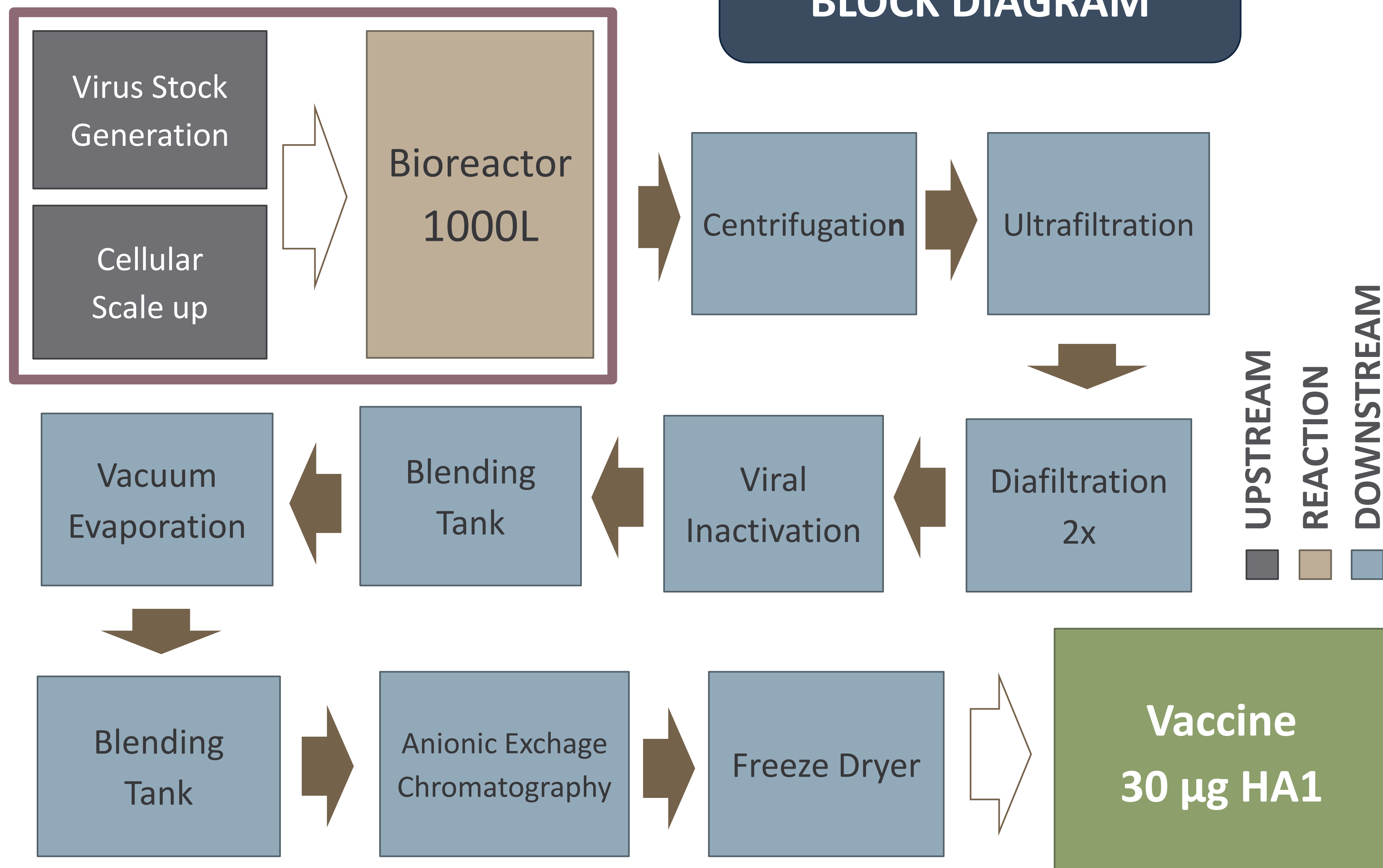


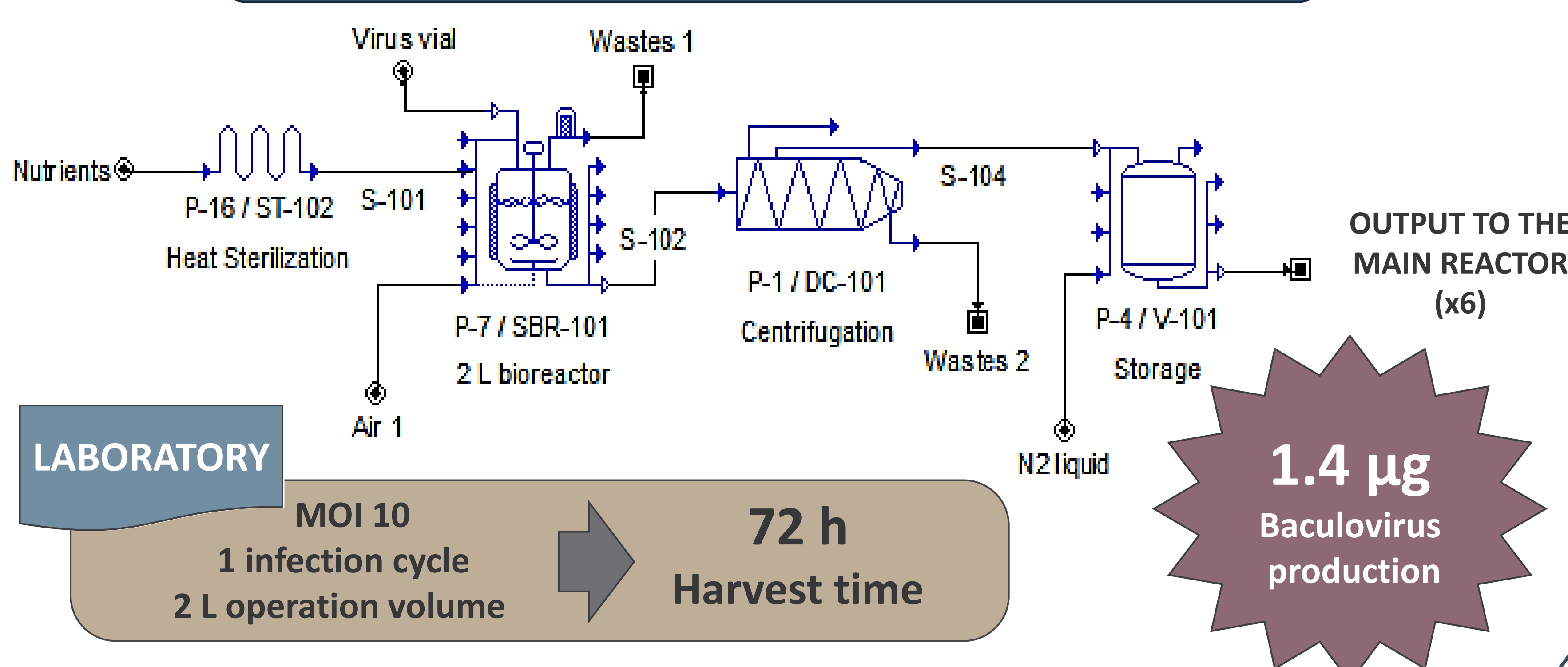
GLOBAL OBJECTIVE

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.

BLOCK DIAGRAM



VIRUS AMPLIFICATION FLOW DIAGRAM



BEVS DEVELOPMENT

Overview

Baculovirus Expression System

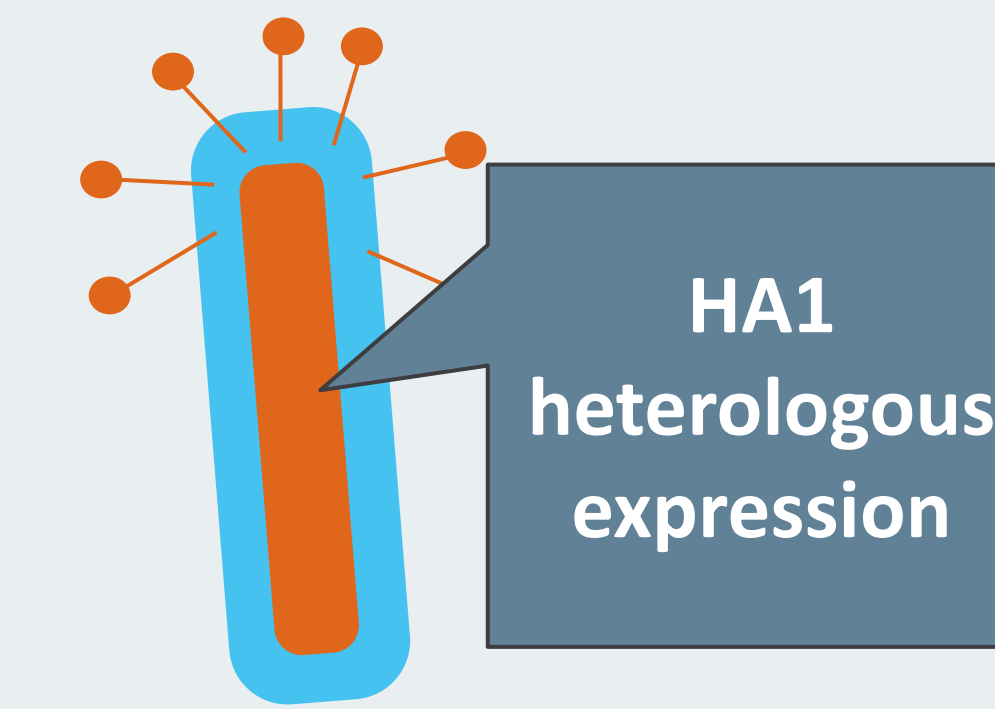
Uses baculovirus as a vector to express heterologous genes in insect cell cultures under the control of strong promoters.

RECOMBINANT BACULOVIRUS:

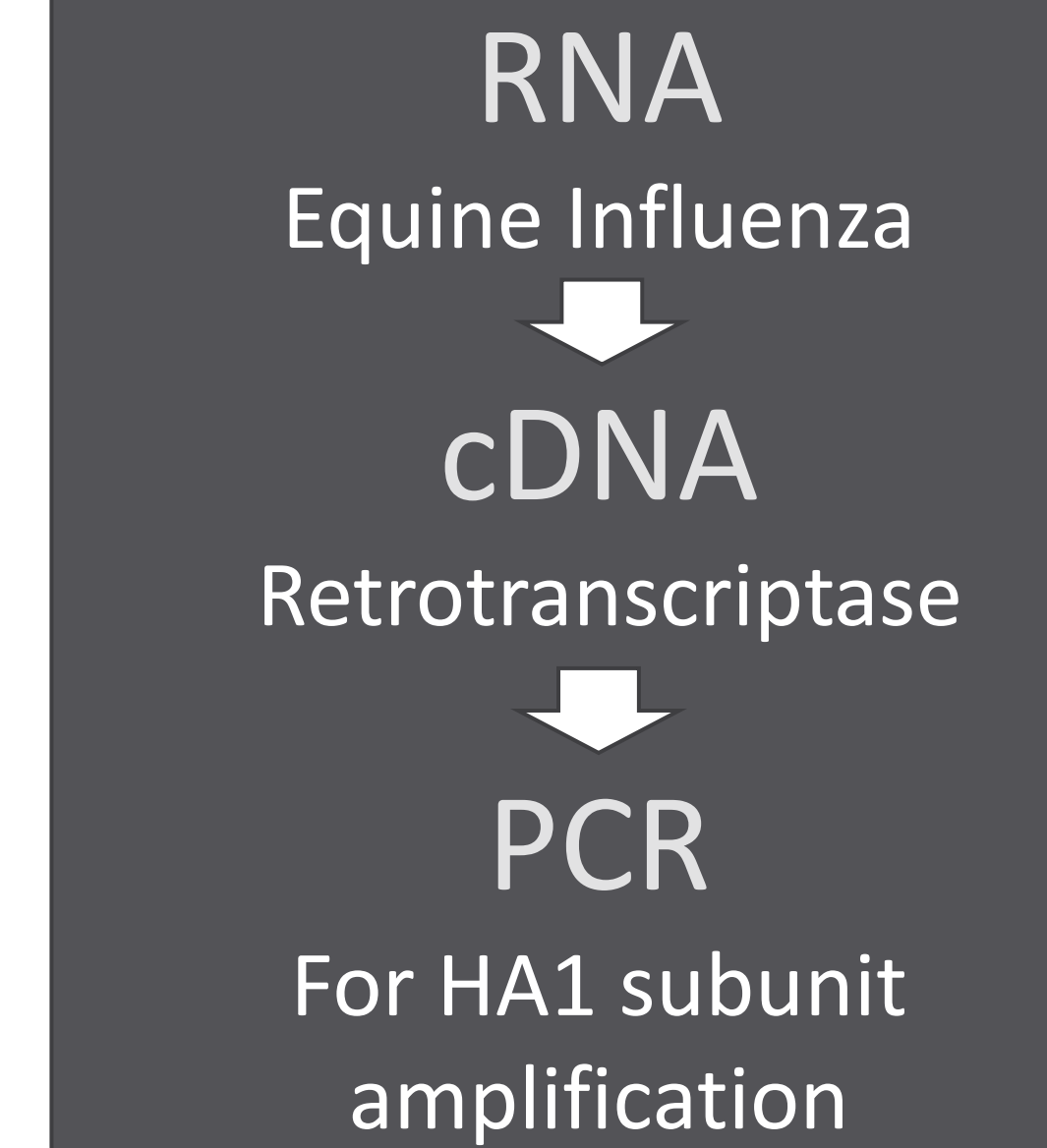
AuNPV

HOSTE RANGE:
Invertebrates

CELL LINE:
BTI-TN-5B1-4
(HighFive Cells)



LABORATORY

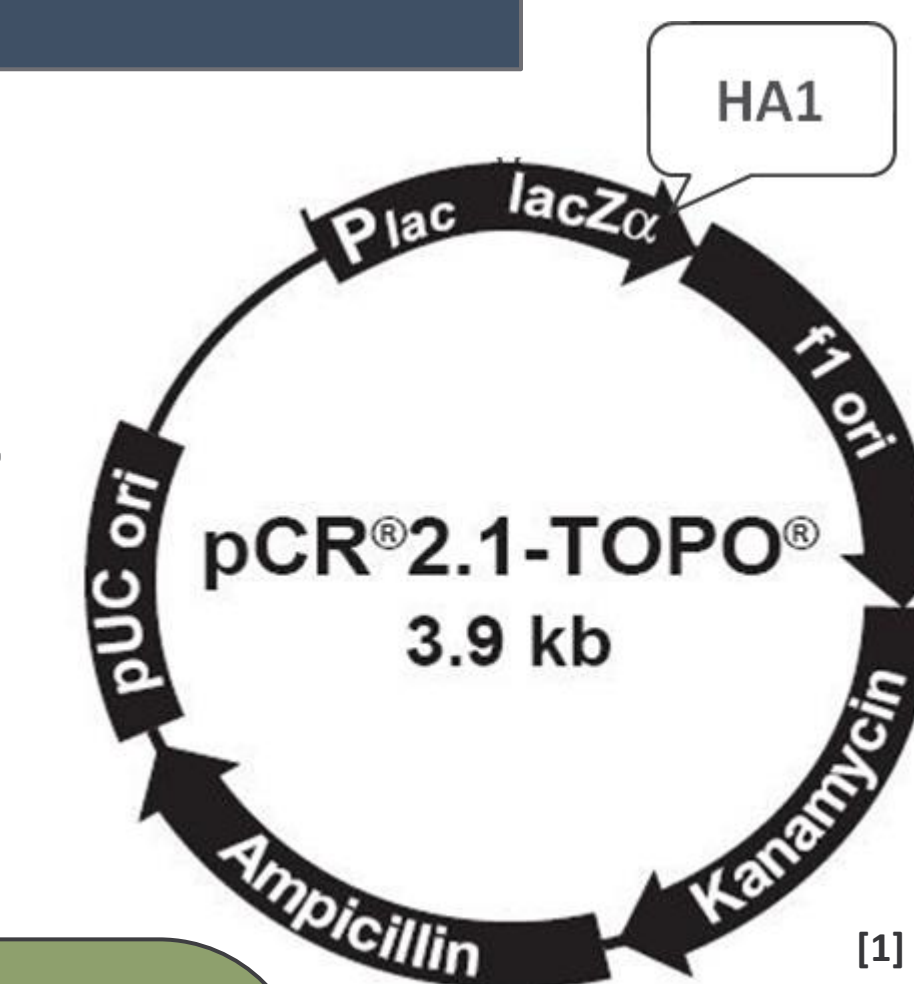


Bac-to-Bac System

Cloning

HA1 in pCR2.1-TOPO vector

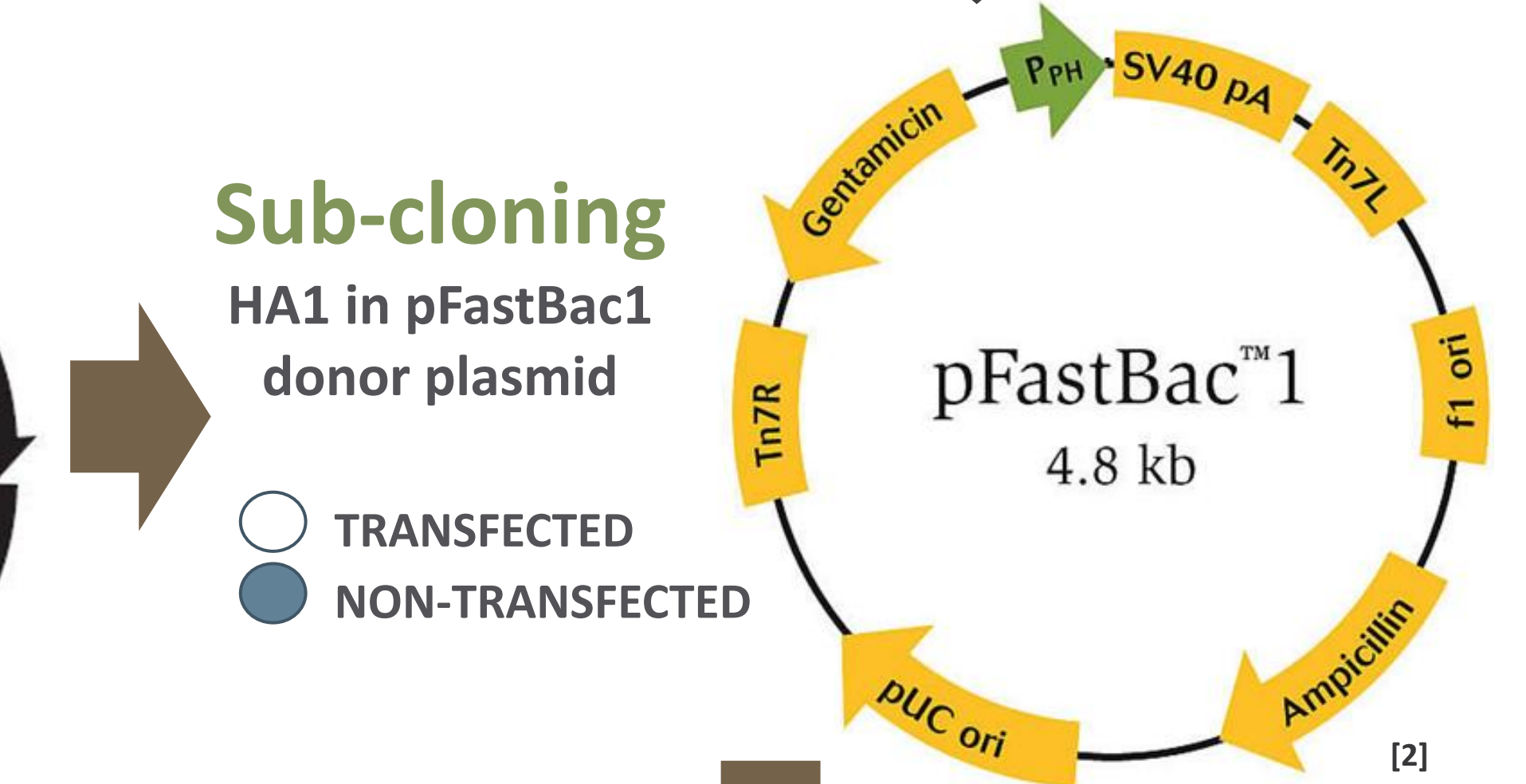
○ TRANSFECTED
● NON-TRANSFECTED



Sub-cloning

HA1 in pFastBac1 donor plasmid

○ TRANSFECTED
● NON-TRANSFECTED



Bacmid bMON14272

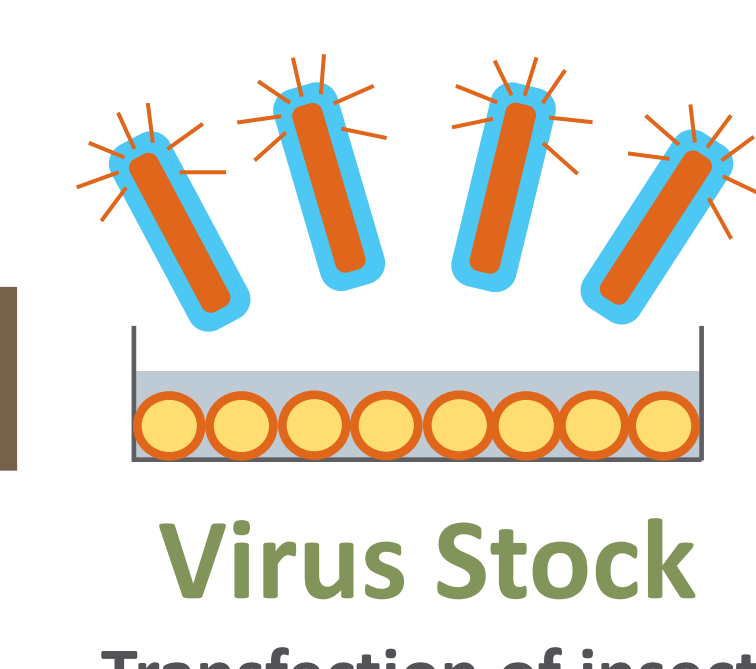
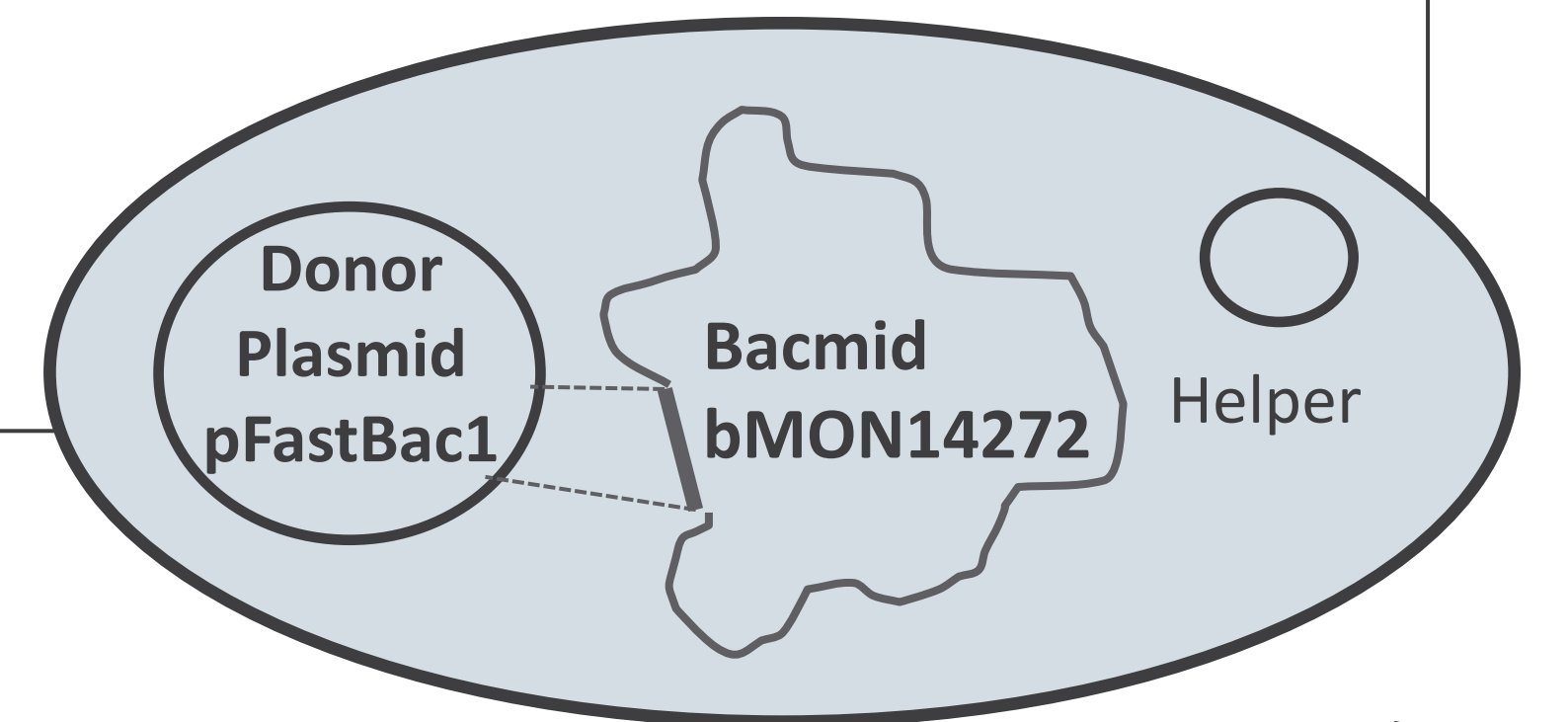
Mini-F replicon
Kanamycin resistance
LacZα peptide
Mini-att Tn7
.....
Helper pMON7124
Tetracycline R.
Transposase

pFastBac 1

Polyhedrin promoter
Cloning sites
Gentamicin R.
Ampicillin R.
Tn7

E.Coli infection

Transfection of competent E.coli DH10Bac

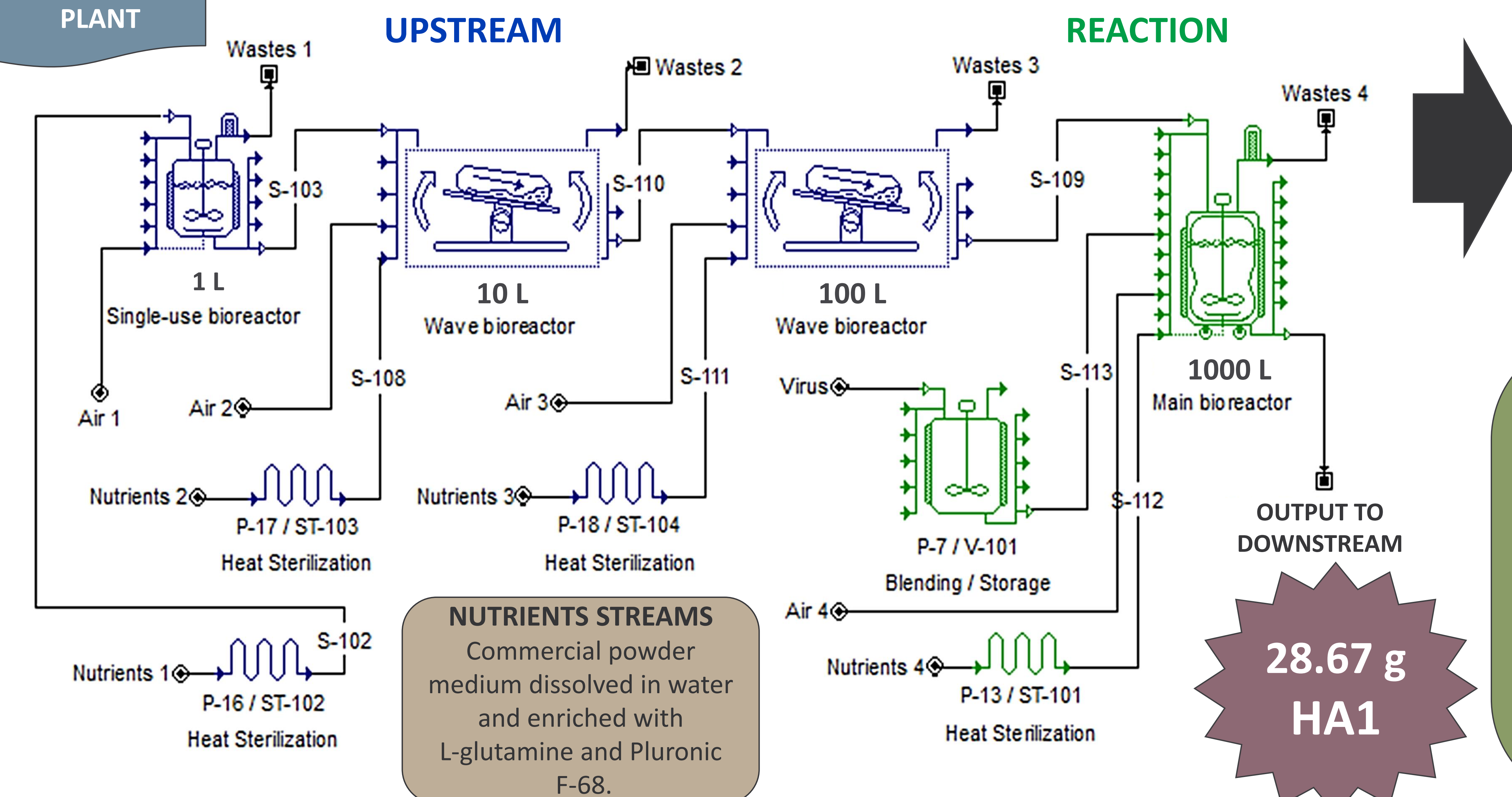


Bacmid DNA isolation



CELLULAR SCALE UP AND REACTION FLOW DIAGRAM

GMP PROCESS PLANT



MAIN BIOREACTOR OPERATING CONDITIONS

| | |
|----------------------|---------------------------|
| Temperature | 27°C |
| Maximum cell density | 2·10 ⁶ cell/mL |
| MOI | 1 |
| Harvest time | 72 h |
| Aeration | 0.05 VVM |
| Reactor type | Disposable |

1 VACCINE → 2 INFLUENZA STRAINS (15 µg HA1/strain)

1 STRAIN ANNUAL PRODUCTION

- 6 batch of cellular scale up and reaction (12 days/batch)
- 1 batch of viral amplification (3 days/batch)

CRITICAL POINTS

- REACTION:
✓ Solution: Parameters control
- CHAIN BATCHES:
✓ Solution: 1 week per month slack time

References

- [1] Life Technologies (2015) pCR21-TOPO. Retrieved May 2, 2015, from: <http://www.xenbase.org/reagents/vectorAction.do?method=displayVectorSummary&vectorId=1221309>
- [2] Life Technologies (2015) pFastBac-1. Retrieved May 2, 2015, from: <https://www.lifetechnologies.com/order/catalog/product/10360014>
- [3] GE Healthcare Life Science (2015) WAVE Bioreactor. Retrieved May 15, 2015, from: <http://www.ecomagination.com/portfolio/wave-bioreactor-for-biotherapeutics-production>