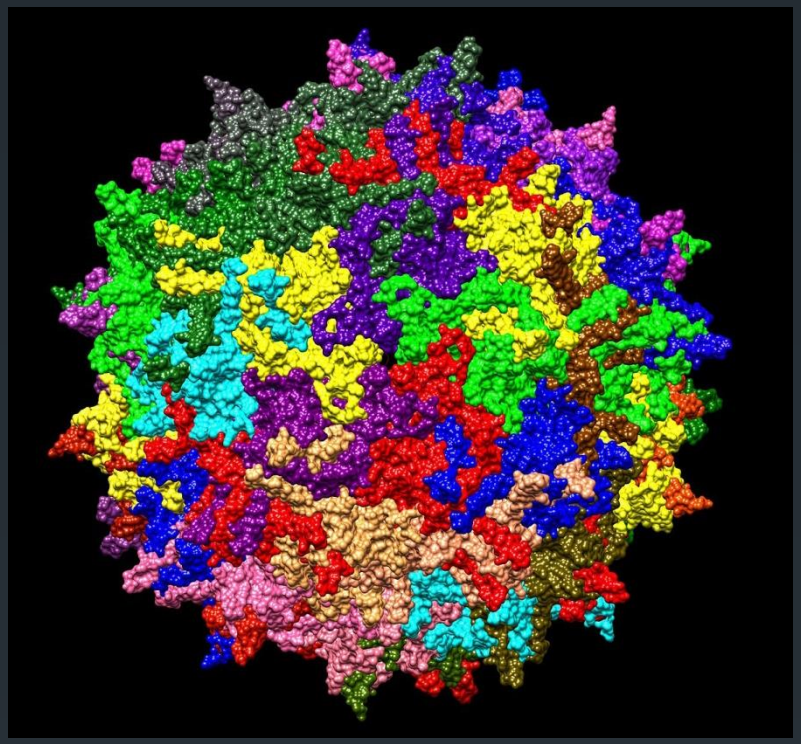
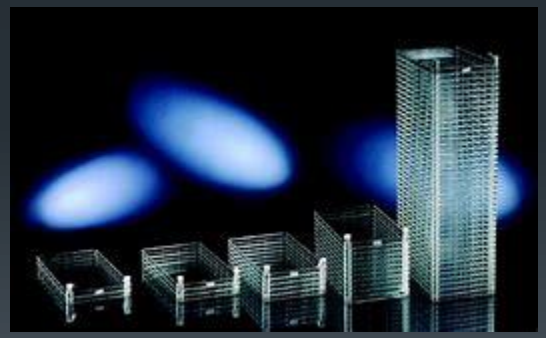


AAV-Process Flow-Chart

Mason Snead

1st Step - Cell Expansion

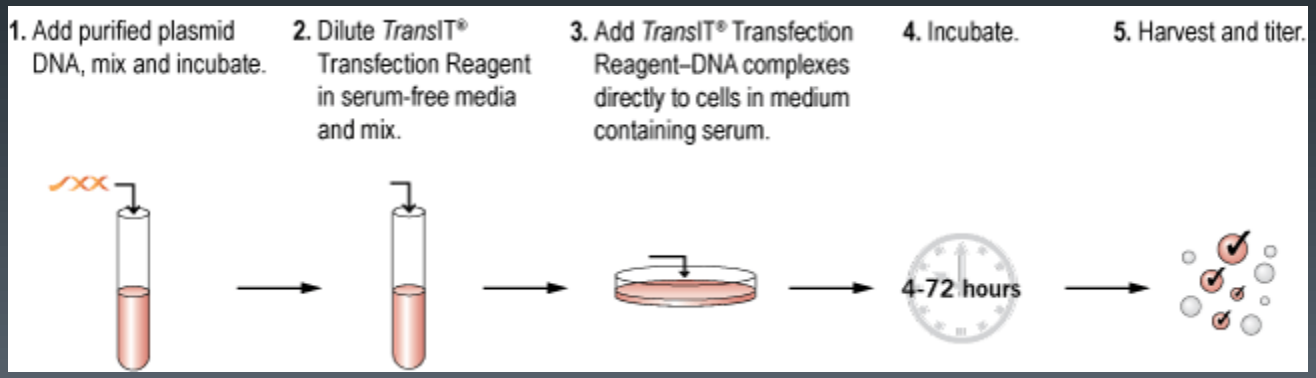
- We first need a master cell bank
- Cell Expansion in adherent cell culture
- Adherent cell factories



- Plate HEK-293 cells
- Plasmid DNA should be of high quality and free of RNA contaminants.

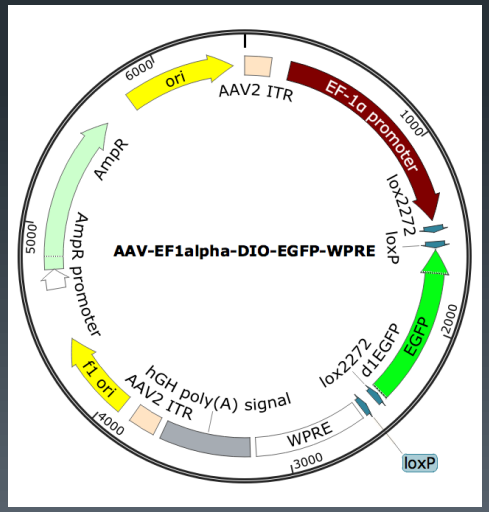
2nd Step – Pre-Plasmid Transfection

- Transfection with 2-4 plasmid vectors
  - pFdelta6, pRV1, pH21, and AAV
- Optimized harvest and transfection



3rd step – Post-Plasmid Transfection

- Virus production phase post-transfection
- Filter the transfection mixture into a 50 ml tube.
- Optimized harvest and transfection



4th Step – Harvest AAV

- 72 hours after transfection, remove media from cell culture plates and discard.
  - All waste should be treated with Virkon solution or other suitable disinfectant.
- Virus extraction, clarification, and concentration
- Recovery and stabilization of virus from cell-culture system
- Incubate at 37°C for 1 hour

5th Step- Purify & Concentrate AAV's

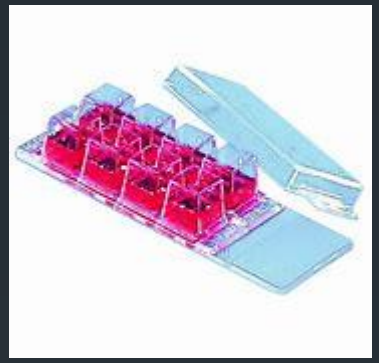
- Setup HiTrap heparin columns using a peristaltic pump so that solutions flow through the column. It is important to ensure no air bubbles are introduced into the heparin column.

- Purification Process ahead of DS Formation

- Concentrate vector using Amicon ultra-4 centrifugal filter units with a 100,000 molecular weight cutoff.

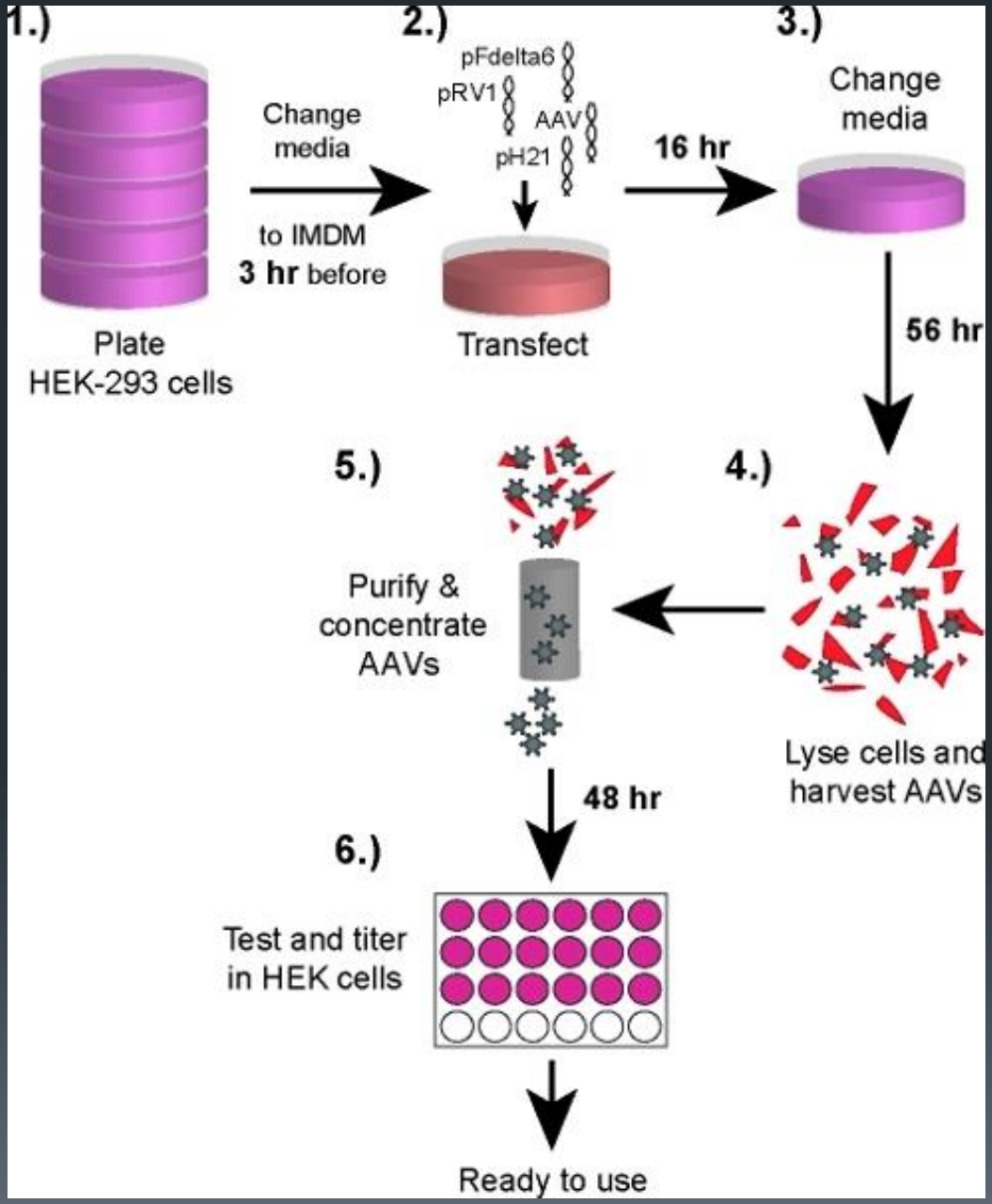
6th step – Test and titer in HEK cells

- Vector should be aliquoted and stored at negative 80°C until required.
- This requires a promoter that is active in Hek293 cells driving a reporter gene or an immunocytochemically detectable gene product.



jove-57-3348.mp4

Summary -


1.)
2.)
3.)
pFdelta6
pRV1
AAV
pH21
Change media
to IMDM
3 hr before
16 hr
Change media
Plate HEK-293 cells
Transfect
56 hr
5.)
4.)
Purify & concentrate AAVs
Lyse cells and harvest AAVs
48 hr
6.)
Test and titer in HEK cells
Ready to use

AAV

1. Add purified plasmid DNA, mix and incubate.
2. Dilute *TransIT*® Transfection Reagent in serum-free media and mix.
3. Add *TransIT*® Transfection Reagent–DNA complexes directly to cells in medium containing serum.
4. iter.