Yeast Directional Clones

Technical Information

Introduction

The information provided below describes the features of the Yeast Directional Clones inserted into the vector pYES2/GS©. General information about propagating the plasmid and the yeast open reading frame (yORF) cloned into this vector is also provided. Specific information about the particular yORF cloned into this vector is provided on the Certificate of Analysis (e. g. size of the DNA fragment) and from the Saccharomyces Genome Database (SGD):

http://genome-www.stanford.edu/Saccharomyces

Use the yORF locus from SGD to obtain the proposed DNA sequence and other information about your particular yORF of interest.

NOTE

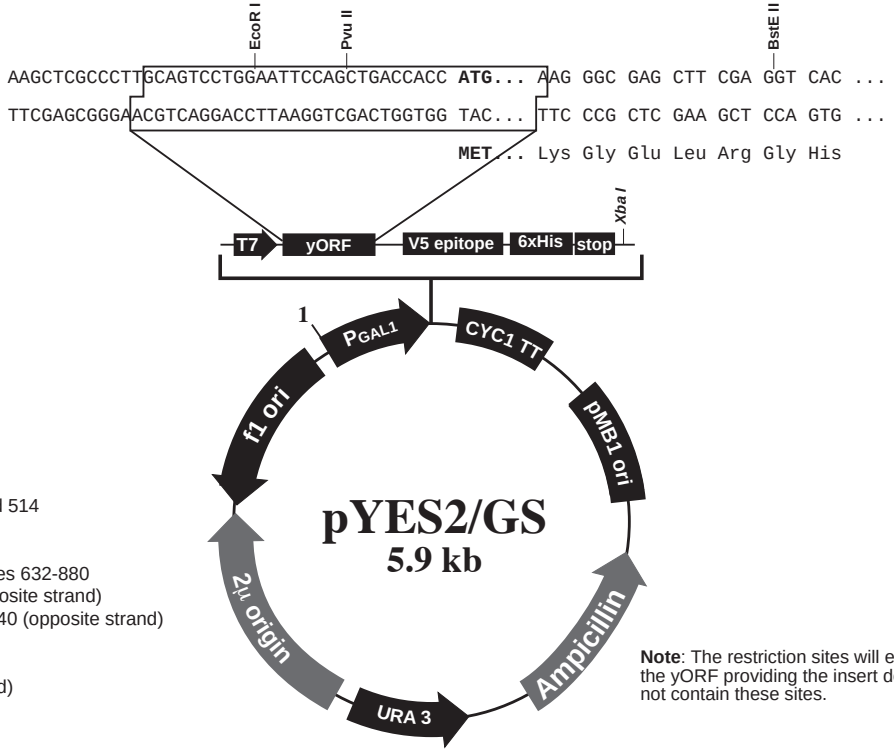
pYES2/GS Yeast Directional Clones have not been demonstrated to express via western blot, but are cloned in the sense orientation. The Yeast Directional Clones have not been sequenced.

Shipping/Storage

50 ng of lyophilized vector containing the yORF of interest is shipped at room temperature. Upon receipt, store at room temperature.

Map of pYES2/GS

The map below shows the elements of pYES2/GS and sequence surrounding the insert (boxed sequence). **Note**: The sequence upstream of the yORF ATG is constant for all inserts. The full sequence of this vector (excluding insert) may be downloaded from our Web site (www.invitrogen.com) or requested from Technical Service (see next page).



Comments for pYES2/GS (no insert)
5880 nucleotides

GAL1 promoter: bases 1-452
T7 promoter/priming site: bases 476-495
yORF cloning site: between bases 513 and 514
V5 epitope: bases 544-585
Polyhistidine region: bases 595-612
CYC1 transcription termination signal: bases 632-880
pMB1 (pUC) origin: bases 1062-1735 (opposite strand)
Ampicillin resistance gene: bases 1880-2740 (opposite strand)
URA3 gene: 2758-3865 (opposite strand)
2 micron origin: 3869-5340
f1 origin: bases 5408-5863 (opposite strand)

Note: The restriction sites will excise the yORF providing the insert does not contain these sites.

continued on next page

Technical Information, continued

Bacterial Transformation

DNA is supplied lyophilized from a sucrose solution (60% sucrose, 0.04% cresol red dye) to allow detection of the nucleic acid pellet after lyophilization. To transform into *E. coli*:

- Resuspend the nucleic acid pellet in 100 µl of sterile water
- Transform 1 to 10 µl (0.5 to 5 ng) into competent *E. coli*

  We recommend TOP10 cells (TOP10 One Shot™ Competent Cells, Catalog no. C4040-03).

  Genotype: F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *deo*R *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G
- Select on LB plates containing 50 µg/ml ampicillin and analyze transformants

Applications

After bacterial transformation and isolation of plasmid DNA, you can--

- Express *in vitro* or *in vivo* transcripts from the T7 promoter
- Use the GeneStorm™ Primer Sets to amplify the yORF for further subcloning

Technical Service

For questions about this product, please call, fax, or E-mail:

U.S. Headquarters:
Toll Free Tel: (800) 955-6288
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The Fine Print