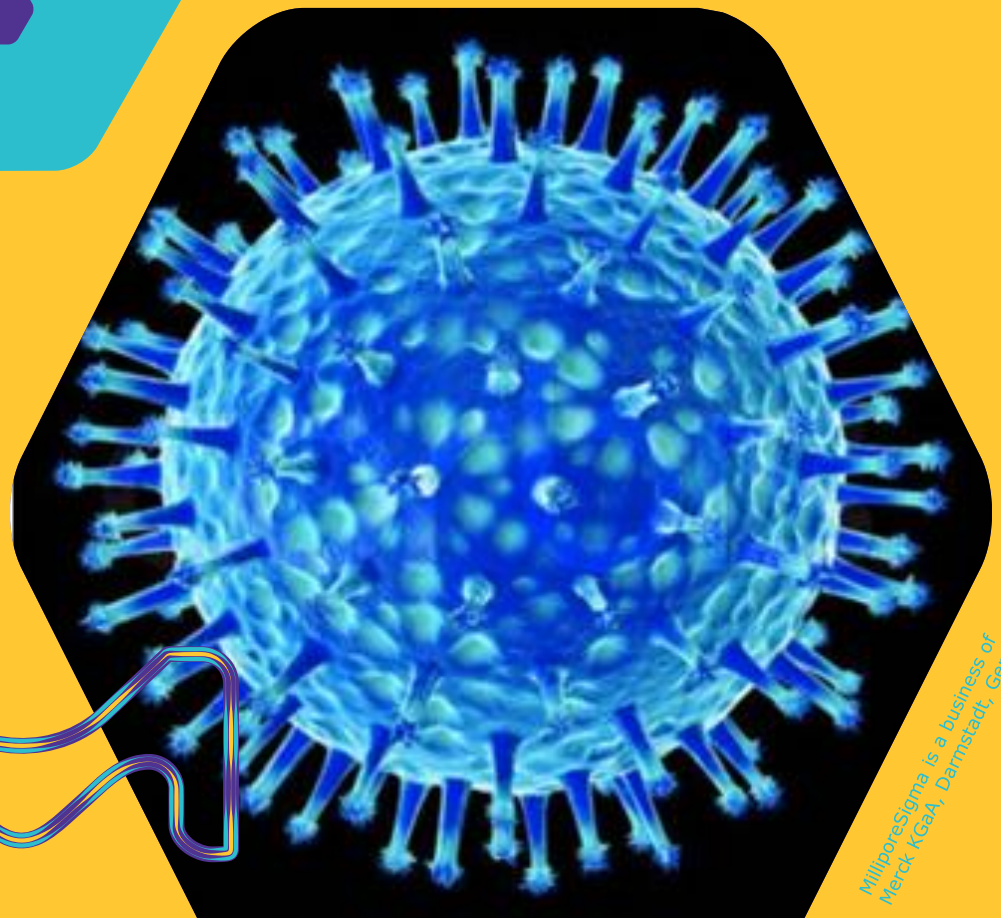
viral vector Manufacturing

Overview of Capabilities



Viral Vector Development & Production Services

- GMP Virus Production in suspension and adherent
- FDA / EMA Inspected
- In-process testing
- Process Development Services

Carlsbad, California, USA

BioReliance® Viral Vector CMO

Bedford, MA, USA

Cell Therapy PD, product development

Glasgow, Scotland, UK

- BioReliance® Viral Vector CMO
- BioReliance® Biosafety testing

Rockville, Maryland, USA

BioReliance® Biosafety testing

St Louis, Missouri, USA

Gene Editing, cell engineering

Martillac, FR

BioReliance® End-to-End Solutions

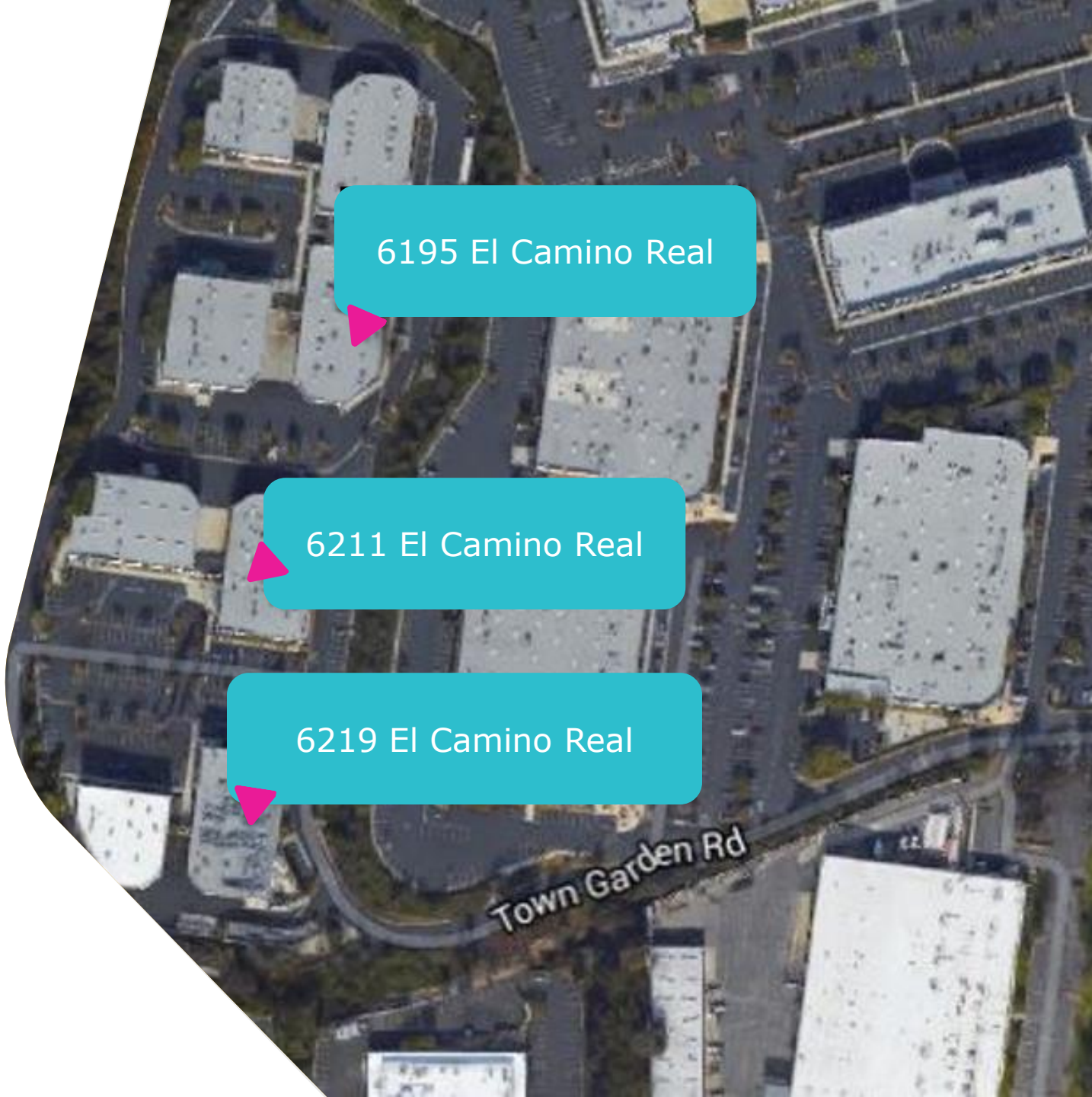
Site History

Carlsbad Facilities

6195 – Manufacturing

6211 – Support

6219 – Manufacturing



Manufacturing Facility Layout

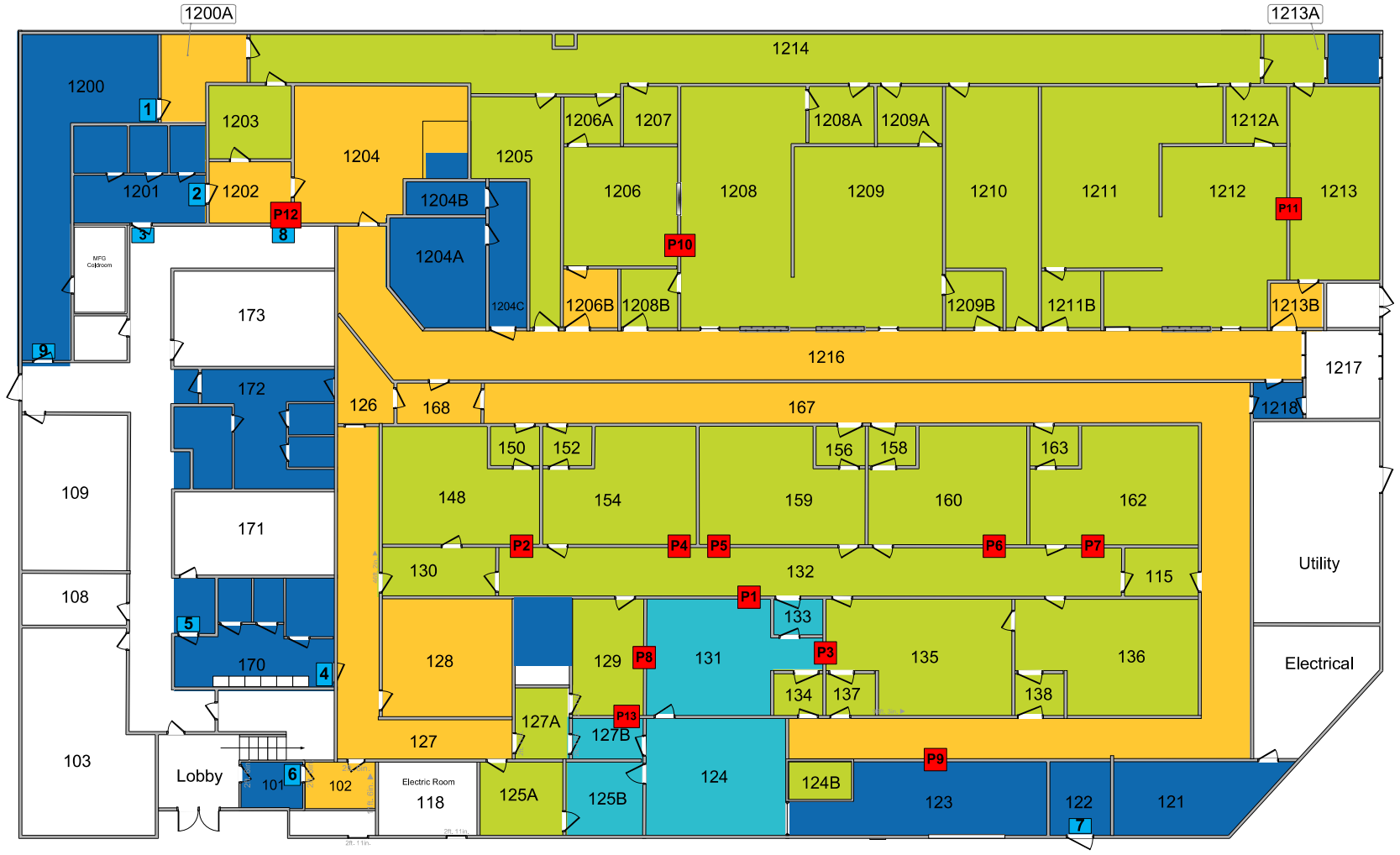
6219 Manufacturing Facility

Grade B

Grade C

Grade D

Controlled/ Unclassified



- **6219 B**
  Two 3 Room Suites for Campaign Manufacturing

  Common Support and Media/Buffer Preparation Areas

- **6219 A**
  Virus Free Cell Expansion Room

  6 Virus Production Suites

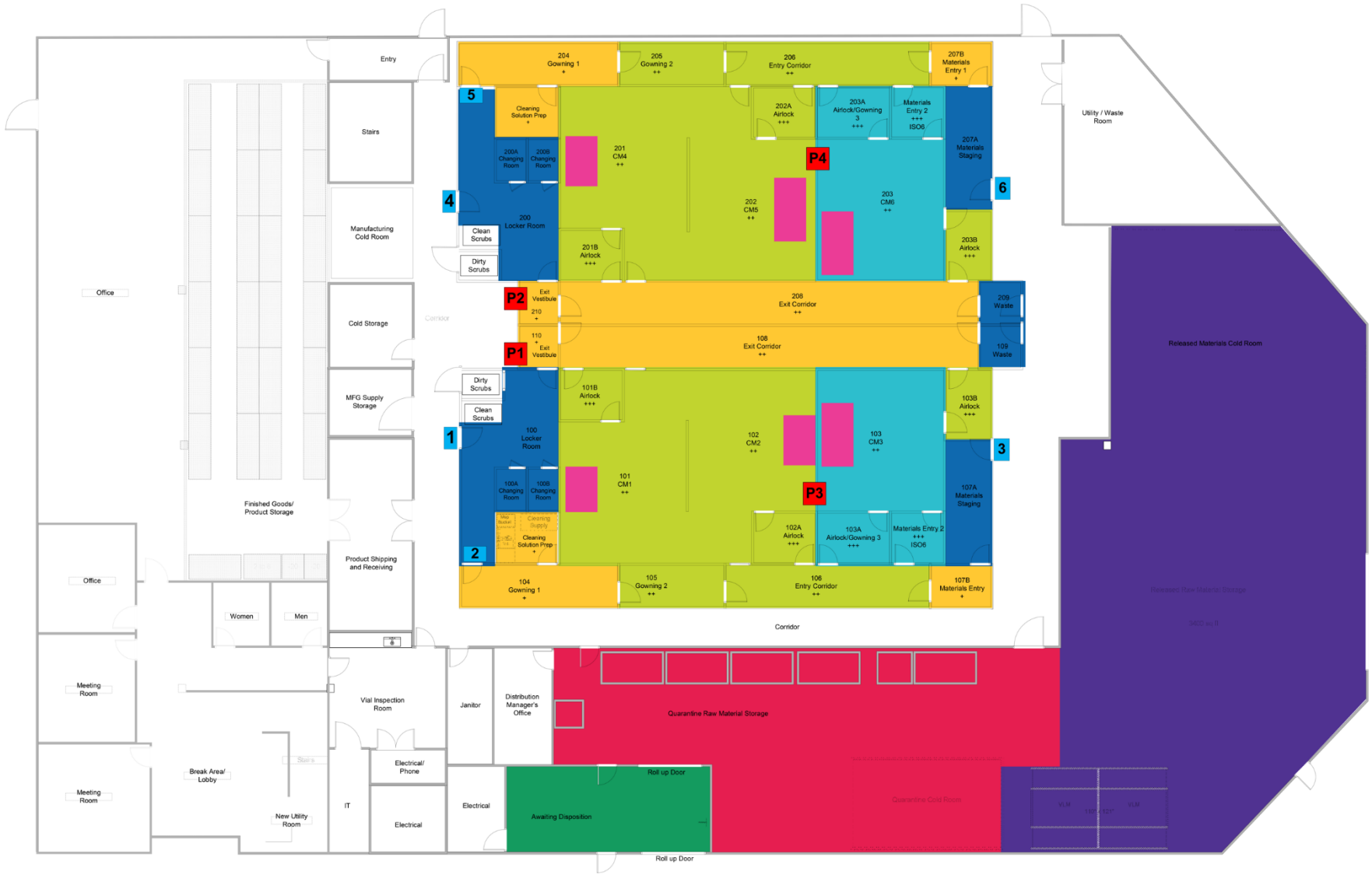
  Grade B Fill Suite

- **6219 C**
  Grade B Fill Suite

Manufacturing Facility Layout

6195: 2 Production Suites & 2 Fill/Finish Suites

- Grade A
- Grade B
- Grade C
- Grade D
- Controlled Unclassified
- Quarantined Raw Materials
- Released Raw Materials
- Receiving



Virus Experience

Cells, Viruses and Batches

Viruses	Experience (batches) 2010-2019
Adeno Associated Virus	72
Adenovirus	80
Lentivirus/Retrovirus	211
Reovirus	20
Dengue Virus	15
Herpes Simplex Virus	13
Coxsackie Virus	19
Others (Sendai, Yellow Fever/West Nile and others)	16

** 3 batches in Glasgow

Virus Experience

Production Scales, Cells and Fills

Viral Production Scales	
Adherent	40x10 layer cell stacks, iCELLis 500
Suspension	50L, 100L, 500L SUB, 100L/1000L perfusion

Cells
293, 293T, BHK, PER.C6, MRC5, HeLa, HT1080, A549, Vero

Fills
Fill/finish: 800 vials/day semi-automated into 2mL to 30mL vials

Our PD capabilities

Our group possesses significant R&D and PD capabilities including "End to End" processing over multiple scales, modalities and vectors

Upstream	• Optimization of (planar) adherent cell culture processes • Development of SUB processes (in microcarrier mode or "single cell" suspension) • Transfection optimization • Development of scaled up cell culture processes
Midstream and Downstream	• Virus clarification • Chromatography development and optimization • TFF development and scale up
Analytical Methods	• P24 & AAV ELISAs • qPCR • Bioassays with imaging (Cell based)

VIAL THAW

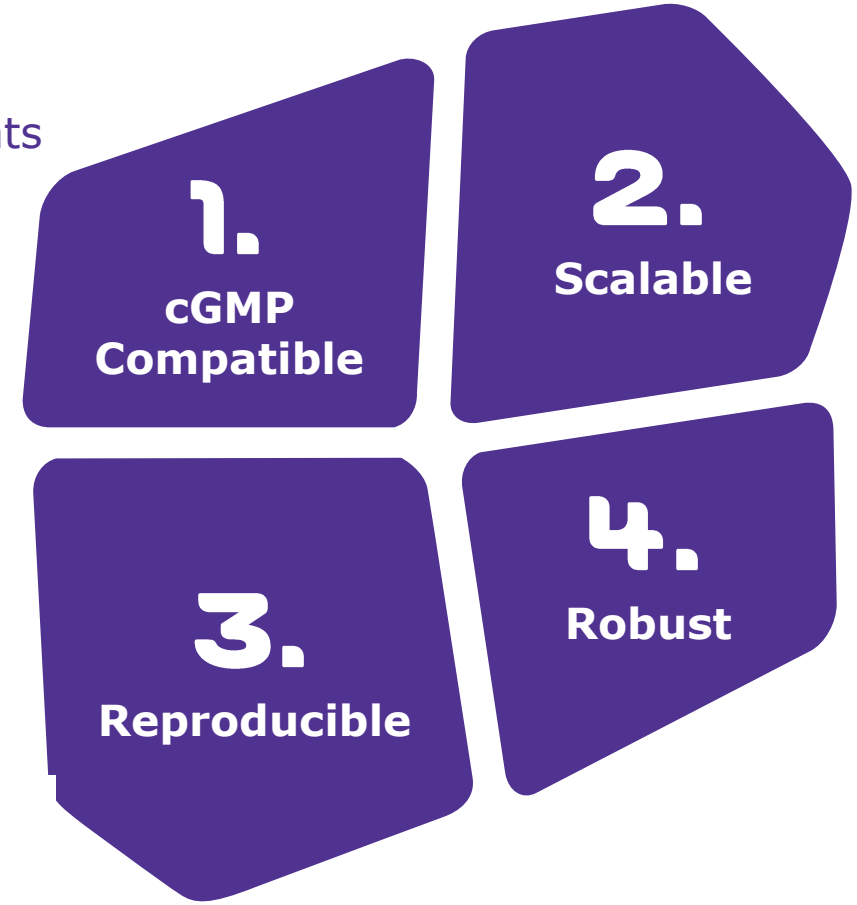
DRUG SUBSTANCE

We fully characterize your product & process

	Early (PD)	Late (ENG/GMP)
Product	• Titer • Infectious titer • Purity	• Contaminants • Residuals • Potency • Stability • Identity • Appearance
Process	• Mass balance • Process appropriateness • Material/supplies appropriateness • Scale assessment	• Safety • Reproducibility • Trending

Critical Factors for Viral Vectors Manufacturing

- Raw Materials and Reagents
- Equipment
- Consumable Sets
- Single Use Technology



- Equipment
- Unit Operations

- Product Quality
- Product Yield
- Titer
- Purity

- Mode of Operation
- Stability
- Tolerance
- Feasible Range
- Process Time

6211 Process Development Laboratory

PD Main Equipment:

- Mobius 50L Bioreactor
- iCELLis nano Fixed Bed Bioreactor
- Eppendorf CelligenBlu Bioreactor
- Eppendorf DASGIP
- AKTA Prime
- AKTA Pilot
- Spectrum TFF system
- PendoTech Filtration System

PD adherent scale:

- 10 x CS10
- iCELLis nano up to 4m^2
- *Access to iCELLis 500m^2*

PD suspension scale:

- Mobius 3L
- Mobius 50L
- Hyclone SUB 50L
- Eppendorf CelligenBlu 50L

Manufacturing

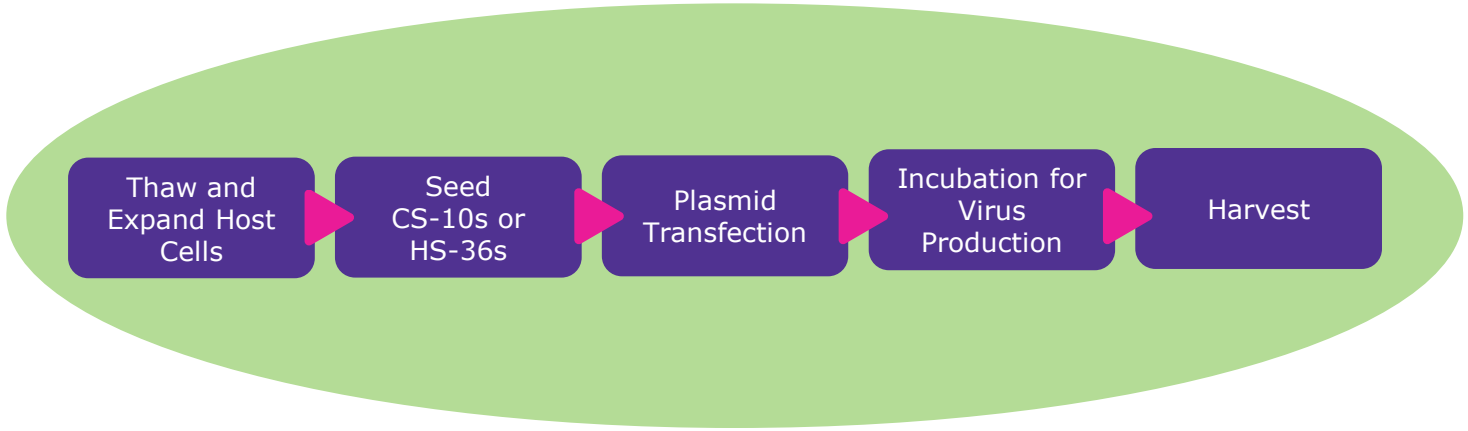
Full service, experienced CDMO designed to meet your rigorous timelines

- Product innovators with >25 years of experience
- Technology transfer partners
  - Ensure cGMP readiness
  - Focus on robust process design and risk reduction
- Produced >500 batches of virus to support development from clinical through commercialization
- cGMP state-of-the-art facility accommodates scale-up and transfer to manufacturing, through to commercial launch
- Viral vector manufacturing at scales of
  - Up to 500m^2 iCELLis for adherent
  - 100 L for suspension (1000L with perfusion processes)
  - 500 L SUB

Typical AAV Process and Recoveries

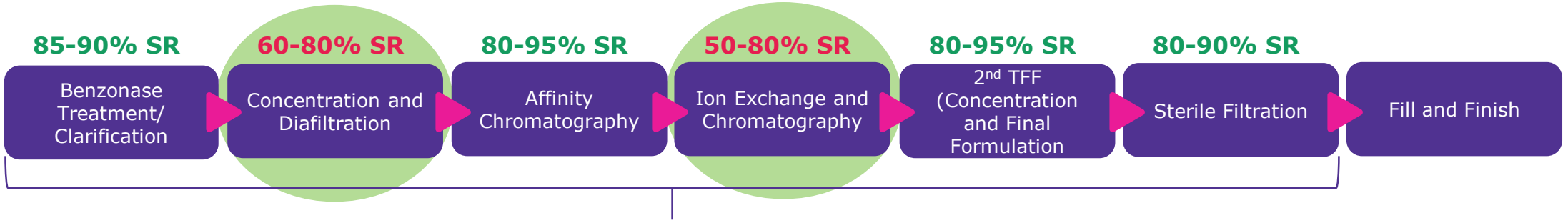
Adherent Process

Upstream



GC/mL
2.0-5.0E+10

Downstream



Typical process recovery from harvest through sterile filtration: 40-60%

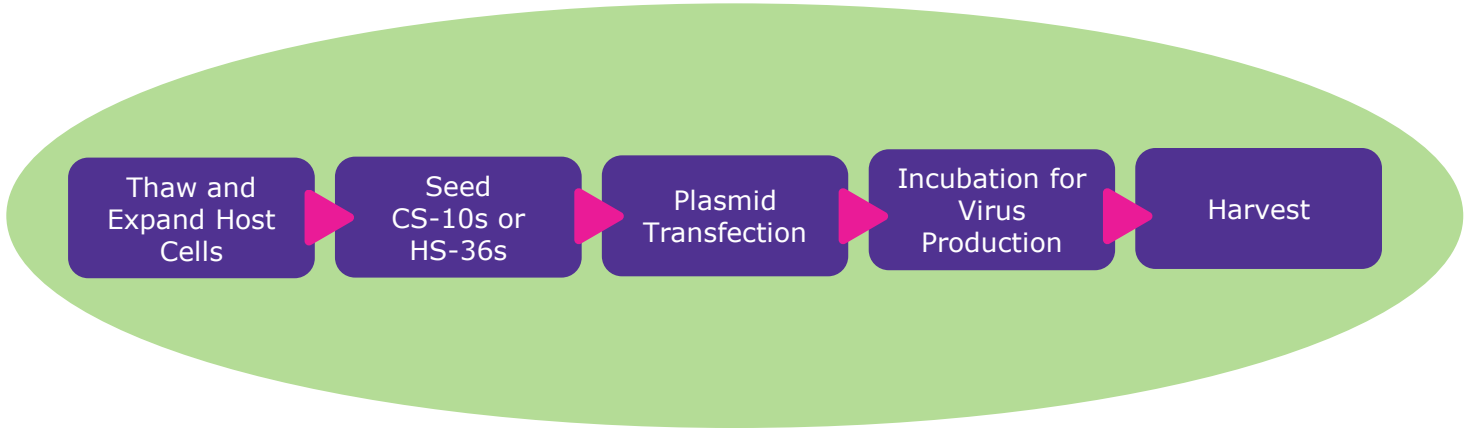
Purified GC/mL
~1.0E+13

Significant optimization opportunity, SR = Step recovery

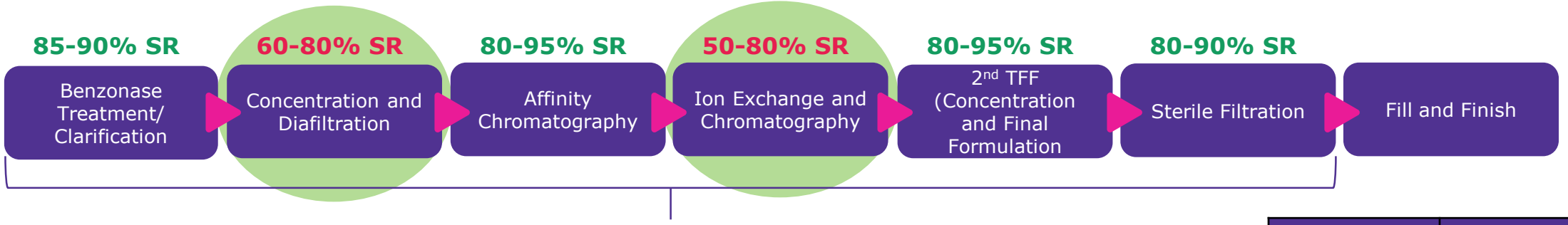
Typical AAV Process and Recoveries

Suspension

Upstream



Downstream



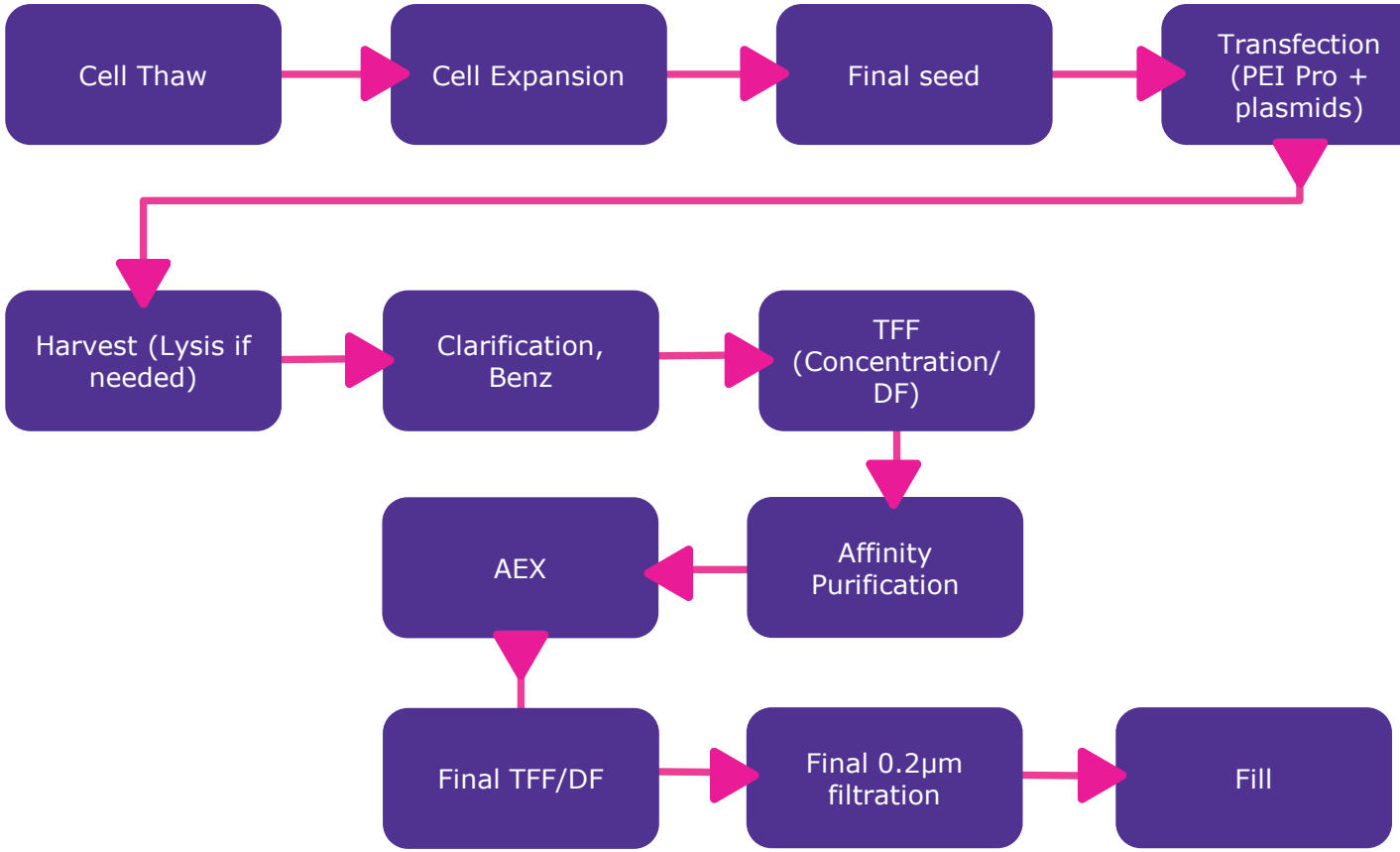
Typical process recovery from harvest through sterile filtration: 40-60%

GC/mL	Full capsids
2.0E+11	≥ 70%

Significant optimization opportunity, SR = Step recovery

Typical AAV Process

Process Flow



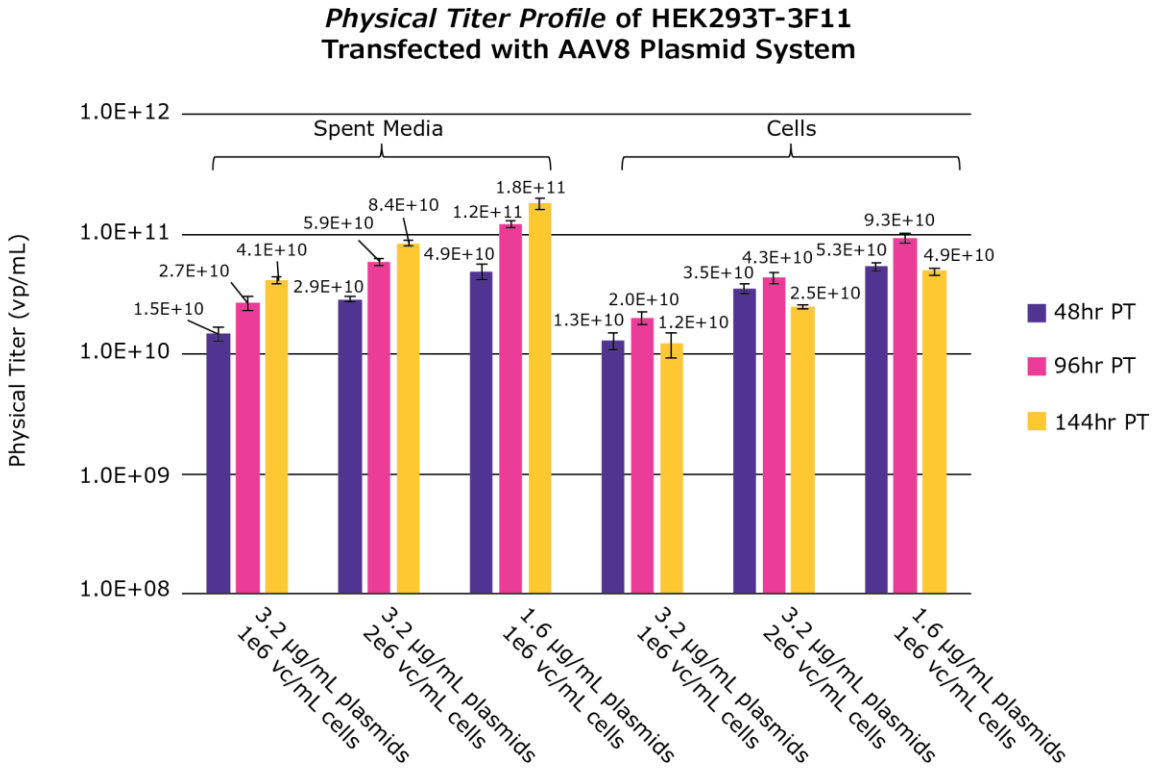
*Typical yields depending on serotype

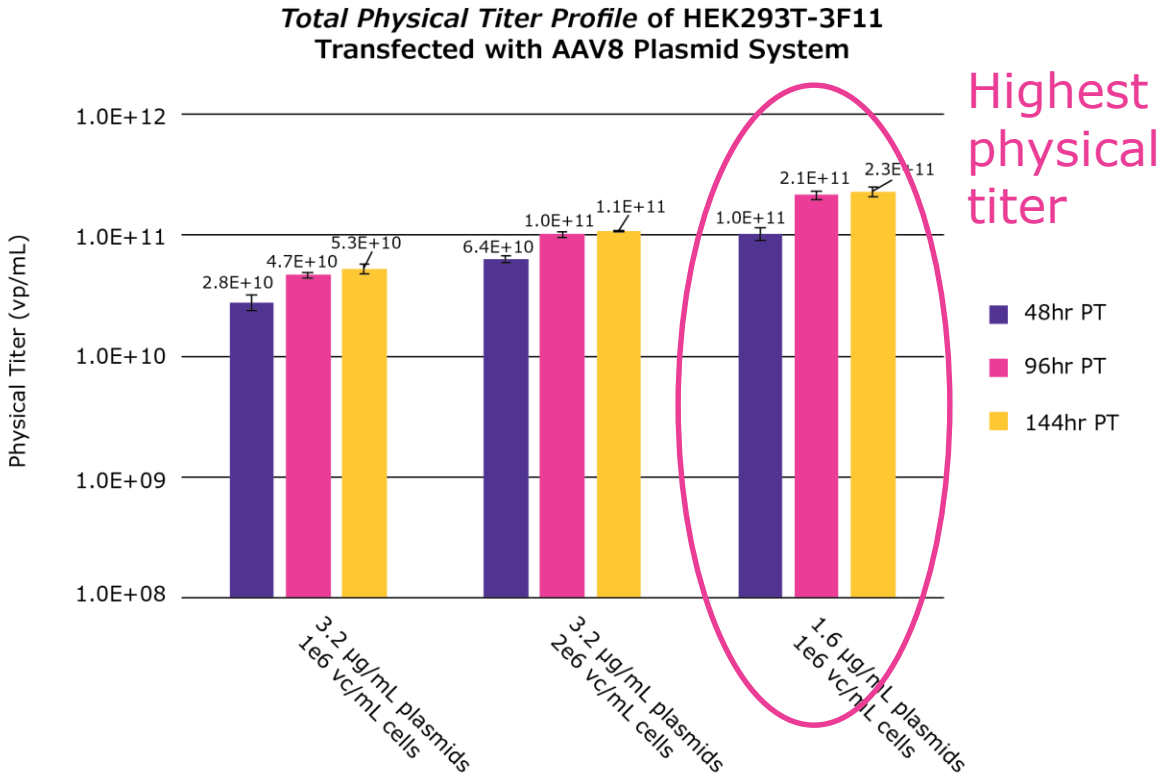
** Current experiments in SF and Brx

Method	GC/mL
Adherent at crude harvest	~2.0-5.0E+10*
Suspension in SF and Brx at crude harvest	~1.4E+11**

AAV production using HEK293T-3F11 cells

Physical Titers





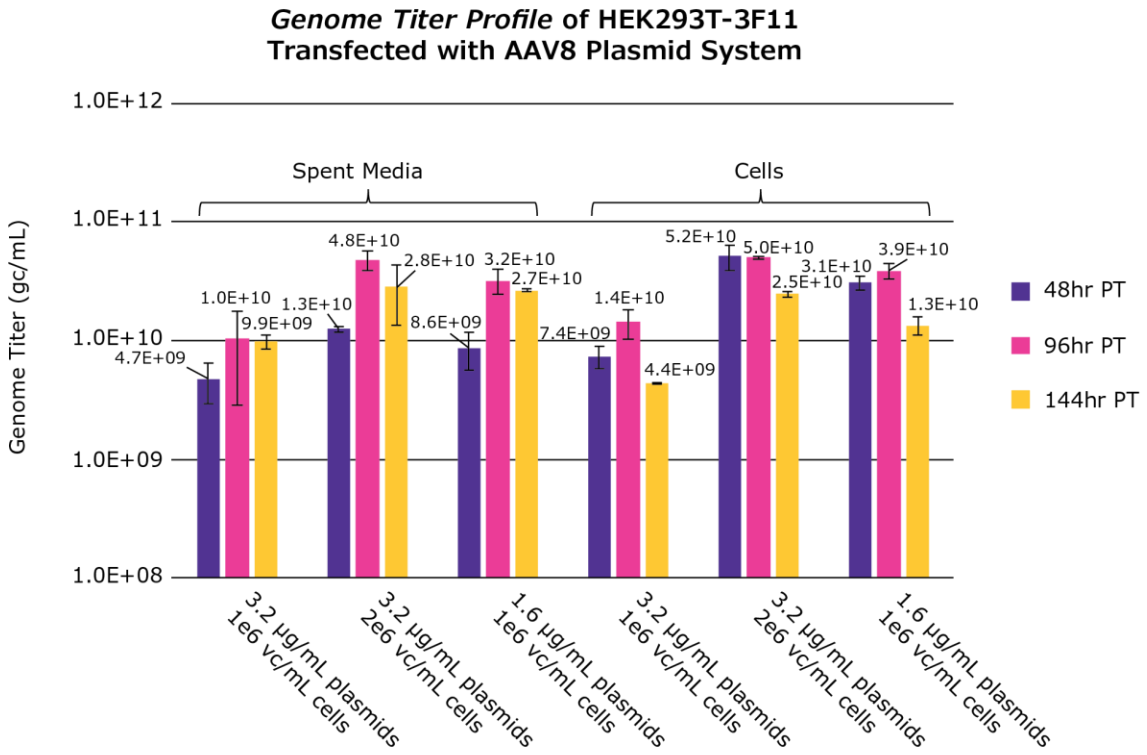
Highest physical titer

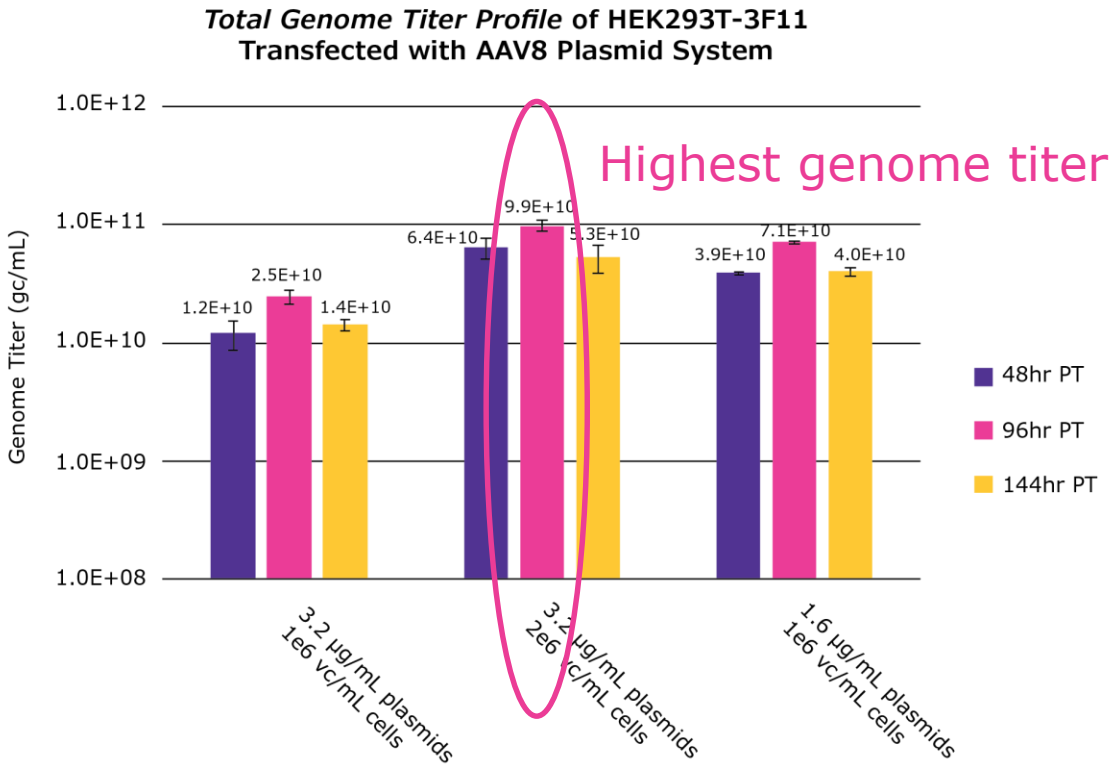
Each bar represents an average of 2 shake flasks x 1 or 2 dilutions x 2 replicate wells and error bars are stdev

- Physical titer of AAV8 increased over time in spent media, but reached the maximum at day 4 PT in cells.
- Total physical titer of AAV8 increased until day 4 PT and then plateaued.

Results using HEK293T-3F11 cells

Genome Titers



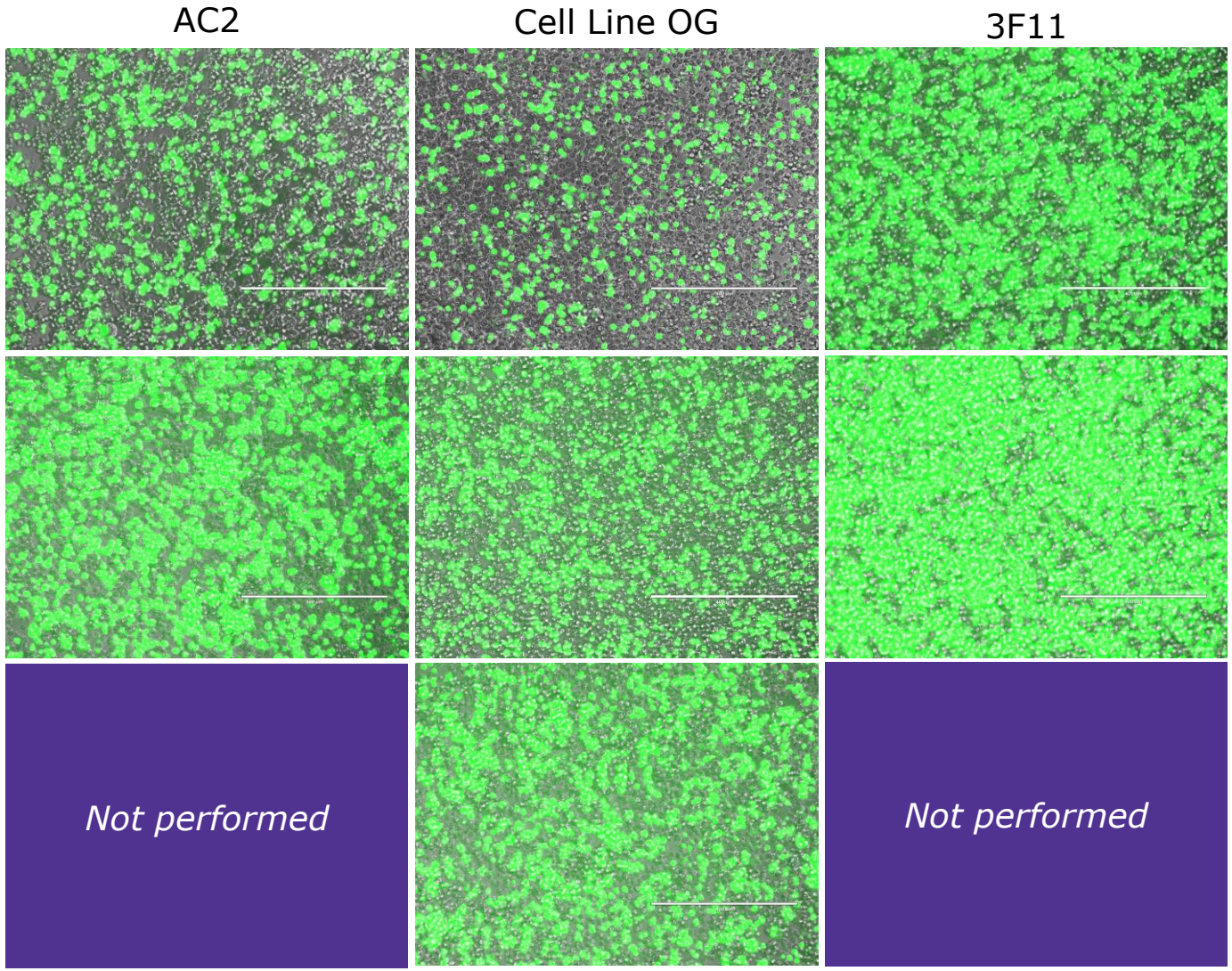


Each bar represents an average of 2 shake flasks x 2 or 3 replicate wells and error bars are stdev

- Genome titer of AAV8 increased until day 4 PT and then plateaued in spent media, but decreased after day 4 PT in cells.
- Total genome titer of AAV8 increased until day 4 PT and then decreased.

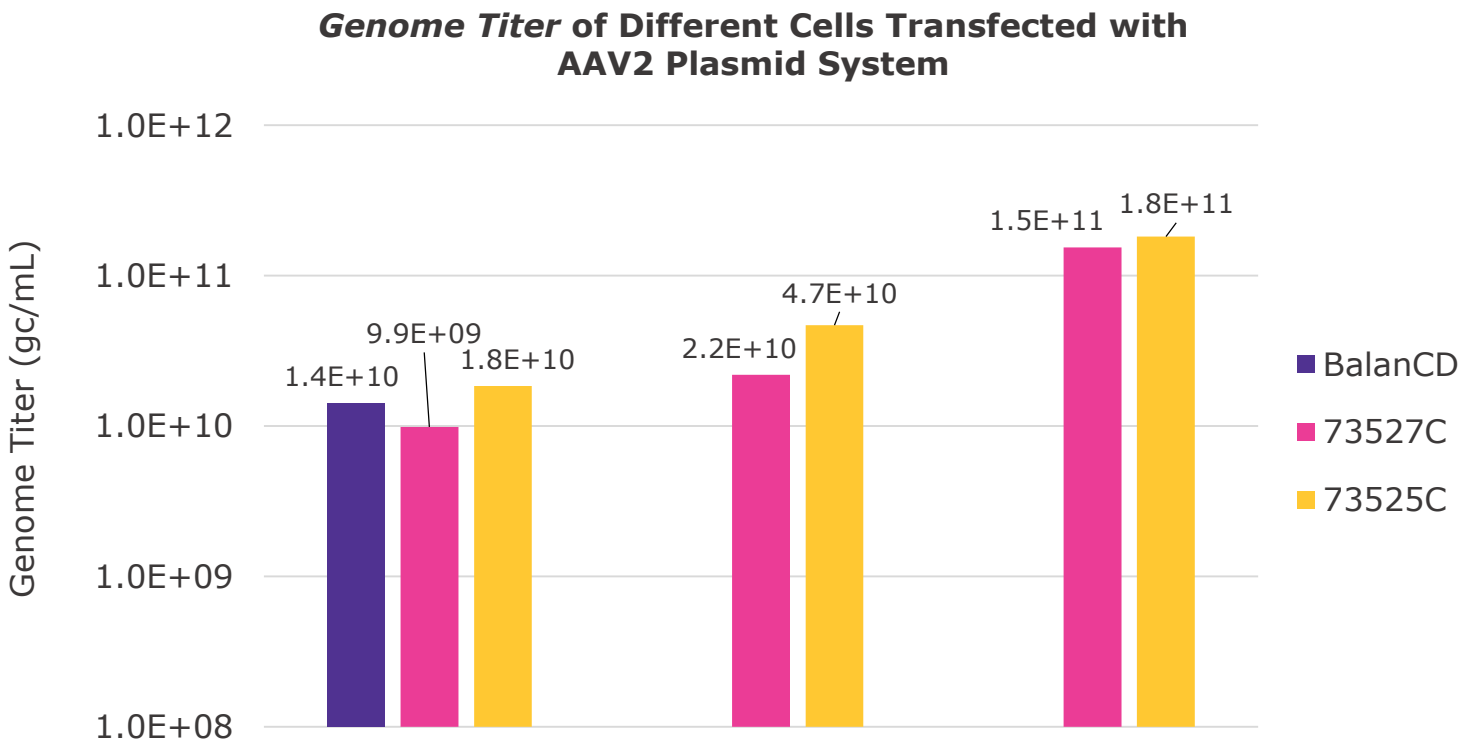
AAV2 Production Transfection Efficiency

24 hrs post transfection



AAV2 Production

AAV2 Production in T and non-T containing HEK293 cells



73527C = V1

73525C = V2

Results:

- Highest titers seen with clone 3F11 (T antigen containing cell line)
- Clone AC2 (non T) also gave good titer, especially in V2 medium
- Clone OG (non T) gave lowest titers

Carlsbad Operations
Process Diversity

Virus	Production Vessel	Purification Method	Fill Scale/Container
Lenti	10 Layer CellStacks	Membrane Absorber	200/Glass
Retro	10 Layer CellStacks	None	800/Bags
Retro	S.U.B.	Column Chromatography	1000/Glass
Reo	SS Bioreactor	Column Chromatography	>10,000/Glass/NA
Adeno	iCellis	Column Chromatography	2000/Glass
Coxsackie	10 Layer CellStacks	CsCl	>4,000/Glass/NA
HD Ad	10 Layer CellStacks	CsCl	2000/Glass
AAV	36 Layer HyperStacks	Column Chromatography	1000/Glass

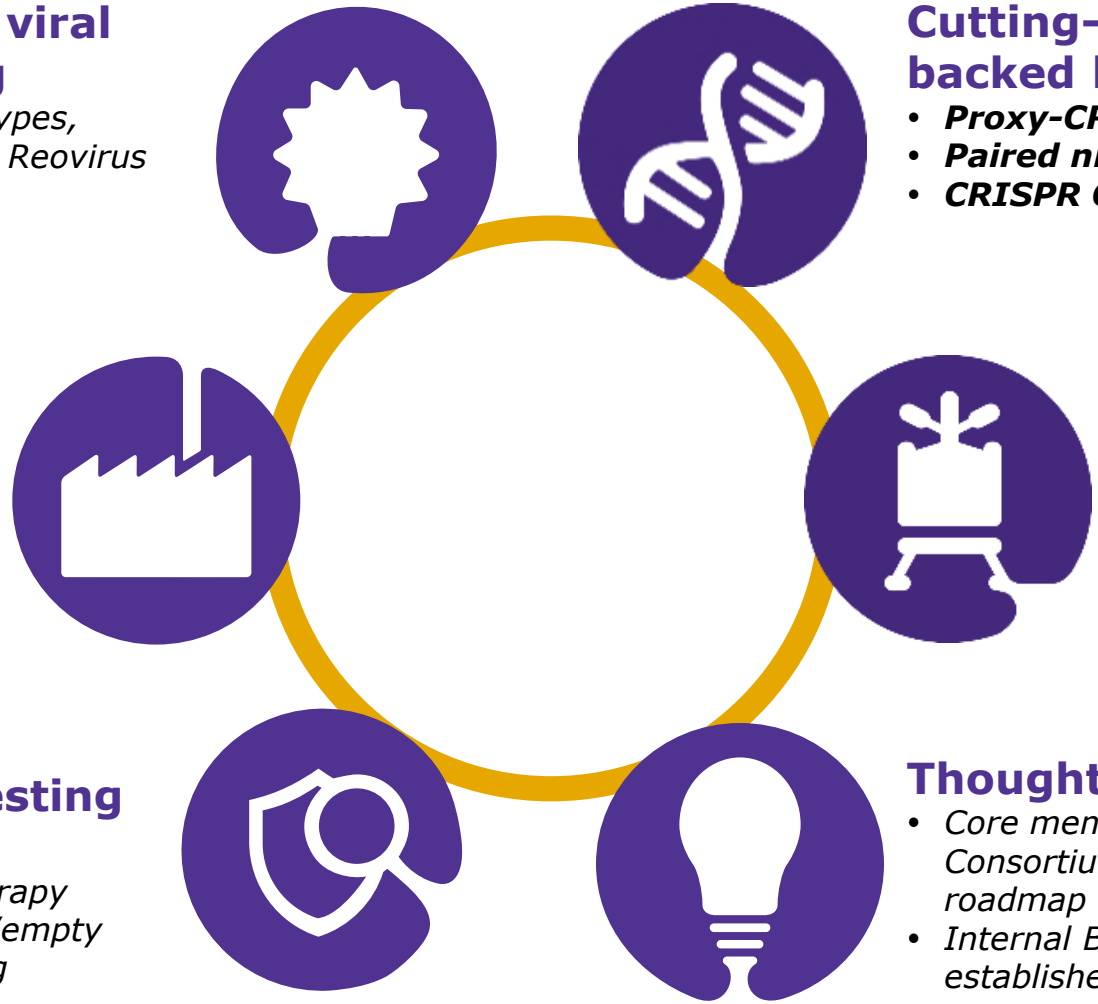
What MilliporeSigma brings to the table

Industrial-scale GMP viral vector manufacturing

Deep know-hows across virus types, including AAV, Lenti/Retro, and Reovirus

Cutting-edge gene editing tools backed by a strong IP portfolio

- ***Proxy-CRISPR:*** *expanded access of genome*
- ***Paired nickase:*** *increased cutting precision*
- ***CRISPR Chrom:*** *tools to modify epigenetics*



GMP manufacturing services

Cell line development, biological production, regulatory support, facility design

Best-in-class bioprocessing reagents and equipment

including upstream and downstream processing

Industry leading testing services

Including viral and gene therapy testing (e.g., off target, full/empty capsid) and biosafety testing

Thought leadership

- *Core member of National Cell Manufacturing Consortium and developed national roadmap on cell therapy manufacturing*
- *Internal Bioethics Advisory Panel established on gene editing*

Our gene therapy testing services

	Virus Seed	Cell Banks	Plasmids	Unprocessed Bulk Harvest	Purified Bulk Harvest	Final Lot
Identity	✓	✓	✓	✓	✓	✓
Titer	✓			✓		
Sterility	✓	✓	✓	✓	✓	✓
Adventitious Agents	✓	✓	✓	✓		
Cell Properties		✓				
Vector Concentration					✓	✓
Expression of Gene					✓	✓
Residuals					✓	✓
Product Characteristics i.e. pH					✓	
Endotoxin			✓			✓