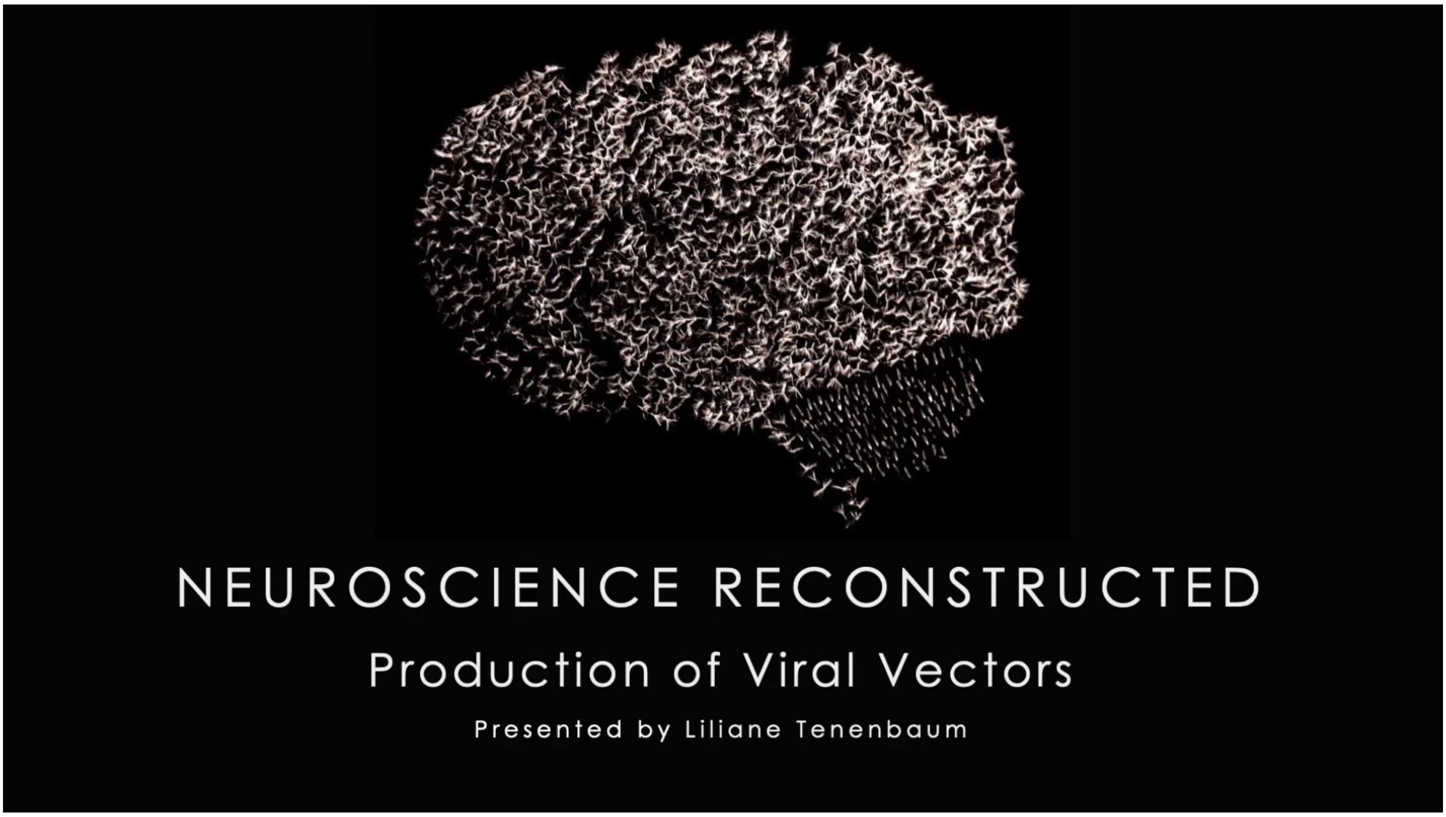

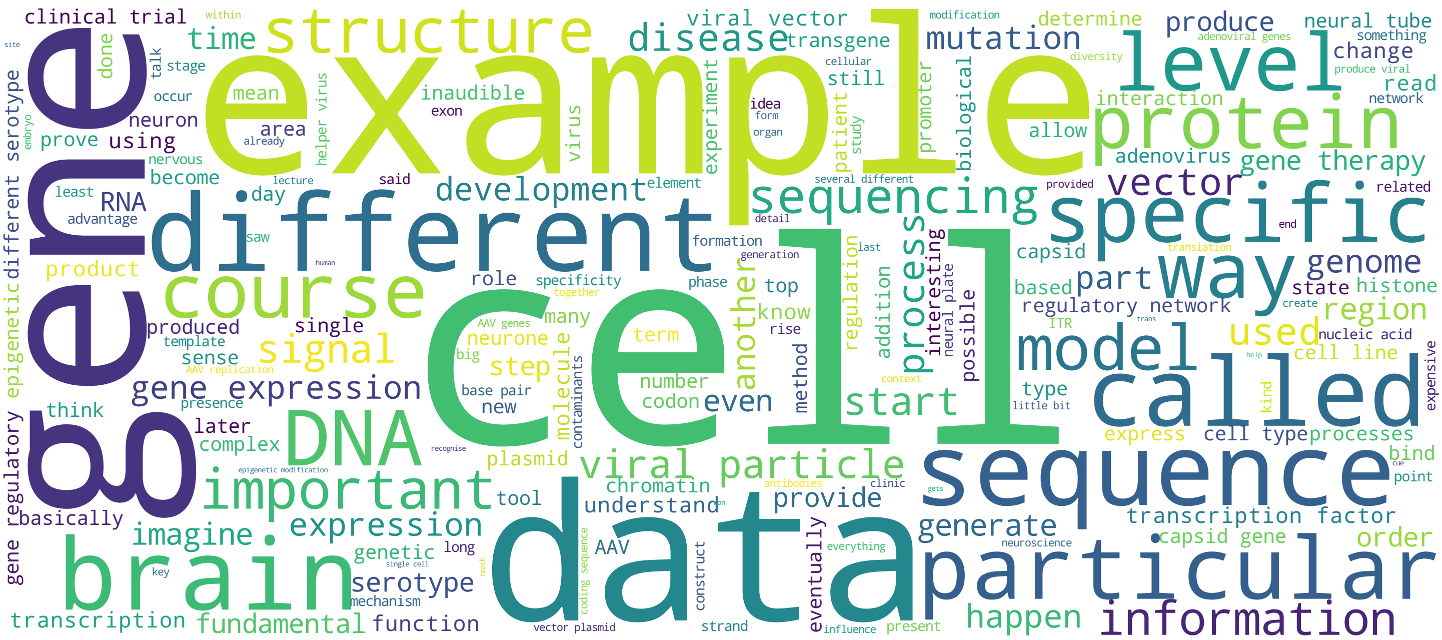

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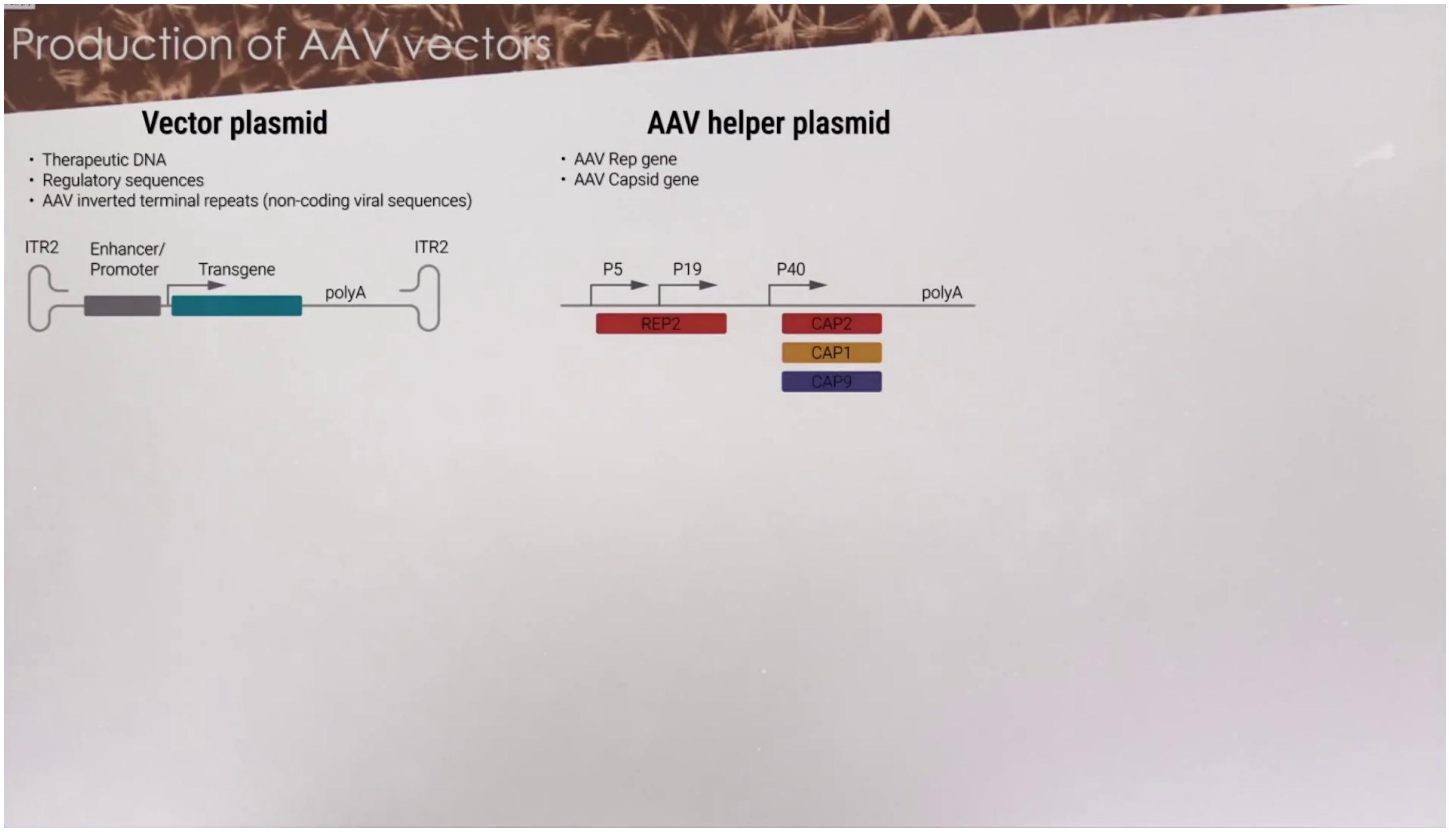
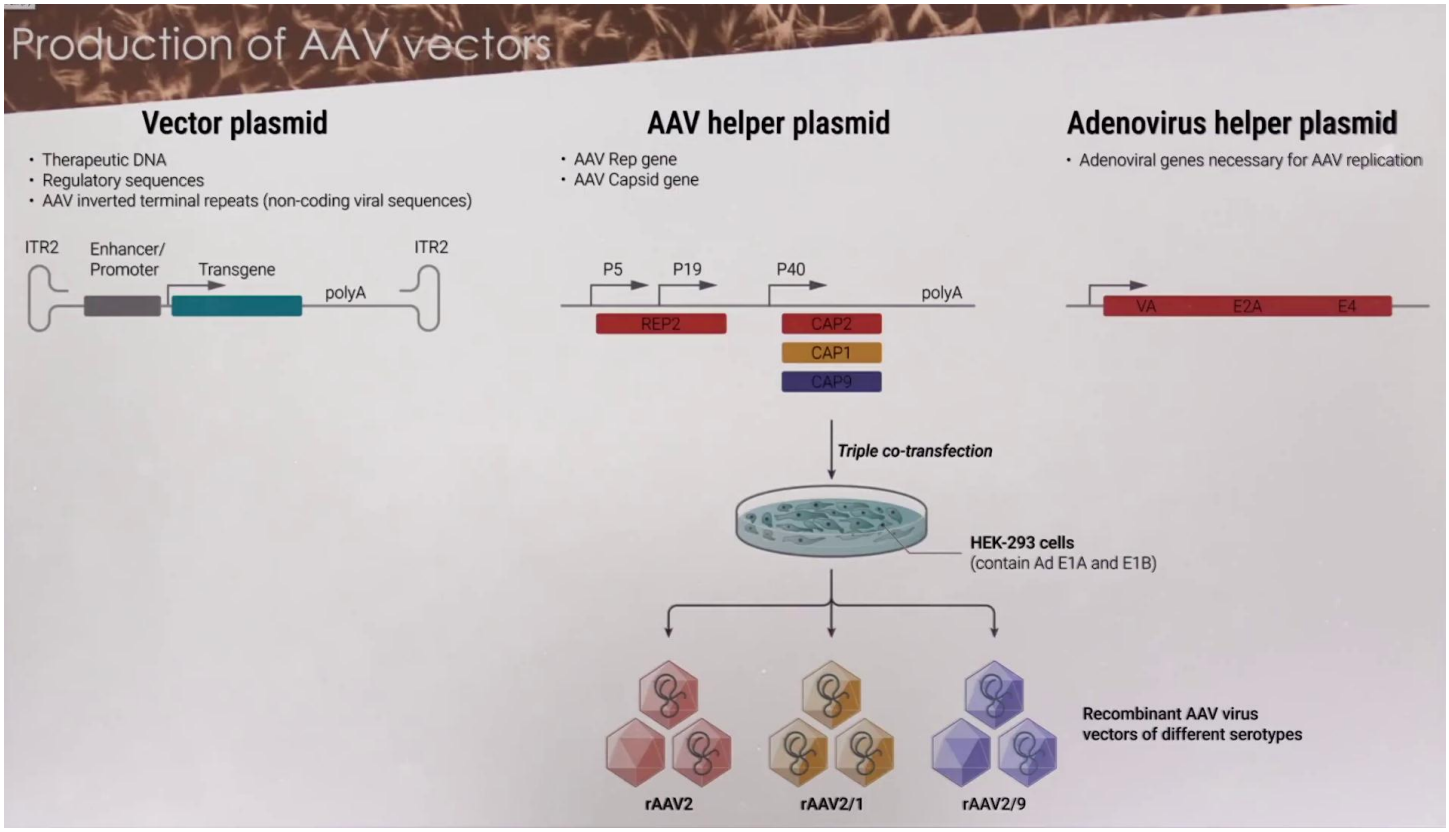


Now, how do we produce viral vectors? First of all, we have to construct a vector plasmid. A plasmid that will have our transgene and the sequences required to express the transgene is a promoter with enhancers and polyadenylation sequence. This plasmid will only return small element from AAV. These are these 145 base pairs inverted terminal repeats. These are noncoding sequences, and they are particular in the sense that they are palindromic. Also the container high percentage of GC base pairs, and this confers to these extremities a very high stability. Now, in addition to this vector, to be able to amplify it to big amounts and package it into viral particles, we will need to use transiently coding sequence from AAV and also from adenovirus, which is the helper virus. These sequences, the AAV sequences were removed from the vector plasmid because as I said before, we don't want the recombinant virus to replicate in AAV. But to produce it, we will have to provide in trans on another plasmid the AAV genes, it's a very simple genome. We have the Rep gene. Here, I have written REP2 because it produces Rep protein from the AAV serotype 2, which recognise and bind to the ITR of AV2.

Notes

Summary

0m 05s

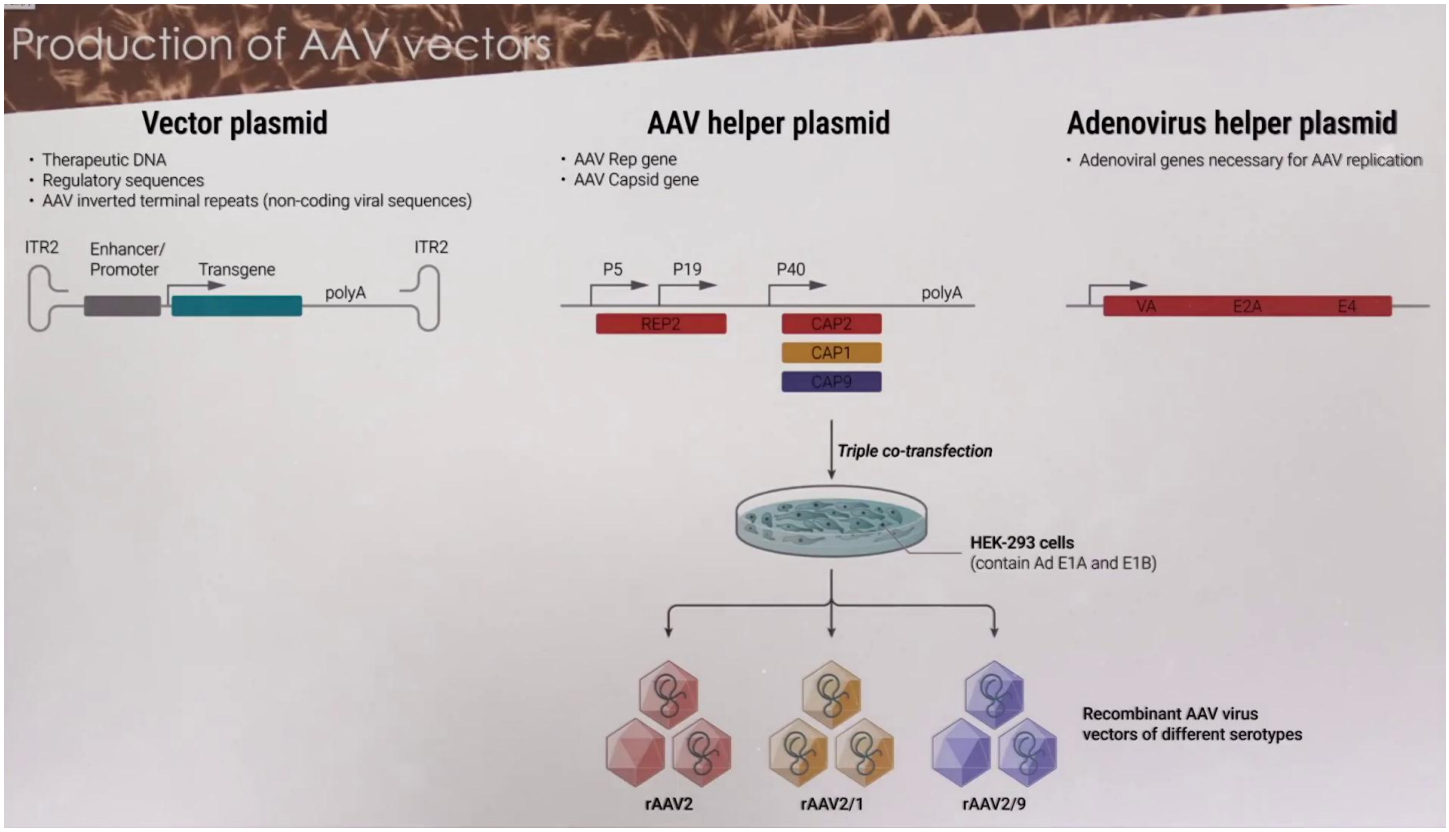


We will need also the capsid gene coding for the three proteins forming the capsid. The interesting thing is that although the RAP gene has to be of the same serotype as the ITR, the capsid gene can be from several different serotype, and so with only one vector, we are able to produce viral particles with different serotypes. In addition to the AAV genes, we need also adenovirus genes, since AAV replication can only occur in the presence of a helper virus such as adenovirus, so we need to provide a third construct, a third plasmid, expressing some adenoviral genes. These three plasmids will be transfected into cultured cells, which contain two other adenoviral genes, E1A and E1B, that are also necessary for AAV replication. This cell line called the producer cell line derives from human embryonic kidney cells and has been immortalised using adenoviral E1A and E1B genes. It's a well-characterised cell line that is widely used in biotechnology, so it can be used for GMP, good manufacturing production, and it's suitable to translate gene therapy protocols for the clinic. When we transfect these three plasmids into 293 cells, the cells will produce in 2-3 days.

Notes

Summary

2m 27s



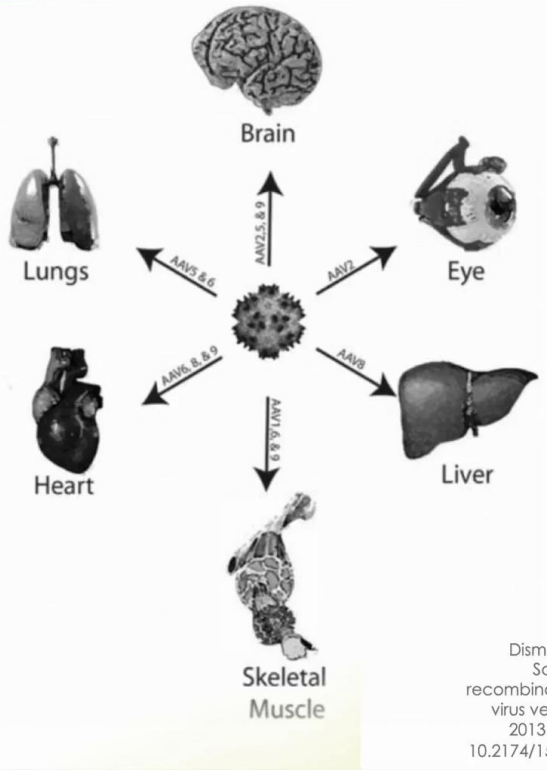
They will produce the recombinant viral particles as of the same serotype as the capsid gene that we have provided. Here, I have skipped numerous steps because as you can imagine, when we will harvest the viral particles after two, three days, the suspension will also contain huge amounts of 2933 cells, proteins, nucleic acids. It will still contain the helper plasmids. We, of course, don't want all these contaminants, so there are several purification steps to reach a purified AAV vector.

Notes

Summary

4m 44s

Efficiency of AAV vectors



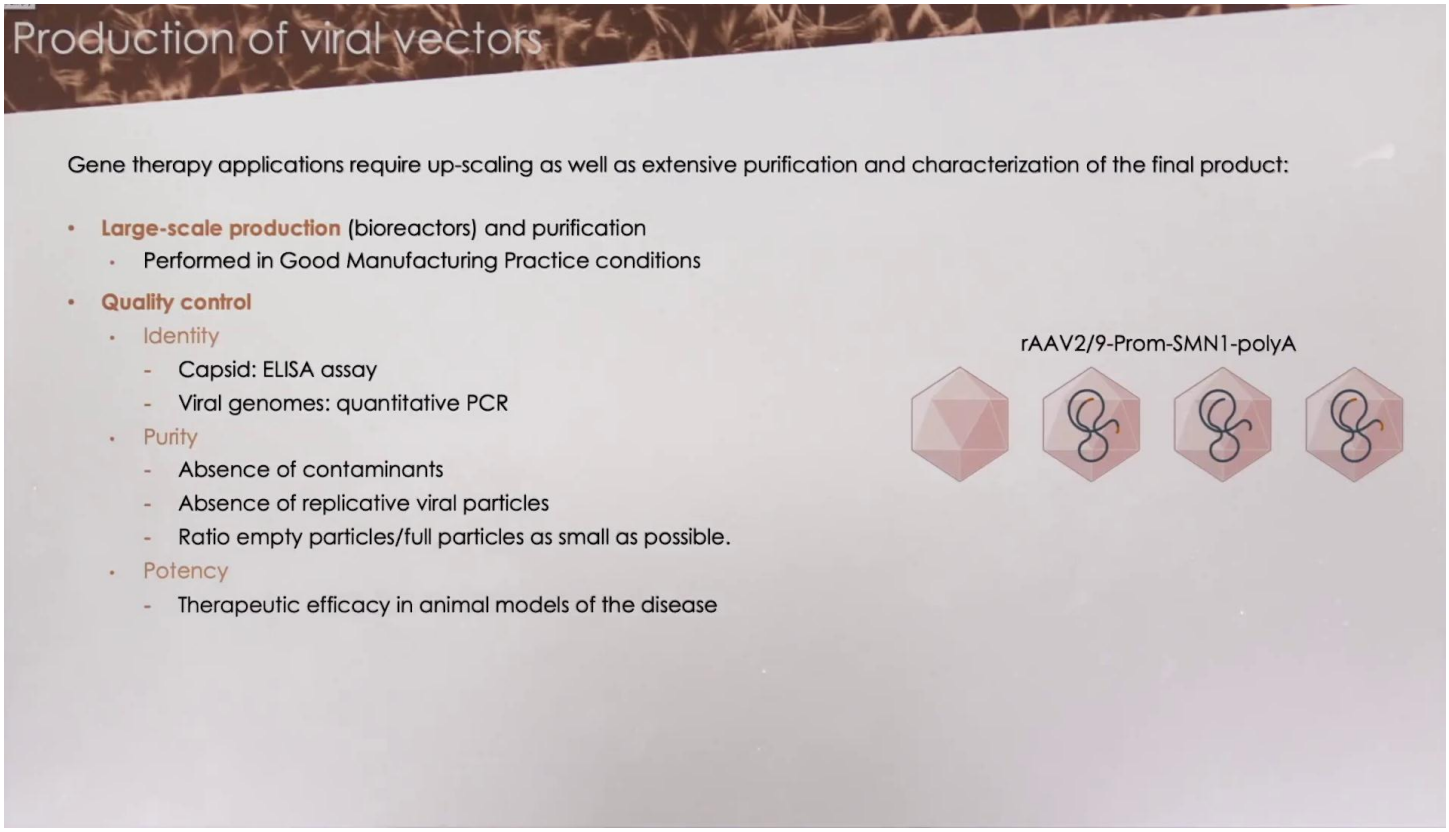
Dismuke DJ, Tenenbaum L, Samulski RJ. Biosafety of recombinant adeno-associated virus vectors. Curr Gene Ther. 2013 Dec;13(6):434-52. doi: 10.2174/15665232113136660007.

This is the advantage of having the possibility of encapsulating or construct into several different serotypes is that each serotype has particular efficiency to transduce different organs. For a given organ, some serotype will be preferred relative to others. It's not 100% specificity, but we can achieve some specificity by using an adequate serotype.

Notes

Summary

5m 44s



In order to have enough virus and preparation, a product that is acceptable for the regulatory authorities, because you have to go through all procedure to have a clinical trial accept. The virus has to be produced in very large scale, not in the small dishes that I showed before but in bioreactor, and purified. You can imagine that these processes are very cumbersome and expensive. What is even more expensive is the quality control. You have to prove the identity of your product to prove that it has the capsid that you expected. You need antibodies to identify the capsid using an ELISA assay. Then you have to prove that the genome is what you say, so you have to characterise it by quantitative polymerous chain reaction. You have to demonstrate the absence of contaminants. Also that the preparation doesn't contain replication-competent viral particles. And last but not least, during the transfection, some particles are produced that don't package DNA. You can imagine that if you have a large amount of these viral particles, this will enhance the immune reaction without any efficiency for gene therapy. The production methods had to be improved in order to have the highest possible percentage of full particle.

Notes

Summary

6m 30s

Production of viral vectors

Gene therapy applications require up-scaling as well as extensive purification and characterization of the final product:

- **Large-scale production** (bioreactors) and purification
  - Performed in Good Manufacturing Practice conditions
- **Quality control**
  - Identity
    - Capsid: ELISA assay
    - Viral genomes: quantitative PCR
  - Purity
    - Absence of contaminants
    - Absence of replicative viral particles
    - Ratio empty particles/full particles as small as possible.
  - Potency
    - Therapeutic efficacy in animal models of the disease

rAAV2/9-Prom-SMN1-polyA

This is also very long development. The final preparation, even if everything was done in pre-clinical settings in animals, with the final viral vector, the therapeutic efficiency has to be again demonstrated.

Notes

Summary

8m 26s