
Cellular Scale-Up

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Cell Lysis

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Ion Exchange Chromatography

DNA Removal

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Ultra and Sterile Filtration

Titration by qPCR

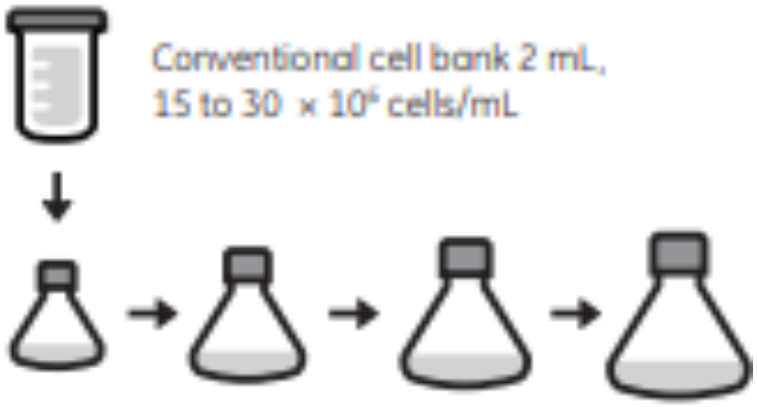
Fill and Finish

## Cellular Scale Up

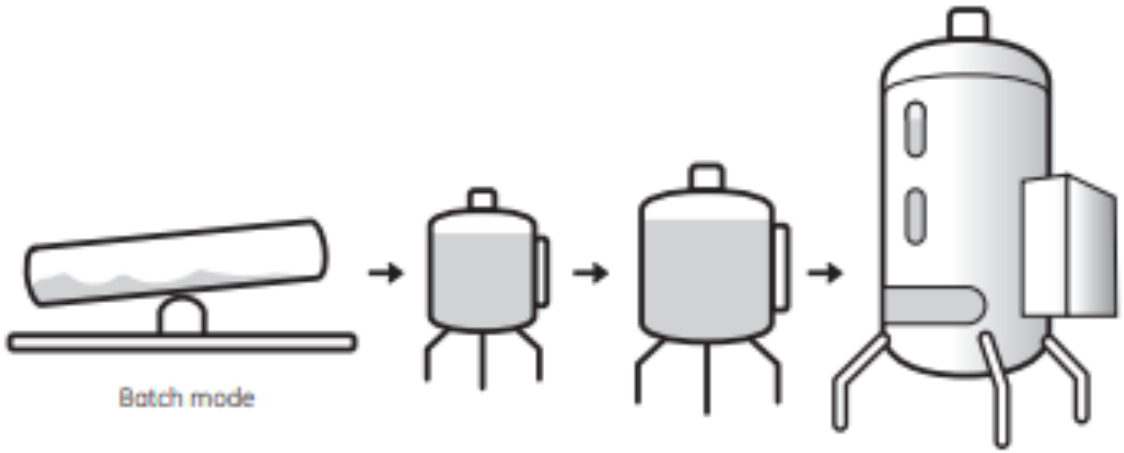
HEK293 cells start in a 2mL cryovial and are moved to a 500mL shaker flask to be scaled up. Scale up is necessary for keeping cell density of media consistent.

Small Scale Bioreactor

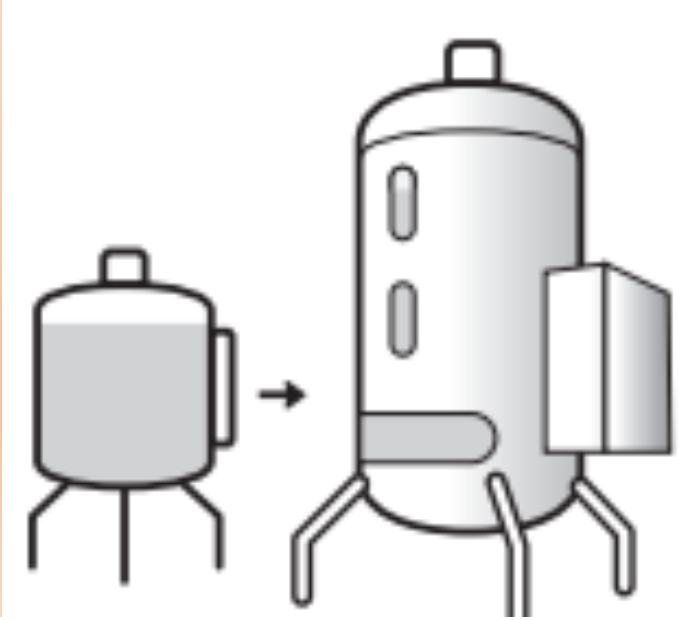
Large Scale Bioreactor



From the shaker flask, cells are moved to cell bags/ bioreactors between 5 and 15L

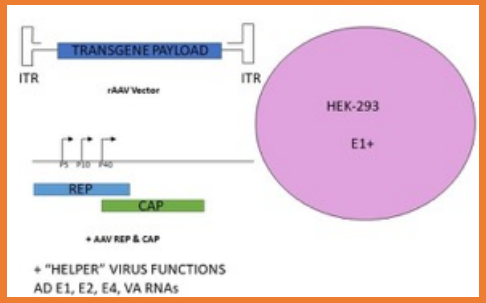


From the smaller scale bioreactor, the cells are scaled up to a larger bioreactor between 50-1000L



## Vector Transfection

HEK 293 cells express adenovirus helper genes E1a and E1b55k, so these cells are used as the viral packaging cell line. Due to the non-pathogenic nature and ability to replicate in many different cell types, adeno-associated viruses are an ideal candidate to be used in viral-vector gene therapy

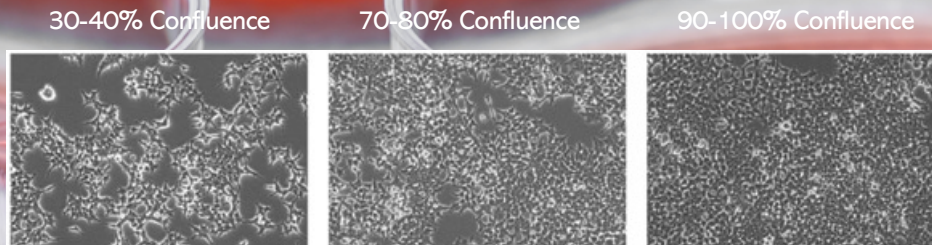



Transfection Process

## Transfection Process

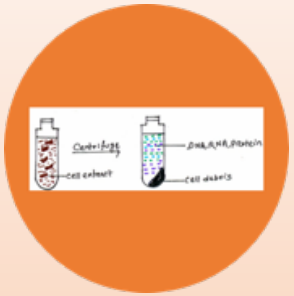
1. For adherent cells, plate between 0.25-1x10^6 cells in 2mL of growth media per well (the goal is to obtain 70-95% confluence); for suspension cells, plate 3.5x10^6 cells in 2ml per well
2. Plasmid DNA is diluted in media and incubated for 5min
3. Mix diluted plasmid DNA with reagent and incubate for 20min
4. Add this mixture to wells containing cells and media, then incubate in CO2 incubator for 24-72 hours

Each plasmid will need to be in a 1:1:1 molar ratio for proper transfection.



## Cell Lysis via Centrifugation

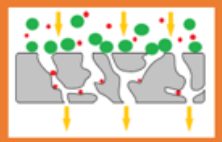
Centrifugation causes the HEK293 cells to lyse (cell walls to break), allowing the virus to be further purified out in future steps

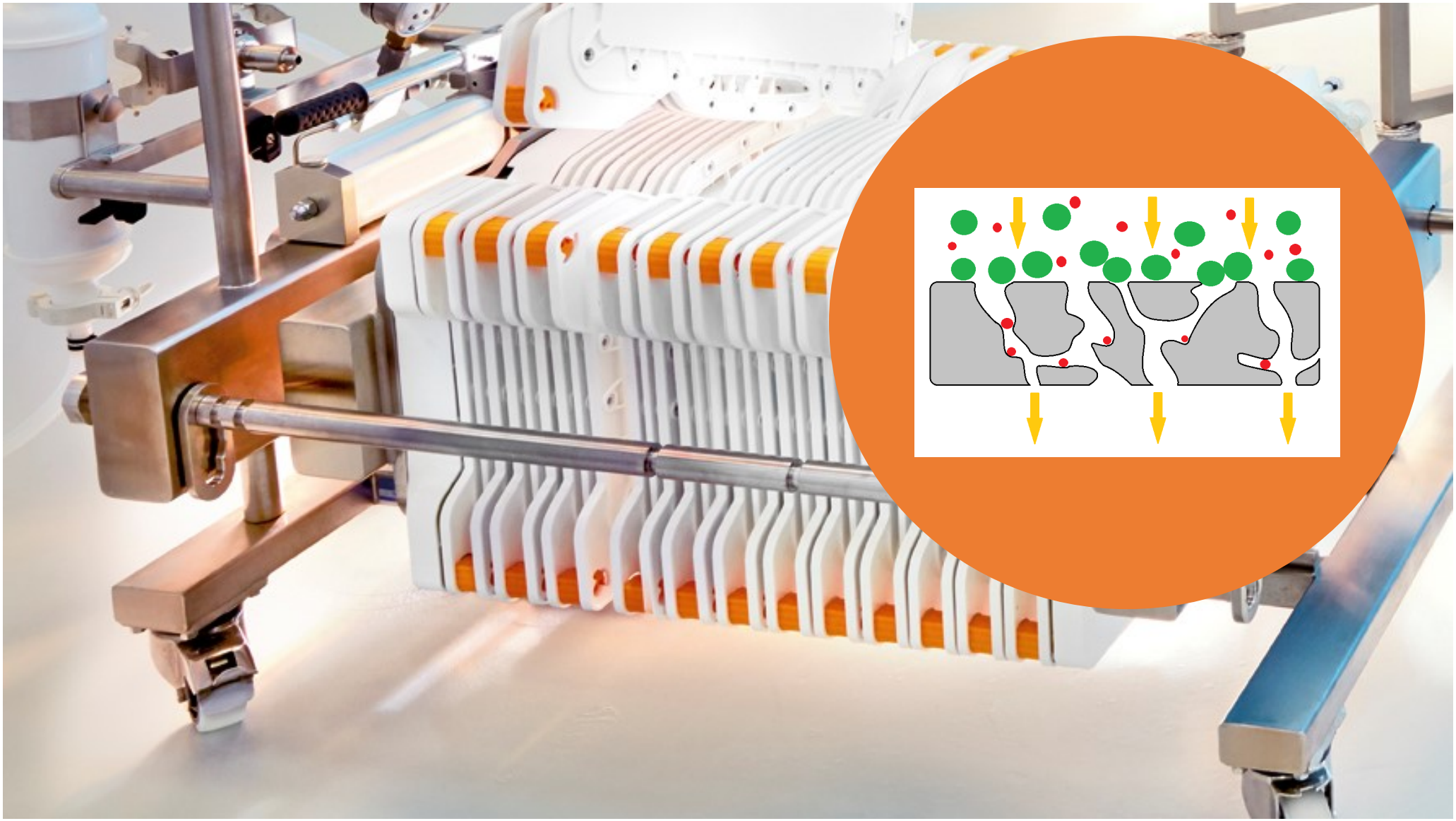



Centrifuge
cell extract
DNA, RNA, Protein
cell debris

## Depth Filtration

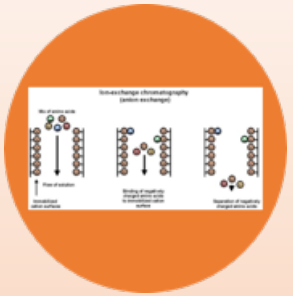
Clarification of the media, with a portion of suspended solids and organic matter being removed from the wastewater

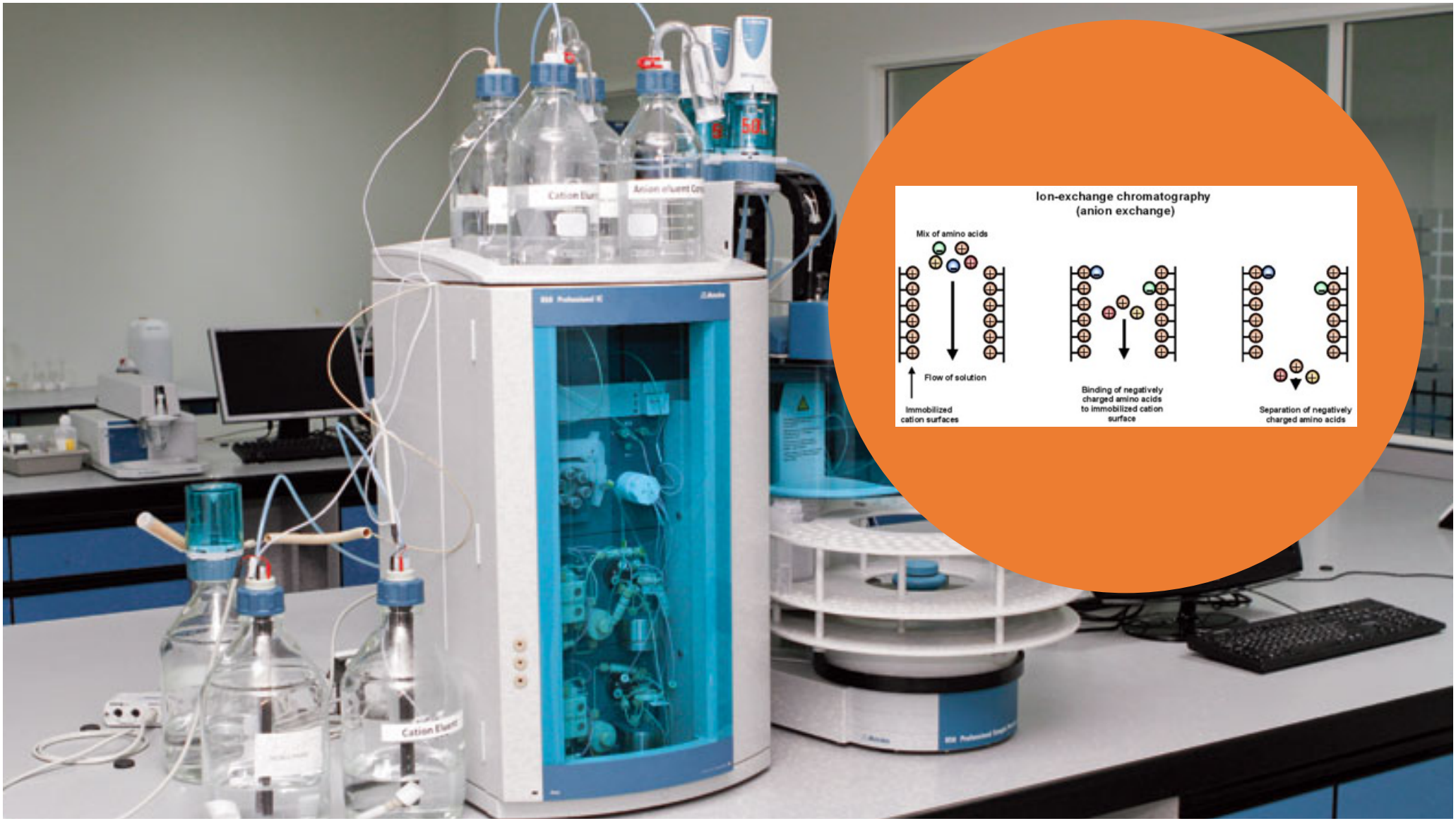




## Ion Exchange Chromatography

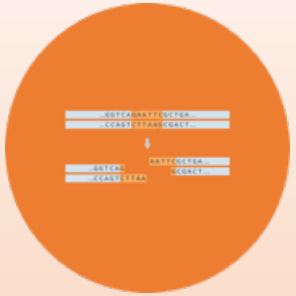
Because rAAV viral particles contain multiple sites to bind anion exchangers, anion exchange chromatography is widely used in the purification of rAAV.

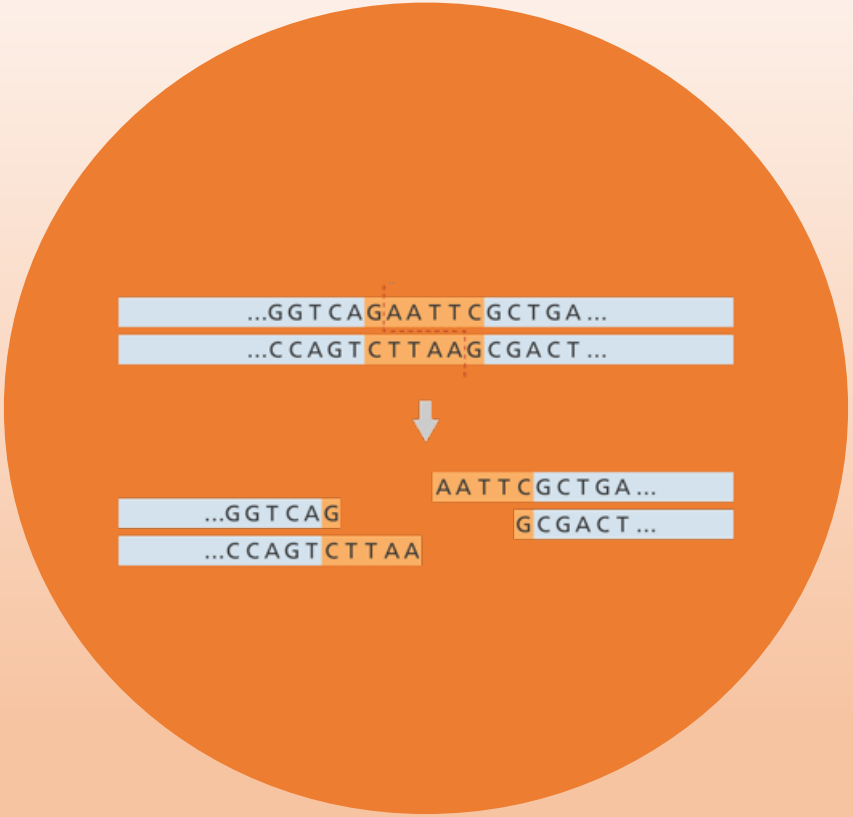



Ion-exchange chromatography
(anion exchange)
Mix of amino acids
Flow of solution
Immobilized
cation surfaces
Binding of negatively
charged amino acids
to immobilized cation
surface
Separation of negatively
charged amino acids

## DNA Removal

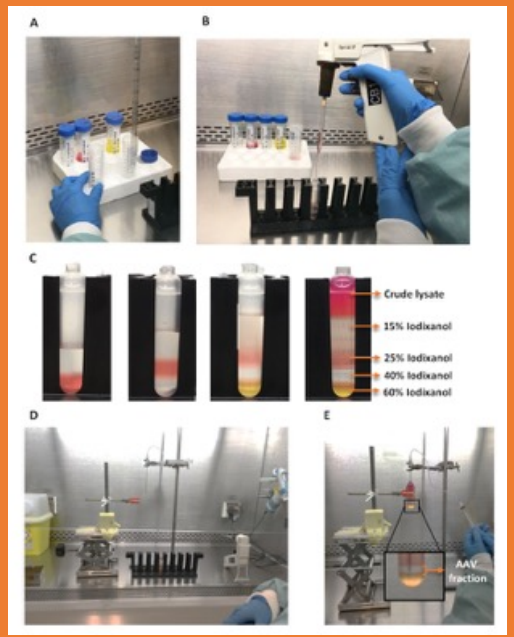
Endonuclease is added to the rAAV solution in order to extract the DNA for isolation of the viral vector




...GGTCAGAATTCGCTGA...
...CCAGTCTTAAGCGACT...
AATTCGCTGA...
...GGTCAG
GCGACT...
...CCAGTCTTAA

## Iodixanol Centrifugation

An iodixanol gradient is created due to a gradually increasing density within the tube. This allows the virus to be isolated and removed for further purification.


A
B
C
Crude lysate
15% Iodixanol
25% Iodixanol
40% Iodixanol
60% Iodixanol
D
E
AAV fraction

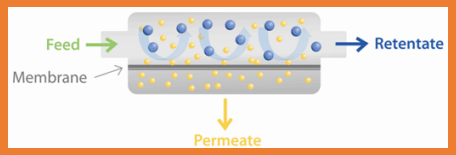
## Filtration

The next step in production is ultra and sterile filtration. Ultrafiltration can be performed via buffer exchange and centrifugation or a tangential flow filtration system. Sterile filtration is then performed with a 0.22-um filter to ensure purity of the product.

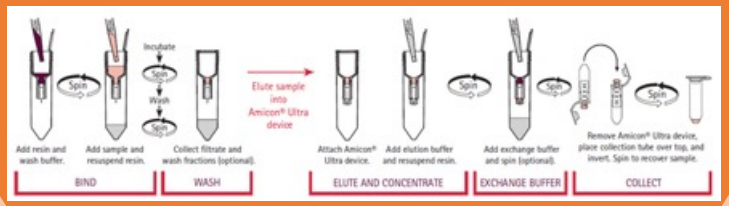
Ultrafiltration

Sterile Filtration

## Tangential Flow Filtration



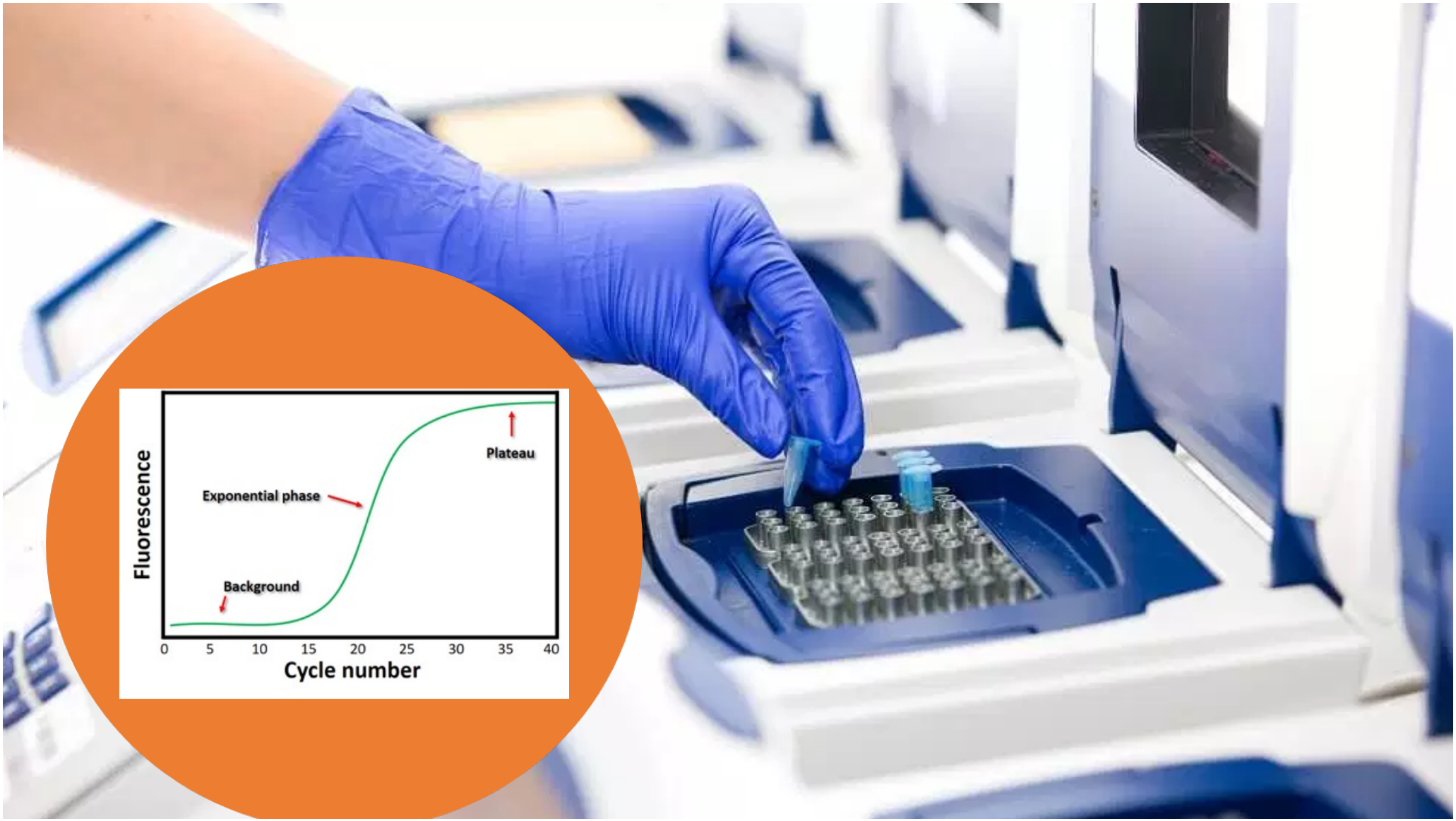

## Buffer Exchange & Centrifugation




## Titration by qPCR

In order to test for residual Plasmid DNA and determine clarity, a qPCR (quantitative Polymerase Chain Reaction) assay is performed.


1 Denaturation
F
F
F
F
2 Annealing
Tag
F
F
F
F
3 Extension
Tag
F
F
F


Fluorescence
Plateau
Exponential phase
Background
0
5
10
15
20
25
30
35
40
Cycle number


Fill and Finish
The final step in rAAV production is aseptic filling into a sterile container (usually glass or plastic sterile vials), sealing with a rubber stopper, and crimping with an aluminum shell.