Gap1 integrative vector

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Dominique Loqué
Carnegie Institution

Wolf Frommer
Carnegie Institution

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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.

References

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A cytosolic trans-activation domain essential for ammonium uptake

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