A Novel Endotoxin-free Microbial Recombinant Protein Expression System: Solutions for High Quality Proteins

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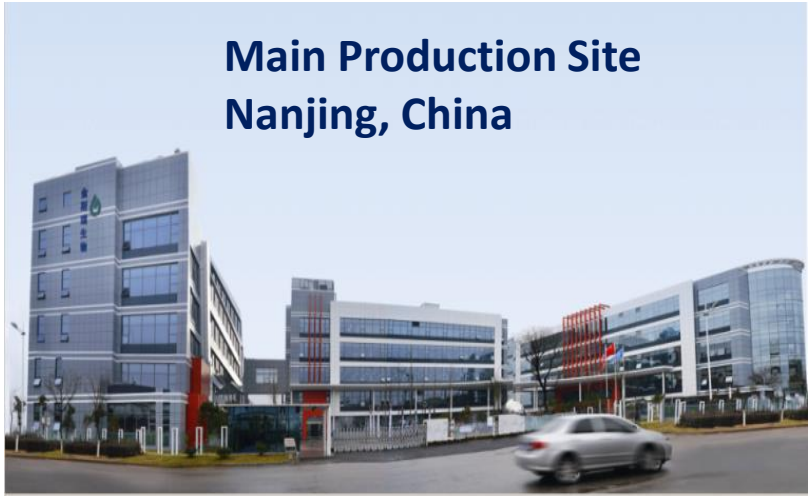
GenScript
Make Research Easy

CONTENTS

GenScript, A Global Bio-CRO

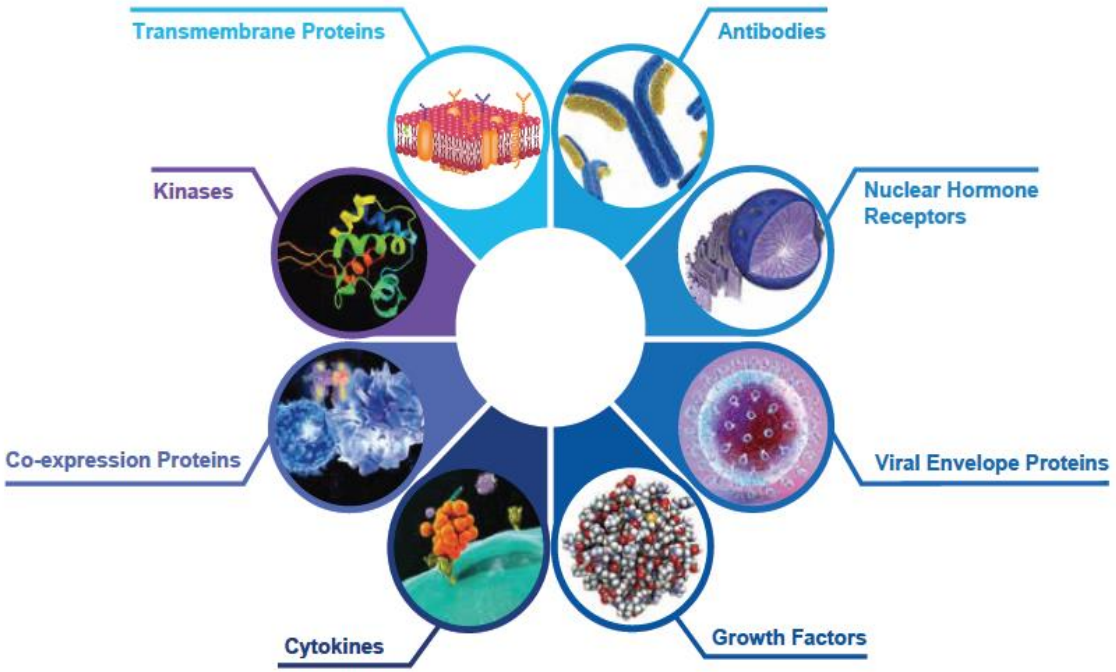
- Founded in 2002
- Publicly traded in the Hong Kong Stock Exchange (HKG:01548)
- Over 2000+ employees
- Top company globally for gene synthesis
- Top Bio-CRO in China
- Offering one-stop service and specialized in biologics discovery & development

- Local technical support
- 24-hour customer service
- Fast global logistics
- Competitive pricing
- Stringent Intellectual Property protection



GenScript Track Record

- GenScript has been producing recombinant proteins since 2004.
- The department has a very diverse portfolio when it comes to producing proteins. The figure on the right shows protein types that we have successfully produced and delivered.
- You can see that the protein department has delivered over 10,000 protein projects, has a success rate of over 95%, and has delivered over 5,000 recombinant antibody projects.



10,000+
High quality Protein orders delivered

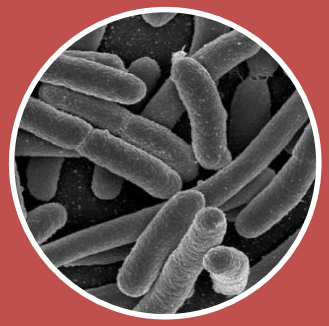
>95%
Success rate for all Protein projects

5,000+
High quality Recombinant Antibody orders delivered

Our track record: Successful track record of delivering high quality proteins.

Types of Expression Systems to Produce Recombinant Proteins

- GenScript offers recombinant protein production in four systems (Bacteria, Insect, Yeast, and Mammalian.
- How does one select which expression system to use? There are various factors that go into that decision making process. Below are some of the key features of each expression system that can help in determining which one would be best for the customers' needs.



Bacteria

E. coli

1. "Work horse"
2. Well established
3. High expression
4. Simple genetics
5. Easy scale up
6. Speed
7. Costs
8. Equipment

Insect

Sf9, High-5

1. Post translational modifications
2. Soluble proteins
3. High expressers

Mammalian

CHO, HEK

1. Post translational modifications
2. Soluble proteins
3. Low expresser
4. Expensive

Expression Systems Overview

Expression System	Advantage	Disadvantage	Recommended Applications	Suitable Targets
Bacteria	• Cost-effective • Fast turnaround time • Simple to culture • Easy to scale up	• Low protein solubility, in vitro folding often necessary • Limited post-translational modifications • Low yield of many eukaryotic proteins	• Structural analysis • Antigen production • Functional assays • Protein interactions	• Antigens, intra-cellular target proteins, tool enzymes
Insect Cell	• Post-translational modifications similar to mammalian system • Suitable for expression of cell toxic products	• Need longer time • High cost of growth medium	• Functional assays • Structural analysis	• Complicated target proteins, such as kinases and proteases
Mammalian Cell	• Comprehensive post-translational modifications • Best method to produce biologically active proteins	• Low volumetric productivity of protein • High cost of media • Significant time investment	• Functional assays • Protein interactions • Antigen production • Therapeutic protein	• Extracellular target proteins, drug candidate

Case Study 1: Expression of Cas9 and Related Protein

- The goal was to successfully deliver eSpCas9(1.1) from *E. coli* system
- Using *E. coli* we were able to improve the expression level and solubility
- In conclusion, we were able to obtain tag-free protein: ~1 mg/L, purity: >95%

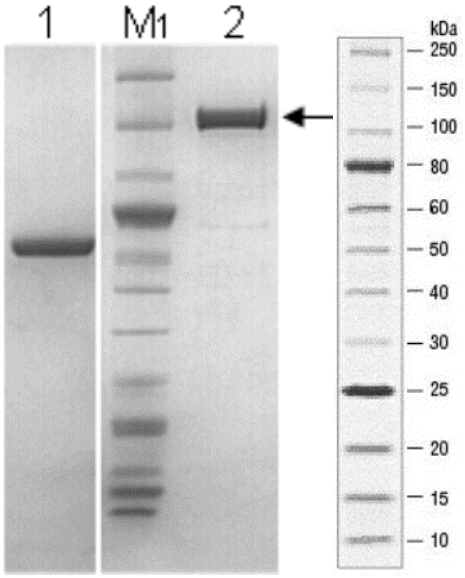
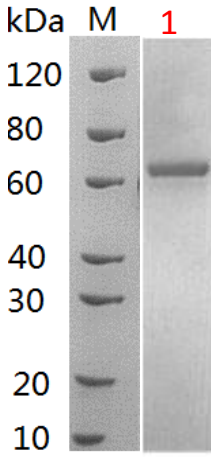


Fig.1 SDS-PAGE analysis
Lane 1: BSA (2.00 μg)
Lane 2: eSpCas9(1.1) (2.00 μg)

Case study 2: Kinase in Insect

- The goal was to successfully express kinase in Insect expression system.
- This protein was labile and easy degraded.
- We were able to successfully receive **Intracellular expression of the Kinase** from 10 liters of Sf9 cell.

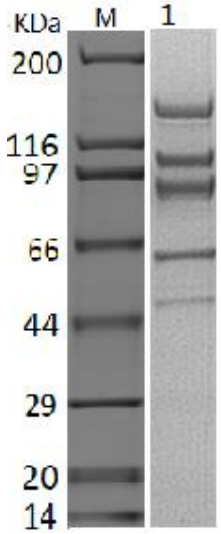


- Purity: 95%
- Yield: 10 mg/L

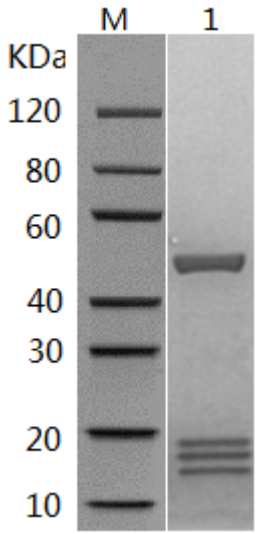
Case study 3: Multi-Subunit Complex in Insect

3 multi- subunit complexes were obtained from our insect expression system successfully

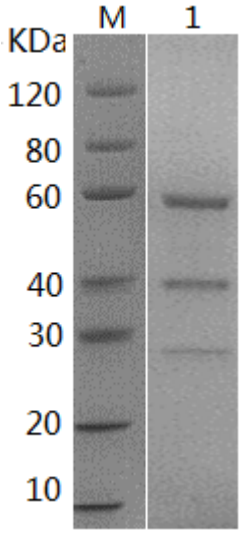
5-subunit complex



4-subunit complex



3-subunit complex



The Insect expression system was an excellent system for multi-subunit complex production

Case Study 4: IgM & IgA in Mammalian

IgM

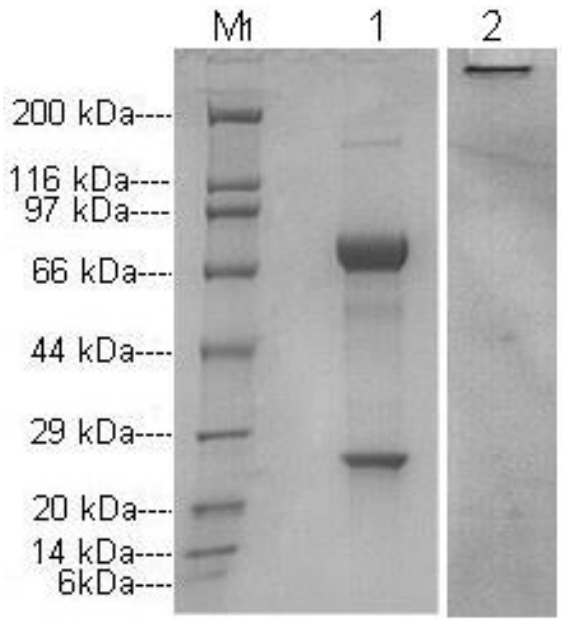


Fig. Left SDS-PAGE analysis of purified IgM
Lane M: Protein Marker
Lane 1: Reducing conditions
Lane 2: Non-Reducing conditions

IgA

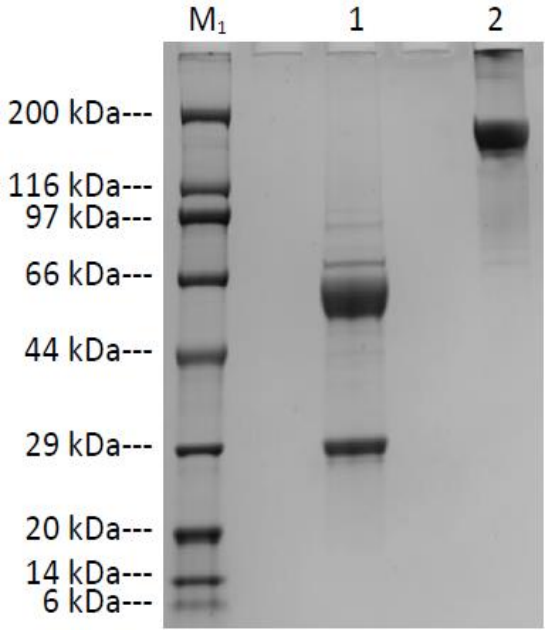
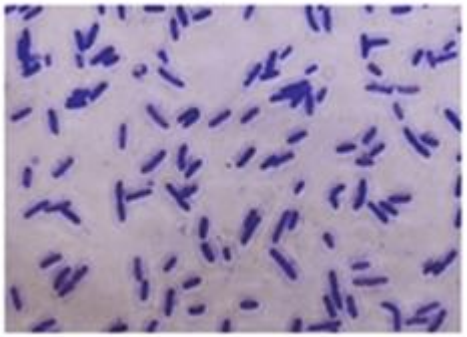


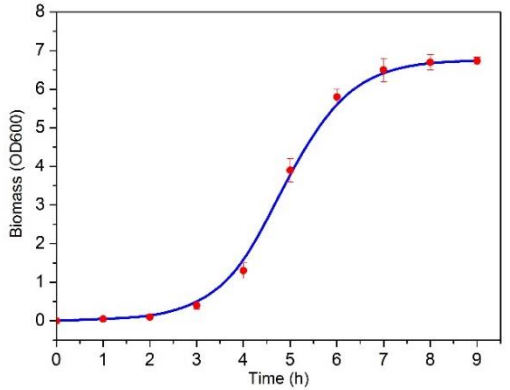
Fig. Right SDS-PAGE analysis of purified IgA
Lane M: Protein Marker
Lane 1: Reducing conditions
Lane 2: Non-Reducing conditions

IgM and IgA were produced in Mammalian cells with a considerable yields

An Introduction of *Bacillus subtilis*



- Model organism of gram-positive bacteria



- Rapid growth rate

- Powerful secretion capacity

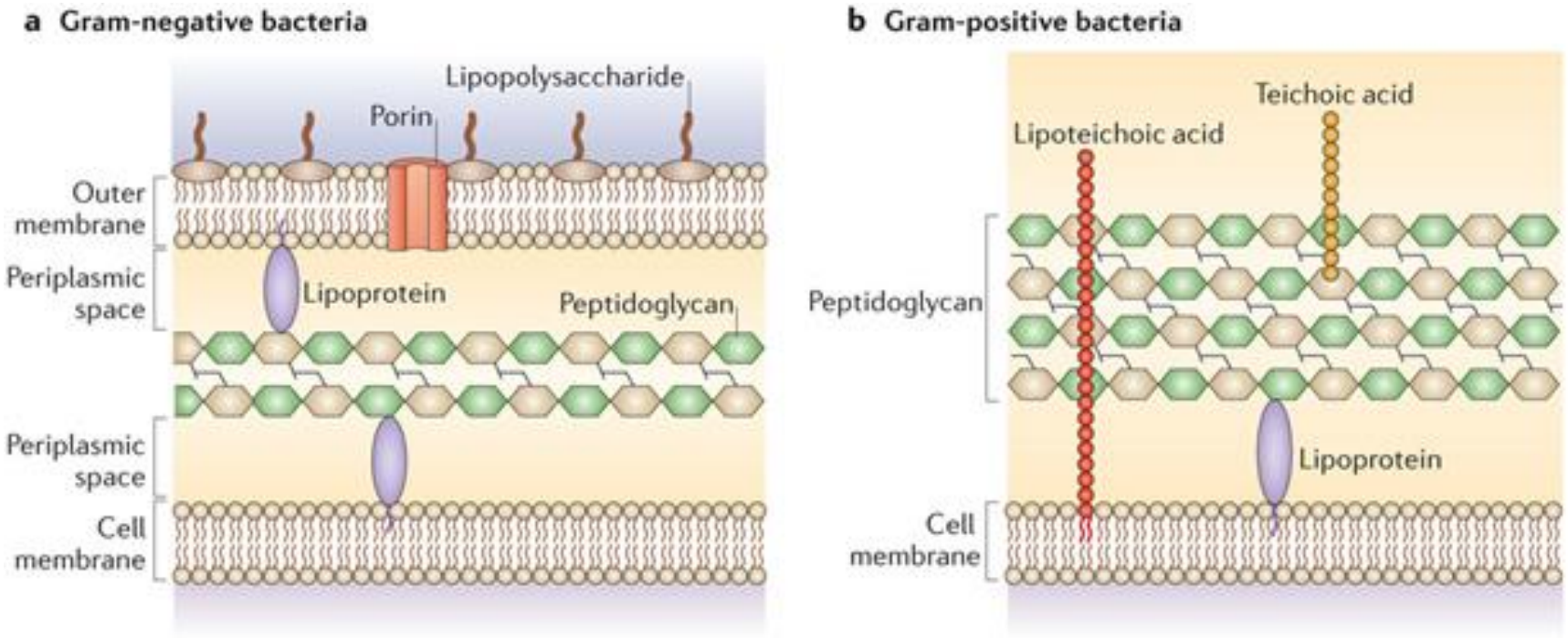
FDA

Ingredients listed as GRAS
(Generally Recognized AS Safe)

- Food-grade microorganism

B. subtilis, which is the second most studied bacteria and has considerable differences in intercellular conditions with *E. coli.* It is supposed to be another excellent bacteria expression host.

The Membrane structure of *B. subtilis*



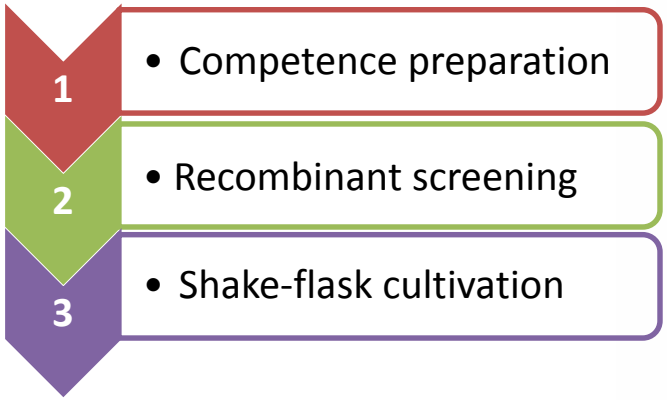
Advantages of *B. subtilis* over *E. coli* in protein production:

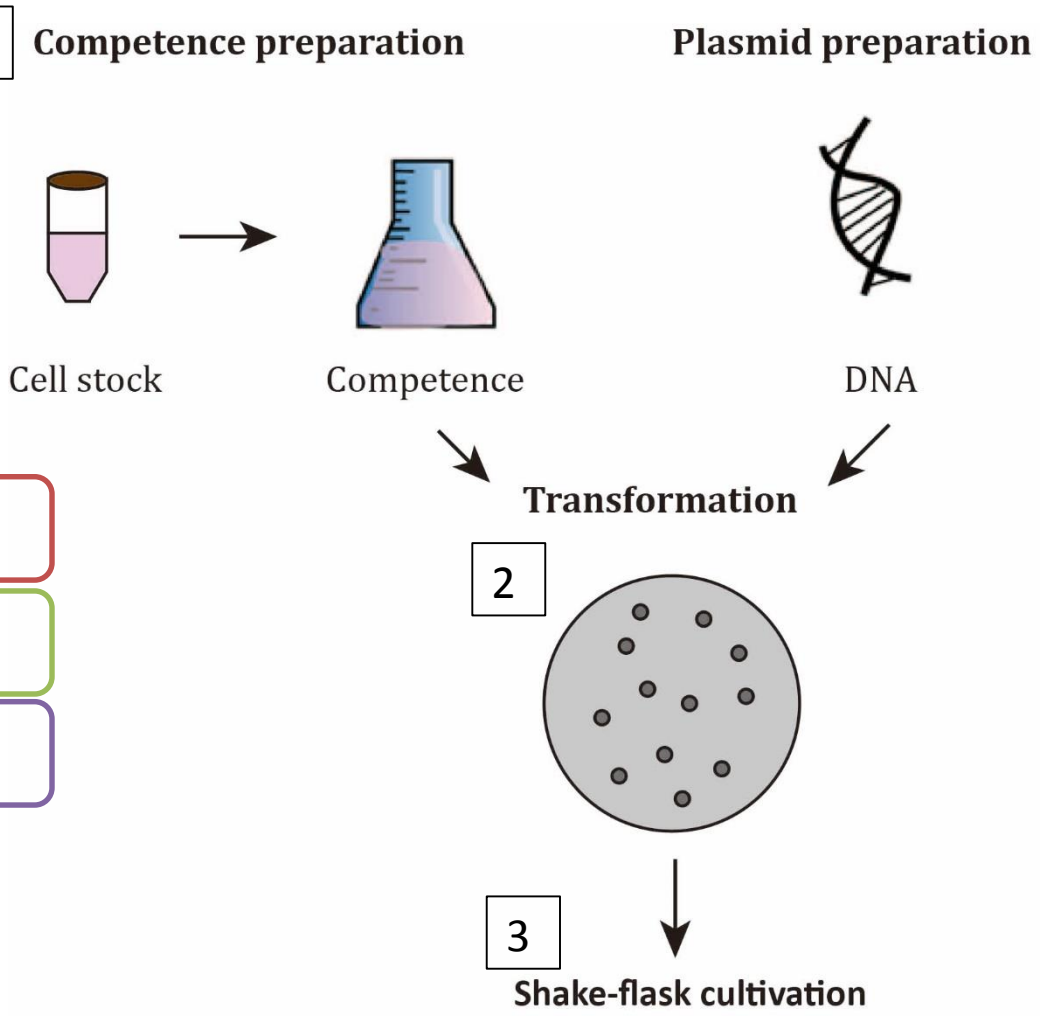
1: Without production of Lipopolysaccharide (endotoxin)

2: Secret protein into medium directly

Citation: Nature Reviews Microbiology volume 13, pages 620–630 (2015)

Bacillus subtilis Protein Production Workflow

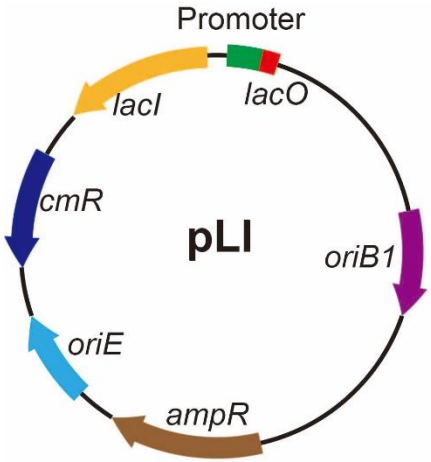




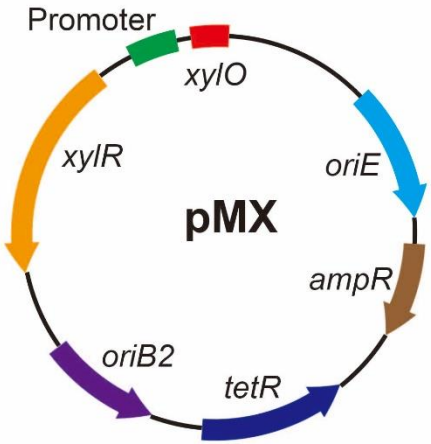
The workflow of *B. subtilis* protein production is quite simple

B. subtilis Expression System

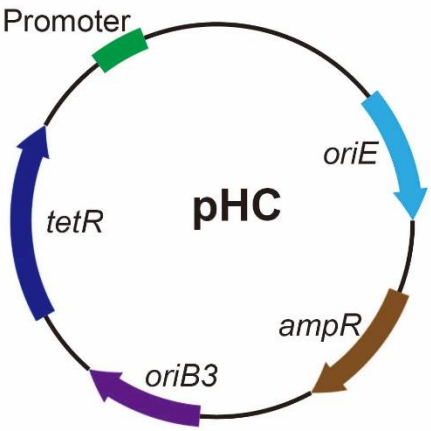
Intracellular expression system



IPTG inducible, low copy number



Xylose inducible, middle copy number



Constitutive, high copy number

Aims:

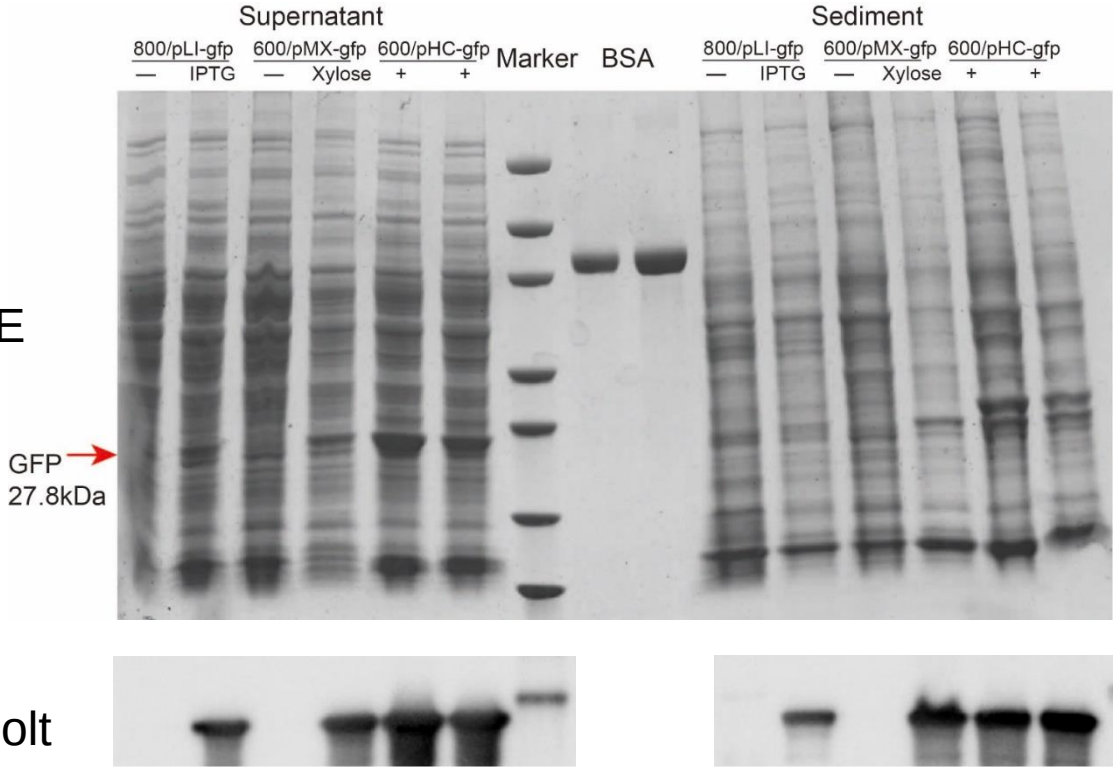
1: pLI and pMX were designed to produce the proteins which were toxic or harmful to the growth of host

2: pHC was designed to improve the expression level of recombinant proteins

Example: GFP Expression Using *B. subtilis*

SDS-PAGE

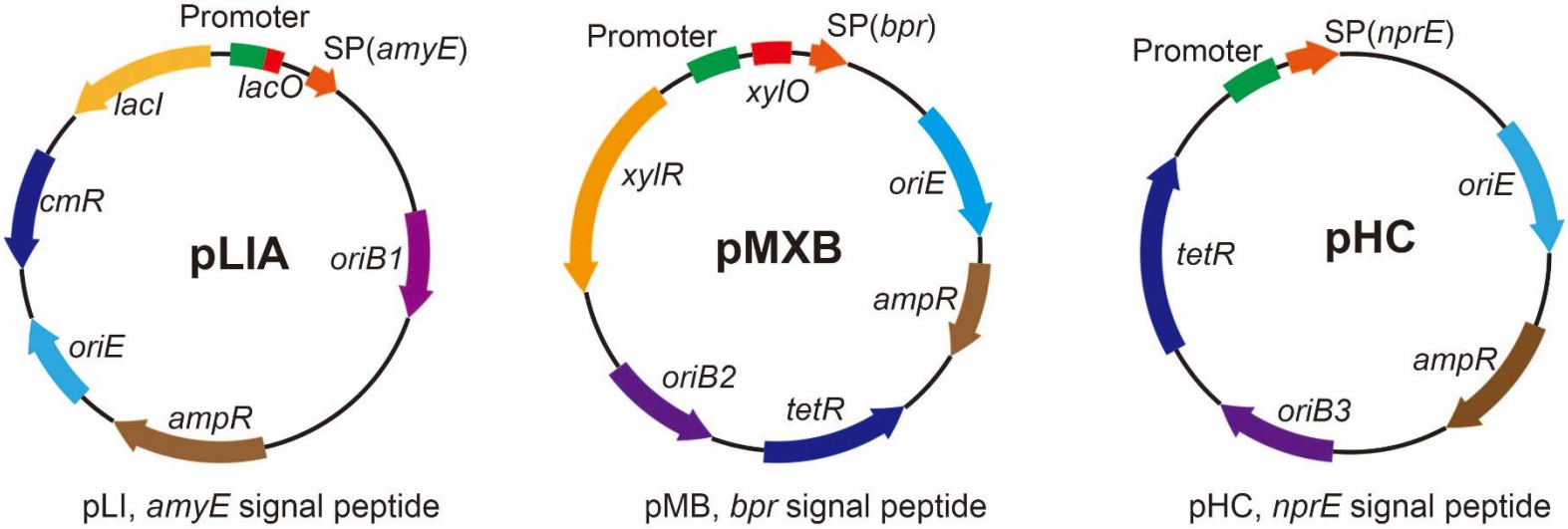
Western-bolt



- GFP was expressed in *B. subtilis*/pLI-*gfp*, B. subtilis/pMB-*gfp*, *B. subtilis*/pHC-*gfp* respectively.
- The inductive effects of pLI and pMB were rigorous. pHC was proved as a high level expression vector

B. subtilis Expression System

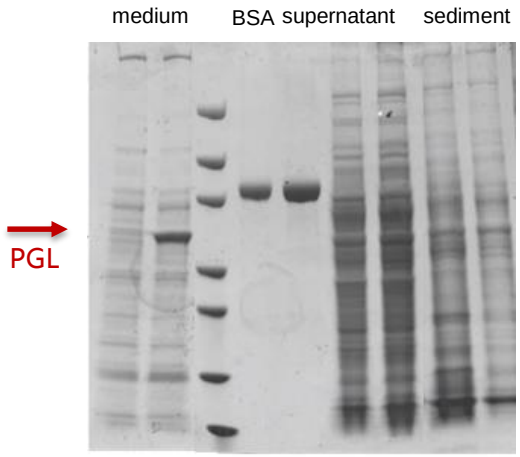
Extracellular expression system



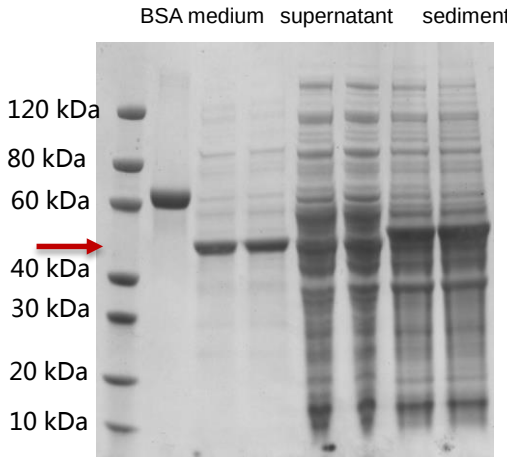
- To secret recombinant protein into medium, 3 frequently-used *B. subtilis* signal peptides, *amyE*, *bpr* and *nprE* are inserted into pLI, pMX and pHC, giving pLIA, pMXB and pHCN respectively.

Example: Pectinase (PGL) Expression Using *B. subtilis*

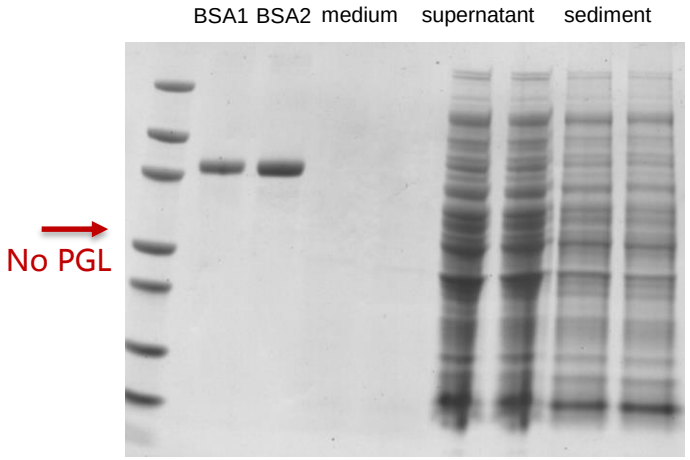
pLIA



pMXB



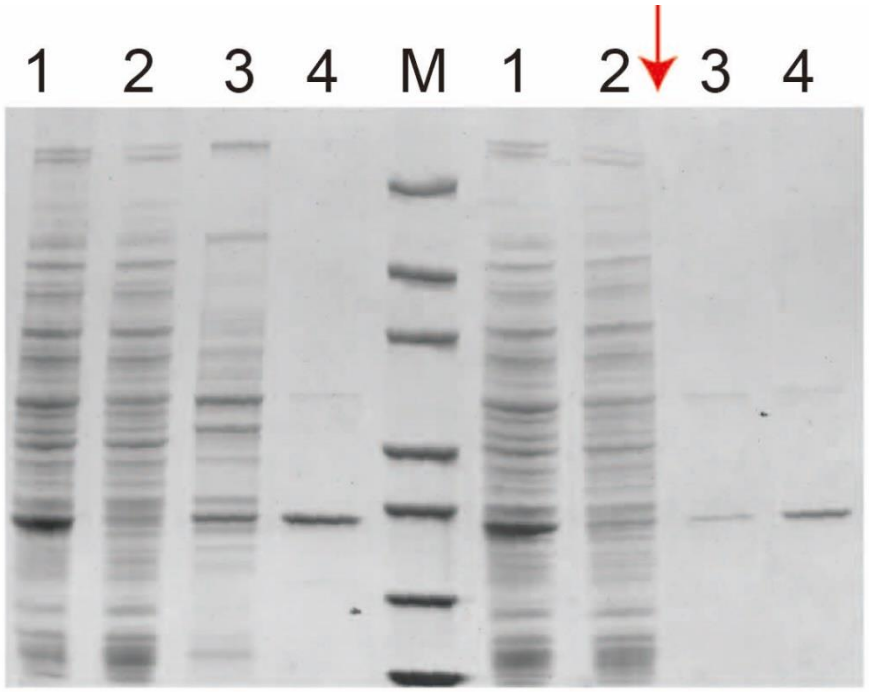
pHCN



- PGL was expressed in *B. subtilis*/pLIA-*pgl*, *B. subtilis*/pMXB-*pgl* and *B.subtilis*/pHCN-*pgl*.
- PGL was secreted into medium with the guidance of *amyE* or *bpr* signal peptide.
- PGL was no expressed in *B. subtilis* with the guidance of *nprE* signal peptide.

GFP Purification and Endotoxin Removal

Purification of GFP by Ni column

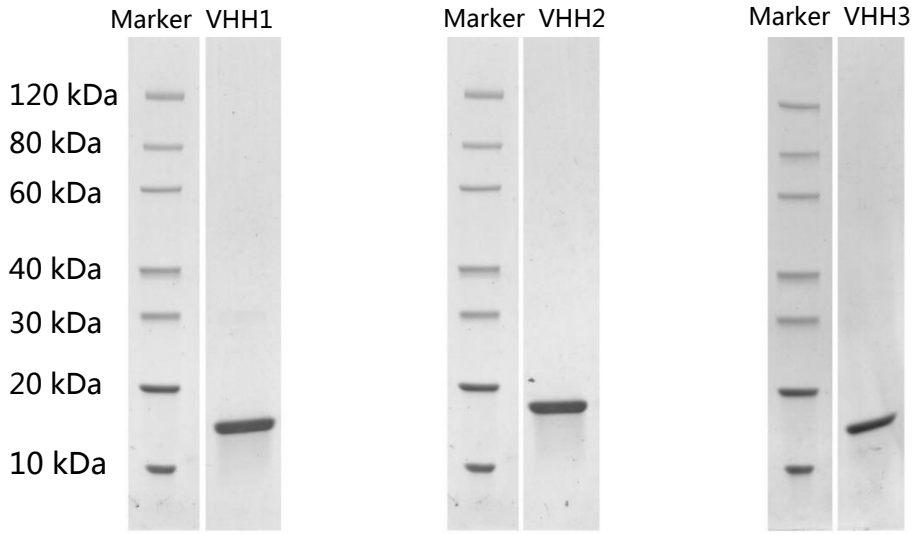


Lane M: Protein marker
Lane 1: Supernatant after cell lysate centrifugation
Lane 2: Flow through
Lane 3: Elution with 20 mM imidazole
Lane 4: Elution with 500mM imidazole
↓ : Wash with 100 mL Trion X-100

Items	Normal	Remove
Yield	8.2 mg/L	5.6 mg/L
Purity	85%	85%
Endotoxin	9.6 Eu/mg	2.4 Eu/mg

- The endotoxin level of GFP was less than 10 Eu/mg protein without any endotoxin removing operation.

Example: VHH Expression in *B. subtilis* System

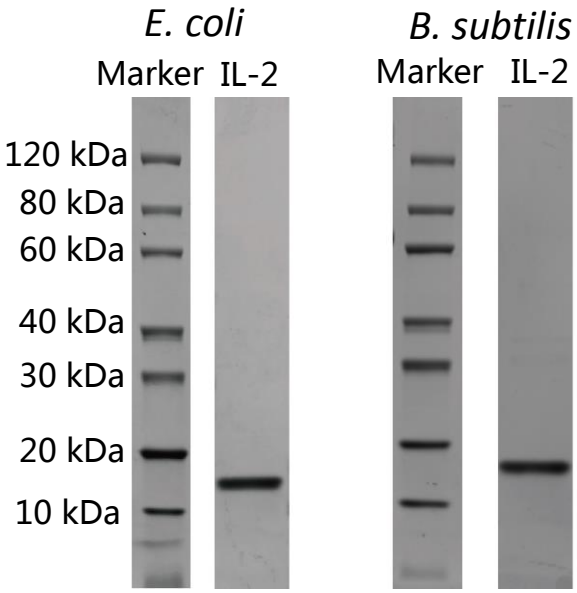


VHH	1	2	3
Yield (mg/L)	5.5	7.0	3.8
Endotoxin (Eu/mg)	4.0	6.3	5.2

- 3 VHH antibody fragments were secreted into medium with the guidance of *amyE* signal peptide. The purities of them were more than 95% after one step purification by Ni column, and the endotoxin were less than 10 Eu/mg protein without any LPS remove.

Example: Interleukin-2 Expression in *B. subtilis*

Sometimes, LPS was hard to be removed, we had tried to produce interleukin-2 in *B. subtilis* expression system.

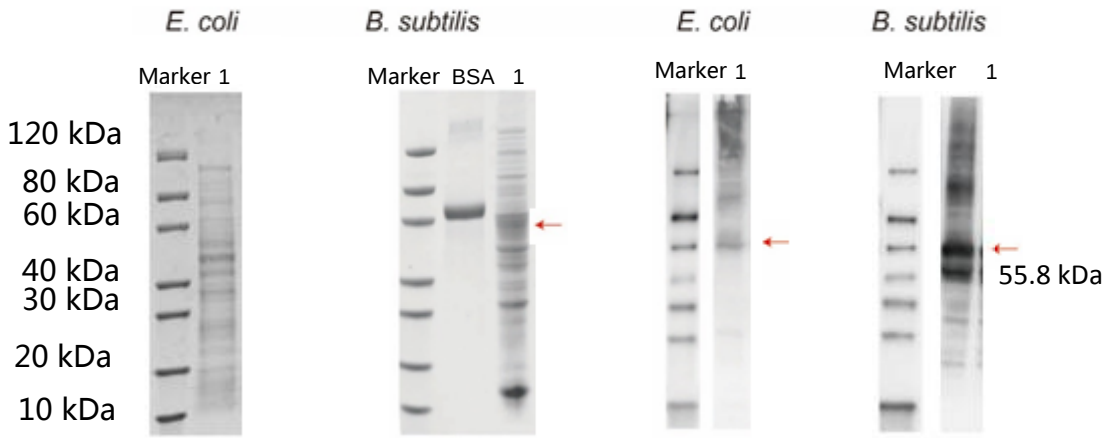


Hosts	*E. coli*	*B. subtilis*
Purification steps	Ni column and endotoxin remove	Ni column
purity	90%	90%
Endotoxin (Eu/mg)	25.6	8.3
Yield (mg/L)	2.5	2.0

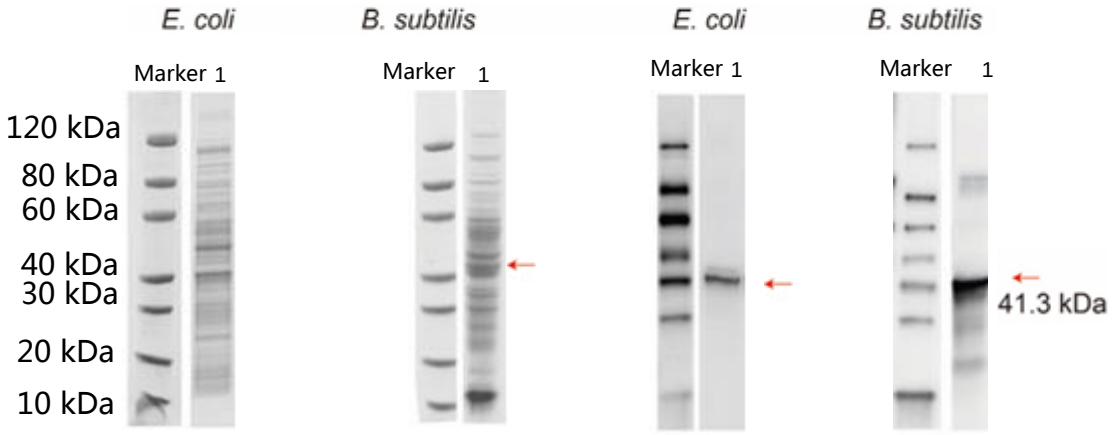
Low Expression Level Proteins in *E. coli*

2 proteins which were low yielding when expressed in *E. coli,* were then expressed in *B. subtilis*.

Adhesin-like protein



Apolipoprotein



B. subtilis expression system can be used to improve the expression level of some recombinant proteins. (Left two lines: SDS-PAGE; right two lines: Western blotting)

Comparison Between *B. subtilis* and Other Expression Systems

Items	*E. coli*	Yeast	Insect	Mammalian	*B. subtilis*
Category	Gram-negative	Fungi	Insect	Mammalian	Gram-positive
Protein location	Intercellular/ Periplasm	Extracellular	Extracellular	Extracellular	Intercellular/ extracellular
Period	3 weeks	5-6 weeks	5-6 weeks	4 weeks	3 weeks
Cost	Low	Middle	High	High	Low
Modification	-	+	+	+	-
Endotoxin	+	-	-	-	-
Technical difficulty	Low	Middle	High	High	Low

Summary

- We have developed *Bacillus subtilis* expression platform for producing recombinant proteins:
  - 3 intercellular expression vectors with low, middle, and high expression levels.
  - 3 extracellular expression vectors harboring *amyE*, *bpr* and *nprE* signal peptides.
  - If low endotoxin is level for the target proteins is necessary, then B. subtilis is a good option.

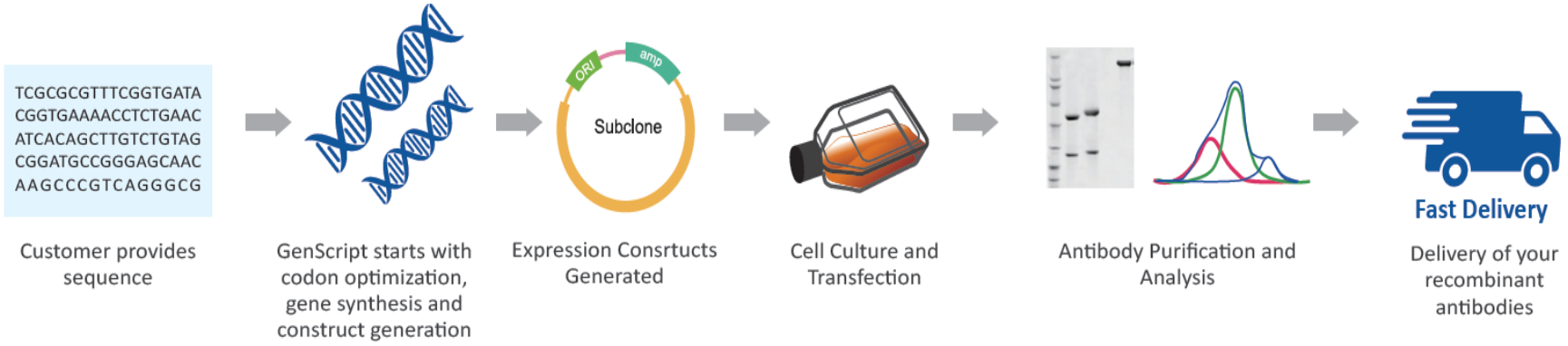
- With our scientific/technical expertise and proven track record, you can trust us to provide your research materials faster and with the highest quality

- We will continue to improve the capability to offer you the best reagents for basic life sciences research, and drug discovery

- With your help, we will ***Make Research Easy***

GenScript-Make Research Easy for People!

GenScript's Protein Expression Workflow



Customer provides sequence

GenScript starts with codon optimization, gene synthesis and construct generation

Expression Consrtucts Generated

Cell Culture and Transfection

Antibody Purification and Analysis

Delivery of your recombinant antibodies

◆ Protein Service Key Features:

- Guaranteed protein/antibody expression in yield, purity, and endotoxin level.
- Proprietary Condon Optimization. Gene synthesis is included, no additional cost.
- Guaranteed delivery: as soon as 4 weeks for most protein projects
- No charge if the project is not successful as outlined in the proposal.

GenScript Key Services

- BacPower™: Guaranteed expression in E. Coli system
- MamPower™: Guaranteed expression in Mammalian system for protein and antibody expression.
- MamPilot™: Guaranteed antibody expression in mammalian system.
    - This can also be ordered as a pilot expression for your scale-up projects down the road!

Order by email: protein@genscript.com

Phone: 1-877-436-7274 (Toll-Free) 1-732-885-9188

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THANKS

from GenScript