

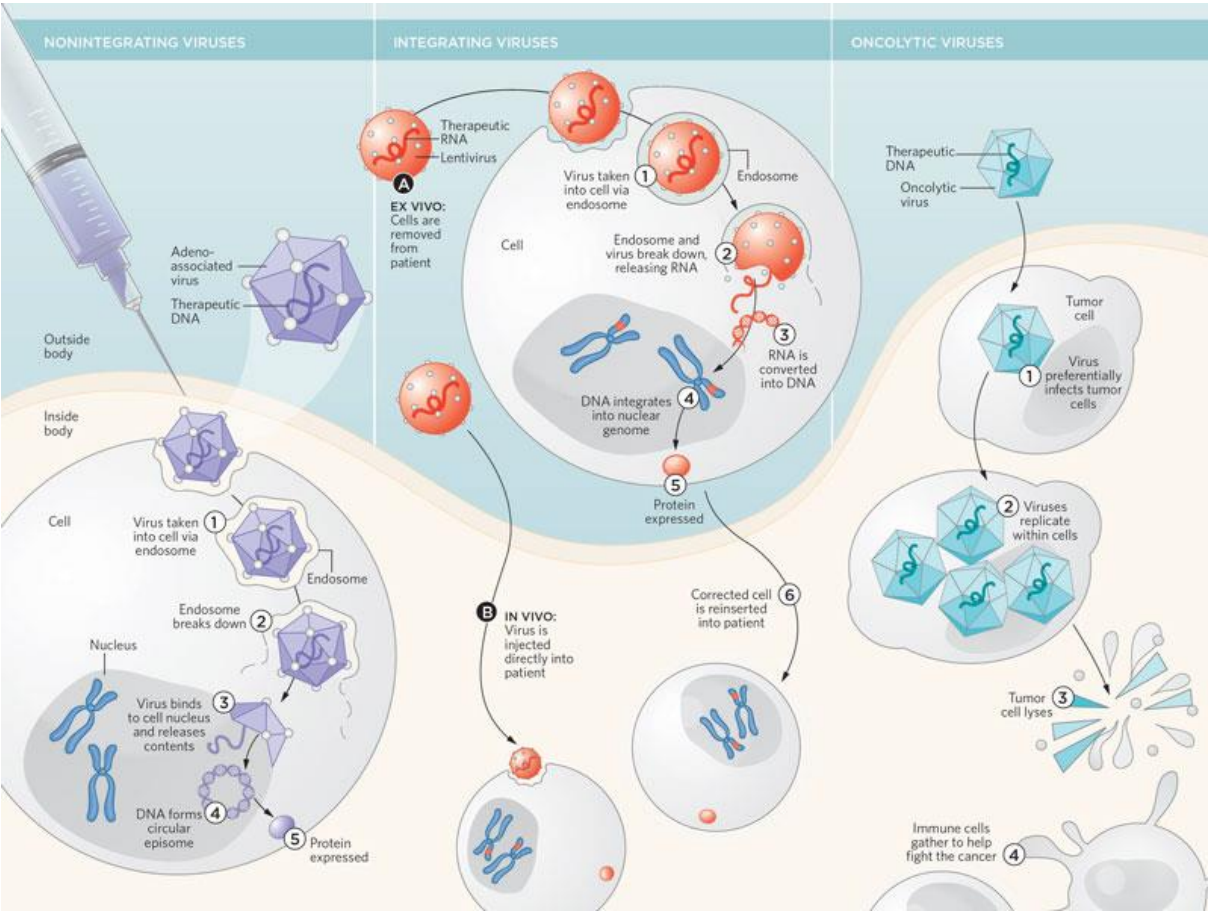
AAV next generation quality control

Tony Bou Kheir

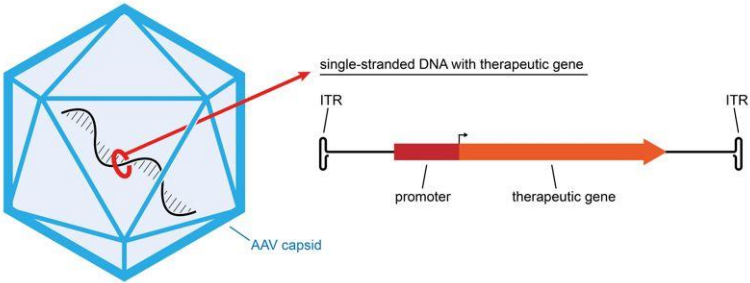
Cell and Gene Therapy Manufacturing Workshop

March 12th 2019

AAV mediated gene therapy



The scientist, Lucy Reading-Ikkanda

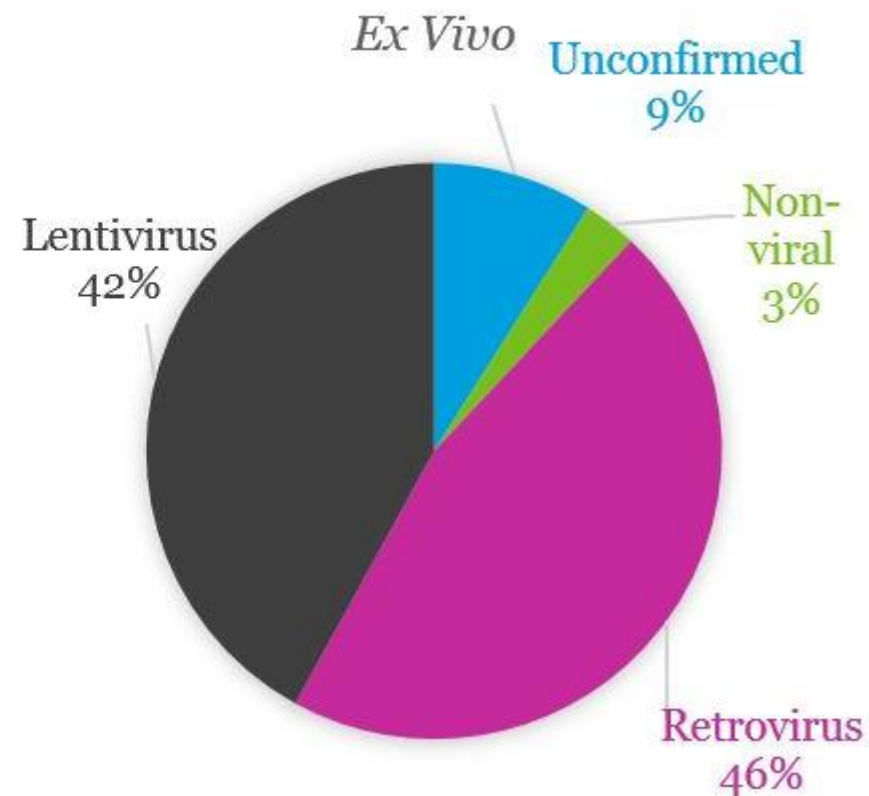
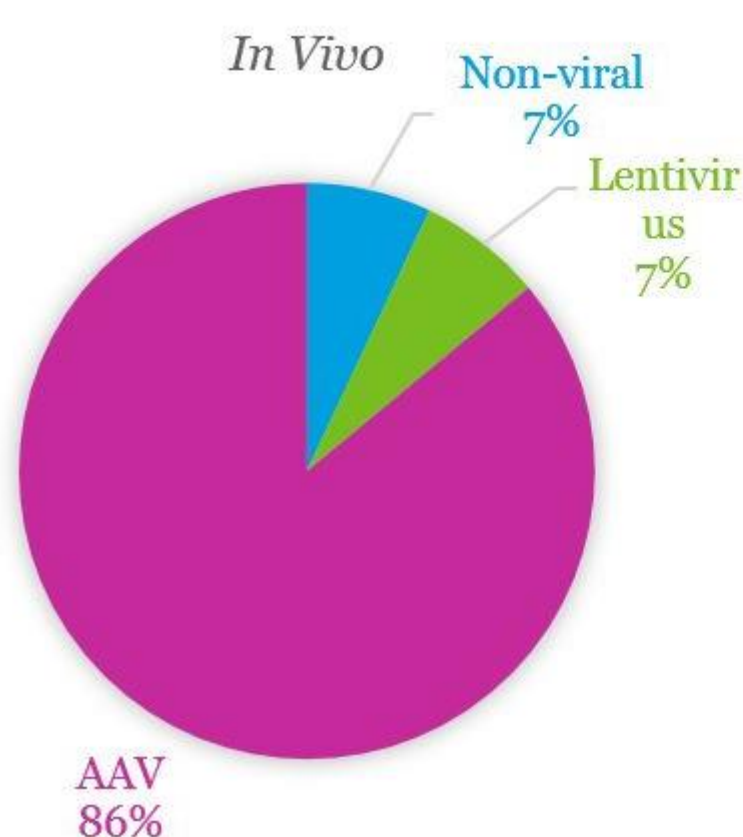


Primary Target Tissues									
Serotype	Retina	Neurons	Brain	Lung	Heart	Liver	Muscle	kidney	Pancreas
AAV-1		✓			✓		✓		✓
AAV-2	✓	✓	✓			✓	✓	✓	
AAV-3	✓			✓		✓	✓		
AAV-4	✓	✓	✓				✓		
AAV-5	✓	✓		✓					
AAV-6				✓	✓	✓	✓		
AAV-7	✓	✓				✓	✓		✓
AAV-8	✓		✓			✓	✓		
AAV-9			✓	✓	✓	✓	✓	✓	✓
AAV-10		✓		✓	✓	✓	✓		
AAV-DJ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

Setback for gene therapy for safety reasons in the 90s

Discovery of novel, safer and more efficient AAV vectors

- Exponential growth
- Funding influx
- High profile deals
- > 100 clinical trial
- 2 launched products
 - Glybera, 2012
 - Luxturna, 2017



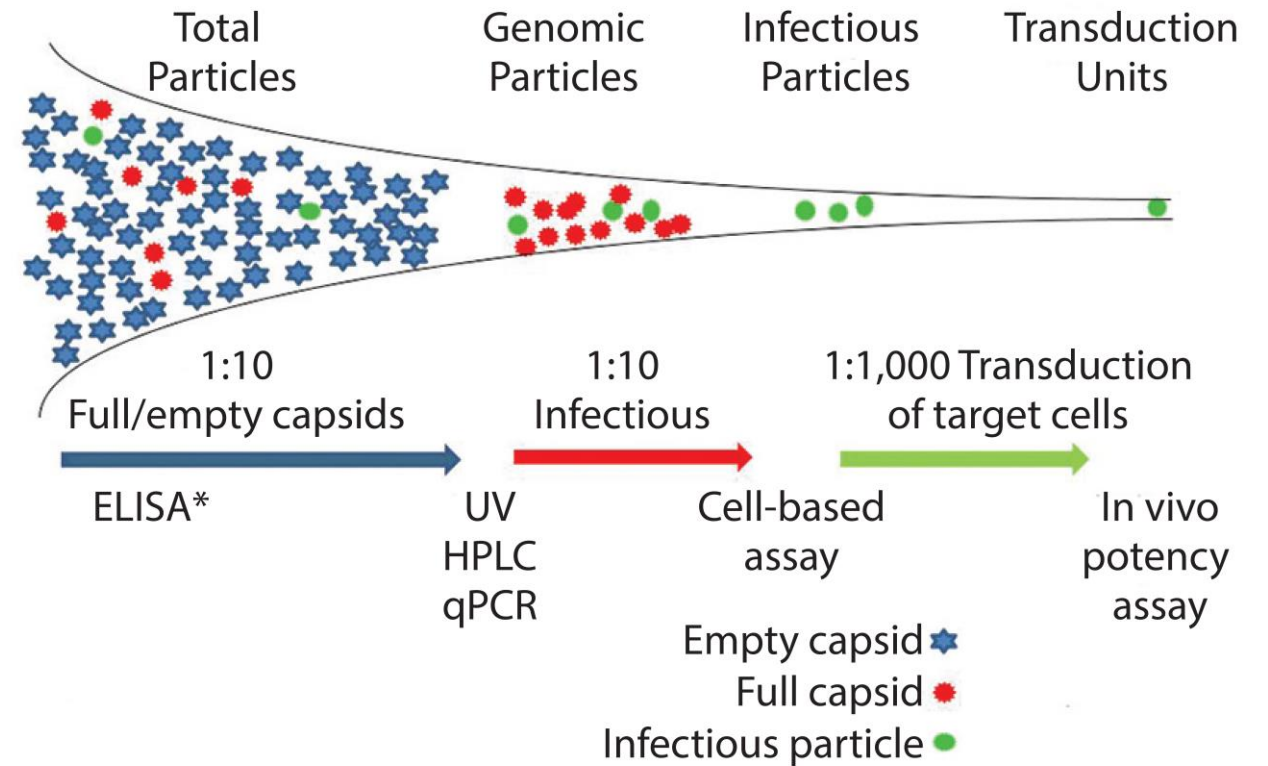
Challenges of AAV gene therapies

Vector Design

IP, capsid, promoter

Manufacture

Scaleup



1:100,000 particles delivered will achieve the desired clinical output

Challenges of AAV gene therapies

Vector Design

IP, capsid, promoter

Manufacture

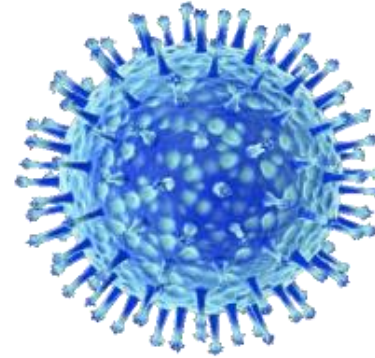
Scaleup

Standardization

International standards

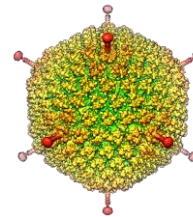
QC (IPC, Product/batch release, process validation)

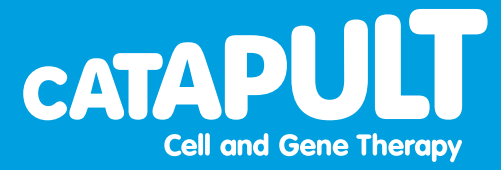
Regulatory



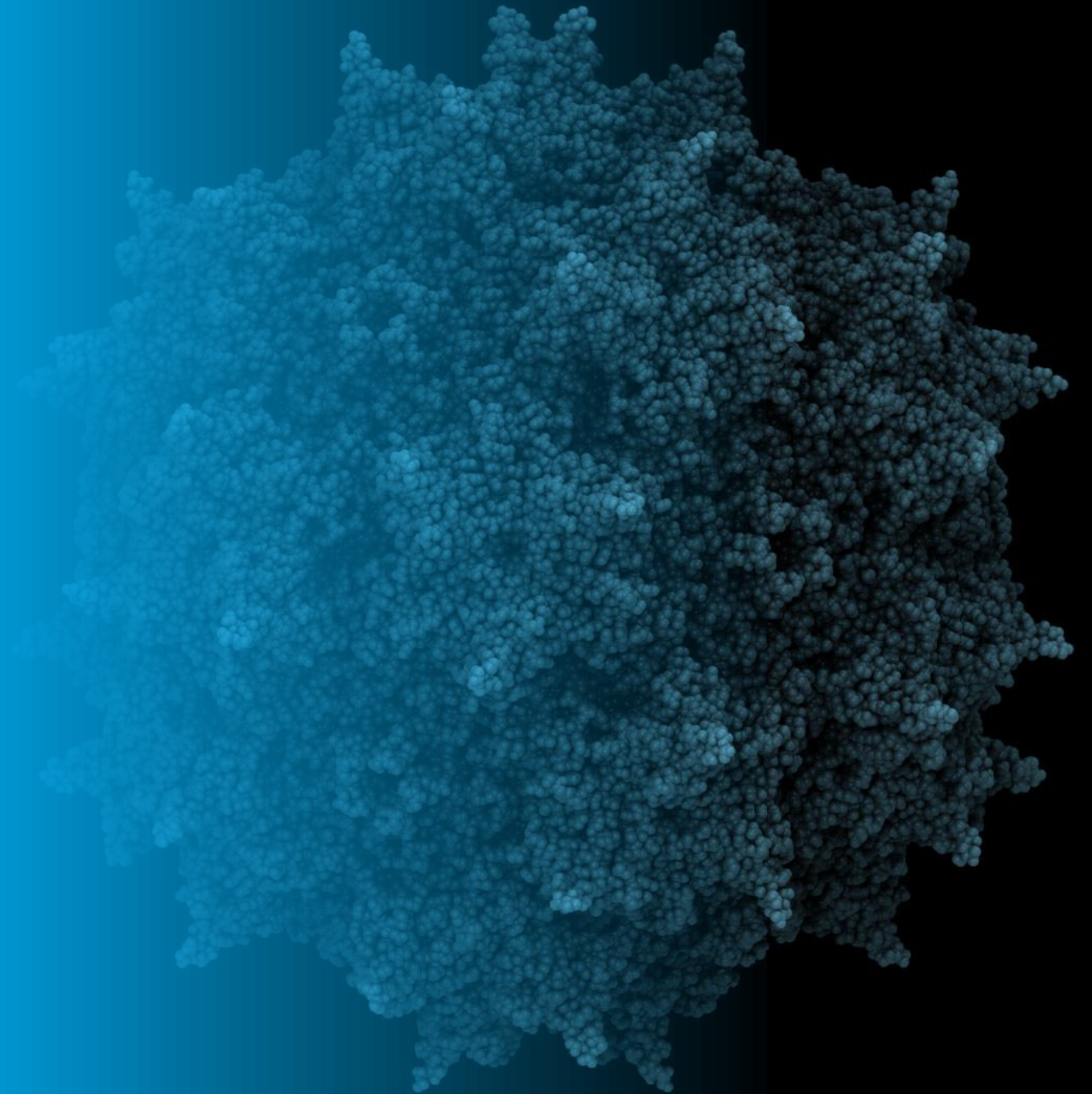
Physical size

120nm Lentivirus

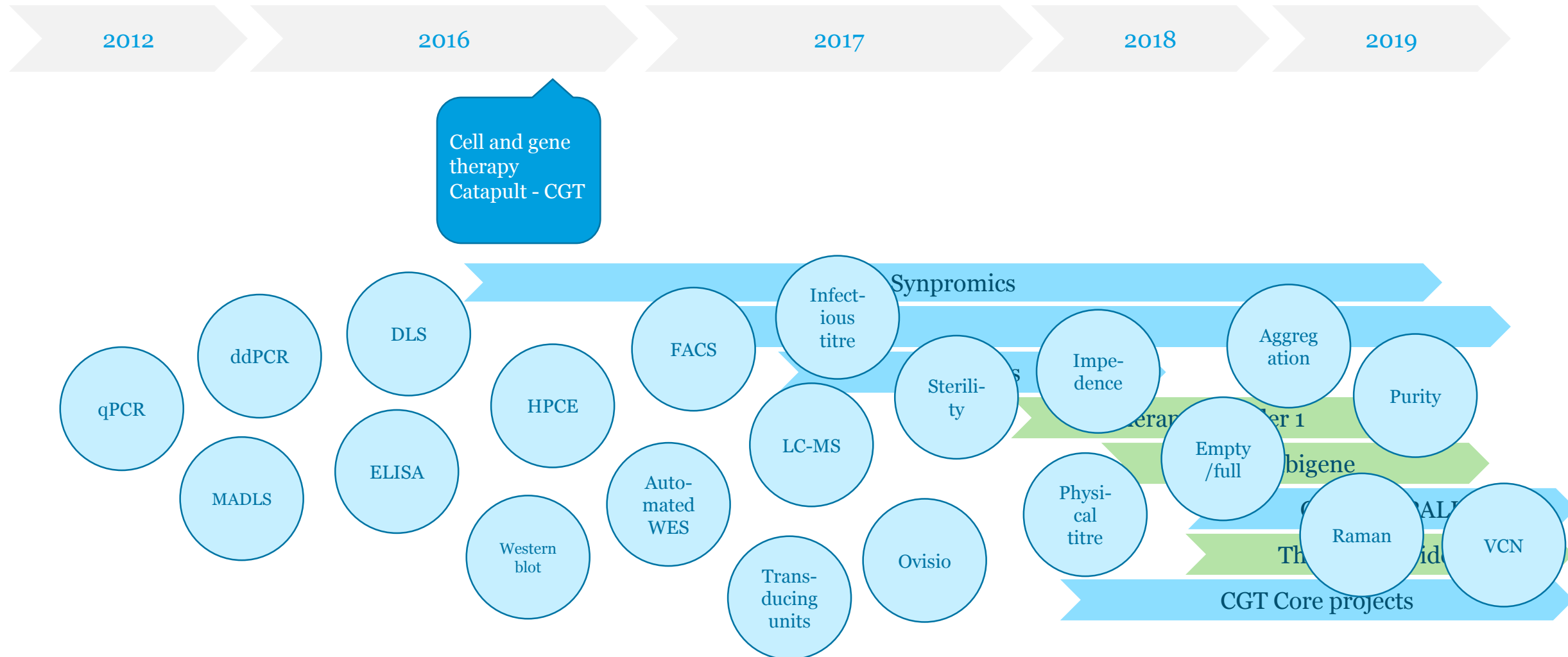


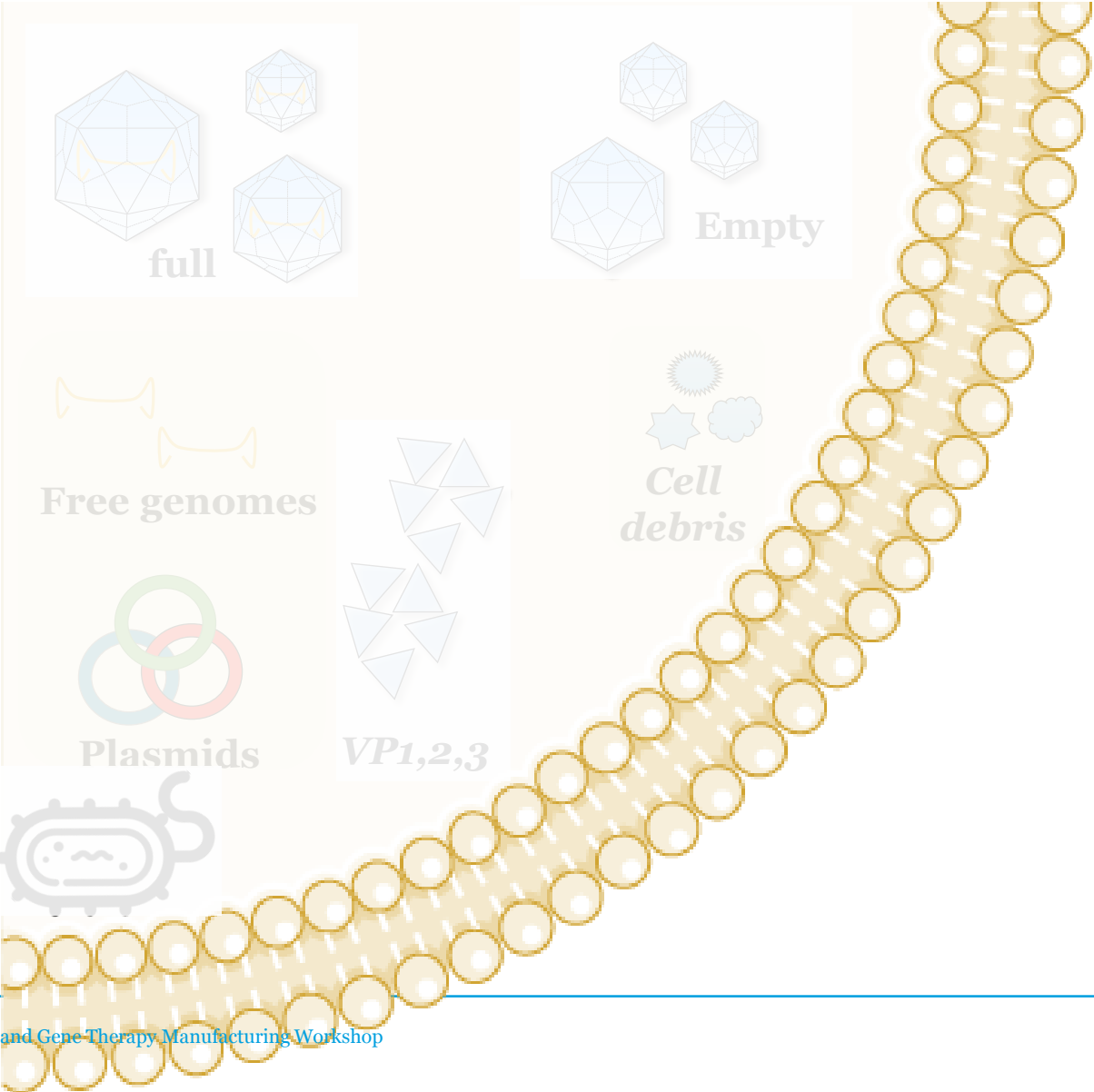


AAV mediated next
generation quality control at
CGT



AAV projects at CGT





	1 st Gen	2 nd Gen
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	<i>In-vitro</i>	FACS/Impedance
Total protein	Coomassie	LC-MS
Sterility	Growth based	ddPCR
Purity		ddPCR / Seq

Traditional methods

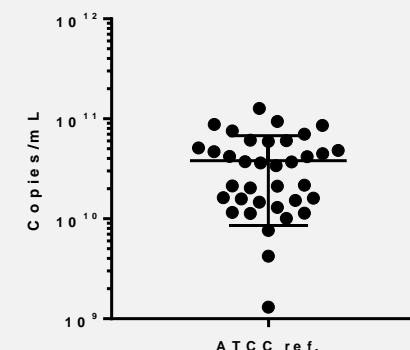
Quantitative real-time PCR (qPCR)

- Primer and probes targeting gene of interest and/or ITRs
- Use of a digested plasmid as a standard curve

Limitations

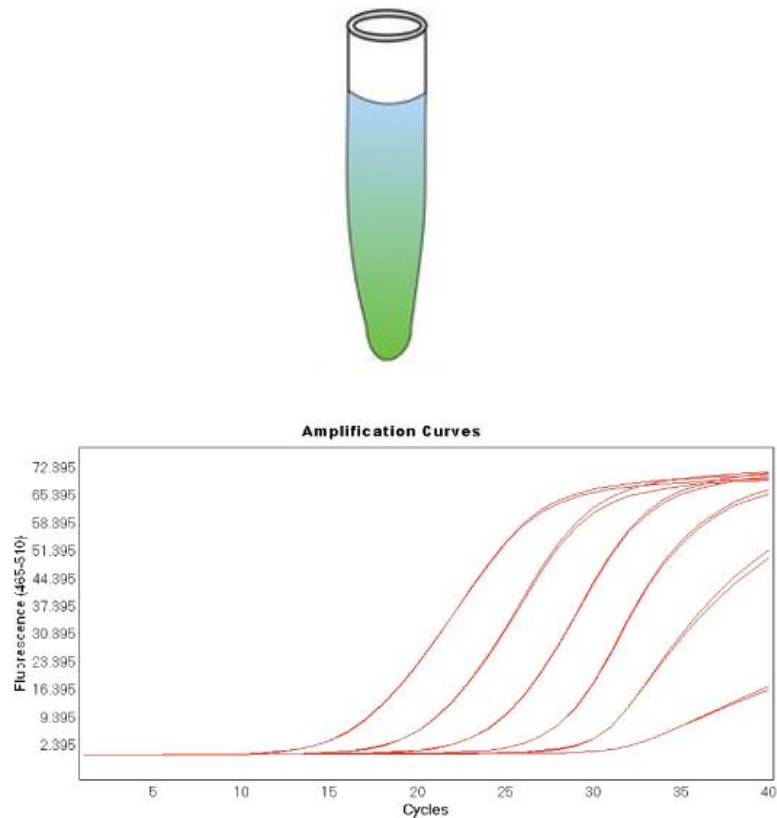
1. Highly sensitive to PCR inhibitors – viral proteins and/or vector diluent → decrease in amplification efficiency → Under-estimation of viral titre
2. Bias from amplification efficiency – especially if targeting ITR region → under-estimation of viral titre
3. Bias introduced from the standard curve – amplification of dsDNA vs ssDNA → Over-estimation of viral titre
4. Steps above → High inter/intra-assay variability

Develop a robust, accurate method for measuring AAV2 vector genomes (and AAV2 derived serotypes)

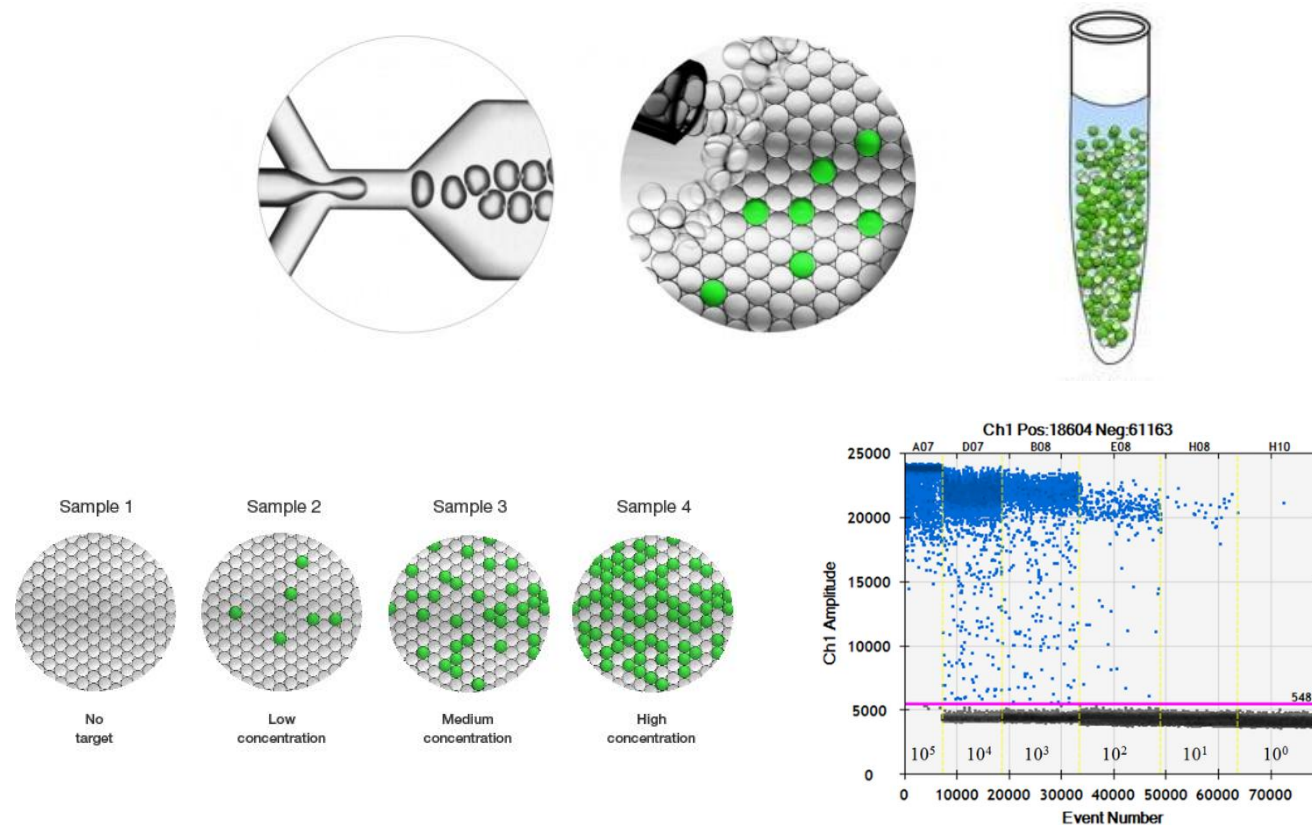


qPCR alternatives - ddPCR

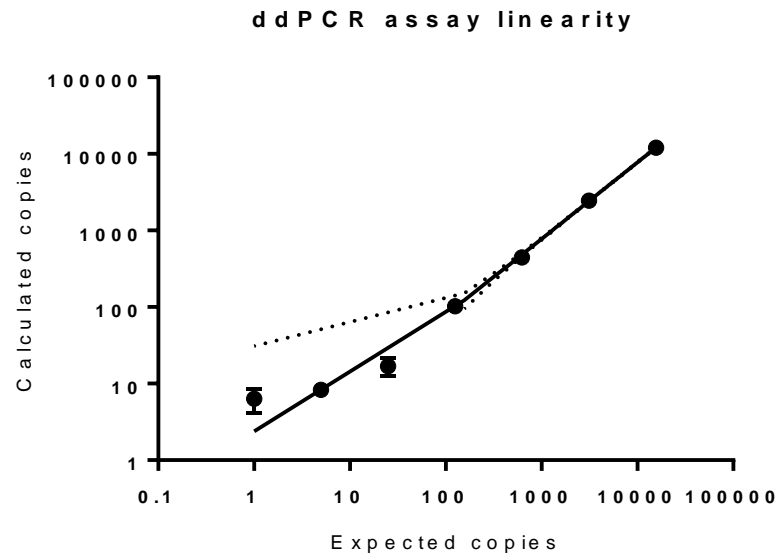
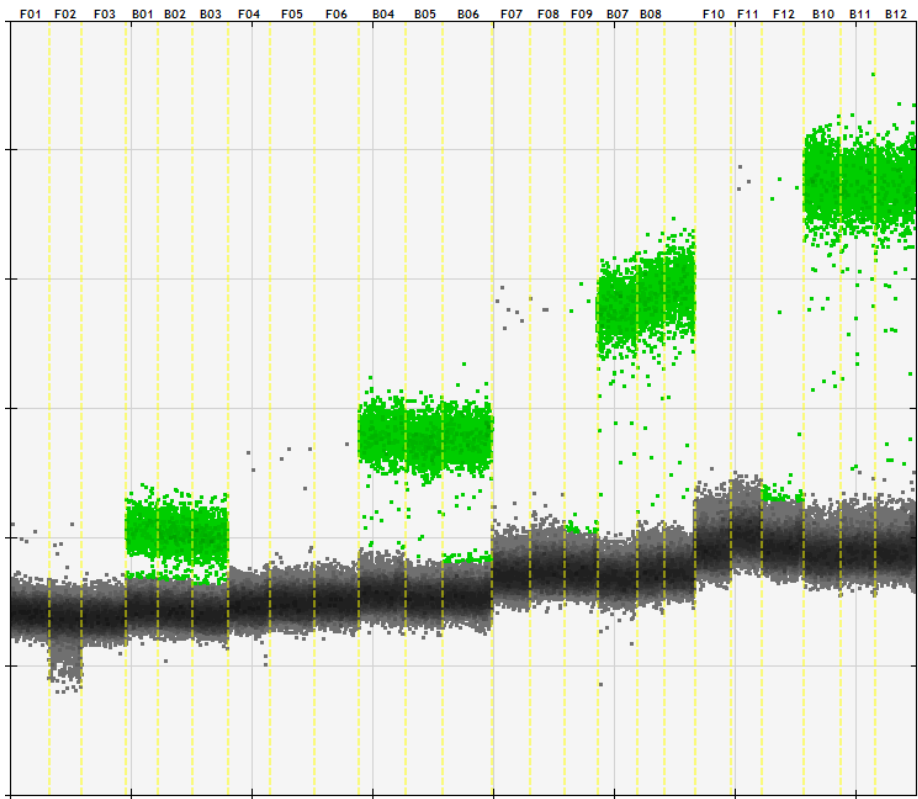
qPCR



ddPCR

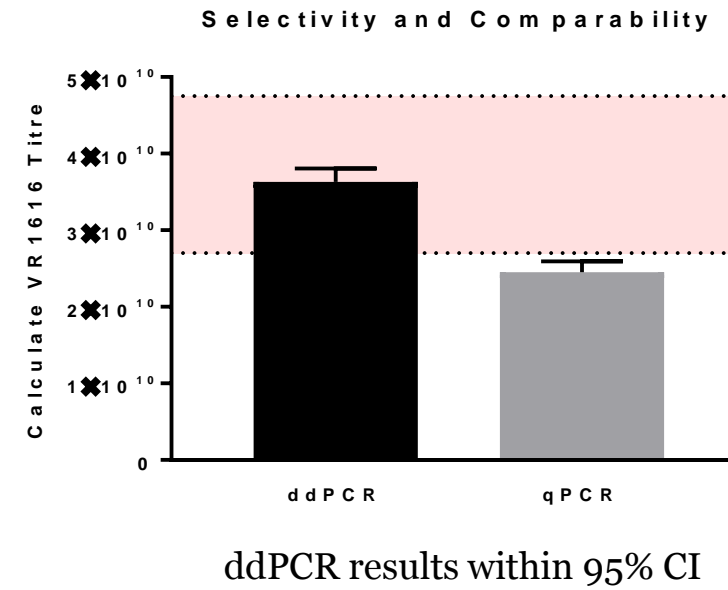
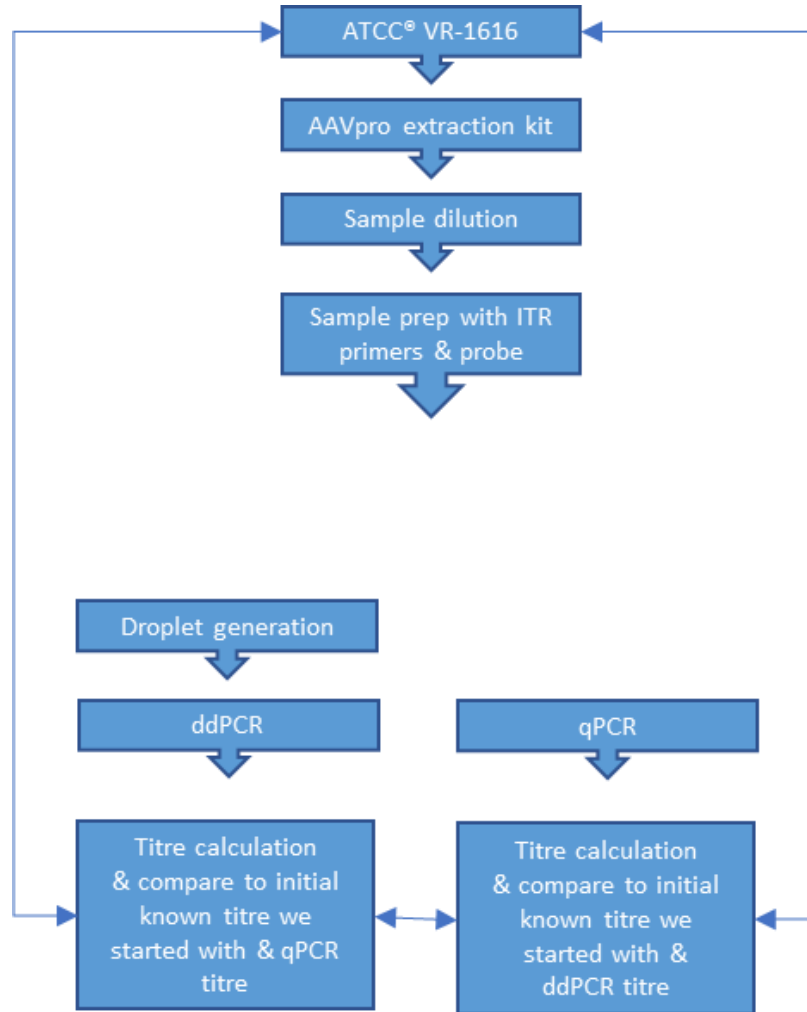


1. Highly sensitive to PCR inhibitors – viral proteins and/or vector diluent → decrease in amplification efficiency → Under-estimation of viral titre
 - ✓ Less sensitive to PCR inhibitors → suitable for in process vector genome measurement
2. Bias from amplification efficiency – especially if targeting ITR region → under-estimation of viral titre
 - ✓ End product measurement – less dependent on amplification efficiency → Suitable for targeting ITRs – Universal assay
3. Bias introduced from the standard curve – amplification of dsDNA vs ssDNA → Over-estimation of viral titre
 - ✓ Absolute quantification – no standard curve required → Improved precision
4. Steps above → High inter/intra-assay variability
 - ✓ Robust and accurate method for in-process control and product characterisation



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

ddPCR and Commercial qPCR method comparability



Fully functional assay offering

1. ITR sequence detection → Applicable to any AAV2 and AAV2 derived serotypes
2. Novel designed primers and extraction method
3. Higher sensitivity → Suitable for in-process sample measurement
4. Increased precision and reproducibility over commercial and current available qPCR titration methods

ELISA

Evaluation of 5 commercial kits

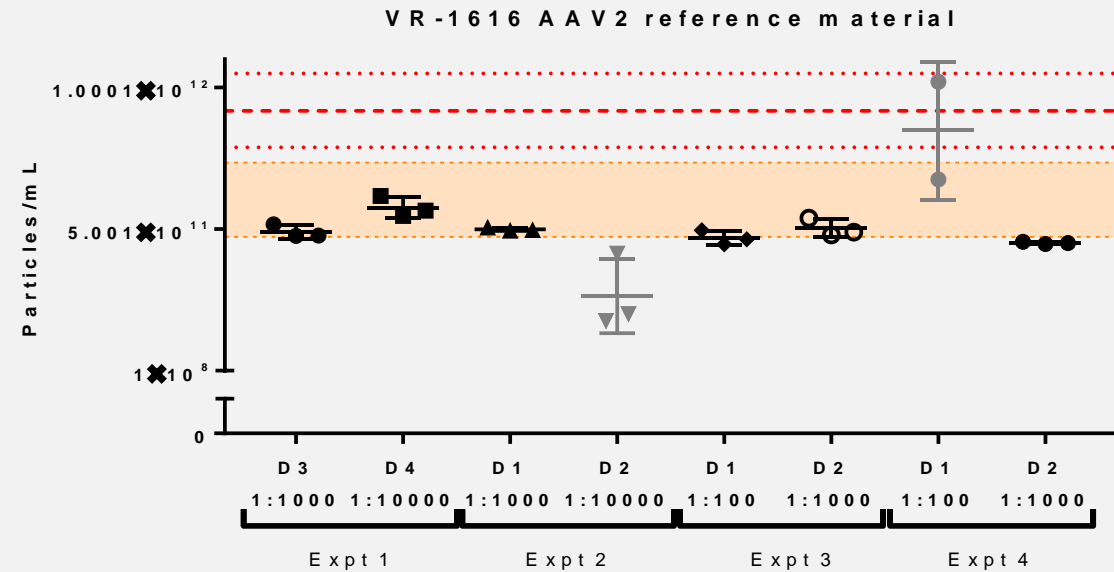
Inter assay CV <8%

Intra assay CV <5%

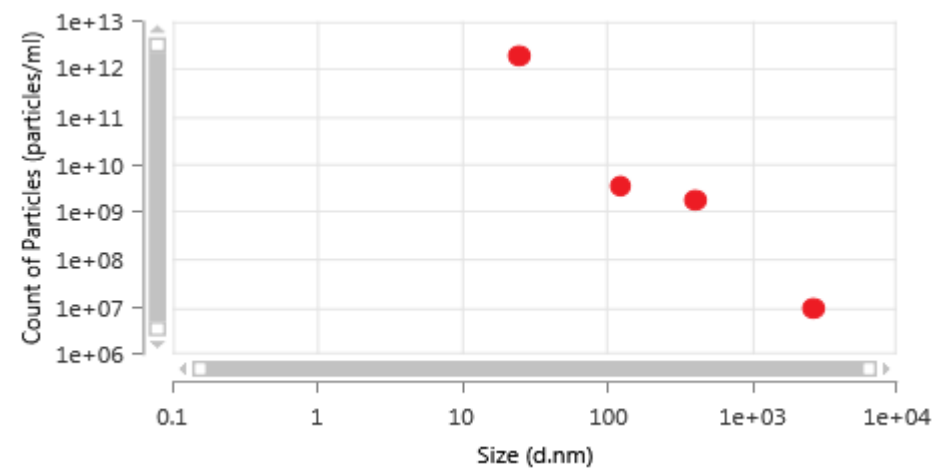
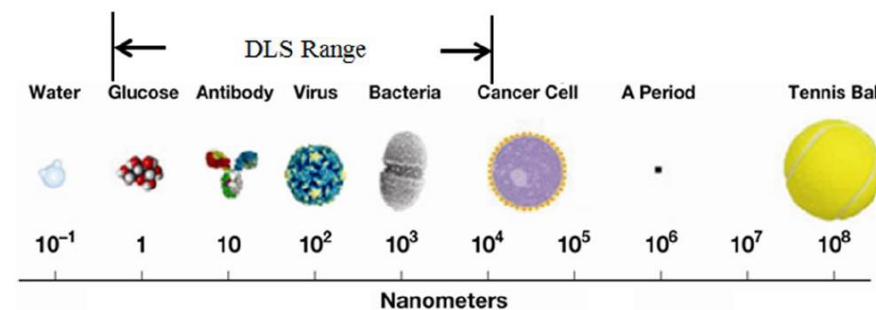
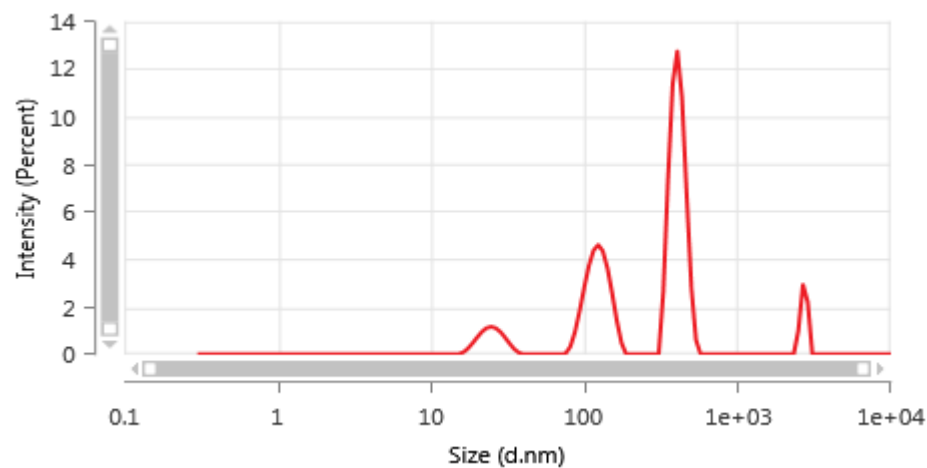
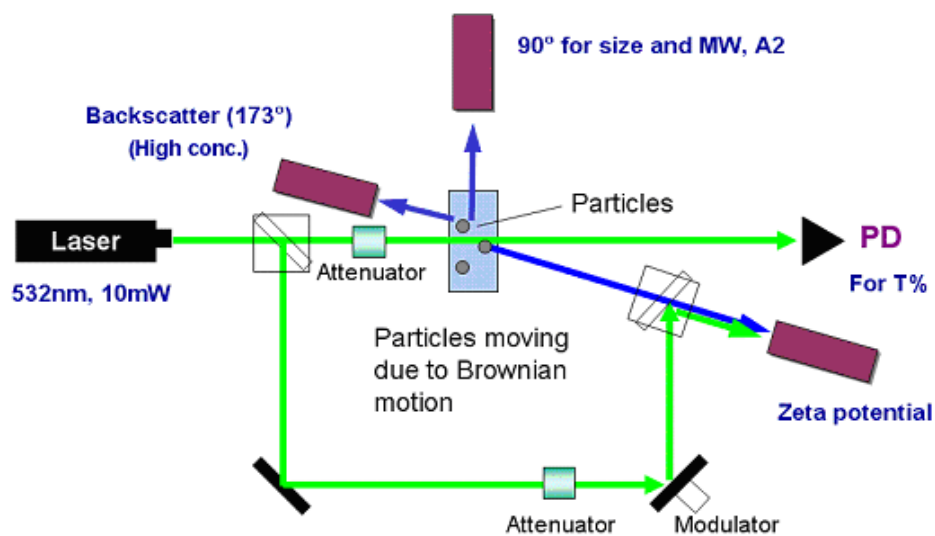
	qPCR	ddPCR
% full particles	13.69%	6.72%

Limitations

1. Expensive
2. Labour intensive
3. Time consuming
4. Antibody specific/serotype dependent



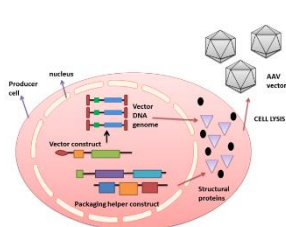
Dynamic Light Scattering technology



Case study



Overview of downstream developmental scope



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



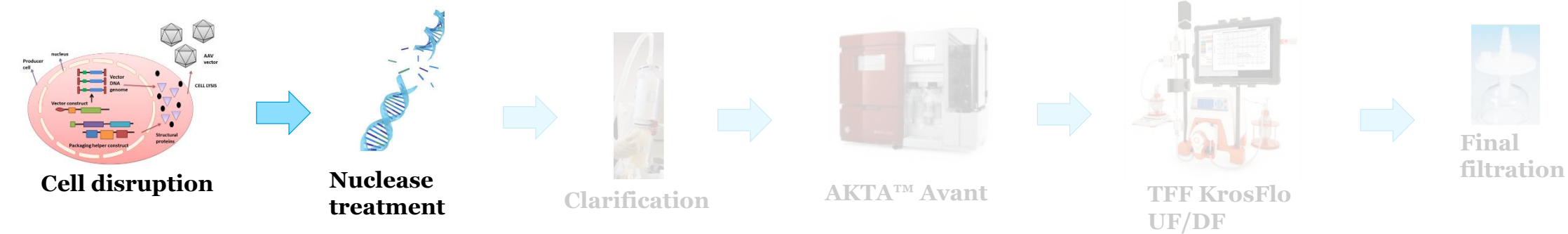
**Final
filtration**
Final
filtration
development

Physical lysis

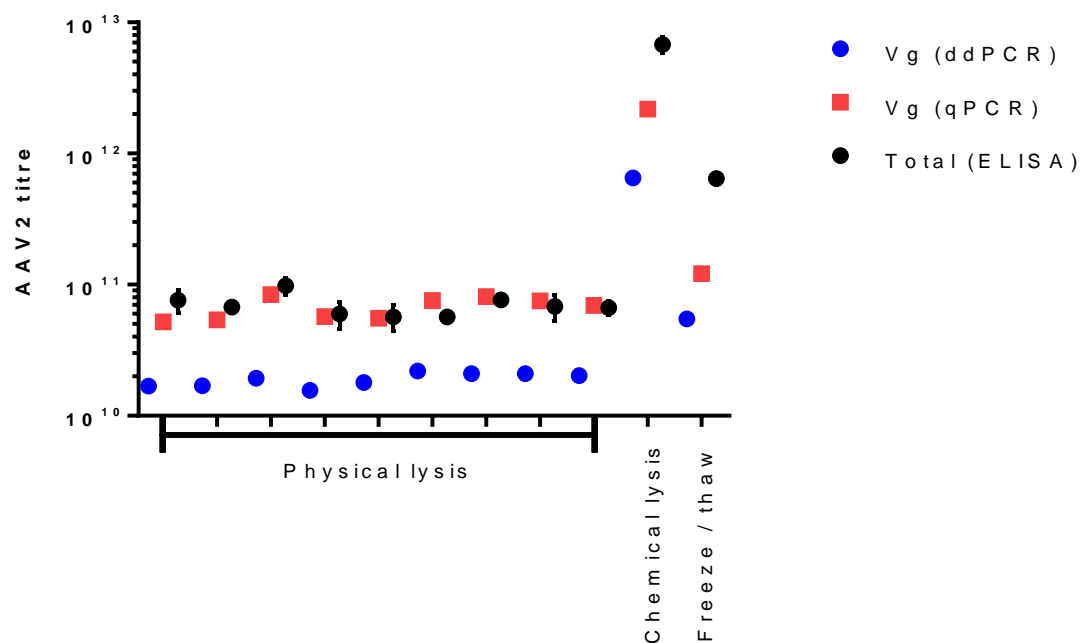
Freeze/thaw lysis

Chemical lysis

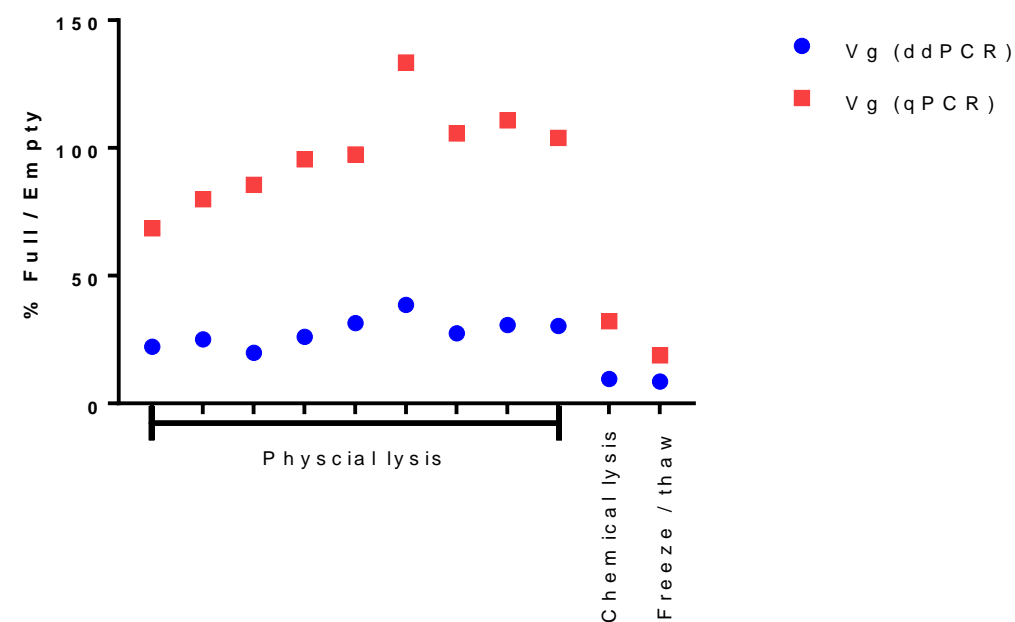
AAV titre and purity check



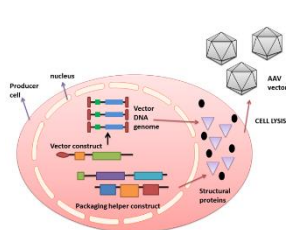
Particle measure



Full to empty ratio



Sample purity - MADLS



Cell disruption



Nuclease treatment



Clarification



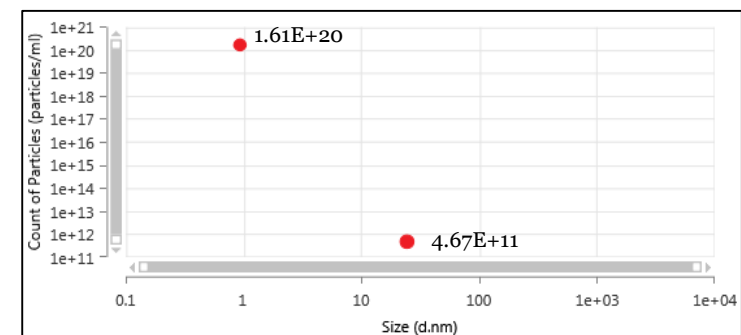
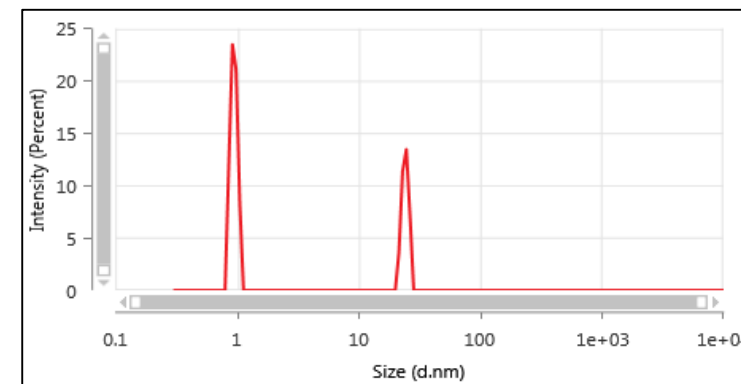
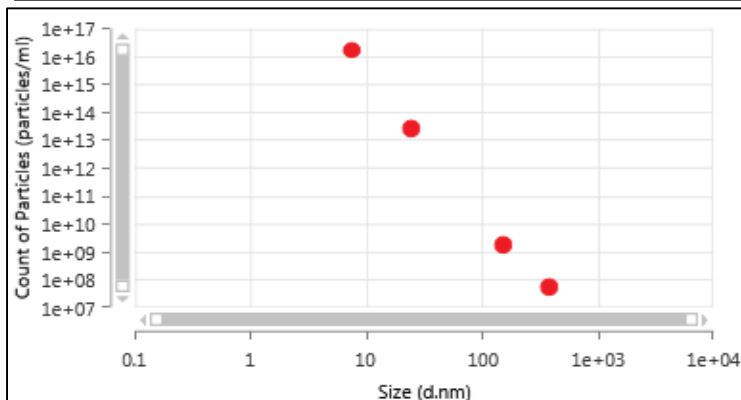
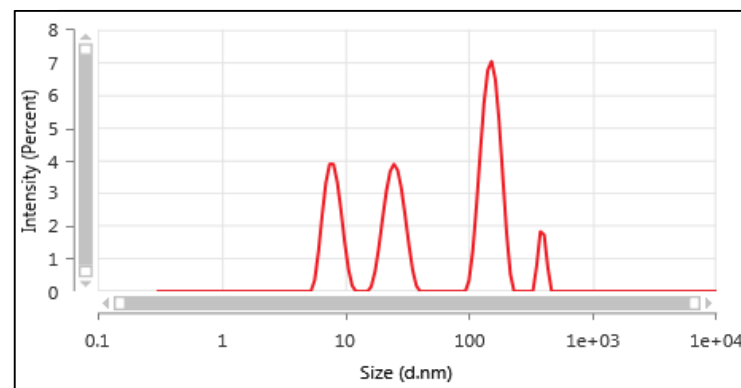
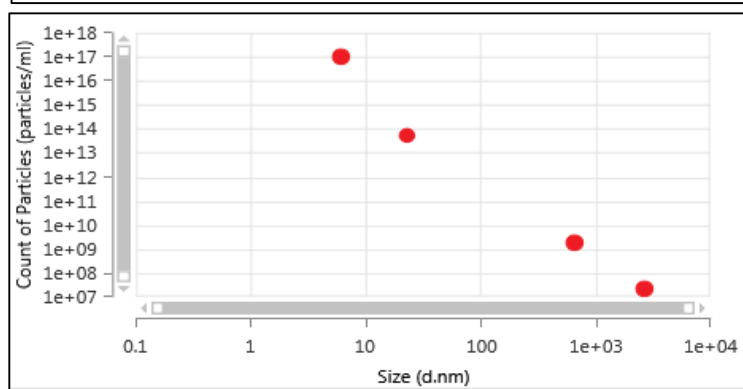
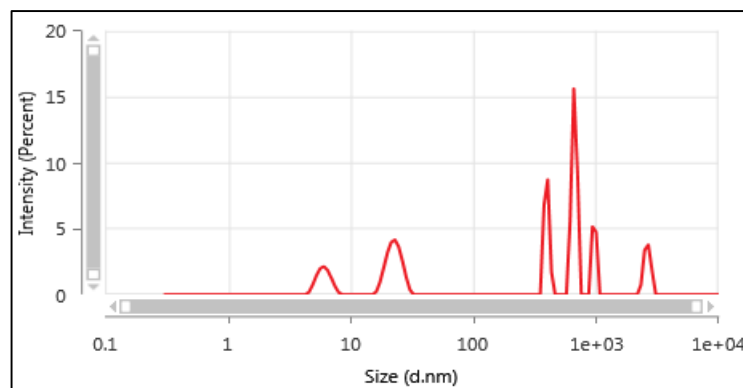
AKTA™ Avant



TFF KrosFlo UP/DF



Final filtration

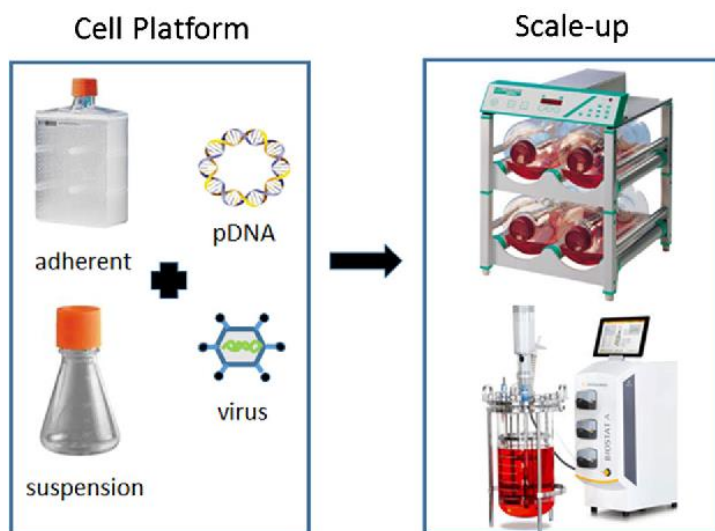


Gene therapy – Overcoming challenges

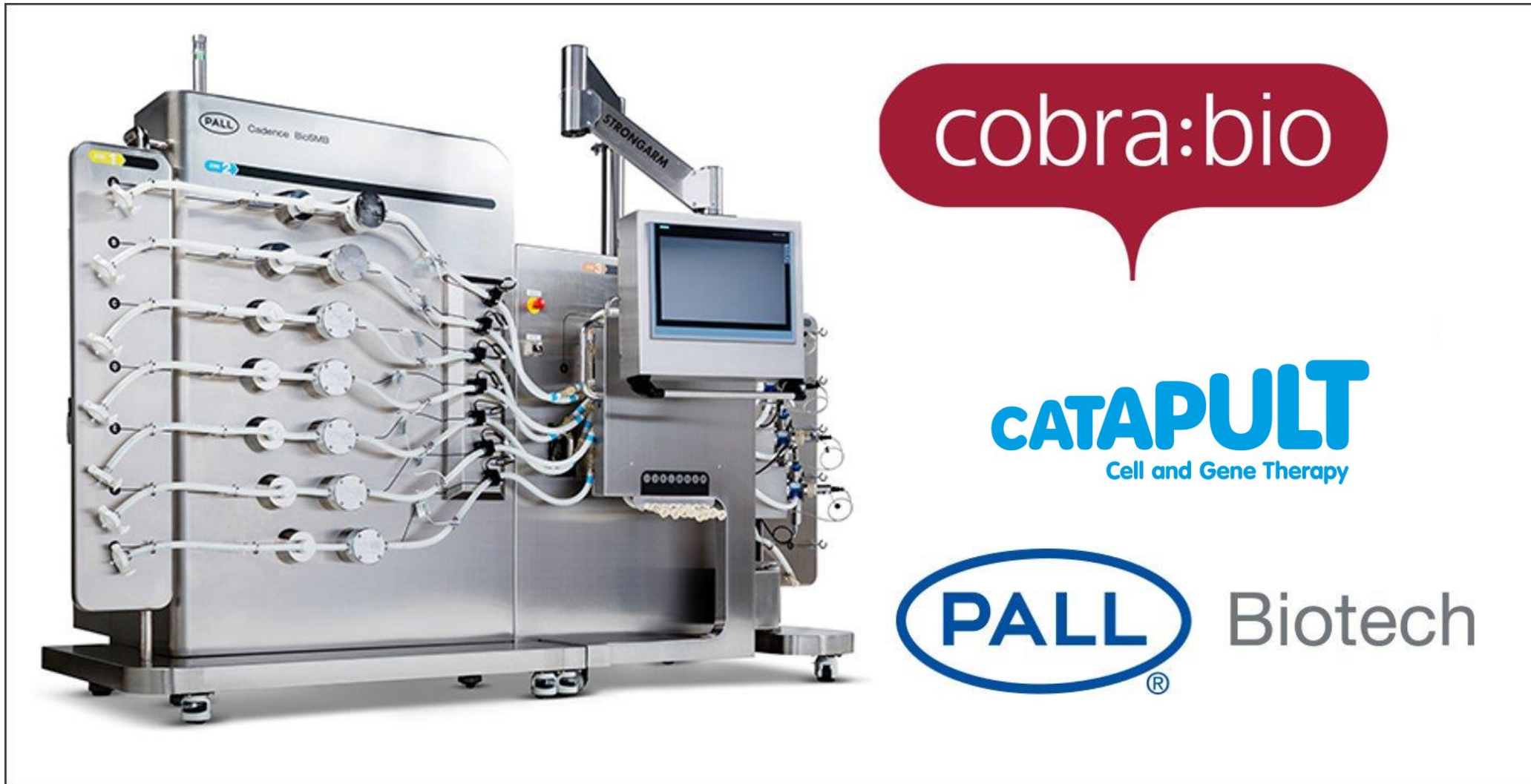


Overcoming AAV therapy challenges

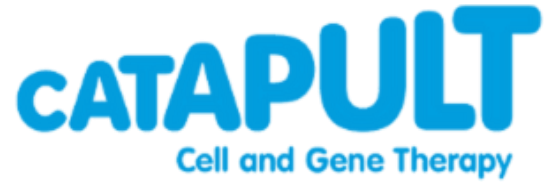
Disease	vg/patient	Estimated patient number (EU/US)	Potential uptake	Estimated Equivalent culture volume (L)
DMD	1.00E+15	1000000	50%	250,000,000
SMA1	6.00E+14	2000	50%	6,000,000
Haemophilia A	4.20E+15	25000	25%	28,000,000
Haemophilia B	7.00E+14	100000	25%	7,000,000
Wet AMD	1.00E+11	1000000	10%	10,000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



Continuous processing - consortium



Acknowledgements



- Julie Kerby
- Damian Marshall
- Mike Delahaye
- Gregory Berger
- Nicole Nicolas
- Anusha Seneviratne
- Nishanthi Weeratunge
- Florian Leseigneur
- Quentin Bazot
- Elena Sokolskaja
- Hadi Mirmalek-Sani

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