pGEX 5X1

Location: Expresión 12
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 σ-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.
pGEX-5X-1 is derivative of pGEX-3X and possess a Factor Xa recognition site.

Complete sequence:
https://www.lablife.org/g?a=seqa&id=vdb_g2.7NGU.gWSAkuLKkgVTR5YEZSM7Dc-_sequence_a93adeb4dcba562f76e5c59d5a2aa2f0255632f1_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)

General Protocols

Prepare fusion construct by inserting gene of interest into the multiple cloning site of pGEX-5X-1 using any one, or combination of unique restriction sites and transform into a host of choice such as *E. coli* BL21.

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.

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4. Centrifuge the crude extract at 10 000 × g for 5 minutes at 4°C.

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.

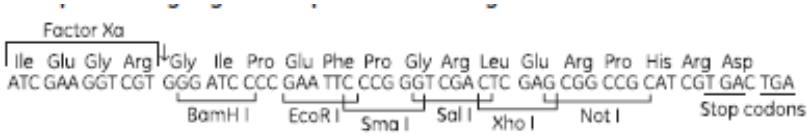
Site-specific proteolysis of fusion proteins:

Separation of the recombinant protein from the glutathione S-transferase moiety may be accomplished by site specific proteolysis using highly-pure factor Xa. 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: factor Xa concentration, 1% (w/w) of fusion protein; reaction buffer, 50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM CaCl2; incubation temperature, 25°C; incubation time, 2–16 hours.

The molecular weight of factor Xa (bovine) is approximately 48 kDa and the protease is available as a stand alone product

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.

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

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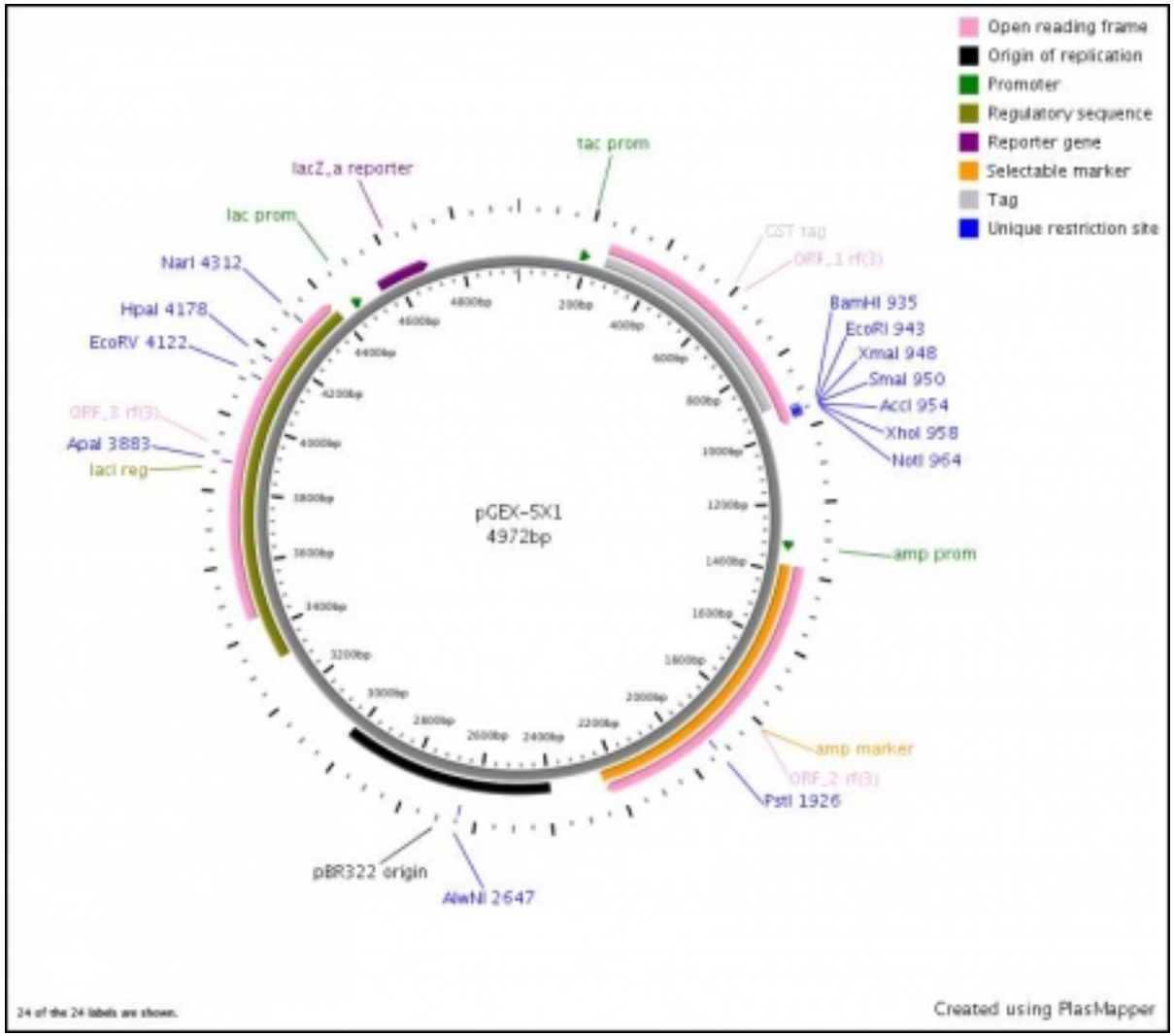

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Feature Name	Start	End
tac_promoter	184	212
M13_pUC_rev_primer	224	246
GST (variant)	258	980
pGEX_3_primer	1044	1022
AmpR_promoter	1310	1338
Ampicillin	1380	2240
pBR322_origin	2395	3014
lacI	3312	4403
lac_promoter	4452	4481
M13_pUC_rev_primer	4495	4517
M13_reverse_primer	4516	4534
lacZ_a	4543	4698
M13_forward20_primer	4562	4546
M13_pUC_fwd_primer	4577	4555

Enzyme Name	Cut
MscI	465
BstBI	655
BamHI	934
EcoRI	942
XmaI	947
SmaI	949
SalI	952
XhoI	957
NotI	963
EagI	963
AatII	1248
PstI	1925
ApaI	3882
EcoRV	4121
HpaI	4177
NarI	4311

ORF	Start	End
ORF frame 3	258	980
ORF frame 3	1380	2240
ORF frame 3	3444	4403
ORF frame 2	4526	81