pGEX 3X

Location: Expresión 10
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.
Nine of the vectors have an expanded MCS that contains six restriction sites. The expanded MCS facilitates the unidirectional cloning of cDNA inserts obtained from libraries constructed using many available lambda vectors. 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._EncMvrUxvqHcls1EOvlQYOYCnw-_sequence_dcec8d52ca89001b260abb4b0a9a9ea20775601c_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-3X 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.

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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.

Purification

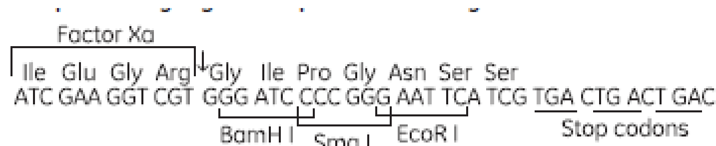
There are a range of Gluthatione Sepharose™ prepacked column and bulk media products available to purify GST Fusion proteins.
For manual purification of sample volumes up to 600 µl use GST SpinTrap™ microspin columns or GST MultiTrap™ 4B 96-well plates.
For sample volumes between 600 µl and 10 ml use 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 (GE Healthcare).

Site-specific proteolysis of fusion proteins:

Site-specific proteolysis of fusion proteins expressed from pGEX-3X may be acomplished using factor Xa via recognition sequence adjacent to the multiple cloning region. Exact reaction conditions for factor Xa cleavage 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, 1 hour.
The molecular weight of factor Xa (bovine) is approximately 48 kDa.

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. 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)

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

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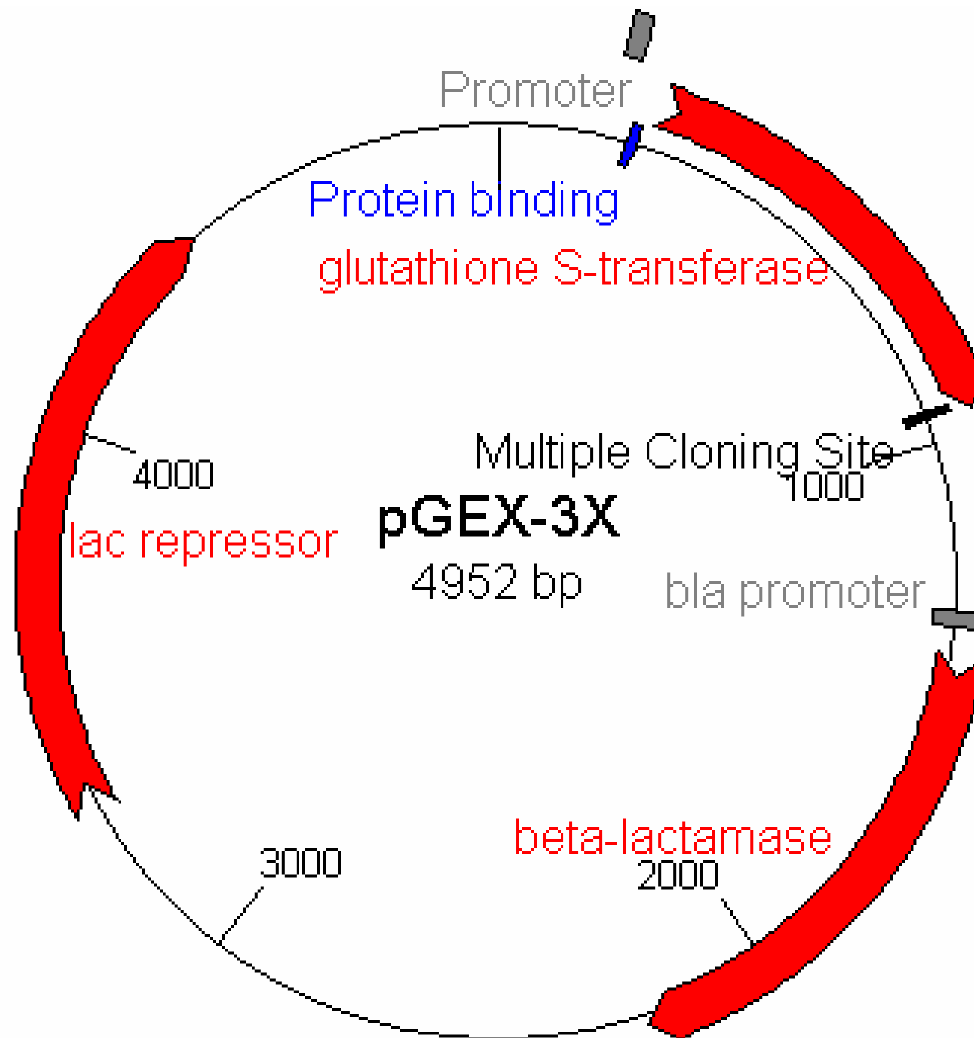

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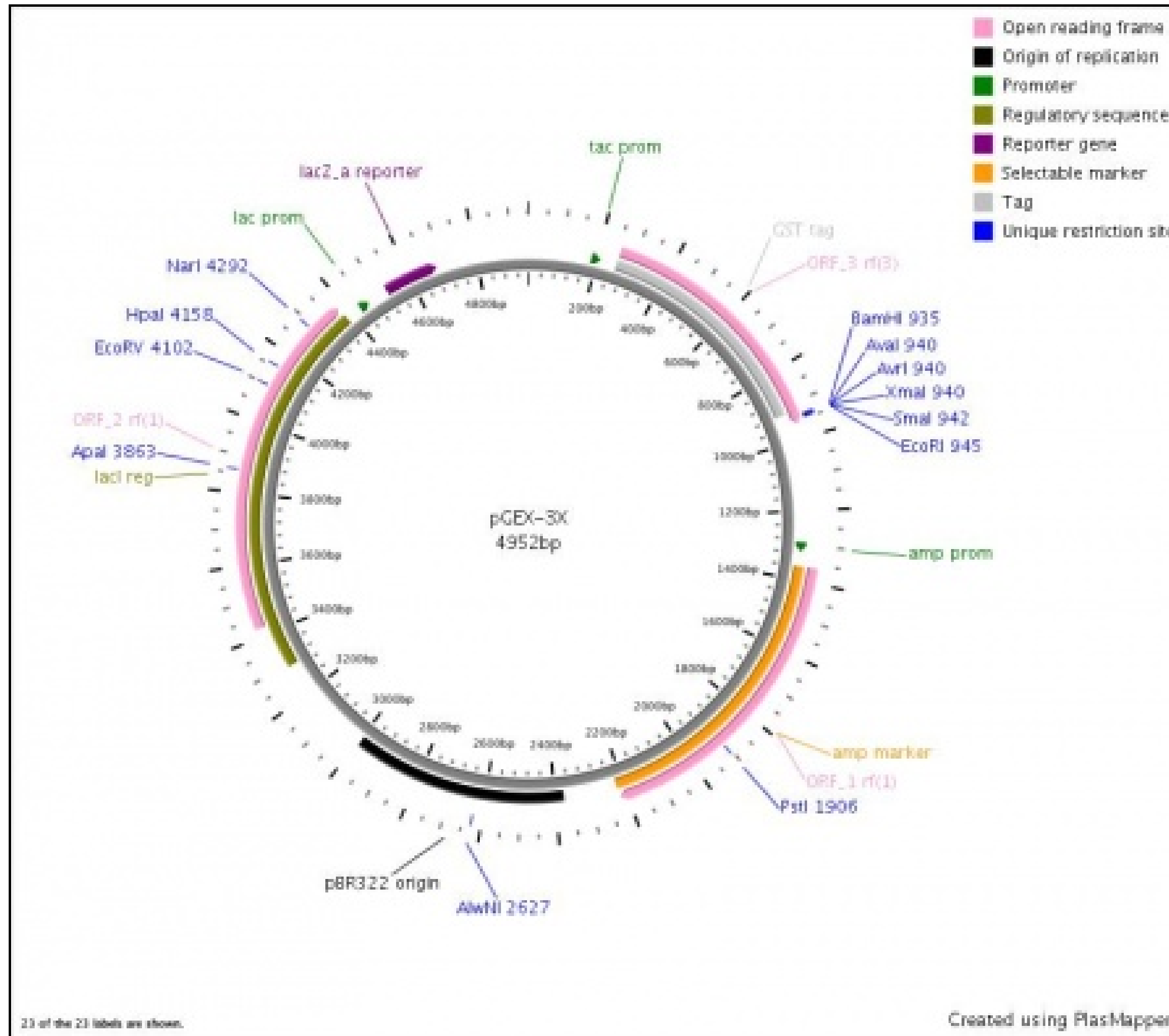

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Feature Name	Start	End
tac_promoter	184	212
M13_pUC_rev_primer	224	246
GST (variant)	258	960
pGEX_3_primer	1024	1002
AmpR_promoter	1290	1318
Ampicillin	1360	2220
pBR322_origin	2375	2994
lacI	3292	4383
lac_promoter	4432	4461
M13_pUC_rev_primer	4475	4497
M13_reverse_primer	4496	4514
lacZ_a	4523	4678
M13_forward20_primer	4542	4526
M13_pUC_fwd_primer	4557	4535

Enzyme Name	Cut
MscI	465
BstBI	655
BamHI	934
SmaI	941
XmaI	939
EcoRI	944
AatII	1228
PstI	1905
ApaI	3862
EcoRV	4101
HpaI	4157
NarI	4291

ORF	Start	End
ORF frame 3	258	956
ORF frame 1	1360	2220
ORF frame 1	3424	4383
ORF frame 3	4506	81