



LakePharma
The Biologics Company



LakePharma Vector Center for Advanced Therapies

LakePharma Worcester
NR-6821
05-11-2021

Viral Vector Systems at LakePharma

Baculovirus



- Bacmid system
- BIIIC storage
- Suspension culture
- Rapid high titer production (2 weeks)

Lentivirus



- Third generation
- Four plasmid system
- HEK293 production

Adeno-Associated Virus



- Insect (Sf9; baculovirus)
- HEK293 helper-free system

Advanced Therapies Services

Vector Engineering

- Vector design
- Gene synthesis
- Genetic cloning
- Plasmid production

Cell Engineering

- Utilize viral vectors
- Variety of cell lines
- Primary cells (e.g. CAR-T)

Upstream Production

- Insect (Baculovirus)
- Mammalian
- Adherent & suspension platforms
- Scale-up production

Downstream Production

- Depth filtration
- Chromatography
- Affinity, IEX, SEC, etc.
- Ultracentrifugation
- Diafiltration

Analytics

- Capsid titer (ELISA)
- Genome titer (ddPCR)
- Infectious titer
- Purity (PAGE)
- Full: Empty capsid (electron microscopy)
- Aggregation (DLS)
- Bioburden/sterility
- Mycoplasma (PCR)
- Host cell DNA/protein



Viral Vector Services: Key Features

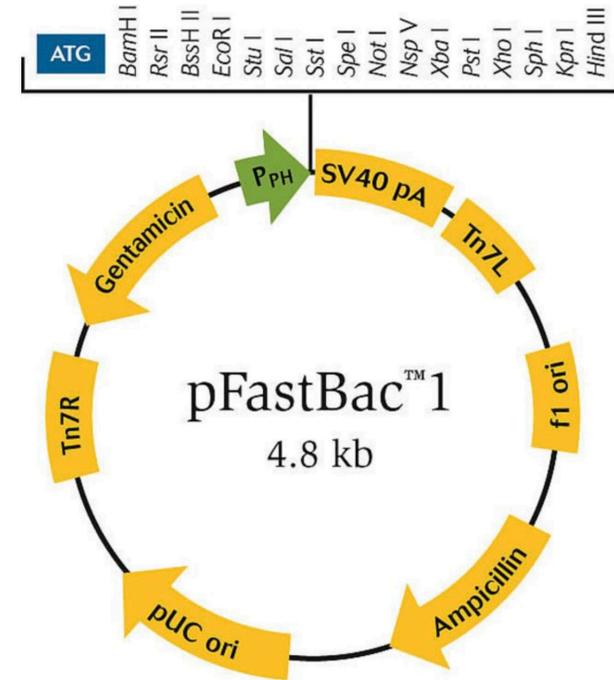
- Construct Design
- DNA Synthesis & Cloning
- Full sequence confirmation
- NGS sequencing of ITR-containing plasmids (AAV)
- Plasmid production
- Production of research grade products for preclinical studies
- Process Development
- Optimization of upstream and downstream unit operations
- FTE Programs available



Gateway Park, Worcester, MA

Baculovirus Platform: Key Features

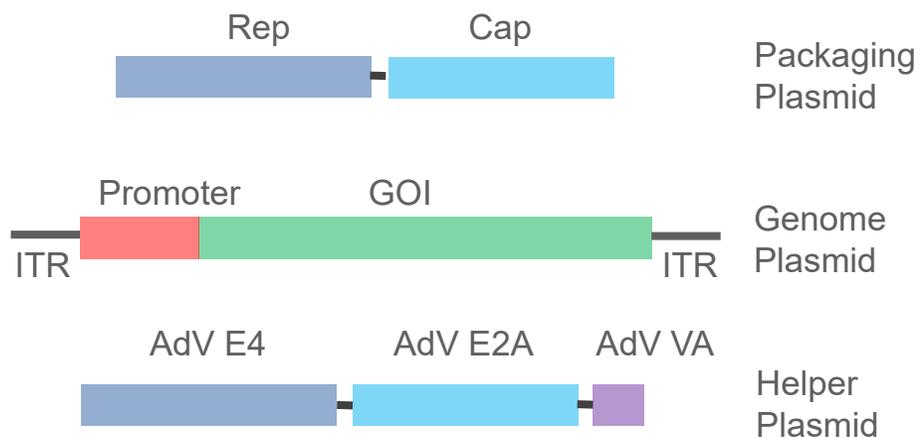
- Suspension-based culture for virus production
- High titer virus production within 2 weeks starting from plasmid
- Rapid titer determination utilizing flow cytometry
- Upstream optimization for viral vector production
 - DOE studies
 - Optimization of culture parameters (e.g. cell density, MOI, virus ratios, incubation time, medium optimization)
- Cryopreserved BIC cell banks for long term storage of baculovirus



AAV Production Platform: Key Features

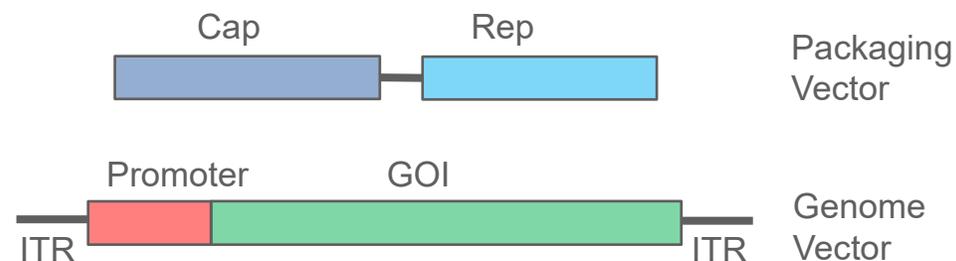
Mammalian

- HEK293 production platform
- Suspension and adherent options
- Helper-free system
- Up to 100L culture production

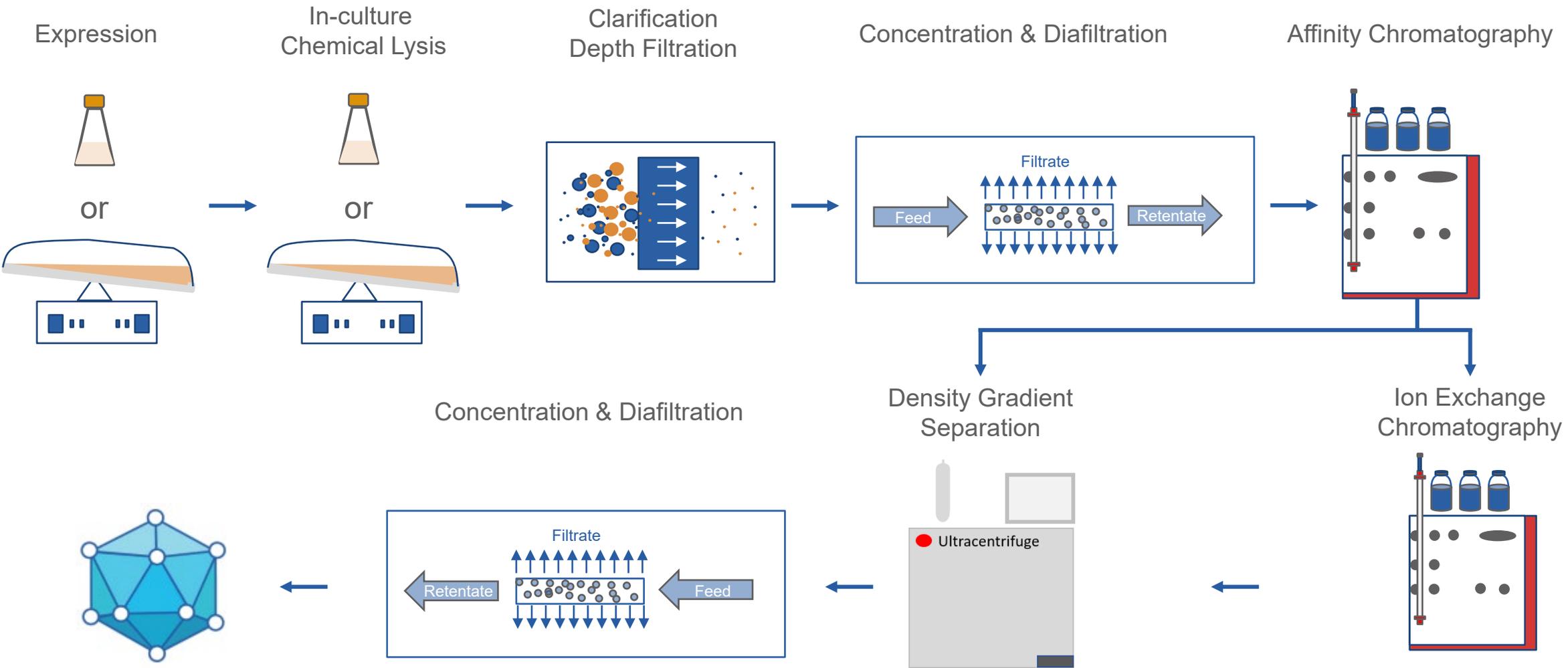


Baculovirus

- Suspension-based culture (Sf9)
- High titer baculovirus production within 2 weeks
- BIIIC research cell banks
- Up to 100L culture production

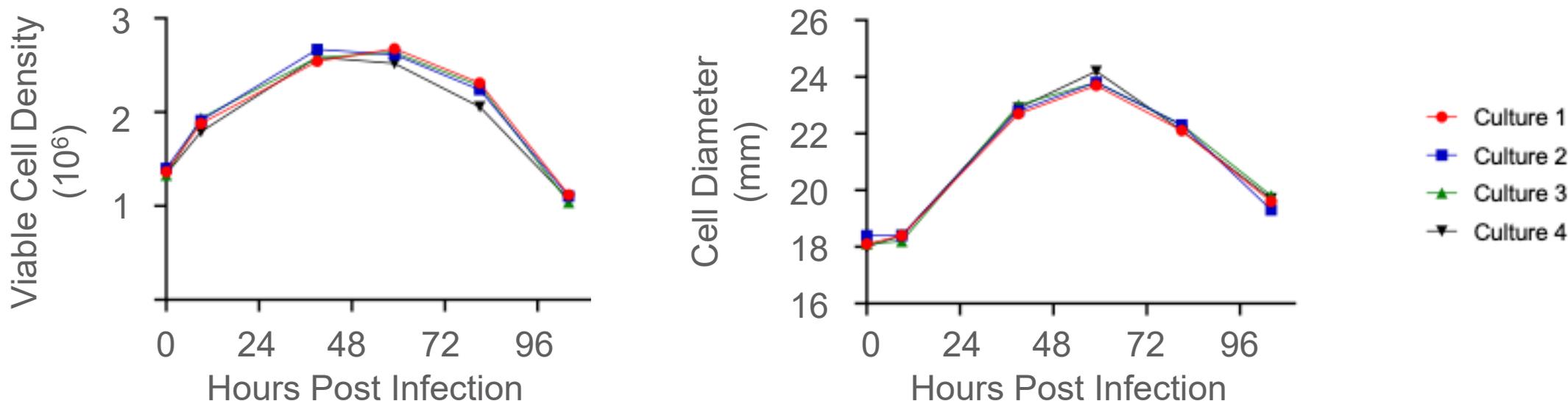


AAV Bioprocess Workflow



Case Study: Production of AAV in Insect Cells (Baculovirus)

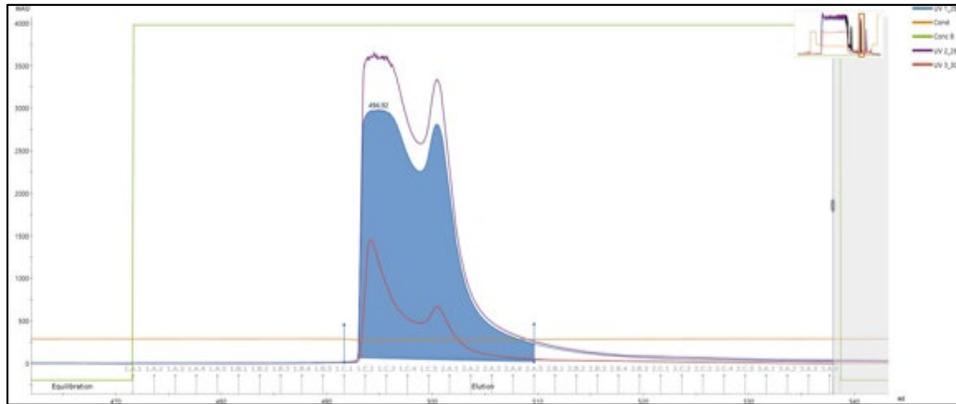
Infection of insect cells with AAV packaging and transfer vectors



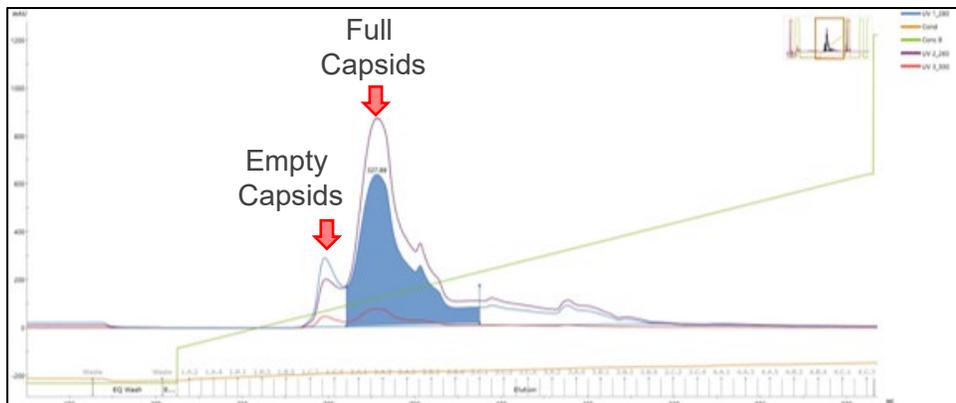
- Four suspension insect cell cultures were infected with baculoviruses
- Viable cell density increases until complete growth arrest, corresponding to peak cell diameter
- The kinetics of infection are highly reproducible

Two-Step Purification of AAV from Insect Cells (Baculovirus)

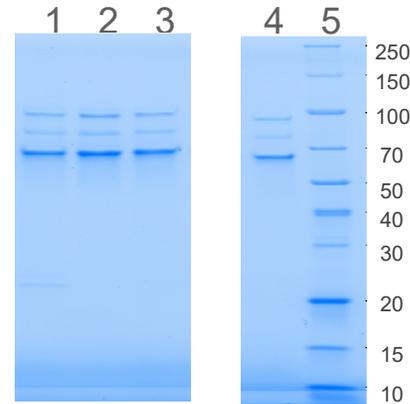
Affinity Chromatography AAV



Ion Exchange Chromatography

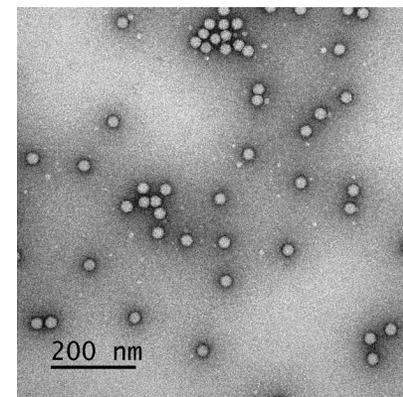


Analysis by SDS-PAGE

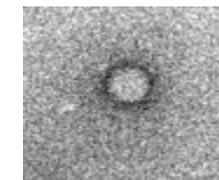


- 1: Affinity Purified
- 2: IEX Peak 1 (Empty)
- 3: IEX Peak 2 (Full)
- 4: IEX Final
- 5: MW Std (kD)

Electron Micrographs of Purified AAV

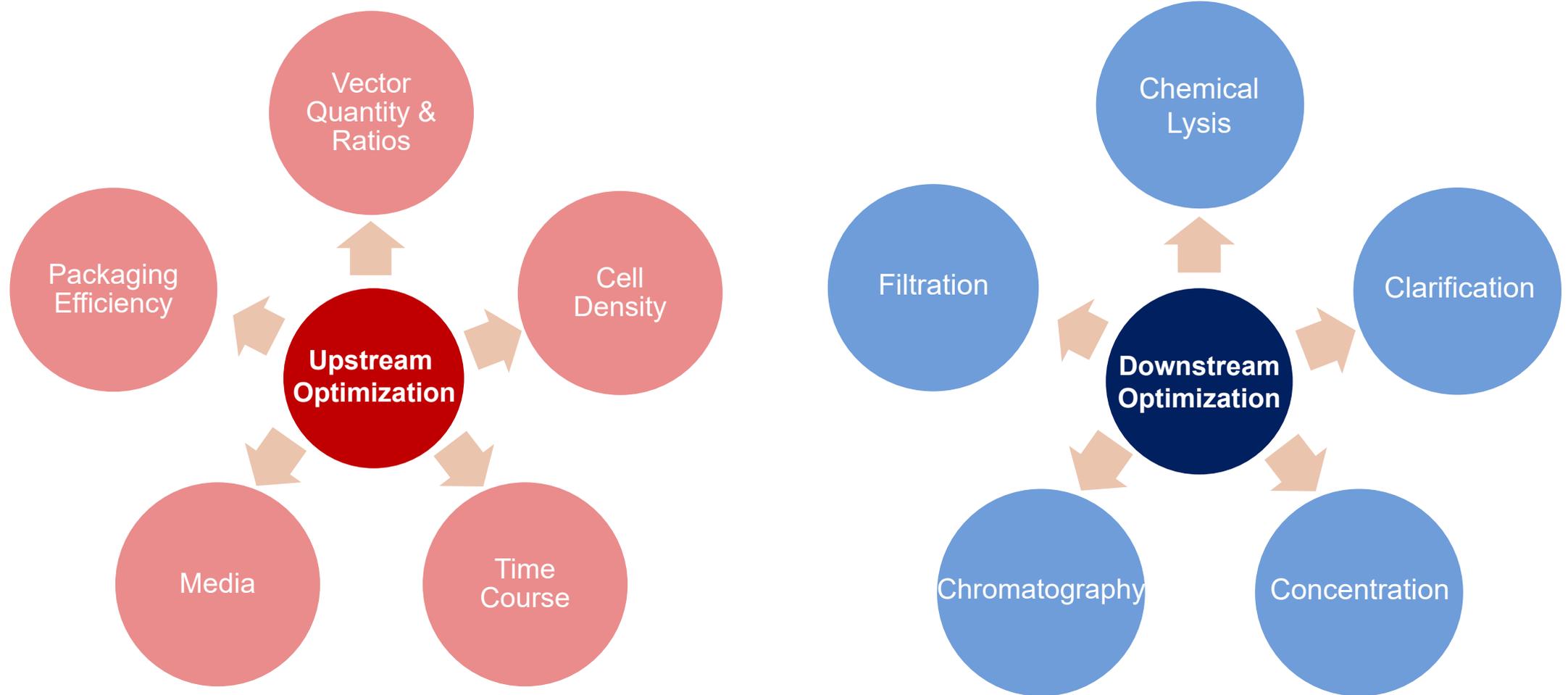


Empty capsid



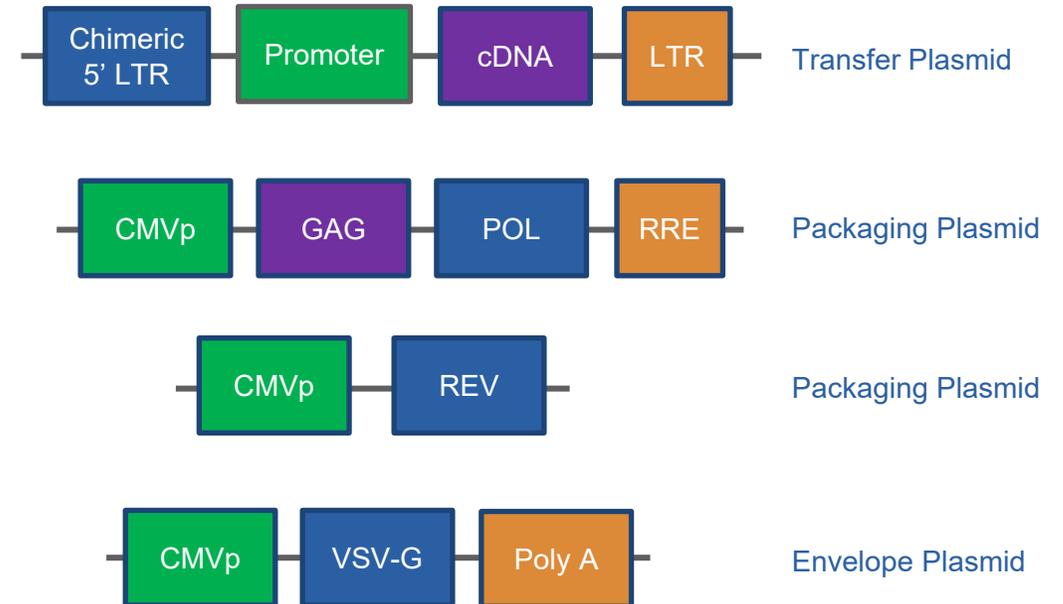
Full capsid

Process Development Services: Key Features



Lentivirus Platform: Key Features

- Used for CAR-T and cell line engineering
- 3rd generation, four plasmid system
- Produced in HEK293-based system
 - Adherent & suspension
- Standard titer: 10^7 to 10^9 IFU/mL
- Pseudotyping



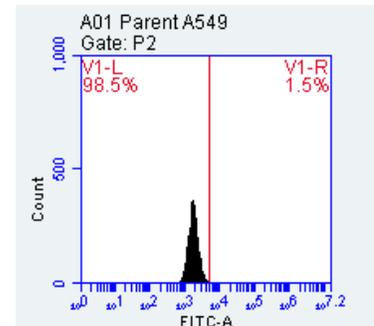
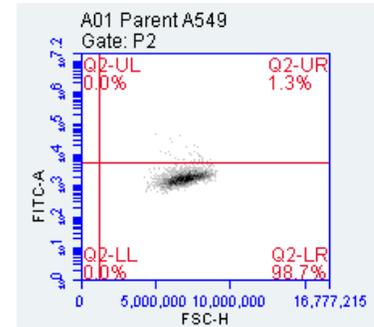
Cell Line Engineering Service: Key Features

- Variety of cell types
 - HEK293, CHO, A549, hematopoietic (T cells, including CAR-T)
 - Hundreds of engineered cell lines created
- Target types
 - Enzymes, antibodies, membrane/structural proteins, reporter proteins, etc.
- Antibiotic titration (kill curve) analysis
- Gene delivery
 - Viral vector mediated transduction
 - Cationic lipid transfection
- Single cell cloning
- Research cell banks
- Cell line characterization
 - Cell surface protein expression: Flow cytometry
 - Intracellular or secreted protein expression: PAGE, western blot, microscopy
 - RNA expression: qPCR or ddPCR
 - Gene copy number: qPCR or ddPCR
 - Functional analysis
 - Reporter assays
 - Cell line stability

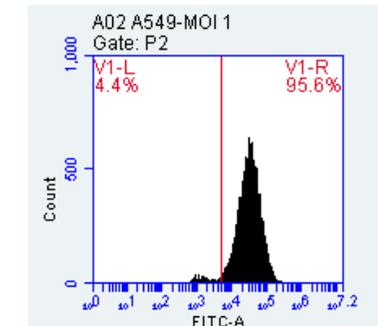
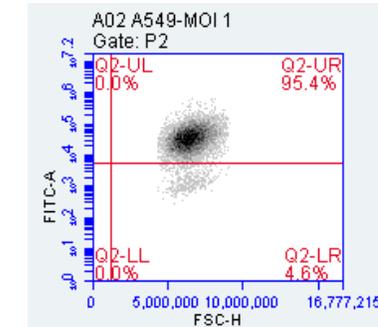
Case Study: Cell Line Engineering Utilizing Lentivirus

- A549 cell line transduced with lentivirus vector encoding target GOI
- MOI = 1 and 20
- Expression analysis by flow cytometry
- Fluorescence increased approximately 100-fold at MOI 20

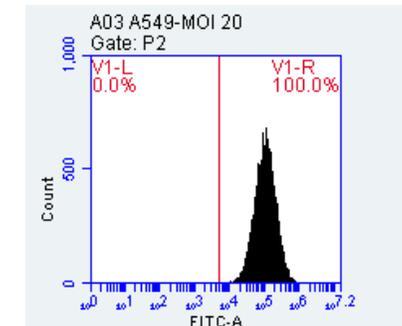
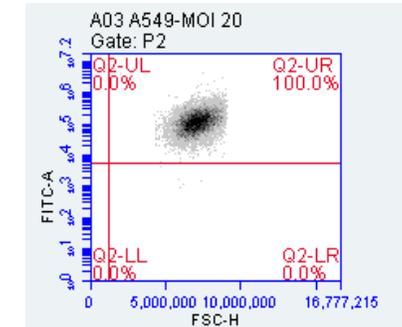
Non-Transduced
Parental A549 Cells
(Background Fluorescence)



Transduced A549 Cells
MOI = 1



MOI = 20



CAR-T Cell Engineering Service

- Lentivirus is engineered and produced
- T cells are activated and transduced with lentivirus encoding Chimeric Antigen Receptor (CAR)
- Cells are characterized by flow cytometry for T cell markers and CAR
- Cells are expanded and cryopreserved
- Engineered cells are for research use only

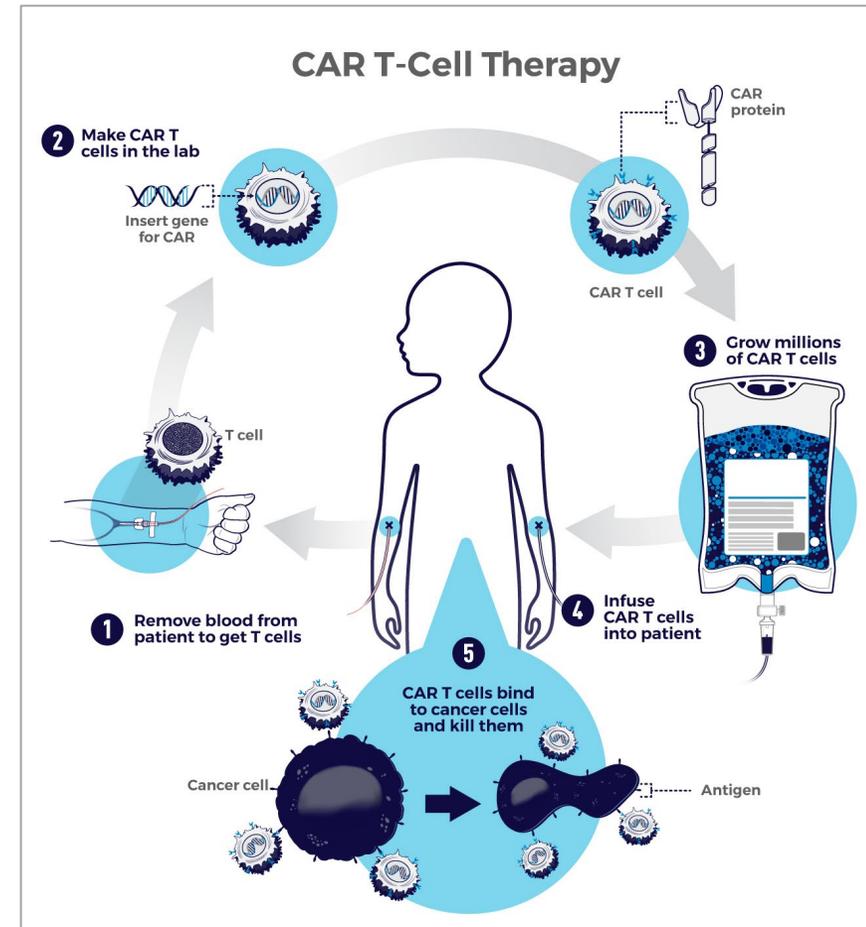
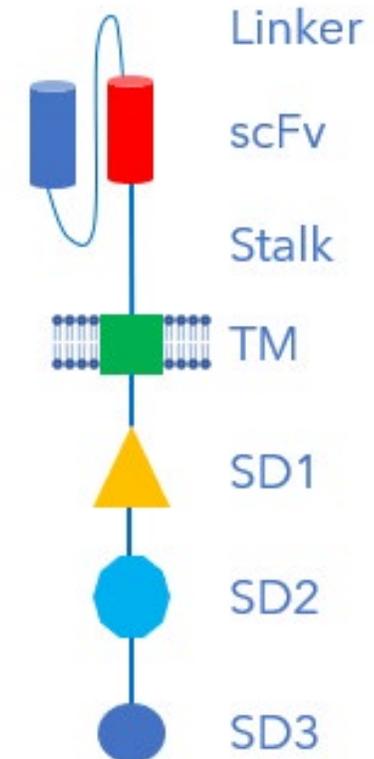


Image source: National Cancer Institute (NCI)

CAR Vector Selections Available from LakePharma

Stalk	TM	Signaling Domain 1	Signaling Domain 2	Signaling Domain 3
IgG1	CD28	4-1BB	CD28	CD3 ζ
IgG1	CD28	OX40	CD28	CD3 ζ
CD8A	CD8A	4-1BB	CD28	CD3 ζ
CD8A	CD8	OX40	CD28	CD3 ζ
IgG1	CD28	IL12	CD28	CD3 ζ
CD8A	CD8A	IL12	CD28	CD3 ζ



Custom design and engineering available upon request

Working with LakePharma

- Complete technology platform provided by LakePharma
- Fee-for-service
- Process development FTE programs available
- Technical consultation
- Online data system for 24-hour access to project information
- Project management
- Collaboration with technical team via email and teleconferences

For inquiries, email us at
Inquiries@LakePharma.com

To learn more visit <https://lakepharma.com/services/viral-vector/overview>

