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Cell and Gene Therapy

Viral Vectors: what are the solutions to current supply challenges?

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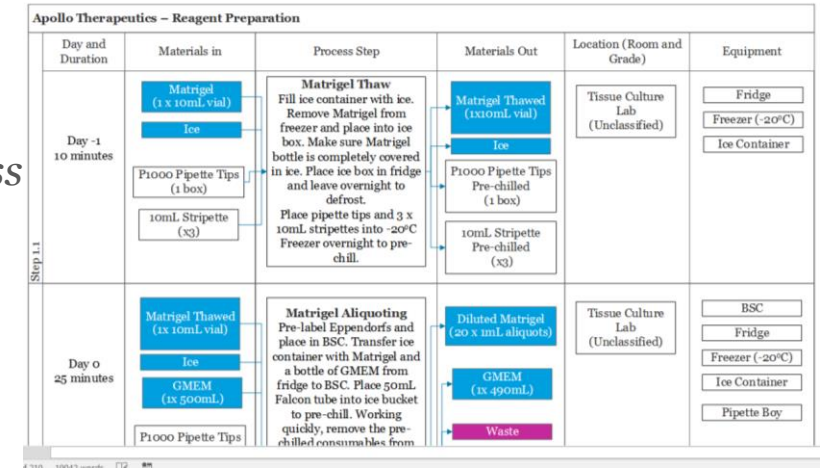
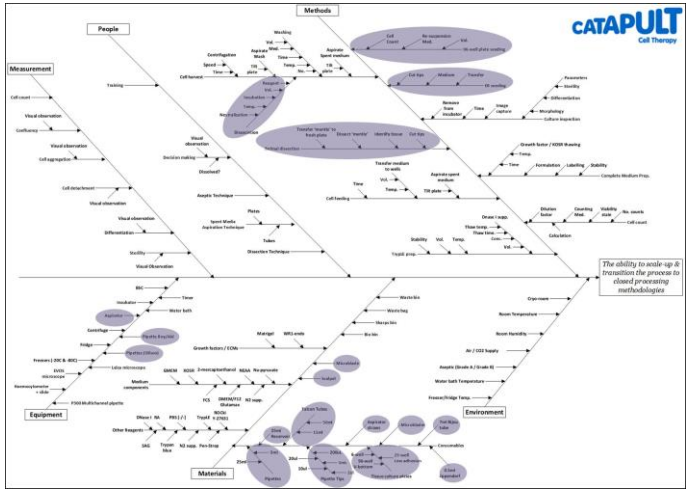
Diagnostics toolkit

Areas of process currently undefined

Process mapping



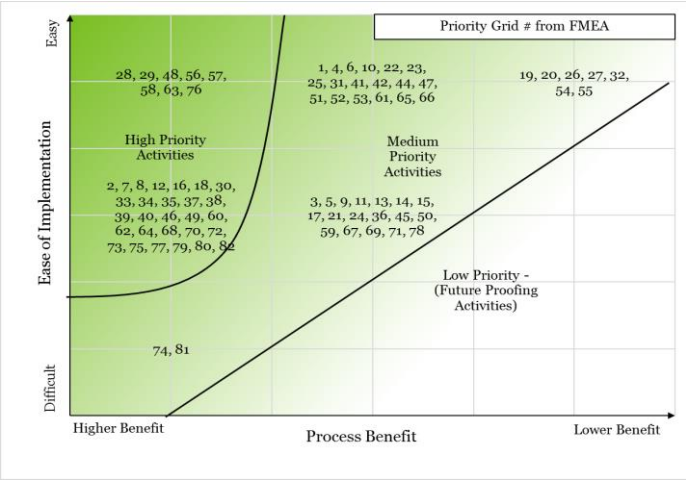
Ishikawa



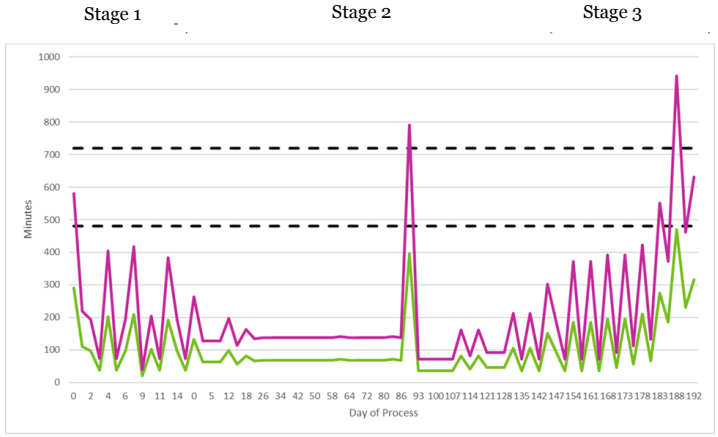
Root cause of failure

Risks and mitigation strategies

FMEA

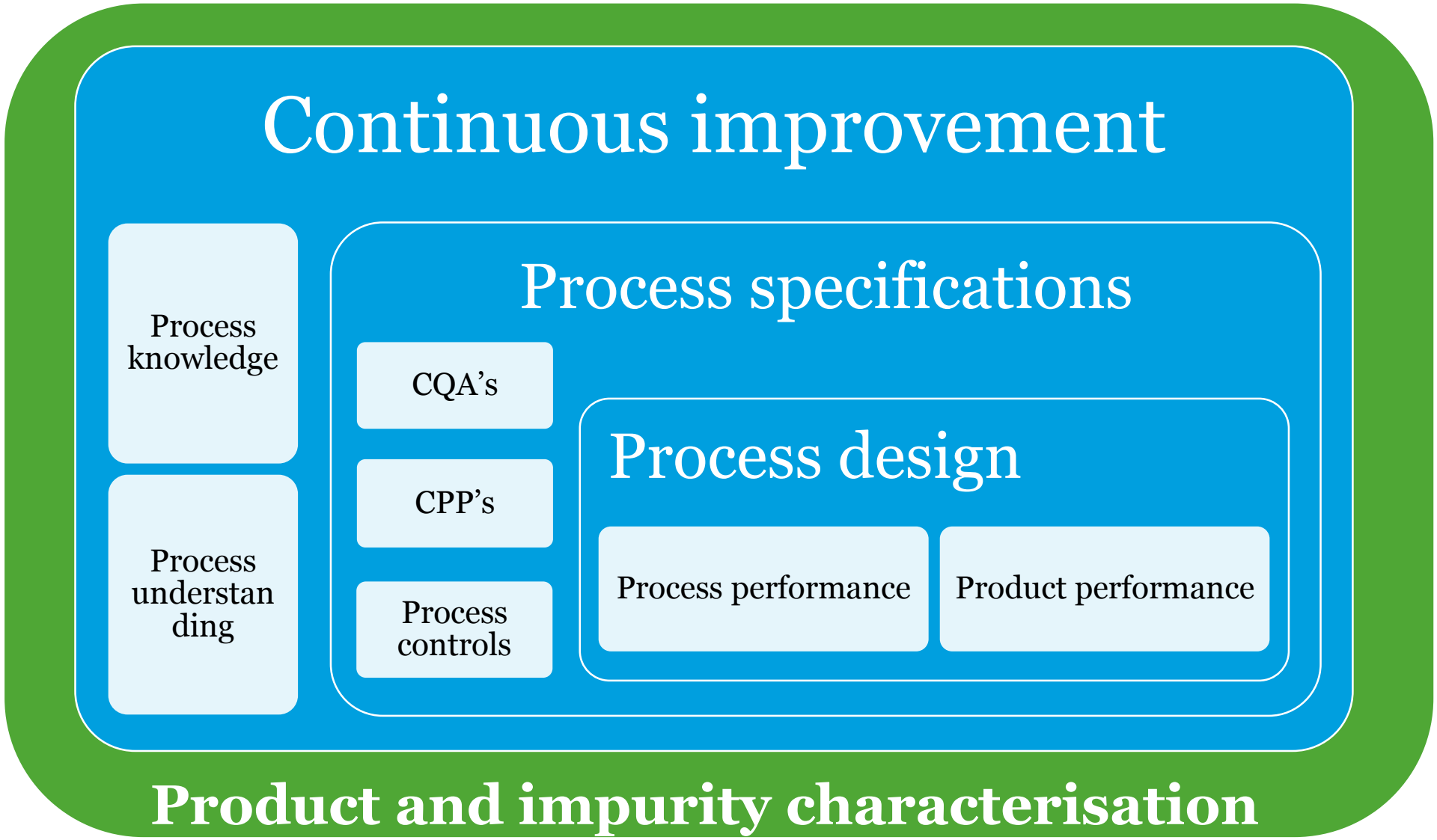


Facility utilisation and CoGs



Facility utilisation profile

Quality by design



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Improving viral vector production...

Viral gene delivery

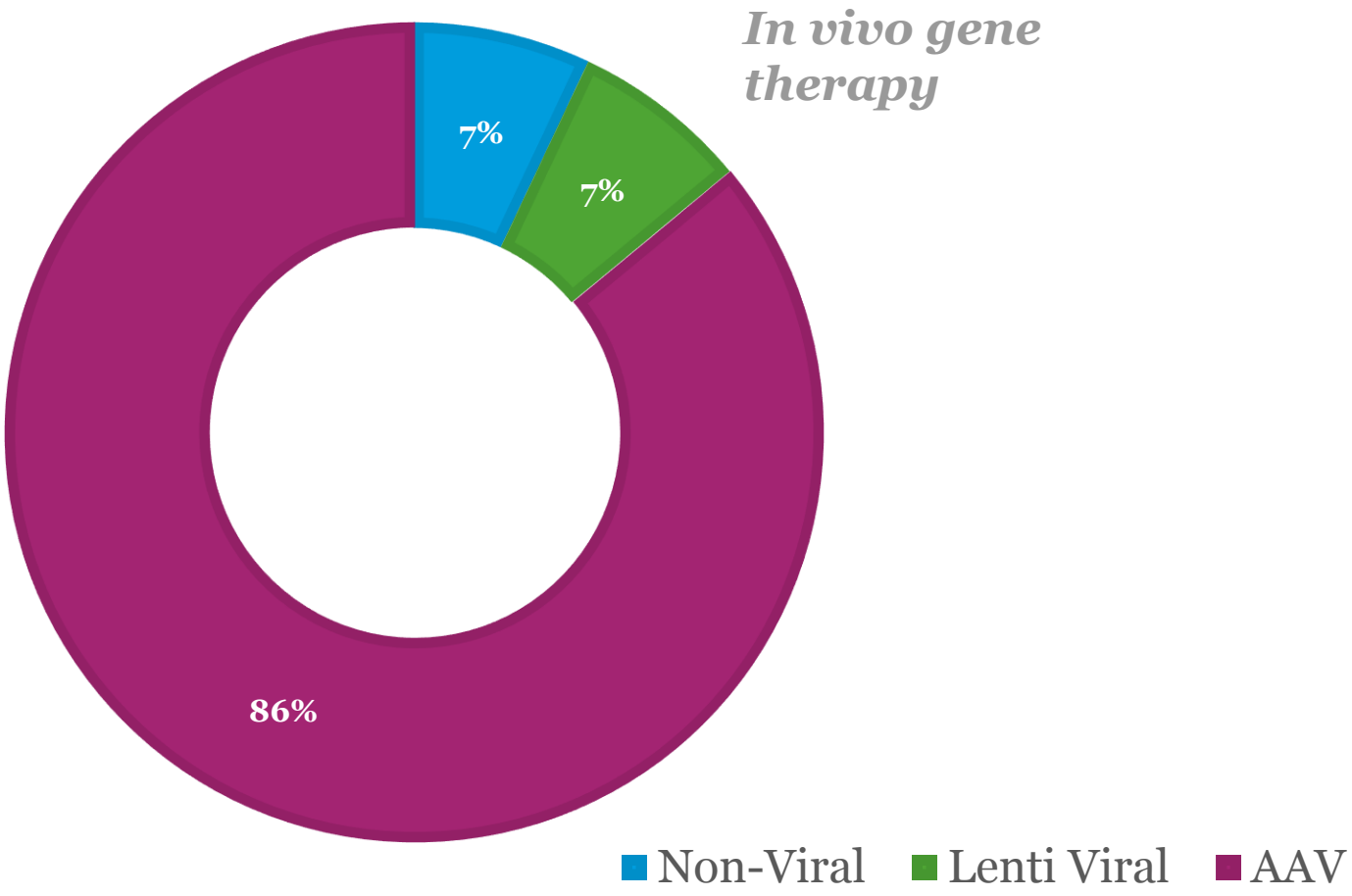
Viral vector manufacturing capacity is a barrier that is limiting the development of therapies and places the UK research pipeline and industry at risk.

We've seen an improvement in capacity but demand has increased'

Industry challenges

- Rapidly growing industry – huge demand
- Low process yields
- Highly variable analytical assays
- Suboptimal unit operations
- High cost of goods
- Lack of clinical grade supply
- Large volume manufacture for early development
- Process development can only detect large changes

Gene therapy landscape



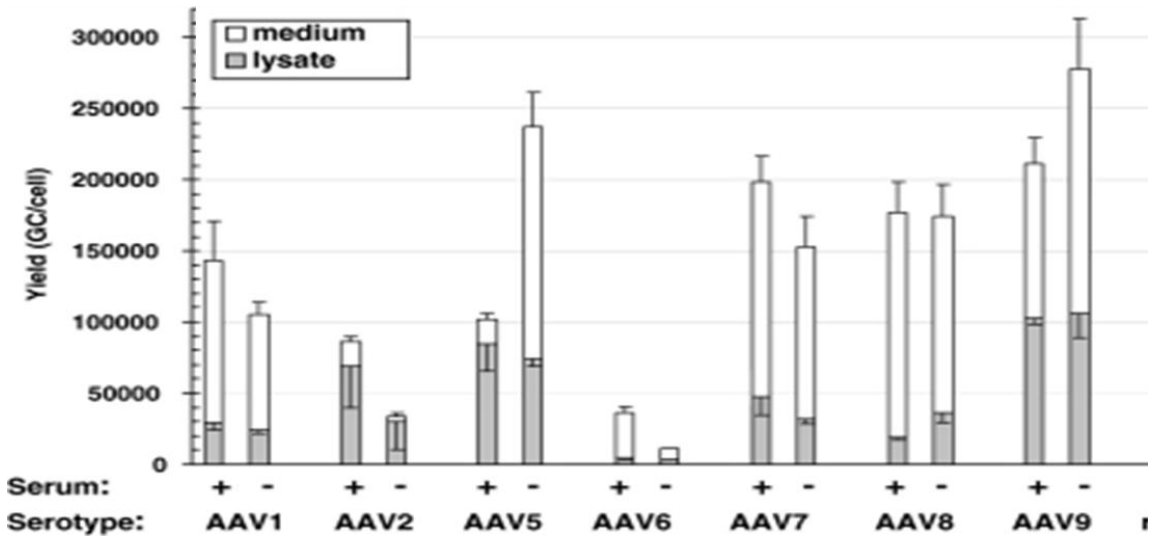
Setback for gene therapy for safety reasons in the 1990s
Discovery of novel, safer and more efficient AAV vectors

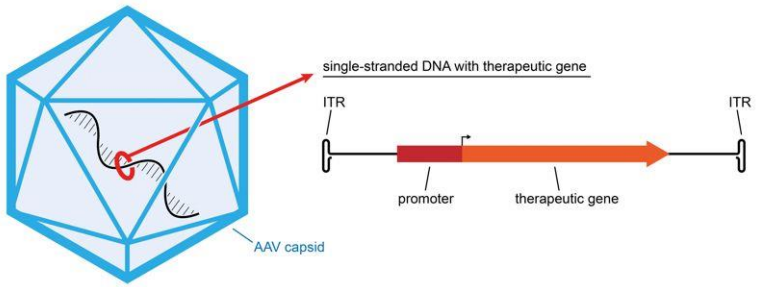
- *Exponential growth*
- *Funding influx*
- *High profile deals*

- *> 25 clinical trials in the UK*
- *2 launched AAV products*
  - ***Glybera, 2012***
  - ***Luxturna, 2017***

https://ct.catapult.org.uk/sites/default/files/publication/Clinical%20Trials%20Database%202018.pdf

AAV mediated gene therapy





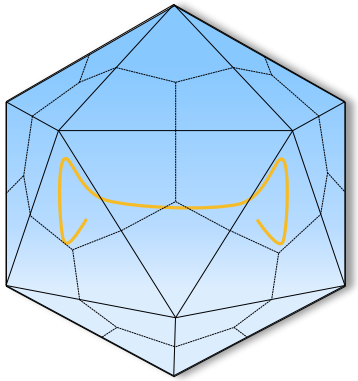
Primary Target Tissues									
Serotype	Retina	Neurons	Brain	Lung	Heart	Liver	Muscle	kidney	Pancreas
AAV-1		√			√		√		√
AAV-2	√	√	√			√	√	√	
AAV-3	√			√		√	√		
AAV-4	√	√	√				√		
AAV-5	√	√		√					
AAV-6				√	√	√	√		
AAV-7	√	√				√	√		√
AAV-8	√		√			√	√		
AAV-9			√	√	√	√	√	√	√
AAV-10		√		√	√	√	√		
AAV-DJ	Efficiently transduces a wide variety of cell types *in vitro*								
AAV-DJ/8	A variant of AAV-DJ that permits infection of liver as well as other tissues *in vivo*								

Vandenberghe et *al.*, Human Gene Ther. 2010

Gene delivery - AAV

Processing challenges

1. Process tools designed for proteins NOT viral particles
2. Efficient production systems
3. Purification operations



AAV
25nm

Gene therapy	Condition	Virus	vg/kg	Est. total dose
RPE65	retinal dystrophy	AAV2	N/A	2.5E+12
Factor IX	hemophilia B	AAV5	2E+13	1.4E+15
SMA 1	Spinal muscular atrophy	AAV9	2E+12	6E+15

 ..... 4 cell factories

 ..... 150 cell factories

 .... 600 cell factories

With a push towards more sophisticated pipelines, analytical tools will be needed to control manufacturing processes

Challenges for AAV manufacture

Vector design

IP, capsid, promoter,

transgene

Manufacture

Unit operation selection

Scale-up

Environmental control

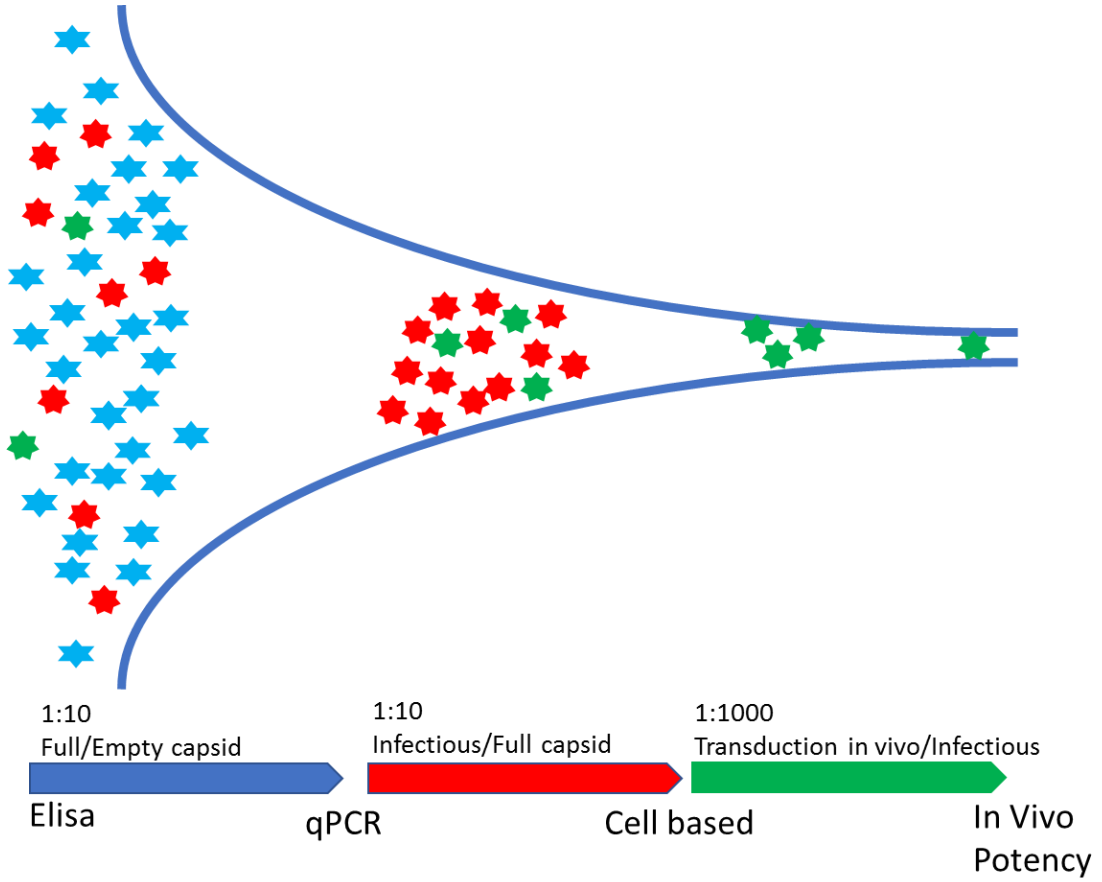
Limiting/removing impurities

Standardization

International standards

QC (IPC, Product/batch release, process validation)

Regulatory



Around 1:100000 particles will achieve clinical output

Adapted from Hitchcock et *al.*, 2017

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The next generation of platform technology...

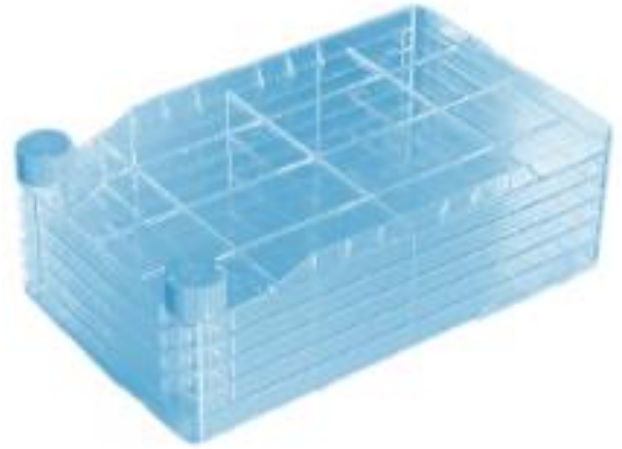
Viral vector manufacture - AAV

1st generation platform

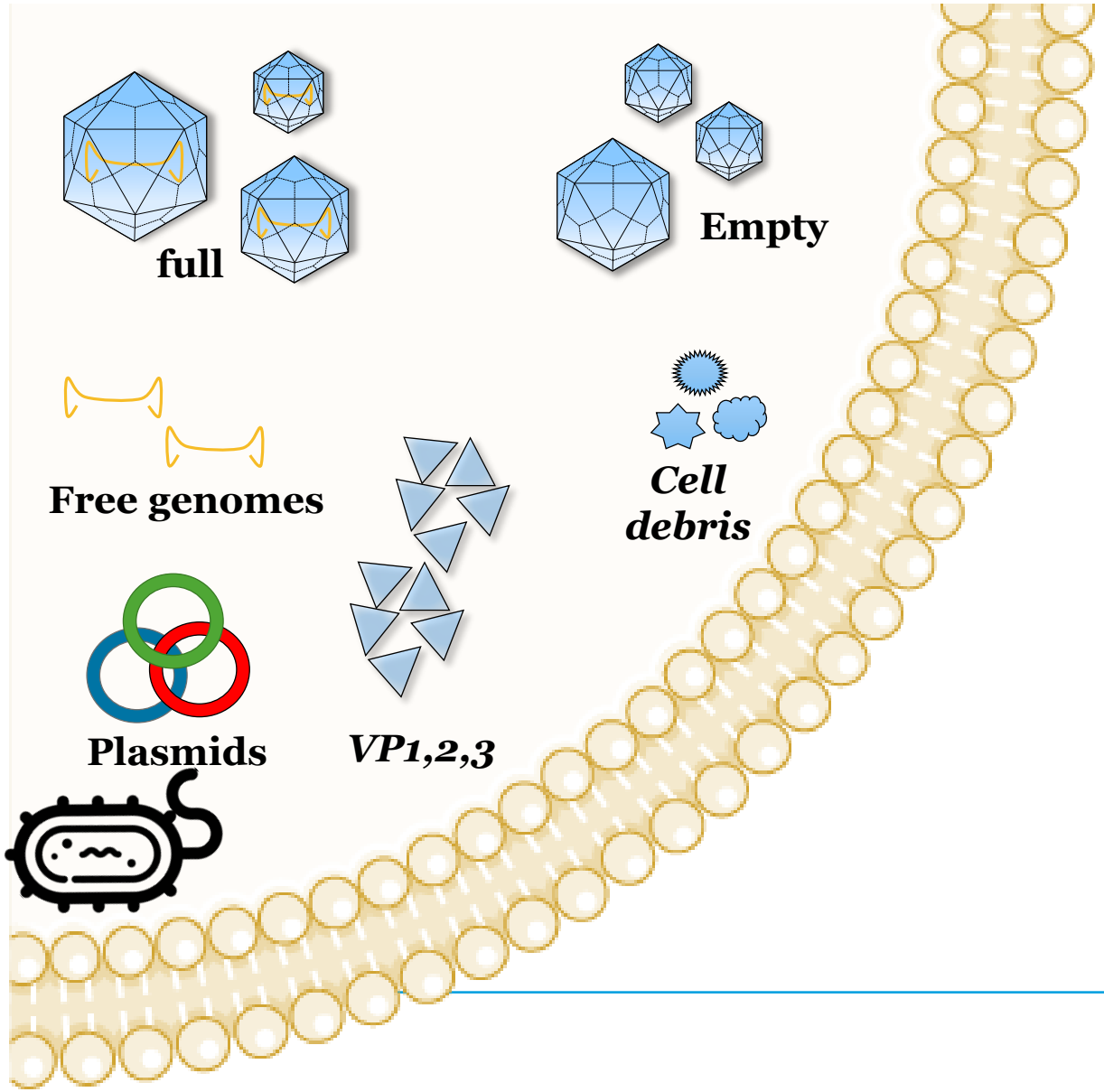
- **Adherent tissue culture plastic** expansion/production
- **Plasmid DNA transfection** - CaCl2
- **Ultra centrifugation** (density gradient)
- **Depth filtration** sterilisation
- **CMO's** moving away from these methods

2nd generation platform

- **Latest adherent tissue culture systems** technology 2D or 3D expansion/production
- **Plasmid DNA transfection** - PEI/Lipofectamine
- **Multi-step chromatographic** purification
- **Single use technologies** from USP to DP



Approach to analytics



	1st Generation	2nd Generation
Physical titre	ELISA	MADLS
Packaged genomes	qRT-PCR	ddPCR
Packaging ratio	ELISA/PCR	HPCE
Viral capsid proteins	Western blot	Automated WES
Aggregation	DLS	MADLS
Infectious titre	FACS	ddPCR
Functional titre	*In-vitro*	FACS/Impedance
Total protein	Coomassie	LC-MS
Sterility	Growth based	ddPCR
Purity		ddPCR / Seq

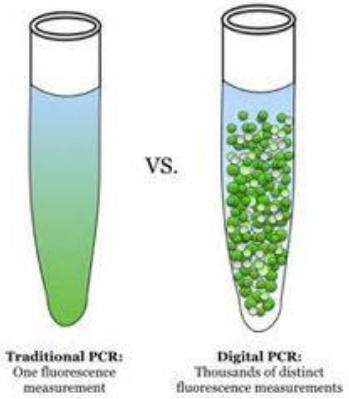
ddPCR and qPCR method comparability

AAV physical titration method

Physical AAV titration is calculated using commercial qPCR kit

- Shelf-life of 6 months after opening
- Requires standard curve

Aim – To create a ddPCR based in-house AAV physical titration protocol

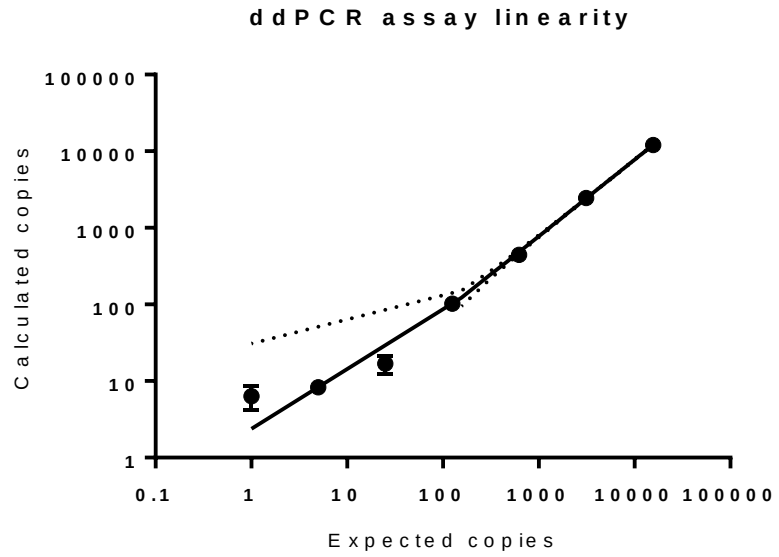


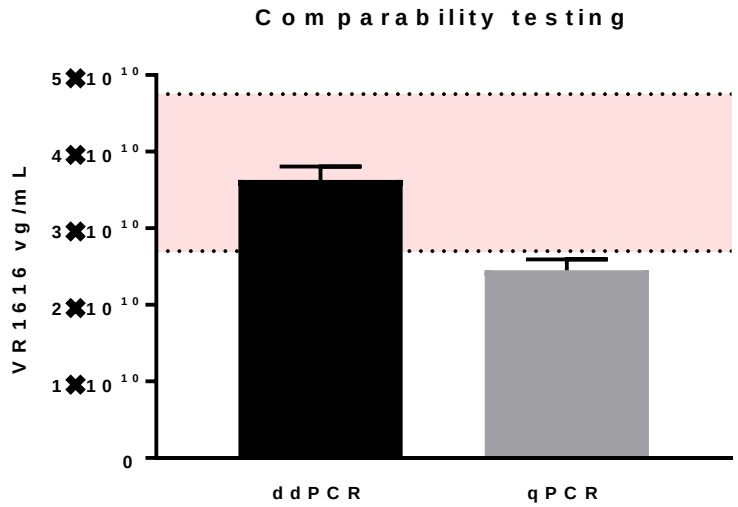
In-house primers targeting the conserved ITRs

- Less sensitive to PCR inhibitors
- Quantification is not dependent on standard curve
- Low assay variability (CV typically <8%)

Sample	Condition	Estimated vg/mL	Calculated vg/mL	SD	CV	Yield
VR1616	ddPCR platform	3.28E+10	3.31E+10	1.02E+09	3%	101%
VR1616	qPCR platform	3.28E+10	6.74E+10			206%

ddPCR and qPCR method comparability





ddPCR results within 95% CI

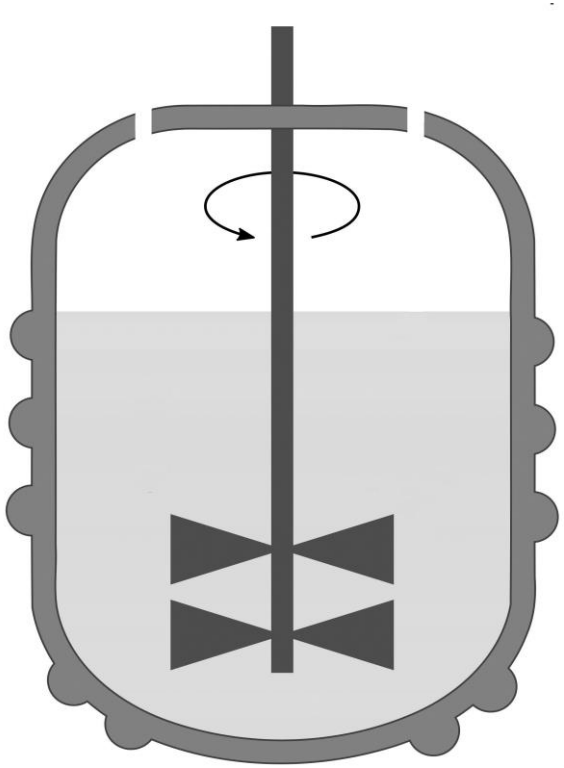
R square	0.9969
Deviation from linearity	non significant
LLoD	16.16 copies
LLoQ	125 copies
Intra/inter assay CV	< 20%

1. Novel primer/probe design → Applicable to any AAV2 and AAV2 derived serotypes
2. Novel genomic extraction method
3. Higher sensitivity → Suitable for in-process sample measurement
4. Increased precision and reproducibility over commercial and current available qPCR titration methods

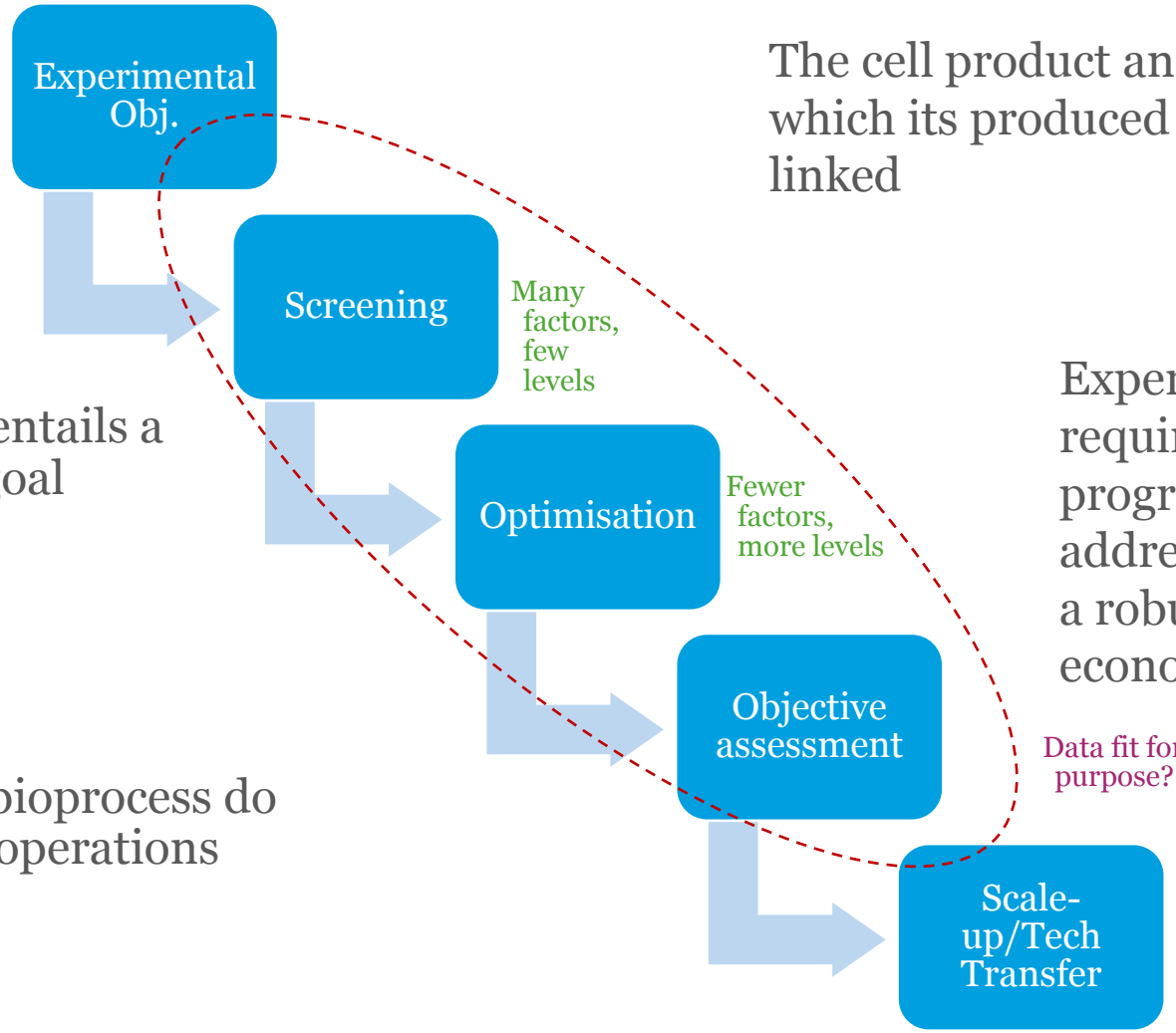
Viral vector manufacture - AAV

3rd generation platform

- **Suspension** based technology for expansion/production
- **Continuous** harvest and clarification e.g. kSep
- **Alternative transfection** methods e.g. high-throughput electroporation
- **Continuous/single step** chromatographic purification
- **Process analytical technologies (PAT)** implementation using Raman, LC/MS, high resolution imaging



Viral vector manufacture: DoE approach



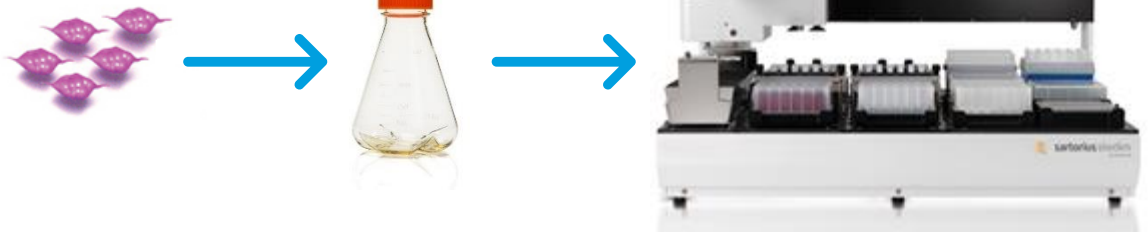
The cell product and the process by which its produced are intricately linked

Bioprocess development entails a progressive approach to goal attainment

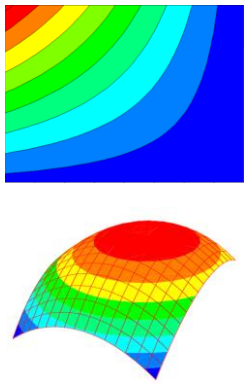
Experimental planning requires a degree of rational progression to successfully address the goal: producing a robust, efficacious and economically viable product

Unit operations within a bioprocess do not reside as stand alone operations within a bioprocess train

Viral vector manufacture: Scaling up production



Screening



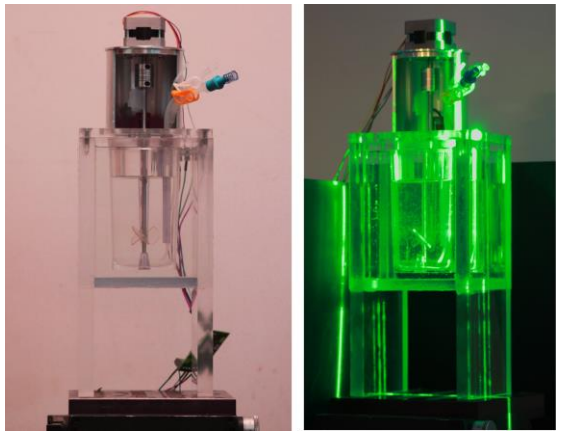
I.D of CPP's

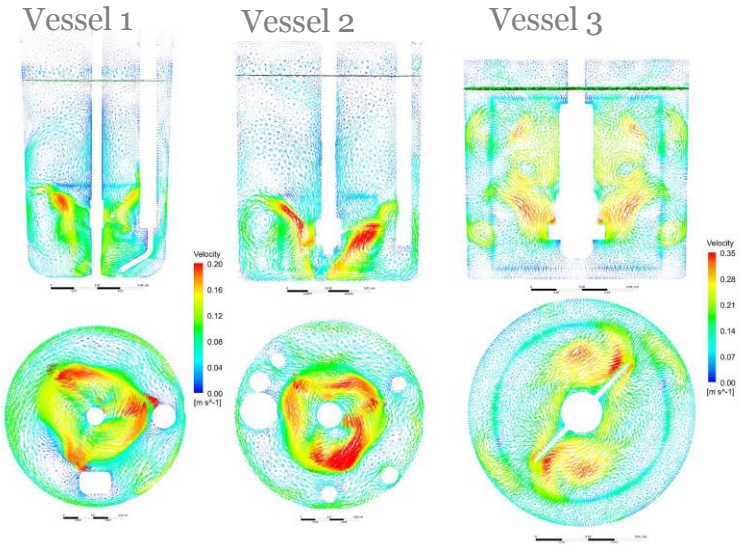
Optimisation

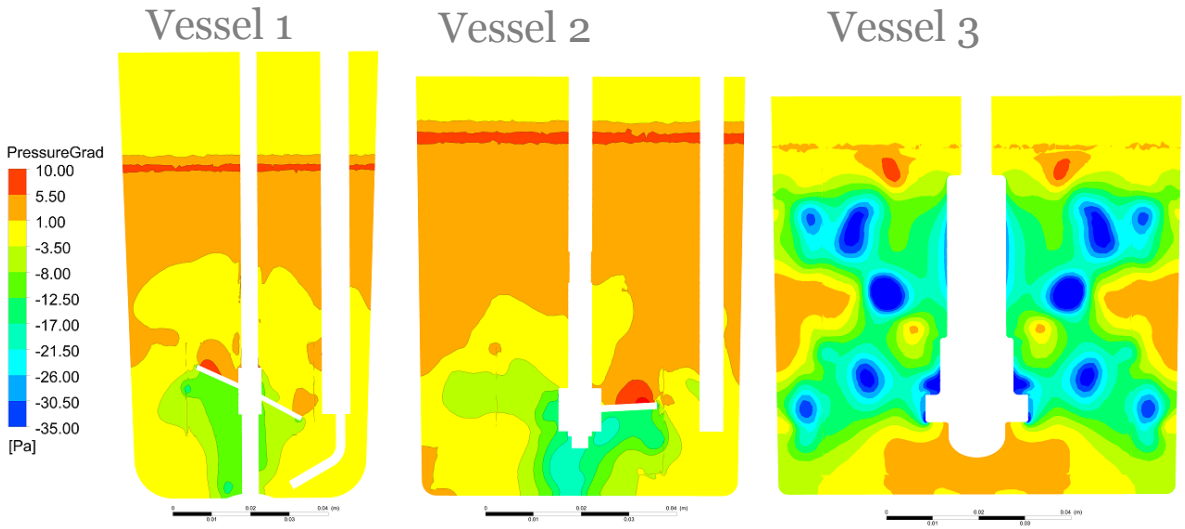
Seed train optimisation

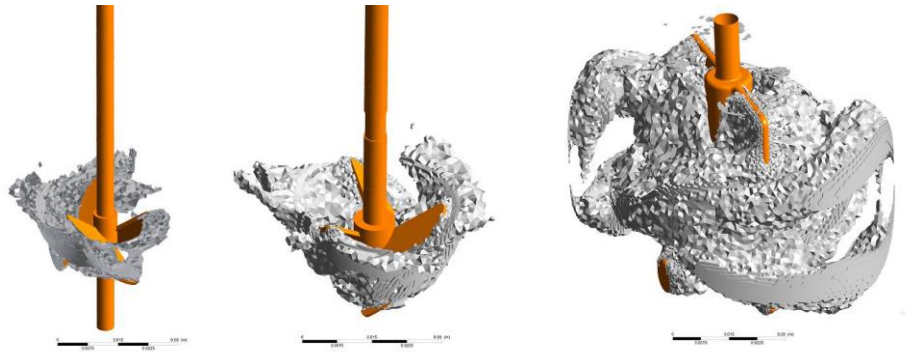
Scale-up (50 L)

Bioprocess development: vessel characterisation









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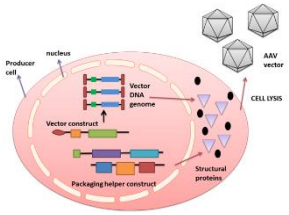
Viral vector manufacture: DSP challenges



Cell disruption
Viral vector release development and optimisation

Nuclease treatment
DNA removal development and optimisation

Clarification
Harvest filtration development

AKTA™ Avant
Chromatography purification development and optimisation

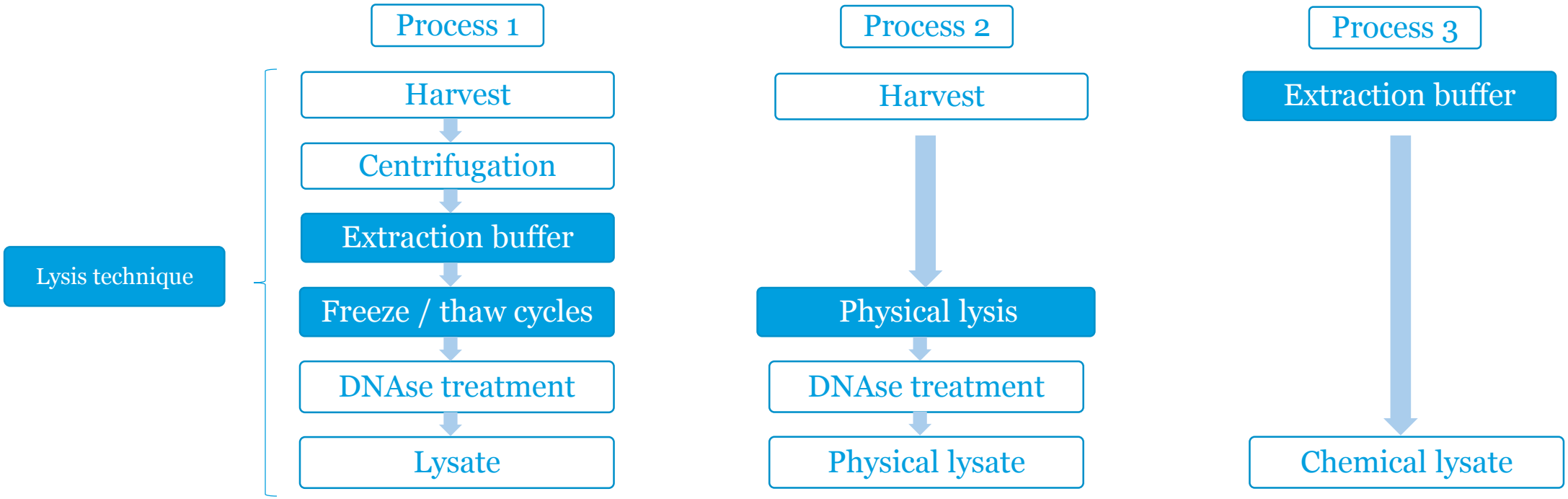
TFF KrosFlo UF/DF
Concentration & buffer exchange development and optimisation

Final filtration
Final filtration development

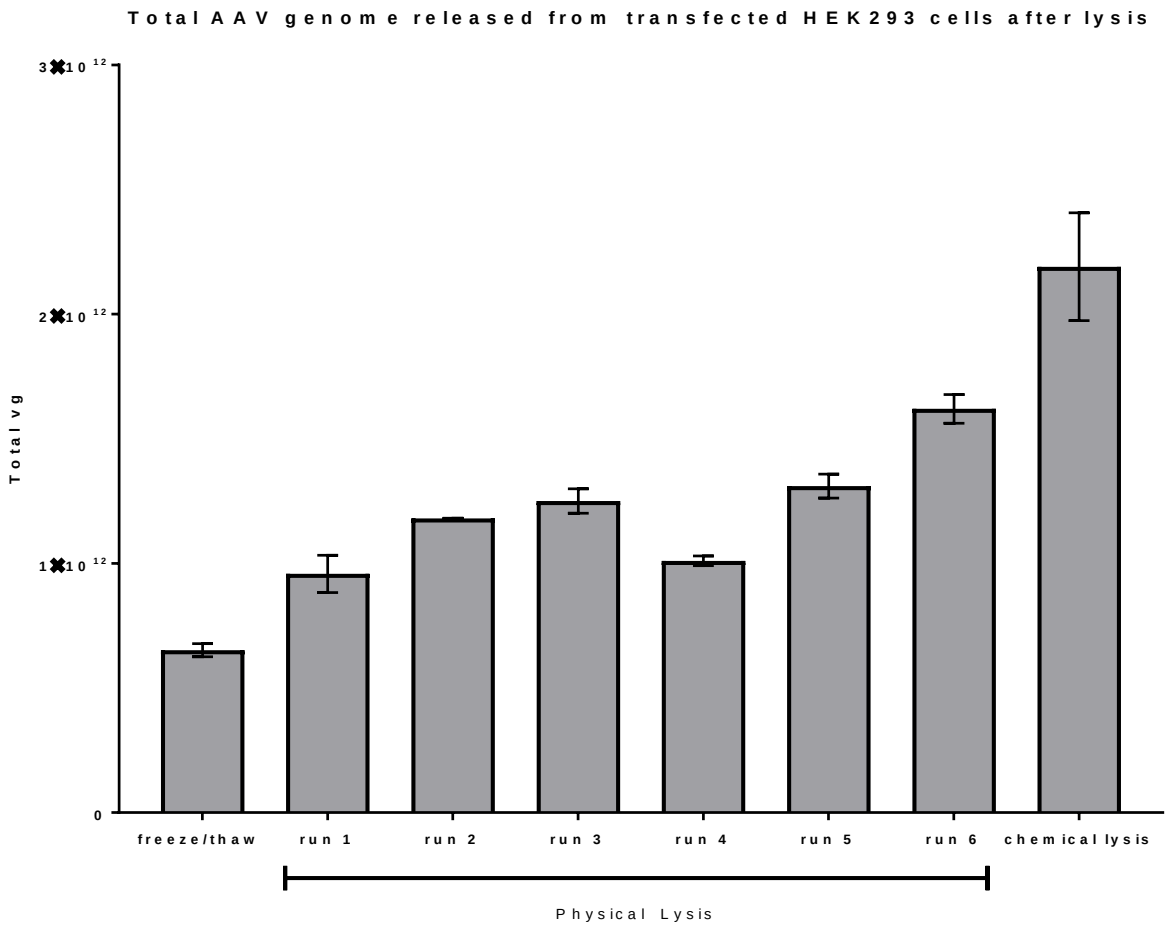
Viral vector manufacture: DSP challenges

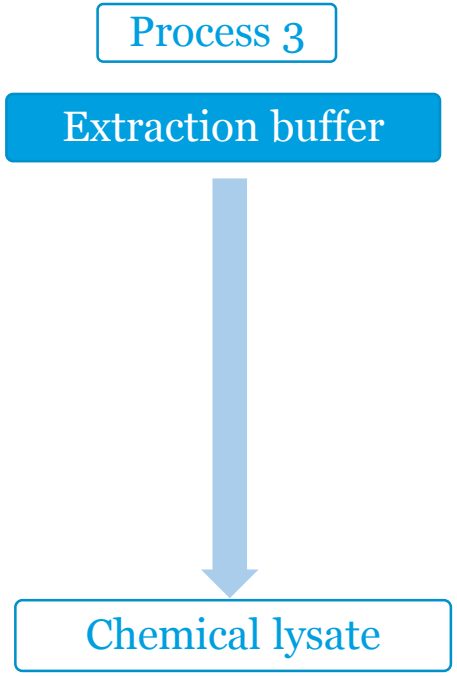
Side-by-side comparison performed between three different cell disruption methods :

- Freeze/Thaw Cycles (gold standard for lab scale cell lysis)
- Physical Lysis Using a High Pressure-shear device
- *In situ* chemical lysis with detergent-based formulation



Viral vector manufacture: DSP challenges





Chemical lysis released more AAV particles

Viral vector manufacture: DSP challenges

Purification process that aims to separate viral vector from the impurities produced during the manufacturing process.

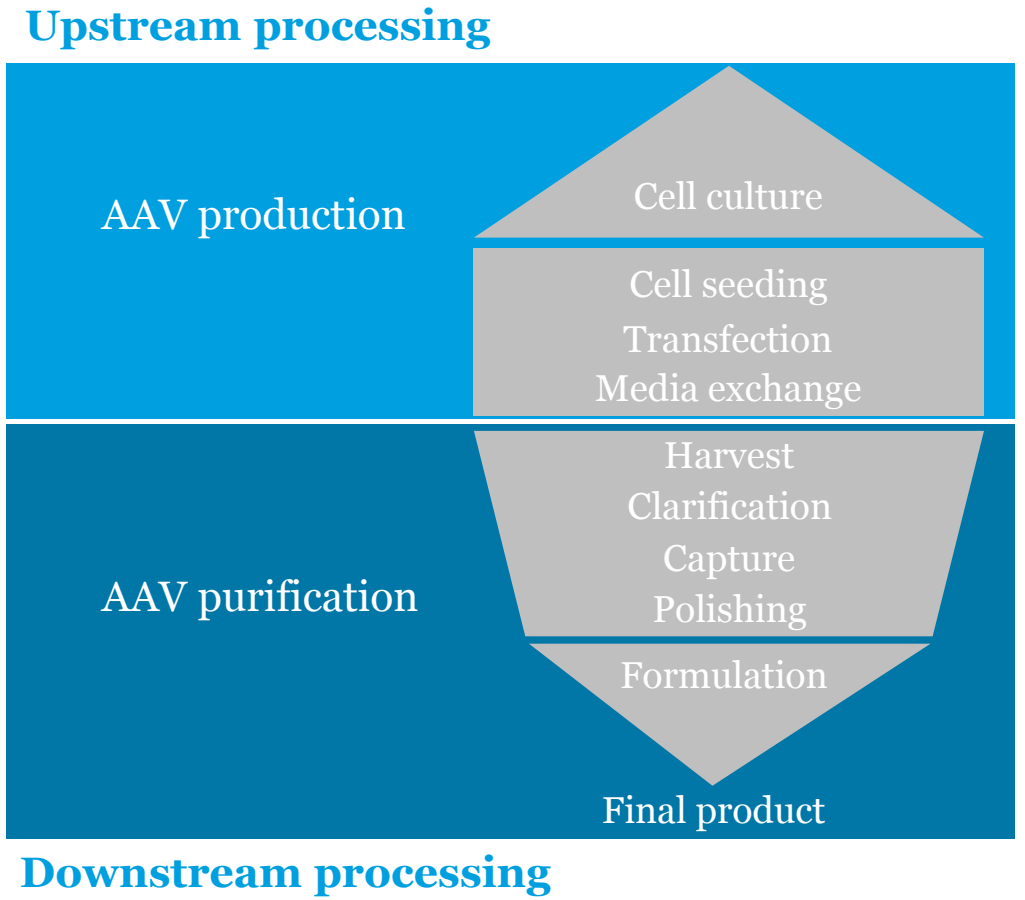
Viral vector manufacture: DSP challenges

What are the impurities ?

- Process-related impurity :

  Producer cells
  Transfection Reagent
  Plasmid DNA
  Host cell DNA/RNA, Host cell proteins
  Cell culture media components

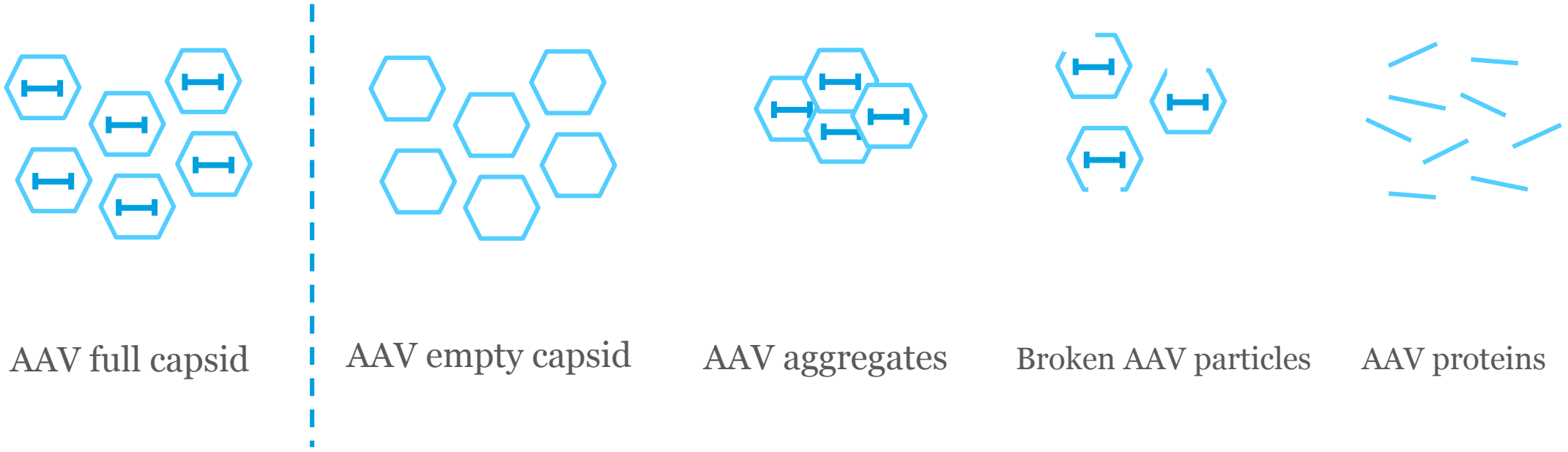
  Purification Buffer
  Chromatography media ligands
  Centrifugation media



Viral vector manufacture: DSP challenges

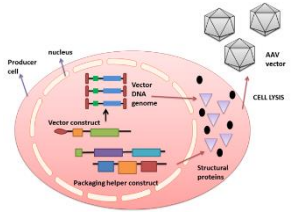
What are the impurities ?

- Product-related impurity :



AAV full capsid | AAV empty capsid | AAV aggregates | Broken AAV particles | AAV proteins

Viral vector manufacture: DSP challenges



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Clarification
microfiltration development

AKTA™ Avant
Chromatography purification development and optimisation

- Capture study
- Polishing study

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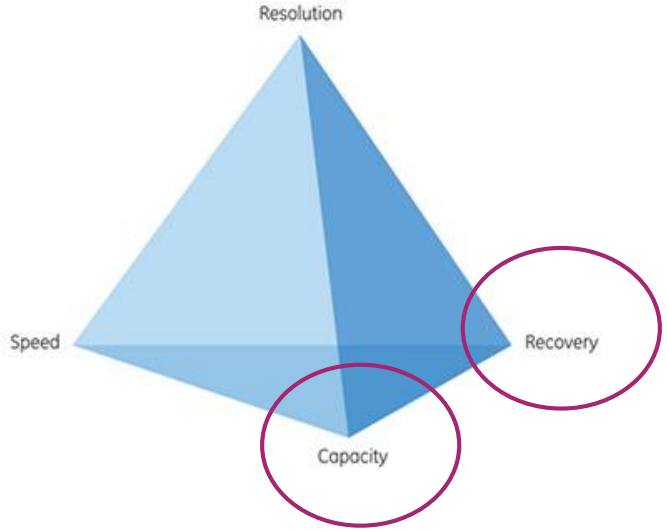
Final filtration development

Viral vector manufacture: DSP challenges

Key Performance Indicators for Capture

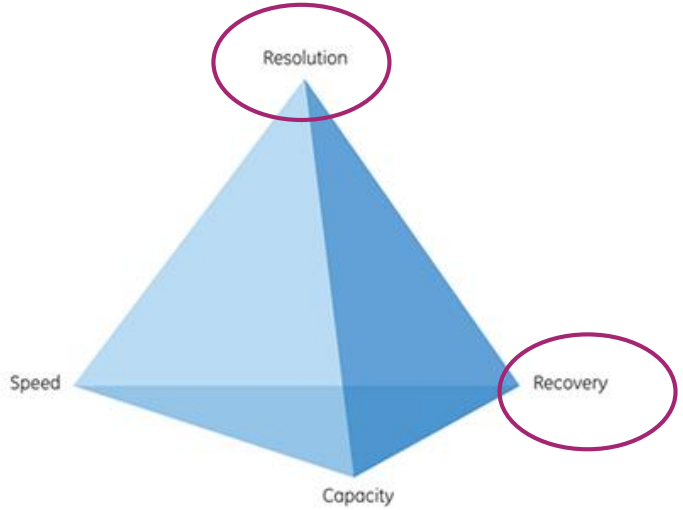


Chromatography

Capture Step

Affinity Chromatography (AC)

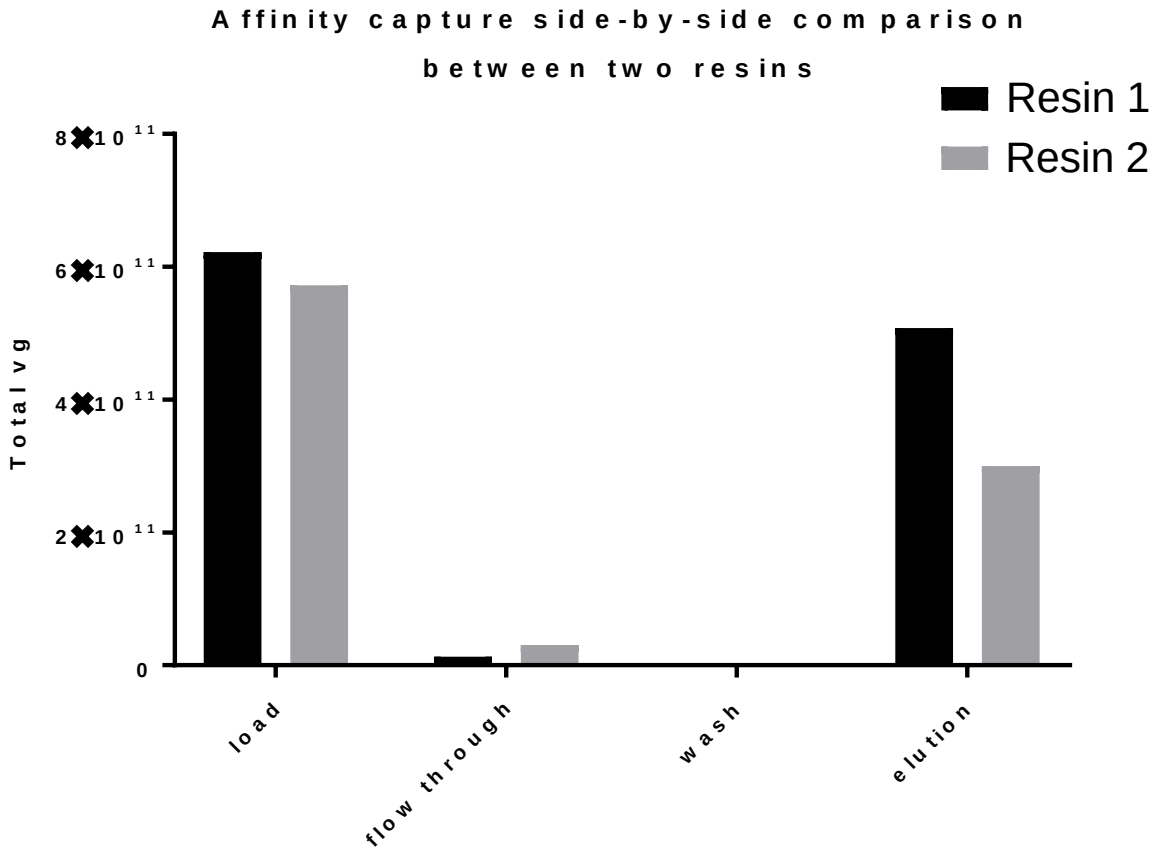
Key Performance Indicators for Polishing



Polishing Step

Anion Exchange Chromatography (AEX)

Viral vector manufacture: DSP challenges



Fraction of interest	Vg/ml (volume)	Total vg	Recovery
Capture runs with resin 1	9.24E+10 4.4 ml	4.06E+11	86.3%
Capture runs with resin 2	5.46E+10 4.4 ml	2.40E+11	51.0%

Recovery > 85%
Concentration factor: 13.6 X

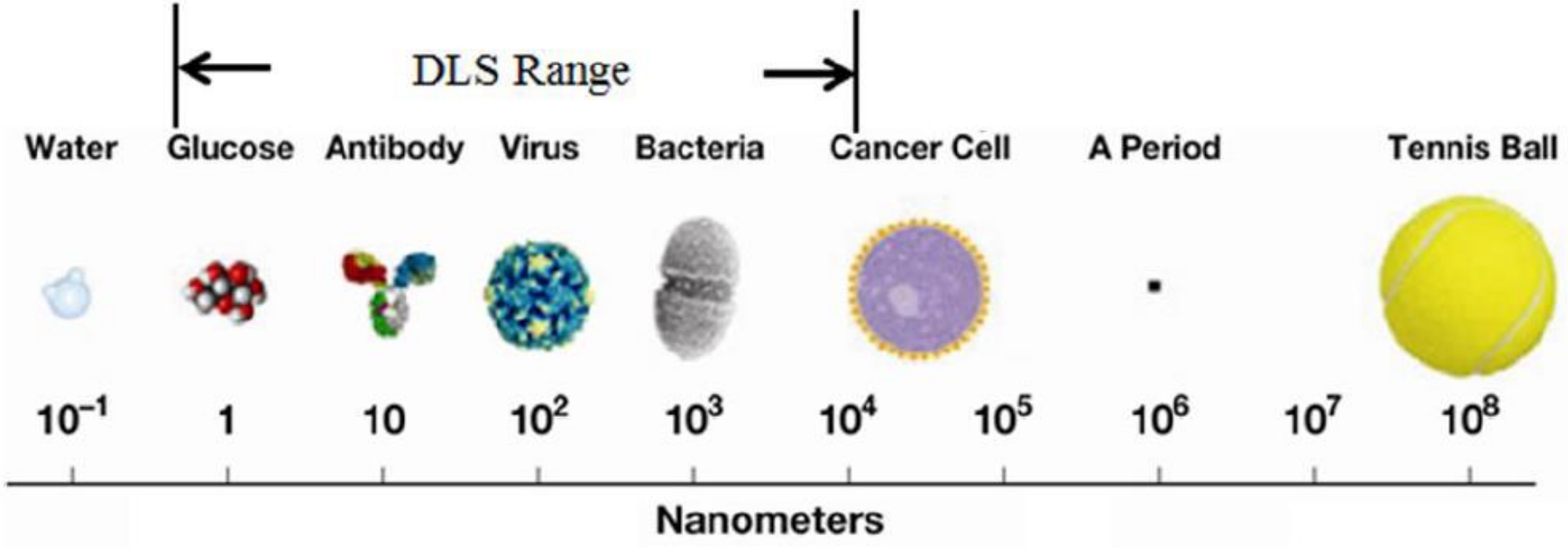
Scale-up challenges

- Processing times
- Large buffer volumes – storage, prep, supply
- Variability between serotype

Viral vector manufacture: Purity profile

	PCR	ELISA
Method	Bio-assay	Bio-assay
Time	4-5h	4-5h
Working volume	20-40 µL	80-100 µL
Potency output	+	+
Purity output	-	+
Complexity Time consuming	Lab experience	Lab experience
Calibration	Calibration	Calibration
Method detects	Vector genome copy	Empty & full particles

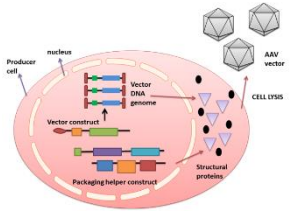
Viral vector manufacture: Purity profile



Viral vector manufacture: Purity profile

	PCR	ELISA	MA-DLS
Method	Bio-assay	Bio-assay	Physical method
Time	4-5h	4-5h	1-3 min (40min)
Working volume	20-40 µL	80-100 µL	3-35 µL
Potency output	+	+	+
Purity output	-	+	+++
Complexity Time consuming	Lab experience	Lab experience	Simple
Calibration	Calibration	Calibration	Calibration-free
Method detects	Vector genome copy	Empty & full particles	Physical particles 1nm-10µm

Viral vector manufacture: Purity profile



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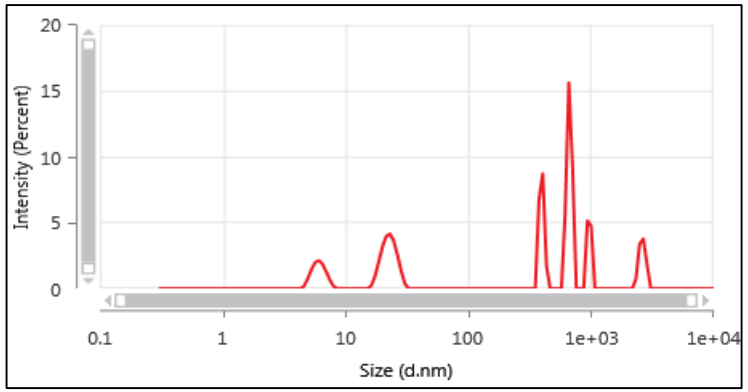
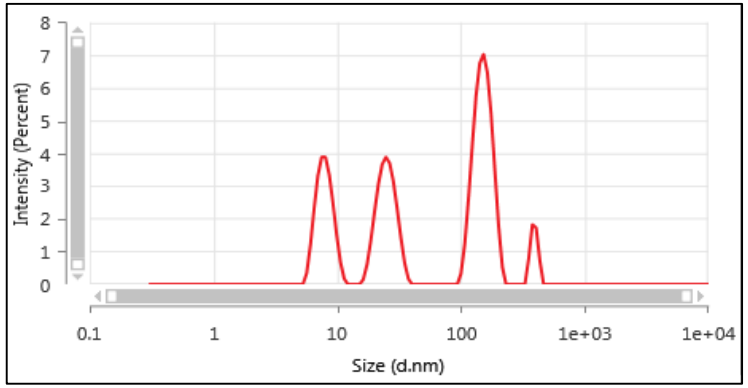
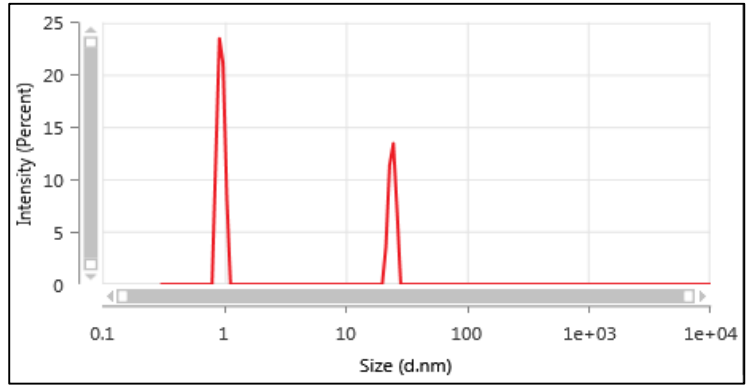
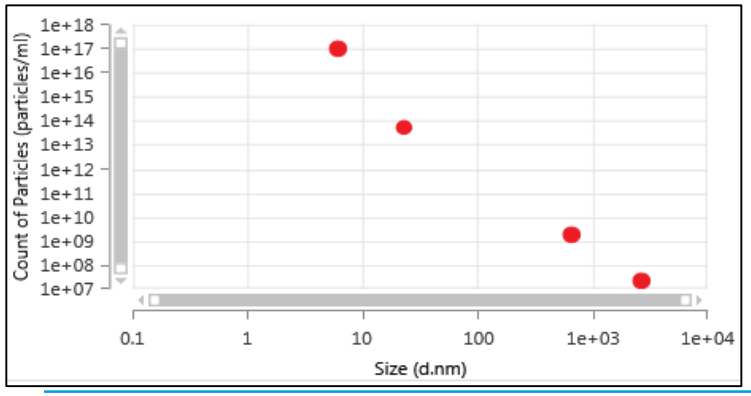
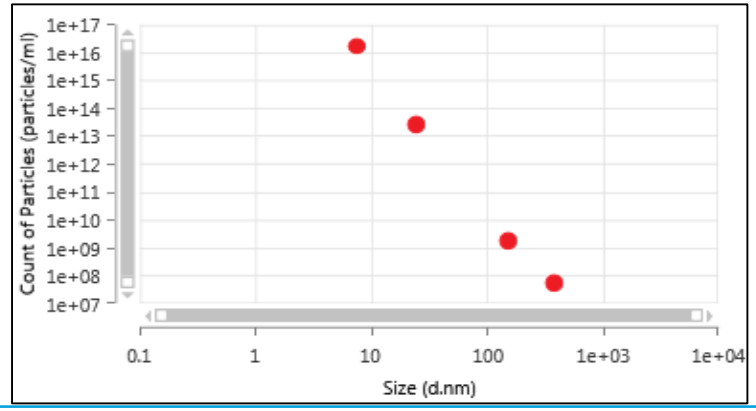
Final filtration

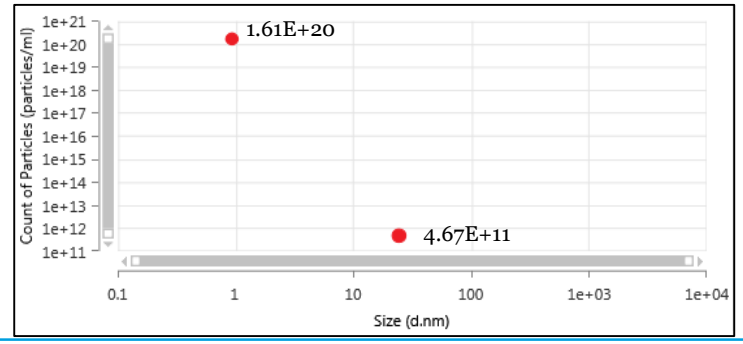












A move towards smart processing…

Viral vector manufacture – PAT for intensification

3rd Generation platform requirements

Disease	vg/patient	Estimated patient number (EU/US)	Potential uptake	Estimated Equivalent culture volume (L)
DMD	1.00E+15	1000000	50%	250,000,000
SMA1	6.00E+14	2000	50%	6,000,000
Haemophilia A	4.20E+15	25000	25%	28,000,000
Haemophilia B	7.00E+14	100000	25%	7,000,000
Wet AMD	1.00E+11	1000000	10%	10,000
Chloroidermia	6.00E+09	25000	50%	750

FDA - PAT—a framework for innovative pharmaceutical development, manufacturing and quality assurance. 2004

Viral vector manufacture – PAT for intensification

PAT is a framework for

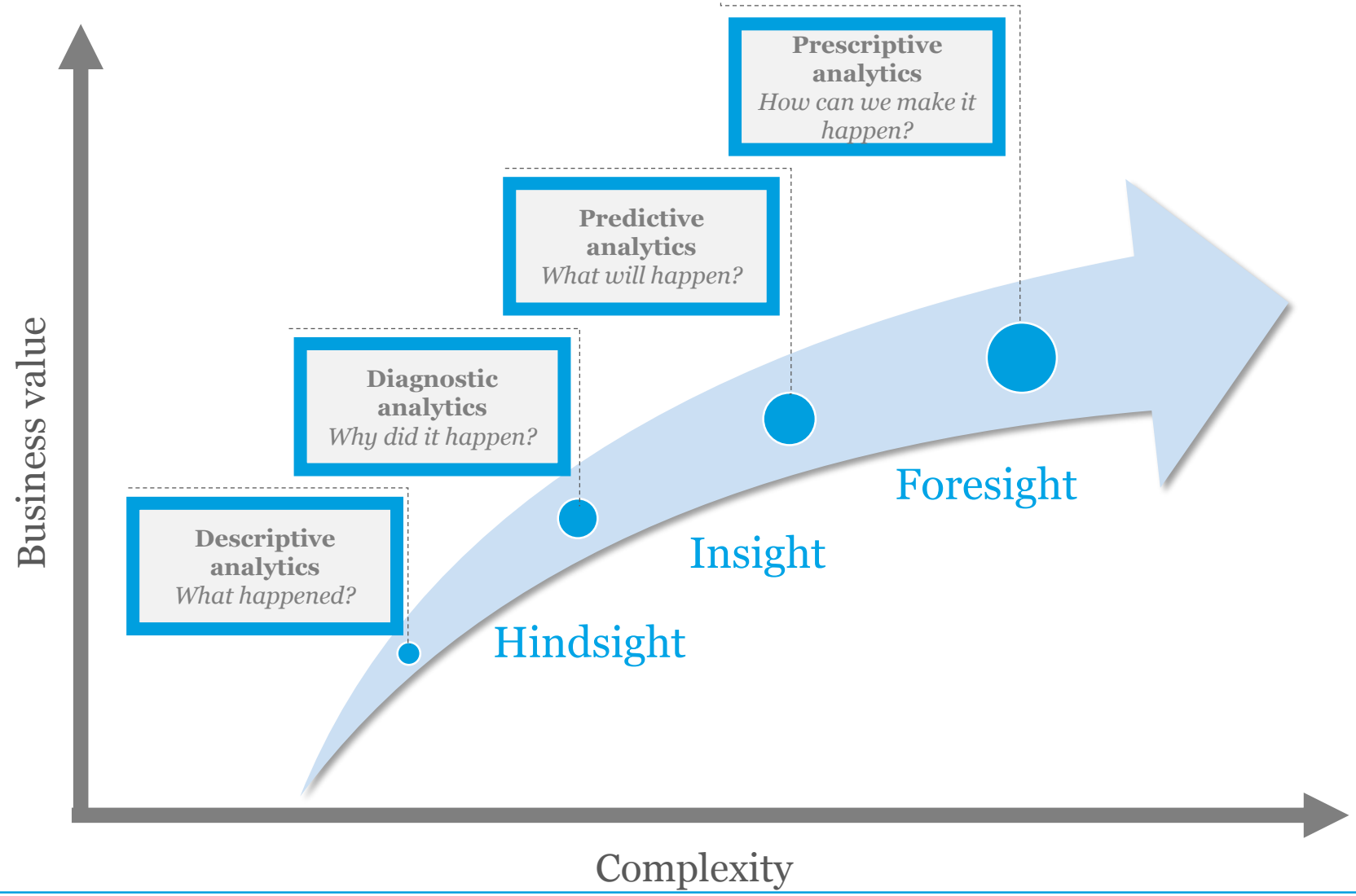
"*designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality*"

The aim of PAT is to obtain better process control by

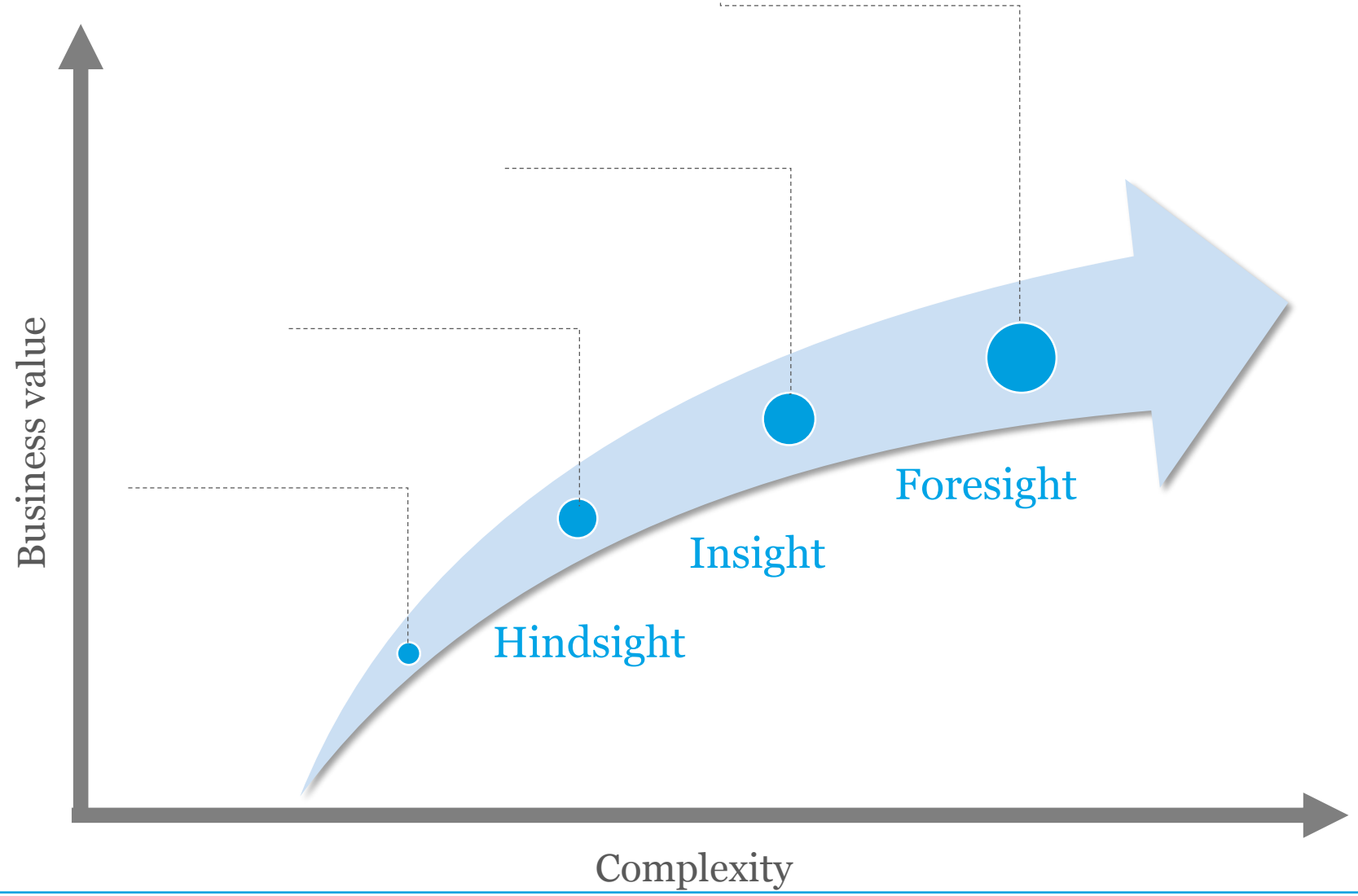
- identifying and managing sources of variability,
- reducing cost by optimising the use of raw materials
- minimising product cycle times through the use of measurements that are
  - in-line (analysed in place),
  - on-line (sample removed, analysed and returned to the process stream)
  - at-line (sample removed and analysed close to the process stream)

FDA - PAT—a framework for innovative pharmaceutical development, manufacturing and quality assurance. 2004

Viral vector manufacture – data and control



Viral vector manufacture – data and control



Descriptive analytics
What happened?

Diagnostic analytics
Why did it happen?

Predictive analytics
What will happen?

Prescriptive analytics
How can we make it happen?

Diagnostic analytics - Transcriptomics

Descriptive understanding → Mechanistic understanding → Functional understanding

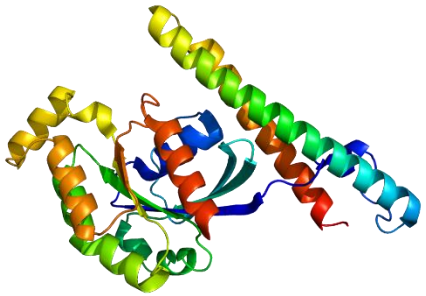
Omics - data rich technologies:

- differential expression and
- understanding cell behaviour during processing

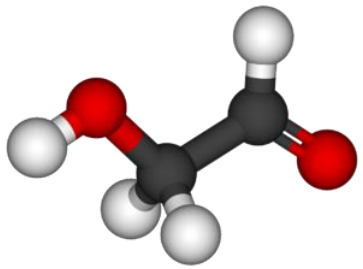
Transcriptomics

Genomics epigenomics

Proteomics



Metabolomics



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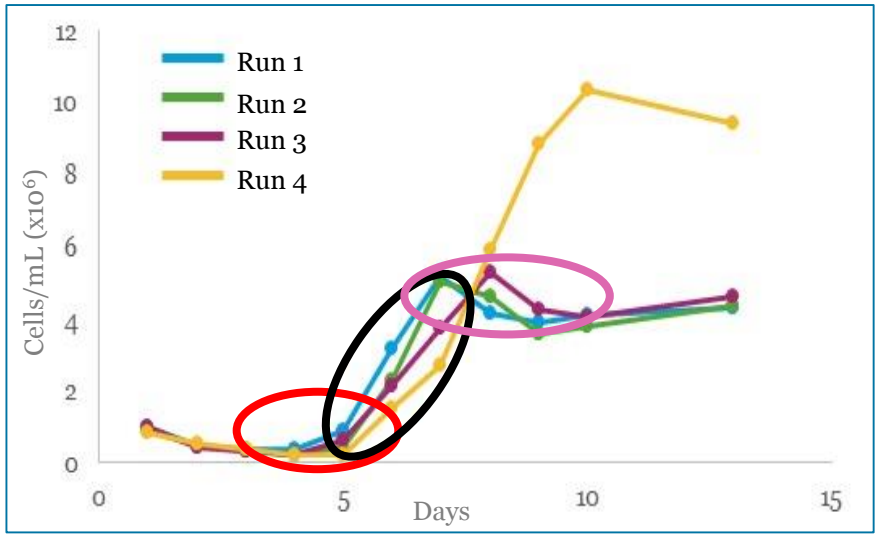
Diagnostic analytics - Metabolomics

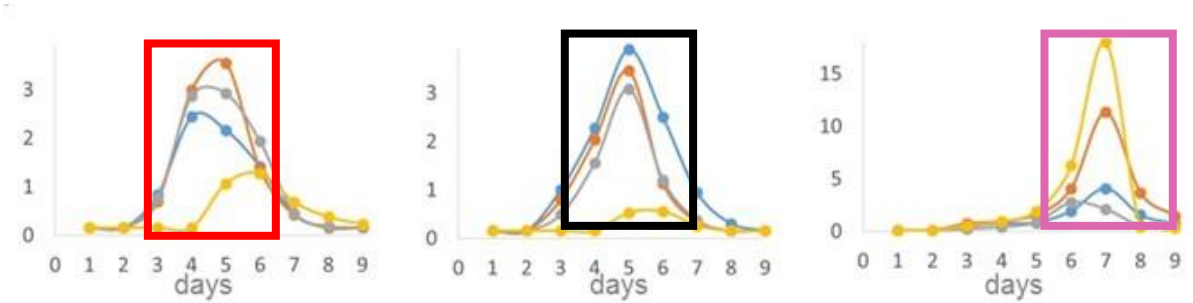
Metabolomics

The systematic identification and quantification of the small molecule metabolic products (the metabolome) produced by cells at a specific point in time.

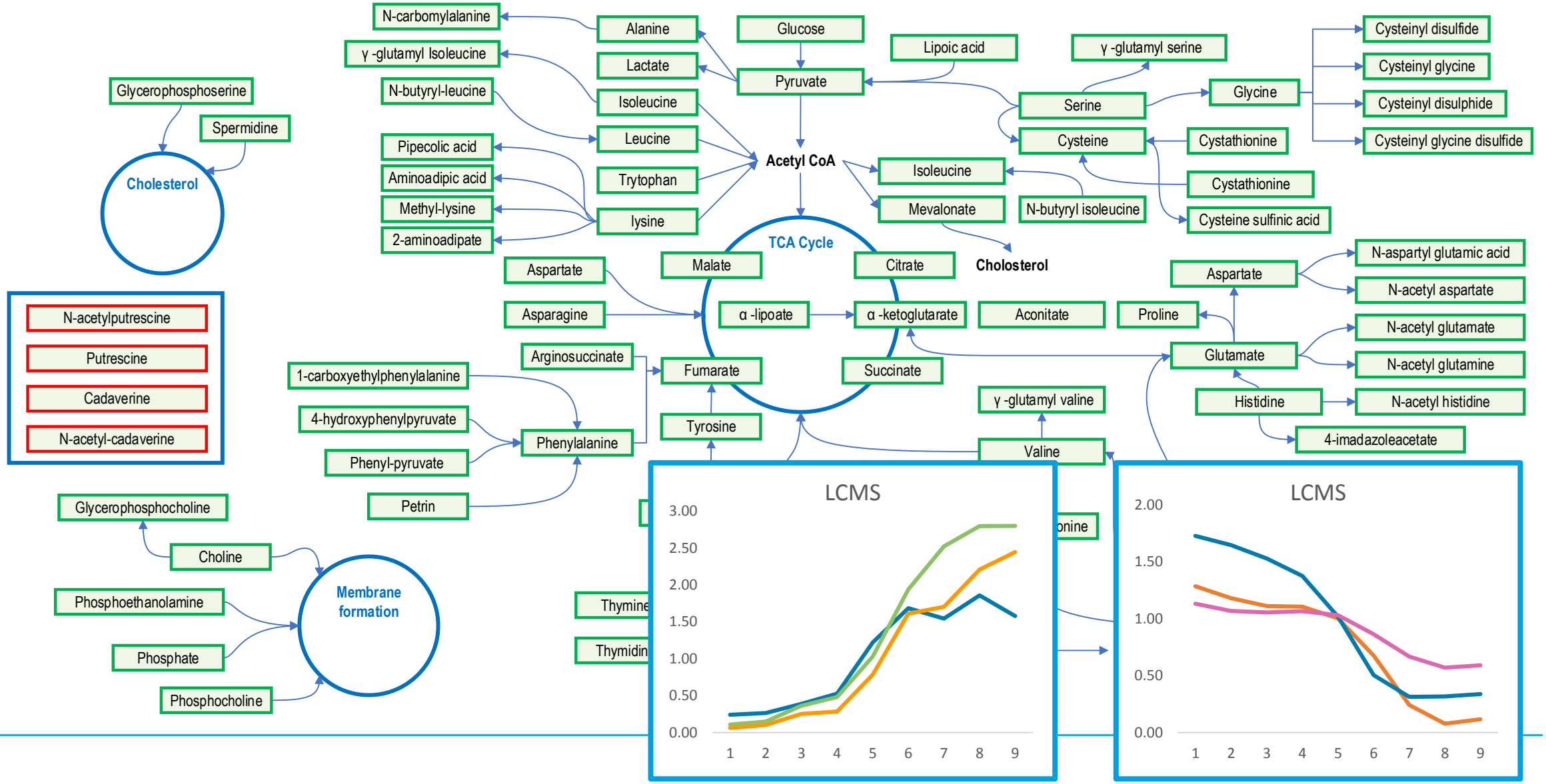
Since the metabolome is the end product of cellular processes it provides a functional fingerprint of cell quality and behaviour.

CGT Catapult has a fully automated LC-MS platform for metabolomic analysis



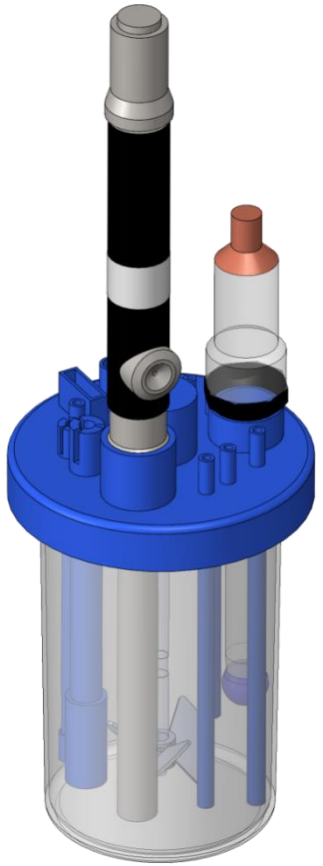


Cell metabolism



Predictive analytics – PAT technology suitability

	Technology	Measurement
In-line	NIR spectroscopy	Glucose/Glutamine/Lactate/Ammonia VCD/TCD/osmolality
	Raman spectroscopy	Glucose/Glutamine/Lactate/Ammonia VCD/TCD/osmolality
	Fluorescent sensors	pH and DO
	Refractive index	Compositional changes
	multiwavelength Fluorimetry	Amino acids
	Holographic imaging	Cell shape/size, cell viability
	Impedance	Biomass / call viability
	Turbidity	Biomass
On/At-line	HPLC	Media (amino acids, sugars, proteins, metabolites)
	LC-MS	Media (amino acids, sugars, proteins, metabolites)
	Cell counter	Biomass / call viability
	Imaging	Cell size/shape, cell viability
	Photometric analysers	Glucose/Glutamine/Lactate/Ammonia



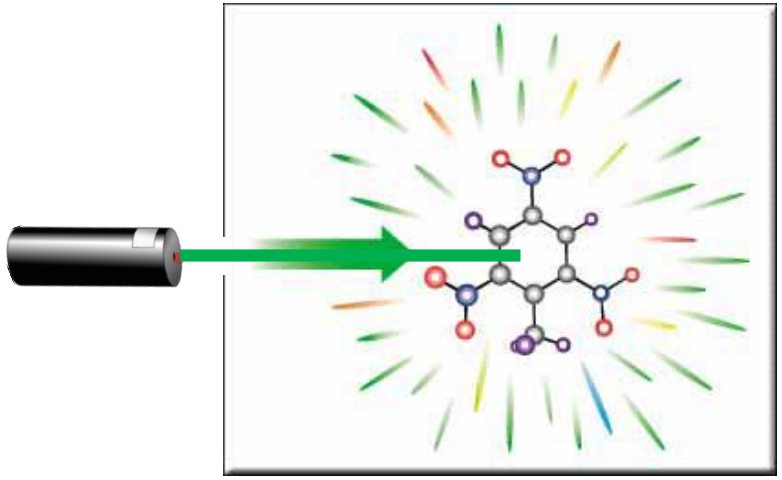
Predictive analytics – PAT Raman spectroscopy

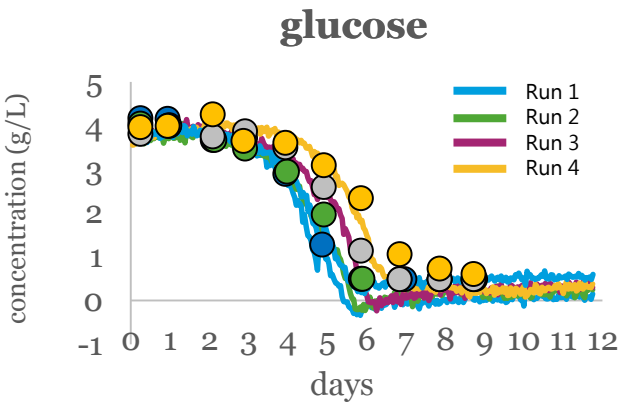
CATAPULT Cell and Gene Therapy

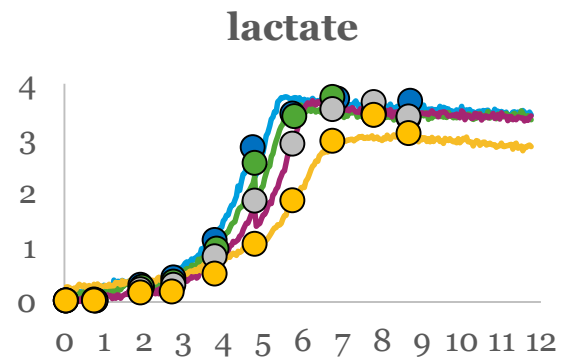
Raman spectroscopy

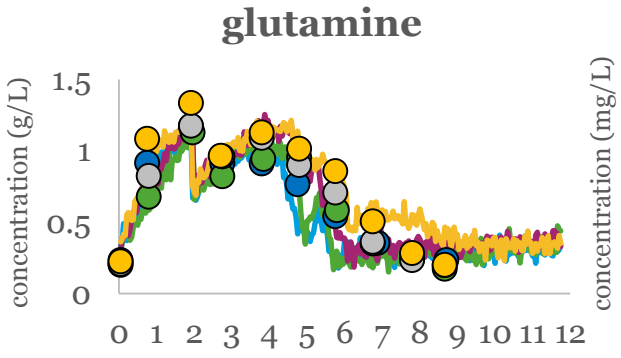
Raman Spectroscopy is a technique used to observe molecular vibrations that can identify and quantitate molecules

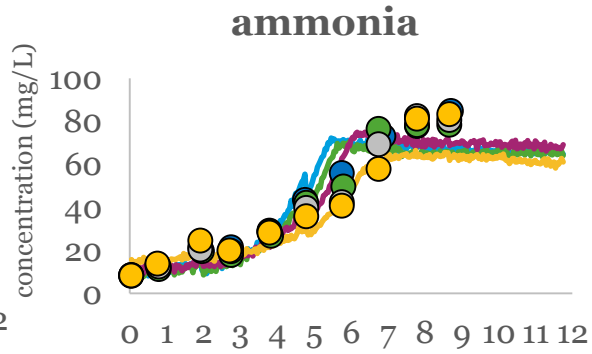
By measuring changes in the wavelength of laser light its possible to identify what molecules are present in the cell culture media



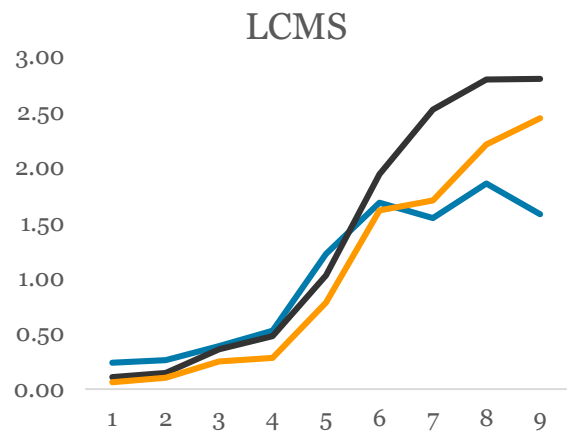


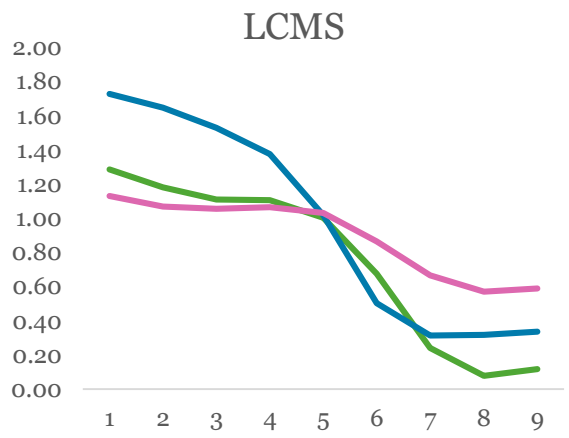


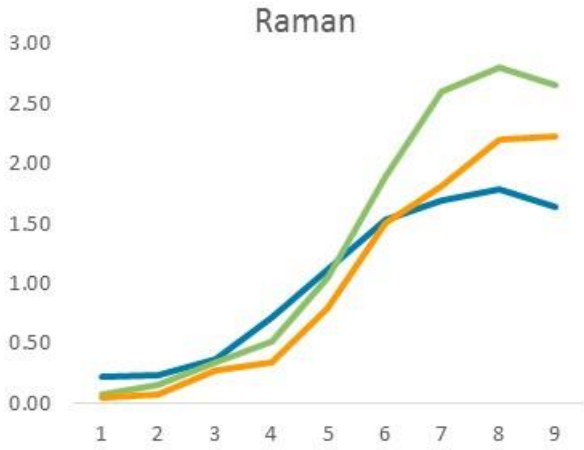


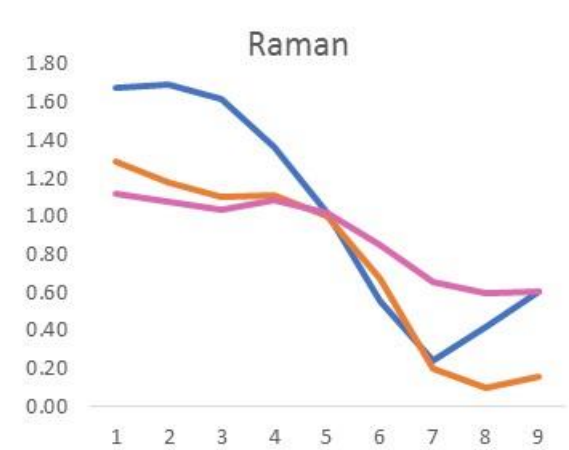


Predictive analytics – PAT Raman spectroscopy

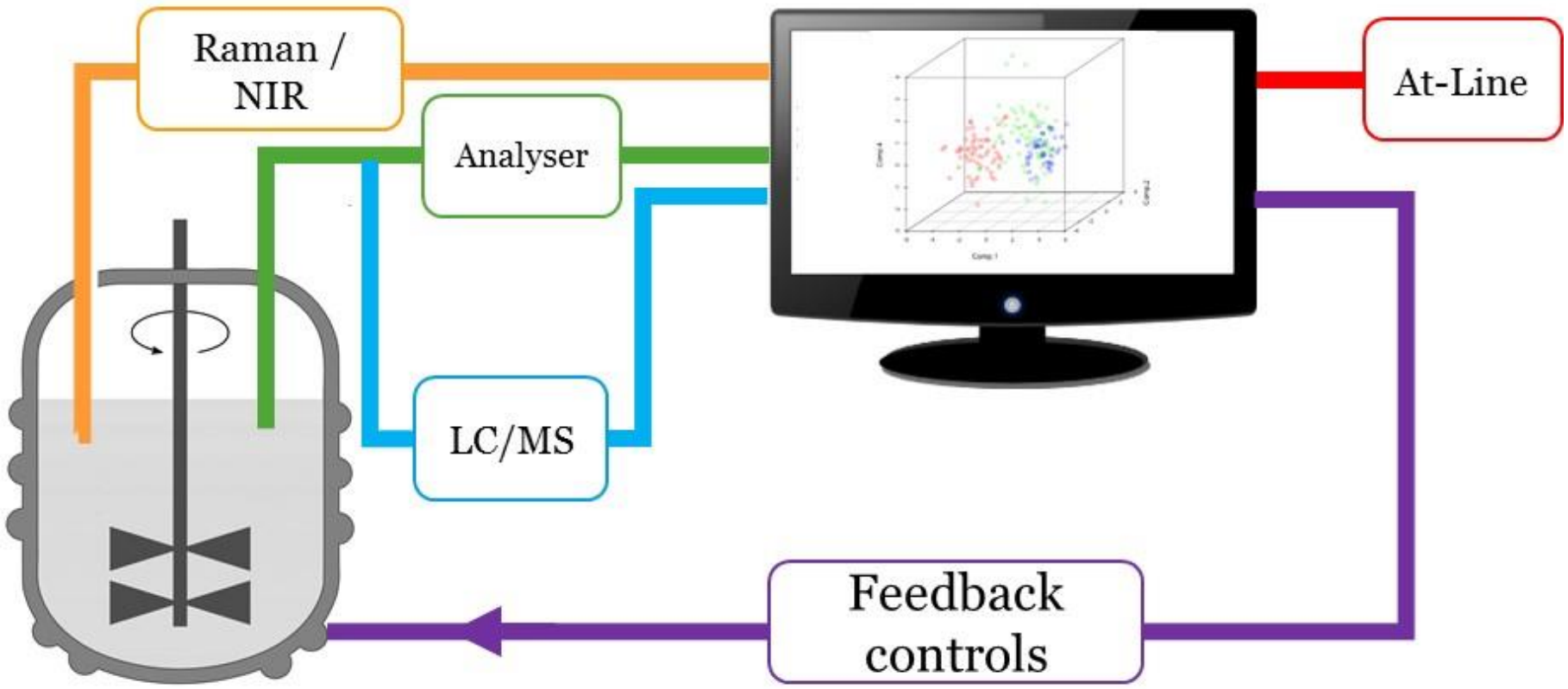








Future manufacturing control strategies



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Continuous processing – 4th generation…?

In summary…

Industry drivers	Technical solutions
1. Next generation analytics for viral characterisation	1. Method for absolute quantification of viral titre, Assays for rapid measurement of functional titre
2. Improving product yield - High titre, high quality viral production	2. Identification of CPPs driven by high resolution characterisation of CQAs
3. CoGs optimisation – more efficient and robust processes	3. Use of QbD and PAT to improve process potential
4. CMC – understanding the regulatory pathway	4. Definition of characterisation required to support scale-up and continuous approach
5. Capacity for production and supply	5. Translation of developed approaches into manufacturing environment

Industrialisation team

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We work with Innovate UK