pGEX 2T

Location: Expresión 11
Resistance: Ampicillin 100 µg/mL

Description pGEX vectors :
GST-tagged proteins are constructed by inserting a gene or gene fragment into the MCS of one of the 13 pGEX vectors. Expression is under the control of the tac promoter, which is induced by the lactose analog isopropyl ơ-D-thiogalactoside (IPTG). All pGEX vectors are also engineered with an internal lacIq gene. The lacIq gene product is a repressor protein that binds to the operator region of the tac promoter, preventing expression until induction by IPTG, thus maintaining tight control over expression of the insert.

Complete sequence:
https://www.lablife.org/g?a=seqa&id=vdb_g2.fpzDkSJdEuqykKDjECRqRQzAQdI-_sequence_f938b0691965416defa7a9dd73fd7e8979f1bfb9_10

Genotype of *E coli* strain BL21(DE3) pLysS : F– *ompT hsdS*(rB– mB–) *gal dcm* λ(DE3) pLysS (Camr)(λ(DE3): *lac*I, *lac*UV5-T7 gene 1, *ind*1, *sam*7, *nin*5)

Protocols

Prepare fusion construct by inserting gene of interest into the multiple cloning site of pGEX 2T using any one, or combination of unique restriction sites and transform into a host of choice such as *E. coli* BL21 (GE Healthcare 27-1542-01) or JM105 (GE Healthcare 27-1550-01).

Growth and Induction:
1. Dilute an overnight culture transformed with pGEX fusion construct, 1:10 in fresh complex medium containing 100 µg/ml ampicillin. Grow the cells at 37°C to mid-log phase (A600 = 0.6–1.0).
2. Induce expression of fusion proteins by adding isopropyl-β D-thiogalactoside (IPTG) to 0.1 mM final concentration and allow the cells to grow for an additional 3–5 hours at 37°C.
3. Expression of GST fusion proteins can be monitored using the Anti-GST Antibody (27-4577-01), GST Detection Modules (GE Healthcare 27-4590-01, 27-4592-01) or ECL GST Western Blotting Detection Kit (GE Healthcare RPN1237).

Preparation of cell extracts:
1. Sediment the cells by centrifugation and resuspend in 1/20 volume of PBS (PBS: 140 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.3).
2. Lyse the cells by mild sonication or chemical lysis.
3. Add Triton X-100 to a final concentration of 1% and mix gently at room temperature (25°C) for 30 minutes to solubilize proteins.
4. Centrifuge the crude extract at 10 000 × g for 5 minutes at 4°C.

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Purification

There are a range of Gluthatione Sepharose™ prepacked column and bulk media products available to purify GST Fusion proteins.

For manual purification of sample volumes up to 600 µl use GST SpinTrap™ microspin columns or GST MultiTrap™ 4B 96-well plates.

For sample volumes between 600 µl and 10 ml use GST GraviTrap™ gravity flow column. Where sample volumes are above 10 ml, use LabMate™ reservoir together with GST GraviTrap. All formats described can be used for preparation of samples in parallel. In addition GST HiTrap™ 1 and 5 ml columns and GST HiPrep™ 16/10 column are available for purification in a chromatography system such as the ÄKTA™ design system. Alternatively, Gluthatione Sepharose bulk media are available from 10 ml up to 500 ml. A GST

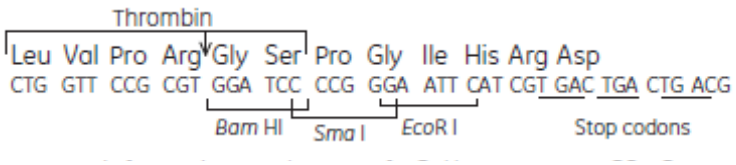
Bulk Kit is also available combining 10 ml Gluthatione Sepharose 4B bulk medium with required buffers. For simplified buffer preparation use the GST Buffer Kit. Ordering information for all associated products is listed below.

Site-specific proteolysis of fusion proteins:

Separation of the recombinant protein from the glutathione S-transferase moiety may be accomplished by site specific proteolysis using bovine thrombin (27-0846-01). Exact reaction conditions will vary with the nature of the fusion protein. The following conditions may be used as a guideline and should be optimized for each fusion protein: thrombin concentration, 0.2% (w/w) of fusion protein; reaction buffer, PBS; incubation temperature, 25°C; incubation time, 2–16 hours

Multiple Cloning region and protease cleavage site

For more information on the use of pGEX vectors, see GST Gene Fusion System Handbook.



Intracellular expression of some eukaryotic proteins in *Escherichia coli* can lead to the formation of inclusion bodies (2). Increased solubilities can be obtained by lowering the growth temperature from 37°C to 28–30°C (3). Shortening the induction period may also improve results. Exact conditions must be established for each protein.

The following primers for double-stranded sequencing of pGEX vectors are avaliable : 5´pGEX sequencing primer (bases 869-891) and 3´sequencing primer (bases 1020-998)

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Further information relating to DNA sequence, restriction maps and control regions can be found at www.gelifescience.com

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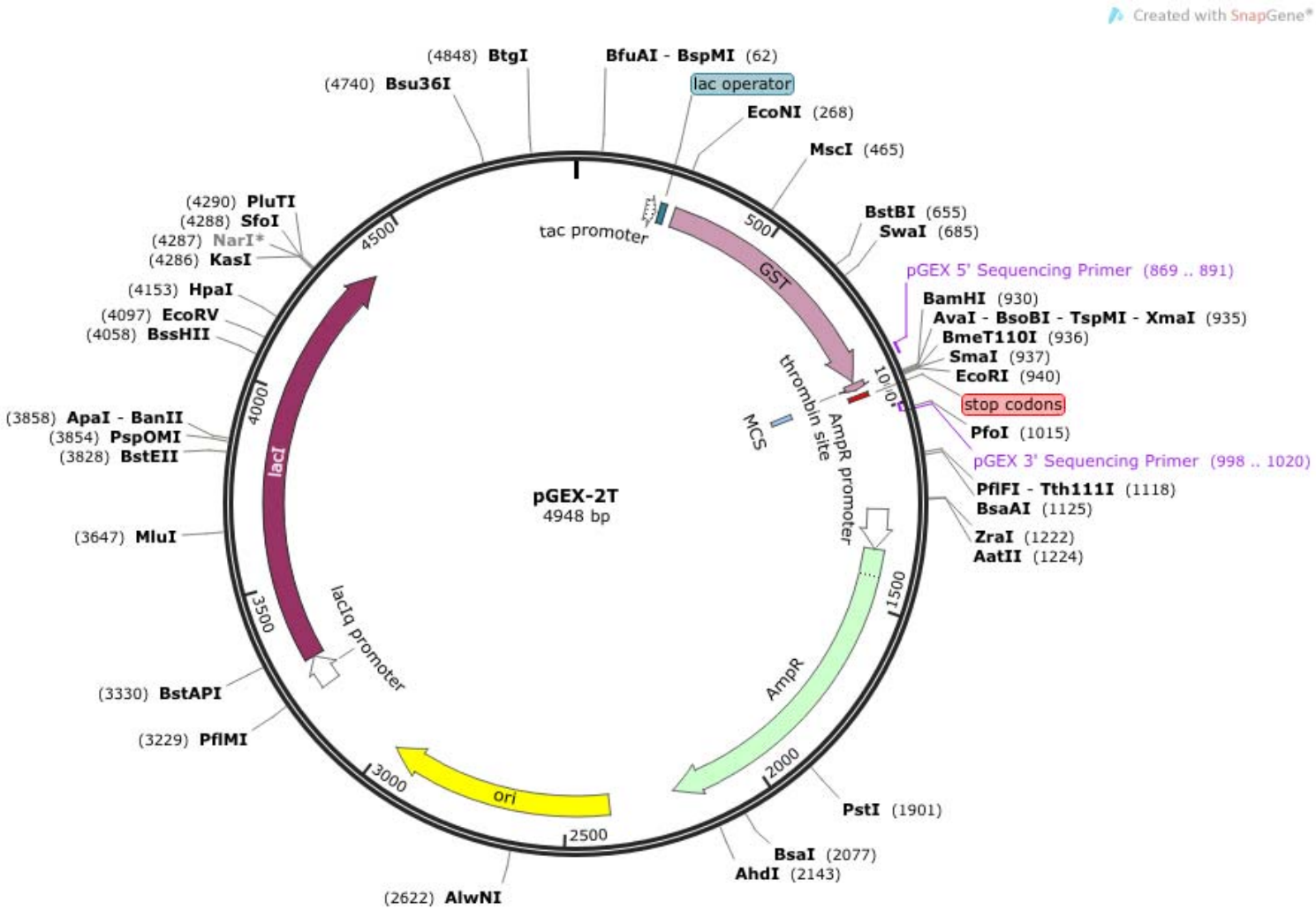

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Feature Name	Start	End
tac_promoter	184	212
M13_pUC_rev_primer	224	246
GST (variant)	258	956
pGEX_3_primer	1020	998
AmpR_promoter	1286	1314
Ampicillin	1356	2216
pBR322_origin	2371	2990
lacI	3288	4379
lac_promoter	4428	4457
M13_pUC_rev_primer	4471	4493
M13_reverse_primer	4492	4510
lacZ_a	4519	4674
M13_forward20_primer	4538	4522
M13_pUC_fwd_primer	4553	4531

Enzyme Name	Cut
MscI	465
BstBI	655
BamHI	930
SmaI	937
XmaI	935
EcoRI	940
AatII	1224
PstI	1901
ApaI	3858
EcoRV	4097
HpaI	4153
NarI	4287

ORF	Start	End
GST frame 3	258	956
AmpR frame 3	1356	2216
lacIq frame 3	3420	4379
LacZ frame 2	4502	81