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

Název: Molecular Biology tools to preparation of fluorescent proteins in In vivo Imaging

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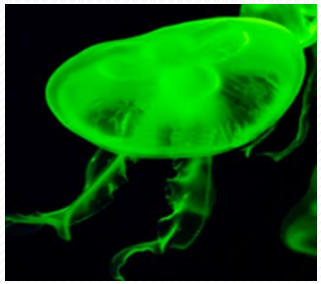
Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

pGLO plasmid



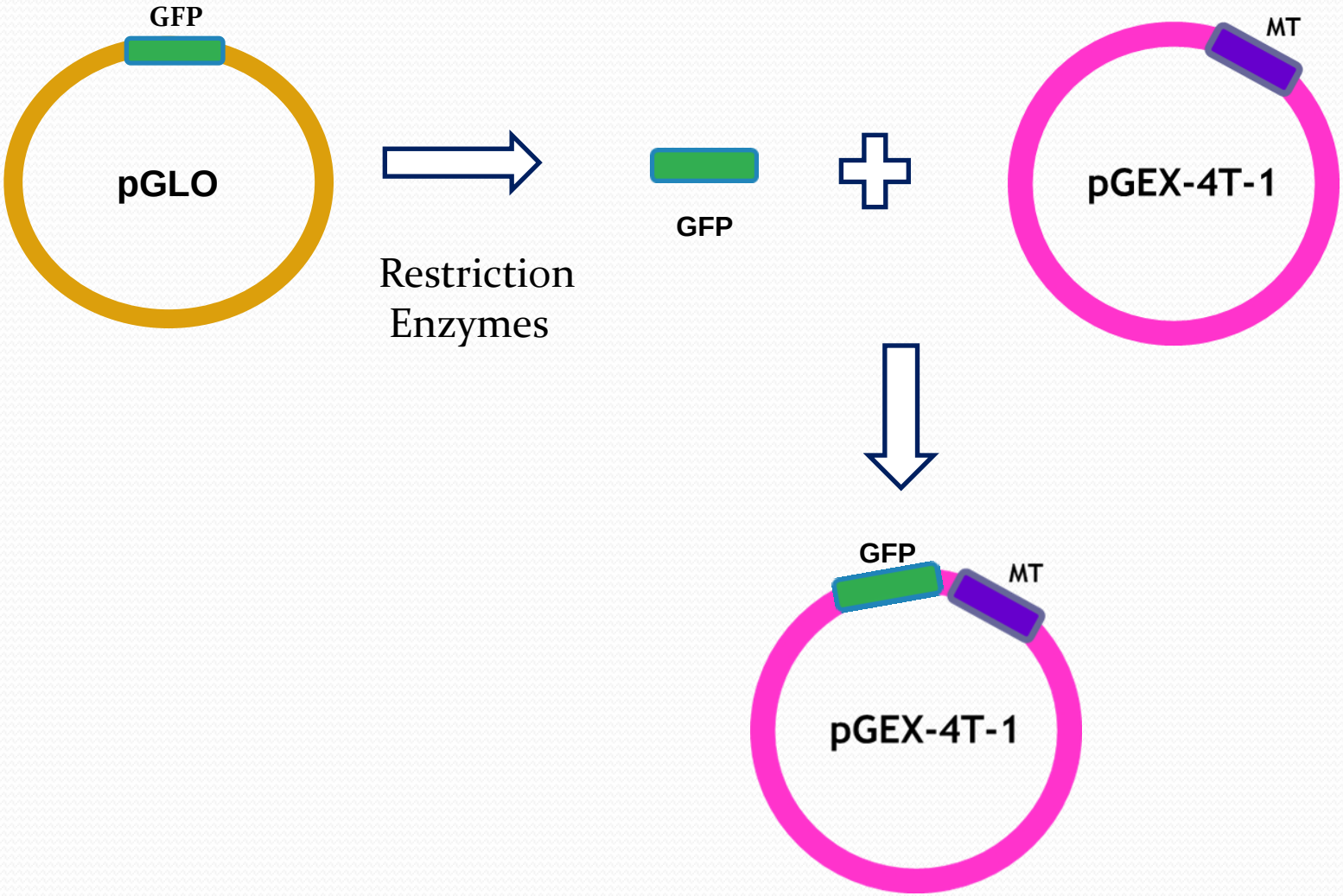
- A protein composed of 238 Aa, exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range
- GFP was isolated from the jellyfish *Aequorea victoria*

- The pGLO plasmid contains the GFP, the ampicillin resistance gene and arabinose promoter

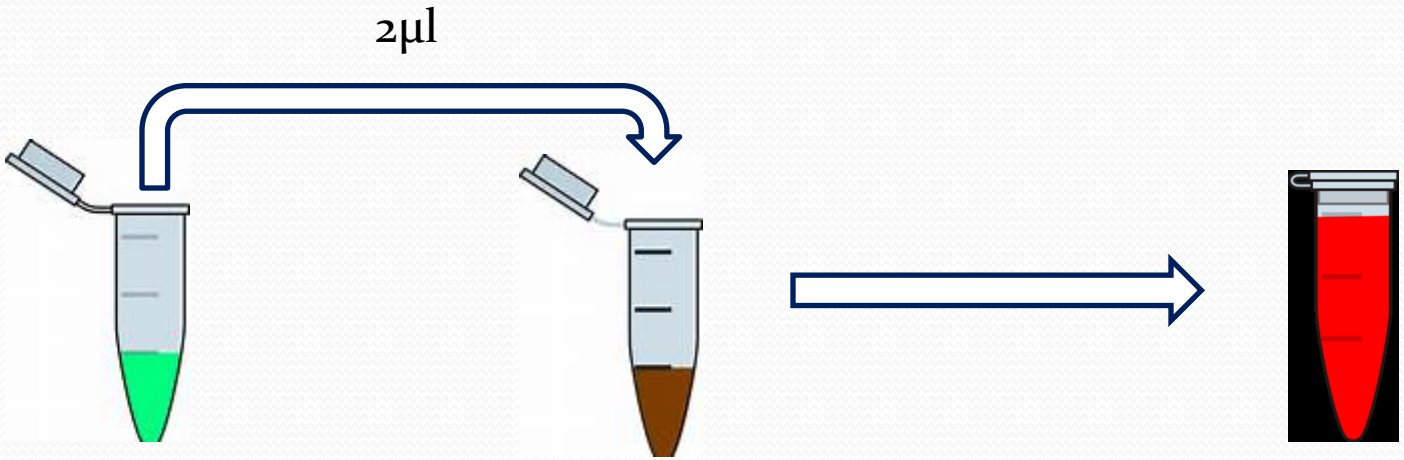
Subcloning GFP in other plasmids

- The GFP gene has been introduced and expressed in many bacteria, yeast and other fungi, fish, plant and mammalian cells

Subcloning

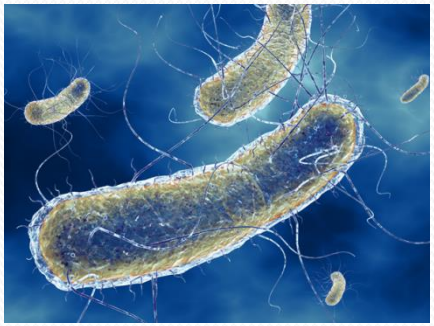


Molecular Cloning

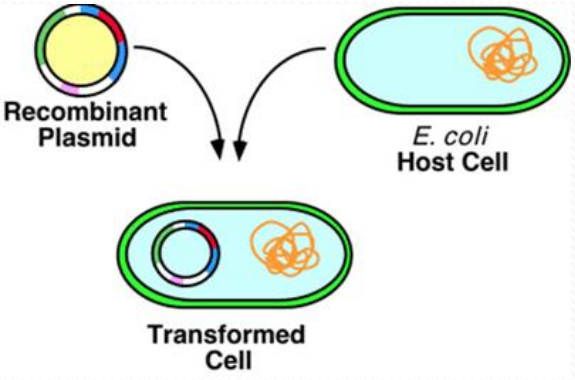


pGLO plasmid

E.Coli (competent cells)



Transformed cells



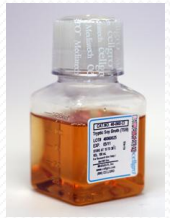
Molecular Cloning

E.coli+GFP plasmid

Incubation in ice 30 min
(no mojito!! ☺)

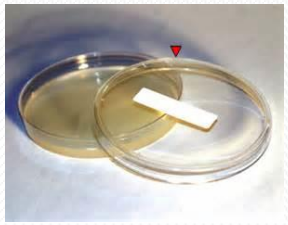
Heat shock 45 seconds at 42°C

Incubation in ice 3 min



Add 250 µl of SOC broth

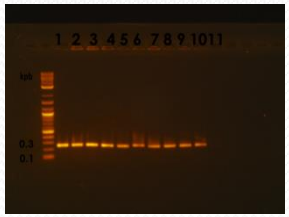
Shaking at 37 °C for 90 min



Spread the transformed cells
on selective plate

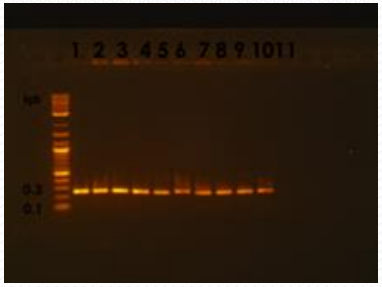
Incubate
overnight at 37 °C

Screening



Bacterial Lysis

Positive transformants with GFP plasmid



LB+ampicillin+arabinose

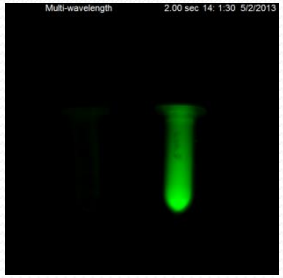
Shaking O/N at 32 °C

Lysozyme

-80°C

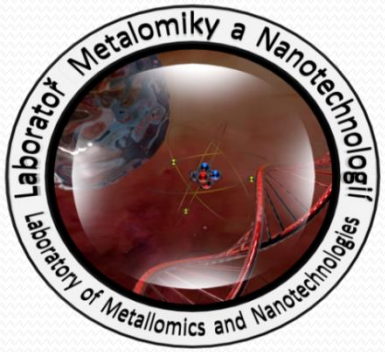
Enzymatic lysis with lysozyme and freezing

HPLC



GFP Protein Isolation with HPLC

Thank you for your attention!



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