

Production of the equine influenza vaccine using a baculovirus expression system in insect cell lines

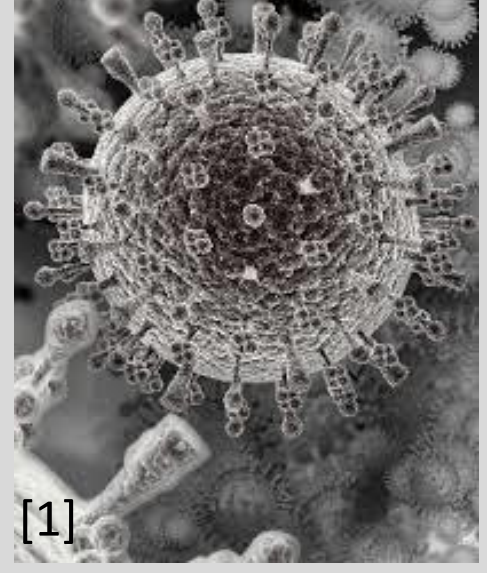
Part I: Design basics

Saioa Arza, Helena García, Oriol Cabau, Laia Puig

GLOBAL OBJECTIVE

Design of an industrial bioprocess plant with the simulator SuperPro Designer for the production of the equine influenza vaccine using a baculovirus expression system in insect cell lines, and subsequent analysis of its sustainability.

THE INFLUENZA VACCINE



The equine influenza disease is a highly contagious respiratory pathology which affects horses, donkeys and mules, among other equines. This disease is caused by two viral subtypes of influenza A virus, H7N7 and H3N8, which belong to the Orthomyxoviridae family.

Production with eggs

Nowadays influenza vaccines are produced using eggs. The vaccine contains inactivated virus. The main drawbacks of this process are:

- Long production time
- Large infrastructure
- Allergies
- Dependence to eggs

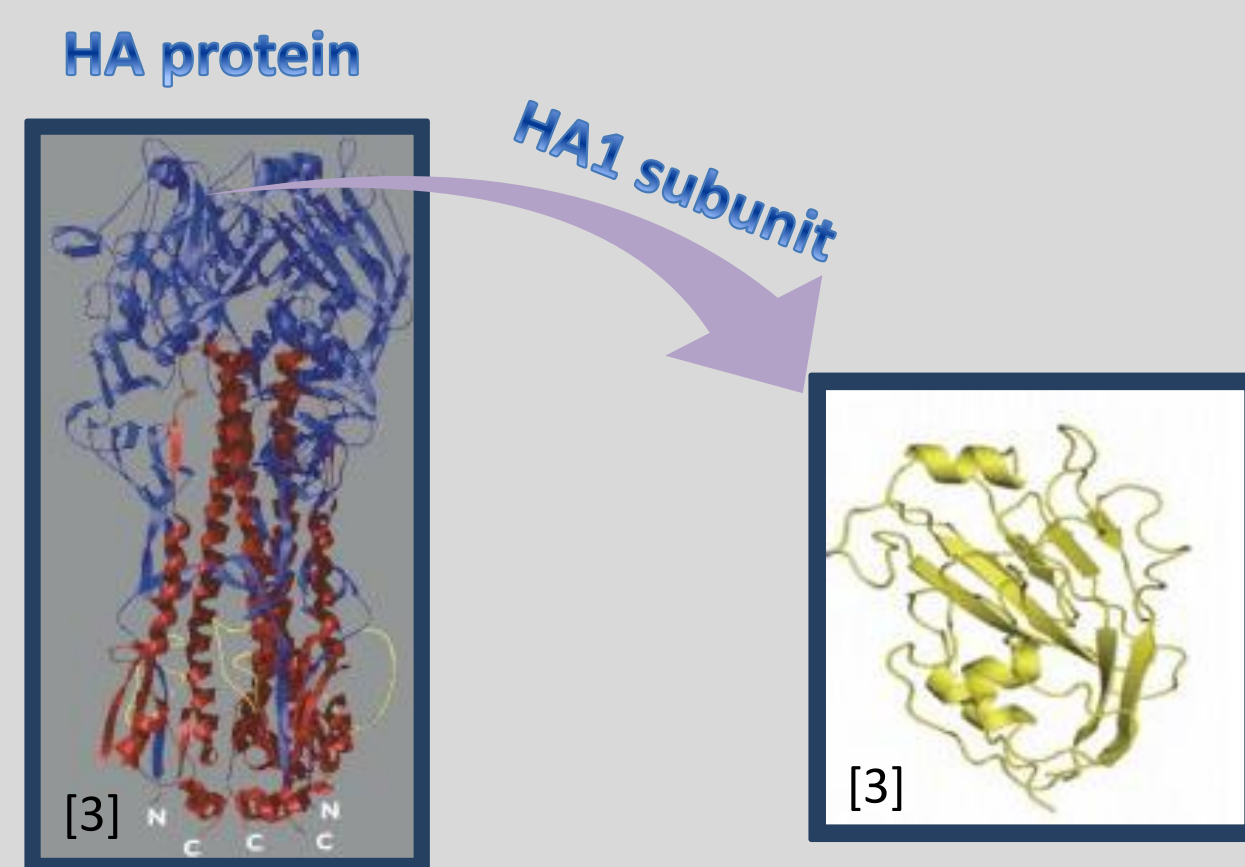


Alternative → cell cultures

The vaccine contains the HA1 subunit of the influenza virus, which is the subunit of the influenza virus hemagglutinin (HA).

The HA is the viral protein that attaches to cell receptors, causing fusion of both, viral and cellular membranes.

The HA on the influenza virus is a *trimer*, it is made of three copies of the HA polypeptide.



The new vaccine

- 15µg of HA1 (H7N7)
- 15µg of HA1 (H3N8)
- Adjuvant

1st THE FIRST EQUINE VACCINE PRODUCED USING CELLS

BACULOVIRUS EXPRESSION SYSTEM

The production of the vaccine using the baculovirus expression system has several advantages

- Reduces vaccine production time
- Growth of all influenza viruses
- No need for chicken eggs
- Animal component free
- High purified and safe vaccine

Insect cells VS mammals

Insect cells are used instead of mammals, due to de fact that are easier to maintain.

Sf9 Vs High five cells

With the addition of some protective agents, high five cells can have high productivity, being the optimum choice for the recombinant protein production.

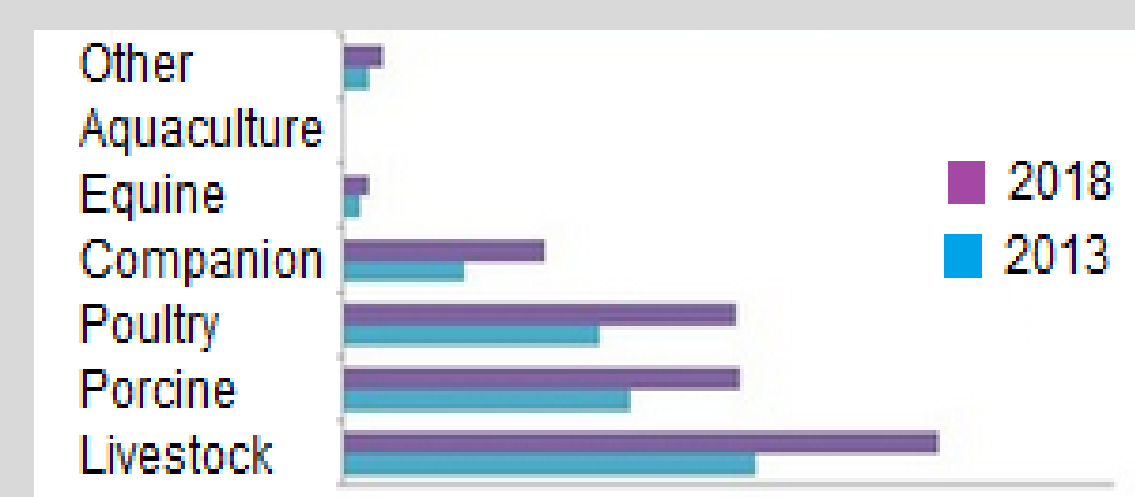
Sf9	HighFive
✓Resilient	Sensible
Lower protein productivity	✓High protein productivity
✓Good in virus amplification	Bad in virus amplification

Baculovirus

- They have a restricted host range, limited to invertebrate species
- Easy to manipulate
- High levels of heterologous gene expression, due to their strong promoters

MARKET

The veterinary equine market is one of the least exploited and so it is a great business opportunity to work on it now and in a near future due to the increase in the next years.



Distribution

A total of 8M doses would be produced and distributed around the world.

8M Doses



Legally all competing horses must be vaccinated twice a year. The USA is the country with the highest number of horses, 9.5M, and among these 3.5M are federated competition horses.

National Equine Use Patterns, 2003 [4]

Use	Percentage of Total	Number of Horses
Recreation	42	3,906,923
Showing/Competition	29	2,718,954
Other	19	1,752,439
Racing	9	844,531
Total		9,222,847

3.5M of horses that are federated and in competition.

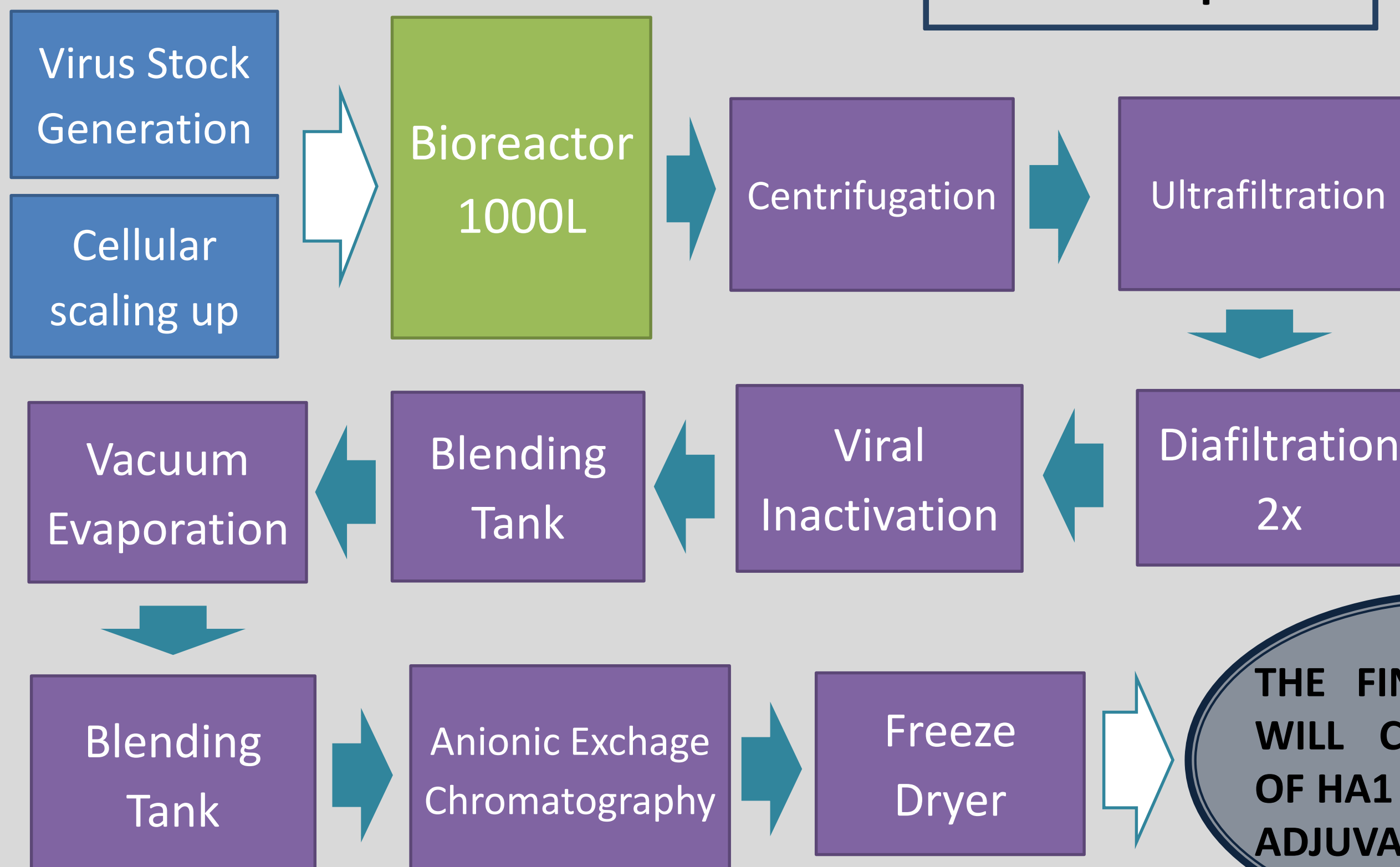
PLANT LOCALITATION AND ORGANIZATION

The plant will be located in the United States of America.



Block diagram

1 month of process



Process distribution

The process is divided in 3 parts :

- **Upstream**
 - Virus stock generation
 - Cellular scaling up
- **Reaction**
 - Protein production
- **Downstream**
 - Separation
 - Concentration
 - Purification
 - Packaging the final product

USA

- Avoid import taxes
- High number of federate horses in competition

PLANT

- Laboratory → Viral generation
- Production process

GMP

- Biosafety level 3
- Under the umbrella of QbD

12 batches/year

- First peak
 - 3 batches for H7N7
 - 3 batches for H3N8
- Second peak
 - 3 batches for H7N7
 - 3 batches for H3N8

REFERENCES

- [1] Accessed 28th May 2015: <http://www.nti.org/gsn/article/scientists-agree-unprecedented-withholding-flu-virus-research/>
- [2] Accessed 27th May 2015: <http://www.sartorius.com/en/products/bioreactors-fermentors/single-use/biostat-str/>
- [3] Chunling Xuan; Yi Shi; Jianxun Qi; Wei Zhang; Haixa Xiao; George F. (2011) Structural vaccinology.: structure-based design of influenza A virus hemagglutinin subtype-specific subunit vaccines. *Protein cell.* Issue (12): 997-1005.
- [4] Kilby, E. R. (2007). The demographics of the U.S equine population. In D.J. Salem & A. N Rowan (Eds.), *The state of the animals 2007* (pp. 175-205). Washington, DC: Humane Society Society Press.