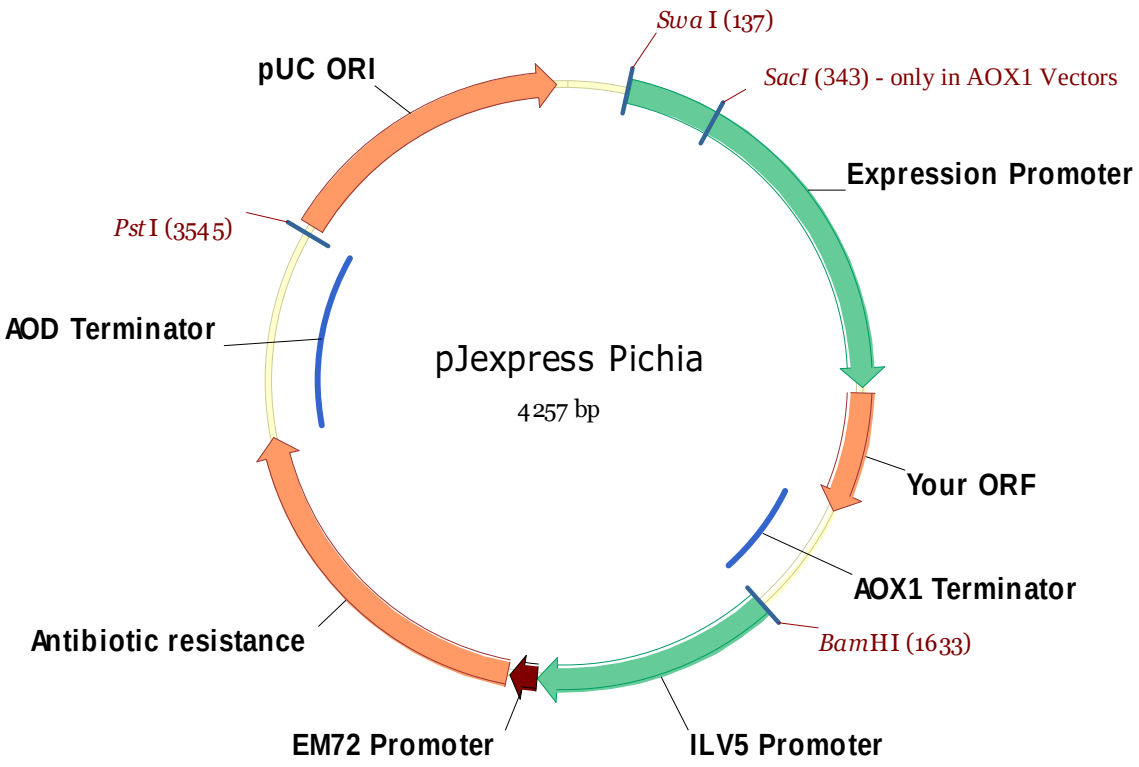
DNA 2.0

pJexpress Pichia Vector Information

Vector	Resistance Marker	Expression Promoter	Induction	Alpha factor Signal Sequence
pJexpress 901	Kan/Gen	AOX1	MeOH Inducible	No
pJexpress 902	Zeocin	AOX1	MeOH Inducible	No
pJexpress 905	Zeocin	GAP1	Constitutive*	No
pJexpress 911	Kan/Gen	AOX1	MeOH Inducible	Yes
pJexpress 912	Zeocin	AOX1	MeOH Inducible	Yes
pJexpress 915	Zeocin	GAP1	Constitutive*	Yes

*Vectors with constitutive promoter result in lower expression levels, but do not require addition of methanol.

pJ901, pJ902, pJ905 Intracellular Expression Vectors:



DNA 2.0

Cloning Site for Pichia intracellular expression vectors:

AAAACGATG.....TAAGGG
Kozak sequence ORF

- Must include N-terminal methionine and stop codon in gene sequence
- Kozak sequence shown above will be added
- Any frame is ok
- Your gene must NOT include BamHI, BsaI, PstI, SacI(AOX1 promoters) or SwaI sites

pJexpress 901

- Kanamycin selection in *E.coli* and Geneticin selection in *Pichia pastoris*
- *AOX1* promoter for methanol inducible expression of gene of interest in *Pichia pastoris*
- For integration at AOX1 locus, linearization with SwaI or SacI
    - Choose SwaI to linearize vector for low to medium expression level; choose SacI to linearize vector for increased expression
- For integration at AOX1 terminator, linearization with BamHI
- To remove *E. coli* sequence from the integrated expression cassette, linearization with SwaI (or SacI) and PstI
- Must include N-terminal methionine and stop codon in gene sequence
- Your gene must NOT include BamHI, BsaI, PstI, SacI or SwaI sites

pJexpress 902

- Zeocin selection in *E.coli* and *Pichia pastoris*
- *AOX1* promoter for methanol inducible expression of gene of interest in *Pichia pastoris*
- For integration at AOX1 locus, linearization with SwaI or SacI
    - Choose SwaI to linearize vector for low to medium expression level; choose SacI to linearize vector for increased expression
- For integration at AOX1 terminator, linearization with BamHI
- To remove *E. coli* sequence from the integrated expression cassette, linearization with SwaI (or SacI) and PstI
- Must include N-terminal methionine and stop codon in gene sequence
- Your gene must NOT include BamHI, BsaI, PstI, SacI or SwaI sites

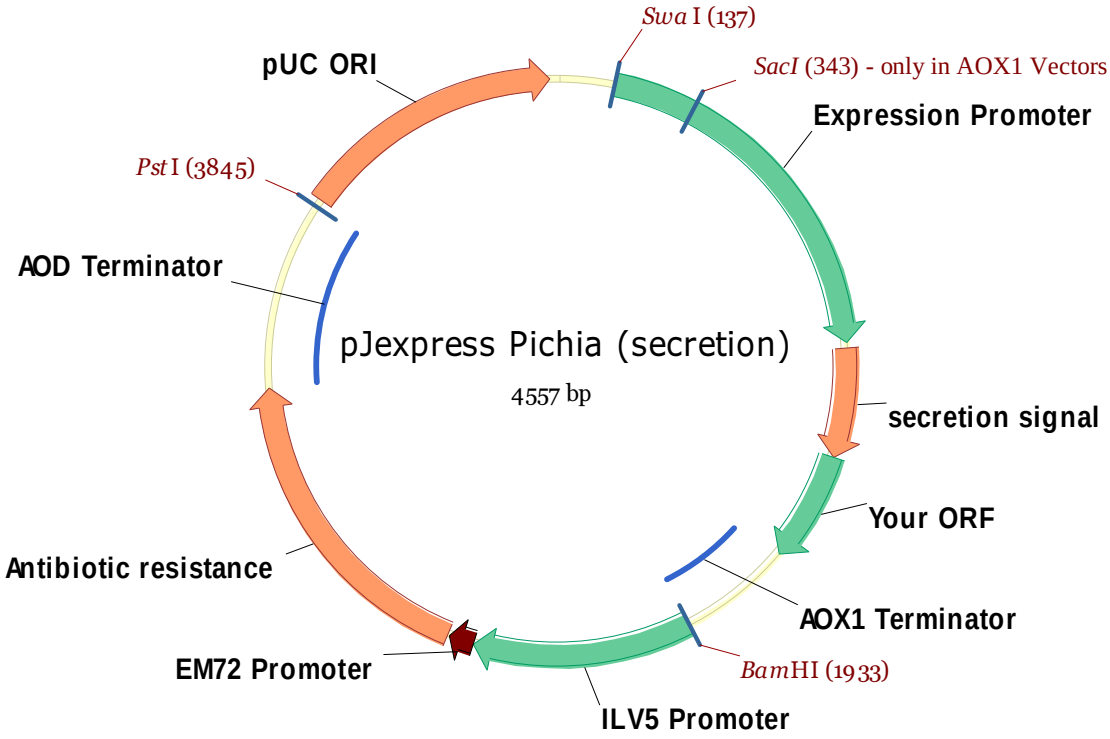
DNA 2.0

pJexpress 905

- Zeocin selection in *E.coli* and *Pichia pastoris*
- *GAP1* promoter for constitutive expression of gene of interest in *Pichia pastoris*
- For integration at GAP1 locus, linearization with SwaI
- For integration at AOX1 terminator, linearization with BamHI
- To remove *E. Coli* sequence from the integrated expression cassette, linearization with SwaI and PstI
- Must include N-terminal methionine and stop codon in gene sequence
- Your gene must NOT include BamHI, BsaI, PstI or SwaI sites

DNA 2.0

pJ911, pJ912, pJ915 Secretion Vectors:



Cloning site for Pichia Secretion Vectors:

AAAAGAGAGGCCGAAGCT.....TAAGGG
Secretion Sequence Gene

- Gene MUST be in frame
- Secretion sequence shown above will be added
- Does not need N-terminal methionine
- Must include stop codon in gene sequence
- Your gene must NOT include BamHI, BsaI, PstI, SacI(AOX1 promoters) or SwaI sites

DNA 2.0

pJexpress 911

- Kanamycin selection in *E.coli* and Geneticin selection in *Pichia pastoris*
- *AOX1* promoter for methanol inducible expression of gene of interest in *Pichia pastoris*
- Alpha factor signal sequence for secreted expression of gene of interest
- For integration at AOX1 locus, linearization with SwaI or SacI
    - Choose SwaI to linearize vector for low to medium expression level; choose SacI to linearize vector for increased expression
- For integration at AOX1 Terminator, linearization with BamHI
- To remove *E. coli* sequence from the integrated expression cassette, linearization with SwaI (or SacI) and PstI
- Your gene must NOT include BamHI, BsaI, PstI, SacI or SwaI sites

pJexpress 912

- Zeocin selection in *E.coli* and *Pichia pastoris*
- *AOX1* promoter for methanol inducible expression of gene of interest in *Pichia pastoris*
- Alpha factor signal sequence for secreted expression of gene of interest
- For integration at AOX1 locus, linearization with SwaI or SacI
    - Choose SwaI to linearize vector for low to medium expression level; choose SacI to linearize vector for increased expression
- For integration at AOX1 Terminator, linearization with BamHI
- To remove *E. coli* sequence from the integrated expression cassette, linearization with SwaI (or SacI) and PstI
- Your gene must NOT include BamHI, BsaI, PstI, SacI or SwaI sites

pJexpress 915

- Zeocin selection in *E.coli* and *Pichia pastoris*
- *GAP1* promoter for constitutive expression of gene of interest in *Pichia pastoris*
- Alpha factor signal sequence for secreted expression of gene of interest
- For integration at GAP1 locus, linearization with SwaI
- For integration at AOX1 Terminator, linearization with BamHI
- To remove *E. coli* sequence from the integrated expression cassette, linearization with SwaI and PstI
- Your gene must NOT include BamHI, BsaI, PstI or SwaI sites

DNA 2.0

Genetic elements used in the vectors

The pJexpress Pichia vectors were constructed by the Glieder lab at the Graz University of Technology in collaboration with the Research Centre of Applied Biocatalysis and VTU Technology, for protein expression in *P. pastoris*. The vectors are designed to be used with no IP restrictions. An expert opinion for full freedom to operate was established by Sonn & Partner Intellectual Property Attorneys. The plasmids neither contain patented elements nor are copied from tools commercially distributed. These new vectors can be used for the production of proteins in research and for commercial applications. Commercial distribution of the cloning vectors is prohibited. All vector components were either constructed synthetically or amplified from plasmids and strains published over 20 years ago, including publicly available *Pichia pastoris* wild-type strains CBS7435 and CBS704, which were purchased from the CBS Yeast Database (Centraalbureau voor Schimmel cultures, Utrecht, the Netherlands). The elements used in the vectors, their origins and functions are described in table 1.

Customers are free to use these vectors for the production of proteins in research and commercial applications without any reach-through of any sort from Glieder lab or DNA2.0. Customers may not resell vector or vector with insert without written permission from Glieder Lab and DNA2.0.

Table 1: The origins and functions of plasmid components

Element	Origin	Function
AOX1 Promoter	Synthetic, gi:2104960	*AOX1* Promoter for methanol induced expression of the target gene in *Pichia pastoris*
GAP Promoter	CBS7435, *Pichia pastoris* strain	*GAP1* Promoter for constitutive expression of the target gene in *Pichia pastoris*
ILV5 Promoter	CBS7435, *Pichia pastoris* strain	*ILV5* Promoter for expression of respective antibiotic resistance gene in *Pichia pastoris*
EM72 Promoter	Synthetic consensus sequence *E.coli* Promoter (designed @IMBT)	Constitutive Promoter for expression of respective antibiotic resistance gene in *E. coli*
AOX1 Terminator	Synthetic, gi:2104960	*AOX1* Transcription Termination (TT)
AOD Terminator	CBS7435, *Pichia pastoris* strain	*AOD* Transcription Termination (TT)
Zeocin CDS	Synthetic, gi:1567211, codon optimized: Gene Designer, Leto 1.0; mixed codon usage (*E. coli, P. pastoris*): Leto	Zeocin resistance, selection marker (*E.coli* and *Pichia pastoris*)
KanMX6 CDS	KanMX6 (Oka et al., 1981;Wach et al., 1994)	Kanamycin and Geneticin resistance in *E. coli* and *Pichia pastoris*, respectively; selection marker
pUC ORI	pBR322, Plasmid	pUC replication origin for *E.Coli*
Alphafactor Signal Sequence	Synthetic, gi: 2168568, codon optimized: Gene Designer, Leto 1.0; based on *Saccharomyces cerevisiae* prepro alpha-factor (MF alpha-2)	Secretion signal sequence

Note: Only vectors with an Alpha factor provide a Kozak-consensus sequence (as published by P., G. R. Davis, et al. (1989)).

Methanol induction protocol: BioTechniques 2005 38:44-48. Condensed protocol for competent cell preparation and transformation of the methylotrophic yeast *Pichia pastoris*. Lin-Cereghino *et al*. http://www.biotechniques.com/multimedia/archive/00003/BTN_A_05381BM04_O_3873a.pdf