Analytical Methodologies for Gene Therapy Products

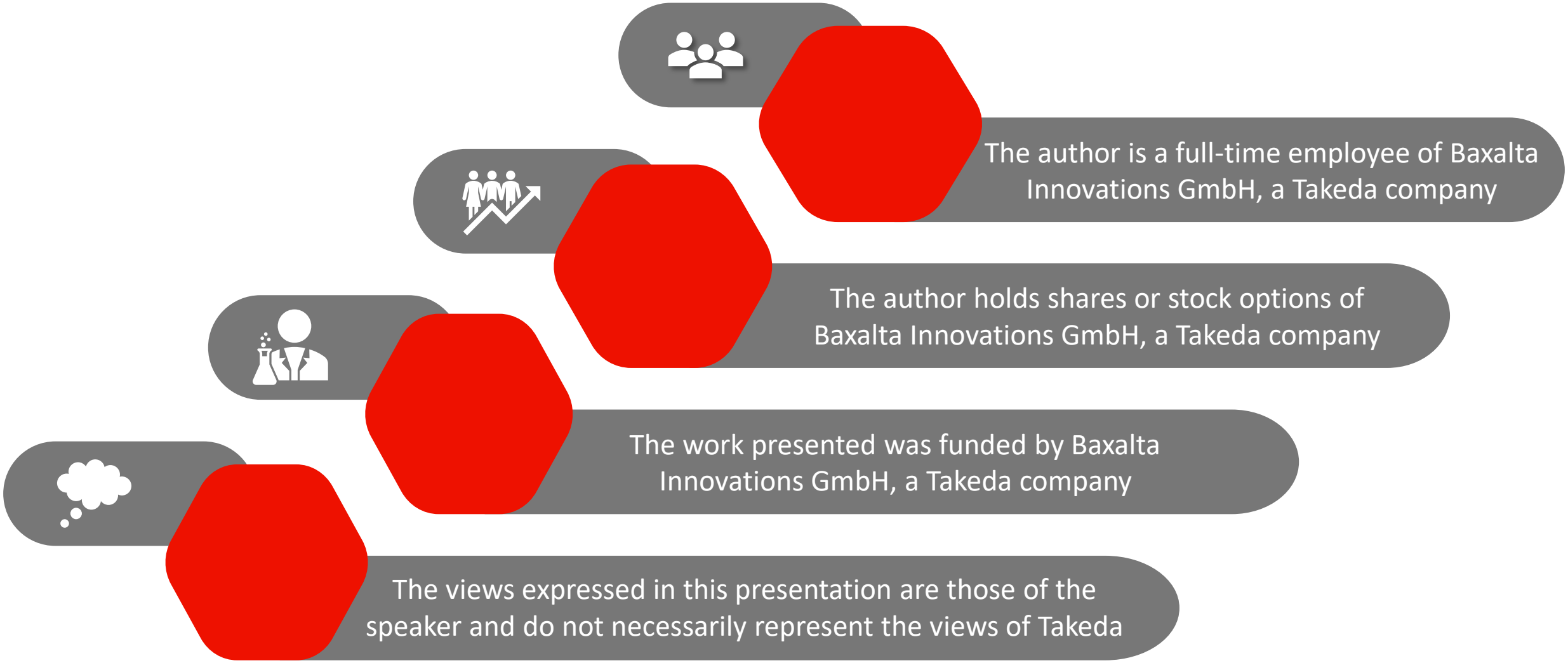
ViruSure 2nd Virus Safety Workshop - Sep 2022

Pletzenauer Robert

Head of Gene Therapy Analytics

Better Health, Brighter Future

Disclaimer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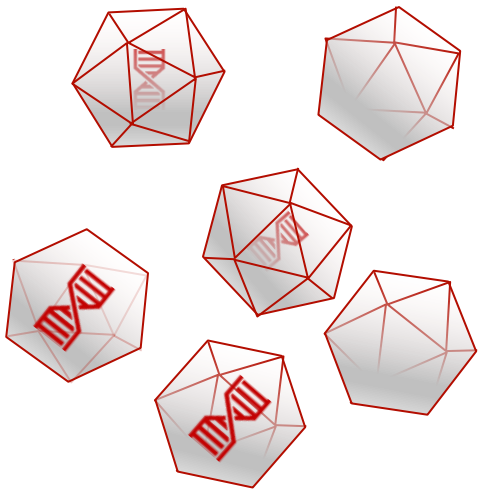
Agenda



- Introduction to AAV Production and Analytics
- Vector Genome Quantification - digital PCR (dPCR).
- Capsid Particle Quantification - ELISA
- Purity determination - Capillary Electrophoresis
- Relative quantification of capsid subspecies - Analytical Ultracentrifugation

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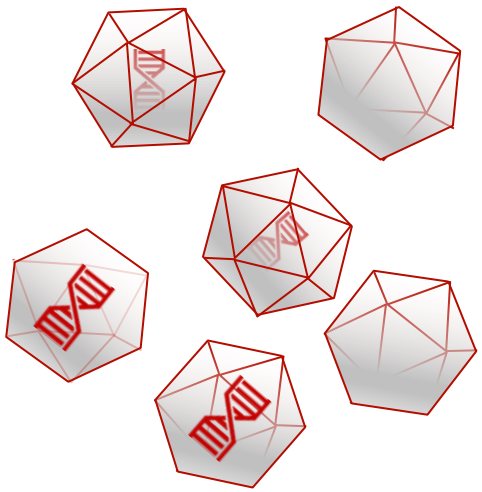

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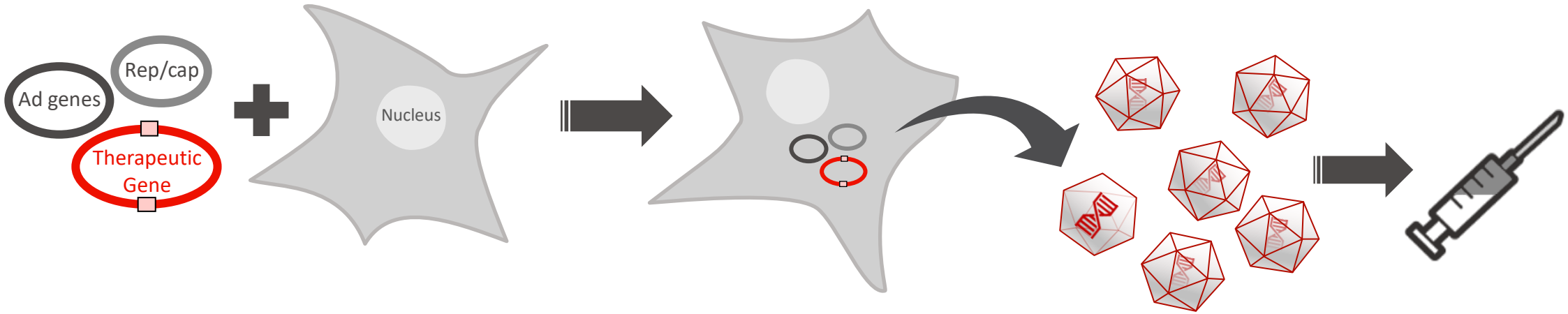
Introduction – AAV Production

Triple Transfection Method for Adeno-Associated Virus (AAV)



Plasmid DNA | **HEK 293 cells** | **Triple transfected HEK 293 cells** | **Adeno-Associated Virus (AAV) containing therapeutic gene**

Introduction – AAV Production

Available for cGMP manufacturing up to 1000 L scale

UPSTREAM

Spinner Flasks

Single-use Bioreactor 50 L

Single-use Bioreactor up to 1000 L

Depth Filtration

DOWNSTREAM

UD Filtration

Affinity/IEX Chroma

Ultracentri-fugation

1. Virus Inactivation

IEX Chroma

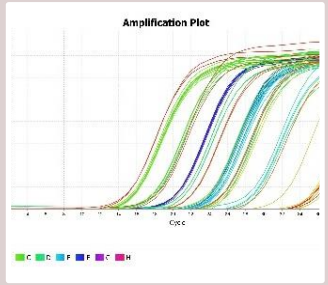
2. Virus Inactivation Nano-filtration

Drug Substance

Introduction – AAV Analytics

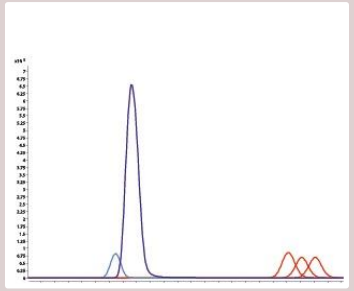
AAV vector quantification

- Vector Genome Titer - Digital PCR
- Vector Genome Titer - Real-Time PCR
- AAV Capsid Titer - ELISA
- Total protein by LabChip
- Total protein by Bradford



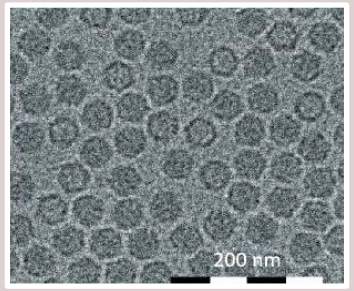
Process related impurities testing

- HEK293 HCP ELISA
- HSP70 ELISA
- Lactate dehydrogenase LDH
- HEK293 DNA
- Residual Plasmid DNA
- Next Generation Sequencing
- Residual solvent detergent chemicals (HPLC)
- PEI (polyethyleneimine)
- AAV Ligand Leakage ELISA
- Osmolality, pH
- Visible particles
- Foreign subvisible particles (MFI, LO)
- Appearance



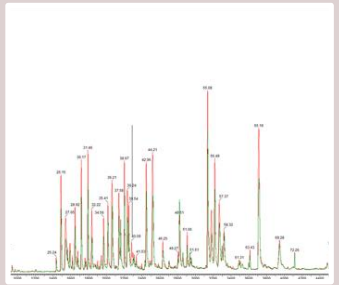
Product related impurities/Safety testing

- High Resolution SDS-PAGE for vector protein integrity
- Aggregates by SEC and FFF (Field Flow Fractionation)
- %full AAV (cryoTEM, HPLC)
- nsTEM for sample
- Bioburden, Endotoxins, Sterility used as safety tests
- Adventitious Agents (in vitro, MMV, Mycoplasma)



AAV particle characterization

- CE - Capillary Electrophoresis
- AUC - Analytical Ultracentrifugation
- LC-MS peptide mapping primary sequence conformation
- in vivo/ in vitro biopotency



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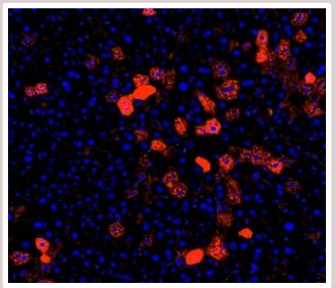
- Virological infectivity assays (TCID50)
- Replicating AAV (rcAAV)
- Analysis of transduced animal organs



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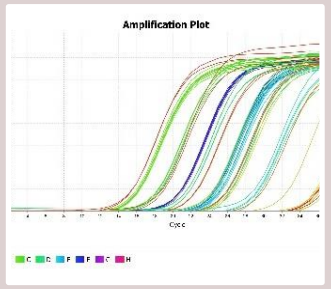

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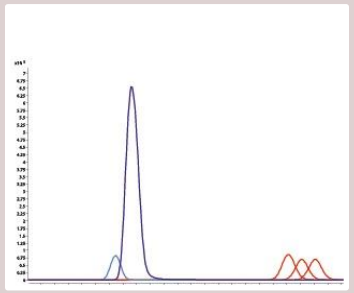

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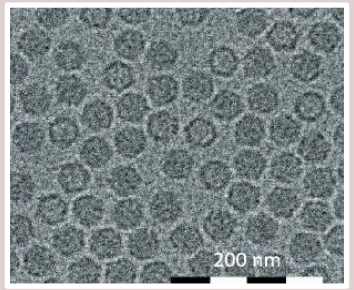

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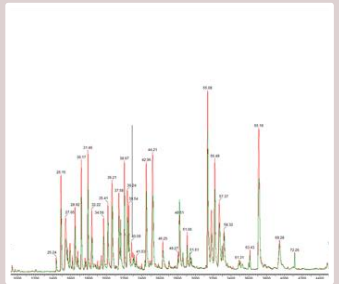

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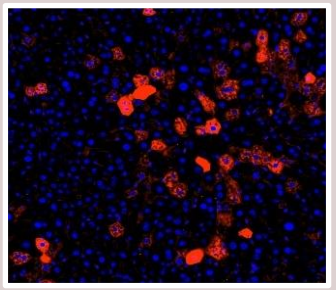

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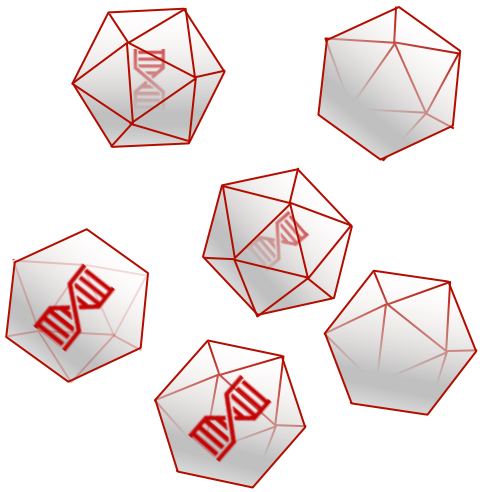

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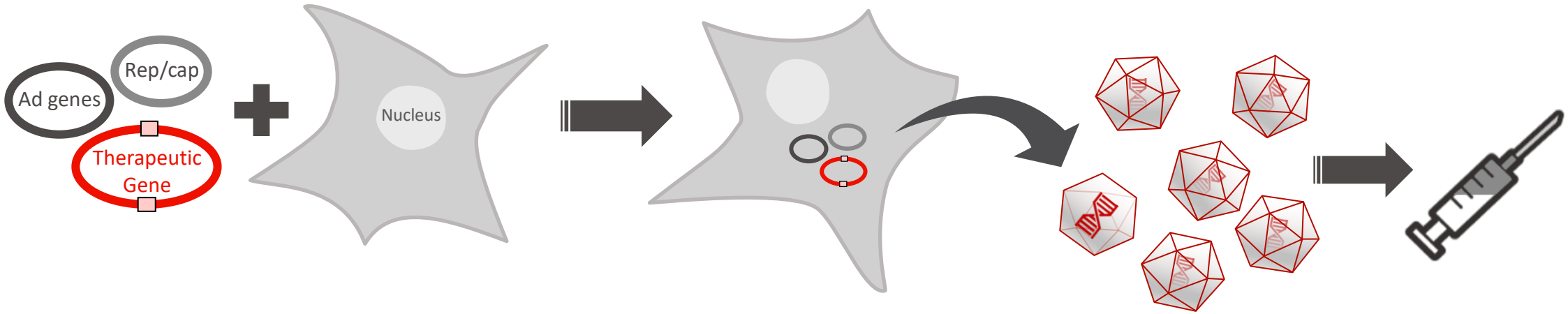

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Vector Genome Quantification

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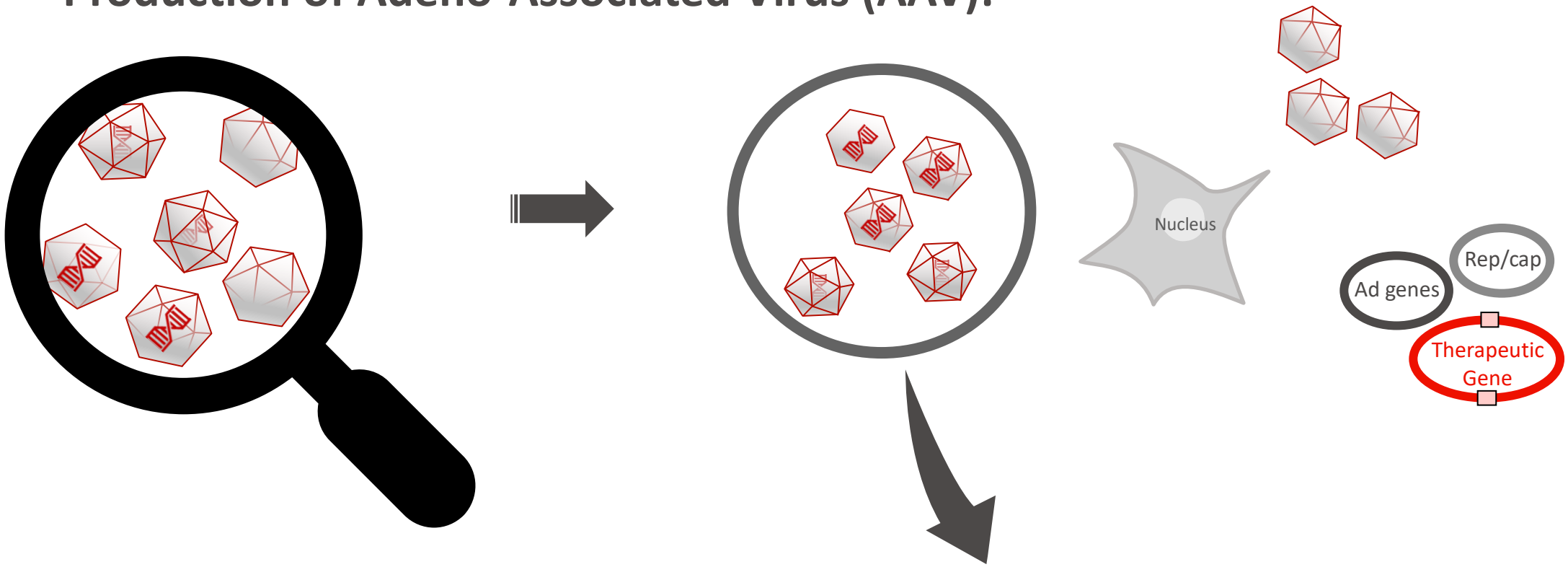
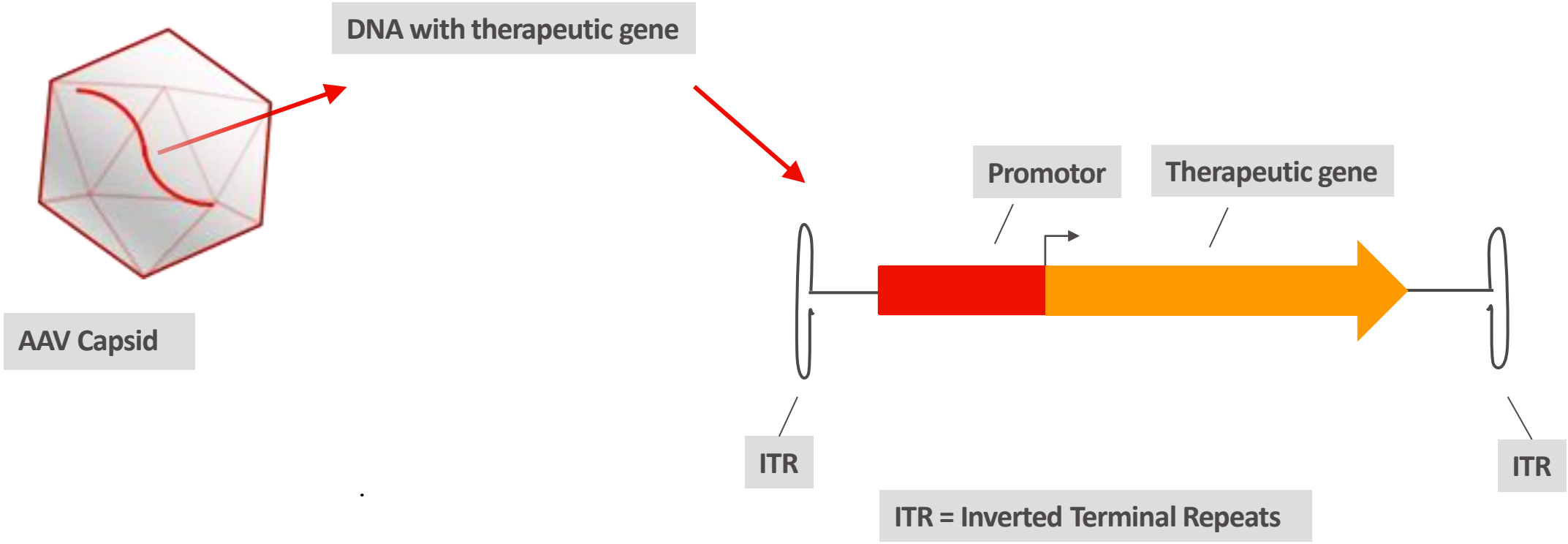


- **Quantification of AAVs containing the therapeutic gene**

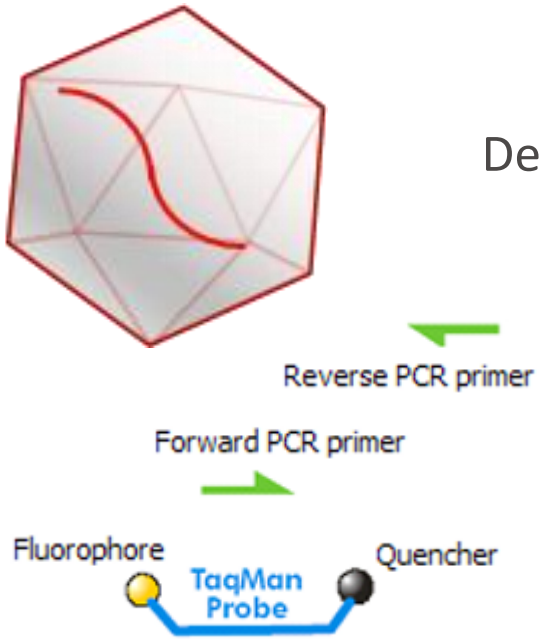
Vector Genome Quantification

- **Target DNA for qPCR and Droplet Digital PCR for Vector Quantification**

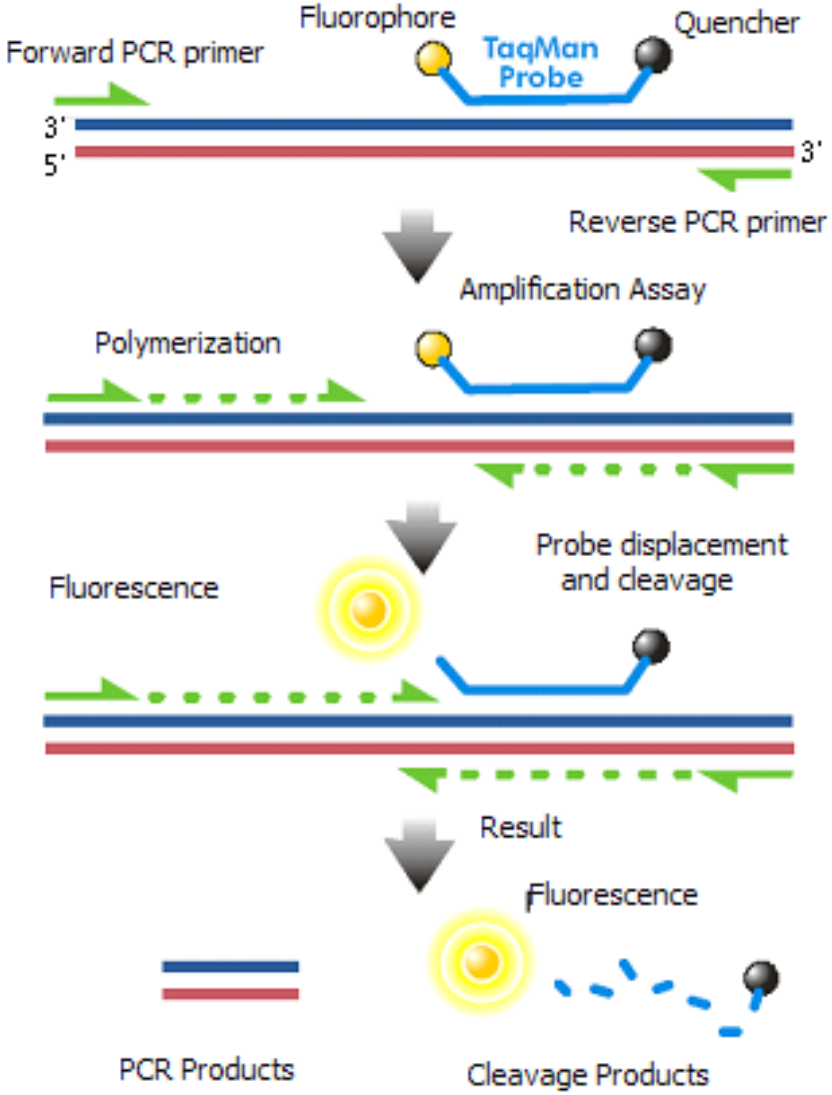


Vector Genome Quantification

TaqMan qPCR for vector DNA



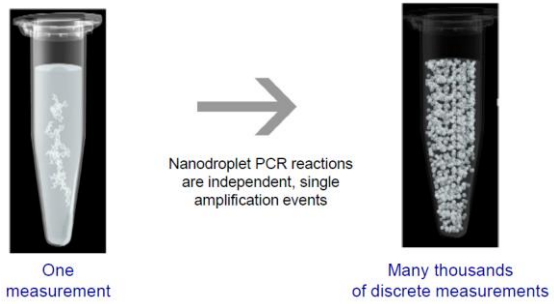
Denturation step

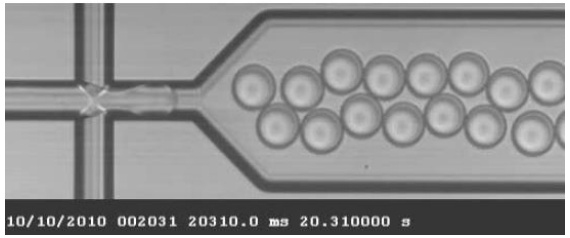


https://en.wikipedia.org/

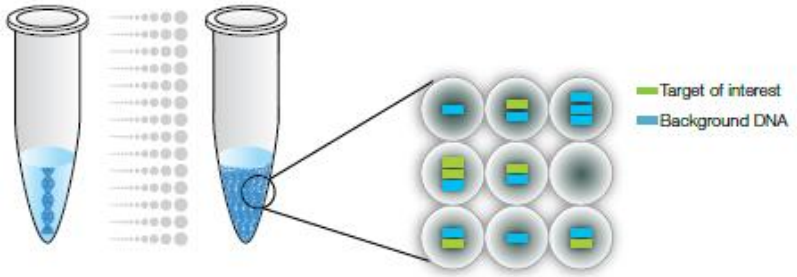
Vector Genome Quantification

- PCR reaction is divided into many compartments (e.g. droplets)





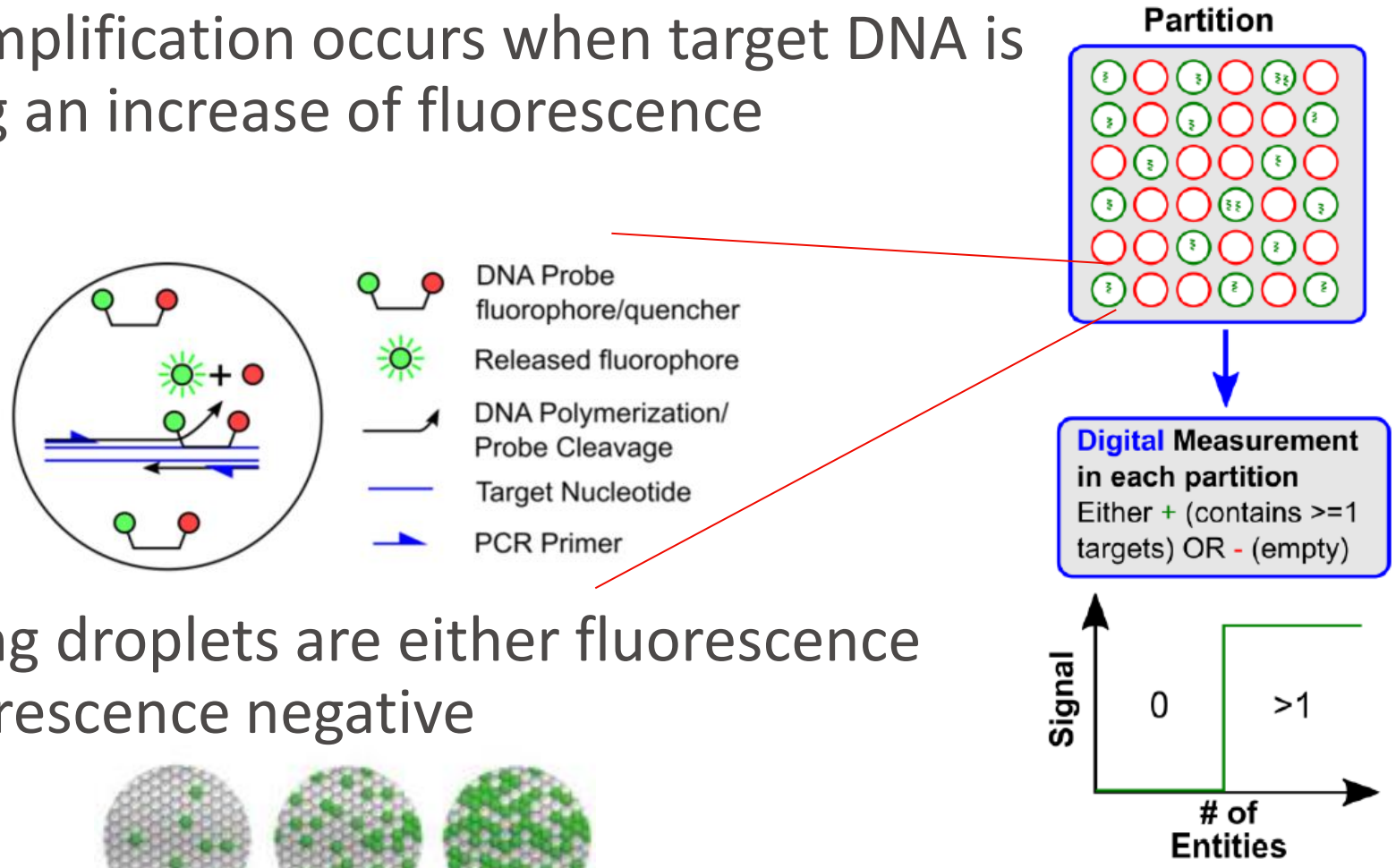
- Target DNA is distributed randomly to the droplets (zero, one or several target DNA molecules per droplet)



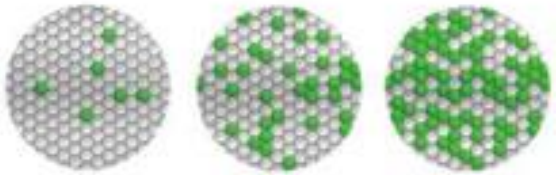
www.Bio-rad.com

Vector Genome Quantification

- Like in qPCR, amplification occurs when target DNA is present causing an increase of fluorescence



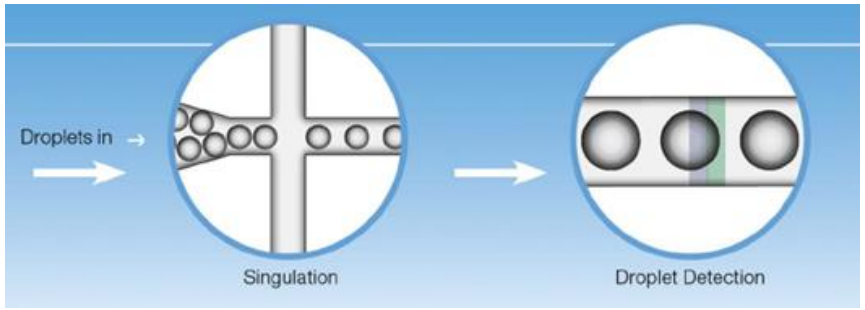
- After PCR cycling droplets are either fluorescence positive or fluorescence negative

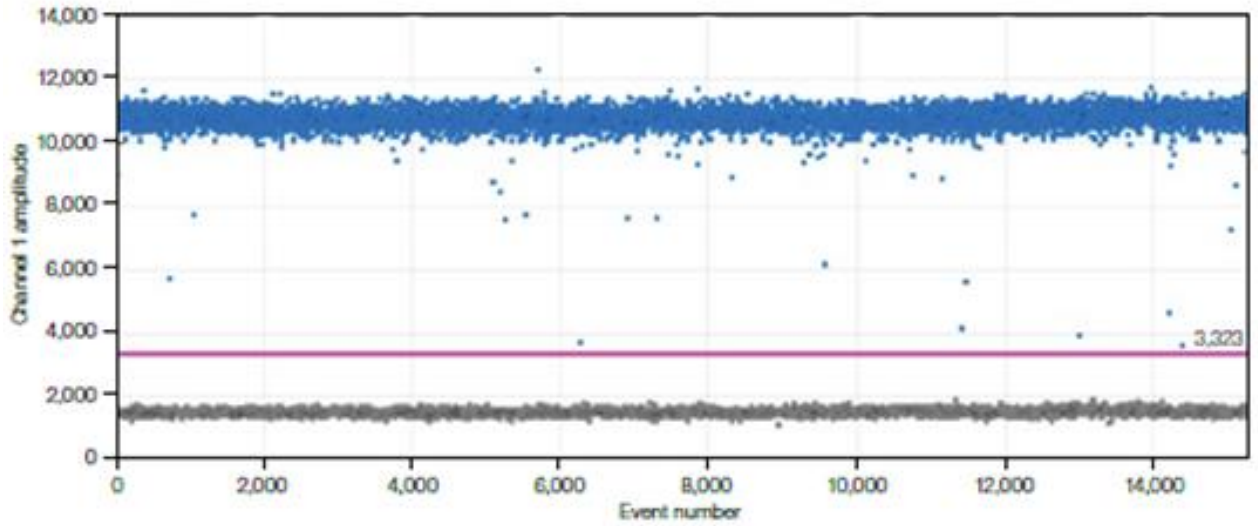


Basu AS. SLAS Technology. 2017. 22(3): 369-386.
www.Bio-rad.com

Vector Genome Quantification

- Droplet Reader – Droplets are scattered and counted





www.Bio-rad.com

- Applying Poisson distribution, the target DNA concentration within the PCR reaction is calculated from the known amount of positive and negative droplets.

→ **absolute Quantification**

Vector Genome Quantification

First Generation

- **QX 200 Manual** Droplet generation
- PCR Cycling
- Droplet Reader

Second Generation

- **QX 200 Auto DG** automated Droplet generation
- PCR cycling
- Droplet Reader

Third Generation

- **QX ONE** All-in-One System for full automation:
  Droplet generation, thermal cycling and droplet read-out within a single system
- **QIAcuity** nanoplate-based All-in-One System: partitioning, thermocycling and imaging

Manual QX 200

PCR Cycler

Auto DG QX 200

Droplet Reader

QX ONE

www.Bio-rad.com

QIAcuity

www.Qiagen.com

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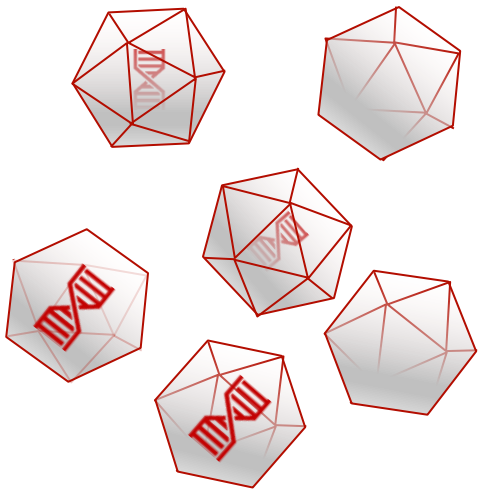

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Capsid Particle Quantification

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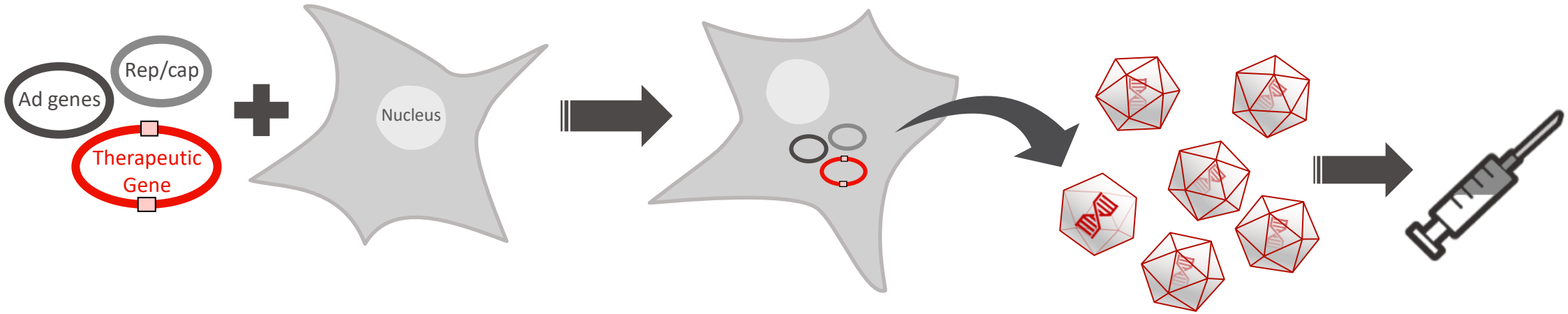

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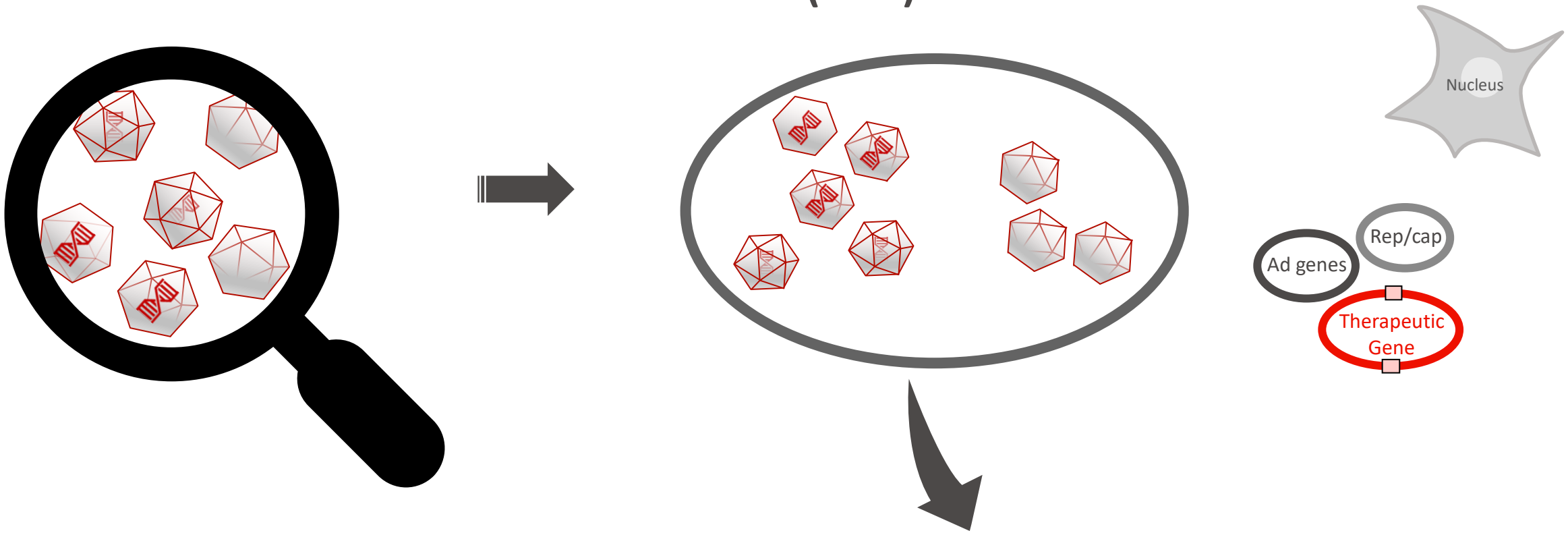
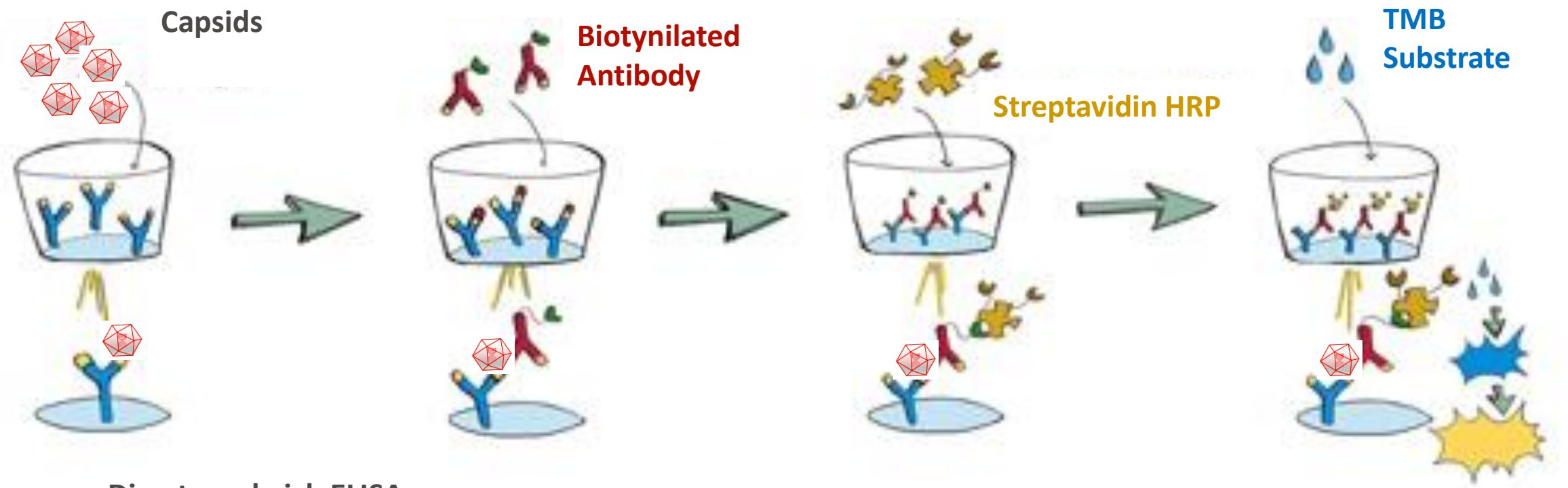


- **Quantification of all AAV particles**

Capsid Particle Quantification - ELISA



modified from www.biossusa.com

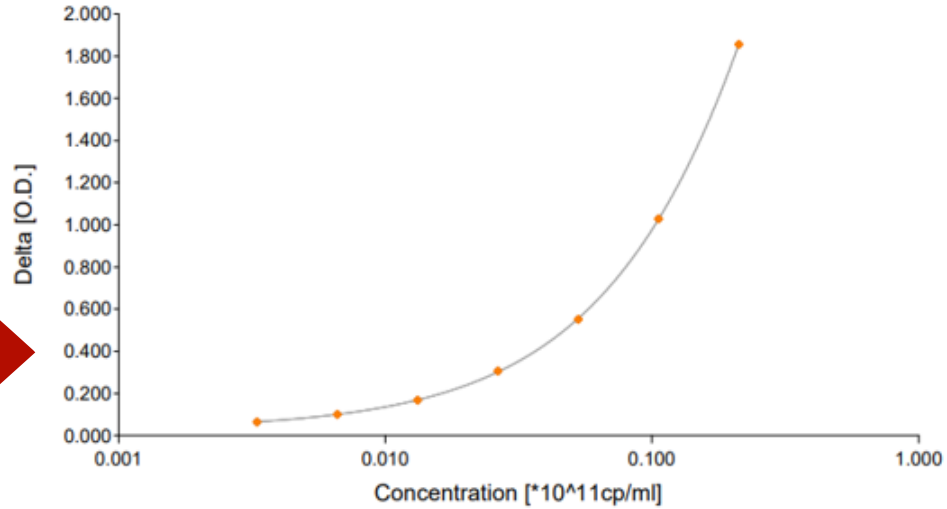
Capsid Particle Quantification - ELISA

www.progen.com

www.eppendorf.com

www.agilent.com

www.hamilton.com



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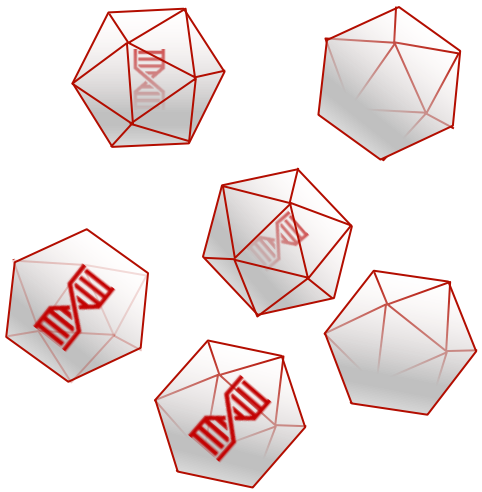

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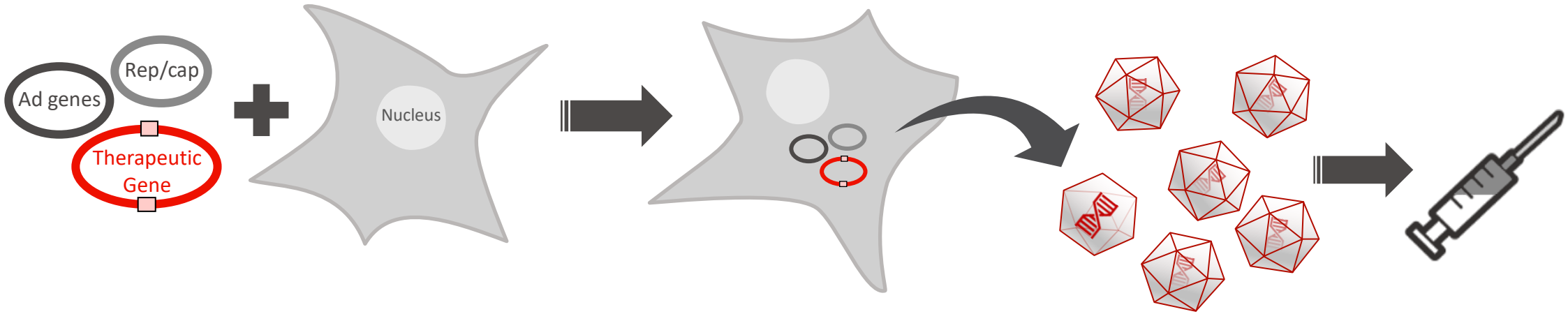

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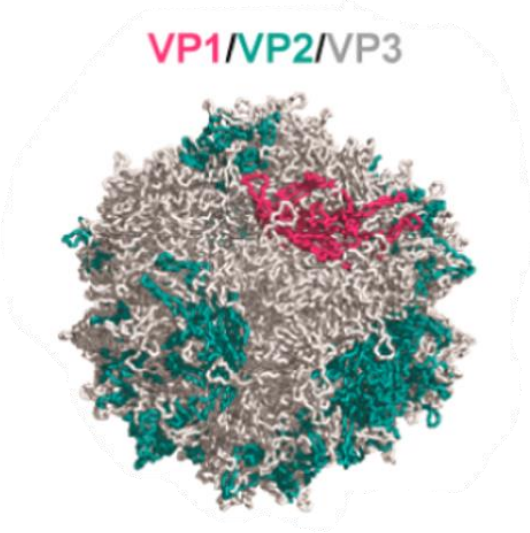
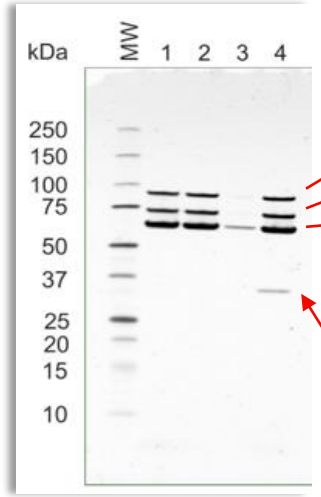
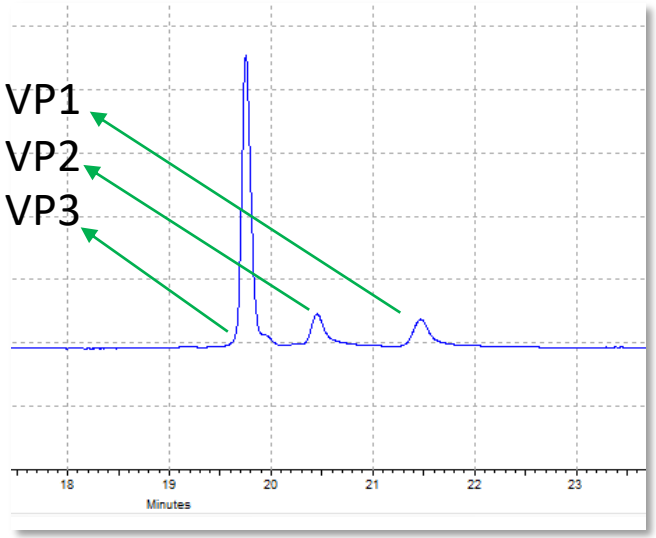






- **Purity Determination**

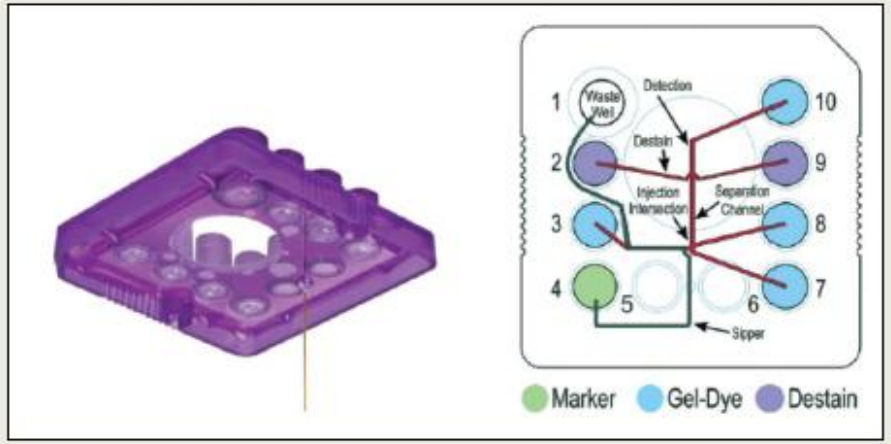
Purity determination - “In Process Samples”

- Silver Stain PAGE

www.thermofisher.com

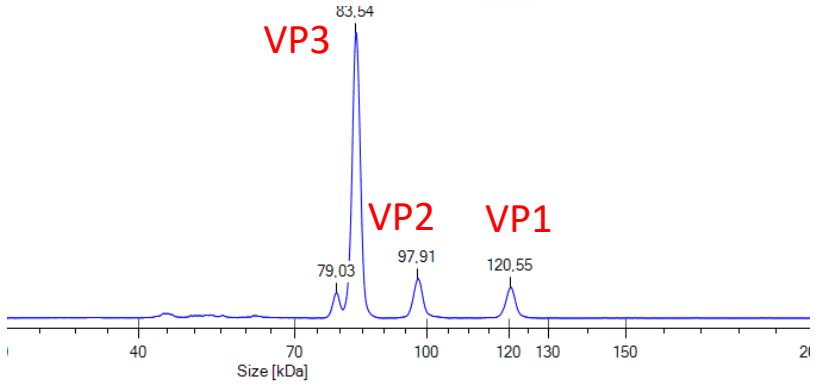
- Labchip CE

LabChip® GXII Touch™ Instrument



Sipper Chip in Quartz

Microfluidic Channels

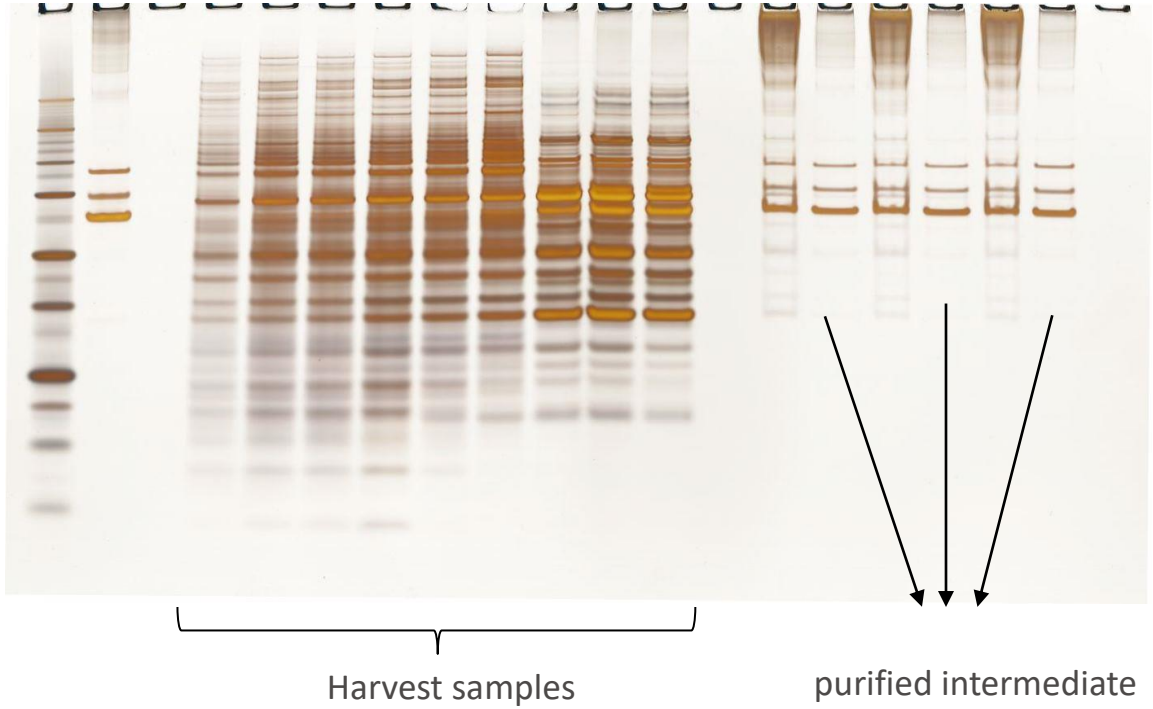


www.perkinelmer.com

Purity determination - Silver Stain PAGE vs. Labchip CE

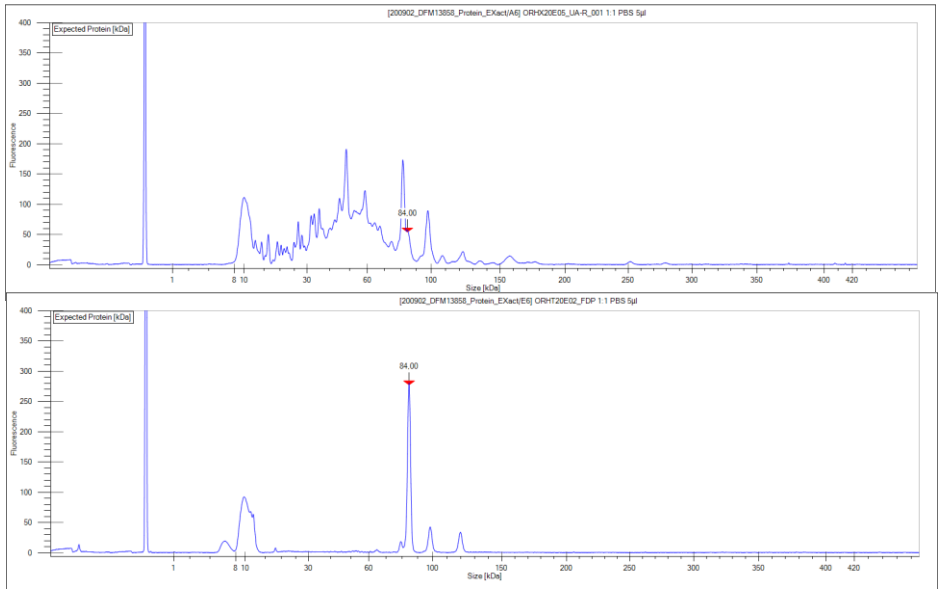
Silver Stain PAGE

- Detection of several proteins in Range
- Qualitative assay
- High sensitivity



LabChip

- Detection of several proteins in Range
- Lower sensitivity compared to silver stain
- Relative quantification of VP´s
- Calculation of Capsid Titer [cp/mL]
- Quantification of total Protein [mg/mL]



Harvest sample

purified intermediate

Purity determination - “BDS, FDP, Stability”

- Flamingo Stain PAGE

www.thermofisher.com

www.Bio-rad.com

- Purity CE

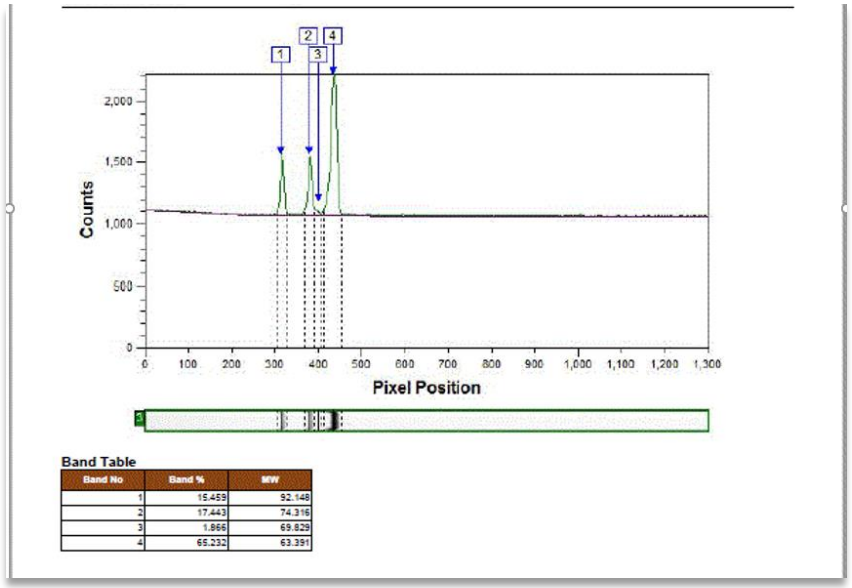
www.sciex.com

www.agilent.com

Purity determination - Flamingo stain PAGE vs. Purity CE

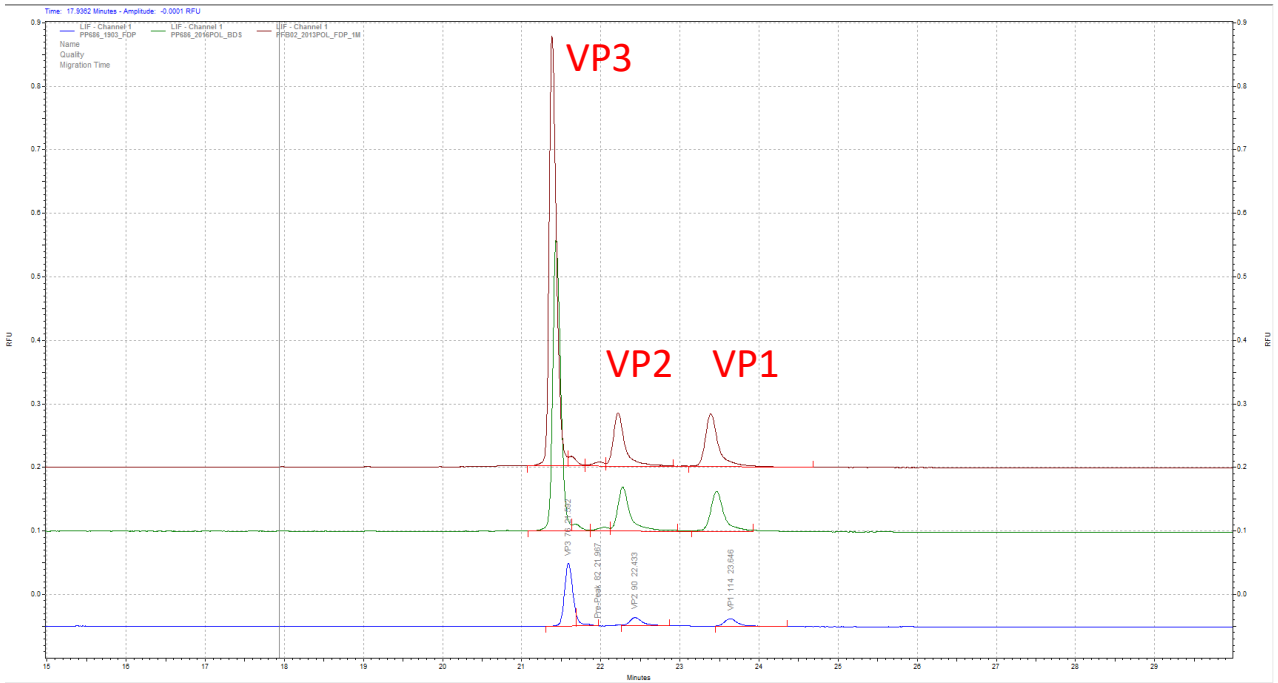
Flamingo stain PAGE

- Detection of several proteins in Range
- Long hands-on time
- Relative quantification of VP's combined with densitometric evaluation
- High sensitivity



Purity CE

- Detection of several proteins in Range
- Automated system
- High sensitivity after labelling
- Relative quantification of VP's



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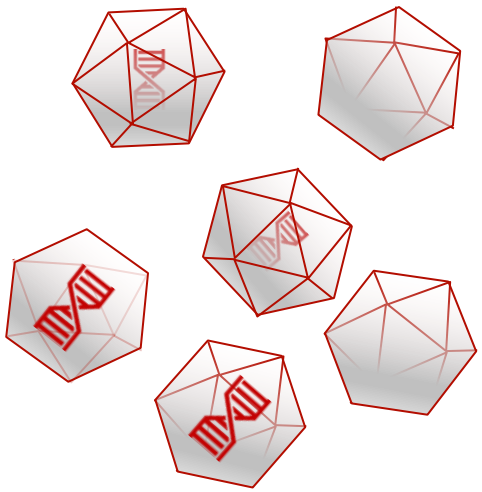

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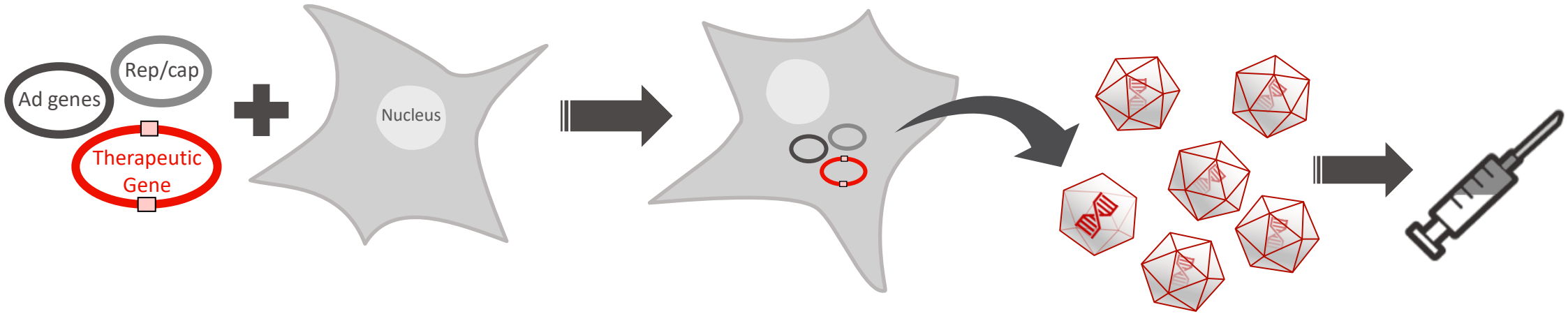
Relative quantification of capsid subspecies - AUC

Triple Transfection Method for Adeno-Associated Virus (AAV)



Plasmid DNA

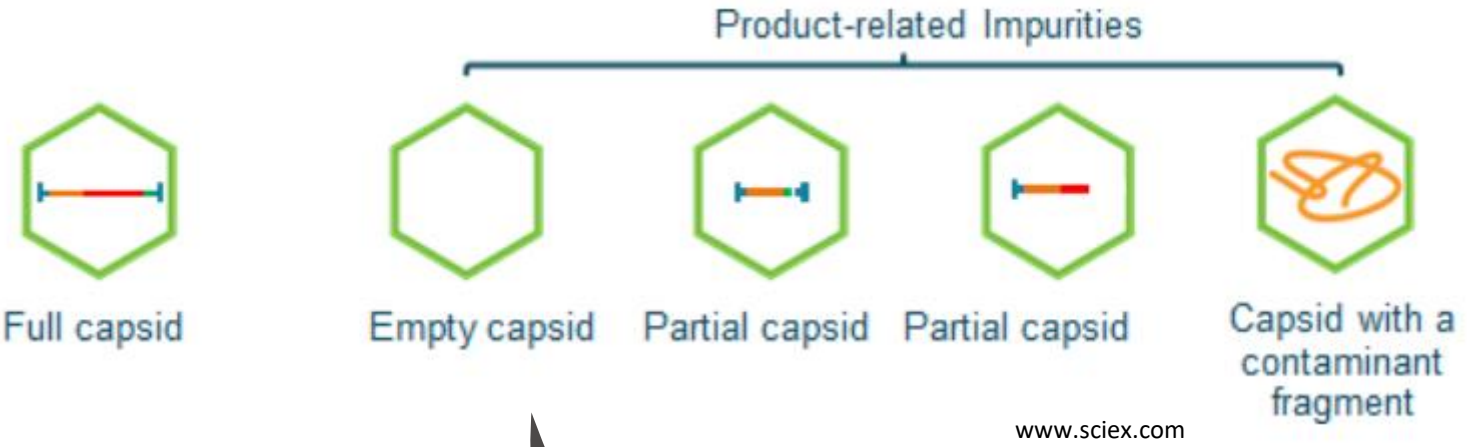
HEK 293 cells

Triple transfected HEK 293 cells

Adeno-Associated Virus (AAV) containing therapeutic gene

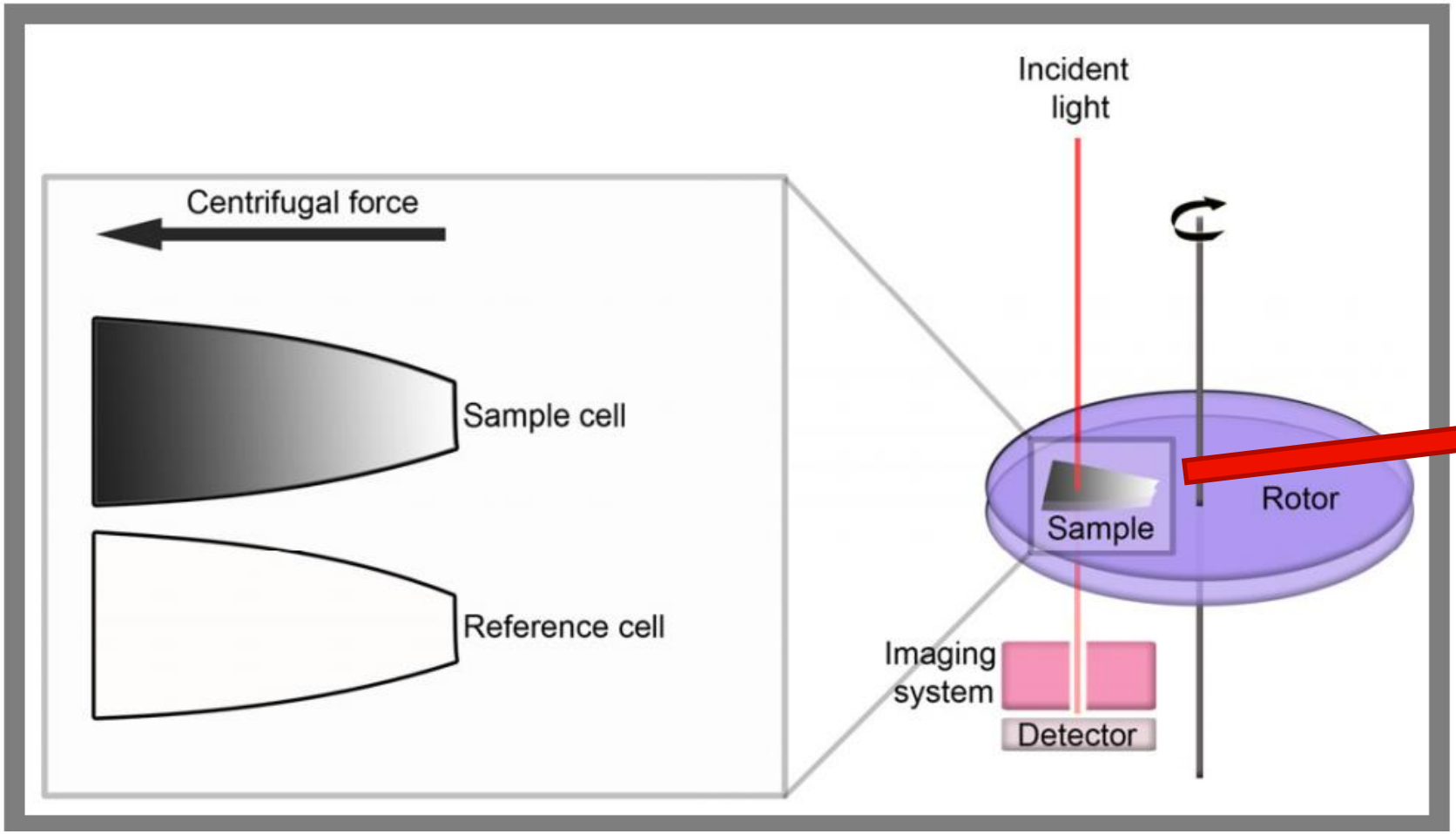
Relative quantification of capsid subspecies - AUC

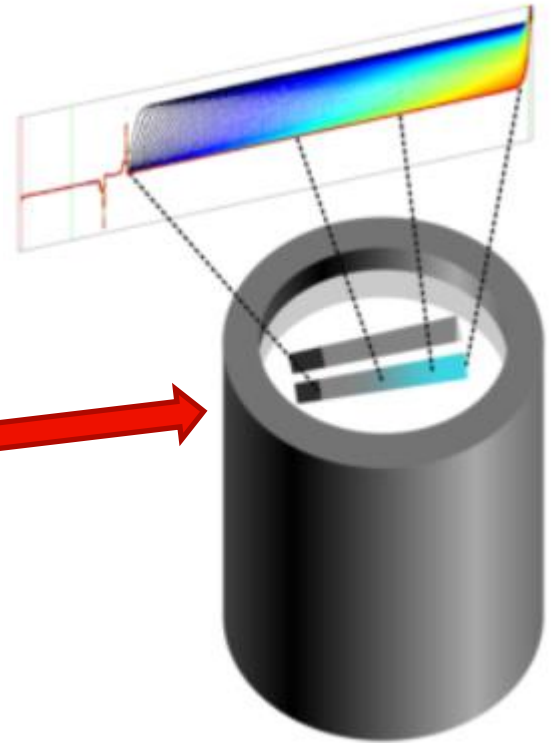
- **Production of Adeno-Associated Virus (AAV):**



Relative Quantification of of Full, Empty and other Capsid subspecies

Relative quantification of capsid subspecies - AUC





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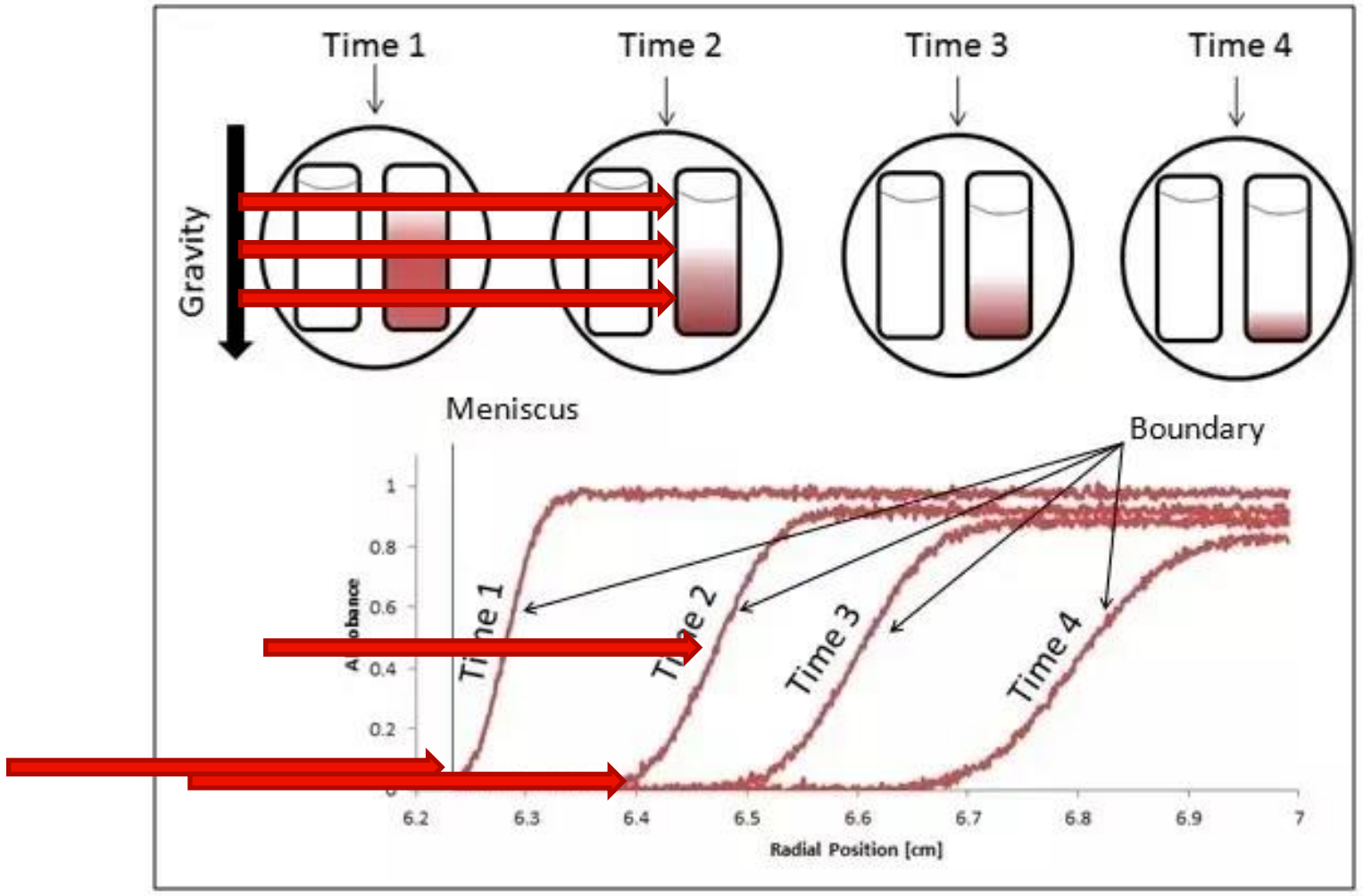
Noy, Elad & Reicher, Barak & Barda-Saad, Mira. (2012). Stoichiometry of Signalling Complexes in Immune Cells: Regulation by the Numbers. 10.5772/33205.

Relative quantification of capsid subspecies - AUC



Van Holde, K.E. and Baldwin, R.L., 1958. Rapid attainment of sedimentation equilibrium. *The Journal of Physical Chemistry*, *62*(6), pp.734-743.

Relative quantification of capsid subspecies - AUC

Analytical Ultracentrifuge

Rotor

Cells

https://www.beckman.de

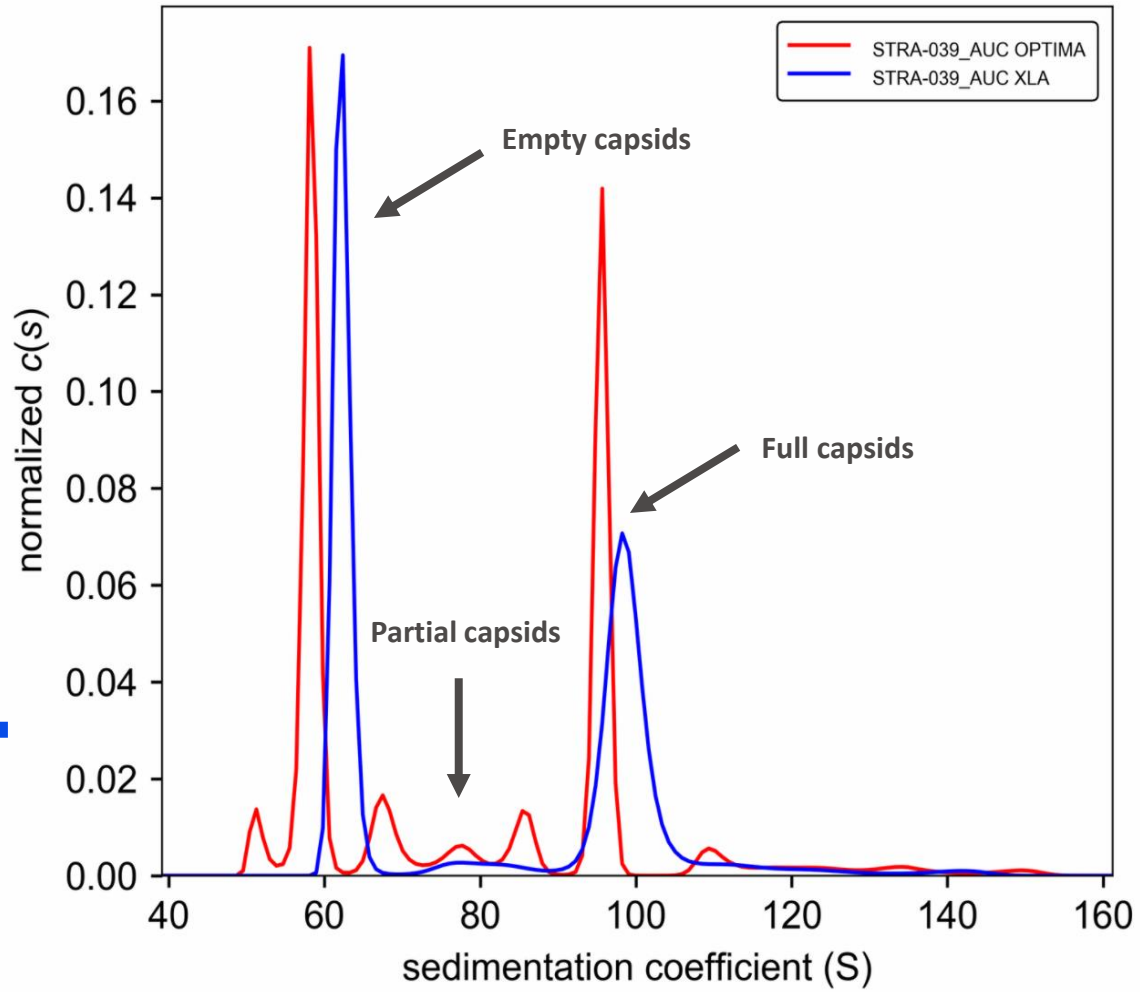
Computer and Software for Evaluation

Relative quantification of capsid subspecies - AUC

AUC Optima

AUC XLA

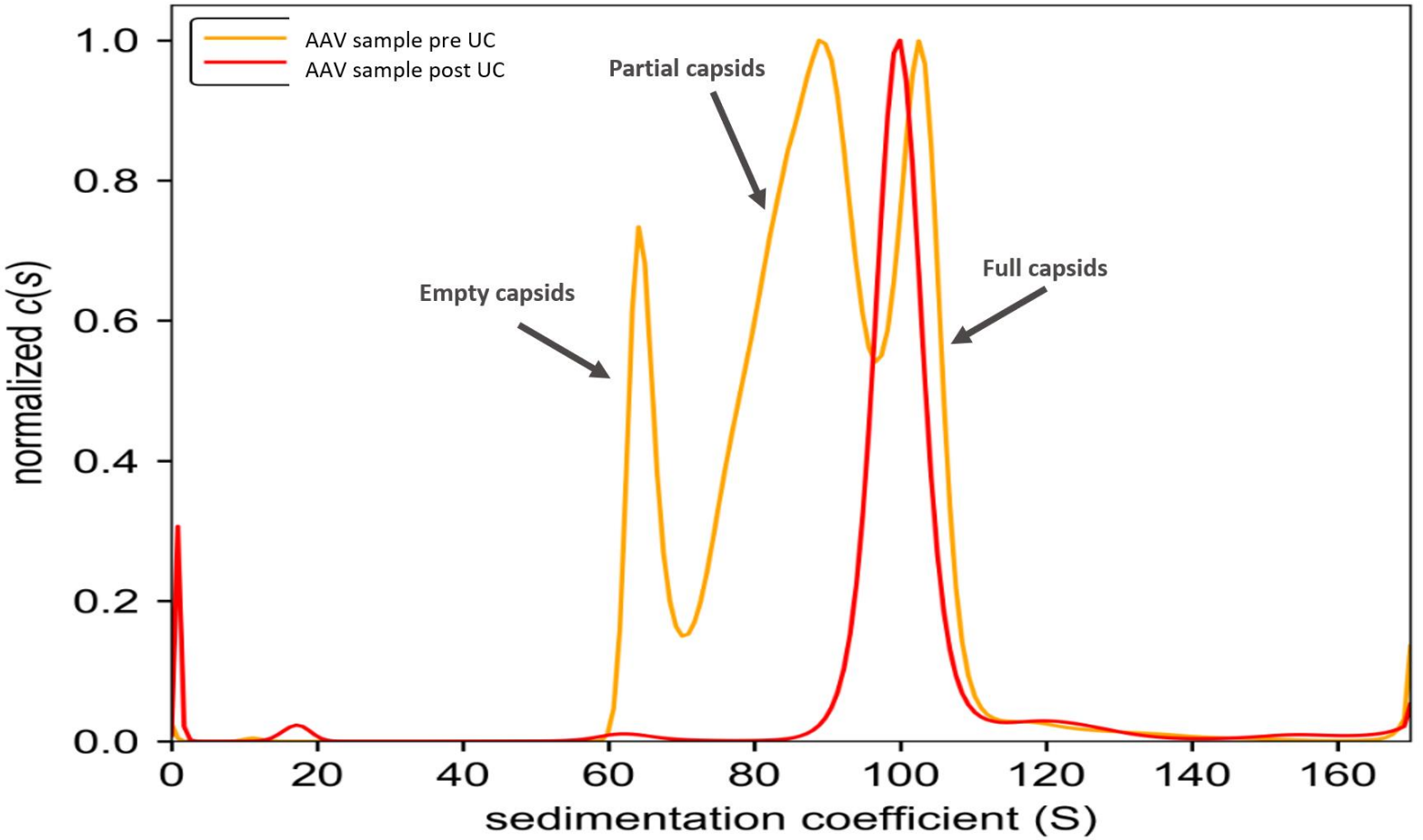
https://www.beckman.de



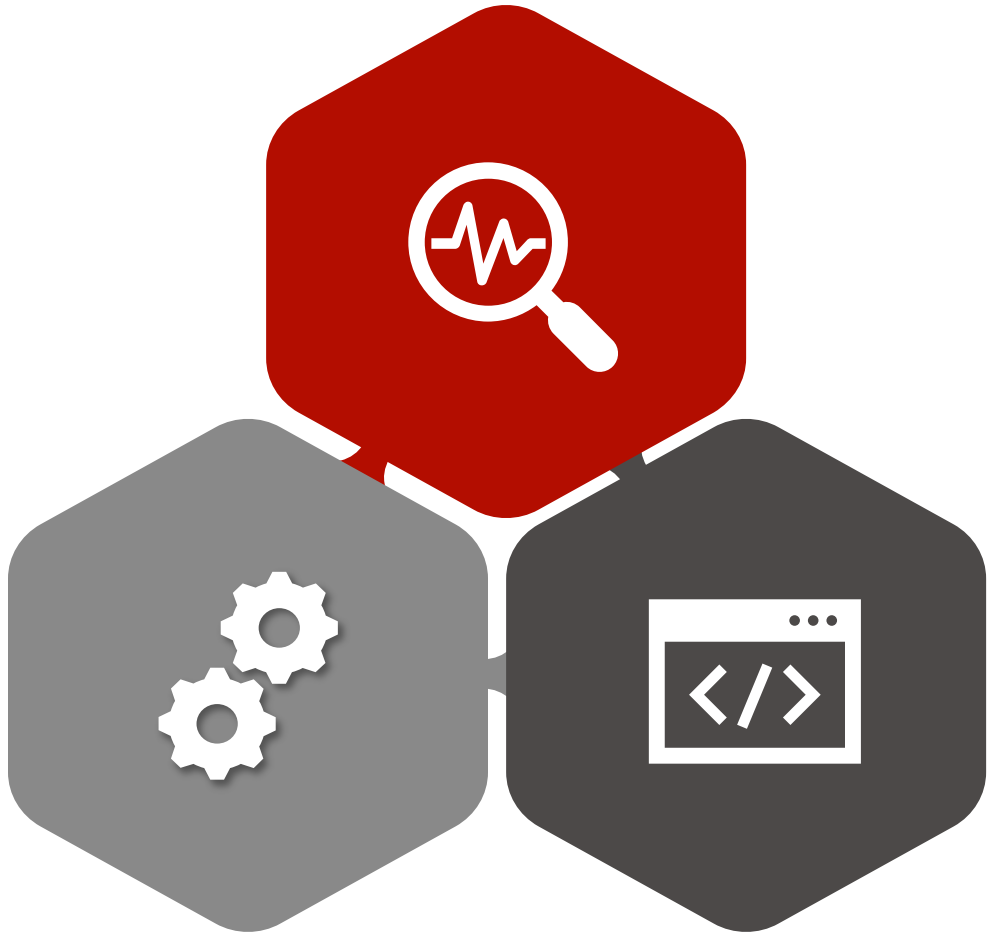
Relative quantification of capsid subspecies - AUC

Full, Empty and others

- **Discrimination between Full and empty Capsids**
- **Partially filled capsids**



Acknowledgements



Gene Therapy Process Development Team

Gene Therapy Analytics Team

Bernd Innthaler
Ewald Kapeller
Sabine Unterthurner
Roman Raim

All Other Support functions

The END…

Thank you for your attention!!!