curia

Vector services for advanced therapies

Viral vector systems at Curia

Baculovirus

- Bacmid system
- BIIC 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

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 BIIC cell banks for long-term storage of baculovirus

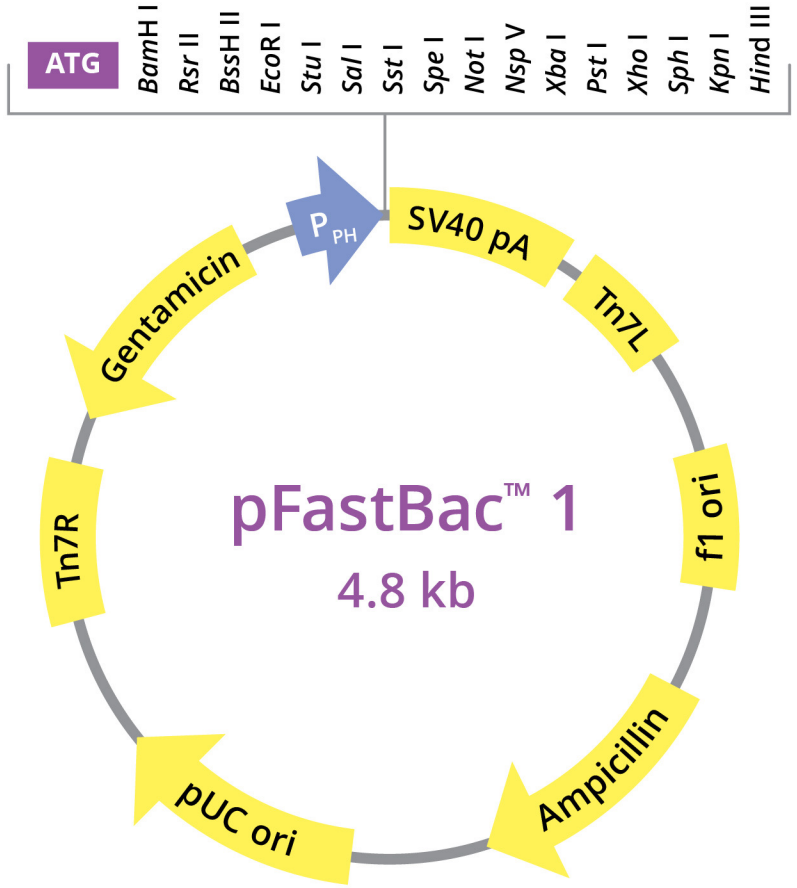
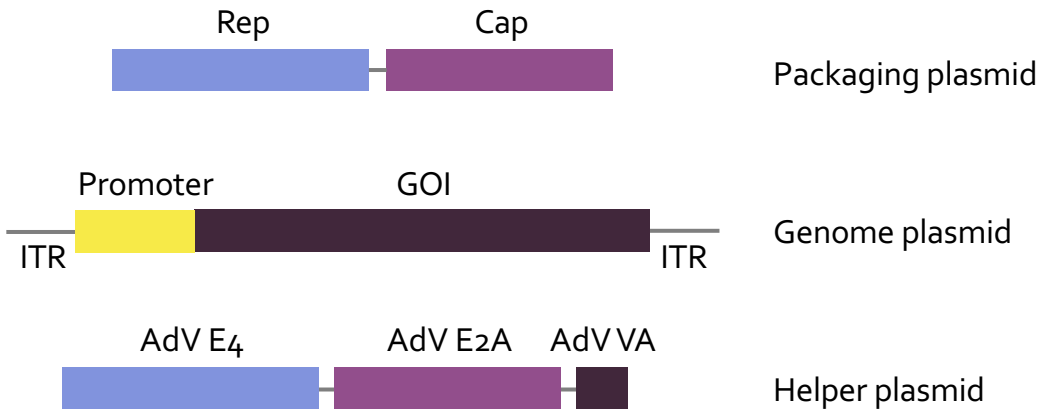


Image source: Thermo Fisher

AAV production platform: Key features

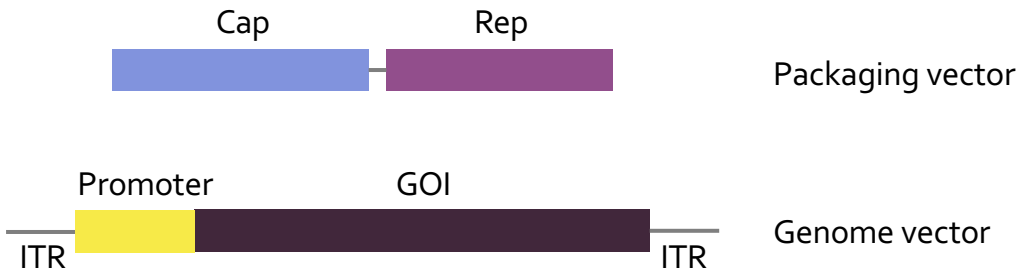
Mammalian

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

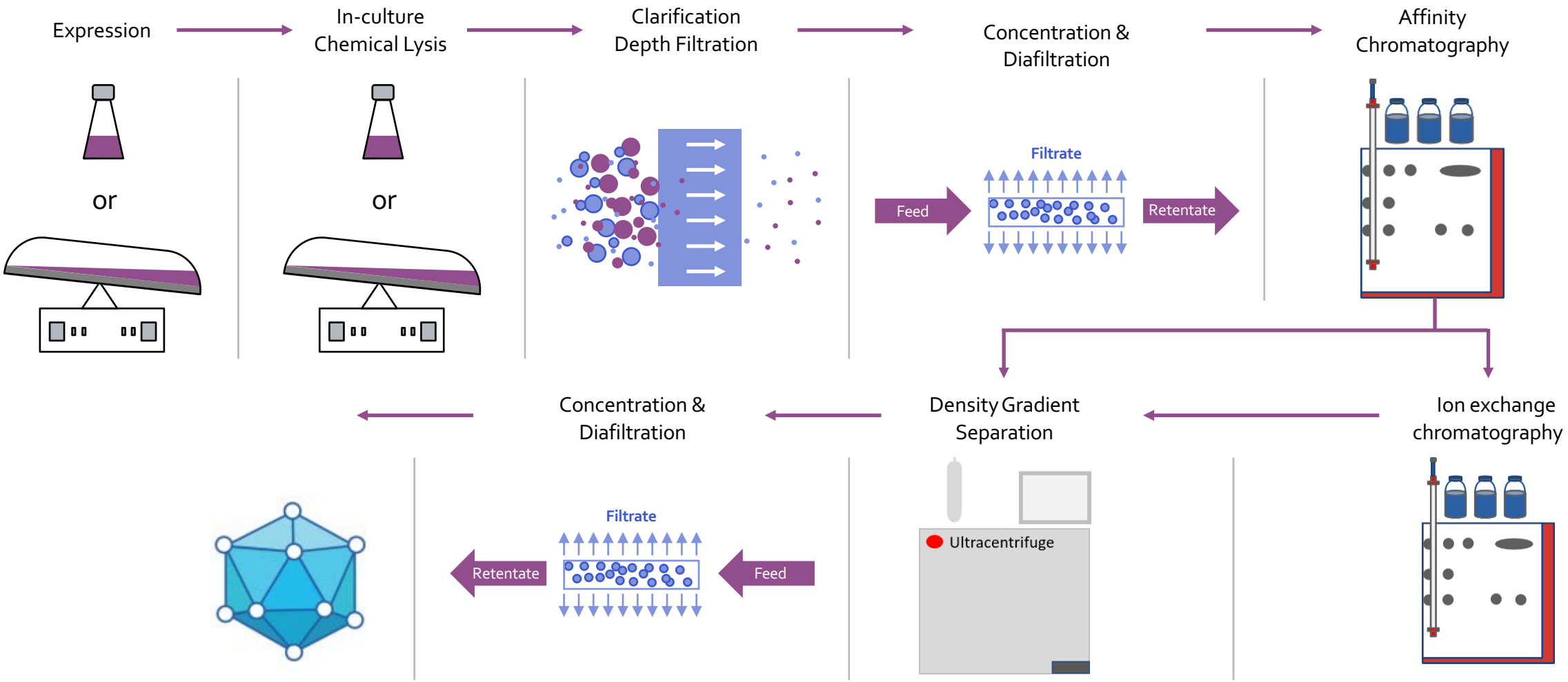


Baculovirus

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



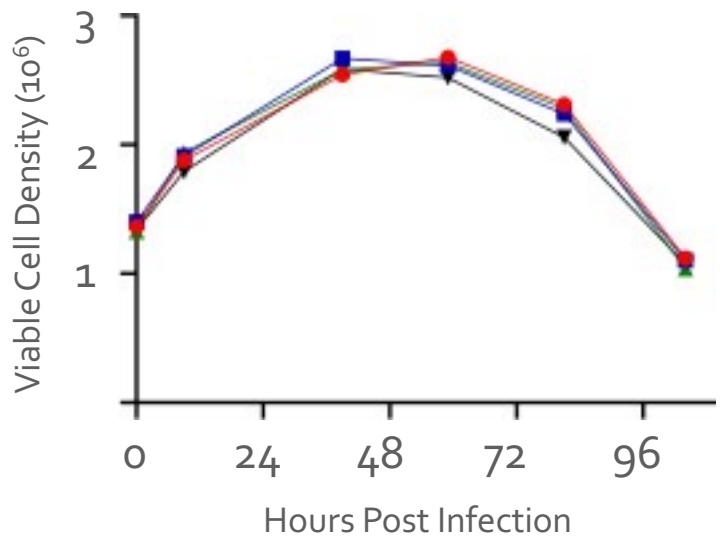
AAV bioprocess workflow

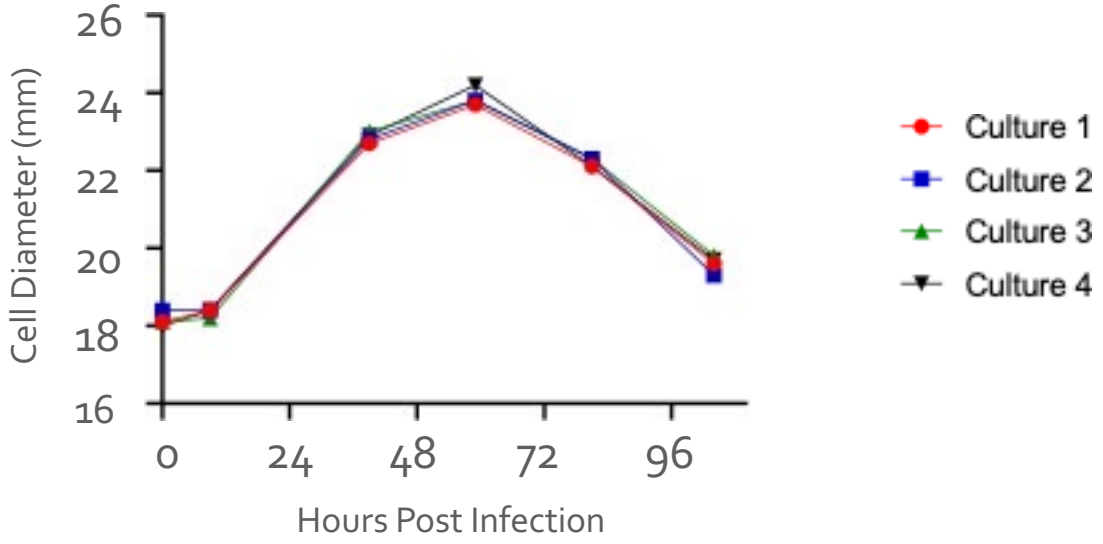


Case study: Production of AAV in insect cells (Baculovirus)

- 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

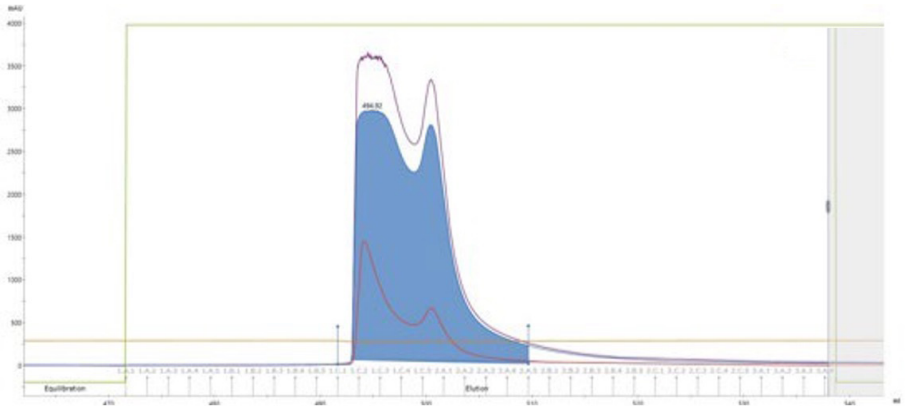
Infection of insect cells with AAV packaging and transfer vectors



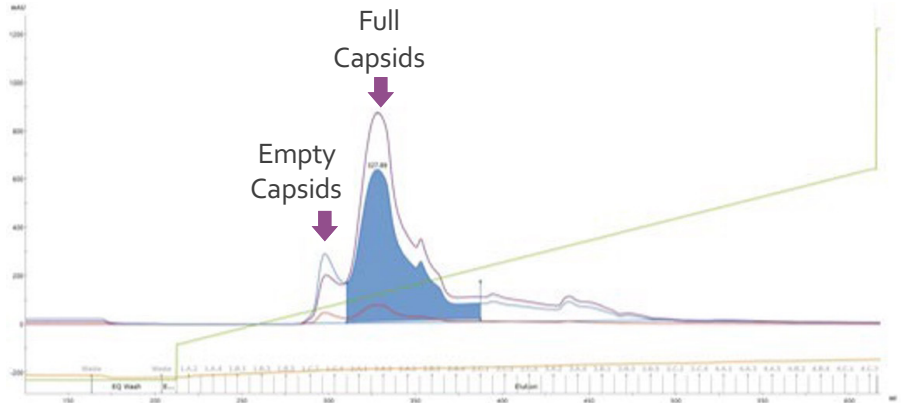


Two-step purification of AAV from insect cells (Baculovirus)

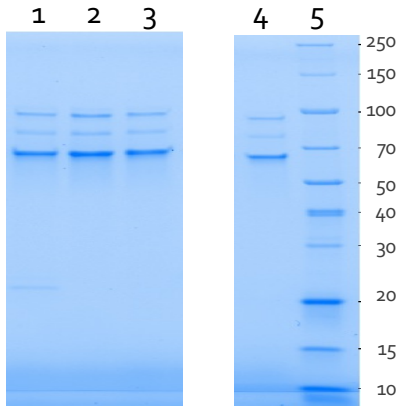
Affinity Chromatography AAV



Ion Exchange Chromatography

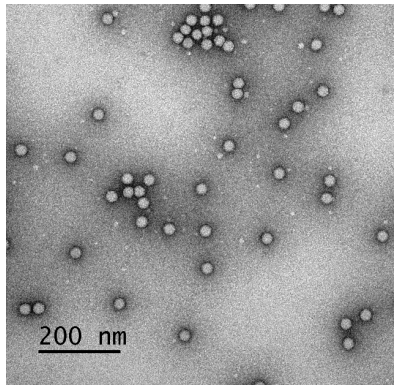


Analysis by SDS-PAGE

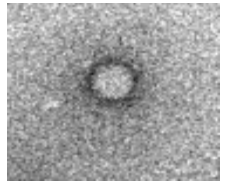


1. Affinity Purified
2. IEX Peak 1 (Empty)
3. IEX Peak 2 (Full)
4. IEX Final
5. MW Std (kD)

Electron Micrographs of Purified AAV

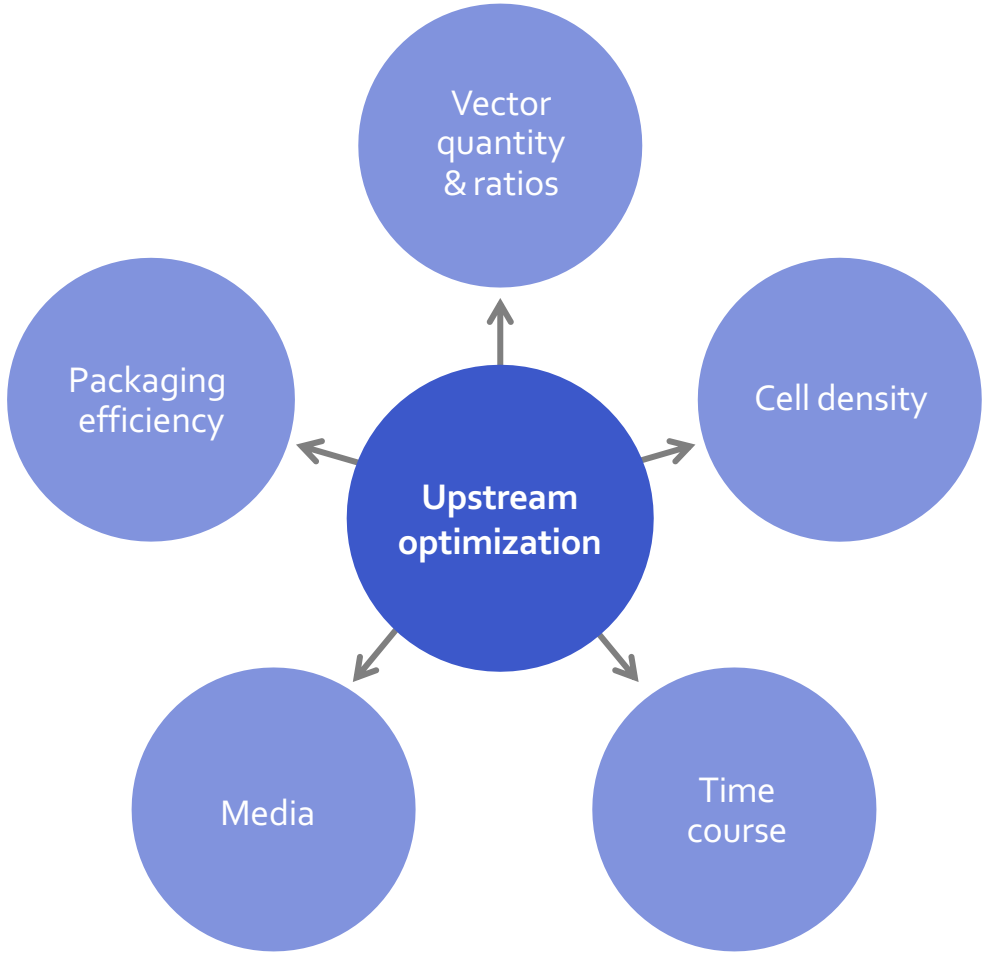


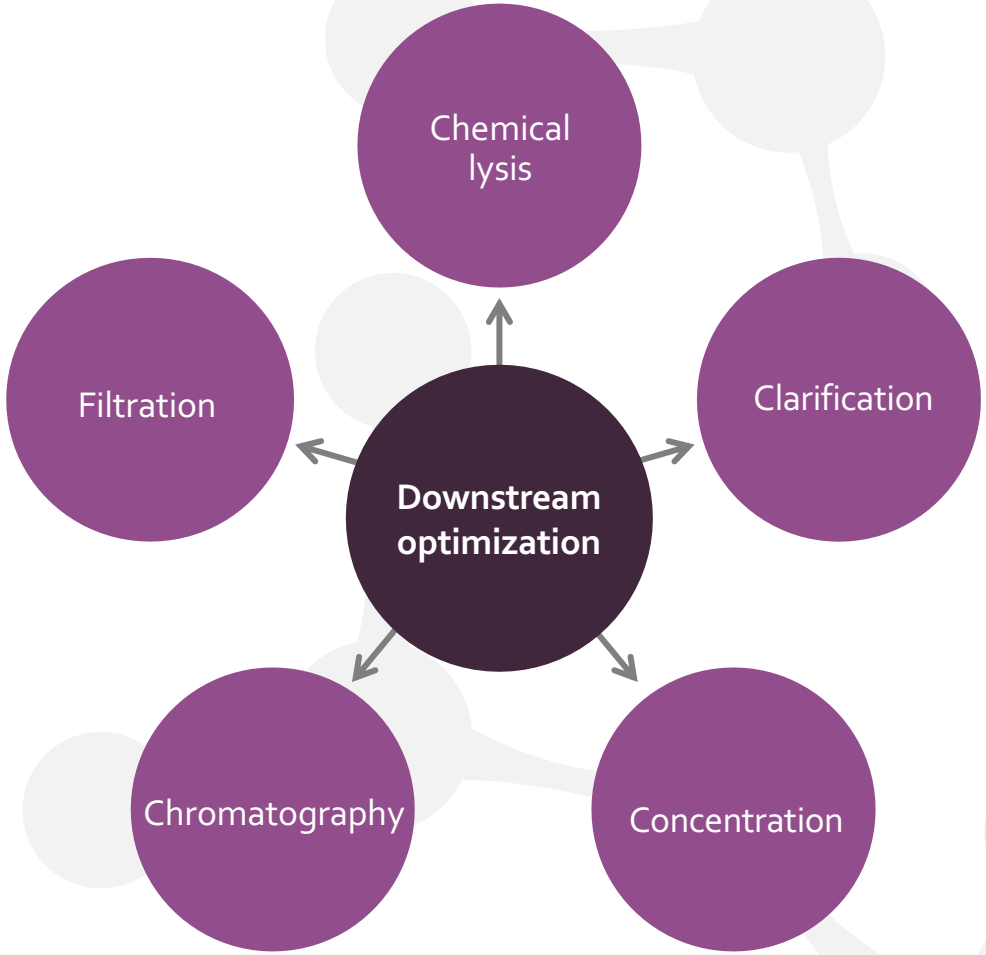
Empty capsid



Full capsid

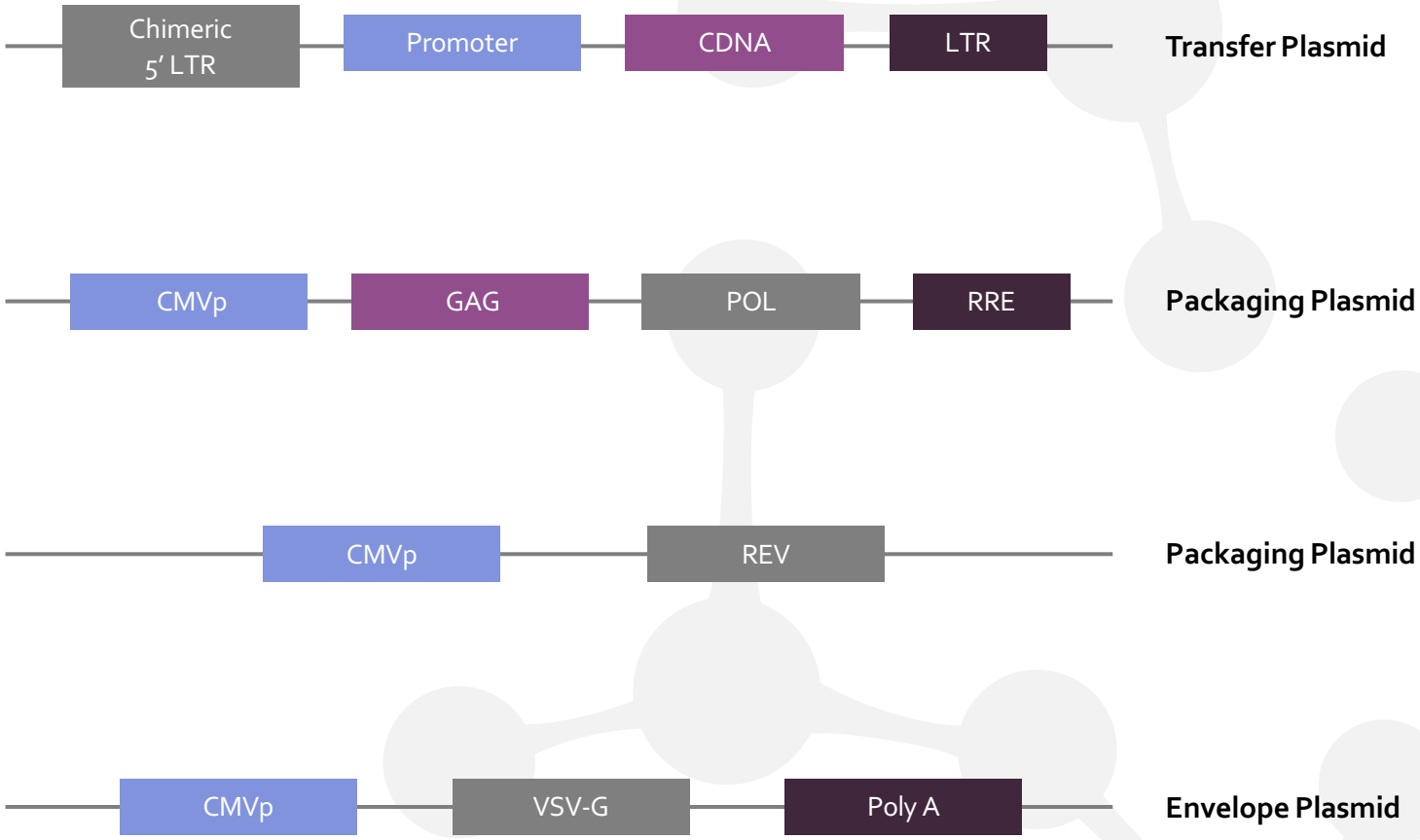
Process development services: Key features





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: 107 to 109 IFU/mL
- Pseudotyping



Cell line engineering service: Key features

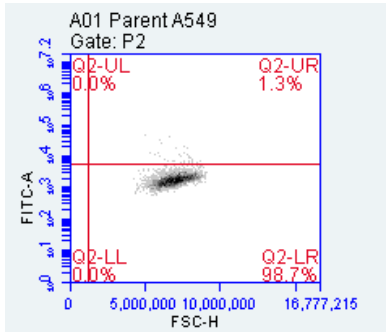
- Variety of cell types
  - » HEK293, CHO, A549, hematopoietic (T cells, including CAR-T)
  - » Hundreds of engineered cell lines created
- Target types
  - » Enzymes, antibodies, membrane/structural proteins, reporter proteins, etc.
- Antibiotic titration (kill curve) analysis
- Gene delivery
  - » 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

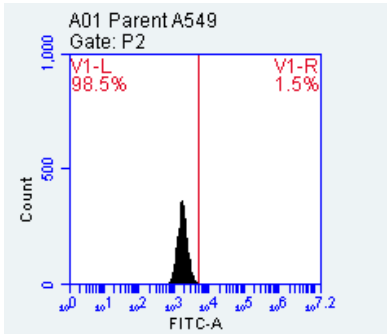
Case study: Cell line engineering utilizing Lentivirus

- 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

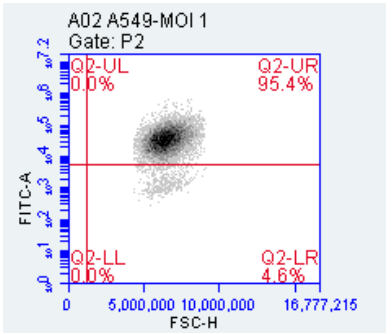
(Background Fluorescence)

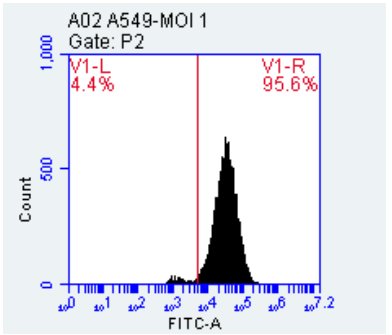




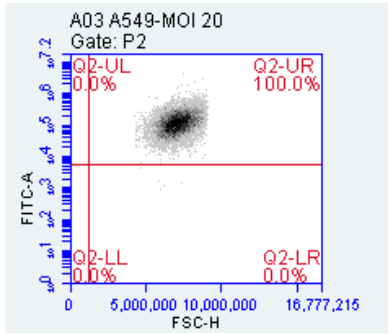
Transduced A549 Cells

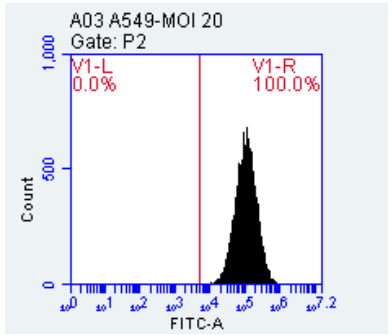
MOI = 1





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

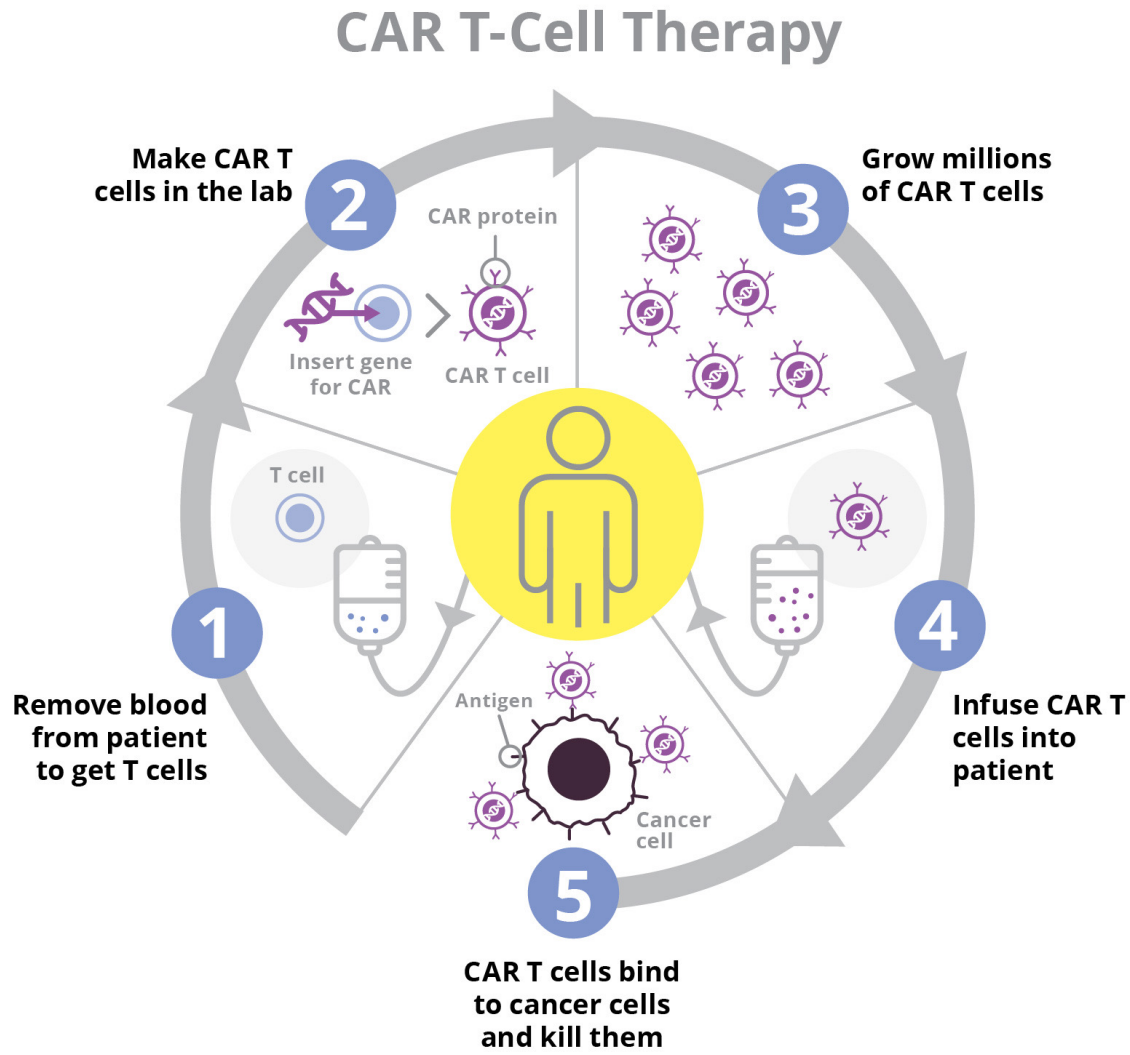
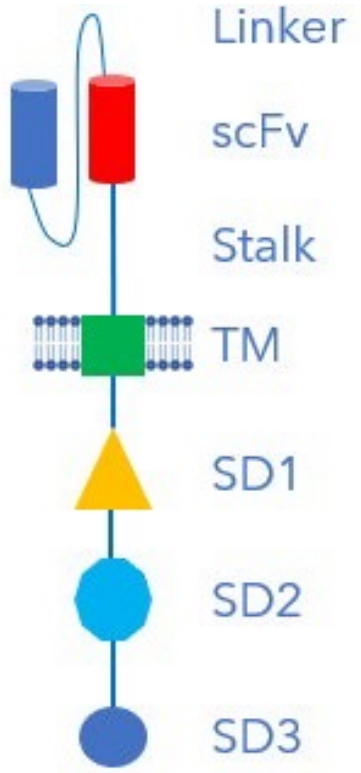


Image source: National Cancer Institute (NCI)

CAR vector selections available from Curia

Stalk	TM	Signaling domain 1	Signaling domain 2	Signaling domain 3
IgG1	CD28	4-1BB	CD28	CD3ζ
IgG1	CD28	OX40	CD28	CD3ζ
CD8A	CD8A	4-1BB	CD28	CD3ζ
CD8A	CD8	OX40	CD28	CD3ζ
IgG1	CD28	IL12	CD28	CD3ζ
CD8A	CD8A	IL12	CD28	CD3ζ



Custom design and engineering available upon request

Working with Curia

- Complete technology platform provided by Curia
- 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

To learn more visit
curiaglobal.com/biologics/viral-vectors-cell-engineering

For inquiries, email us at bio_inquiries@curiaglobal.com