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Gap1 integrative vector

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Method Article

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Abstract

Introduction

Genes can be integrated into the genome of yeast. This protocol describes how to generate a vector that contains sequences that allow integratioon of any gene into the GAP1 locus of yeast.

Procedure

1) Amplify a pUC fragment containing the bacterial origin of replication and the kanamycin resistance marker by PCR from PCR-BluntII-TOPO \(Invitrogen, Carlsbad, CA) and ligate with a PvuII fragment of pDRf1 containing the f1 origin of replication, the PMA1 promoter and ADH3 terminator producing pDL001. 2) Subclone a synthetic oligonucleotide containing 55 bp upstream of the start \(-55 to 0) and 55 bp downstream of the stop \(+1810 to 1865) of Gap1 ORF with HpaI and AscI restriction sites into pGEM-T-easy. 3) Amplify the hphMX3 cassette \(for hygromycin B selection in yeast) by PCR from pAG34[1] and clone into the SpeI site located in the 5'-part of the Gap1 cassette. 4) Amplify this cassette by PCR and clone into a blunted BglII of pDL001. 5) Subclone AtAMT1;1, AtAMT1;1-T460A and AtAMT1;1-eGFP from pDR vectors into the KpnI site of the Gap1 integrative plasmid. 6) Use the yeast strain 31019b \(Δ::LEU2 ::KanMX2 ura3; see reference 2) to generate DL1 \(Δ Δ) mutant strain and the versions in which AtAMT1;1 or its mutants are integrated. 7) Transform yeast strain 31019b using a LiAc protocol\(3) with an AscI linearized Gap1 integrative vector containing either AtAMT1;1, AtAMT1;1-T460A, AtAMT1;1-eGFP or the empty vector, to generate gap1::WT, gap1::T460A, gap1::AMT1;1-eGFP or Δgap1 strains respectively. 8) Select transformants on solid YPD medium supplemented with 300 µg/mL hygromycin. 9) Amplify colonies in liquid YPD and reselect on solid YPD supplemented with 300 µg/mL hygromycin. 10) Confirm the insertion at the Gap1 locus by PCR and by complementation of functional proteins.

Timing

References

\(1) Goldstein, A. L. & McCusker, J. M. Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. _Yeast_ **15**, 1541-1553 \(1999). \(2) Marini, A. M., Soussi-Boudekou, S., Vissers, S. & André, B. A family of ammonium transporters in Saccharomyces cerevisiae. _Mol. Cell. Biol._ **17**, 4282-4293 \(1997). \(3) Gietz, R. D. & Woods, R. A. Tranformation of yeast by the LiAc/SS carrier DNA/PEG method. _Methods Enzymol._ **350**, 87-96 \(2002).