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Recombinant protein expression systems- Yeast

BT 207

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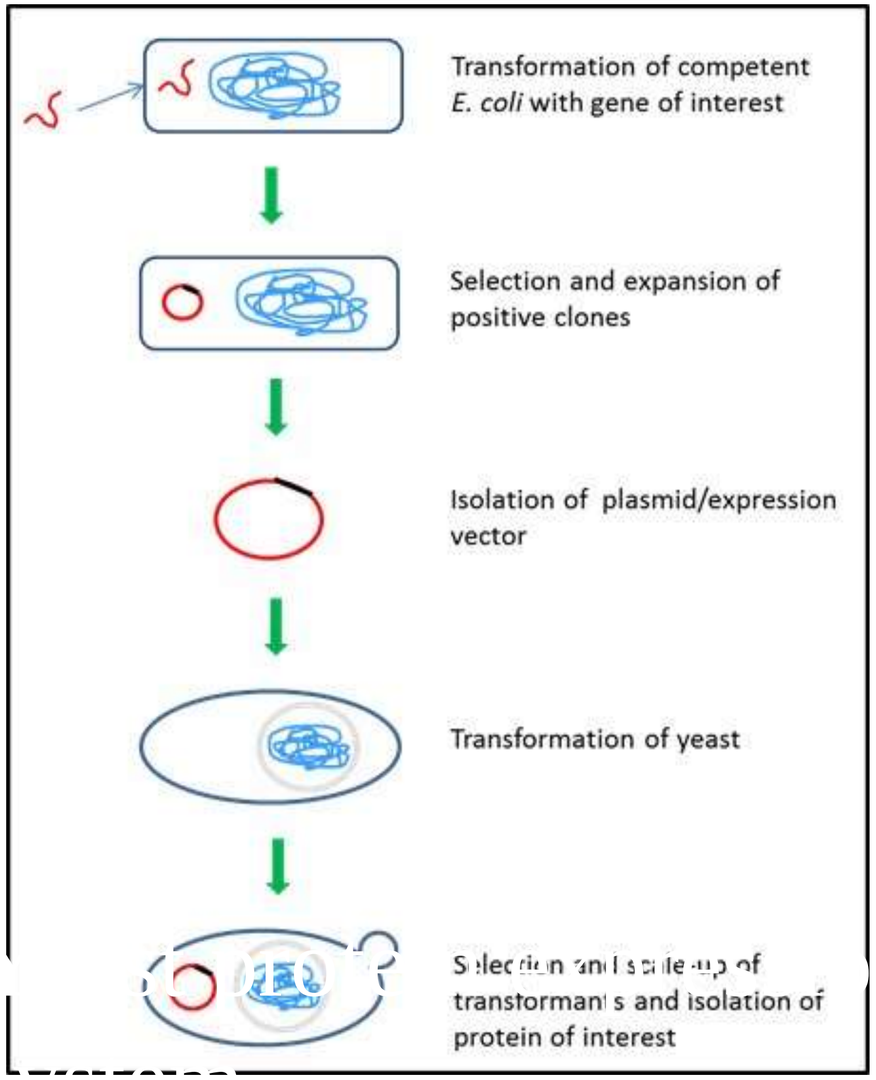


Features

- low cost culture methods
- suitable for both intracellular and secreted proteins
- provides eukaryotic post-translational glycosylation of proteins

Advantages

- Moderately rapid expression
- Works well for secreted and intracellular proteins
- Less expensive
- Most protein folding and post-translational modifications are possible

Disadvantages

- Characteristic N-linked glycan structures of proteins are different when compared to the typical mammalian proteins
- Requires use of yeast secretion signal peptides
- Enhanced safety precautions are required

Several yeast expression systems are used for recombinant protein expression, including

- *Sacharomyces cerevisae*
- *Scizosacchromyces pombe*
- *Pichia pastoris*
- *Hansanuela polymorpha*

Pichia pastoris expression system

Pichia pastoris	
Scientific Classification	
Kingdom	Fungi
Phylum	Ascomycota
Class	Saccharomycetes
Order	Saccaromycetales
Family	Saccharomycetaceae
Genus	*Pichia*
Species	*pastoris*

Pichia pastoris

- In recent years, to solve the problem of protein expression, methylotrophic yeasts such as *Hansenulla polymorpha* and *Pichia pastoris* (*P. pastoris*; syn. *Komagataella phaffii*) have been developed.
- Among these, *P. pastoris* has become the most popular for its cost and expression host system.
- This microorganism can produce high yields of recombinant proteins with the high similarity of glycosylation to the mammalian cells.
- The benefits of protein production by *P. pastoris* system include appropriate folding (in the endoplasmic reticulum [ER]) and secretion (by Kex2 as signal peptidase) of recombinant proteins to the external environment of the cell.
- Given the fact that some proteins produced by their original host are secreted out of the cell; *P. pastoris* is suitable for the production of recombinant proteins since it is equipped with a secretion system.

Basic characteristics of different host systems for the expression of recombinant proteins

Characteristics	*Escherichia coli*	Pichia pastoris	CHO cell
Doubling time	30 min	60–120 min	24 hr
Cost of growth medium	Low	Low	High
Complexity of growth medium	Minimum	Minimum	Complex
Expression level	High	Low to high	Low to moderate
Extracellular expression	Secretion to periplasm	Secretion to medium	Secretion to medium
Protein folding	Refolding usually required	Refolding may be required	Proper folding
N-linked glycosylation	None	High mannose	Complex
O-linked glycosylation	No	Yes	Yes
Phosphorylation & acetylation	No	Yes	Yes
Drawback	Accumulation of LPS	Codon bias	Contamination with animal viruses

- Abbreviations: CHO, Chinese hamster ovary; LPS, lipopolysaccharide.

Pichia pastoris

Distinctions between Yeast and other expression systems.

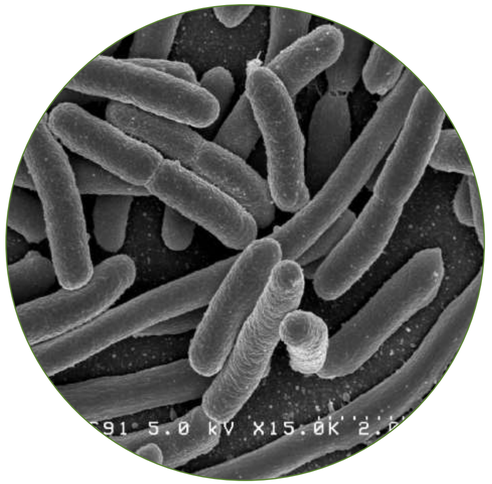
The methylotrophic yeast.

- One of the most important branches of genetic engineering is the expression of recombinant proteins using biological expression systems.
- Nowadays, different expression systems are used for the production of recombinant proteins including bacteria, yeasts, molds, mammals, plants, and insects.
- *Pichia pastoris* is a species of methylotrophic yeast. It was found in the 1960s, with its feature of using methanol, as a source of carbon and energy.

Pichia pastoris

Problems with bacterial expression system

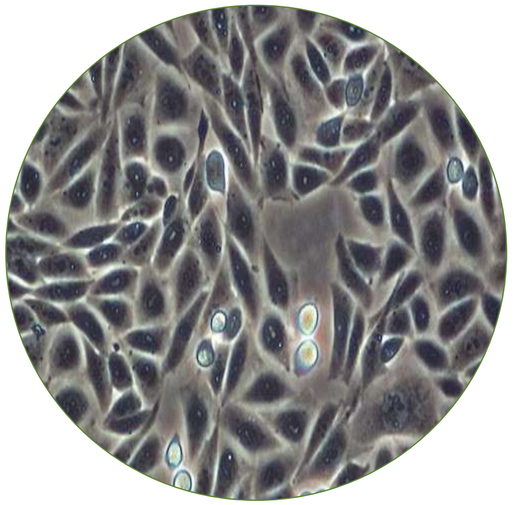
- Bacterial expression system has several advantages including rapid multiplication, simple and inexpensive nutritional requirements, high-level expression, and fast and easy transformation process.
- However, this cell factory has some limitations such as intracellular aggregation and misfolding of heterologous proteins, production of lipopolysaccharide, lack of posttranslational modification, and protein degradation due to proteases.



Bacterial cells

Limitation with mammalian expression system

- Another part of expression systems is the eukaryotic cells which include mammalian cells.
- The most common mammalian cell lines are Chinese hamster ovary (CHO) cells. Currently, CHO cells are used to produce biopharmaceutical compounds, monoclonal antibodies, and Fc-fusion proteins.
- Apart from this, baby hamster kidney, human embryonic kidney 293 and NS0, SP2/0 (mouse-derived myeloma) cell lines have also received legal permissions.
- Significant advantages of this system include proper protein folding, posttranslational modifications, and glycosylation of recombinant proteins in the correct sites which is important for protein stability.
- Besides, mammalian expression systems grow slowly and the relevant nutrient requirement is costly. On the other hand, potential contamination of culture medium with some viruses has limited its use in large-scale production .



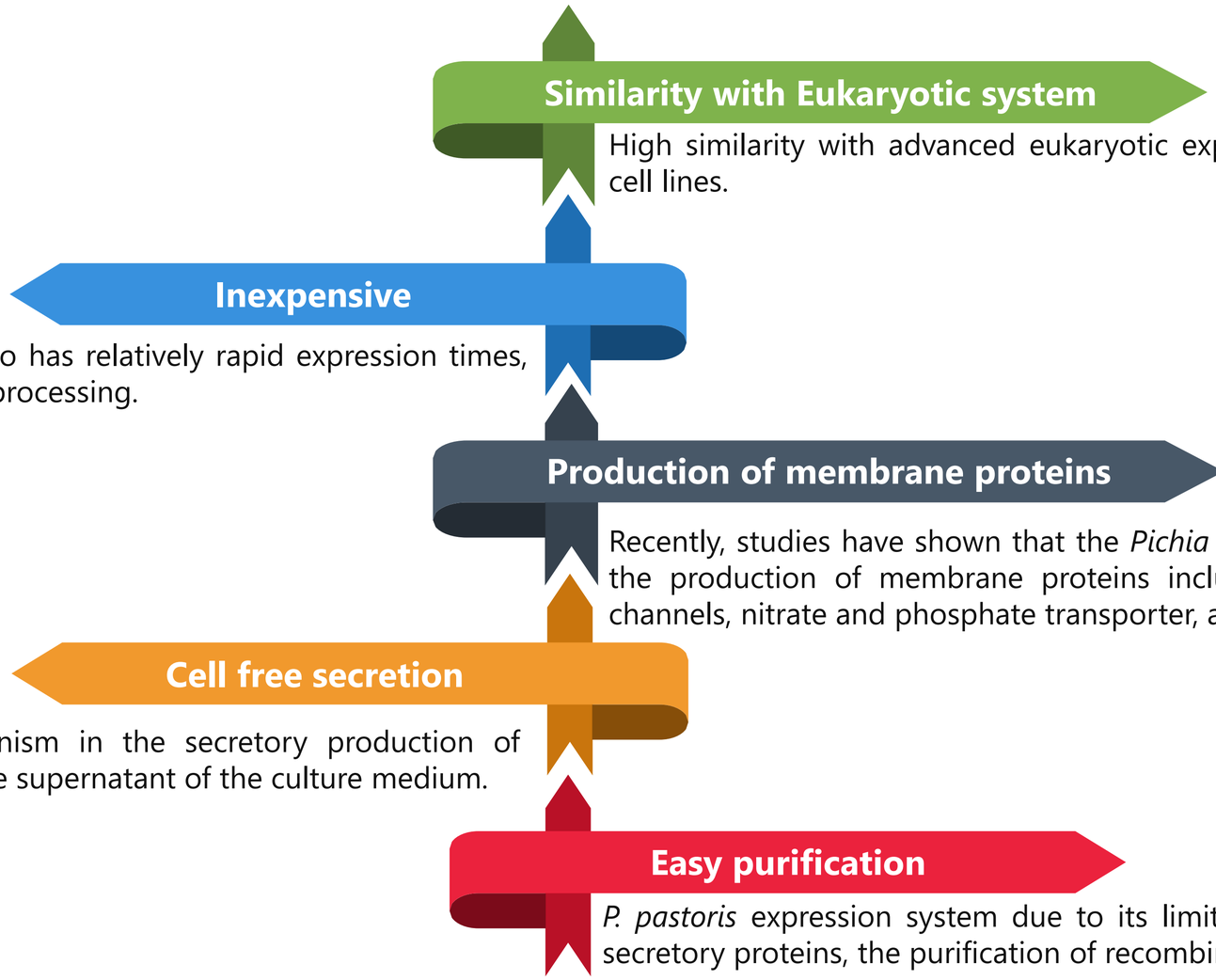
CHO CELLS

Yeast expression system

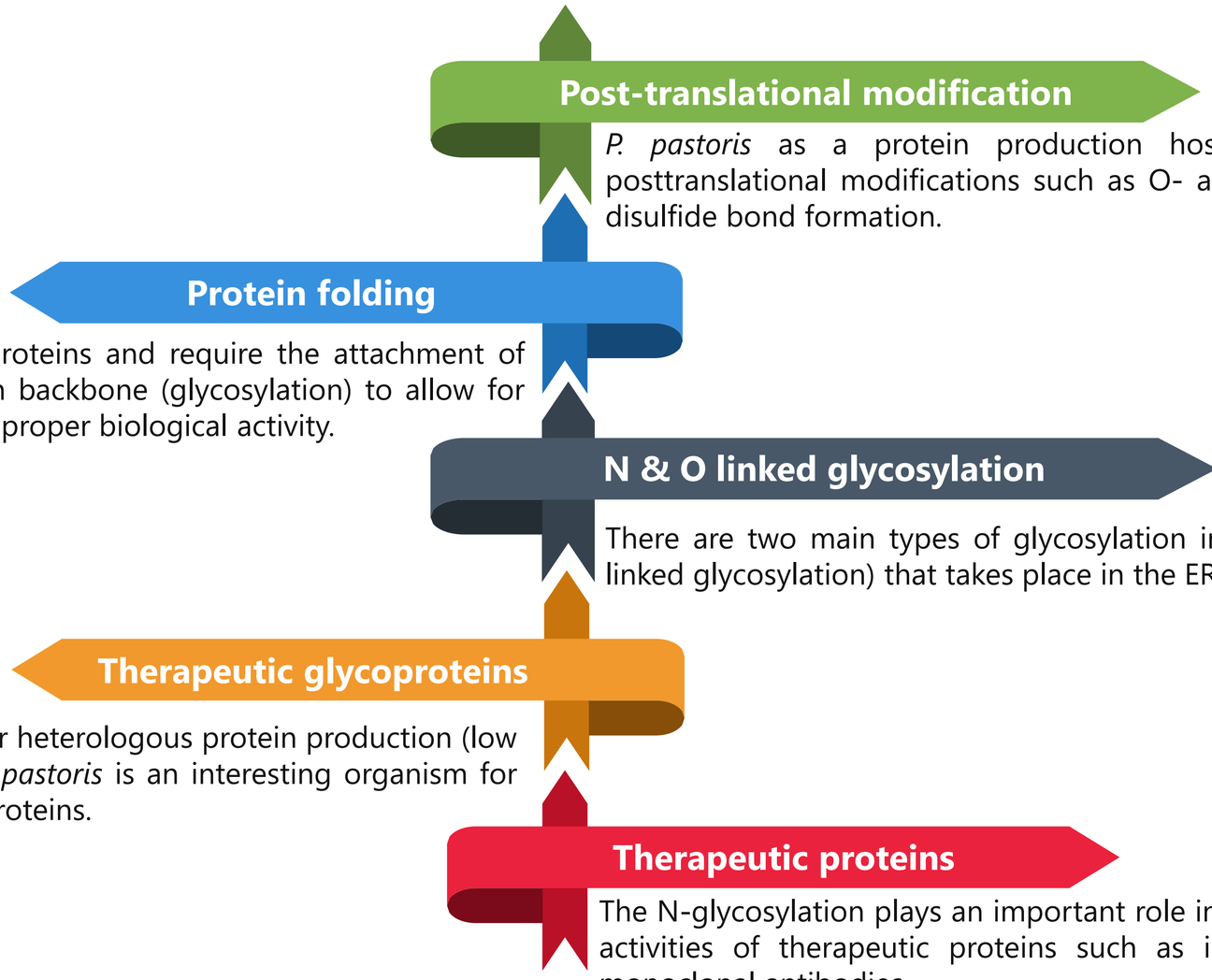
- Yeasts are other eukaryotic cells that are widely used for the expression of several proteins in vaccine and pharmaceutical production.
- The mechanism of protein expression in these microorganisms is close to the ones in mammalian cells. Compared with bacteria, yeast cells have significant advantages including growth speed, post translational modification, secretory expression, and easy genetic manipulation.
- Furthermore, linearized foreign DNA can be inserted in a chromosome in high efficiency via cross recombination phenomena to generate stable cell lines.
- Among yeast cells, *Saccharomyces cerevisiae* is used in the manufacture of hepatitis B and human papillomavirus vaccines, both of which produce a protective immune response against wild-type viruses.
- The expression proteins in *S. cerevisiae* are often N and O-hyperglycosylated, which may affect protein immunogenicity

Saccharomyces cerevisiae

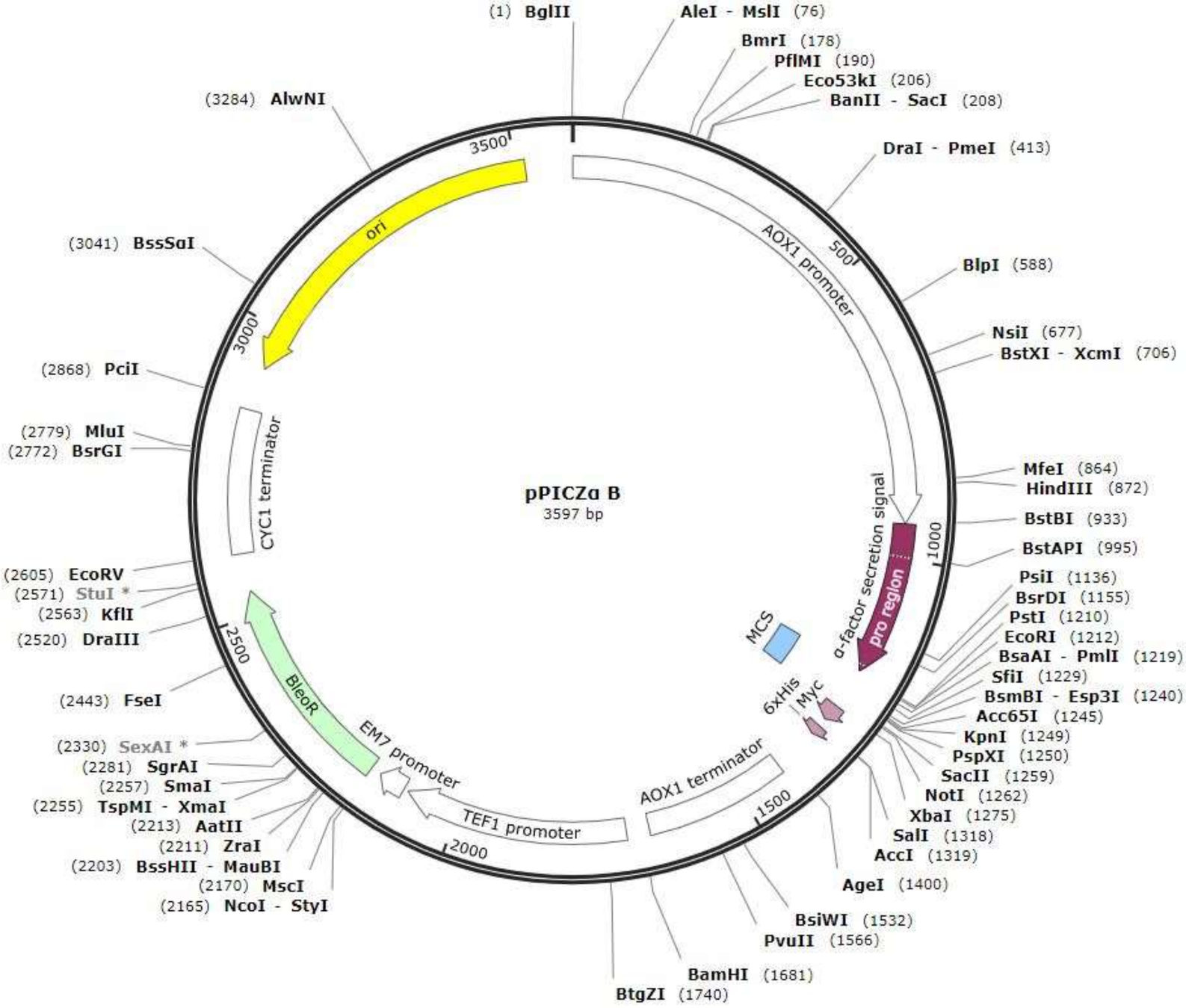
The Characteristic Features of *P. pastoris* Expression System



The Characteristic Features of *P. pastoris* Expression System



pPICZαB


pPICZα B
3597 bp
(1) BglII
AleI - MslI (76)
BmrI (178)
PflMI (190)
Eco53kI (206)
BanII - SacI (208)
DraI - PmeI (413)
BlpI (588)
NsiI (677)
BstXI - XcmI (706)
MfeI (864)
HindIII (872)
BstBI (933)
BstAPI (995)
PsiI (1136)
BsrDI (1155)
PstI (1210)
EcoRI (1212)
BsaAI - PmlI (1219)
SfiI (1229)
BsmBI - Esp3I (1240)
Acc65I (1245)
KpnI (1249)
PspXI (1250)
SacII (1259)
NotI (1262)
XbaI (1275)
SalI (1318)
AccI (1319)
AgeI (1400)
BsiWI (1532)
PvuII (1566)
BamHI (1681)
BtgZI (1740)
AOX1 terminator
TEF1 promoter
EM7 promoter
BleoR
(2330) SexAI *
(2281) SgrAI
(2257) SmaI
(2255) TspMI - XmaI
(2213) AatII
(2211) ZraI
(2203) BssHII - MauBI
(2170) MscI
(2165) NcoI - StyI
(2443) FseI
(2605) EcoRV
(2571) StuI *
(2563) KflI
(2520) DraIII
(2779) MluI
(2772) BsrGI
CYC1 terminator
(2868) PciI
(3041) BssSαI
(3284) AlwNI
ori
MCS
6xHis
Myc
α-factor secretion signal
pro region
AOX1 promoter
500
1000
1500
2000
2500
3000
3500

Common *Pichia pastoris* expression vectors for the production of secretory proteins

Vector name	Marker gene	Used strain	Recombinant protein	Reference
pPIC9K	His4, Kan, Amp	GS115	Xylanase	Fu, Zhao, Xiong, Tian, and Peng (2011)
		GS115	Porcine circovirus type 2	Tu et al. (2013)
		GS115	Endo-1,3(4)-b-d-glucanase	X. Chen et al. (2012)
		GS115	Staphylokinase	Apte-Deshpnade, Mandal, Soorapaneni, Prasad, Kumar, and Padmanabhan (2009)
pPICZα	*Shble*	SMD1168	Human chitinase	Goodrick et al. (2001)
		GS115	Human topoisomerase I	Chan et al. (2018)
		GS115	Human interferon gamma	Prabhu, Veeranki, and Dsilva (2016)
		X-33	C-reactive protein	J. Li et al. (2017)
		SuperMan5	Insulin	Baeshen et al. (2016)
		X-33	Human RNase4	Bardiya and Chang (2017)
pHIL-S1	His4, Amp	GS115	Rabies virus glycoprotein	Ben Azoun, Belhaj, Göngrich, Gasser, and Kallel (2016)
pGAPZα	Shble	GS115	Acyl homoserine lactonase	J. Wu et al. (2016)

Common Pichia pastoris expression vectors for the production of intracellular proteins

Vector name	Marker gene	Used strain	Recombinant protein	Reference
pPIC3.5K	His4, Kan, Amp	KM71	Maltooligosyltrehalose synthase	Han, Su, Hong, Wu, and Wu (2017)
		SMD1168	Camellia sinensis heat shock protein	Wang, Zou et al. (2017)
		GS115	Pleurotus ostreatus laccases	Zhuo et al. (2018)
		GS115	Rhizopus oryzae Lipase	Jiao, Zhou, Su, Xu, and Yan (2018)
		GS115	HSA/GH fusion protein	M. Wu et al. (2014)
pPICZ	Shble	X-33	Aquaporin	Nordén et al. (2011)
		KM71	Membrane protein	J. Y. Lee, Chen, Liu, Alba, and Lim (2017)
		KM71	Dengue virus envelope glycoprotein	Khetarpal et al. (2017)
pHIL-D2	His4, Amp	GS115	Prostaglandin H synthase-2	Kukk and Samel (2016)
		GS115	CatA1 and SODC	Mina et al. (2017)
		KM71	Rhodococcus nitrile hydratase	Pratush, Seth, and Bhalla (2017)
		GS115	Feline serum albumin	Yokomaku, Akiyama, Morita, Kihira, and Komatsu (2018)
pJL-IX	FLD1, Amp	MS105	Formaldehyde dehydrogenase	Sunga and Cregg (2004)
pBLHIS-IX	His4, Amp	KM71	L1-L2 proteins of HPV virus type 16	Bredell, Smith, Görgens, and van Zyl (2018)

Recombinant subunit vaccine expressed in *Pichia pastoris*

Construct name	Used strain	Used vector	Targeted disease	Reference
BoNT Hc	FLD1, Amp	pPICZ-A	Botulism	Webb et al. (2017)
Tc52	X-33	pPICZαA	Chagas	Matos, Alberti, Morales, Cazorla, and Malchiodi (2016)
Apa	GS115	pPIC9K	Tuberculosis	S. Wang, Wang, Wang, Chen, and Kong (2018)
RBD219-N1	X-33	pPICZαA	SARS	W.-H. Chen et al. (2017)
DENV-3 E	KM71	pPICZ-A	Dengue	Tripathi et al. (2015)
CHIKV-C-E3-E2-6K-E1	GS115	pPIC9K	Chikungunya	Saraswat et al. (2016)
PIMP-V1 and PIMP-V2	KM71	pPICZαA	Malaria	Spiegel et al. (2015)
Gp350	GS115	pPICZαA	EBV infection	Wang et al. (2016)
BoNT Hc	X-33	pPICZ-A	Botulism	Webb et al. (2017)
Glycoprotein D	GS115	pPIC9K	HSV-2 infection	Wang, Jiang et al. (2017)
OmpA-Fc	GS115	pPIC9K	Bordetellosis	Dong et al. (2016)

Pichia Compatible Vector

Feature	Benefit
5´ AOX1 promoter	A 942 bp fragment containing the AOX1 promoter that allows methanol-inducible, high level expression of the gene of interest in *Pichia*. Targets plasmid integration to the AOX1 locus.
α-factor secretion signal (from Saccharomyces cerevisiae)	Allows for efficient secretion of most proteins from *Pichia*.
Multiple cloning site	Allows insertion of your gene into the expression vector.
c-myc epitope (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu)	Permits detection of your recombinant fusion protein with the Anti-myc Antibody or Anti-myc-HRP Antibody (Evans et al., 1985).
C-terminal polyhistidine (6xHis) tag	Permits purification of your recombinant fusion protein on metal-chelating resin such as ProBond™. In addition, the C-terminal polyhistidine tag is the epitope for the Anti-His(C-term) Antibody (Lindner et al., 1997) and the Anti-His(C-term)- HRP Antibody.
AOX1 transcription termination (TT) region	Native transcription termination and polyadenylation signal from AOX1 gene (~260 bp) that permits efficient 3´ mRNA processing, including polyadenylation, for increased mRNA stability.
TEF1 promoter (GenBank accession nos. D12478, D01130)	Transcription elongation factor 1 gene promoter from Saccharomyces cerevisiae that drives expression of the Zeocin™ resistance gene in Pichia.
EM7 promoter	Synthetic prokaryotic promoter that drives constitutive expression of the Zeocin™ resistance gene in *E. coli.*
Zeocin™ resistance gene (Sh ble)	Allows selection of transformants in *E. coli* and *Pichia*.
CYC1 transcription termination region (GenBank accession no. M34014)	3´ end of the Saccharomyces cerevisiae CYC1 gene that allows efficient 3´ mRNA processing of the Zeocin™ resistance gene for increased stability.
pUC origin	Allows replication and maintenance of the plasmid in *E. coli.*

Problems with yeast expression system

- Unlike bacteria there are limited selection technique in yeast expression system.
- Extracellular expression of protein size greater than 150KDa is often problematic. In such case whole cell lysate is used to isolate protein of interest.
- The secretion efficiency is low in case of high density cultivation.
- Not suitable for high density cultivation.

Thank you