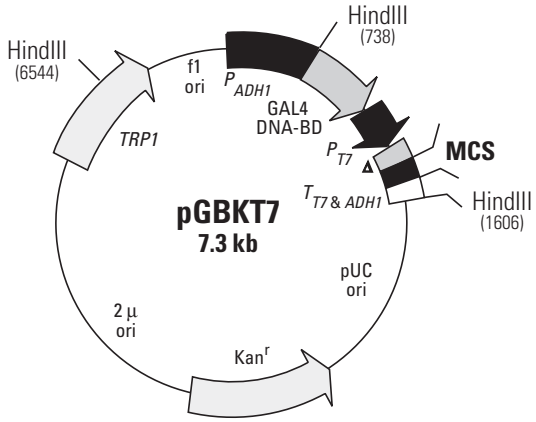
Vector Information

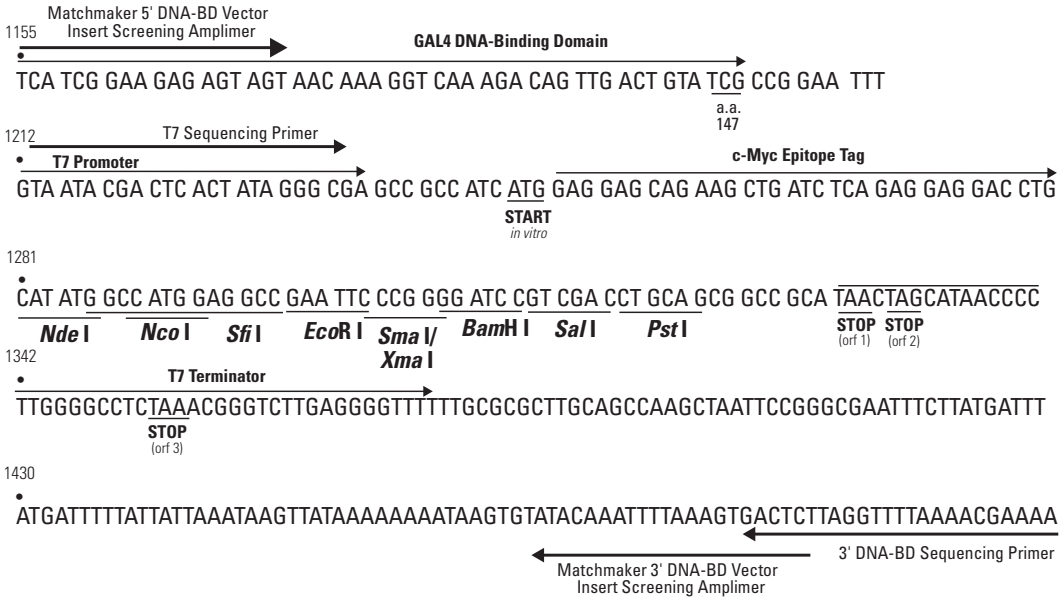
pGBKT7 Vector Information

PT3248-5

Cat. No. 630489
630443



▲ c-Myc epitope tag



Restriction Map and Multiple Cloning Site (MCS) of pGBKT7. Unique restriction sites are in bold.

Description:

The pGBKT7 vector expresses proteins fused to amino acids 1–147 of the GAL4 DNA binding domain (DNA-BD). In yeast, fusion proteins are expressed at high levels from the constitutive *ADH1* promoter (P_{ADH1}); transcription is terminated by the T7 and *ADH1* transcription termination signals ($T_{T7\ \&\ ADH1}$). pGBKT7 also contains the T7 promoter, a c-Myc epitope tag, and a MCS. pGBKT7 replicates autonomously in both *E. coli* and *S. cerevisiae* from the pUC and 2 µ ori, respectively. The vector carries the Kan^r for selection in *E. coli* and the *TRP1* nutritional marker for selection in yeast. Yeast strains containing pGBKT7 exhibit a higher transformation efficiency than strains carrying other DNA-BD domain vectors (1).

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Use:
pGBKT7 is the DNA-BD Vector included with Clontech's Matchmaker™ Systems. The MCS of pGBKT7 contains unique restriction sites in frame with the 3' end of the GAL4 DNA-BD for constructing fusion proteins with a bait protein. The bait protein is also expressed as a fusion to a c-Myc epitope tag. c-Myc tagged proteins can be identified with antibodies raised to this common epitope, eliminating the need to generate specific antibodies to new proteins. The T7 promoter is used for *in vitro* transcription and translation of the epitope tagged fusion protein. Note that the DNA-BD is not expressed during the *in vitro* transcription and translation reactions.

The MCS in pGBKT7 is compatible with those in pMyc-CMV and pHA-CMV, Clontech's epitope tagged mammalian expression vector set (Cat. No. 631604). As a result, the target gene can be shuttled into these vectors in order to confirm protein interactions *in vivo*.

Location of features:

- Truncated *S. cerevisiae ADH1* promoter (P_{ADH1}): 30–736
- GAL4 DNA binding domain (DNA-BD) polypeptide amino acids 1–147: 762–1202
- T7 RNA polymerase promoter: 1212–1235
- c-Myc epitope tag: 1248–1280
- Multiple Cloning Site: 1281–1334
- Transcription termination signals
  T7 terminator: 1335–1381
  ADH1 terminator: 1414–1610
- pUC plasmid replication origin: 1838–2636
- Kanamycin resistance gene: 4144–3222
- Yeast 2 μ replication origin: 4148–5493
- *TRP1* coding sequences
  promoter: 5559–6755
  gene: 6031–6705
- f1 bacteriophage origin of replication: 6756–29

Location of primers:

- T7 Sequencing Primer: 1213–1233
- 3' DNA-BD Sequencing Primer: 1510–1487

Propagation in *E. coli*:

- Suitable host strains: DH5α, DH10 & other general purpose strains
- Selectable marker: plasmid confers resistance to kanamycin (50 μg/ml) in *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

Propagation in *S. cerevisiae*:

- Suitable host strains: AH109(*MAT***a**), Y187(*MAT*α), Y190(*MAT***a**), SFY526(*MAT***a**), CG1945(*MAT***a**), HF7c(*MAT***a**)
- Selectable marker: *TRP1*
- *S. cerevisiae* origin: 2 μ

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.

Reference:

1. Louret, O. F., *et al.* (1997) *BioTechniques* **23**: 816–819.