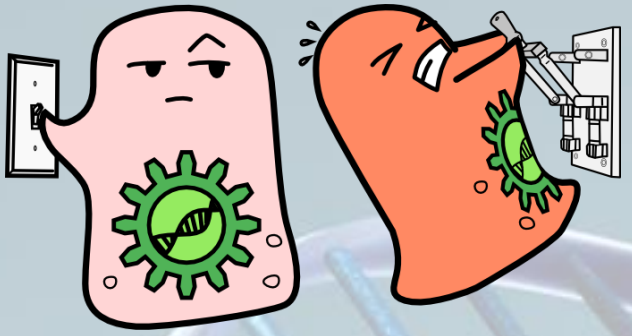


EPFL iGEM Project

switch

A light switch for mammalian cells based on LovTAP

iGEM

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

What's synthetic Biology?

Synthetic biology, field of research in which the main objective is to create fully operational biological systems from the smallest constituent parts possible, including DNA, proteins, and other organic molecules.

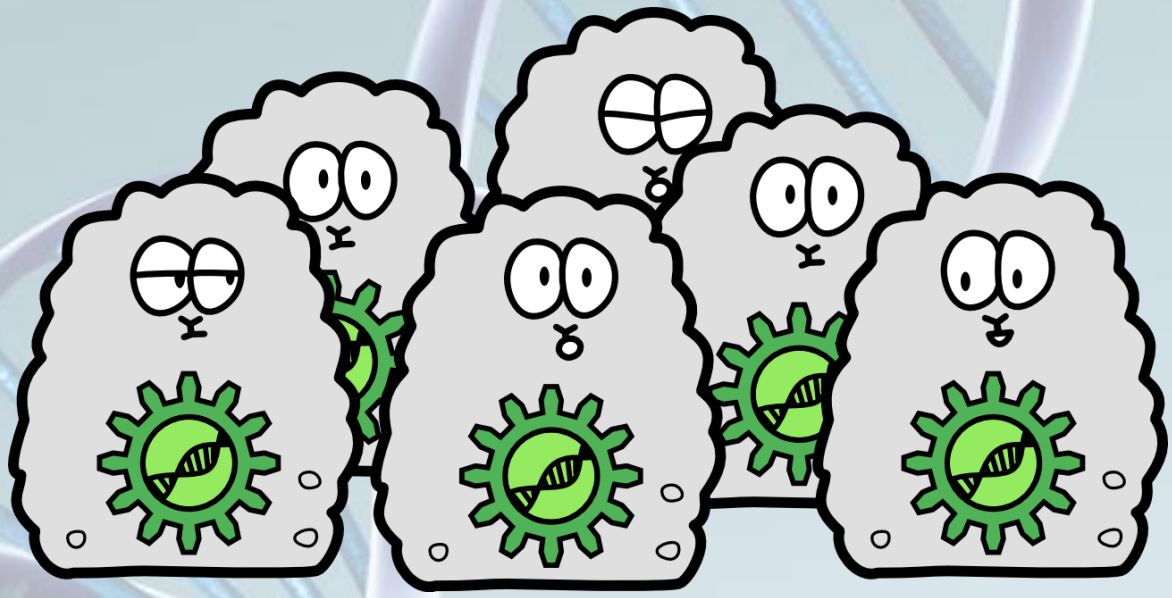
Britannica

iGEM

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

What's iGEM?

- World-wide synthetic biology contest
- More than 170 teams this year



iGEM

EPFL
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

International Genetically Engineered Machine (iGEM)

- **2012 teams:**
- US East Coast: 43
- US West Coast: 24
- Asia: 55
- **Europe: 53**
- South America: 16

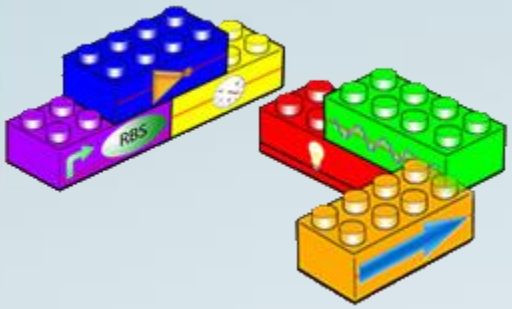
Course/project available as an option in the Masters program of the school of Life Sciences.
Open to all students regardless of their major.

iGEM

EPFL
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Biobricks

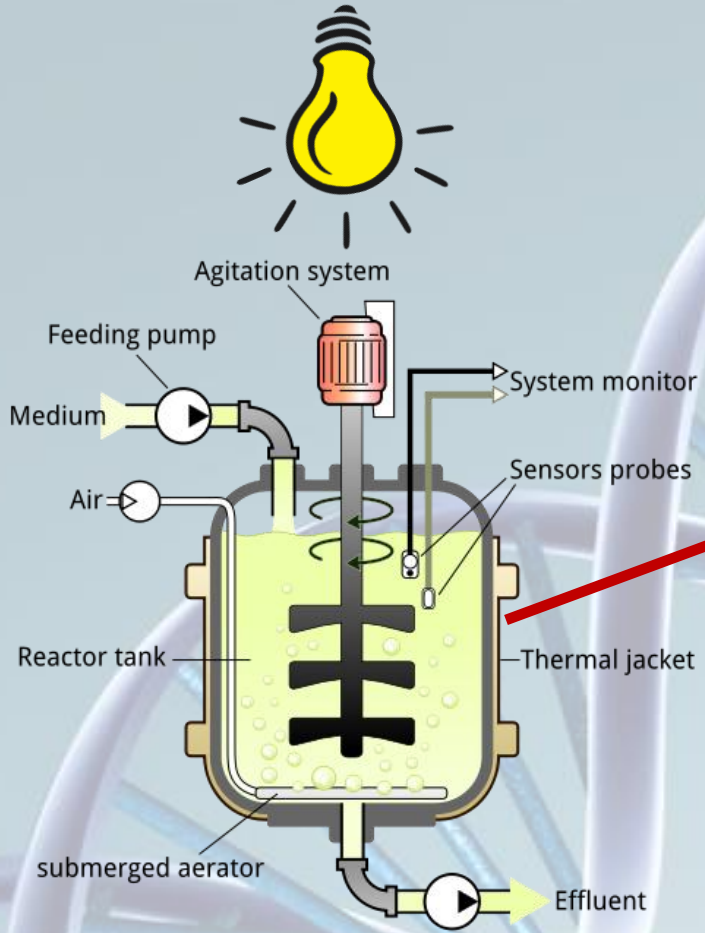
- Use genes like Lego bricks, aim for a maximal compatibility
- Reshuffle genes according to our needs
- Develop functionalities that might not have been present in these living organisms originally

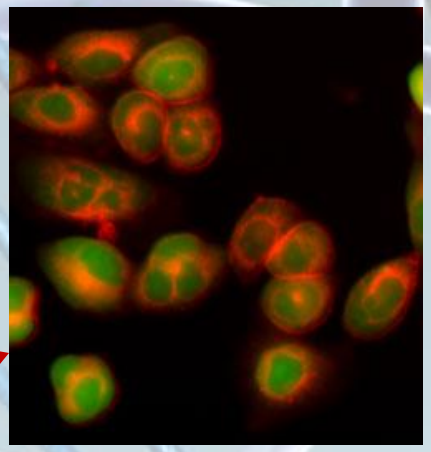


iGEM

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Our aim



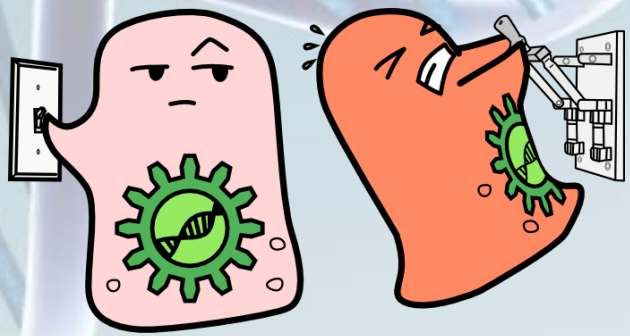


iGEM

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Our project: implement 2 switches

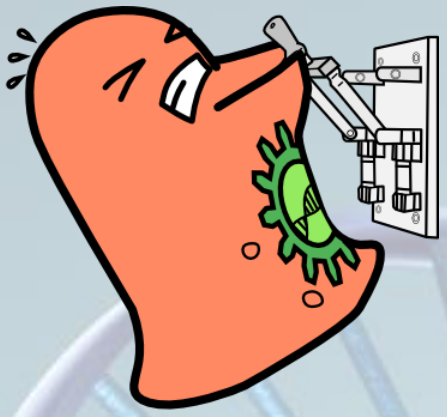
- LovTAP-VP16 switch in CHO cells
- Melanopsin cascade:
  - Repeat M.Fussenegger et al.'s experiment in CHO cells

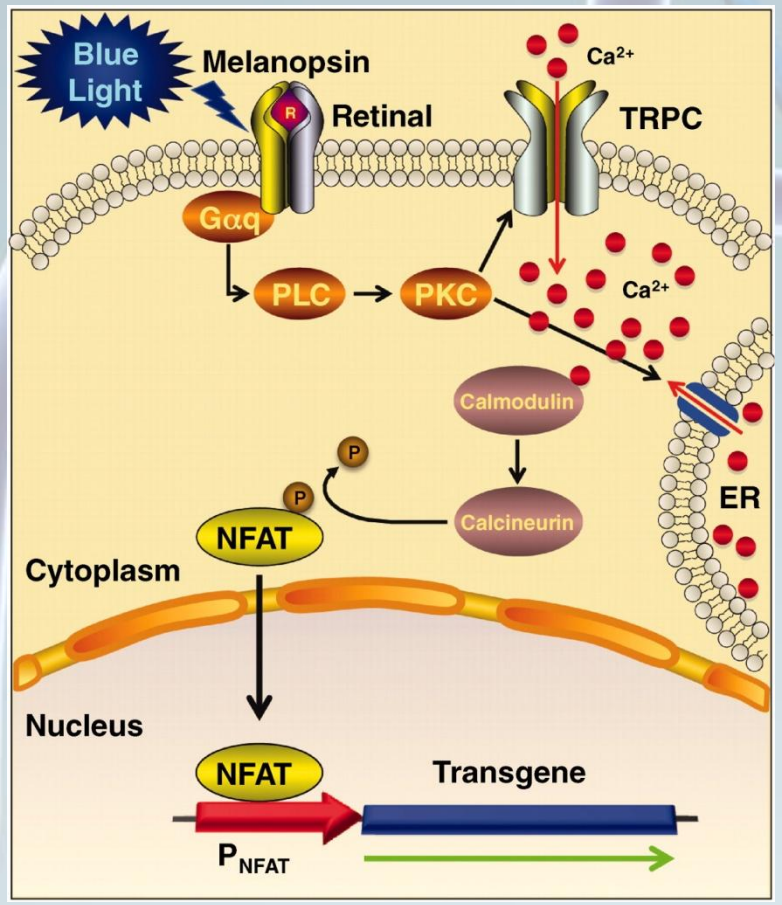


iGEM

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Melanopsin cascade

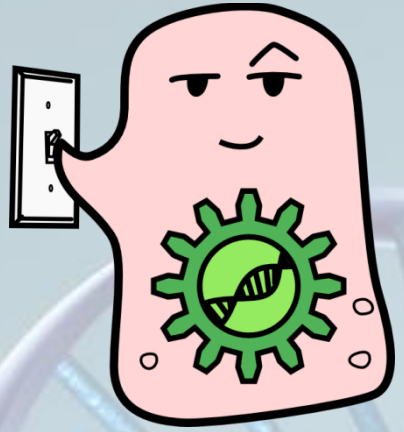


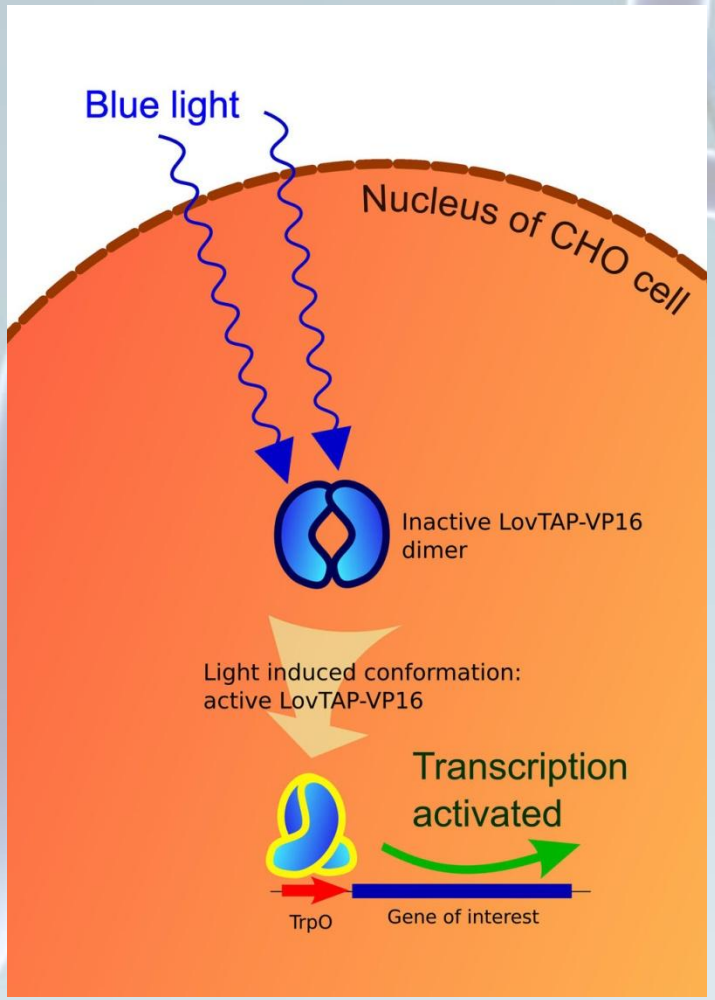


***M. Fussenegger et al**; A Synthetic Optogenetic Transcription Device Enhances Blood-Glucose Homeostasis in Mice. . Science (2011) Vol.23:1-8.*

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

LovTAP-VP16





iGEM

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

LovTAP-VP16

- LovTAP, developed in 2008 at University of Chicago:
  - Light triggered repressor in vitro based on TrpR
- LovTAP biobricking, by EPFL 2009 iGEM team:
  - Light triggered repressor system in bacteria
- LovTAP-VP16, by EPFL 2012 iGEM team:
  - Light triggered activator in mammalian cells

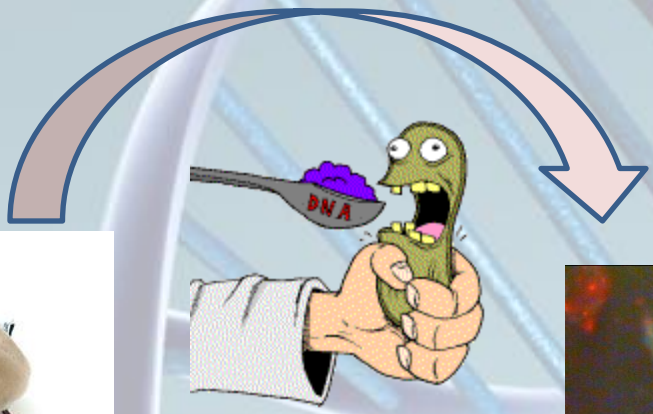
iGEM

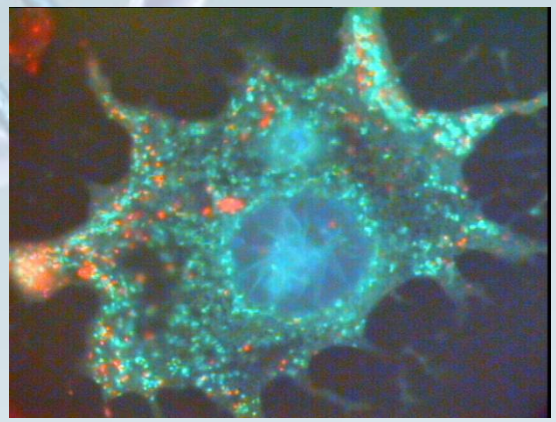
EPFL
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

The SWITCH project

Our project consists of two distinct parts:

Cloning in bacteria: E.Coli





Mammalian expression : CHO cells

iGEM

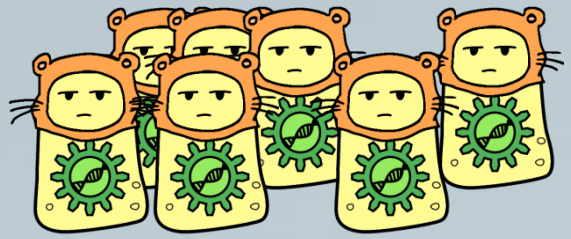
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Our summer

- Find/Synthesize and clone all plasmids for expression in mammalian cells using E.Coli
- Transfection of CHO cells with both switches and expression of the protein
- Build a computer model of LovTAP-VP16 to check if adding VP16 changes folding capacities
- Build a custom bioreactor

iGEM

EPFL
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE



Let's DisCHO!

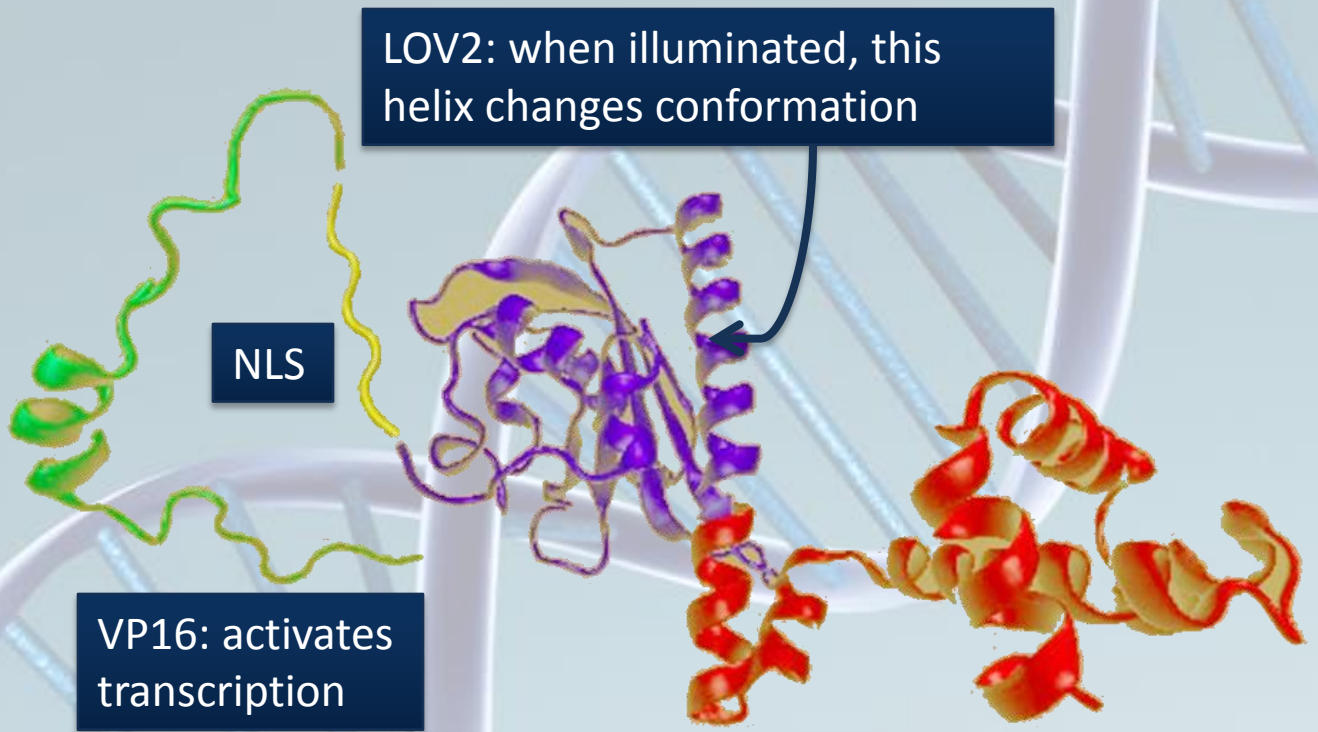
- The goal: insert a genetic «switch » into mammalian cells in order to control them with light
- A protein that reacts to light with a conformational change, which in turn induces the transcription of a gene (ex. for a therapeutic protein)

  → Light induced transcription means we can control the amount of protein produced easily

iGEM

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

LovTAP-VP16



iGEM

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Experimental techniques: Cloning

PCR amplification or digestion of selected genes (LovTAP and read-outs) out of the original plasmid

+

Digestion of the receiving backbone plasmid

↓

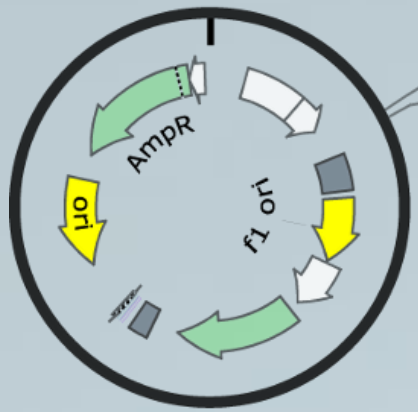
Ligation of the digested product(s) into the backbone plasmid

↓

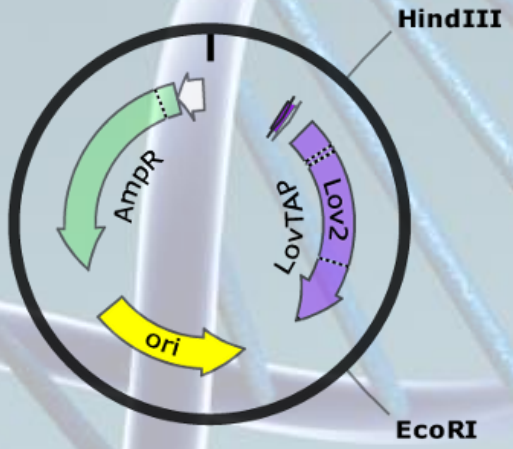
Transformation of the resulting ligated plasmid

iGEM

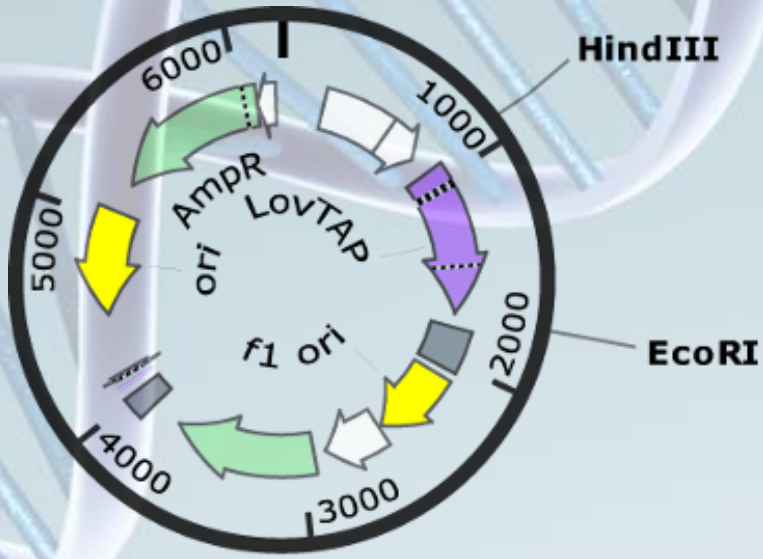
EPFL
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE



pcDNA3.1 (+)
5428 bp



pMA-LovTAP
3216 bp



Cloned
6223 bp

iGEM

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Experimental Techniques: mammalian expression

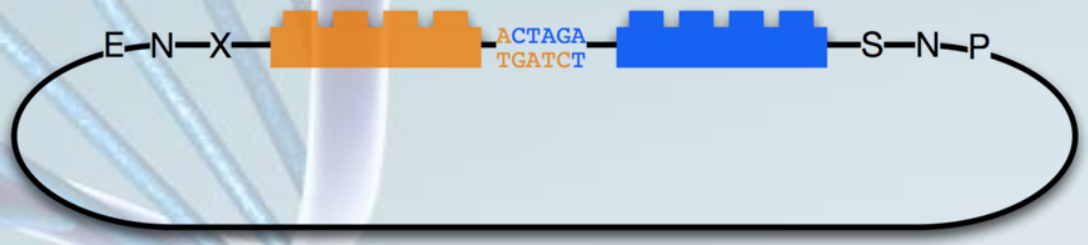
- Plasmid preparation (Mini/Maxipreparation)
- Transfection of CHO cells
- Quantification of messenger RNA - qPCR
- To measure protein expression :
  - Flow cytometry: measure fluorescence
  - Western Blot: detect the protein
  - ELISA: detect and quantify the read-outs

iGEM

EPFL
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Biobricks

- Biobricking uses the same experimental techniques as for the cloning with different specificities for primer design :
  - introduction of the iGEM biobrick restriction sites which enables us to insert them onto the gene which will be amplified by PCR



ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Progress

- Solid evidence of the LovTAP-VP16 gene being in the correct plasmid
- Cloned most of the other required plasmids
- Tried mammalian expression, then came back to cloning
- Hope to be able to transfect and express our proteins in mammalian cells during the next week

iGEM

EPFL
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Acknowledgements

Instructors: Deplancke Bart, Hacker David, Maerkl Sebastian

Teaching Assistants: Balasubramanian Sowmya, Blackburn Matthew Christopher, Odermatt Pascal Damian

KontaktGruppe für Forschungsfragen
Contact Group for Research Matters

NOVARTIS

Roche

syngenta

life technologies™

Merck Serono
Living science, transforming lives

BASF
The Chemical Company

iGEM

EPFL
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE