pGBT9

Catalog No.

Not sold separately. Sold as part of 630439.

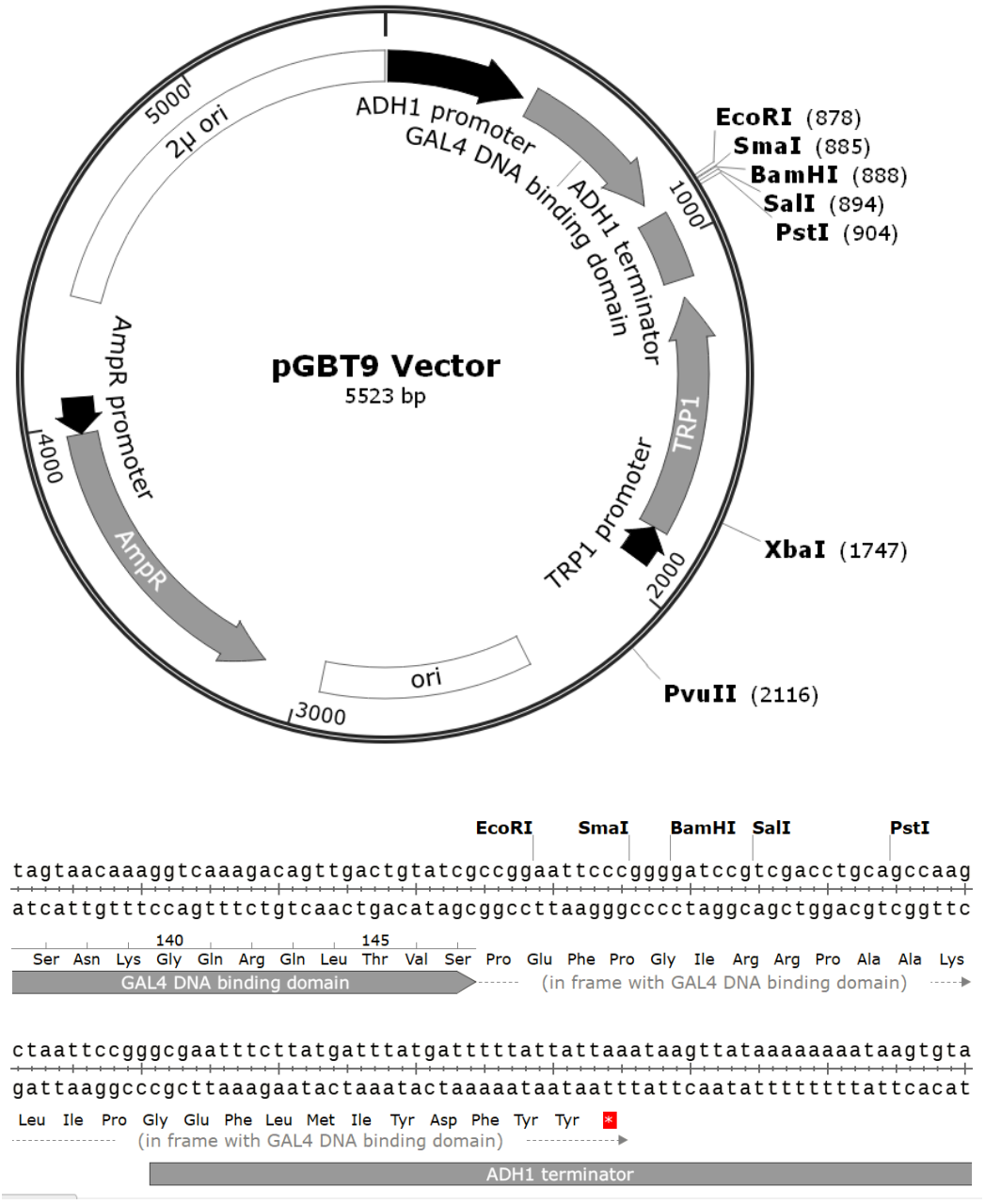

Figure 1. pGBT9 vector map and multiple cloning site.

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Vector Map

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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 (Bartel et al. 1993, Silver et al. 1984). pGBT9 is a shuttle vector that replicates autonomously in both *E. coli* and *S. cerevisiae*. It carries the AMP^r 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.

Location of Features

- *ADH1* promoter: 10–406
- GAL4 DNA binding domain: 434–874
- Multiple cloning site: 878–905
- *ADH1* terminator: 921–1108
- *TRP1*: 1161–1835
- P_{TRP1} (TRP1 promoter): 1836–1937
- Col E1 origin of replication: 2353–2941
- Amp^r (ampicillin resistance gene): 3112–3972 (complementary)
- P_{Amp} (Amp promoter): 3973–4077
- Yeast 2 µ origin of replication: 4359–5523

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

Bartel, P. L., *et al*. (1993) In Cellular Interactions in Development: A Practical Approach (Oxford University Press, Oxford) pp. 135–179.

Silver, P. A., *et al*. *PNAS* **91,** 5951–5955 (1984).

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