財團法人生物技術開發中心
Development Center for Biotechnology

Microbial Secretion Expression System

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Microbial Secretion System

P. Pastoris Secretion System

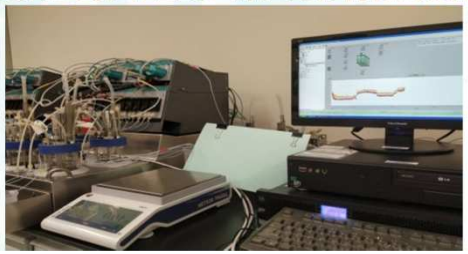
- Protein yields up to **1 g/L ScFv**
- Expressed protein: ScFv, Fab, cytokine
- Number of secretion signal peptides: 6 sequences
- Number of promoters: 2
- Selection method: Zeocin
- Time: 6 weeks

E. coli Secretion System

- Protein yields up to **0.1 g/L Fab**
- Expressed protein: Fab, light chain, ScFv, therapeutic enzyme, vaccine
- Number of secretion signal peptides: 3 sequences
- Number of promoters: 3 (T7, PhoA, Tac1)
- Host: DE3 and non-DE3
- Time: 7~8 weeks

DoE mediated Process Development

DASGIP: 250 ml Fermenter



pH | Dissolve oxygen | Medium | Feeding strategy | Temperature

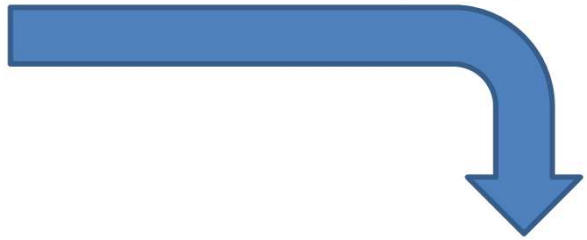
5L Fermenter

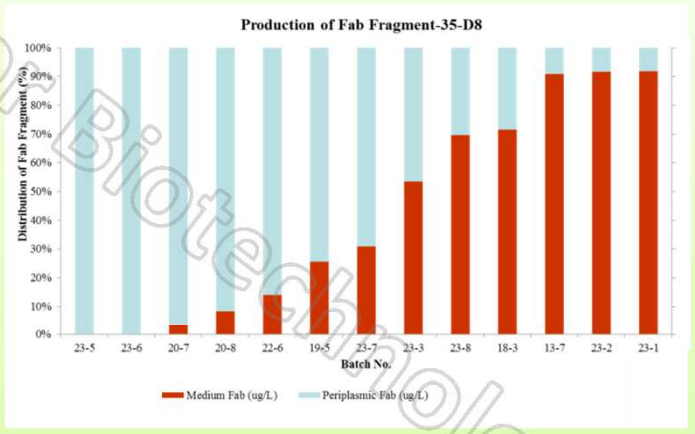
E. coli Expression Strain Construction and Screening

Vector	Promoter	Signal peptide
pET 23a	T7	pel B
pET 27b	Pho A	STII
	Tac 1	Omp A

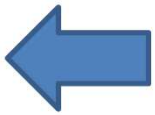
Host:
DE3 & non-DE3

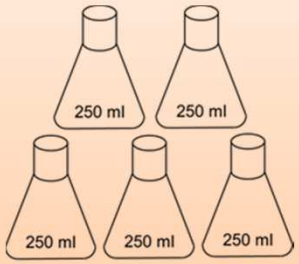
Transformation in to *E. coli*





Screening higher production clone

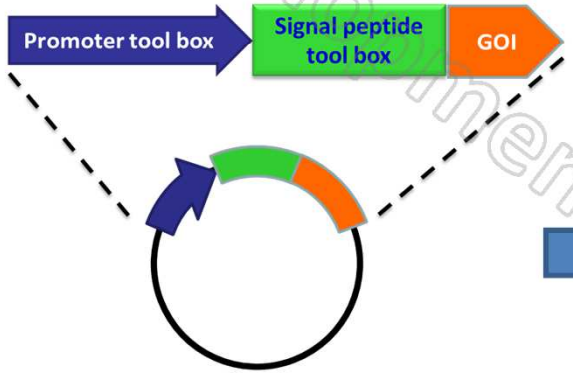




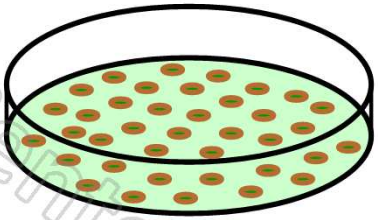
Flask induction test (ELISA, Dot blotting, SDS-PAGE)

6-8 weeks

Pichia patoris Expression Strain Construction and Screening

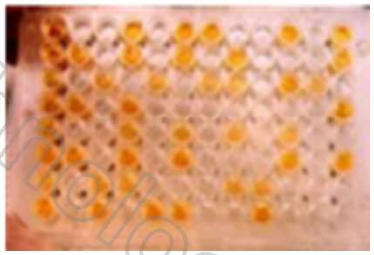


Expression plasmid construction (Zeocine)



Drug selection

Deep well induction



Titer analysis (ELISA, Dot blotting)

Pick up Top 5

Flask induction (ELISA, Dot blotting, SDS-PAGE)

Top three production strains

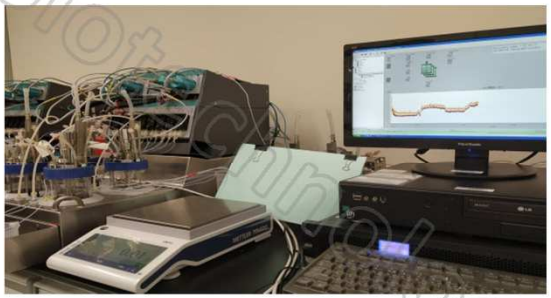
8-10 weeks

Process Development for Microbial Expression System

Cell line generation
High throughput screening
Top 3 clones

Optimize fermentation parameter by 250 ml mini- fermentor system

pH | Dissolve oxygen | Medium | Feeding strategy | Temperature



250 ml Mini-fermentor system

4-8 weeks

Scale up

Scale down

Optimized fermentation parameter scale up to 5L fermentor

2-4 weeks

At Glycerol Feeding Phase Nitrogen Supplementation can Benefite Protein Expression in *P. pastoris*

Typical *P. Pastoris* expression stage:

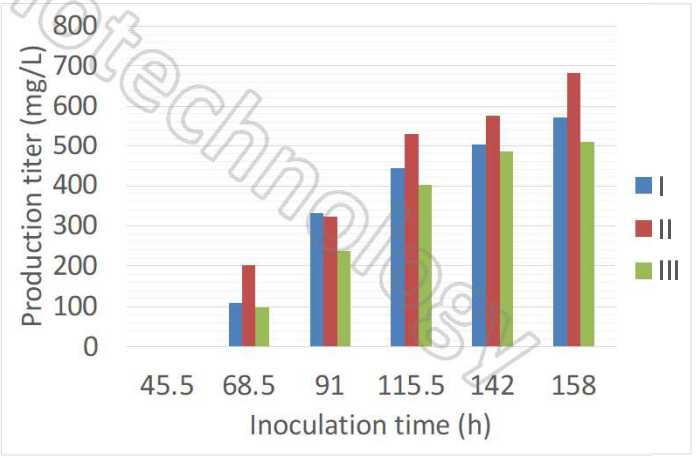
Batch → Glycerol feeding → Methanol Induction

	I	II	III
Glycerol feeding phase*	Non-nitrogen	2X-nitrogen	2X-nitrogen
Methanol feeding phase*	1X-nitrogen	Non-nitrogen	1X-nitrogen
Fermentation time (h)	158	158	158
Induction time (h)	144	144	144
Final OD600nm	529.1	554.1	511.5
Production titer (mg/L)	572.5	682.4	510.3
Specific production titer (mg/L/OD)	1.082	1.232	0.998

pH = 6.0
DO = 30% (cascade)
Agi.: 800~1500 rpm
Temp.: 30℃

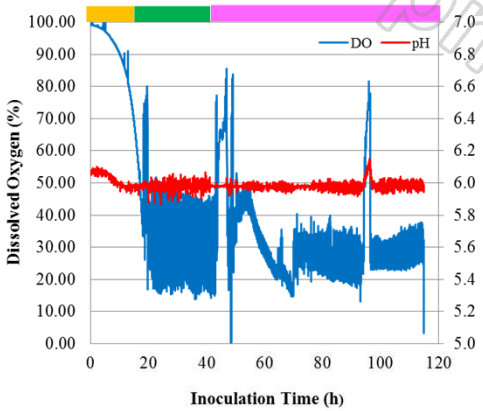
- The glycerol and methanol medium were fed by using DO-stat.
- Methanol induction medium contain methanol and sorbitol.

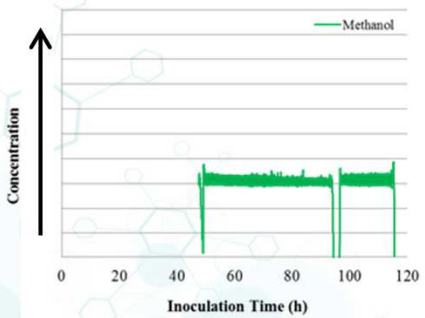
The nitrogen supply at glycerol feeding phase can achieve highest production titer, 682.4 mg/L at 158 hour.



Induction Strategies of *Pichia pastoris* in 5L fermentor

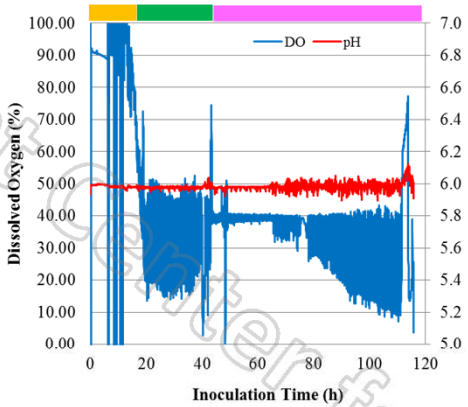
Methanol feedback control system

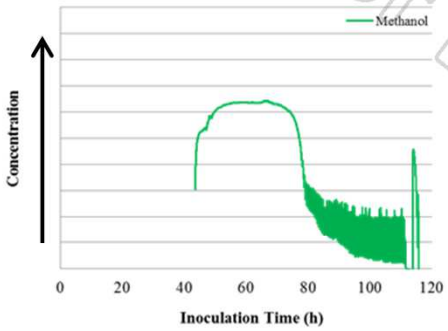




Production titer at 120 h is 1.01 g/L

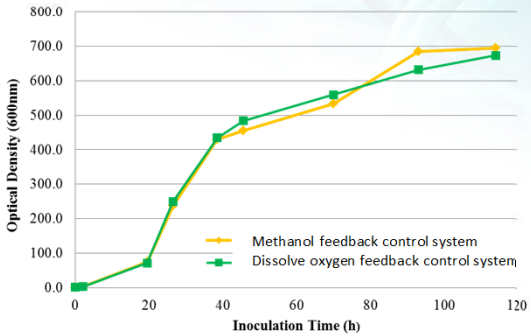
Dissolve oxygen feedback control system





Production titer at 120 h is 0.80g/L

The optical density profile of different induction strategy by 5 L fermentation



The amount of induction medium consumption:
Methanol feedback control: 1440 ml.
Dissolve oxygen feedback control: 1118 ml.

Methanol feedback control as induction medium feeding strategy can produce higher titer then dissolve oxygen feedback control system.

Batch
Glycerol feeding
Methanol induction