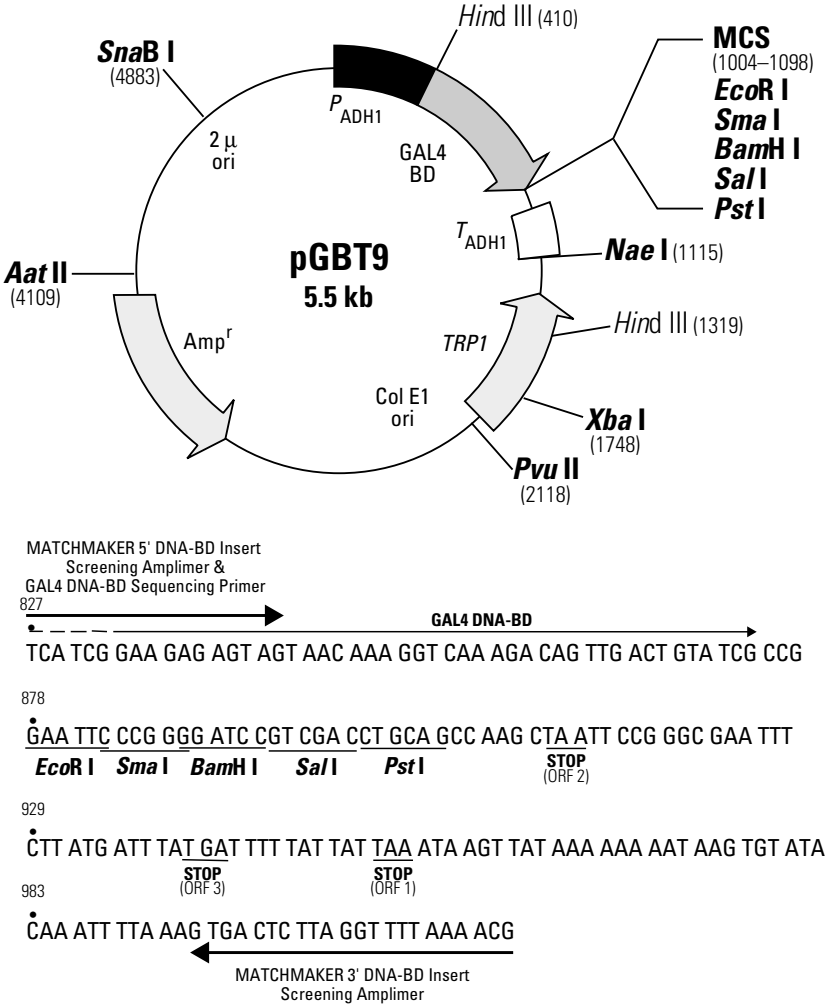
Vector Information

pGBT9 DNA-BD Vector Information PT1021-5

GenBank Accession #: U07646 Catalog #K1605-A



Restriction map and multiple cloning site (MCS) of ppGBT9 DNA-BD Vector. Unique restriction sites are in bold.

Description:

pGBT9 generates a hybrid protein that contains the sequences for the GAL4 DNA-binding domain (DNA-BD; a.a. 1–147). For the construction of a hybrid protein, the gene encoding the protein of interest is ligated into the MCS in the correct orientation and in the correct reading frame such that a fusion protein is generated. The fusion protein is expressed in yeast host cells from the constitutive *ADH1* promoter; transcription is terminated at the *ADH1* transcription termination signal. The hybrid protein is targeted to the yeast nucleus by nuclear localization sequences that are an intrinsic part of the GAL4 DNA-BD (2). pGBT9 is a shuttle vector that replicates autonomously in both *E. coli* and *S. cerevisiae*. It carries the *bla* gene (for ampicillin resistance in *E. coli*) and the *TRP1* nutritional marker that allow yeast auxotrophs carrying pGBT9 to grow on limiting synthetic medium lacking Trp.

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(PR81583)

Location of features

- Promoter
  Fragment containing the *S. cerevisiae ADH1* promoter: 10–406
- GAL4 DNA-binding domain polypeptide
  Start codon: 434–436; stop codon: 953–955
  GAL4 codons 1–147: 434–874
- Multiple cloning site: 878–905
- Transcription termination signal
  Fragment carrying the *S. cerevisiae ADH1* terminator: 921–1112
- *TRP1* coding sequence
  Start codon: 1835–1833; stop codon: 1163–1161
- Col E1 origin of plasmid replication: 2322–2965
- Ampicillin resistance gene
  Promoter: –35 region: 4043–4038; –10 region: 4020–4015
  Transcription start point: 4008
  Ribosome binding site: 3985–3981
  β-lactamase coding sequences
  Start codon: 3973–3971; stop codon: 3115–3113
  Signal peptide: 3973–3905
  Mature protein: 3904–3116
- Fragment containing the 2 μ origin of replication: 1–1348

Primer locations:

- MATCHMAKER DNA-BD 5' Insert Screening Amplimer (#5417-1) or GAL4 BD Sequencing Primer (#6474-1): 827—843
- MATCHMAKER DNA-BD 3' Insert Screening Amplimer (#5417-1): 1015–994

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: 15–20

Propagation in *S. cerevisiae*

- Suitable host strains: Y187(α), Y190(a), HF7c(a), CG1945(a), PJ69-2A, PJ69-4A, and SFY526(a)
- Selectable marker: *TRP1*
- *S. cerevisiae* replication origin: 2 μ
- Copy number: multiple copy

References

1. Bartel, P. L., *et al.* (1993) In *Cellular Interactions in Development: A Practical Approach* (Oxford University Press, Oxford) pp. 135–179.
2. Silver, P. A., *et al.* (1984) *Proc. Natl. Acad. Sci. USA* **91**:5951–5955.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by CLONTECH. This vector has not been completely sequenced.