Plasmid Name: pTF162-13

Location: 884

Concentration of DNA: ~60 ng/μl

Plasmid Size: 6490 bp

Source: Tim Formosa

Stock Prepared by: Tim Formosa

Vector: pTF131/YEplac181

Selectable Markers, Yeast: LEU2

Replication Origin (Yeast): 2 micron (YEpl)

Selectable Markers, Bacteria: Amp

Date: 6/18/2003

Plasmid type: YEpl

Construction Notes:

pTF131 was digested with BamHI and XbaI, then ligated with the annealed product of oligos TF03-07 and TF03-08. This makes a vector with the GAL1 promoter, a 12X histidine tag followed by the TEV protease recognition site, followed by a polylinker. The optimal site for insertion is an NdeI site adjacent to the TEV site, but the linker provides several unique sites for other strategies (linker is NdeI KpnI BamHI SacI XhoI XbaI SalI PstI SphI HindIII).

Most minipreps had the correct restriction pattern, but we had trouble retaining the plasmid during growth of cultures for maxipreps. Worked fine upon restreaking the cells under selection and starting cultures directly from colonies on Amp plates (2/26/2003). Susan maxipreps two (one originally called pTF162-1) and Alison sequences it with M13R. The sequence is correct in the engineered region representing TF03-07, -08 and the junctions flanking this region so this one was saved and used for subcloning.

5/14/2003 We later found that pTF162-1 is correct in the engineered region, but does not transform yeast to Leu+. Restriction digestions with several enzymes give the correct pattern so the error is subtle. Susan's other maxiprep (labeled pTF162-col; her notes say it is from miniprep 6?, an independent clone) was found and tested: it gives the expected number of Leu+ colonies in yeast so pTF162-1 was just an unfortunate mishap not a systematic error. However, pTF162-col has a 9 bp deletion including the NdeI site.

6/18/03: Repeat pTF162 construction from scratch with a fresh batch of annealed oligo, but get a guy with a frameshift. Try subcloning RI-H3 from pTF162-1 into YEplac181 instead. This works and gives the 3 saved isolates with the correct sequence that transform yeast. Save them as pTF162-13, -14, -15 (interchangeable)

For sequencing, TF98-19 and M13 Reverse anneal at 586+ and 833-, respectively, with the NdeI and BamHI sites at 747 and 759.

4/12/2004; prep ran low, store original in archive and retransform/reprep. Gave 1 ml; OD says about 300 ng/μl but gel shows genomic DNA and 2 mystery bands in RI+KpnI digest (same/similar to other preps done same time) and concentration of correct band is lower. Estimate ~60 ng/μl.