ATUM

Yeast Expression Vectors

ATUM offers vectors for protein expression in the yeasts *Saccharomyces cerevisiae* and *Pichia pastoris*.

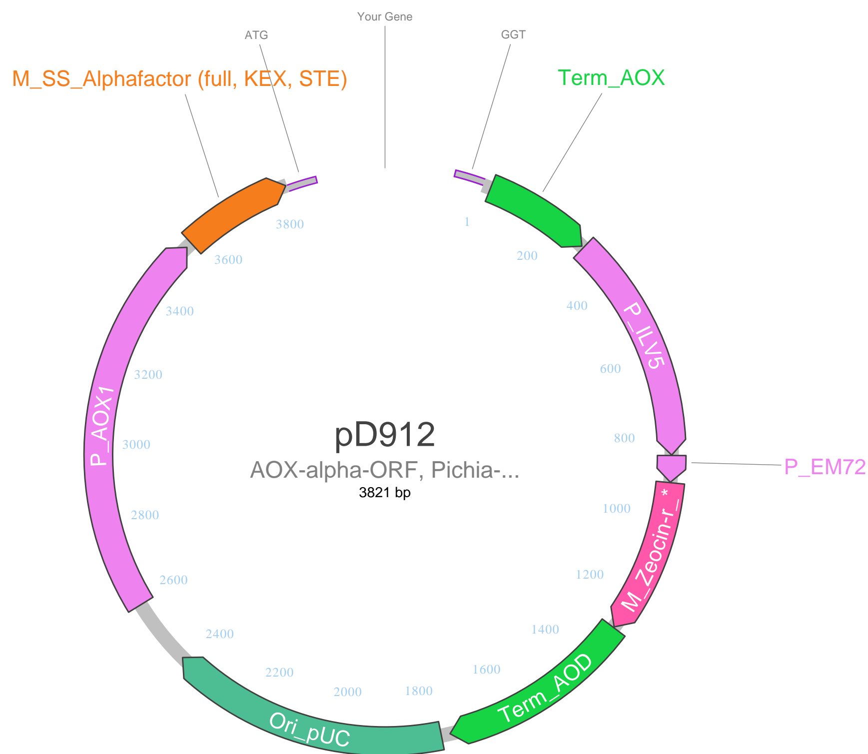
Yeast Secretion Signals

Production of recombinant proteins in the media greatly simplifies purification. It can also reduce protease attack and improve correct protein folding. Our yeast expression vectors are available with a number of eukaryotic secretion signals to enable empirical identification of the best signal for a particular protein. These signals are derived from yeast and other fungi, as well as birds and mammals.

Pichia Expression Vectors

These vectors are available with or without secretions signals. They have either an inducible (AOX) or constitutive (GAP) promoter and carry zeocin resistance. The vectors will integrate into the *Pichia* genome.

Plasmid Map



Name	Qty	Storage
pD912	10Rx	−20℃

Genomic Integration Protocol

Linearize 10-20 µg of expression vector with either PmeI or SacI (AOX promoter) or AvrII (GAP promoter). <u>THE RESTRICTION SITE SHOULD NOT BE PRESENT IN THE EXPRESSED GENE</u>. Ethanol precipitate restriction digests to concentrate the DNA. Transform electro-competent *Pichia* cells with 5-10 µg of linearized plasmid. Add 0.5 x YPD broth with 0.5M sorbitol. Incubate the transformed cells for 1 hour at 30℃ in a shaking or rolling incubator. Spread 50-200 µL of out-grown cells onto YPD agar with 1M sorbitol and 1 mg/ml Zeocin. Incubate at 30℃ until colonies arise in 2-3 days.

Expression Protocol

Pick colonies into BMGY broth with 250 µg/ml zeocin and grow at 28–30℃ shaking at 250 rpm. For AOX promoter: after 2 days of incubation, add 300 µL of BMMY broth to each well, and continue incubation for an additional 2-4 days. Check supernatant for secreted protein expression or cell pellets for cytoplasmic protein expression.

Pichia Protocols are at: https://www.atum.bio/wp-content/uploads/2013/04/Pichia_culture_induction_protocol.pdf

Pichia Vector Controls

Protein Paintbox genes are available in the AOX MeOH-inducible *Pichia* intracellular vectors to serve as controls. In addition, any *Pichia*-optimized Protein Paintbox gene in an Electra MOTHER vector can be cloned into any Electra *Pichia* DAUGHTER vector. Paintbox genes are not secreted, so we recommend a naturally secreted protein (cutinase) as a control for vectors with secretion signals.

Electra Cloning System

Electra is a simple one-tube universal cloning process that can be performed in a 5 minute bench-top reaction with the fidelity of a restriction-based cloning system. A gene from one MOTHER vector is compatible with all DAUGHTER vectors, allowing rapid testing of many different sequence contexts simultaneously.

Reagents

The Electra Reagents kit contains all necessary components to facilitate cloning a gene from a MOTHER into a DAUGHTER vector. The Electra reaction can also be used to clone a PCR product into either a MOTHER or a DAUGHTER vector.

Electra Buffer Mix is supplied at 10X final concentration (use 2 µl in a 20 µl reaction)

Electra Enzyme Mix is supplied at 20X final concentration (use 1 µl in a 20 µl reaction)

Cloning Protocol

Component	Volume (µl)
MOTHER DNA / Positive control (20 ng)	1
DAUGHTER Vector (20 ng)	1
Electra Buffer (10x)	2
Electra Enzyme (20x)	1
Water	15
Total	**20**

1. Combine components and incubate at 25-37°C for 5-20 minutes.

2. Transform 1-2 µl into chemically competent E. coli. (DH10B cells recommended)

3. Recover cells for 45 minutes and then plate on appropriate antibiotic for the DAUGHTER.

3a. Optionally include streptomycin at 100 µg/ml (for selection against pMOTHER with rpsL); or plate on YEG with antibiotic plus p-chloro phenylalanine at 10mM (for selection against pMOTHER with pheS).

Positive Control

A positive control MOTHER vector carries a gene in which Ptet drives expression of green fluorescent protein (DasherGFP). A successful Electra reaction will produce green fluorescent colonies from the DAUGHTER vector.

Electra DAUGHTER Vectors

Electra DAUGHTER vectors are supplied as linearized DNA, with overhangs compatible with an ATG (encoding methionine) at the 5' end and GGT (encoding glycine) at the 3' end.

ATUM
37950 Central Court
Newark, CA 94560

1 877 362 8646 (Toll free)
info@atum.bio
www.atum.bio

Genes in MOTHER vectors have adjacent restriction sites that produce overhangs compatible with an ATG at the 5' end and GGT at the 3' end upon digestion with SapI. Alternatively Electra ends can be added to any gene* by PCR. We recommend you add the following ends to your PCR primers:

5'-TACACGTACTTAGTCGCTGAAGCTCTTCTATG....(ORF)....-3'

5'-TAGGTACGAACTCGATTGACGGCTCTTCTACC....(ORF Reverse Complement)....-3'

*Your gene must not contain any internal SapI recognition sites, since the Electra cloning process utilizes the typeIIs enzyme SapI.

MOTHER vectors also contain a counter-selection gene. This can be used to eliminate any residual gene propagating in the MOTHER.

Feature list descriptions

Ori_pUC	The origin of replication is a sequence in a genome at which replication is initiated. The pUC ori is a mutated form of origin derived from *E. coli* plasmid pBR322 which allows production of greater than 500 copies of plasmid per cell. (www.en.wikipedia.org/wiki/Origin_of_replication)
P_AOX1	A strong and tightly regulated methanol inducible alcohol oxidase promoter in *Pichia pastoris*. The AOX1 promoter is induced by methanol and repressed by glucose. (www.ncbi.nlm.nih.gov/pubmed/16233151 www.link.springer.com/article/10.1007/s11033-008-9359-4#page-2)
P_EM72	The EM7 promoter is a synthetic bacterial promoter derived from the T7 promoter that enables the constitutive expression of the antibiotic resistance gene in *E.coli*. (www.google.com/patents/US7244609)
P_ILV5	The ILV5 promoter from the ILV5 gene is capable of driving strong expression. (www.ncbi.nlm.nih.gov/pmc/articles/PMC341325/)
SS_Alphafactor (full, KEX, STE)	A secretion signal derived from the yeast mating pheromone alpha-factor in *Saccharomyces cerevisiae*, facilitates secretion of heterologous proteins in yeast. A full length form of the full alpha-factor protein with *kex* and *ste13* protease cleavage sites. (www.pnas.org/content/81/15/4642.short)
Zeocin-r	Resistance to zeocin is conferred by the product of the *Sh ble* gene. The *Sh ble* gene product binds the antibiotic so it can no longer cause cleavage of DNA. Zeocin is blue in color due to the presence of copper ion Cu^{2+}. The action of zeocin is effective on most aerobic cells. Typically 10-30 µg/ml is used in mammalian and yeast cells, and 25 µg/ml in bacteria. (www.en.wikipedia.org/wiki/Zeocin)

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