

CLONING OF NUCLEOCAPSID PROTEIN INFLUENZA A VIRUS

Name:

Autor:

Date:

DEA. MSc. Miguel Angel Merlos Rodrigo

15.1.2014

CLONING OF NUCLEOCAPSID PROTEIN INFLUENZA A VIRUS

AIM

The main aim is production of artificial influenza viruses



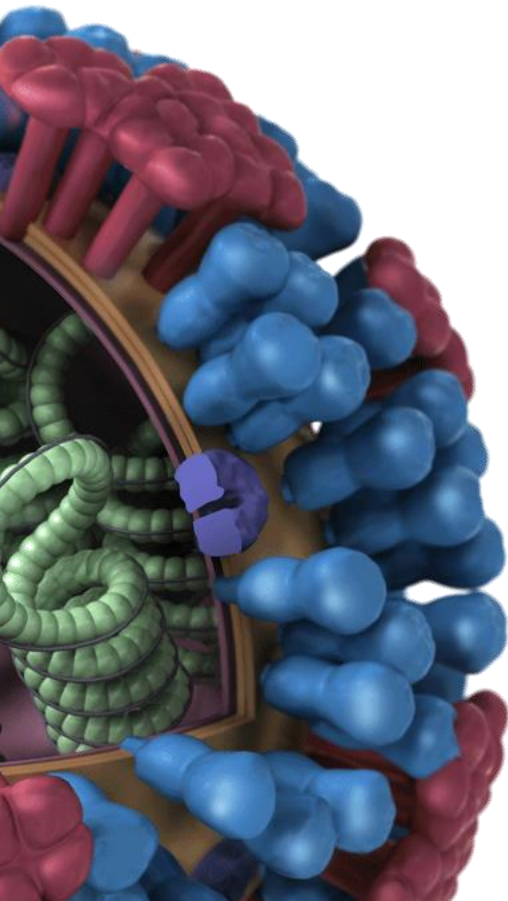
Study the interaction of the virus with drugs



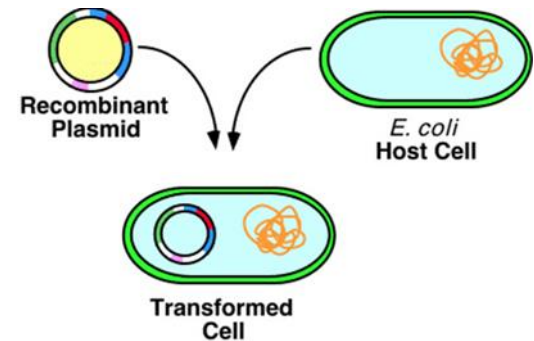
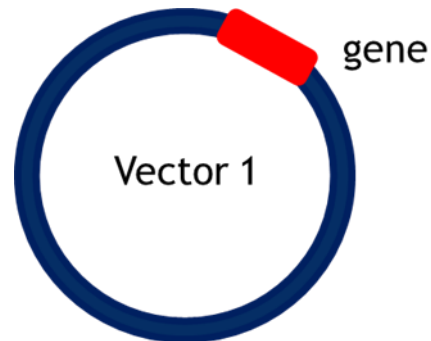
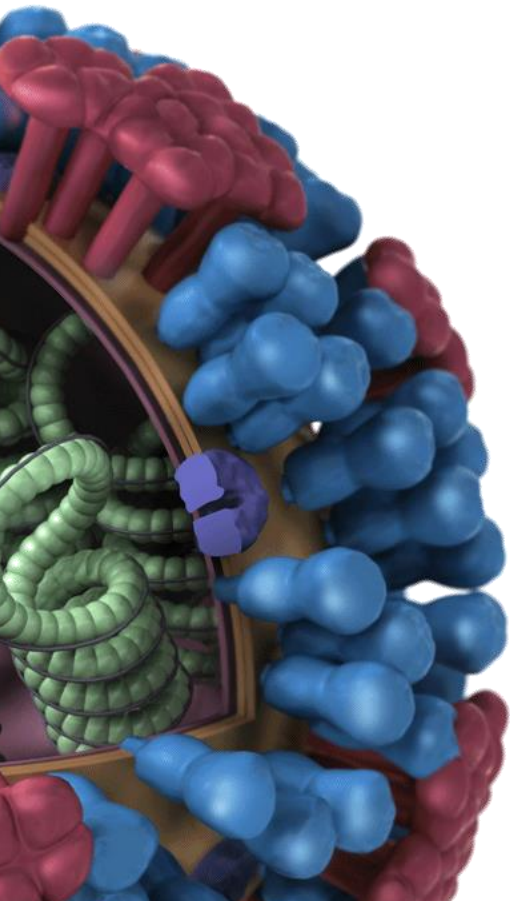
Vaccine Production ?????

Cloning and expression of nucleocapsid protein of Influenza A virus using different plasmid and *E.coli* competent cells.

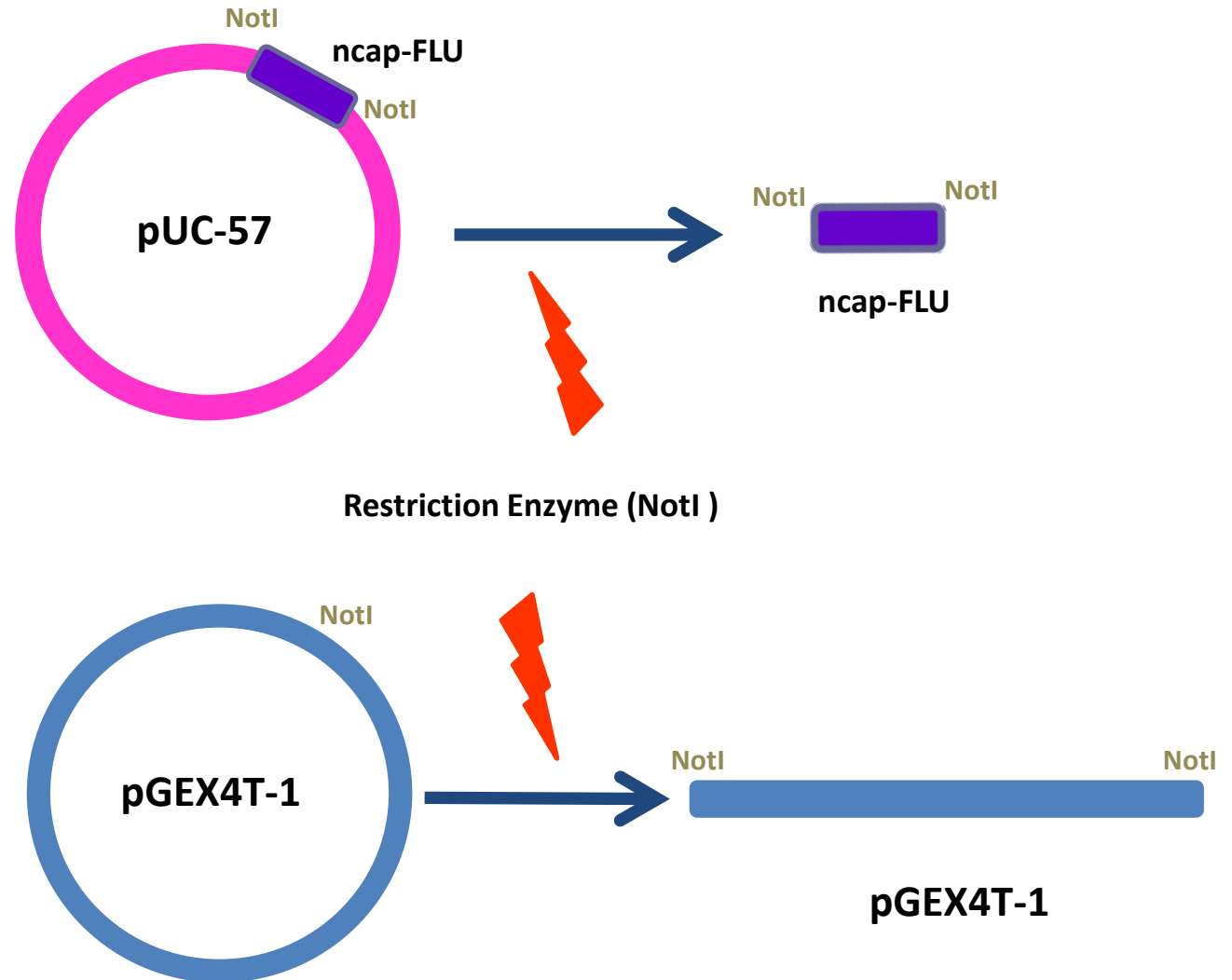
The nucleocapsid protein of Influenza A virus was synthesized and was cloned into the plasmid pUC57 -Amp (GENEWIZ Gene Synthesis, Sigma -Aldrich) resulting a **pUC57-ncapFlu**



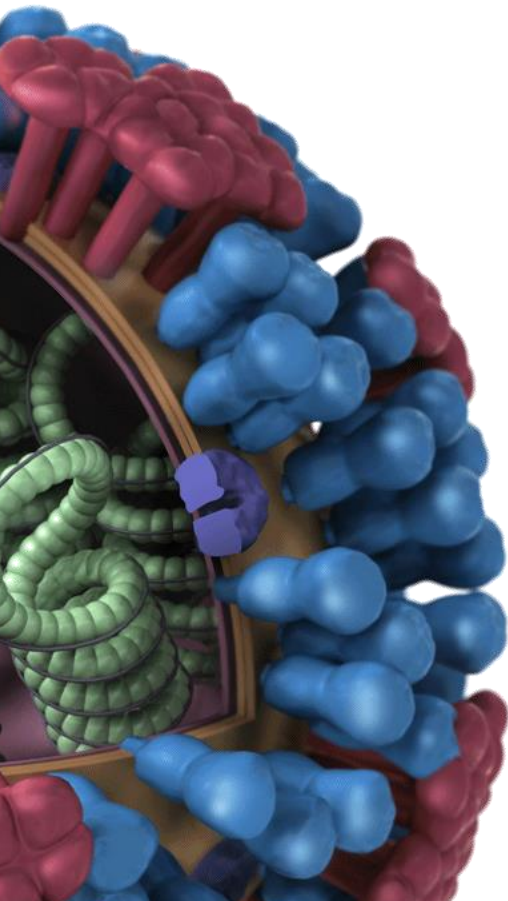
MOLECULAR CLONING



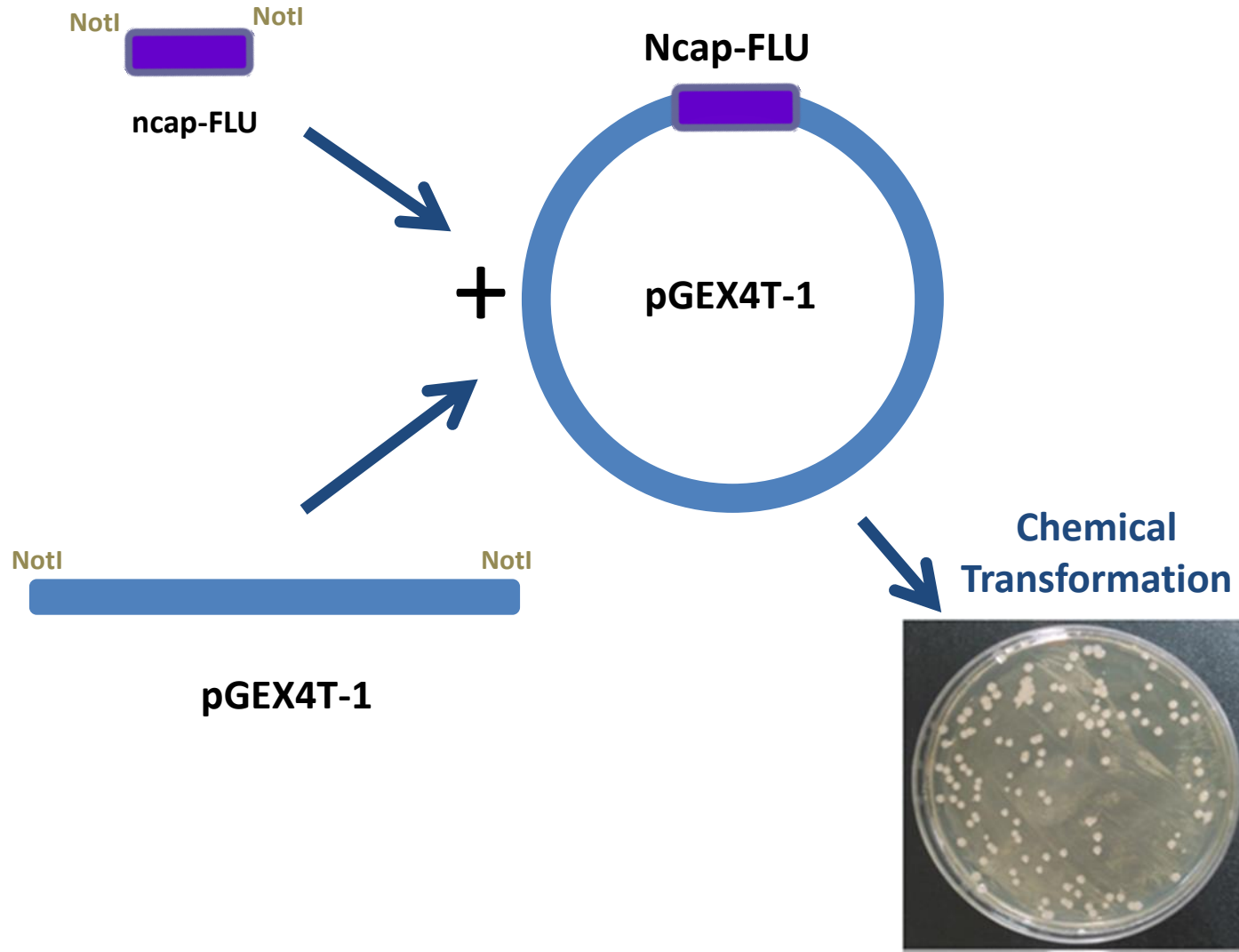
SUBCLONING



CLONING OF NUCLEOCAPSID PROTEIN INFLUENZA A VIRUS



LIGATION



CLONING OF NUCLEOCAPSID PROTEIN INFLUENZA A VIRUS

- **Analysis of Positive Clones**

PCR

4 min 95°C
1 min 95°C
30s 58°C
1:30 min 72°C
10min 72°C
10min 72°C
10 min 10°C
PCR product 1400pb



Primers: pGex 5 forward
ncap-FLU reverse



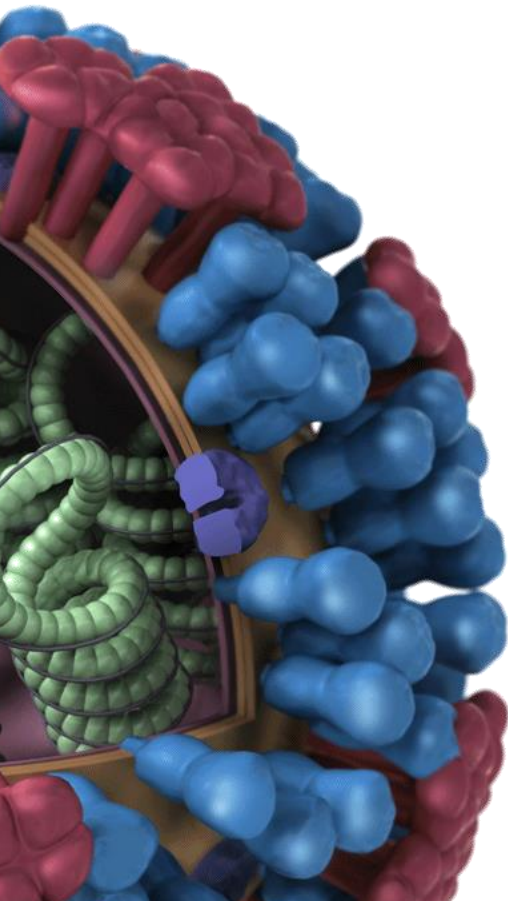
Correct
orientation

Electrophoresis

1% AgarGel
Ethidium Bromide 5 µl/100µl
TAE Buffer, 100v
60min, UV

- **Isolation of Protein**

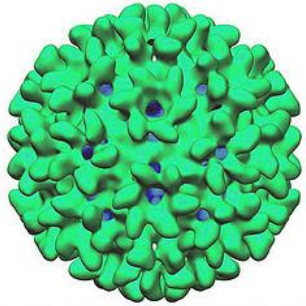
Bacterial Lysis and Protein Chromatography



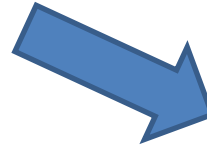
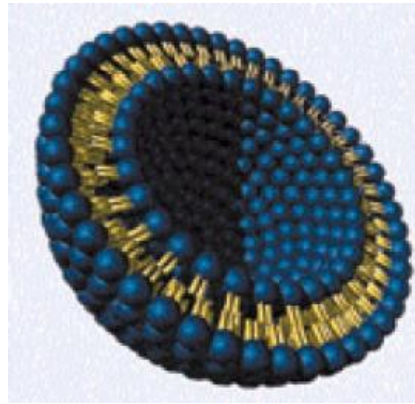
CLONING OF NUCLEOCAPSID PROTEIN INFLUENZA A VIRUS

Future Work:

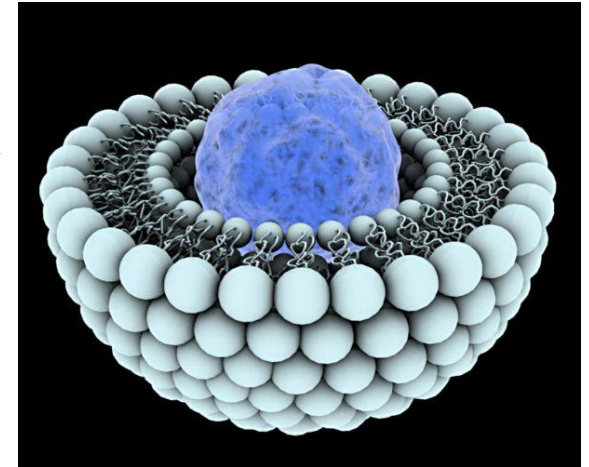
Nucleocapsid protein



Liposome



Artificial virus



Thank you for your attention

Reg.č.projektu: CZ.1.07/2.4.00/31.0023

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu