

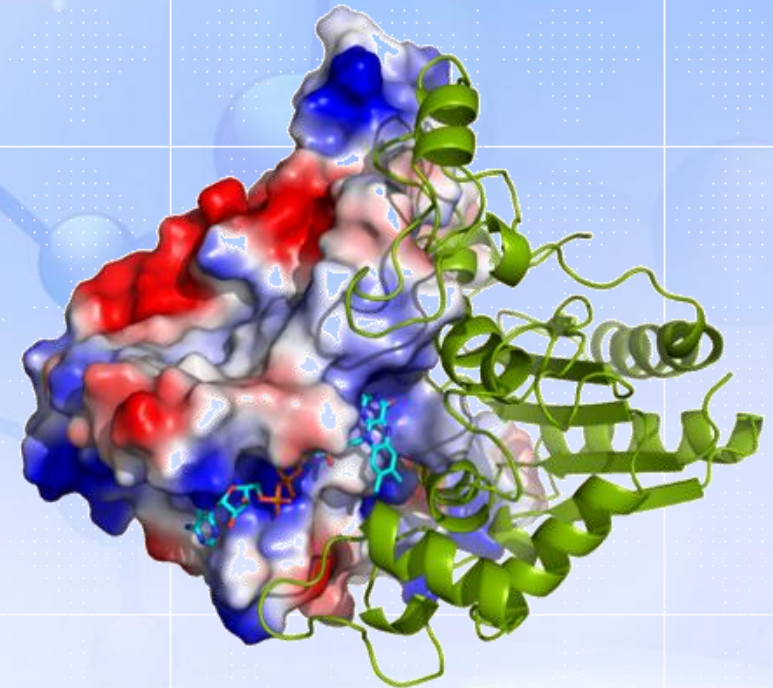


Our Protein Expression Platform

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February, 2025

Web: en.biortus.bio



Multiple expression systems available

BIORTUS

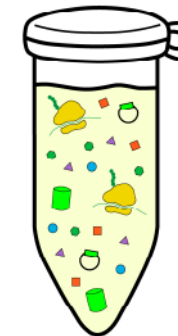
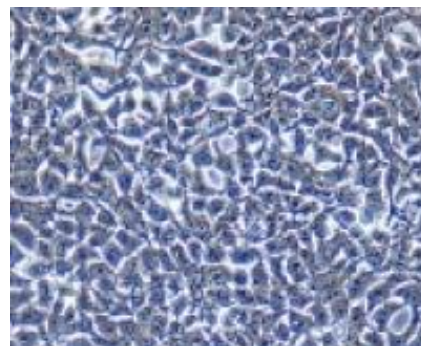
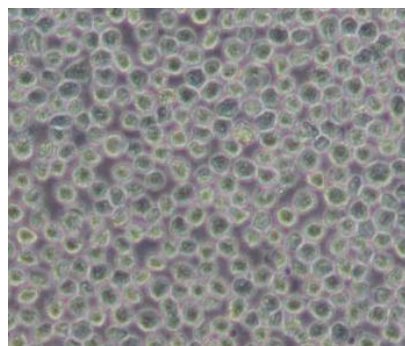
E.coli

Insect cells

Mammalian
cells

Yeast

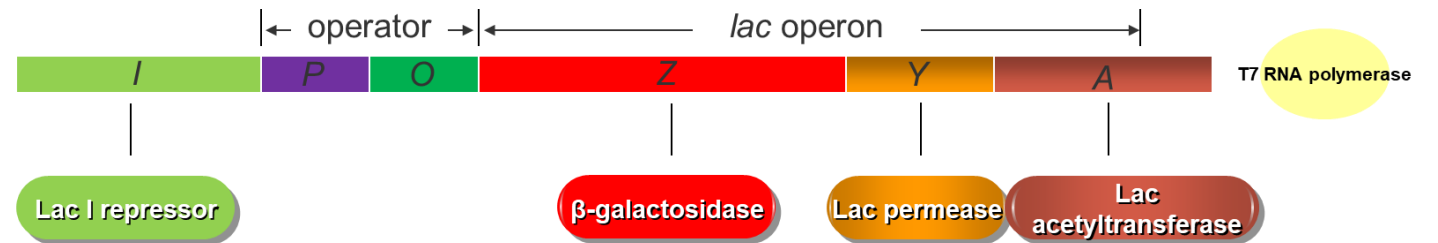
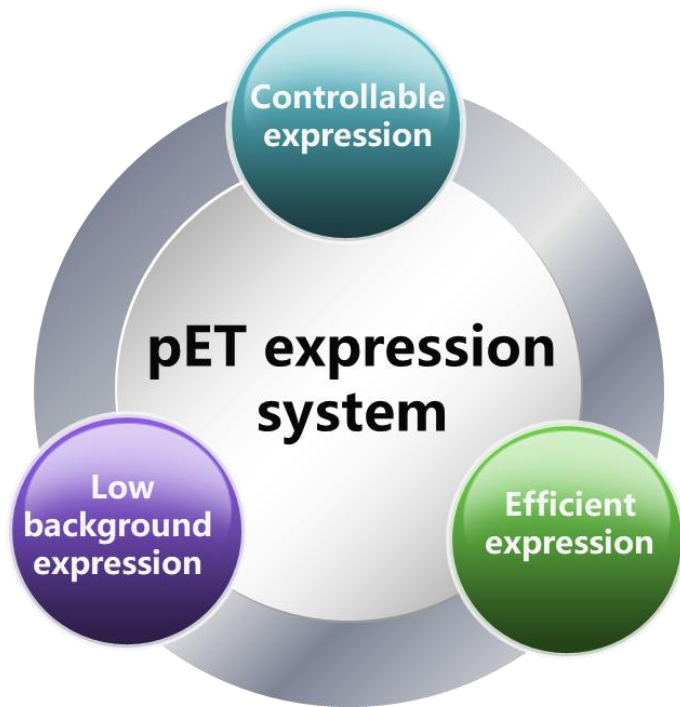
Cell-Free
system



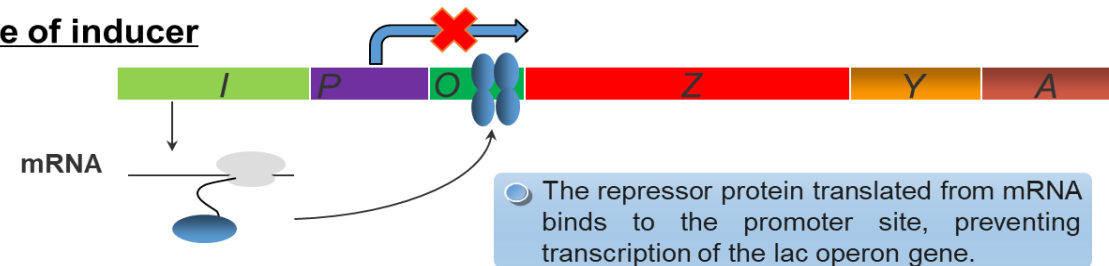
Multiple expression systems available

Expression System	<i>E. coli</i>	Insect cell	Mammalian	Yeast	Cell free
General Applications	Scalable Fastest turnaround Cost effective	Simple PTMs Scalable Membrane proteins	Comprehensive PTM; Membrane proteins; Antibodies	Low cost, eukaryotic PTMs	High throughput; Unnatural amino acids (UAAs) incorporation
Approximate Expression Time	1 week for small scale expression test and expression optimization; O/N expression	2 weeks for virus generation; 1 week for virus titer determination and small scale expression test; 2-3 days for expression	1 week for small scale expression test; 3-5 days for expression	4-6 days	~ 4 hours
Capacity	5 - 10L/batch 1000L/week (max)	0.8 L - 9.6L/batch 400L/week (max)	0.2 L - 9.6L /batch 200L/week (max)		
Strains available	BL21 (DE3), T7 Express, Rosetta, Artic, etc. More than 20 choices	Sf9, Sf21, Hi-5	ExpiCHO-S, Expi293, Expi293 GnTI-,	Pichia pastoris SMD116, SuperMan5-24	

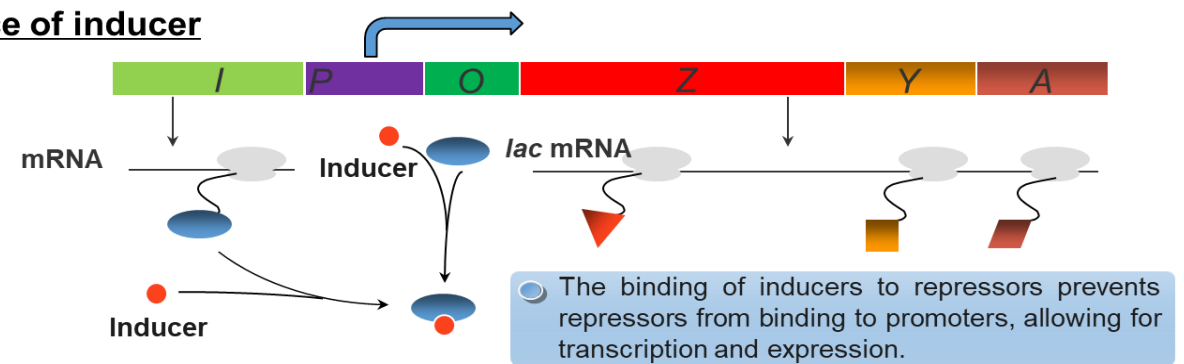
Principle of *E.coli* expression



In absence of inducer



In presence of inducer



- **Key equipment**

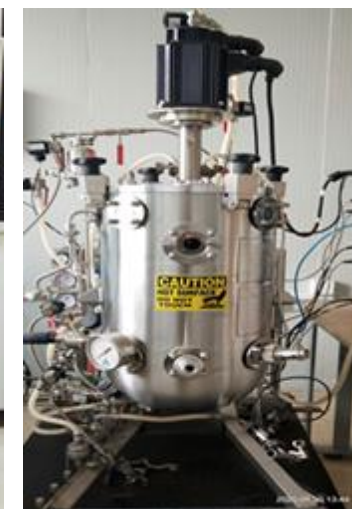
- ✓ 15 Shakers
- ✓ 1x40L fermenters
- ✓ 2x5L fermenters

- **Small and Large Scale Protein Expression and Purification**

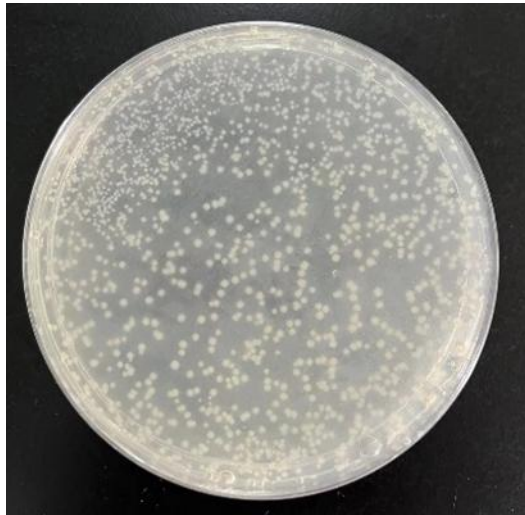
- ✓ Variety of commercial strains (such as BL21(DE3), BL21 (DE3) Gold, BL21 (DE3) CodonPlus RIPL, and Rosetta);
- ✓ Different culture mediums (LB, TB, 2xYT, M9) can be selected;
- ✓ Different induction methods (IPTG induction, arabinose induction, autoinduction) can be selected;
- ✓ Coexpression chaperone (pG-TF2、 pGro7、 pTf16、 pG-KJE8) can be selected to improve soluble expression;
- ✓ Expression of isotope labeled proteins (such as ^{15}N labeling, $^{15}\text{N}^{13}\text{C}$ double labeling, Se-Met labeling)

- **Capabilities**

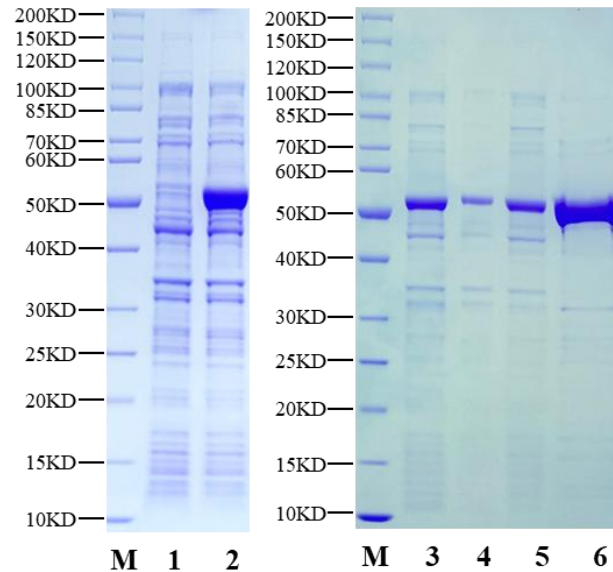
- ✓ Up to 1000L *E. coli* Expression per week



E.coli expression at Biortus



Transformation
2 days



5ml expression test
1-2 days

Sample information	#23161
Competent cell	BL21(DE3)
Medium	LB
IPTG	0.5 mM
Temperature	15 °C
Induction time	16 h

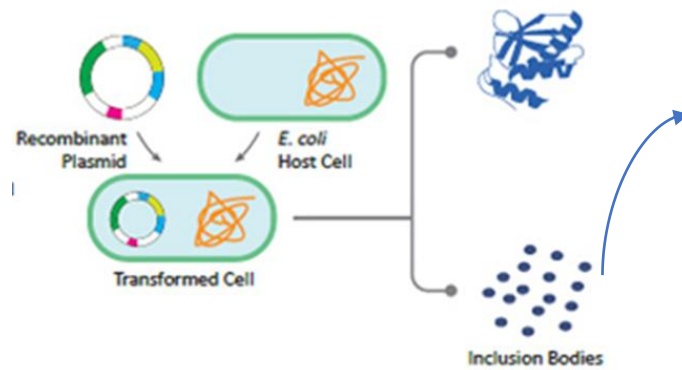
M = Marker
Lane 1 = Uninduced
Lane 2 = Induced at 15 °C, 16 h, 0.5 mM IPTG
Lane 3 = Total cells
Lane 4 = Precipitate
Lane 5 = Supernatant
Lane 6 = Elution



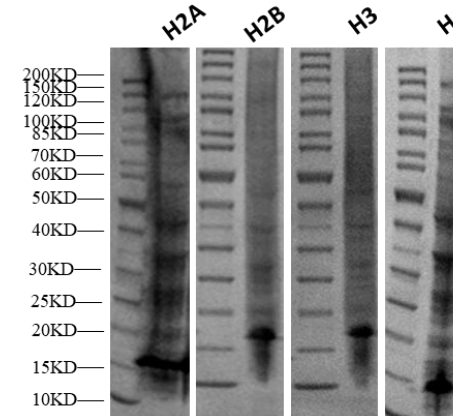
Large scale expression
1-2 days

E.coli expression showcase-Histone production

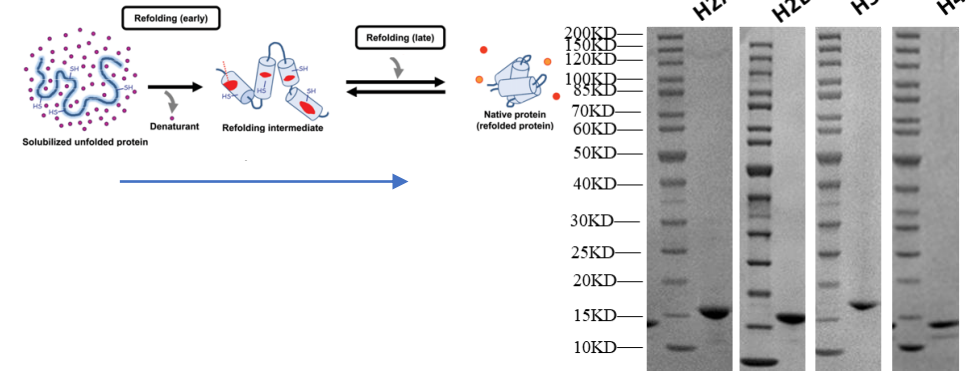
1. Expression



2. Inclusion body

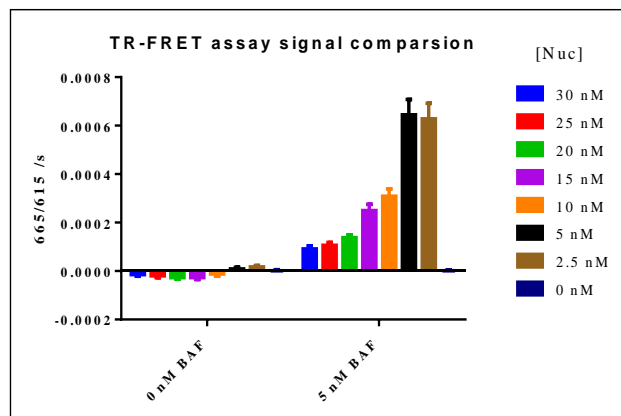


3. Refolding & Purification



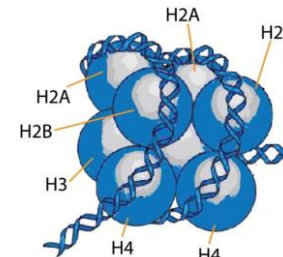
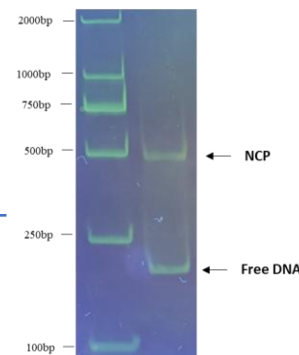
6. Activity test

Activity test



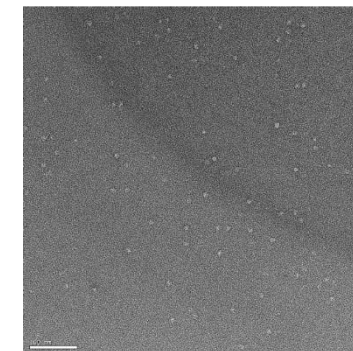
5. Reconstitution of Nucleosome Core Particles

Nucleosome core particle

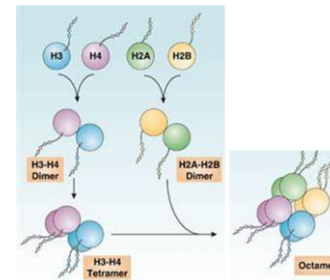
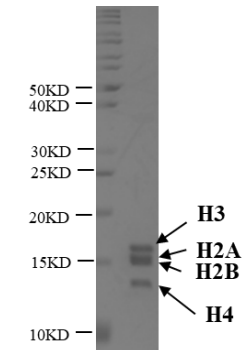


4. Refolding of Histone Octamer

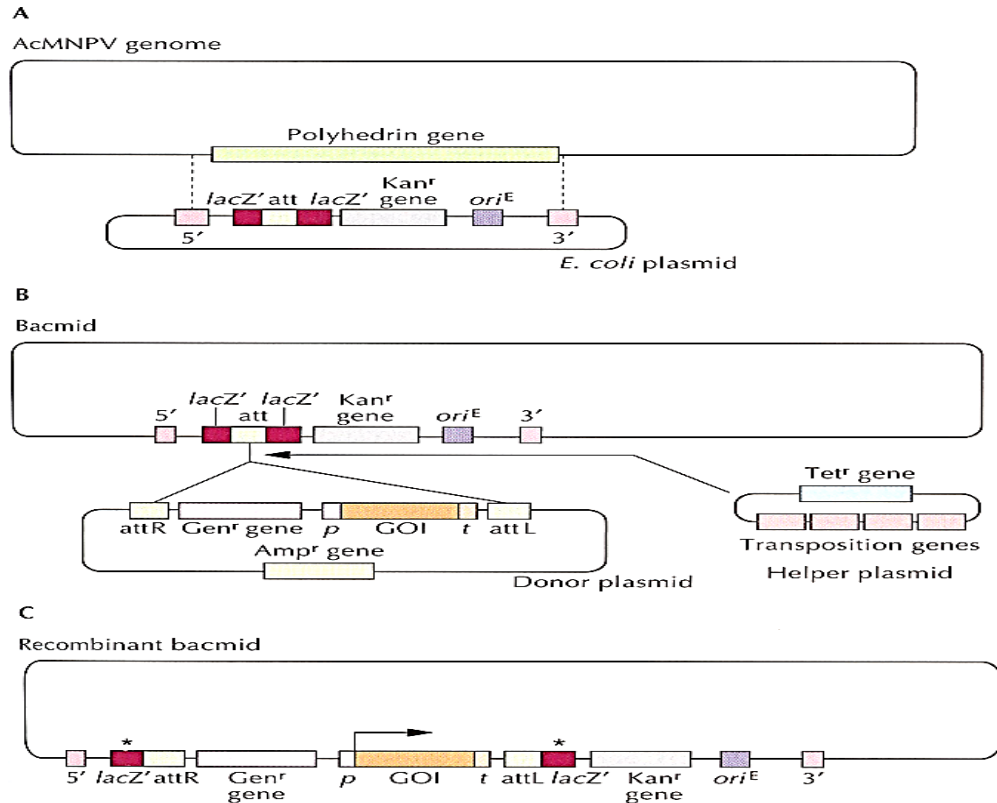
Negative staining micrographs



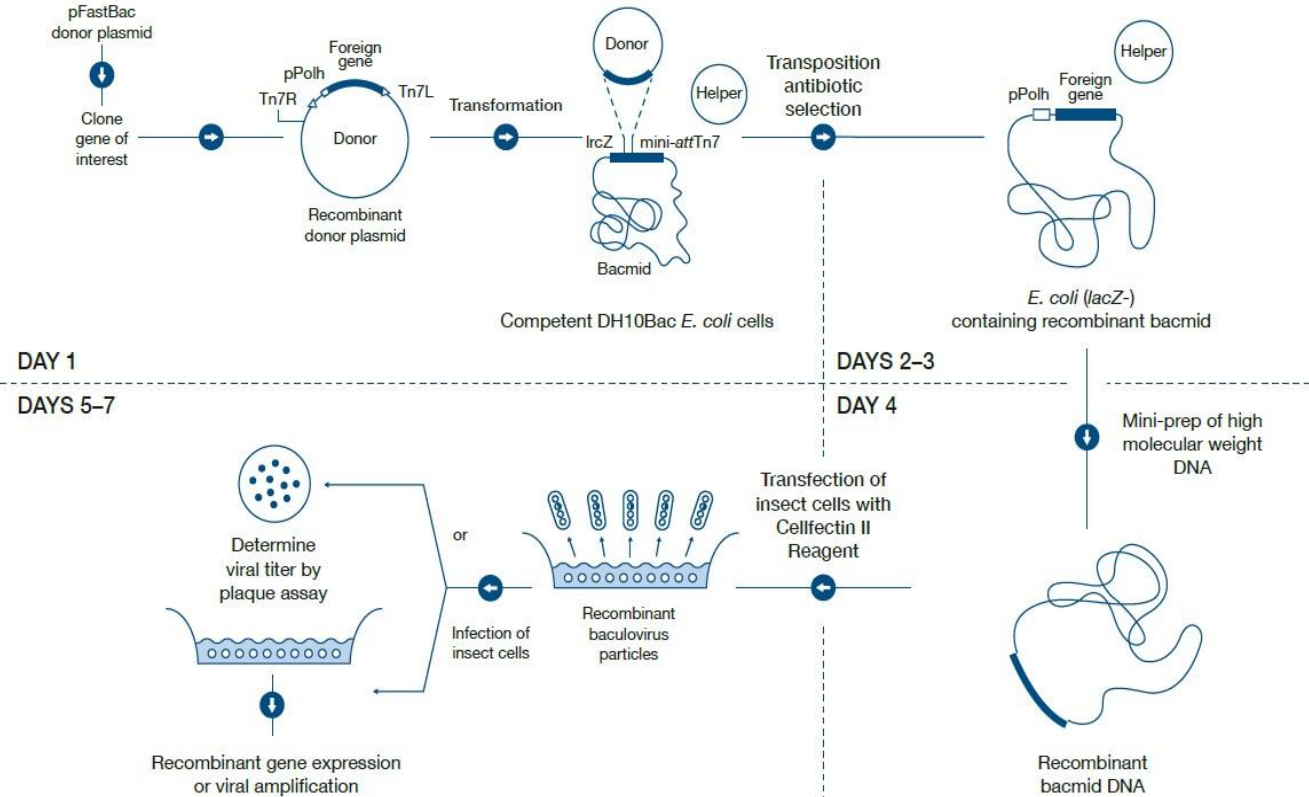
Octamer



Principle of Bac-to-Bac expression system (insect cell) BIORTUS



Recombinant bacmid



Virus preparation process

Insect cell expression at Biortus

- **Key equipment**

- ✓ 50 Shakers
- ✓ 3x15L fermenters
- ✓ 1x50L WAVE

- **Small and Large Scale Protein Expression and Purification**

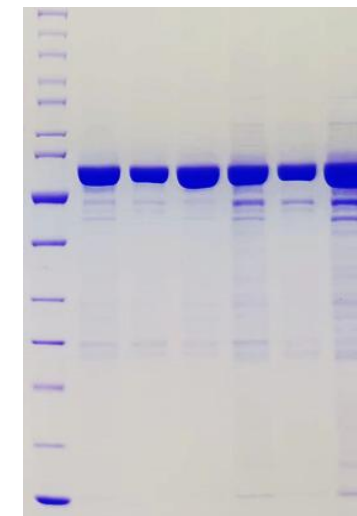
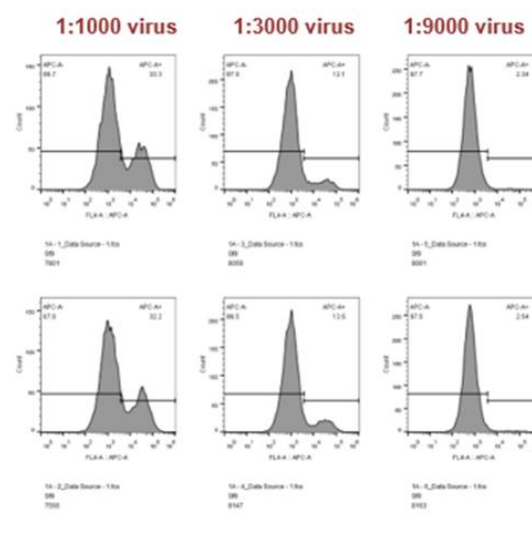
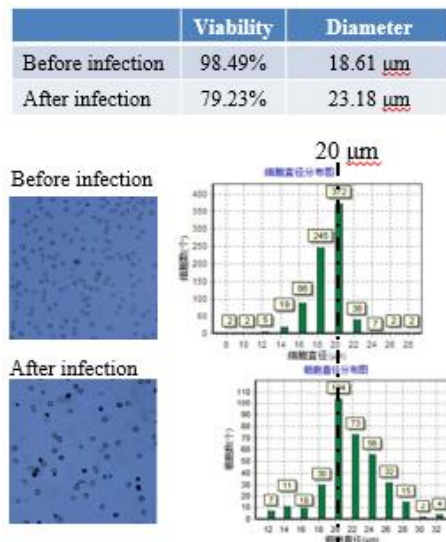
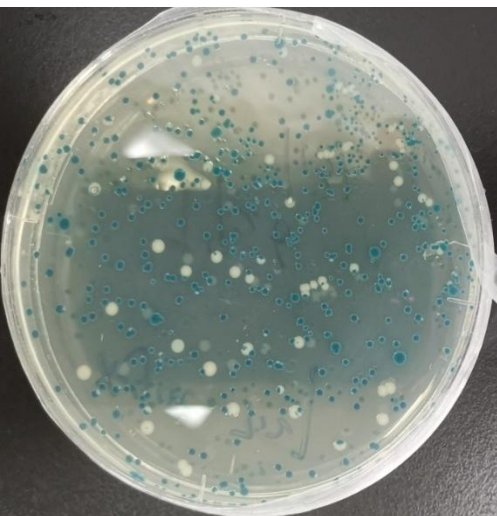
- ✓ Variety of commercial cells (such as Sf9, ExpiSf9, Sf21, High five, S2 cells);
- ✓ Baculovirus systems: Bac-to-Bac system; Bacmam system;
- ✓ Virus titer test by Flow Cytometer;
- ✓ Optimization of expression: cell lines, infection time, infection ratio (MOI), expression temperature...;
- ✓ Insect cell transient experiment

- **Capabilities**

- ✓ Up to 400L insect cell Expression per week



Insect cell expression at Biortus



Bacmid preparation
3 days

P0-P2 virus preparation
10 days

Virus titer
Determination
2 days

Small scale
expression test
3-4 days

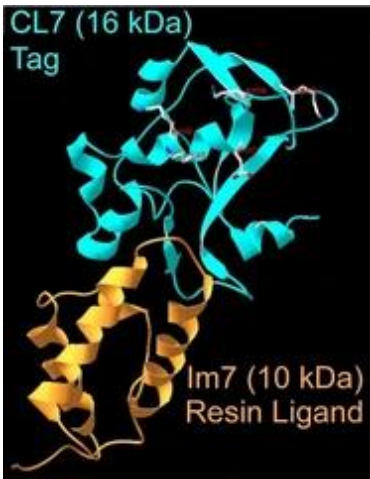
Large scale expression
2-3days



Insect cell expression showcase- CL7-IM7 mediated one-step protein preparation

BIORTUS

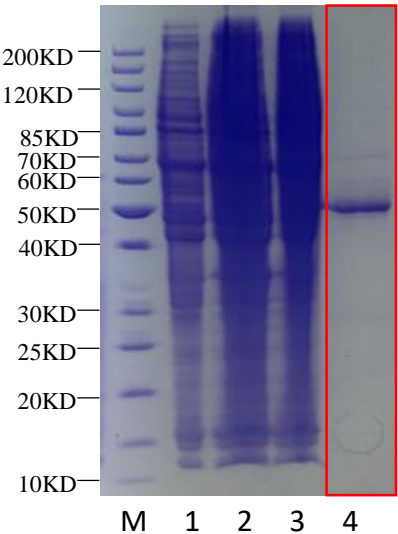
❖ **CL7 tag:** K_D : $10^{-14/-17}$ M, infinity affinity tag!



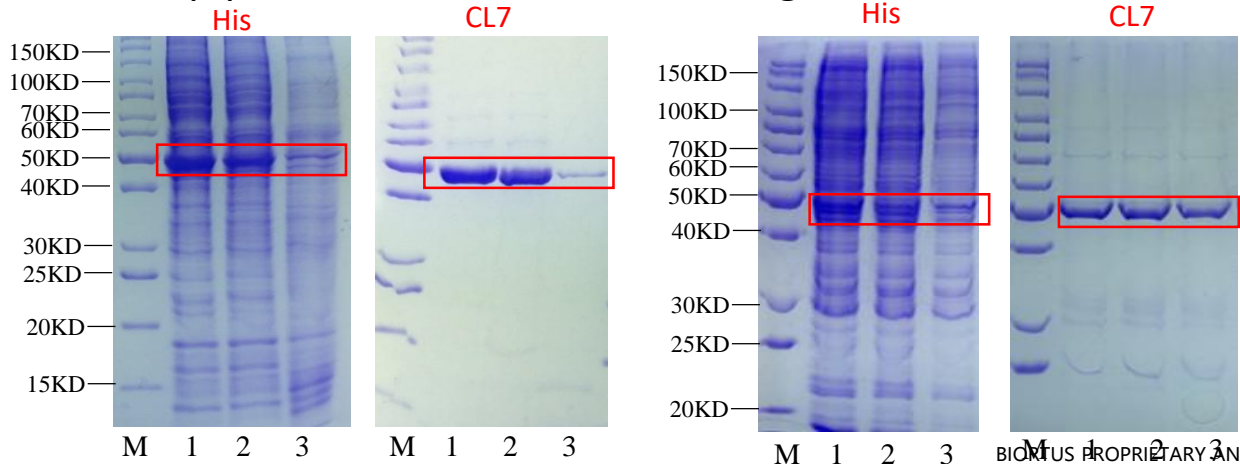
CHR Type*	Affinity K_D^* (M)	Loading NaCl* (M)	Capacity (mg/ml)
FLAG	$10^{-8/-9}$	< 0.2	0.6
CalBD	10^{-9}	< 0.3	1.5
ChBD	10^{-6}	~ 1	2
Pr-A	$10^{-8/-10}$	< 0.2	2-3
Strep	10^{-6}	< 0.2	6
Halo	COV	< 0.2	7
MBP	10^{-6}	< 0.2	6-10
GST	$10^{-6/-7}$	< 0.2	10
His	$10^{-8/-9}$	1+	20-40
CL7	$10^{-14/-17}$	1+	15-20
IEQ/S	N/A	< 0.2	40
HEP	N/A	< 0.2	5

❖ More cases of one step purification with CL7 tags

Membrane protein



❖ One step purification with His / CL7 tags

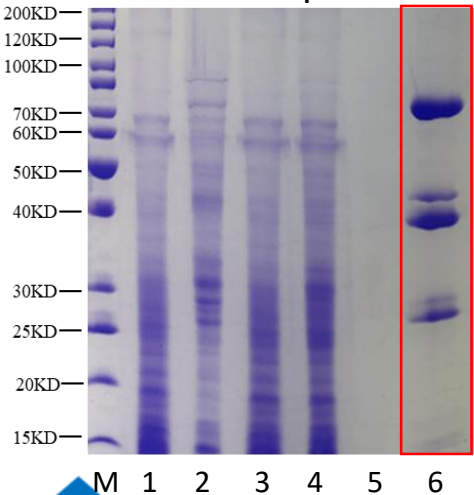


Proc Natl Acad Sci U S A. 2017 Jun 27;114(26):E5138-E5147. doi: 10.1073/pnas.1704872114. **Efficient, ultra-high-affinity chromatography in a one-step purification of complex proteins.**

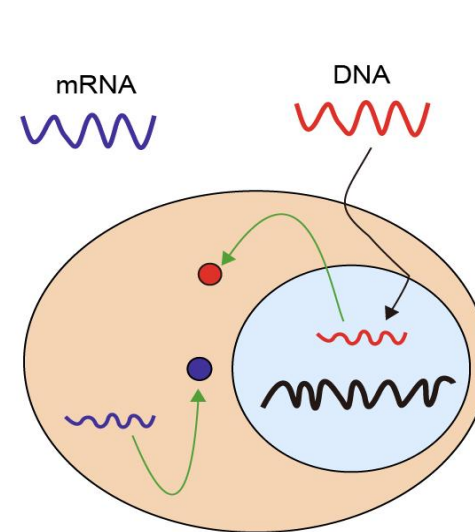
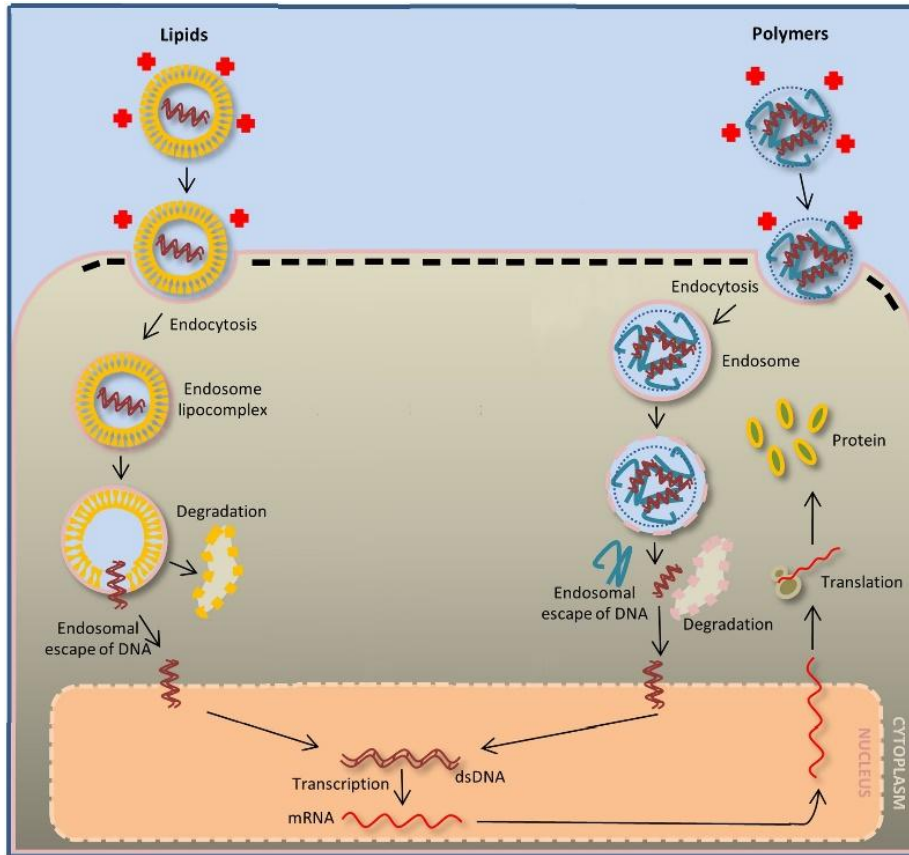
<https://pubmed.ncbi.nlm.nih.gov/28607052/>

M = Marker
Lane 1 = Sf21, 48h, 1 μ l/0.5 ml,
Lane 2 = Sf21, 48h, 3 μ l/0.5 ml,
Lane 3 = Sf21, 48h, 8 μ l/0.5 ml,

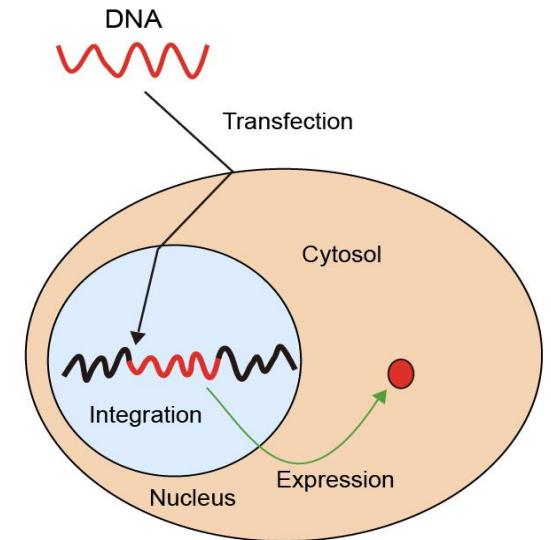
Protein complex



Principle of mammalian cell expression system



Transient transfection



Stable transfection

Cationic lipids or polymers mediated transfection

Mammalian cell expression at Biortus

- **Key equipment**

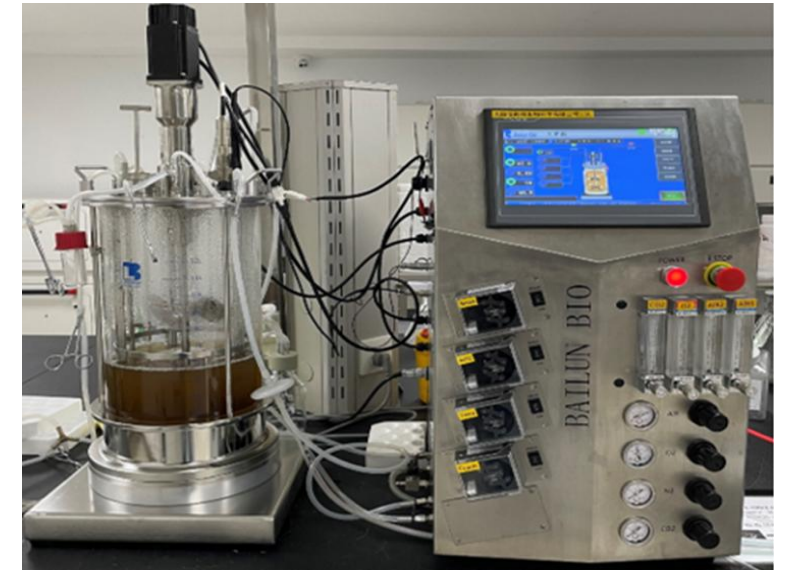
- ✓ 26 Shakers
- ✓ 3x15L fermenters
- ✓ 1x50L WAVE

- **Small and Large Scale Protein Expression and Purification**

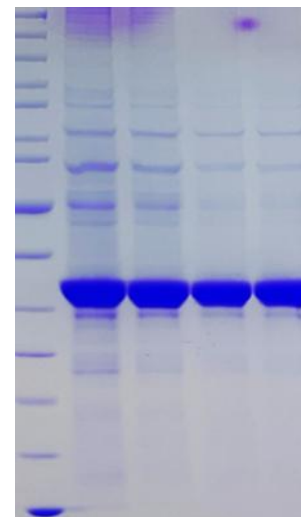
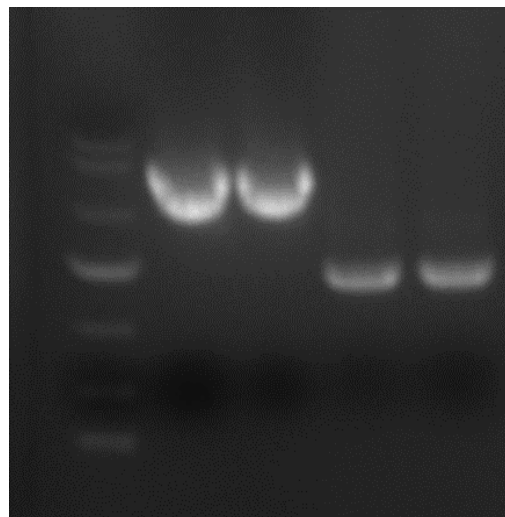
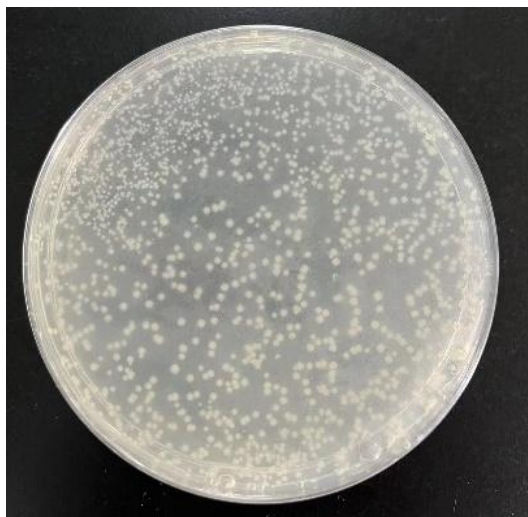
- ✓ Variety of commercial cells lines (such as Expi293F, ExpiCHO-S, Freestyle CHO-S, Freestyle 293F, and Expi293 GnTI- cells);
- ✓ Cell culture service with suspension mammalian cell including transient transfection, viral infection;
- ✓ Optimization of expression: cell lines, transfection time, transfection ratio (DNA/PEI), expression temperature...;
- ✓ Stable cell line generation for protein expression.

- **Capabilities**

- ✓ Up to 200L Mammalian cell Expression per week



Mammalian cell expression at Biortus



Transformation and small
scale plasmid extraction
3 days

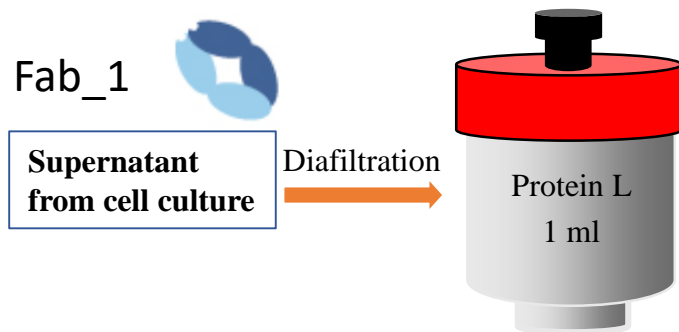
Large scale plasmid extraction
and PCR verification
2 days

10ml expression test
3-4 days

Large scale expression
2-3 days

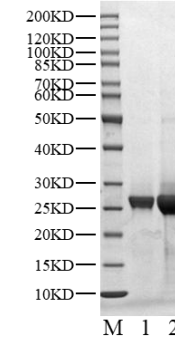
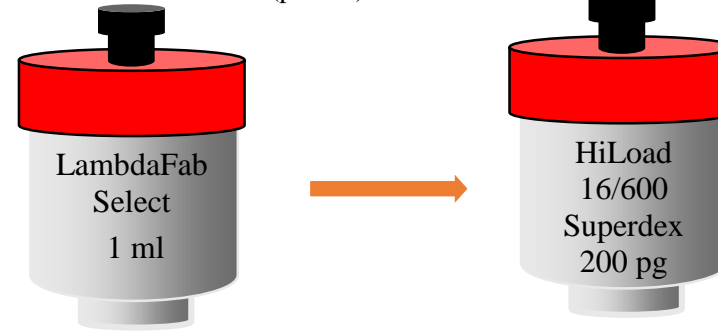
Mammalian cell expression showcase-antibody generation

Elution buffer: 0.1M Glycine (pH 2.8).
Neutralization Buffer: 1M Tris (pH 8.0).

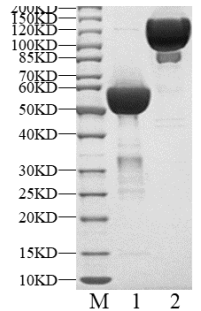


OR

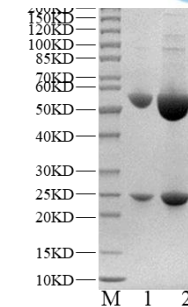
Elution buffer: 0.1 M acetate buffer(pH 3.5).
Neutralization Buffer: 1M Tris (pH 8.5).



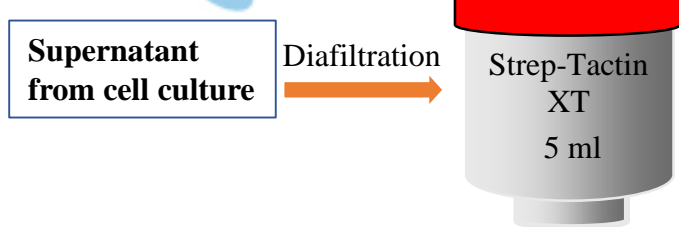
Fc-dimer-fusion



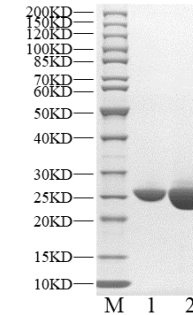
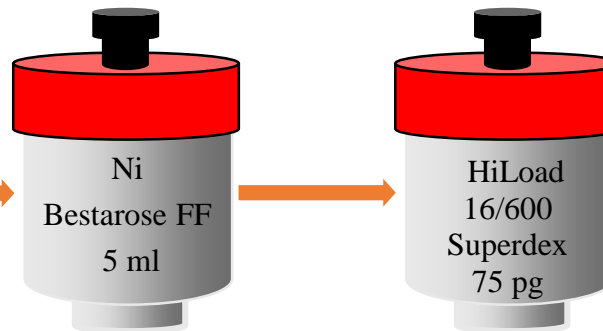
Full-length IgG



8His-Strep II-
TEV-GG-scFv

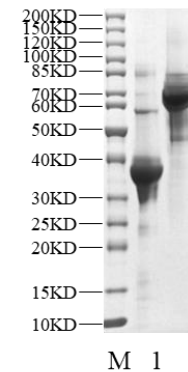
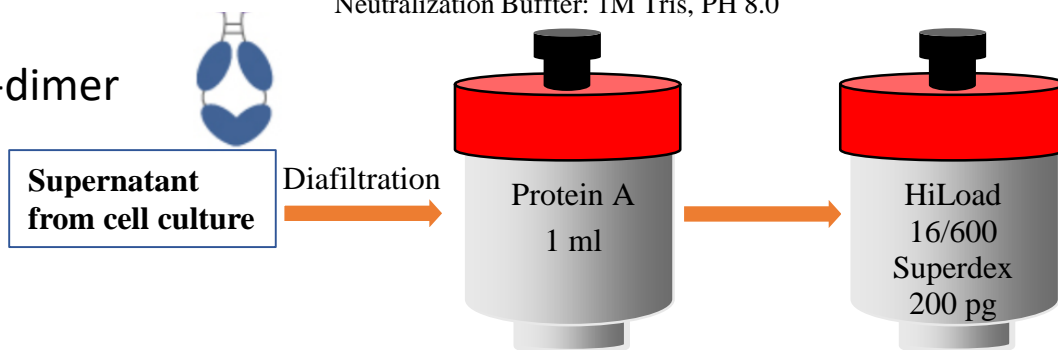


Digestion



Elution buffer: 50mM Glycine, 100mM Arginine (pH 3.3).
Neutralization Buffer: 1M Tris, PH 8.0

Fc-dimer





Protein Expression in *Pichia pastoris*

- *Pichia pastoris* is widely used for the production of **biopharmaceutical, industrial enzymes** and other **heterologous protein**.

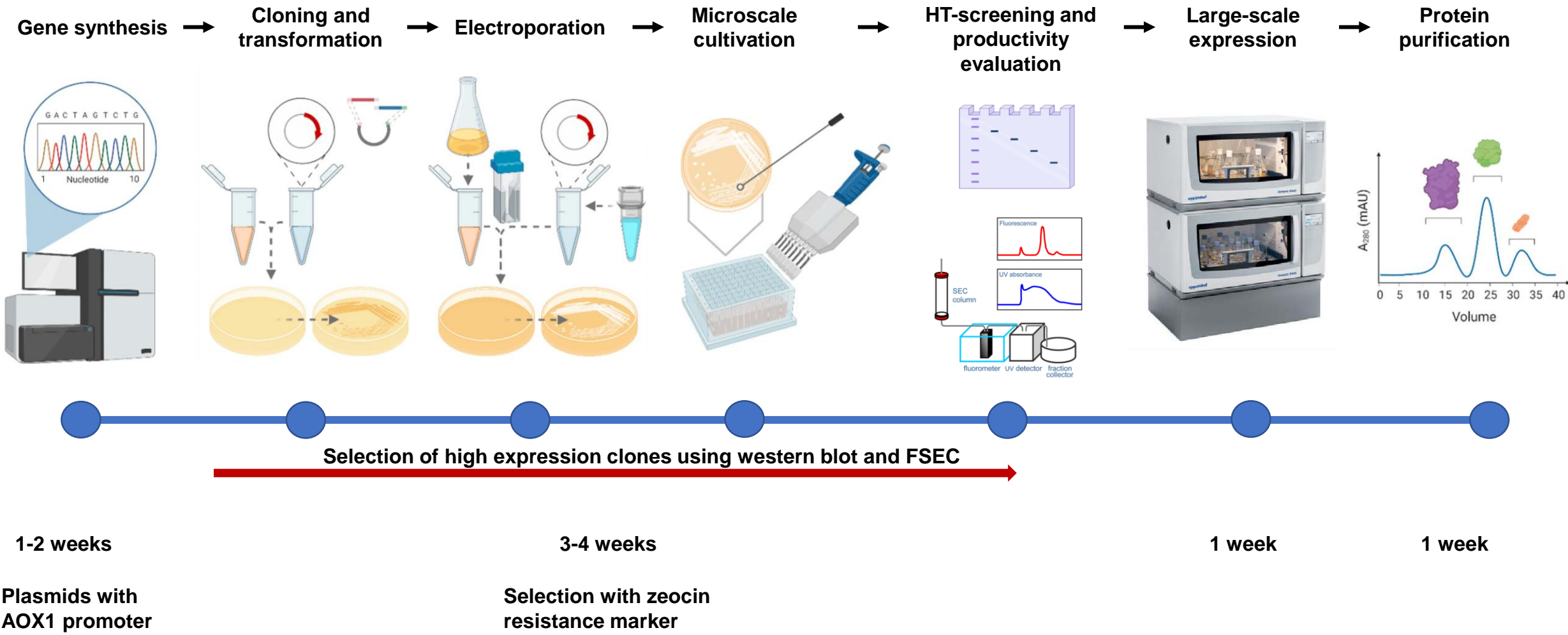
Advantages of using *Pichia pastoris*

- High growth rate, posttranslational modification, secretory expression, and easy genetic manipulation
- High efficiency transformation and ability to generate **stable cell lines**
- Production of heterologous proteins of pro and eukaryotes
- Soluble proteins and membrane proteins
- High cell densities/high protein yield
- Products similar to those produced in mammalian systems





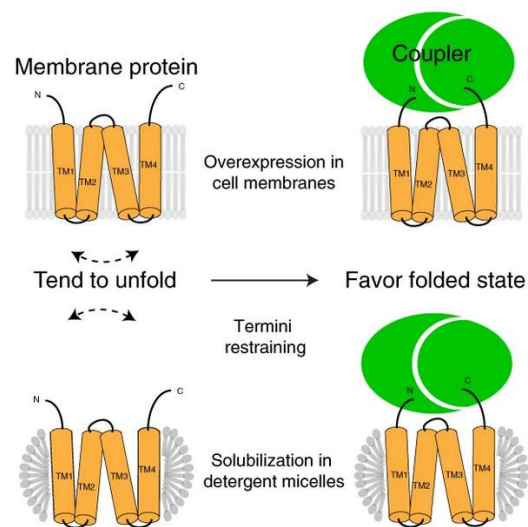
General workflow



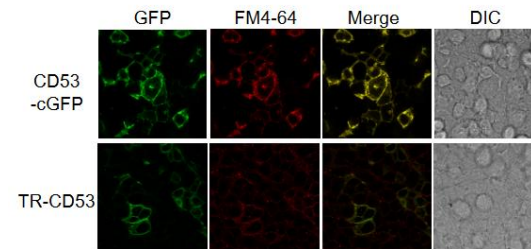


Yeast expression showcase-

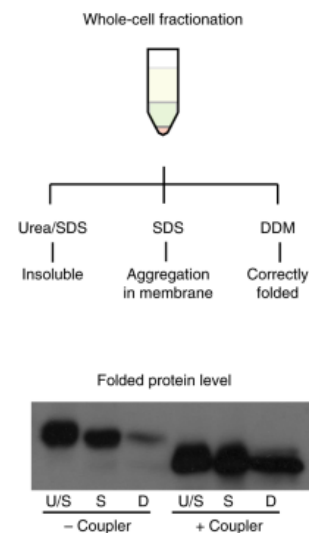
Termini restraining of small membrane proteins



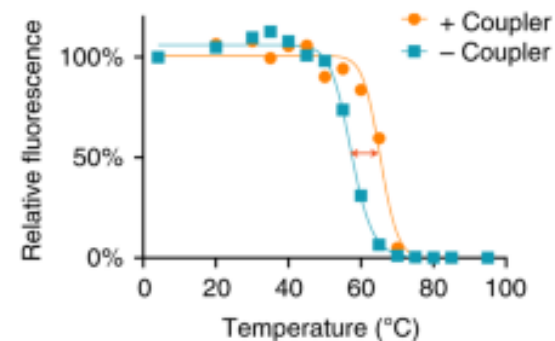
Termini restraining



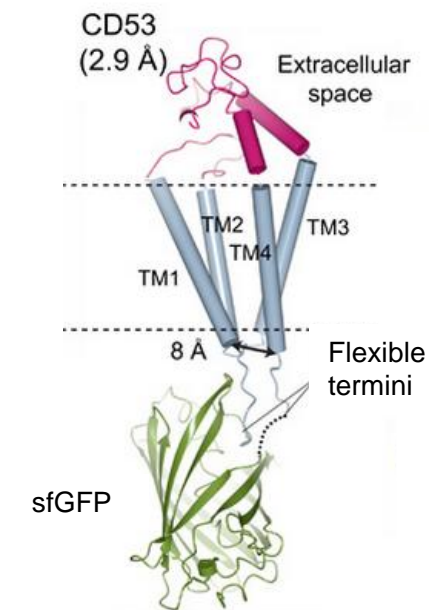
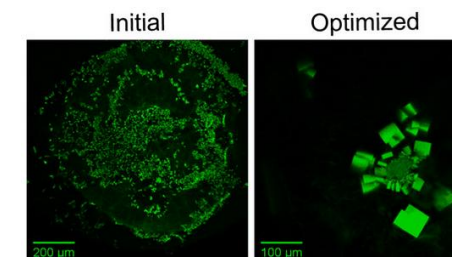
Subcellular location



Protein folding

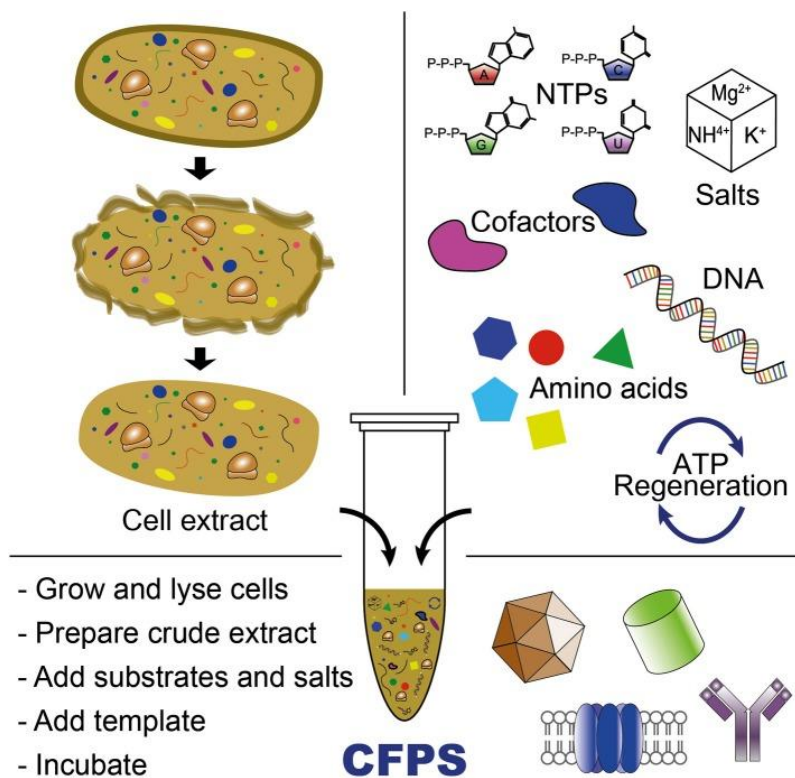


Thermostability

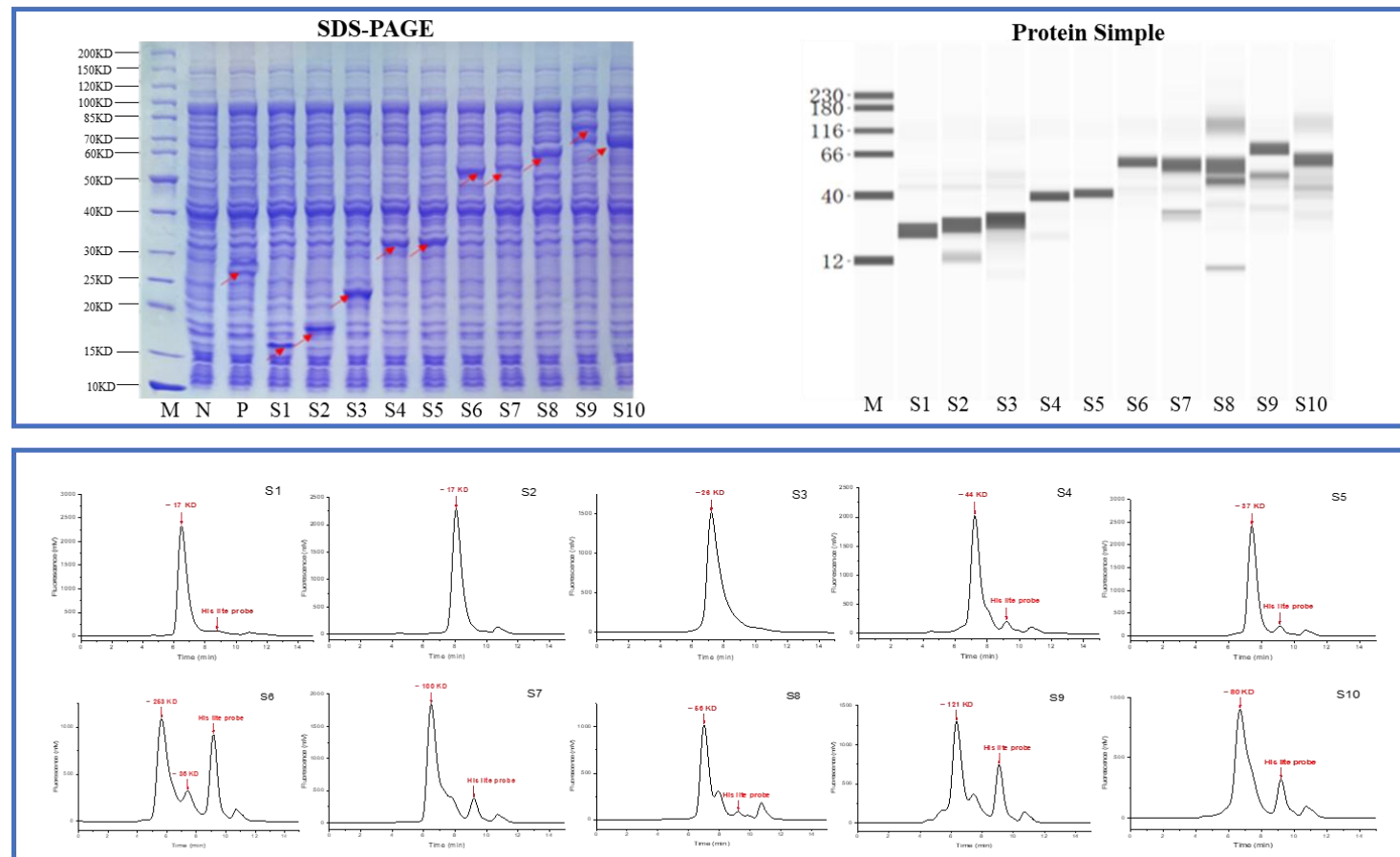


Xtal structure

Cell-Free Expression (CFS): Generate protein in 4 hours!



Hong, Seok Hoon & Kwon, Yong-Chan & Jewett, Michael. (2014). Non-standard amino acid incorporation into proteins using *Escherichia coli* cell-free protein synthesis. *Frontiers in chemistry*. 2. 34. 10.3389/fchem.2014.00034.

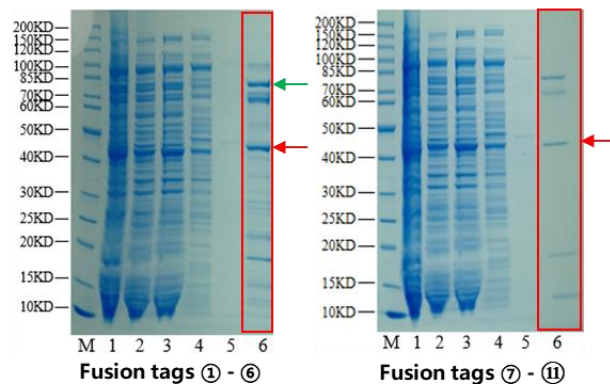


A variety of proteins produced at Biortus with Cell-Free Expression System.

Cell-Free Expression (CFS) Applications

- High-throughput screening
- Varied boundaries; Multiple fusion tags

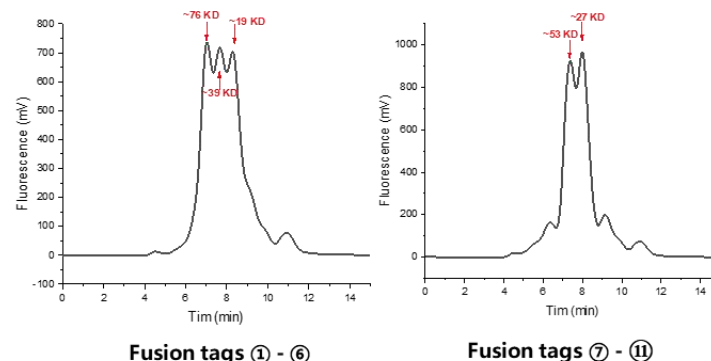
➤ Screening results (CFPS small scale test)



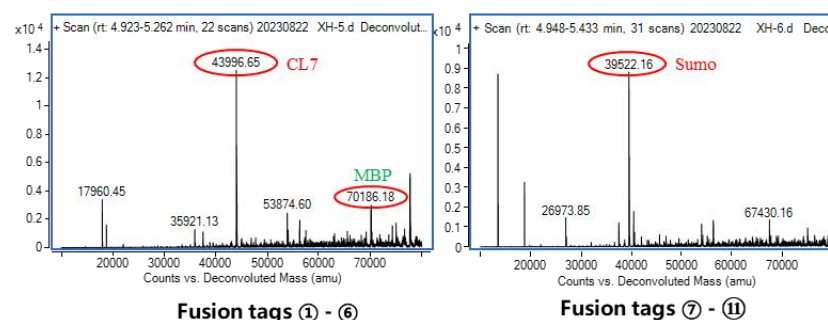
M = Marker
Lane 1 = Total cells
Lane 2 = Flow through
Lane 3 = Buffer A wash
Lane 4 = 8% Buffer B wash
Lane 5 = 8% Buffer B wash
Lane 6 = 400 mM Imidazole Elution

	Plasmid	Theoretical MW (Da)
①	pET28a-8His-Strep II-CL7-TEV-GG-POI	44007.68
②	pET28a-8His-Strep II-MBP-TEV-GG-POI	70119.47
③	pET28a-8His-Strep II-Gbeta-TEV-GG-POI	35247.09
④	pET28a-8His-Strep II-TF-TEV-GG-POI	77689.26
⑤	pET28a-8His-Strep II-GST-TEV-GG-POI	54851.49
⑥	pET28a-8His-Strep II-TrxA-TEV-GG-POI	40958.92
⑦	pET28a-8His-Strep II-3Flag-TEV-GG-POI	32014.41
⑧	pET28a-8His-Strep II-Dsbc-TEV-GG-POI	54904.41
⑨	pET28a-8His-Strep II-HSP90-TEV-GG-POI	113806.99
⑩	pET28a-8His-Strep II-NusA-TEV-GG-POI	83962.99
⑪	pET28a-8His-Strep II-Sumo-POI	39535.26

➤ Screening results (CFPS FSEC test)

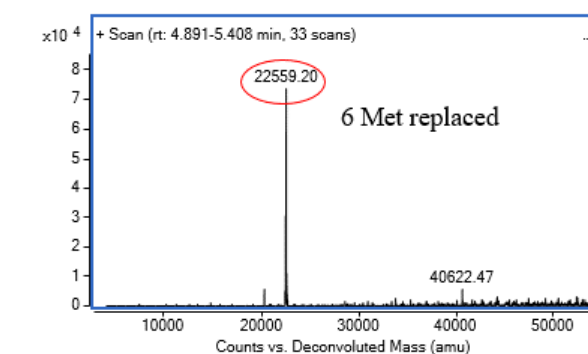
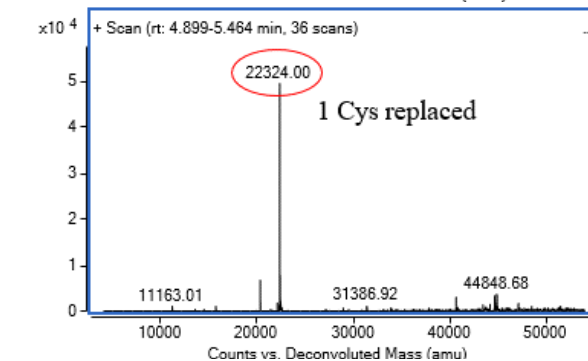
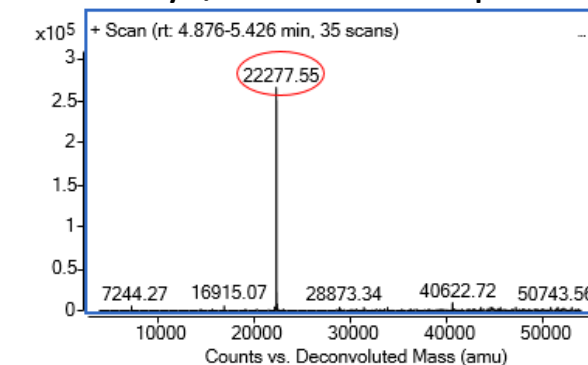


➤ Screening (CFPS LC-MS)



- Preferred fusion tag: Sumo & CL7
- StrepII-8His-Sumo-GGG-POI & 8His-CL7-TEV-GG-POI

- UAA incorporation
- Se-Cys/Se-Met incorporation



Specialize in Drug Targets

- Current inventory at over 2500 and expanding
- Focus on Drug Targets (E1, E2, E3, DUB, Ras, CDK, GPCR, SLC, etc.)
- High standards for QC
- >600 proteins, 17 assay kits are available on VWR and Chem-space and more products are coming.



@Biortus



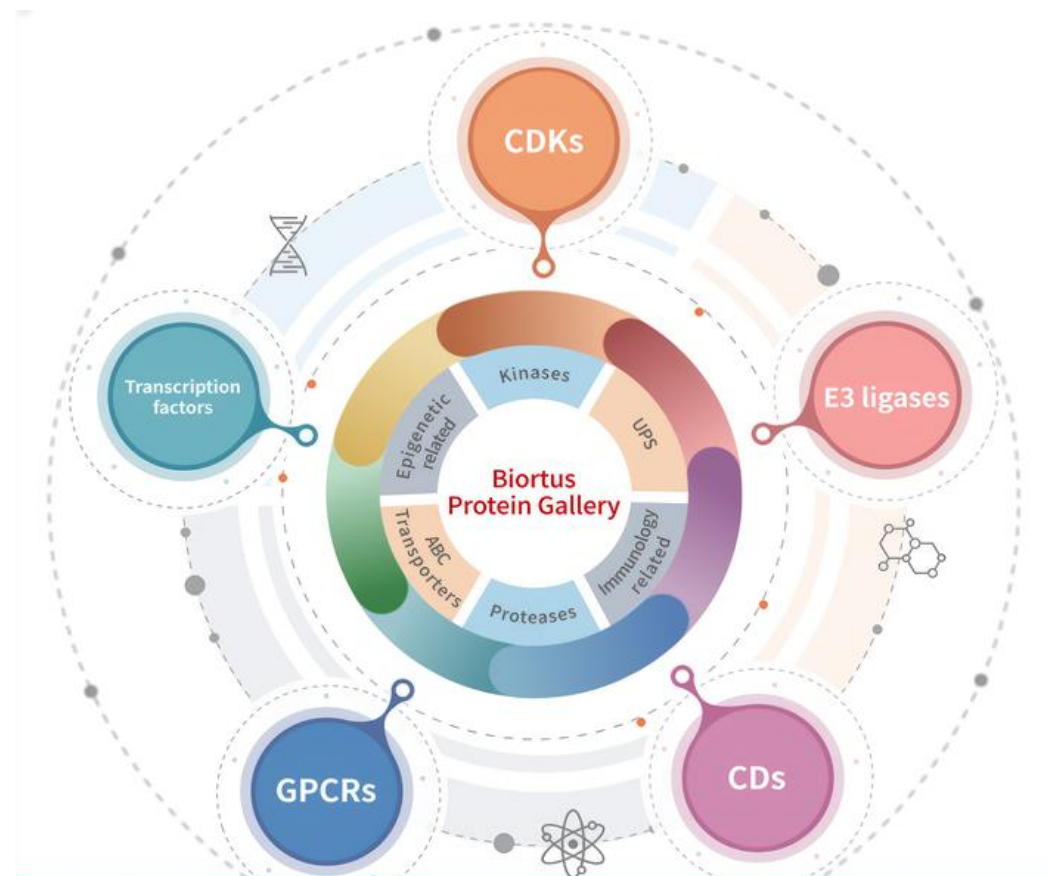
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Protein Focused Services for Drug Discovery
From Gene to Structure



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