evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

CLONING OF CAPSID PROTEIN OF HUMAN IMMUNODEFICIENCY VIRUS

Name:

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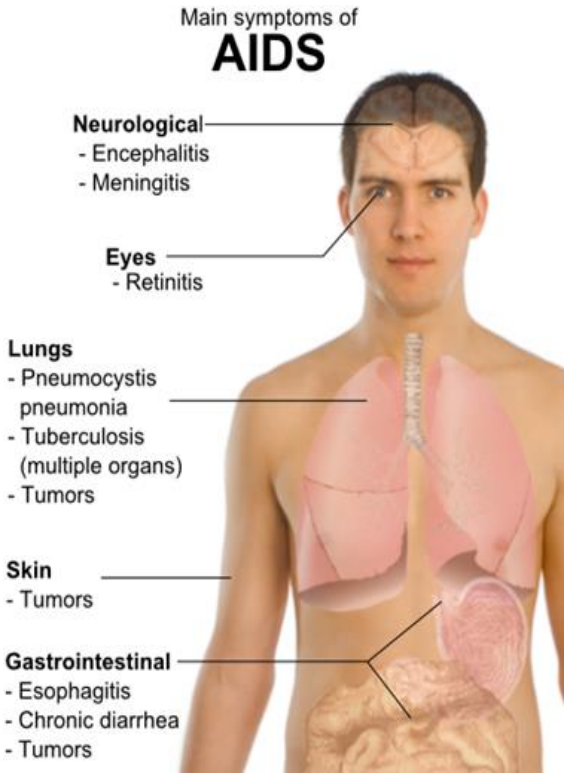
Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

NanoBioMetalNet

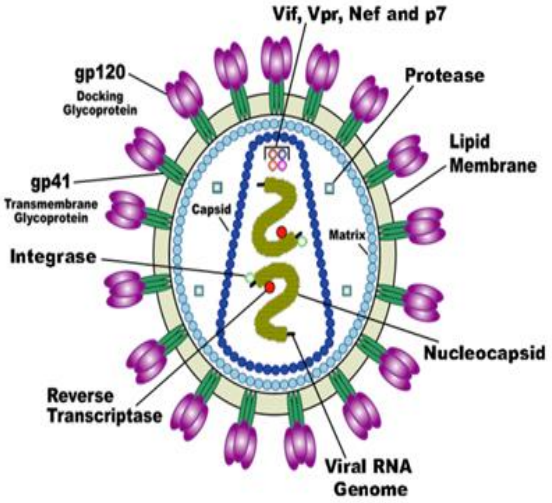
INTRODUCTION

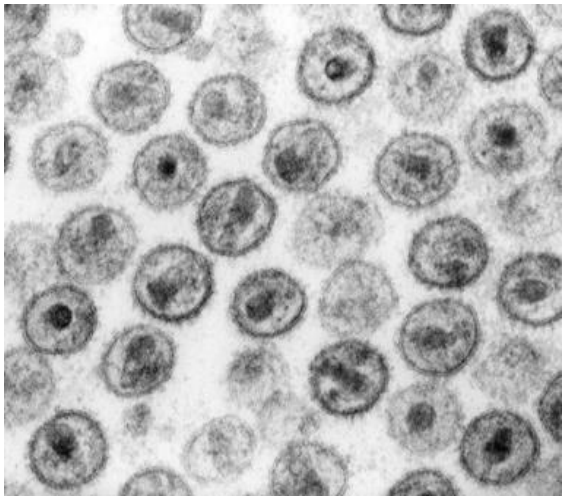
- Human immunodeficiency syndrome (AIDS) is a disease of the human immune system caused by infection with human immunodeficiency virus (HIV).
- This disease was discovered by the team of Luc Montagnier in France in 1983. Since its discovery, AIDS has caused nearly 30 million deaths.
- As of 2012, approximately 35 million people are living with HIV globally.
- This illness produces a progressive failure of the immune system. As the illness progresses, making the person much more likely to get infections, including opportunistic infections and tumours.
- Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, or breast milk.

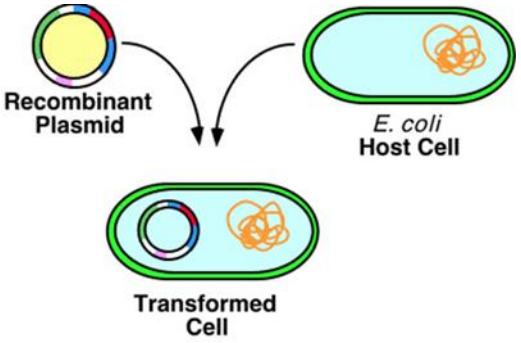


INTRODUCTION

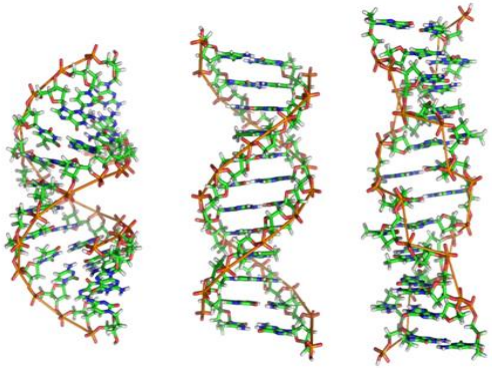
- Virion contains genetic information and has a spherical shape. It consists of three layers.
- The genome is a single stranded RNA strand to be copied to multiply DNA and integrated into the genome of the cell it infects, by the action of reverse transcriptase.
- The virus genome is composed of three genes gag, pol and env. Each of these genes encodes proteins that help the virus from reproducing. The structural proteins are also encoded by these genes.
- A capsid is the protein shell of a virus. It consists of several oligomeric structural subunits and encloses the genetic material of the virus.
- Our work was developed about the **capsid protein** of human immunodeficiency virus.

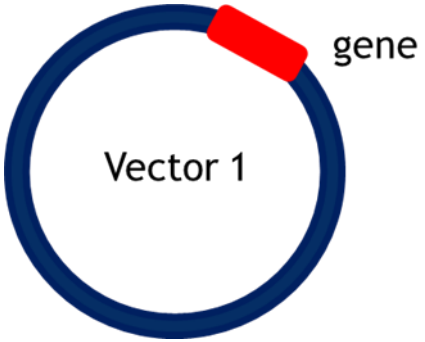






MOLECULAR CLONING

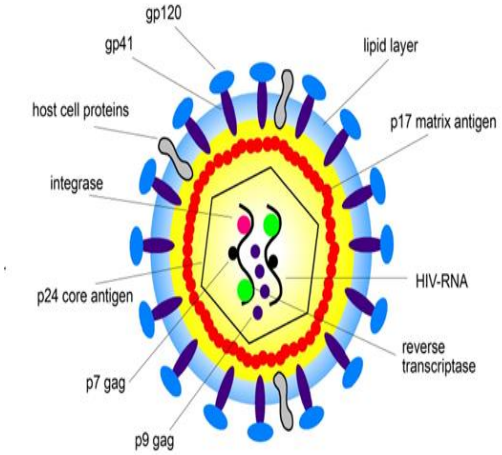


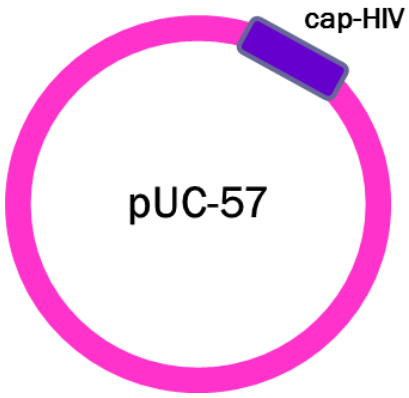


AIM

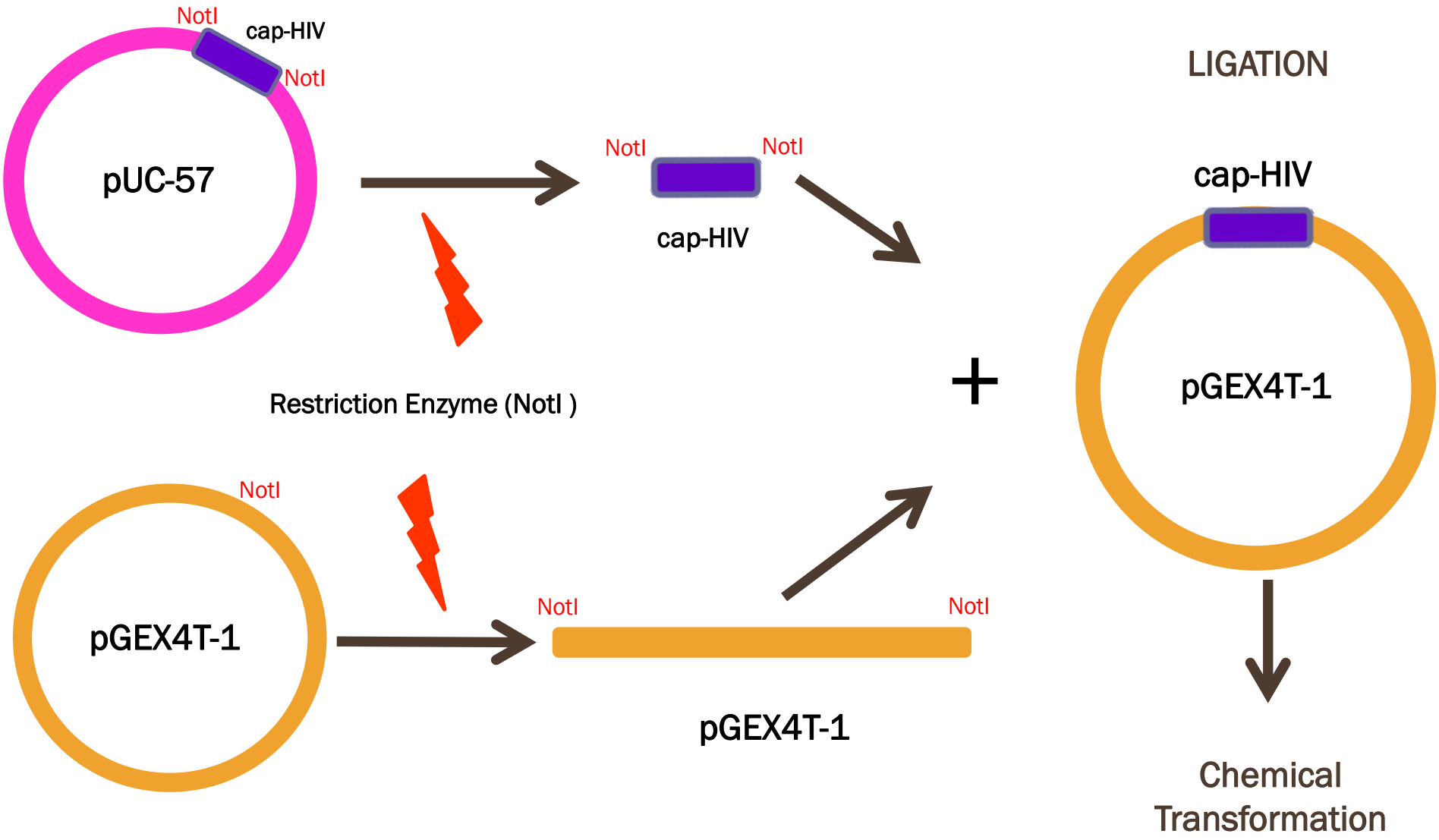
- Cloning and expression of capsid protein of HIV using different plasmid and *E.coli* competent cells.

- The capsid protein of HIV was synthesized and was cloned into the plasmid pUC57-Amp (GENEWIZ Gene Synthesis, Sigma - Aldrich) resulting a **pUC57-capHIV.**



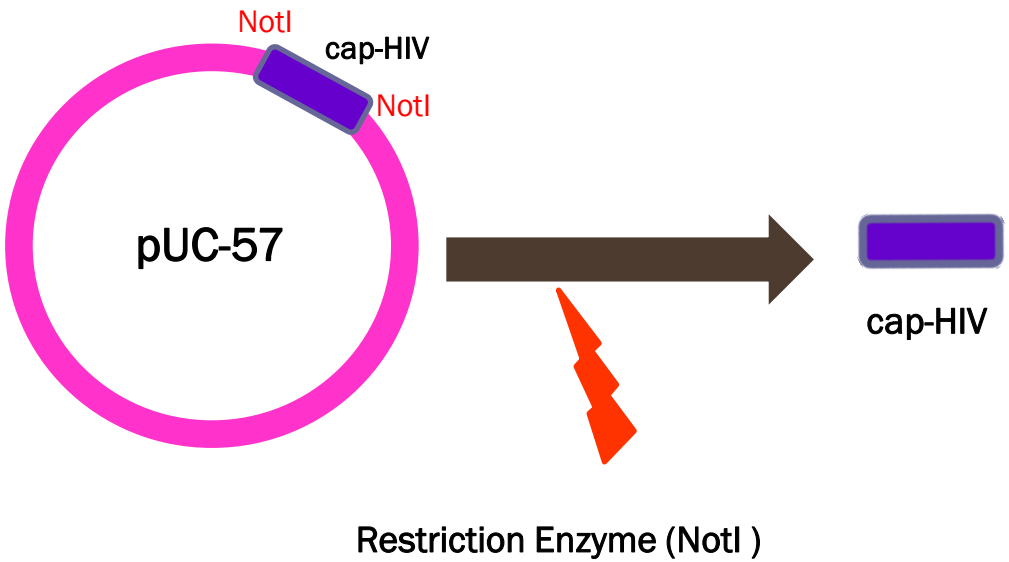


SUBCLONING


NotI
cap-HIV
NotI
pUC-57
NotI
NotI
cap-HIV
Restriction Enzyme (NotI)
NotI
pGEX4T-1
NotI
NotI
pGEX4T-1
+
LIGATION
cap-HIV
pGEX4T-1
Chemical
Transformation

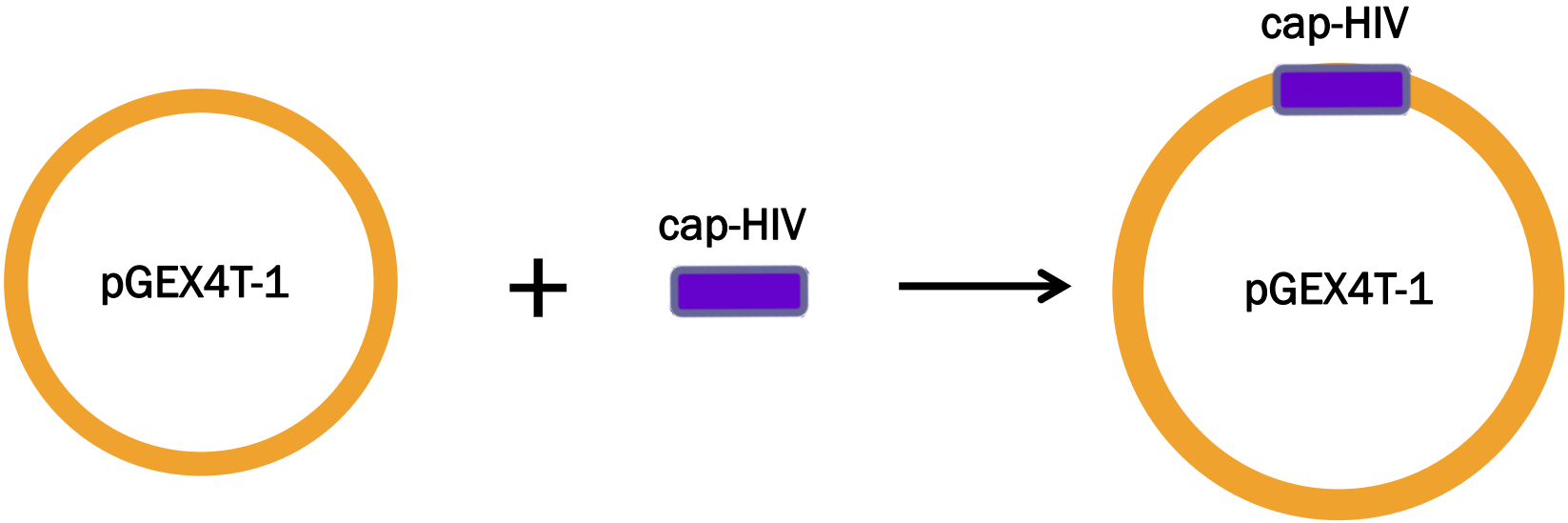
ENZYME DIGESTION

- 2 µg of DNA miniprep of the plasmid
- 1x NEB Buffer 3.1
- 1.5 µl of NotI (10000 units/µl)
- mQH2O (until 30µl)
- 37°C 2 hours+ 65°C 20 min



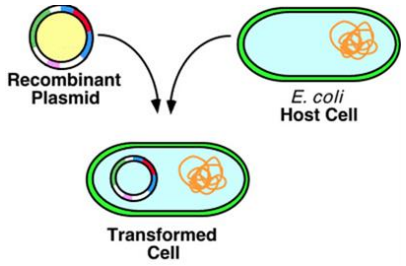
LIGATION

- Ligation of PCR purification product (cap-HIV) with pGEX4T-1 plasmid.
- Relation Insert:Vector 3:1
- Reaction:
  - DNA from Gel Purification
  - pGEX4T-1 Vector (50ng)
  - 2x Quick Ligation Reaction Buffer
  - Quick T4 DNA Ligase (1µl)
  - Deionized water to a final volume of 10 µl
- Mix reaction gently and incubate the reactions 5 minutes at 25°C



CHEMICAL TRANSFORMATION

- Add 2µl of the cap-HIV-pGEX4T-1 into a vial of chemically competent cells (BL21 strain, New England Biolabs)
- Incubate on ice for 30 minutes.
- Heat-shock the cells for 10 seconds at 42°C.
- Transfer the tubes to ice for 5 minutes.
- Add 950µl of SOC medium.
- Shake the tube horizontally (200 rpm) at 37°C for 90 minutes.
- Spread 50-100µl from transformation on a selective plate (LB agar+Amp).
- Incubate overnight at 37°C.
- Pick colonies for analysis (screening).

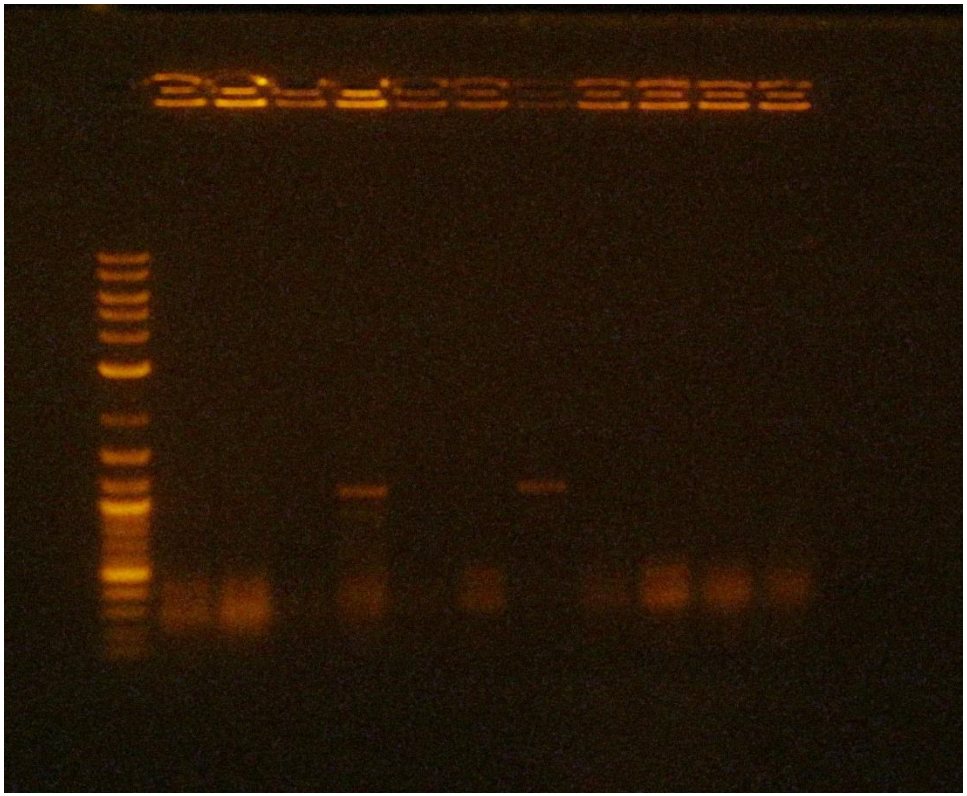


ANALYSIS OF POSITIVE CLONES

PCR SCREENING 35x
4 min 95°C
1 min 95°C
30s 58°C
1 min 72°C
10min 72°C
10min 72°C
10 min 10°C
PCR product 981pb

Primers: pGex 5 forward
cap-HIV reverse → correct orientation

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



ISOLATION OF THE PROTEIN

BACTERIAL LYSIS AND PROTEIN CHROMATOGRAPHY

- The positive transformants were grown in LB broth with 100 mg/liter ampicillin. Shaking the culture overnight at 37 °C.
- The next day, the culture was grown to 0.1 of OD 600nm
- When the culture reached the exponential phase (0.4-0.6) were added IPTG to a final concentration of 0.5mM and continued to grow the cells during 4 to 6 hours
- The pellet was resuspended, after centrifugation at 4000 rpm for 10min, with PBS (0.14M NaCl, 2.7mM KCl, 10mM Na2HPO4, 1.8mM KH2PO4, pH 7.3).
- The lysis was done by sonication in ice with 5 pulses of 80% during 10 seconds.
- The protein fraction was harvested by centrifugation for 10 minutes at 4000 rpm at +4°C.

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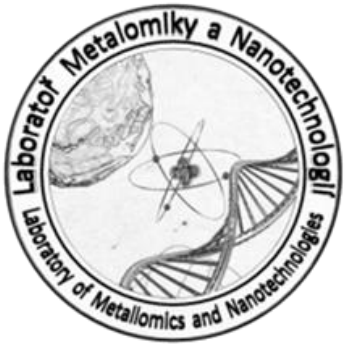
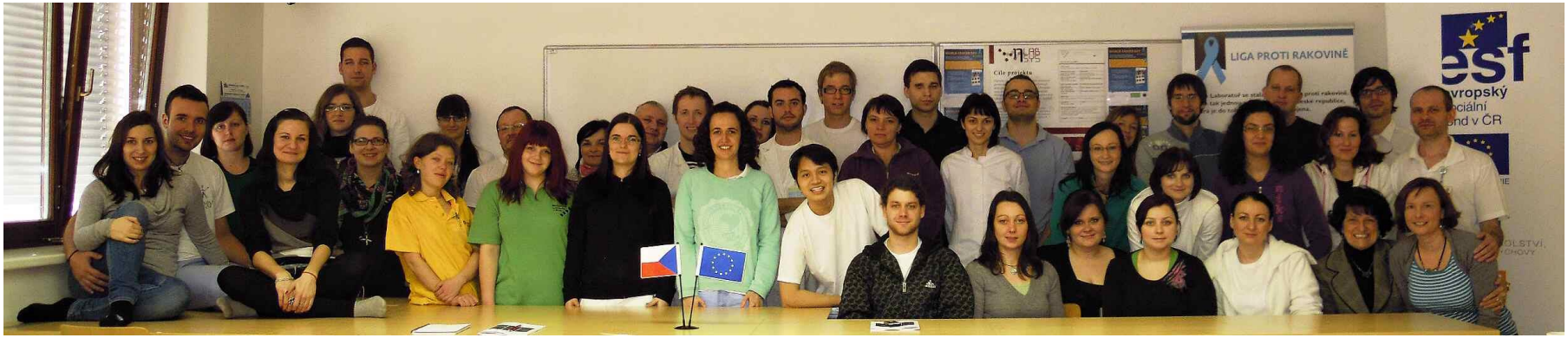
- Merlos Rodrigo Miguel Angel, DEA
- Ing. Kristýna Číhalová



THANK YOU FOR YOUR ATTENTION
DĚKUJI VÁM ZA POZORNOST ☺



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Thank you for your attention

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