BIOPROCESS FOR THE PRODUCTION OF GENE THERAPY FOR HAEMOPHILIA A:

Bioprocess design part I

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AUTHORS: Beatriz García and **Ana Marrodan**
TUTOR: Carles Solà Ferrando

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1. BACK GROUND

Why is Haemophilia type A an attractive candidate for gene therapy ?

Haemophilia type A is a life threatening X-linked genetic disease. Patients have a defective form of the gene that codes for Factor VIII in the coagulation cascade, causing recurrent hemorrhages and inflammation of tissues.

- There are around 400,000 people in the world (~50,000 in the EU) who suffer from hemophilia A.
- There is no cure.
- Symptomatic treatments cost 50,000-150,000 € per patient annually to the Social Security System.

Why Adeno-Associated Virus (AAV)?

- Small size: mild immune response
- Strong liver tropism (serotype 8)
- Safe DNA transduction: episomes
- Gene size limitation: 4.7kb. FVIII gene:11.8kb long (new research suggests it can be cut to fit the capsid)

2. BASES OF DESIGN

Objective: 48,000 doses in 3 years (EU Market)

Product Critical Quality Attributes:

- Dose: 10^{15} AAV
- 80% Full AAV capsids
- Purity and sterility

3. ALTERNATIVES

- **Biocatalyst:** *HEK* vs. *Sf9*
- **Operational:** Fed Batch vs. Batch
- **Gradient:** CsCl vs. Iodixanol

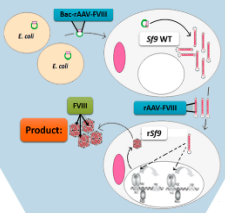
4. MOLECULAR BASES

Sf9 / *Baculovirus*

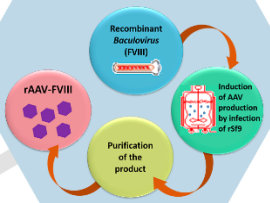
***Sf9* as biocatalyst**

- Biosafety: *Sf9* are insect cells, therefore infected by non-human viruses.
- Efficient genetic material duplication.
- Suspension culture: easier operation and reduction of facility costs.
- High cell density achievable.
- Easily transduceable by *Baculovirus*.

Genetic flow:



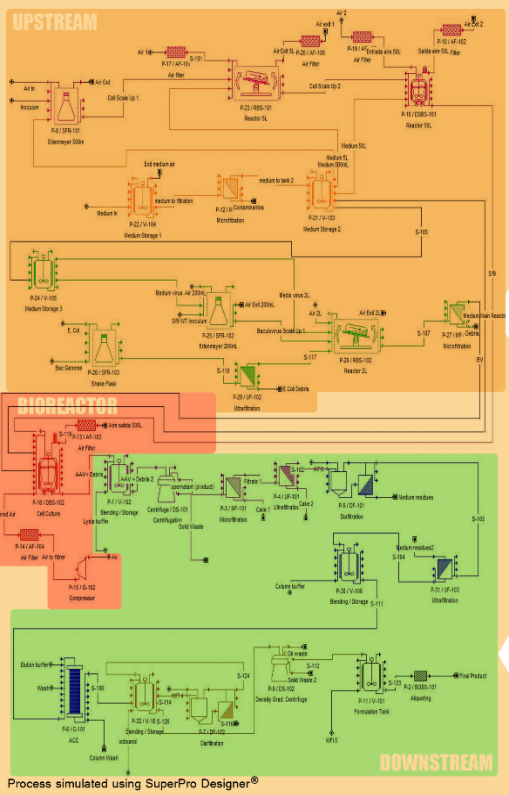
***Sf9*/*Baculovirus* system of production of AAV**



5. BLOCK FLOW DIAGRAM (BFD)

6. PROCESS FLOW DIAGRAM (PFD)

PFD



Process simulated using SuperPro Designer®

Upstream

- Cell scale up: from a 500 mL Erlenmeyer to a 50 L bioreactor cells are grown until a density of 10^9 cells/L is achieved.
- Medium storage and distribution
- *Baculovirus* scale up: the genome of the recombinant *baculovirus* are cloned into a strain of *Escherichia coli* and amplified. After a plasmid extraction *Sf9* wildtype cells are transfected with those genomes and start to produce virions thereafter. This process is continued until the cells lyse and degrade. Finally, the virions are filtered and stored .

The bioreactor

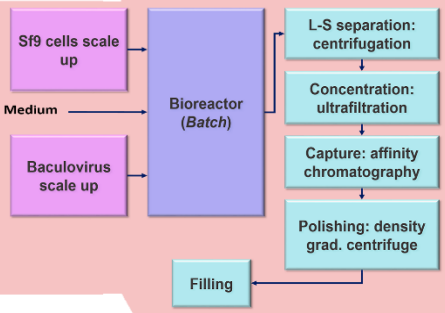
The following steps take place in a 700L single-use stirred bioreactor. It is operated discontinuously for 144h, being the process bottleneck. Three processes take place in this unit:

- Cell growth
- AAV production induction
- Cell lysis

Downstream

- Solid-liquid separation: centrifuge and microfiltration. Bulk separation.
- Product concentration: ultrafiltration allows *Baculovirus* clearance.
- Affinity chromatography: final purification from nucleic acids.
- Density gradient centrifuge: separation of full and empty capsids based on differential densities using a gradient of iodixanol.

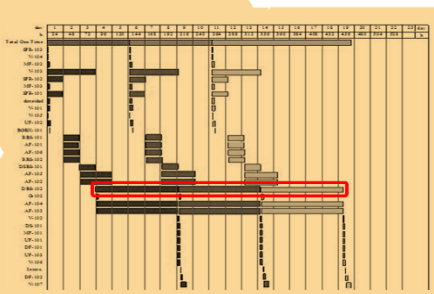
BFD



The process end up divided in three primary blocks: the upstream, which includes the scale up of cells and *baculovirus*.

Gantt: Operation Scheduling

In the Gantt diagram we can easily identify the events in the bioreactor as the process bottleneck.



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