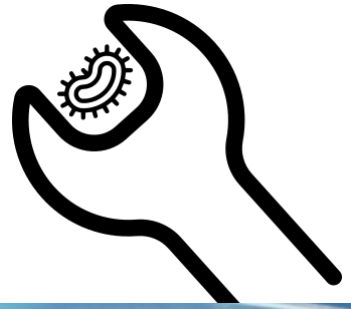
Xpress Biologics

Proteins & DNA for pre-clinical applications

A molecular biology toolbox for rapid selection of E. coli expression strains

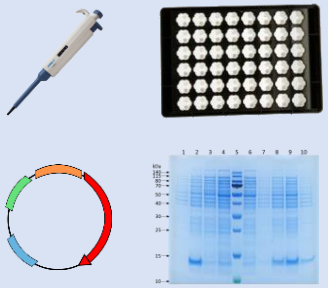


Cell Factories for Industrial Bioproduction
CFIB - Selecting and enhancing expression systems for biomolecules production
Biocitech Paris-Romainville, March 29 and 30th, 2022

Xpress Biologics

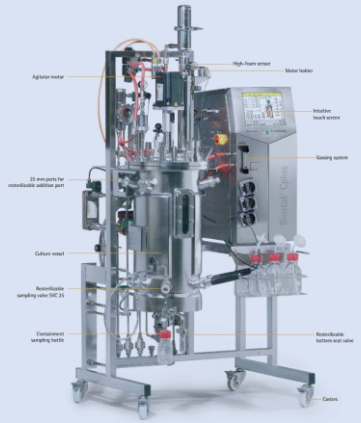
- ✓ C**D**MO company based in Liège (Belgium)
- ✓ 33 people at the moment
- ✓ **Production** and **purification** of biomolecules: **pDNA** and **proteins** (antibody fragments, vaccines, enzymes)
- ✓ From gene to multi-gram industrial process for pre-clinical and clinical trials (GMP facility to be operational next year)
- ✓ **Platform-based** process development (possibility to start at any stage of a process development)

Molecular biology



Escherichia coli
Pichia pastoris

USP

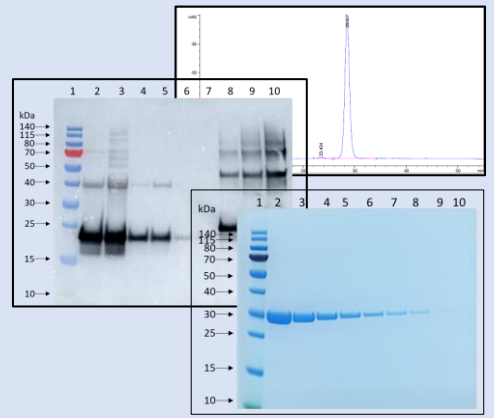


5-50L Bioreactors

DSP

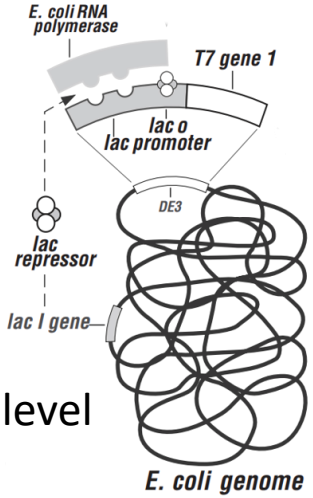
AKTA systems
Avant-Pilot-Process

QC



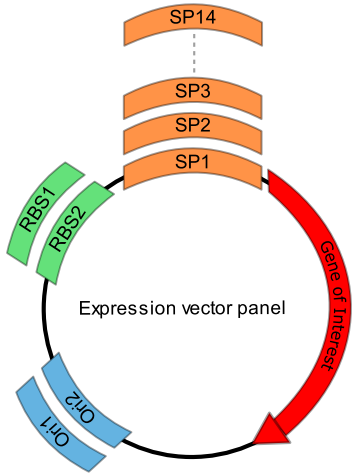
IPC/QC method development

E. coli strain development – Levels of optimisation

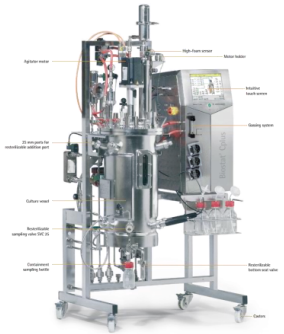


Expression host level

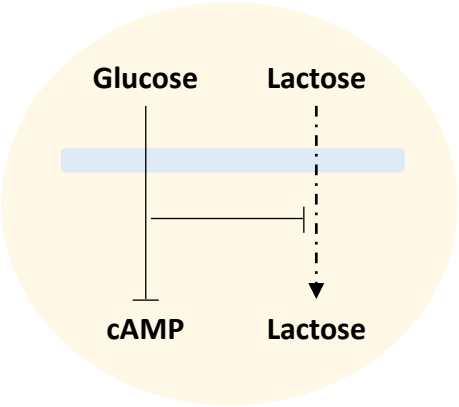
Optimized strain



Expression vector level



Bioreactor production level



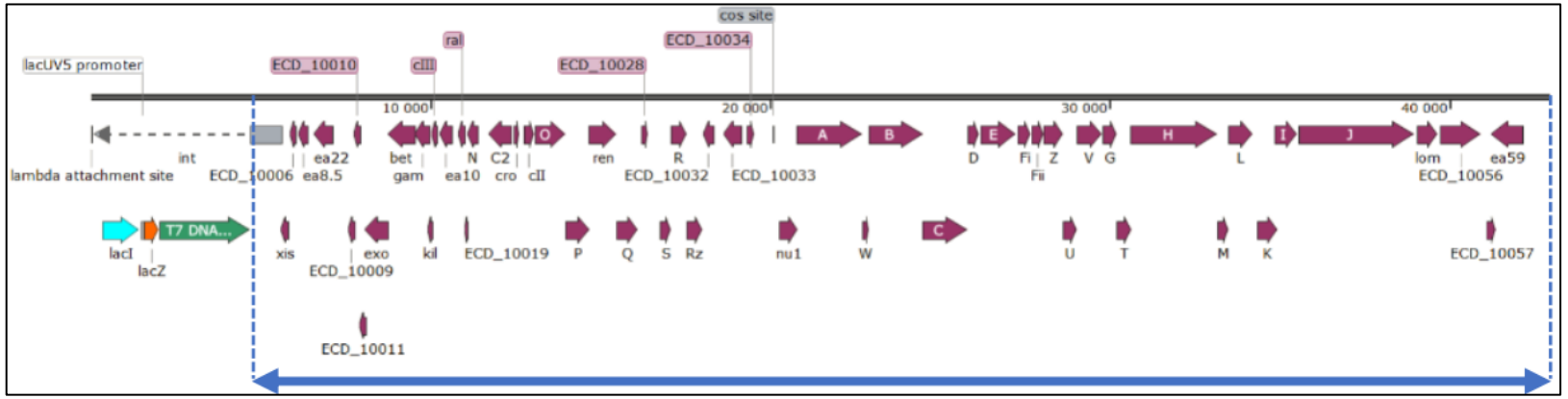
Expression screening method level

Escherichia coli platform – Strains

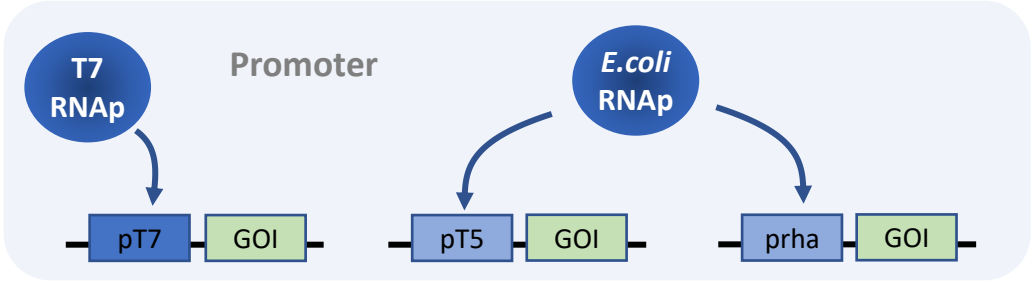
IP-free *E. coli* strains used for expression system development

- ***E. coli* BL21**:
  - **Protease-deficient** (Lon and OmpT)
  - used in expression systems not requiring the T7 RNA polymerase

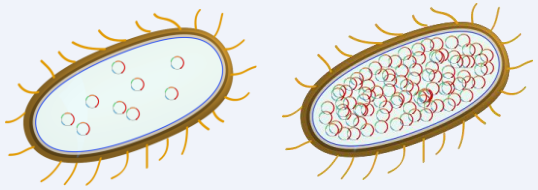
- ***E. coli* BL21 XT7**: BL21 (DE3) derivative obtained after the removal **prophage** elements **inducing lysis in stressful conditions**



Escherichia coli platform – Expression vectors



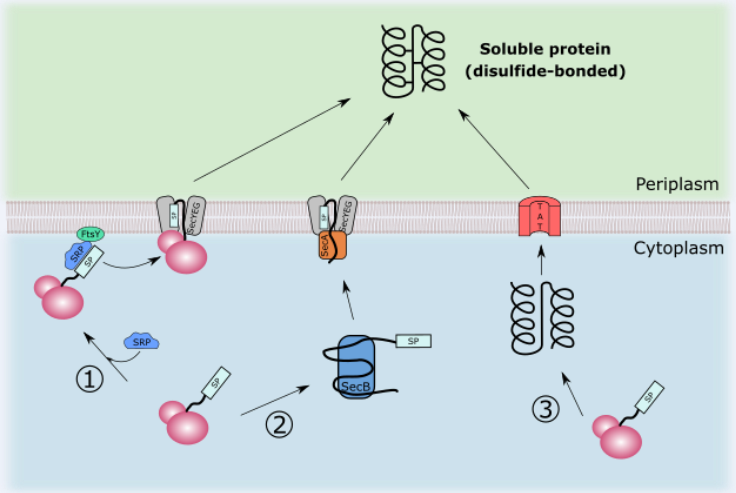
Plasmid copy number



10-12 copies >500 copies

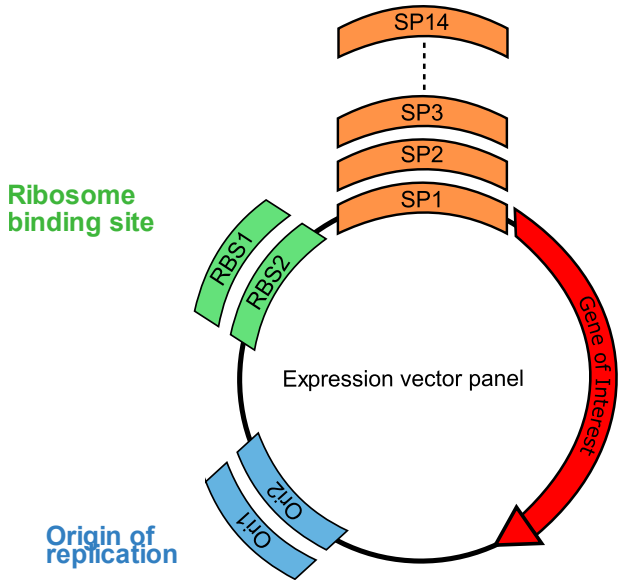
Periplasmic expression

Screening of up to **14 signal sequences**

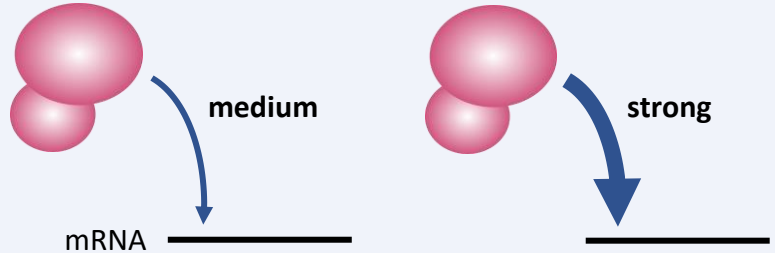


Adapted from Natale *et al.*, 2008

Signal sequences



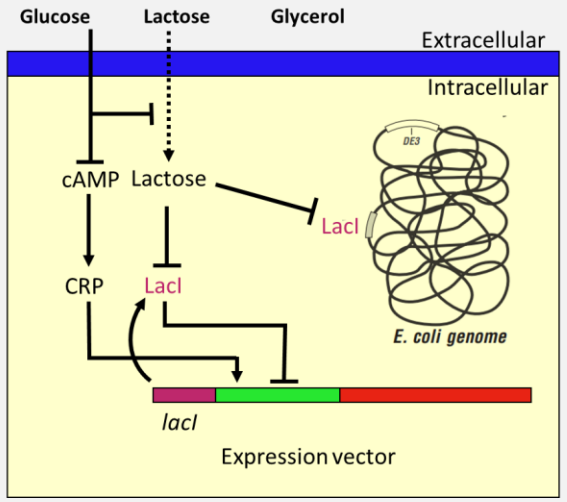
Strength of Ribosome-RBS interaction



Up to **112** possible combinations for a single target gene

Escherichia coli platform – Microplate expression method and objective

Autoinduction – Principle and method

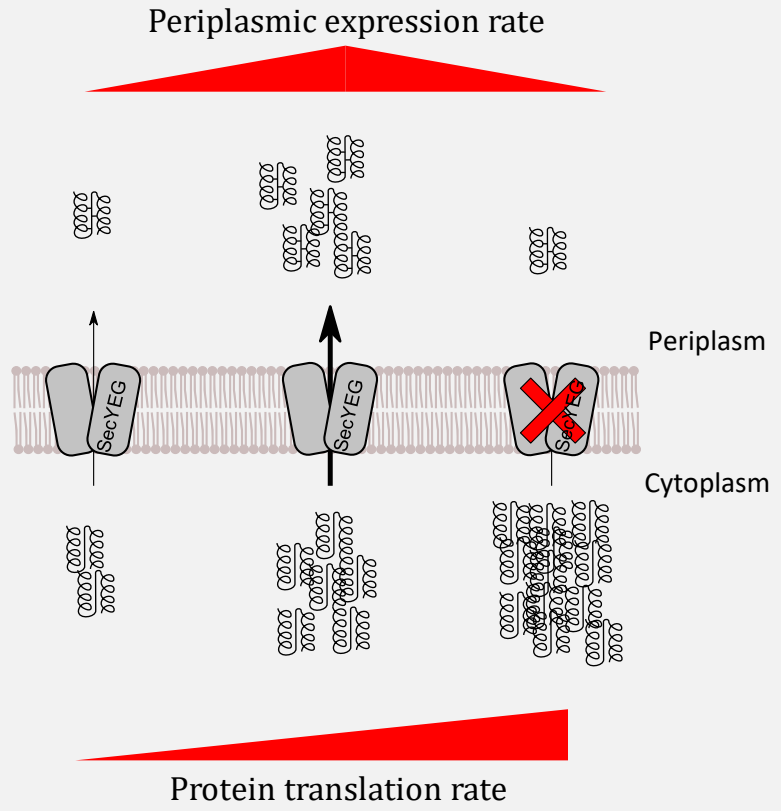


Adapted from :
www3.imperial.ac.uk/pls/portallive/docs/1/15699698.PPT

Autoinduction method

- Inducer (lactose/rhamnose) present in the culture medium
- **No OD_{600} monitoring required**
- Catabolic repression of glucose limiting target protein production
- Depletion of glucose before intake of inducer (**late induction**)
- rhamnose concentration to **modulate expression rate**
- Final OD_{600} : 20-25

Autoinduction – Translocation optimisation

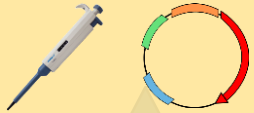


Objectives

- Selection of the **best signal sequence candidates**
- Collect information about **expression/translocation efficiency**

Escherichia coli platform – Protein production – Timelines

Week one



Ligation of target gene in expression vectors with Electra system (**14 signal sequences screened**)
Transformation in expression strain (***E.coli* BL21**)
Generation of backup plates as starting material for expression screening
Minipreps and Restriction mapping QC

Week two

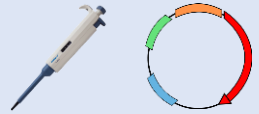
Expression screening in microplate by **autoinduction** mode with **three levels of expression strength** thanks to a tunable **rhamnose-inducible promoter** at **20-37°C**
Harvest + periplasmic extraction + SDS-PAGE QC
Clone selection with client
PCR amplification of target gene + selected signal sequence

From **target gene** to **GCB** for fermentation development in **4 weeks**

Week four

Expression screening in microplate by **lactose autoinduction or IPTG pulse mode** at **20-37°C**
Harvest + periplasmic extraction + SDS-PAGE QC
Clone selection with client
GCB manufacturing to initiate bioreactor productions

Week three

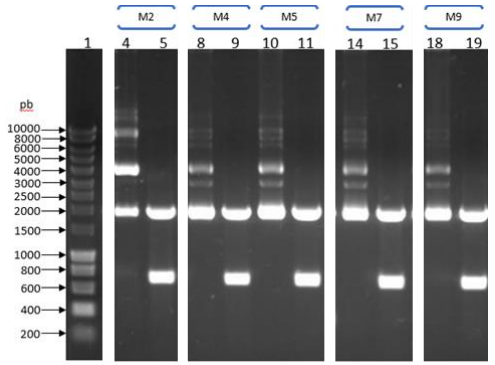


Ligation of target gene with Electra system + selected signal sequences in **IPTG-inducible vectors** (**low/high plasmid copy number** and **medium/strong RBS**)
Transformation in expression strain (***E.coli* BL21 XT7 or BL21**)
Generation of backup plates as starting material for expression screening
Restriction mapping QC and **DNA sequencing**

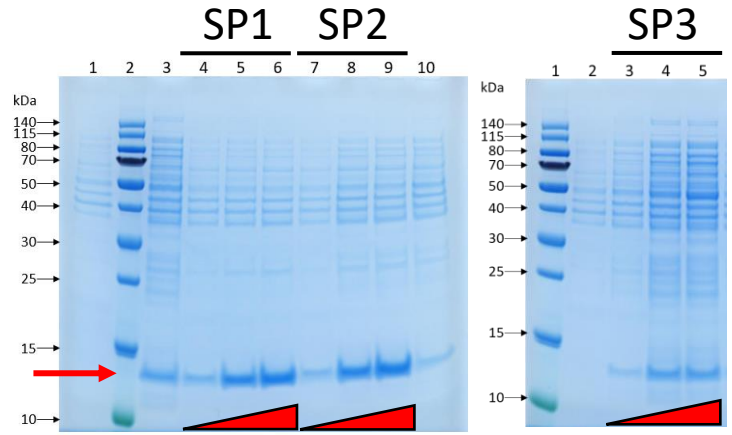
Escherichia coli platform – signal peptide screening

Case study I: Nanobody project (periplasmic expression)

Signal sequences screening



Week one

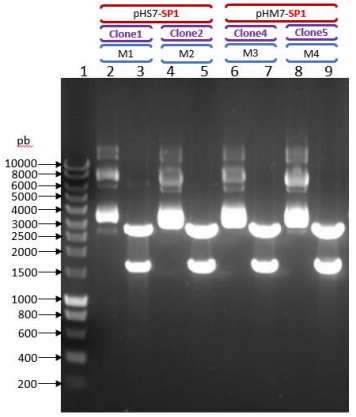


Week two

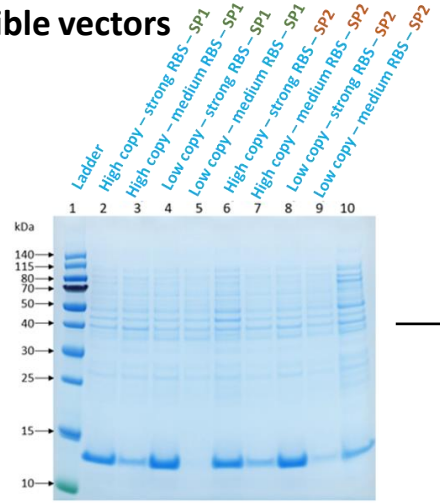
[rhamnose]

- Target gene subcloning in up to 14 rhamnose-inducible vectors
- Induction modulation with [rhamnose]
- **Rhamnose autoinduction** to limit plate manipulation
- ~13kDa target protein detected in periplasmic extracts generated by osmotic shock
- First look at the **expression/translocation potential** of the target protein

Copy-number & RBS screening in IPTG-inducible vectors



Week three



Week four

GCB manufacturing

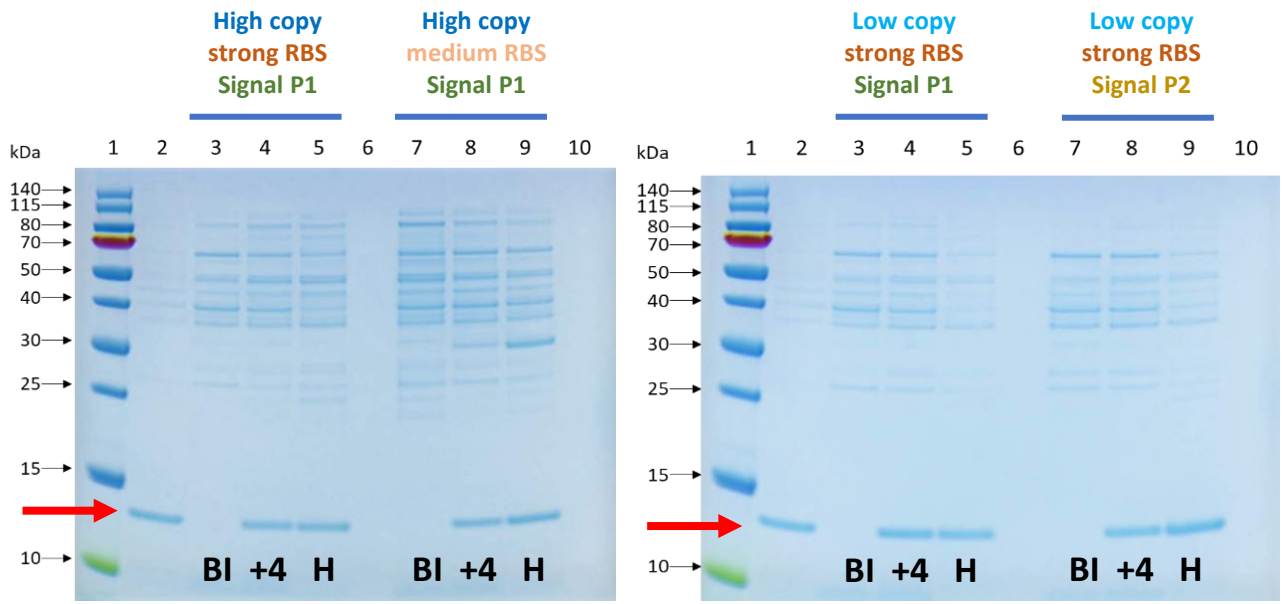
- Selected signal sequence(s)/target gene in IPTG-inducible vectors : **low/high copy number** and **medium/high RBS-ribosome affinity**
- IPTG induction (1mM) at required temperature
- Clone selection based on **expression yield** in the periplasmic space and presence of the target protein in the insoluble fraction (inclusion bodies)
- **GCB manufacturing** to generate starting material for **fermentation development**

Escherichia coli platform – signal peptide screening

Case study I: Nanobody project (periplasmic expression)

Clone selection by fermentation

High-density Fed-batch fermentation
- Uncoupling of biomass growth and target protein induction
- Controlled cellular growth to **minimize metabolic burden** before and during induction
- Harvest data: **OD_{600} 100-200** at harvest (**200-250 g/L** WCW)



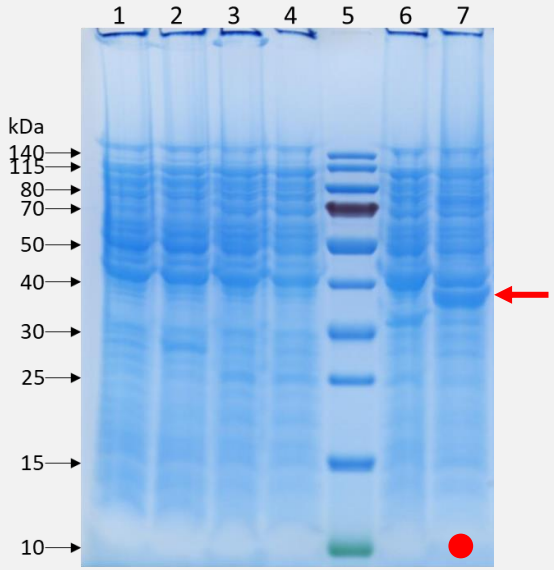
Factors for clone selection (four candidates):
- Production yield
- Protein integrity
- Antifoam pulses until process completion
- pDNA stability (>80% preferred)

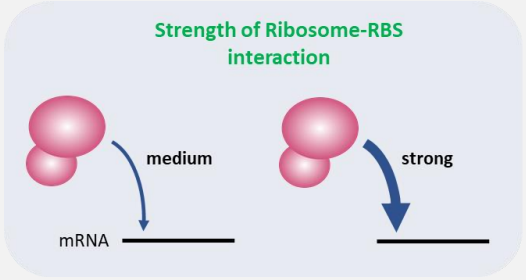
Relevant data from bioreactor harvest	High copy / strong RBS / Signal P1	High copy / medium RBS/ Signal P1	Low copy / strong RBS / Signal P1	Low copy / strong RBS / Signal P2
OD_{600} at harvest	186	124	197	127
Volume harvested (L)	4,4	4,4	4,5	4,4
N pulses antifoam	4	4	1	1
µ (h-1) induction	0,055	0,035	0,059	0,035
pDNA stab. at harvest (%)	55	37	94	93
Viable cell concentration (VCC) at harvest (CFU/mL)	$8,8x10^{10}$	$2,7x10^{10}$	$1,25x10^{11}$	$1,35x10^{11}$
Cell paste mass at harvest (g)	905	1051	840	928
Yield in periplasmic fraction at harvest	2,32	1,2	2,79	3,54
Protein amount estimation (g)	10,21	5,28	12,56	15,58

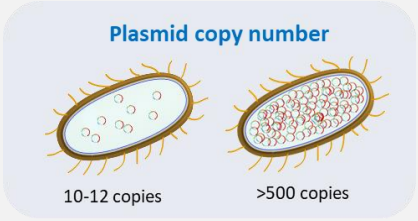
Escherichia coli platform – inclusion body screening

Case study II: vaccine production

Microplate expression screening

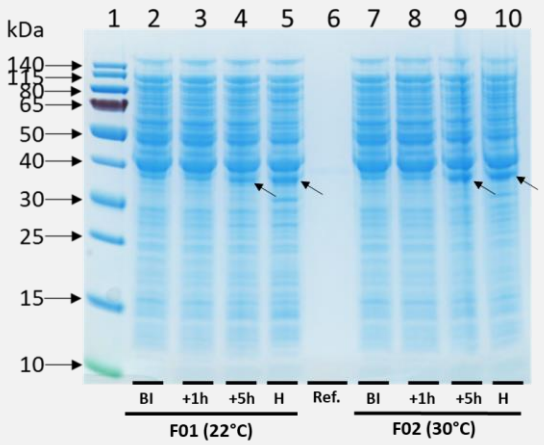


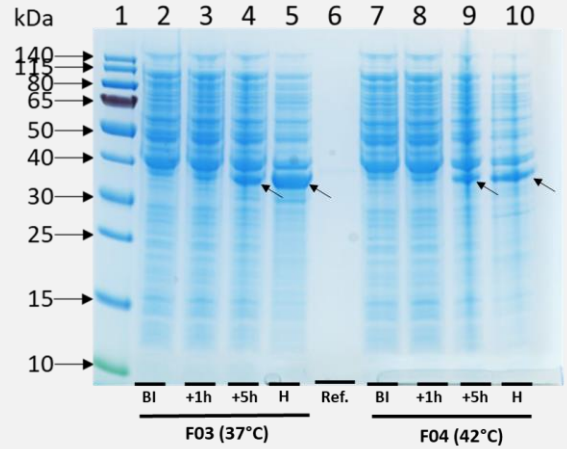




Lane #	Sample name
1	High-copy / medium RBS / non-induced
2	GFP
3	Low-copy / medium RBS / induced
4	Low-copy / strong RBS / induced
5	PageRuler
6	High-copy / medium RBS / induced
7	**High-copy / strong RBS / induced**

Bioreactor production development (5-L scale)



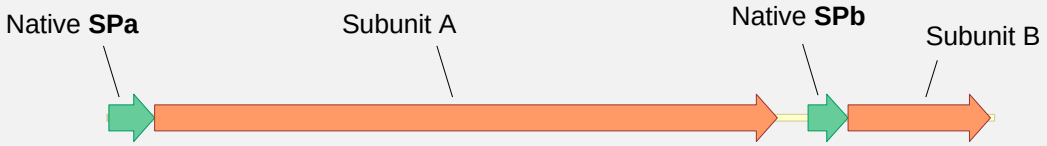


Run #	OD_{600} harvest	**Induction T(°C)**	Yield (g/L)	pDNA stab. (%)
F01	150,5	**22**	3.3	96
F02	136,5	**30**	3.7	83
F03	132,5	**37**	12.5	**82**
F04	113,5	**42**	7.1	92

Escherichia coli expression screening platform – protein complex production

Case study III: hetero complex 1:5 (A:B) ratio

Microplate expression screening



Signal peptide (3 + 1 native) + expression rate screening (rhamnose-inducible vectors)
= 30 conditions tested

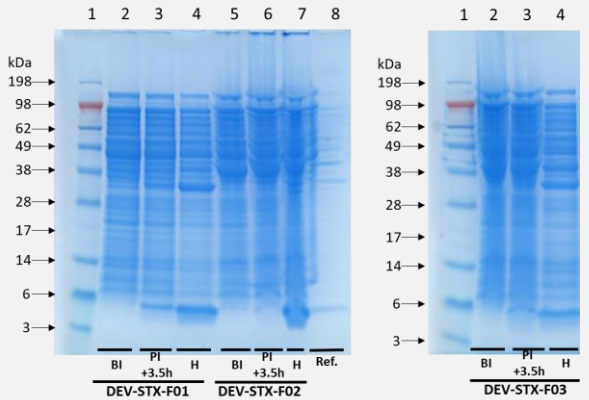
⬇

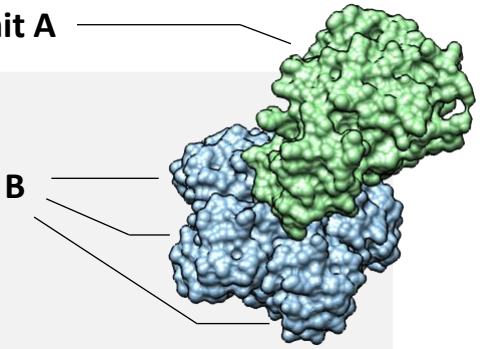
Test of plasmid copy number + RBS/ribosome affinity (IPTG-inducible vectors)
= 8 combinations tested

⬇

Selection of one couple of signal sequences
= **3 IPTG-inducible vectors** to be tested in **bioreactor**

Bioreactor production development (5-L scale)





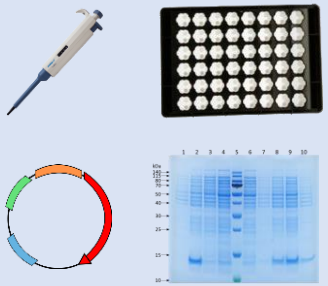
Relevant data from bioreactor harvest	Low copy / strong RBS / Signal P1+P1	Medium copy / medium RBS / Signal P1+P1	High copy / medium RBS / Signal P1+P1
OD_{600} at harvest	209	115	216
pDNA stab. at harvest (%)	96	75	98
Cell paste mass at harvest (g)	868	956	832
Ratio subunitA/subunitB	+	++	++

Factors involved in the clone selection:

- Production yield
- Plasmid stability
- Ratio between subunits

Xpress Biologics

Molecular biology



Escherichia coli
Pichia pastoris

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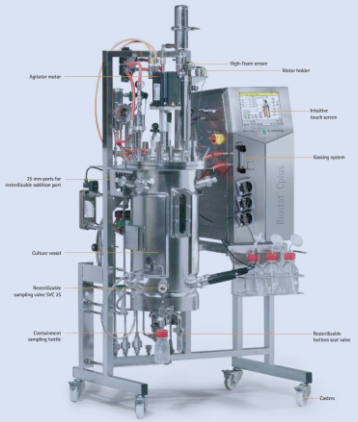
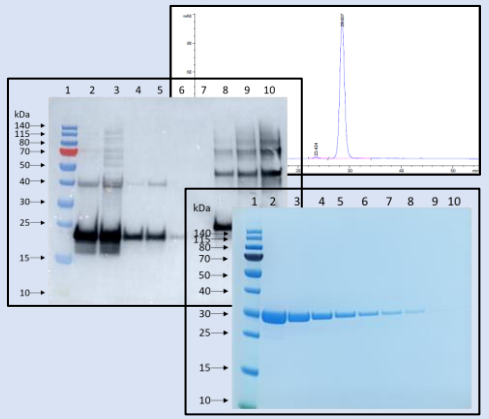


5-50L Bioreactors

DSP

AKTA systems
Avant-Pilot-Process

QC



IPC/QC method development

Platform approach for DSP development

- Small scale screening of chromatographic sorbent
- Scale-up within an industrial setup
- Sizing of clarification/filtration to easily scale-up purification process

IPC/QC method development

- Performed along the process development
- Quality criteria to be discussed with client beforehand to rationalize the purification steps
- Extended experience to challenge/optimize purification strategy to be able to deliver products in agreement with requirements of regulatory agencies (purity, safety, efficacy)

Contact: m.rocaboy@xpress-biologics.com