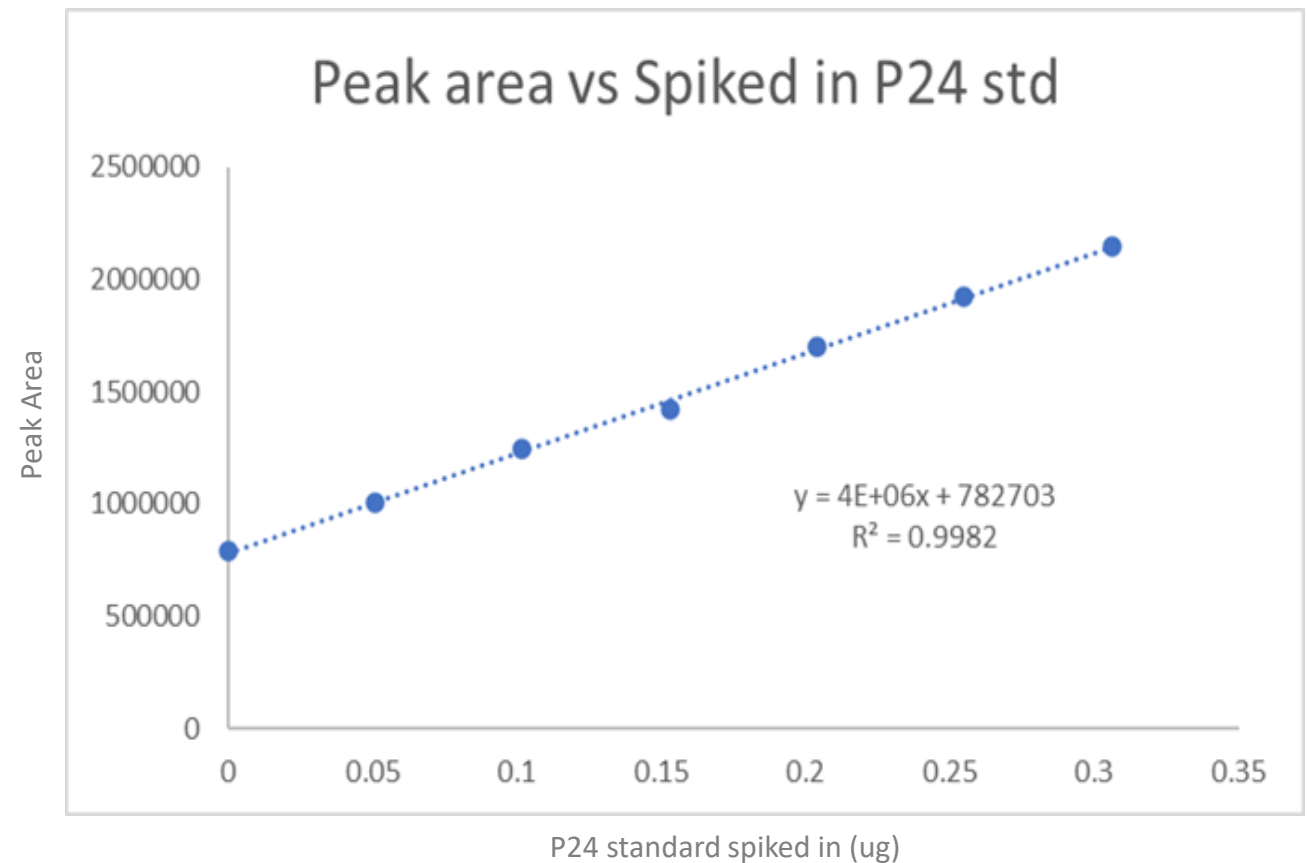
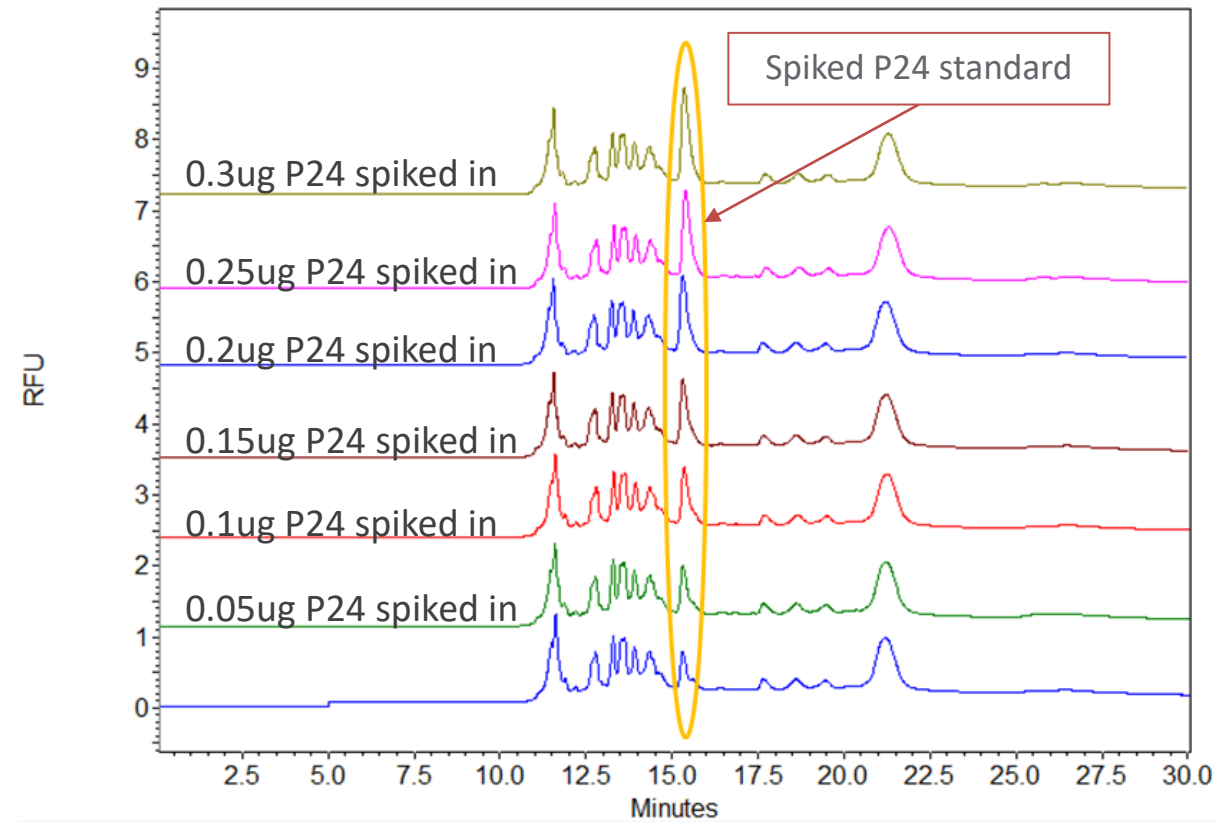


- Titer determination: quantifying P24
- Confirm batch to batch reproducibility
- Lentiviral protein purity assessment



# Identification of the P24 peak

## P24 protein standard addition method



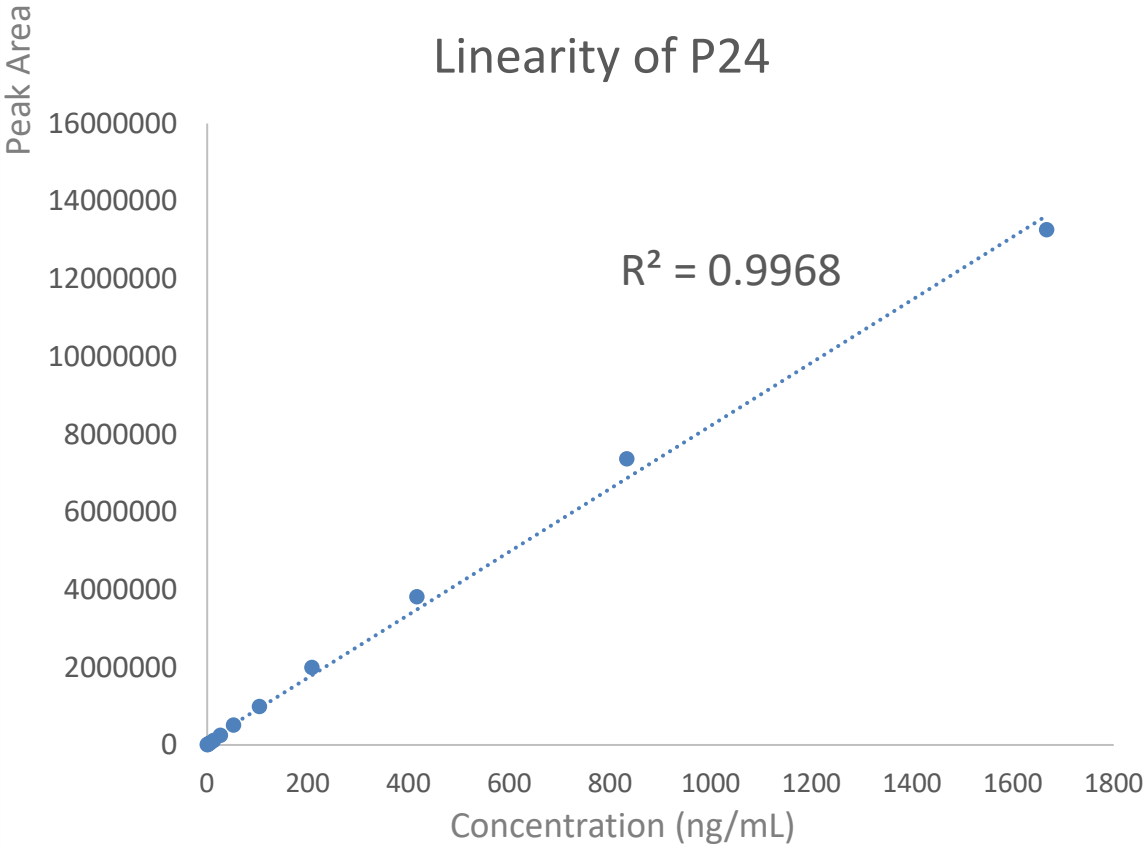
- Identification of the P24 peak by standard addition to lentivirus sample of  $1 \times 10^9$  TU/mL



# Linearity, LOQ, and repeatability of the P24

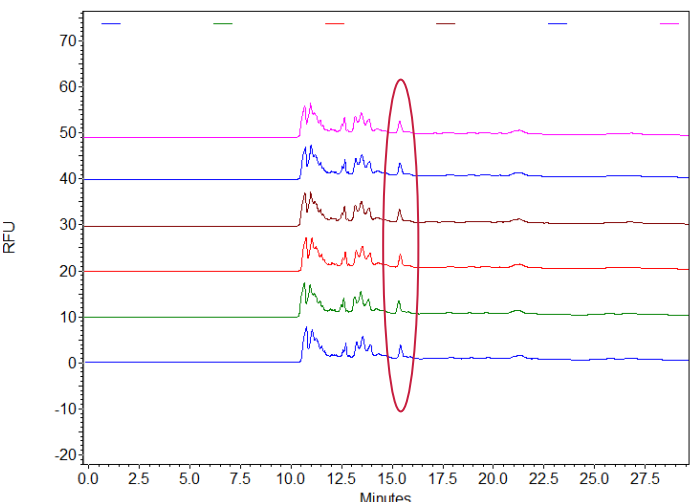
## Linearity and LOQ

Limit of quantification of 8 ng/mL



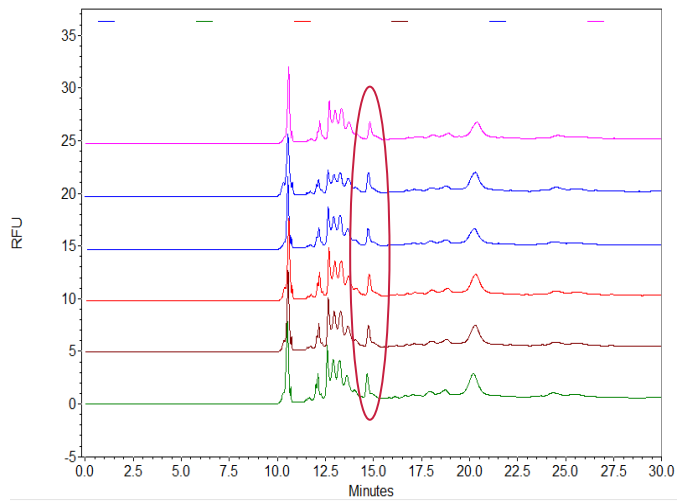
## Repeatability assessment

Six consecutive injections



Injection	MT (Min)	Peak area
1	15.57	3594174
2	15.60	3615001
3	15.57	3621318
4	15.61	3594266
5	15.62	3565088
6	15.65	3535125
RSD%	0.20	0.91

Six sample preparations



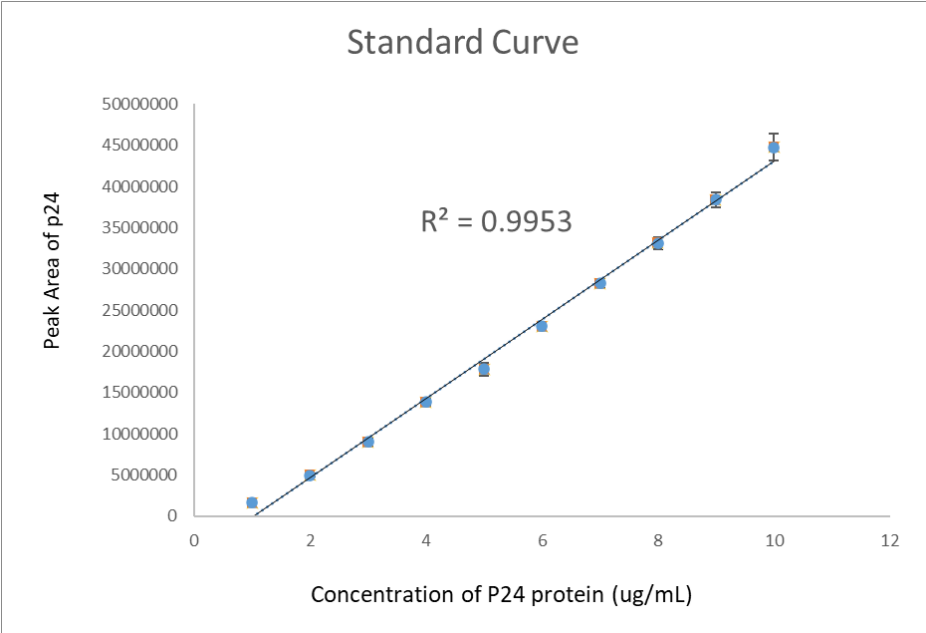
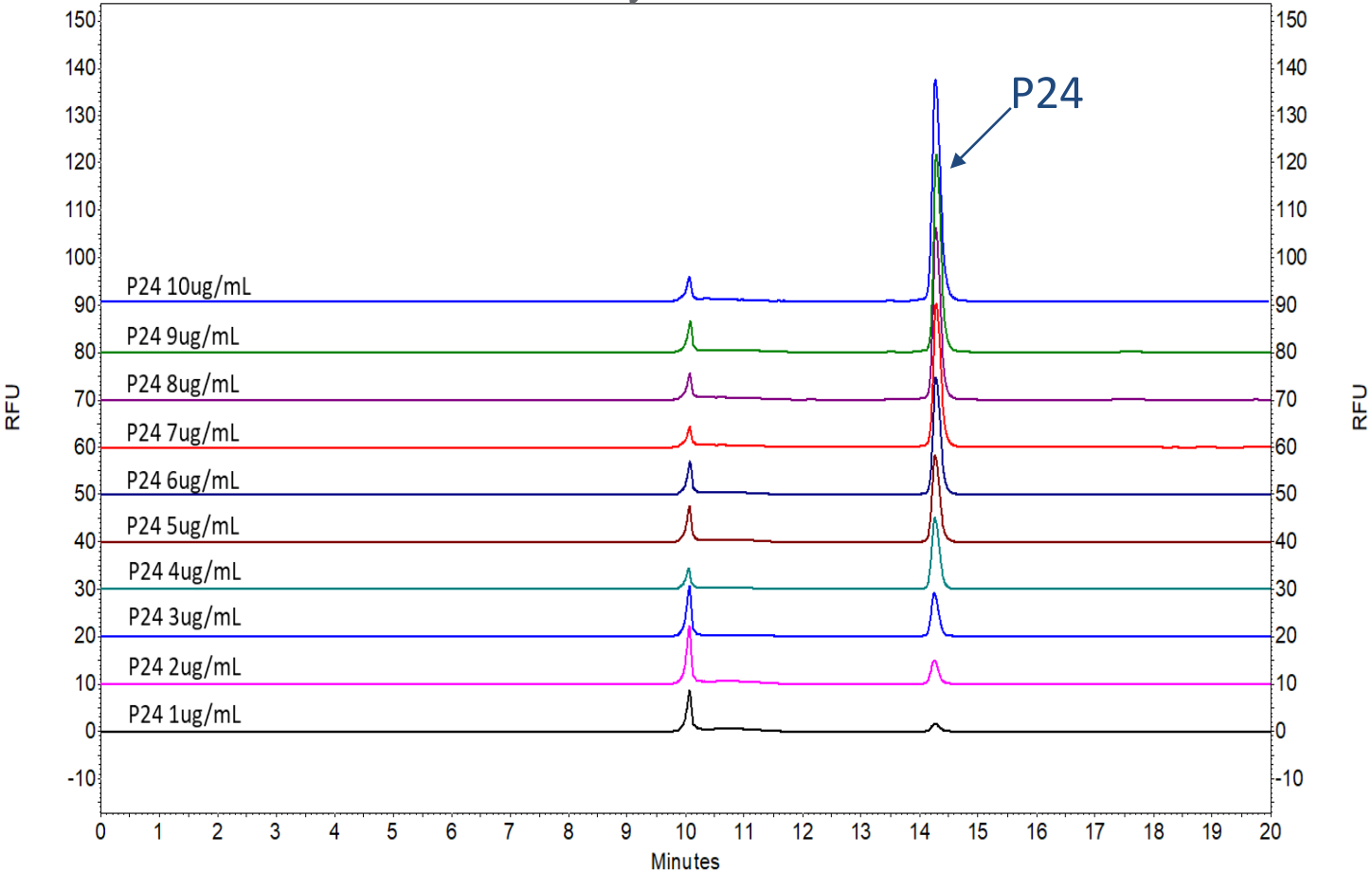
Injection	MT (Min)	Peak area
1	14.62	1874688
2	14.62	2145261
3	14.68	2013849
4	14.69	1974002
5	14.73	1965482
6	14.76	1996383
RSD%	0.39	4.41



# Calibration Curve and P24 titer determination

## P24 standard curve from 3 sample preparations

Results from CE titer method  
across 3 days and ELISA



### Comparison between LVV P24 titer methods

Methods	Titer (TU/mL)
Titer calculated (CE Method) - Day 1	1.45 X 10 <sup>9</sup>
Titer calculated (CE Method) - Day 2	1.45 X 10 <sup>9</sup>
Titer calculated (CE Method) - Day 3	1.46 X 10 <sup>9</sup>
Titer from vendor (ELISA P24 kit)	1.5 X 10 <sup>9</sup>

- CE quantitation demonstrated consistency across 3 preparations on 3 separate days
- P24 titer by CE is comparable to the p24 titer by ELISA



## Analysis of Lentiviral Proteins

- Titer determination from p24 protein
- **Lentiviral vector protein profiling**

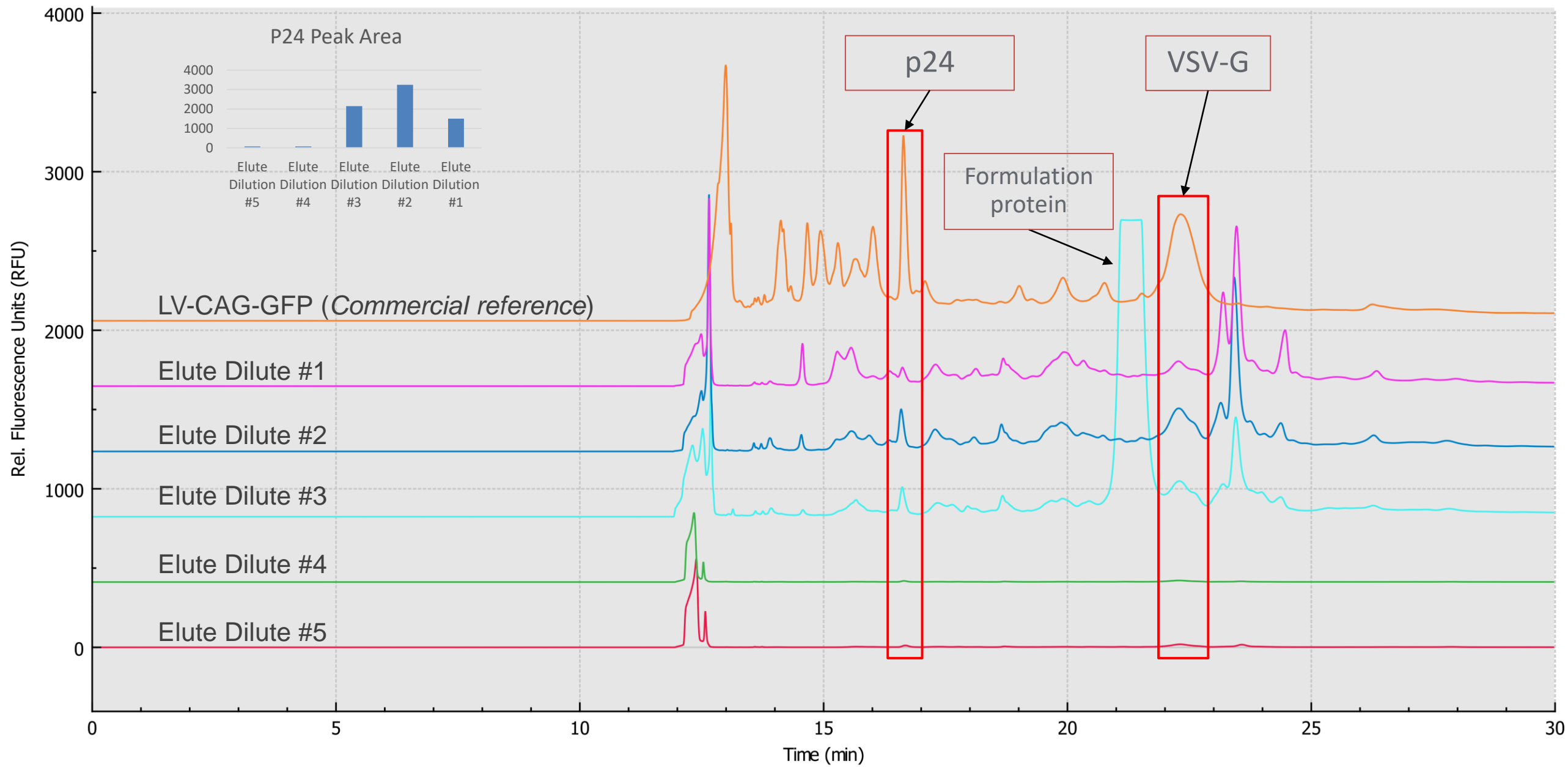
## Analysis of Lentiviral Genome

- Genome sizing and impurity screening



# Protein profiling to assess in-process samples

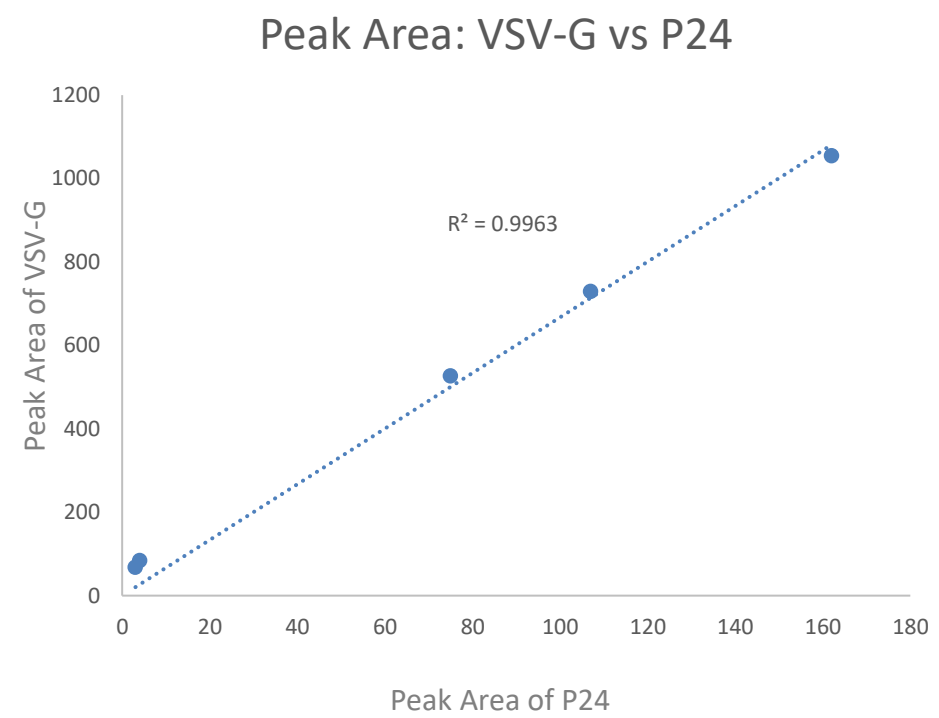
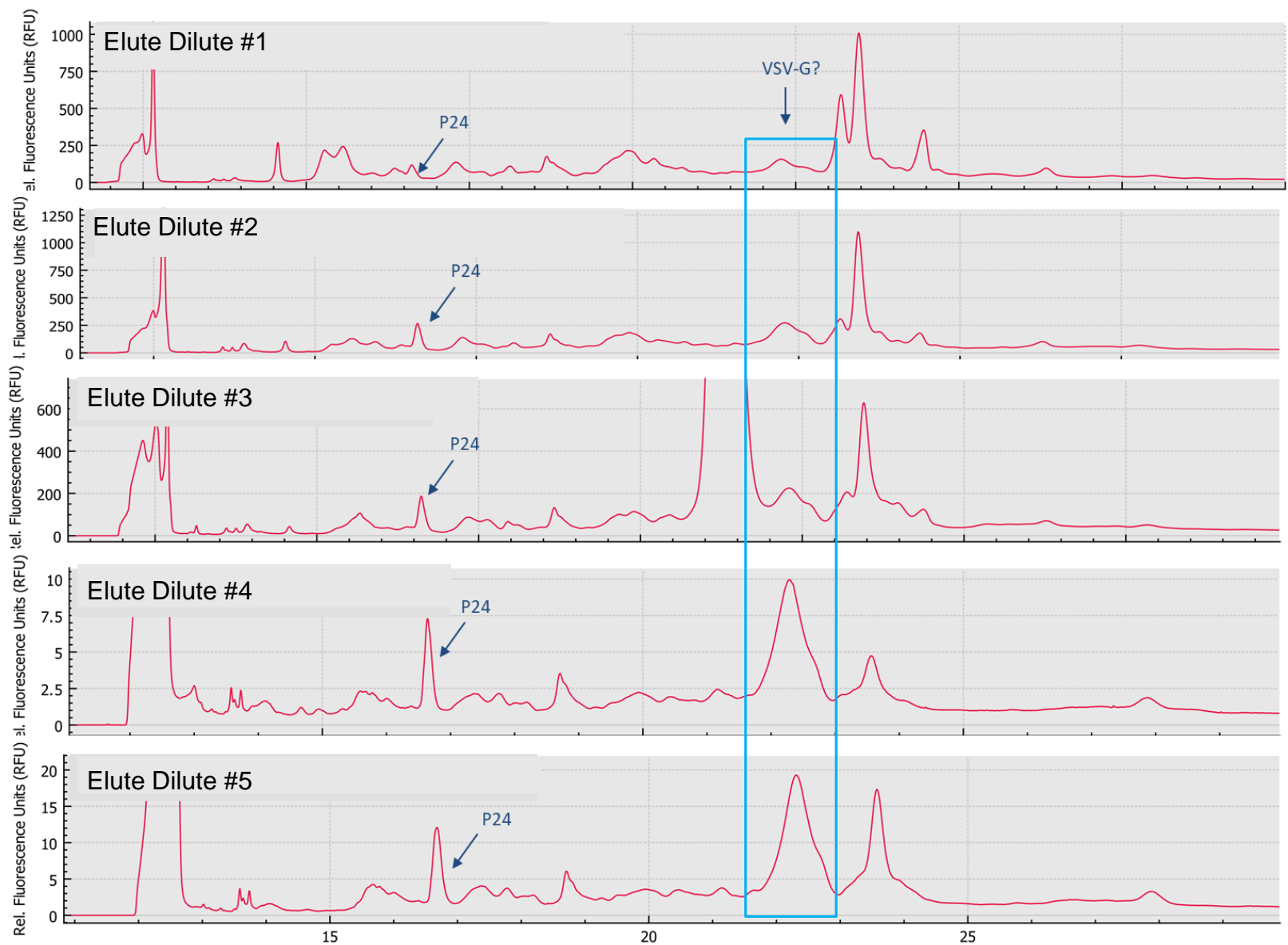
In-process samples compared to a commercial reference material





# Protein profiling to assess in-process samples

## Linear correlation of VSV-G to the P24 LVV proteins





## Analysis of Lentiviral Proteins

- Titer determination from p24 protein
- Lentiviral vector protein profiling

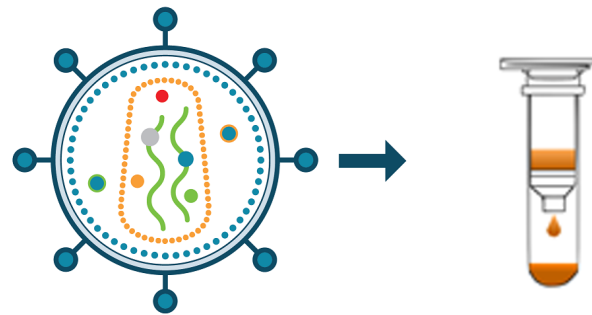
## Analysis of Lentiviral Genome

- Genome sizing and impurity screening



# Workflow for genome integrity analysis

## Easy sample preparation



RNA extraction from LVV < 20 min

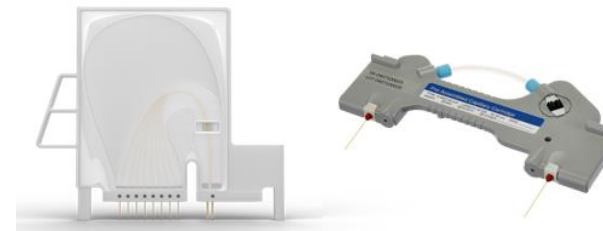
## CGE-LIF analysis



BioPhase 8800 system

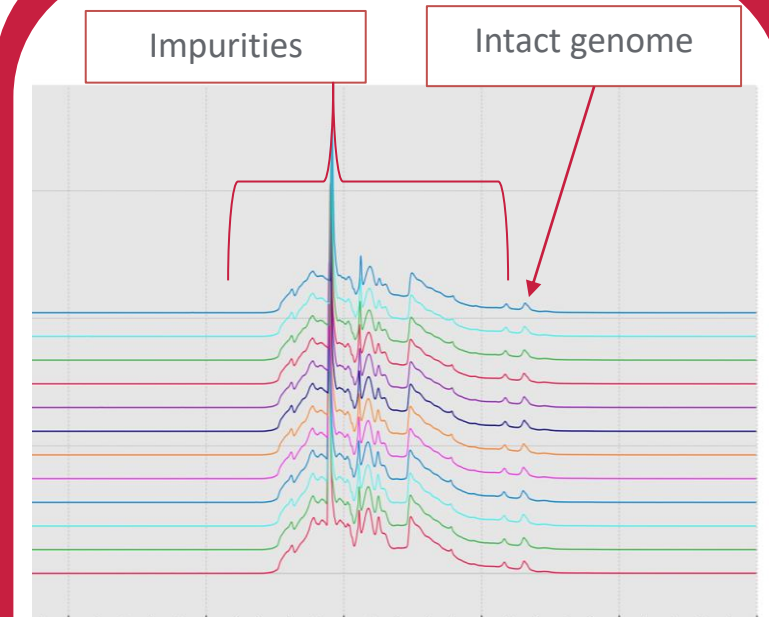


PA 800 Plus



PVP Gel, SYBR Green II Dye & BFS cartridge

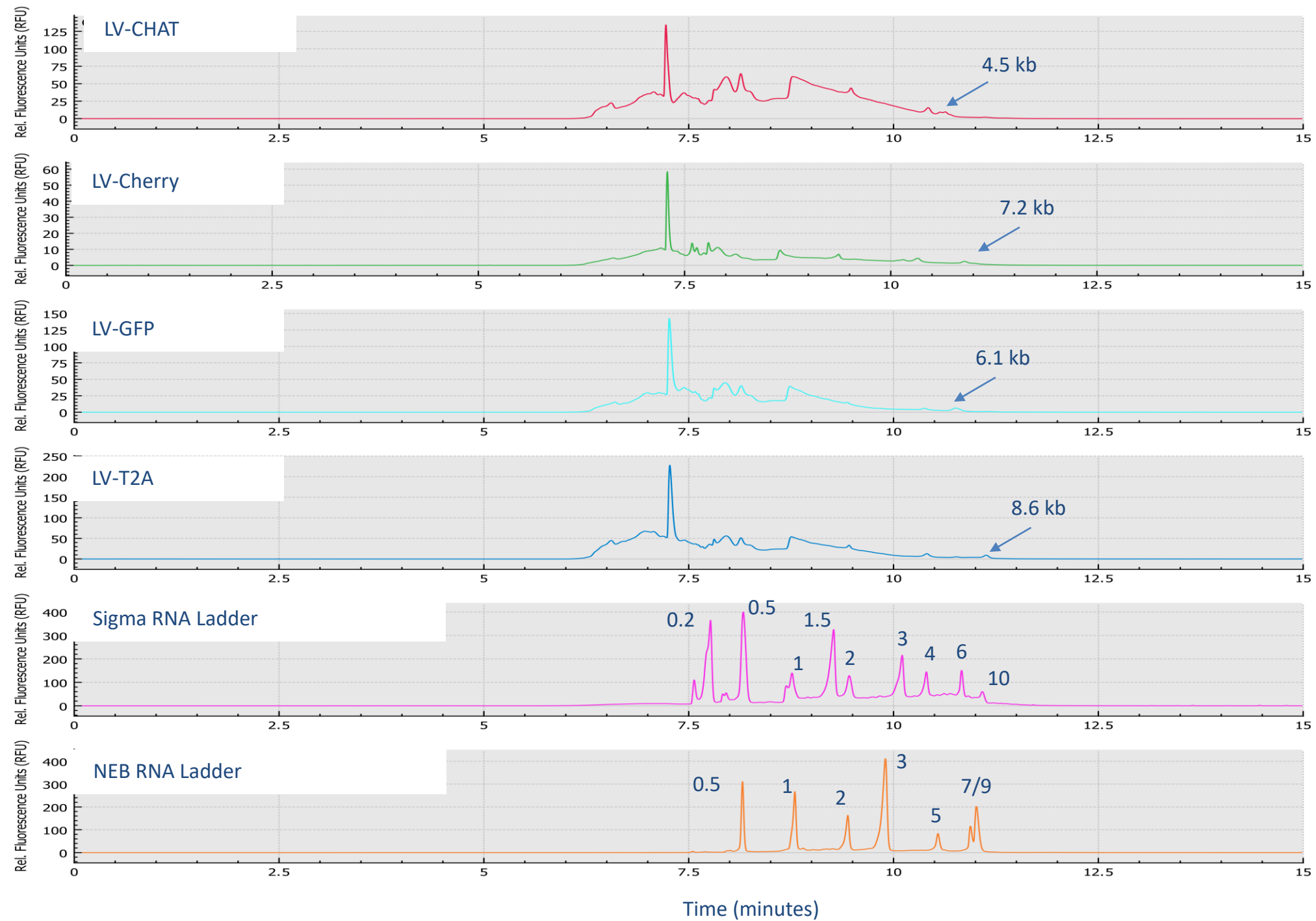
## Automated result generation



- Determine intact genome size
- Identify product-related impurities
- Residual host nucleic acid screening



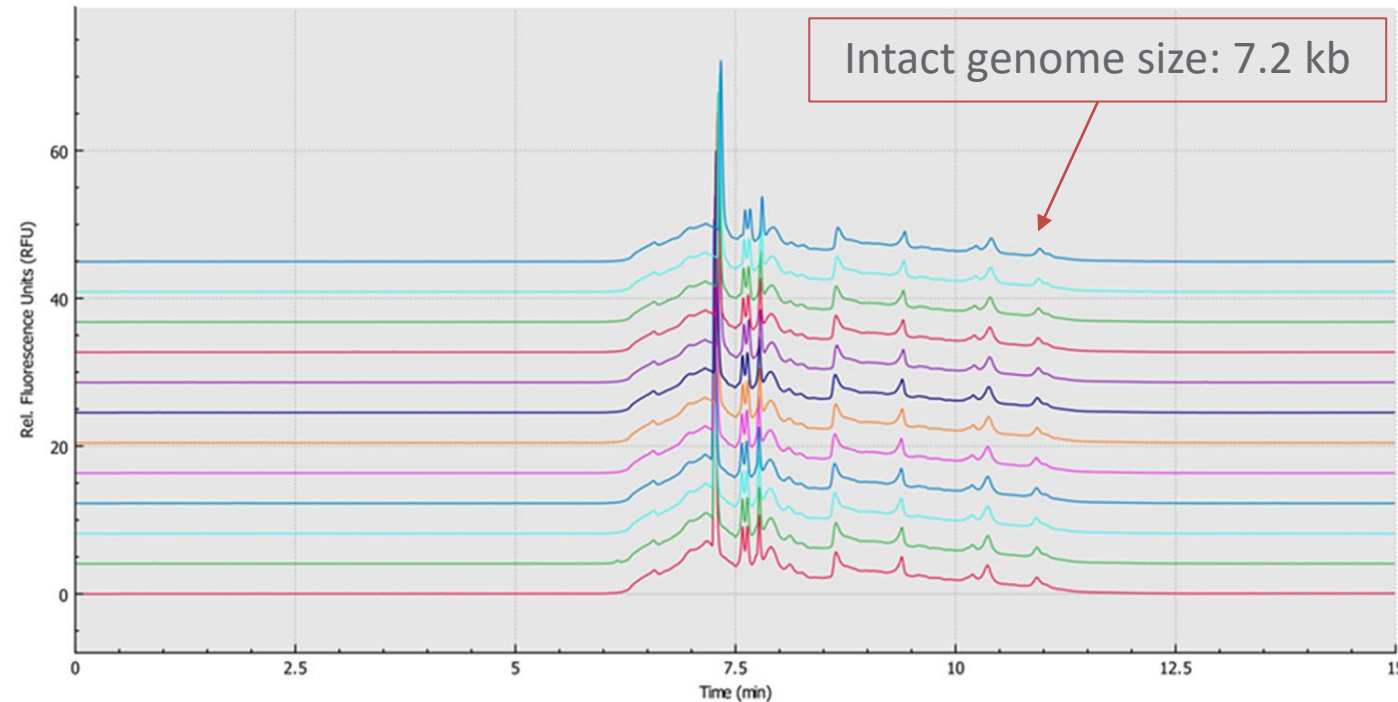
# Analysis of LVVs with various genome sizes



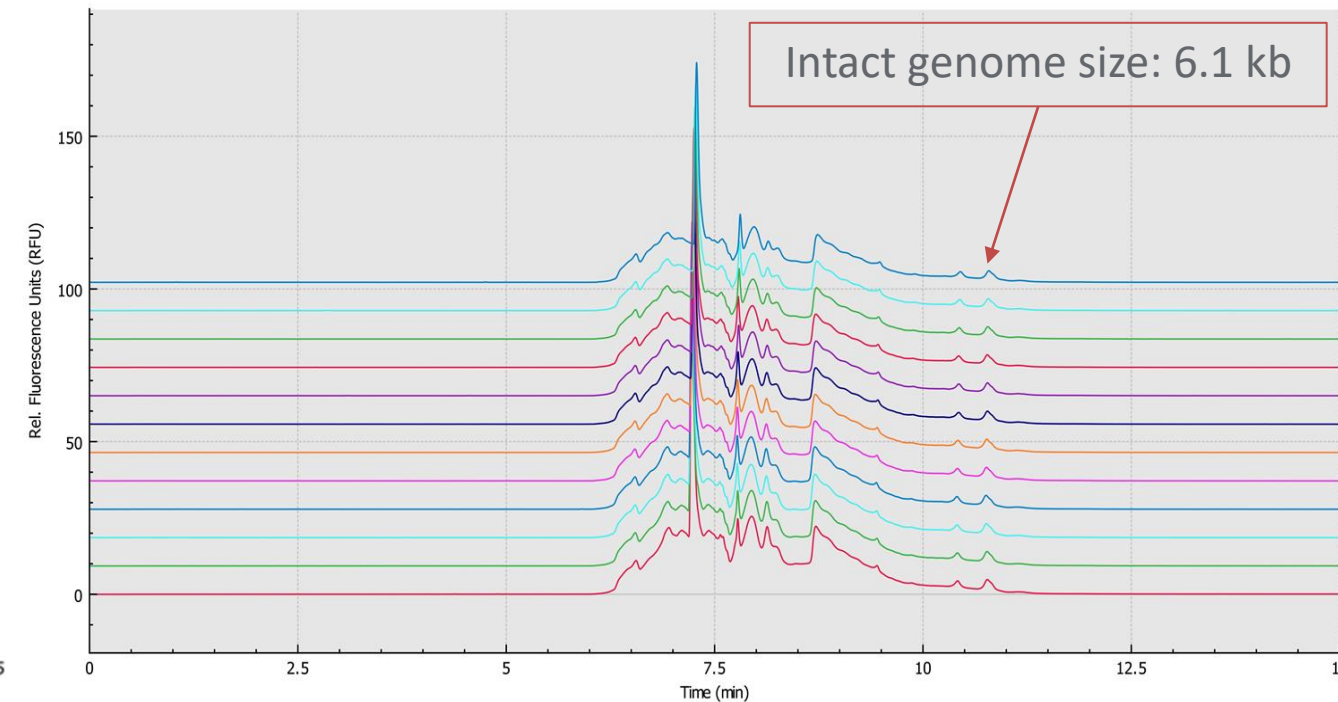


# Genome integrity workflow consistency

## LVV-Cherry



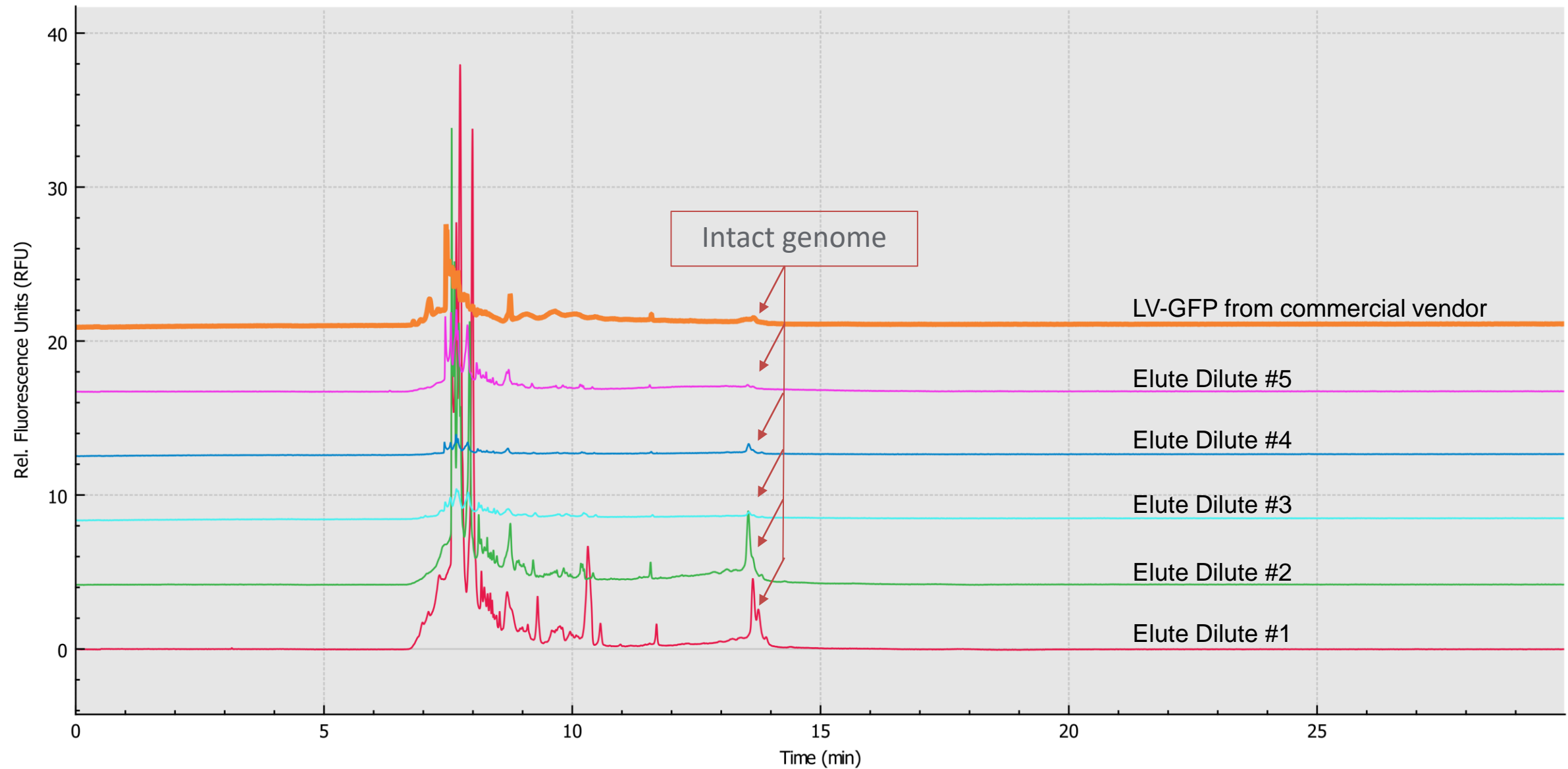
## LVV-GFP



- Consistent migration time for the intact genome peak across 12 consecutive injections of 2 commercial samples

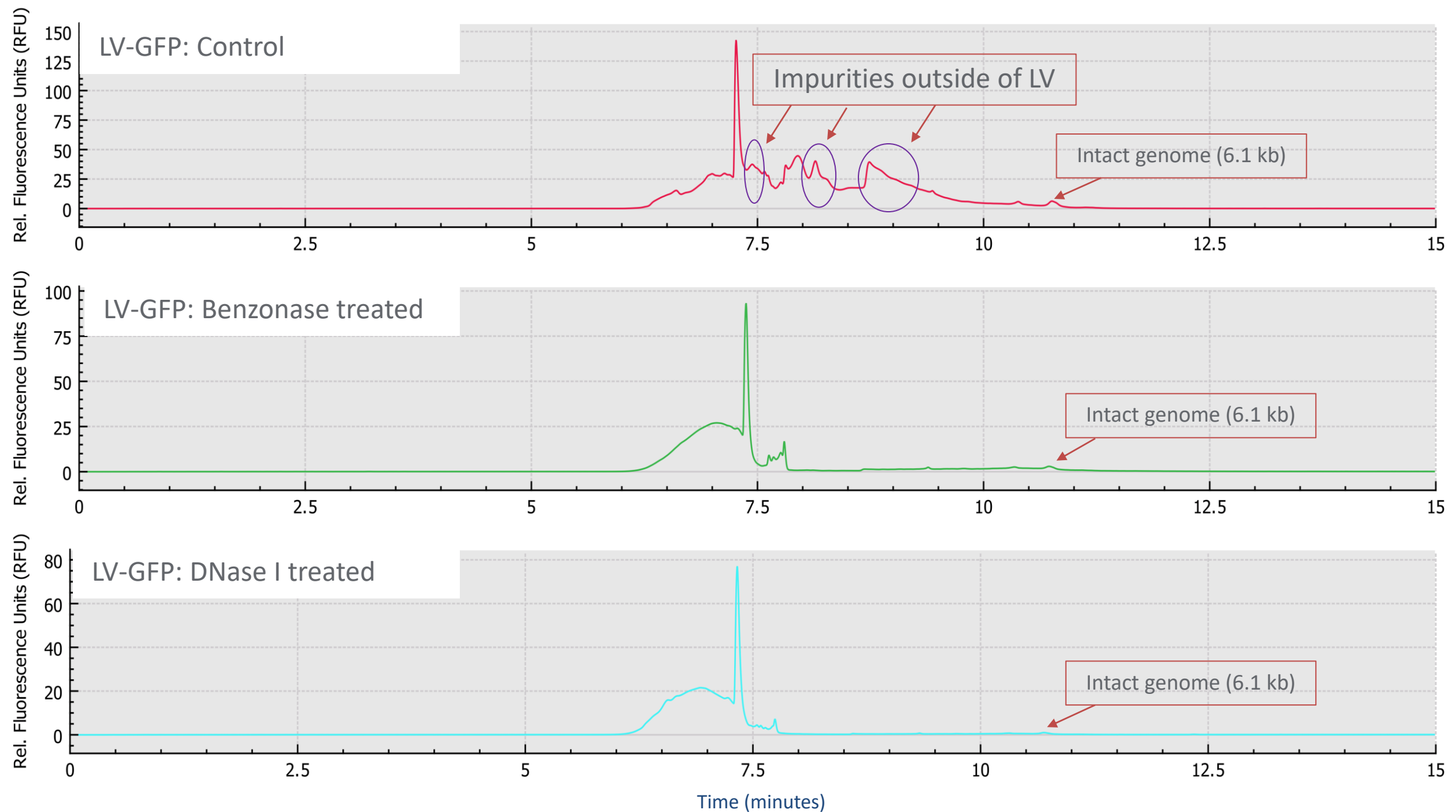


# Genome integrity analysis of in-process samples





# Residual nucleic acids screening workflow





# Conclusion

The CE platform technology demonstrates unique capabilities for quality attribute monitoring of lentiviral vectors

- Sensitive titer determination based on precise quantification of the separated p24 protein peak with 4 magnitude orders of detection linearity of  $r^2 = 0.9968$  and LOQ of 8 ng/mL
- Easy to assess batch-to-batch reproducibility and protein purity based on the resulting proteome profile
- Good linear relationship of p24 and VSV-G indicates CE titer method is not measuring free p24.
- High sensitivity of CE-LIF enables genome integrity analysis of lentiviral vectors at a titer of  $1 \times 10^9$  TU/ml without amplification, providing high resolution of intact LV genome from various impurities with good repeatability



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