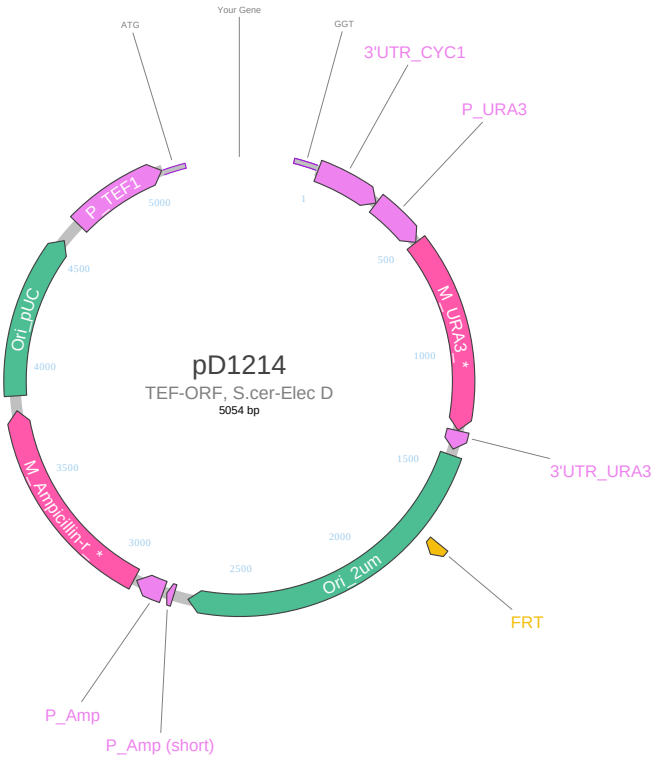
Yeast Expression Vectors

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

Saccharomyces Expression Vectors

These vectors are available with or without secretions signals. They have either an inducible (Gal), strong constitutive (GPD, TEF) or weaker constitutive (ADH) promoter. The vectors carry LEU2, HIS3, URA3 or TRP1 markers or geneticin resistance. They are maintained on 2µm ori plasmids.

Plasmid Map



Name	Qty	Storage
pD1214	10Rx	−20℃

Expression Protocol

Transform plasmid into appropriate auxotrophic strain, plate onto minimal media lacking the appropriate nutrient and incubate at 30℃ until heterotrophic colonies arise in 2-3 days. Pick colonies into minimal media or YPD and grow for 24-90 hours at 28–30℃. Check supernatant for secreted protein expression or cell pellets for cytoplasmic protein expression.

Saccharomyces Vector Controls

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

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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)

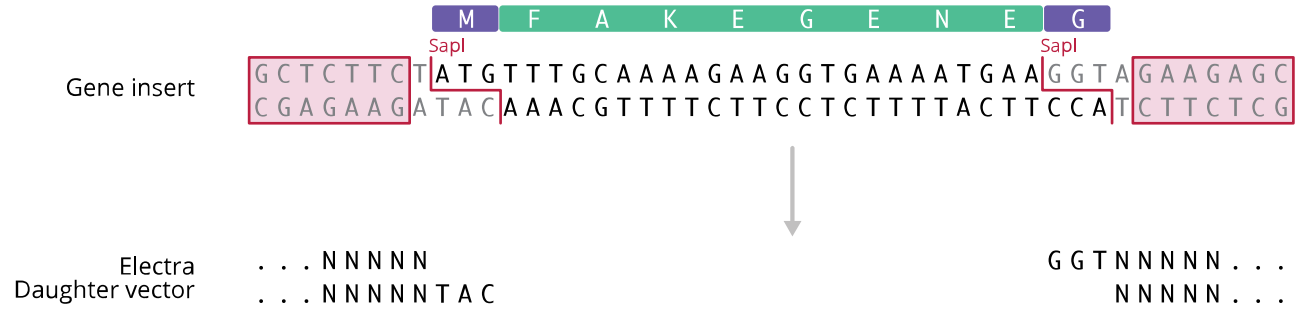
3. Recover cells for 45 minutes and then plate on appropriate antibiotic for the DAUGHTER.

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.

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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

3'UTR_CYC1	The 3'UTR controls post transcriptional regulation. CYC1 is the heme-containing component of the cytochrome b-c1 complex, which accepts electrons from Rieske protein and transfers electrons to cytochrome c in the mitochondrial respiratory chain. (www.genecards.org/cgi-bin/carddisp.pl?gene=CYC1 www.genesdev.cshlp.org/content/9/23/2997.full.pdf+html)
3'UTR_URA3	The 3'UTR of the URA3 gene controls post transcriptional regulation. The URA3 gene encodes orotidine-5' phosphate decarboxylase, an enzyme that is required for the biosynthesis of uracil. (www.genesdev.cshlp.org/content/9/23/2997.full.pdf+html www.protocols.mmml.nl/protocols/yeast/yeast_selectable_markers.php)
Ampicillin-r	A semi-synthetic penicillin derived from 6-amino-penicillanic acid causes cell death by inhibiting cell wall biosynthesis. The gene coding for ampicillin resistance (*bla*) is a beta lactamase which is secreted into the periplasmic space where it catalyzes hydrolysis of the beta-lactam ring of ampicillin. *E.coli* transformed with plasmid containing the ampicillin resistance gene can grow on media containing 50-100 µg/ml ampicillin. (www.jac.oxfordjournals.org/content/43/5/699.full)
Ori_2um	The 2µ plasmid encodes proteins that allow cells to maintain 20-50 copies of any plasmid carrying the 2µ origin of replication. Because 2µ plasmids are maintained at such high copy numbers, they provide a convenient way to monitor the effects of overproduction of a particular gene product. (www.ncbi.nlm.nih.gov/pubmed/2691836)
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_TEF1	A pyrimidine rich promoter from the TEF1 gene encoding translation-elongation factor 1 alpha demonstrates strong promoter activity. (www.ncbi.nlm.nih.gov/pubmed/9720204)
P_URA3	A promoter from the yeast pyrimidine biosynthetic gene, URA3. (www.ncbi.nlm.nih.gov/pubmed/2204810)
URA3	The URA3 gene encodes orotidine-5' phosphate decarboxylase, an enzyme that is required for the biosynthesis of uracil. (www.genesdev.cshlp.org/content/9/23/2997.full.pdf+html)

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