Antibody Design LABORATORIES

TGEX-SCblue Mammalian Expression Vector

INSTRUCTION MANUAL

TGEX-SCblue Mammalian Expression Vector

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Description

Introduction

The **TGEX™** vector series is designed for the rapid expression of antibody molecules by transient gene expression in mammalian cell suspension culture. The vector series features a CMV promoter, the adenovirus tripartite leader sequence (TPL) (Logan 1984, Mariati 2010), a variable antibody domain leader sequence with its intron and convenient cloning sites to insert antibody variable regions. The constant regions are derived from the human IgG1 and kappa sequences. There is no selection marker and the overall small size of the vectors is optimized for transient transfection; expensive antibiotics to prepare large quantities of plasmid for transient transfections are not required. Using widely available cell lines and large scale transfection technologies (see experimental procedure) yields of antibody between 10 mg/L and 100 mg/L in serum-free conditions are routinely achieved in the laboratory in just a few days.

The **TGEX™-SCblue** vector is designed for the transient mammalian expression of scFv-Fc fusions after transfer of scFv fragments from the **PADL™** phagemid vector series. **TGEX™-SCblue** vector enables rapid and convenient expression of scFv fragment isolated by phage display as dimeric scFv-Fc fusion with all the advantages conferred by the Fc fragment for detection using commercially available secondary antibodies. The pelBK signal peptide responsible for the secretion of the Fc fusion in the culture supernatant is a composite between a mammalian kappa leader sequence and the bacterial pelB leader sequence with a compatible SfiI restriction site for the transfer (Valadon 2006). Proper recombinant clones can be isolated using a blue/white colony screening.

Content, Shipping & Storage

Content

VECTOR	COMPOSITION	AMOUNT
TGEX-SCblue	20 µl at 0.5 µg/µl of DNA vector in DNA Conservation Buffer (Tris-HCL 5 mM, EDTA 0.1 mM, pH 8.5)	10 µg

Shipping & Storage

TGEX-SCblue vector is shipped on wet ice. Upon receipt, store the vector at -20ºC.

Limited Product Warranty

This warranty limits our liability to the replacement of this product. No other warranties of any kind express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Antibody Design Labs. Antibody Design Labs shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

For research use only; not intended for any animal or human therapeutic or diagnostic use.

TGEX Vector Series

Characteristics of the TGEX vector series

VECTOR	CATALOG	USE	DESCRIPTION
TGEX™-HC	MX001	Cloning of VH domain	For the expression of human IgG1 heavy chain
TGEX™-LC	MX002	Cloning of VL domain	For the expression of human Kappa light chain
TGEX™-FC	MX003	Cloning of VH domain	For the expression of human IgG1 Fc fusion.
TGEX™-FH	MX004	Cloning of VH domain	For the expression of human IgG1 Fab fragment
TGEX™-HChis	MX005	Cloning of VH domain	For the expression of human IgG1 heavy chain with a HIS tag
TGEX™-SCblue	MX006	scFv cloning vector	For the transfer of scFv from any PADL phagemid vector and expression as an scFv-Fc fusion

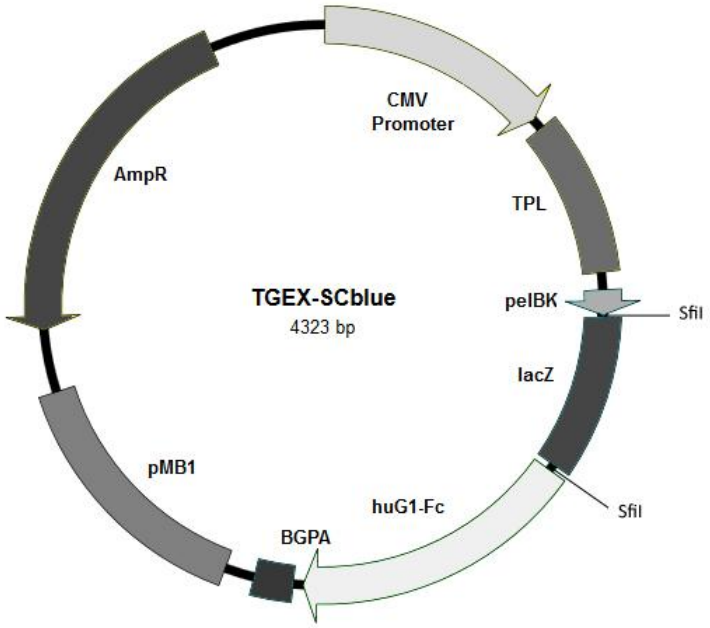
Combination of vectors to desired antibody format

COMBINATION	FORMAT	PURIFICATION[1]
TGEX™-HC + TGEX™-LC	Full length human or chimeric human IgG1/K	Protein A or G
TGEX™-HChis + TGEX™-LC	Full length human or chimeric human IgG1/K	Protein A , G or IMAC
TGEX™-FH + TGEX™-LC	Human or chimeric IgG1/K Fab fragment	Protein L, G or IMAC
TGEX™-FC	Fc fusion	Protein A or G
TGEX™-SCblue	scFv-Fc fusion	Protein A or G

1. Purification by protein A, G and L may require testing.

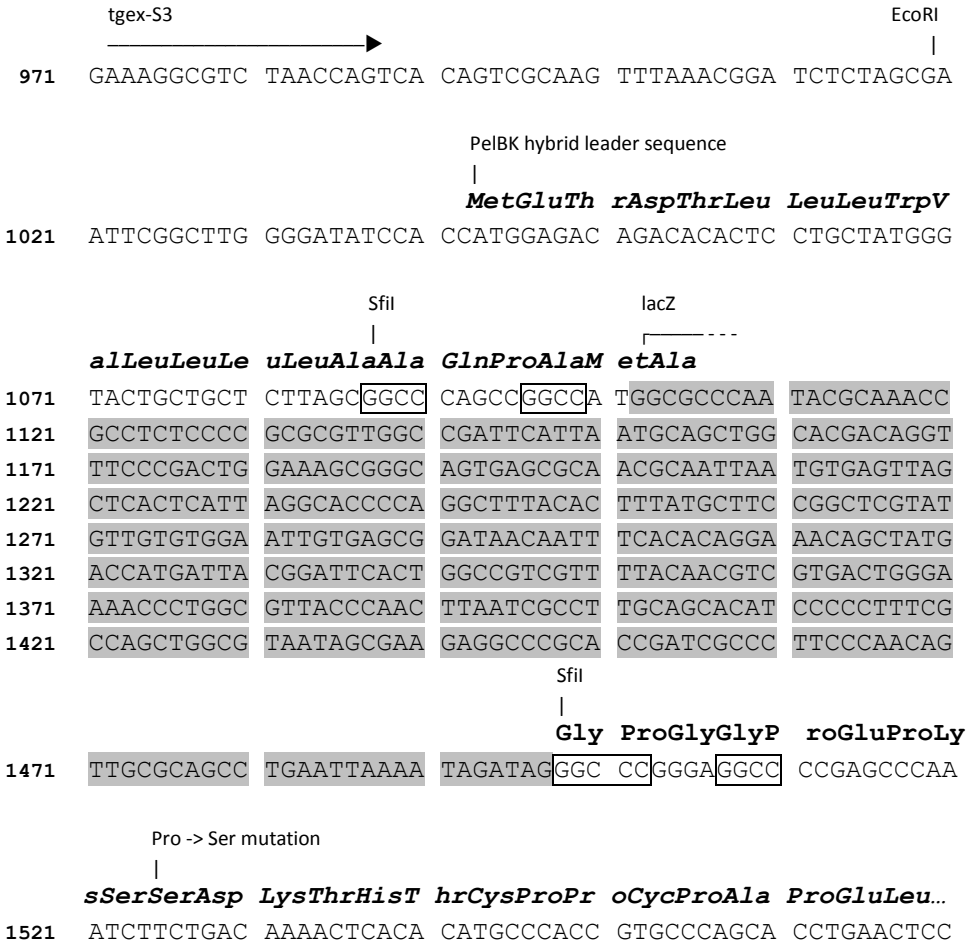
Vector Map

The figure below illustrates the main features of **TGEX-SCblue** expression vector. The full vector sequence is available online for download in varied formats on the product web page; the total length of the vector is 4323 bp.



Cloning Site

Following is an illustration of **TGEX-SCblue** cloning site from the EcoRI site and onward. The scFv fragment is inserted between the two SfiI sites; the lacZ fragment with the lac promoter has been grayed.



Feature Table

The features of **TGEX-HC** transient expression vector are highlighted in the following table.

FEATURE	LOCATION	DESCRIPTION
Promoter	5-585	CMV promoter.
TPL	612-1000	Adenovirus tripartite leader sequence (Logan 1984, Mariati 2010)
pelBK leader	1019-1024	Composite leader sequence between mammalian kappa leader and bacterial pelB (Valadon 2006).
lacZ	1203-1497	LacZ fragment with lac promoter.
BGpA	2235 -2333	Rabbit beta-globin polyadenylation signal sequence.
pMB1 origin	2407 - 3026	pBR322 origin for replication in *E. coli* with a temperature-sensitive high copy-number phenotype (Lin-Chao 1992).
TEM1 beta-lactamase	3181 - 4041	Ampicillin resistance for selection in *E. coli*.

Restriction Site Summary

Enzyme	Site	Nb	Position	Strand	Isoschizomers
AarI	CACCTGC(4/8)	1	2111	-	
ApaI	GGGCC^C	1	1497		Bsp120I PspOMI
ArsI	(8/13)GACNNNNNNTTYG(11/6)	1	851		
BalI	TGG^CCA	1	4251		MlsI MluNI MscI Msp20I
BcgI	(10/12)CGANNNNNNTGC(12/10)	1	3770		
Bpu10I	CCTNAGC(-5/-2)	1	930		
BtrI	CACGTC(-3/-3)	1	1660	-	AjiI BmgBI
CspCI	(11/13)CAANNNNNGTGG(12/10)	1	407		
EagI	C^GGCCG	1	2213		BseX3I BstZI EclXI Eco52I
EcoNI	CCTNN^NNNAGG	1	1791		BstENI XagI
EcoRI	G^AATTC	1	1019		
EcoRV	GAT^ATC	1	1033		Eco32I
FalI	(8/13)AAGNNNNNCTT(13/8)	1	784		
GsuI	CTGGAG(16/14)	1	3339	-	BpmI
HindIII	A^AGCTT	1	2356		
NaeI	GCC^GGC	1	1093		PdiI NgoMIV MroNI
NarI	GG^CGCC	1	1102		DinI EgeI EheI KasI Mly113I SfoI SspDI
NmeAIII	GCCGAG(21/19)	1	3377		
NotI	GC^GGCCGC	1	2212		CciNI
NsiI	ATGCA^T	1	2149		EcoT22I Mph1103I Zsp2I
OliI	CACNN^NNGTG	1	1644		AleI
PflMI	CCANNNN^NTGG	1	641		AccB7I BasI Van91I
PfoI	T^CCNGGA	1	1627		
PmeI	GTTT^AAAC	1	1000		MssI
SacI	GAGCT^C	1	583		Ecl136II EcoICRI Eco53kI Psp124BI SstI
SapI	GCTCTTC(1/4)	1	2181	-	BspQI LguI PciSI
SexAI	A^CCWGGT	1	1949		CsiI MabI
SnaBI	TAC^GTA	1	357		BstSNI Eco105I
SpeI	A^CTAGT	1	18		AhlI BcuI
XhoI	C^TCGAG	1	966		StrI TliI Sfr274I PaeR7I SlaI
AclI	AA^CGTT	2	3479		Psp1406I
		2	3852		
AlwNI	CAGNNN^CTG	2	1556		CaiI PstNI
		2	2772		
ApaLI	G^TGCAC	2	2675		Alw44I VneI
		2	3921		
BciVI	GTATCC(6/5)	2	2570	-	BfuI BsuI
		2	4097		
BglII	A^GATCT	2	2350		
		2	4174		
BseRI	GAGGAG(10/8)	2	715		
		2	1744		
BsgI	GTGCAG(16/14)	2	1792	-	
		2	2161		
Bsp1407I	T^GTACA	2	1910		BsrGI BstAUI
		2	4285		
BspMI	ACCTGC(4/8)	2	1963		Acc36I BfuAI BveI
		2	2111		
BssSI	CACGAG(-5/-1)	2	2534	-	BauI Bst2BI
		2	3918		
Bsu36I	CC^TNAGG	2	1636		Eco81I AxyI Bse21I
		2	1678		

DrdI	GACNNNN^NNGTC	2	1675	AasI DseDI
		2	2463	
Eam1105I	GACNNN^NNGTC	2	1577	AhdI AspEI BmeRI DriI
		2	3249	
FspI	TGC^GCA	2	1472	Acc16I AviII NsbI
		2	3474	
NdeI	CA^TATG	2	252	FauNDI
		2	2312	
PvuI	CGAT^CG	2	1452	Ple19I BpvUI MvrI
		2	3621	
SacII	CCGC^GG	2	740	Sfr303I KspI SgrBI Cfr42I SstII
		2	1738	
SfiI	GGCCNNNN^NGGCC	2	1087	
		2	1498	
SmaI	CCC^GGG	2	1500	Cfr9I TspMI XmaI
		2	1928	
TstI	(8/13)CACNNNNNNTCC(12/7)	2	1559	
		2	1818	
XmnI	GAANN^NNTTC	2	2127	Asp700I MroXI PdmI
		2	3849	

Absent Sites:

AbsI, AflII, AgeI, AjuI, AlfI, AloI, AscI, AsuII, AvrII, BaeI, BamHI, BarI, BbvCI, BclI, BlpI, BplI, BsaBI, BsePI, BsiWI, BsmI, BspEI, BstAPI, BstEII, BstXI, BstZ17I, ClaI, DraIII, Eco31I, Eco47III, Esp3I, FseI, FspAI, HpaI, KflI, KpnI, MauBI, MfeI, MluI, MreI, NheI, NruI, PacI, PasI, PciI, PmaCI, PshAI, PsiI, PspXI, PsrI, PstI, RsrII, SalI, SgfI, SgrAI, SgrDI, SphI, SrfI, Sse8387I, StuI, SwaI, Tth111I, XbaI, XcmI.

Experimental Procedures

General Molecular Biology Techniques

Molecular biology should be conducted under the supervision of a qualified instructor trained to standard safety practice in a molecular biology laboratory environment. Standard molecular biology procedures can be found in a general molecular biology handbook such as Sambrook (1989).

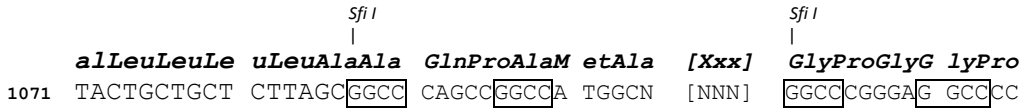
Plasmid Maintenance

Propagation and maintenance of **TGEX-SCblue** is obtained on any *recA1, endA1 E. coli* strain using LB or 2xYT medium supplemented with ampicillin (100 µg/ml) as a selection marker and incubated at 37ºC with agitation. **TGEX- SCblue** is a derivative of pBR322 with a high copy number origin of replication and usually gives high yields of plasmid DNA with most standard laboratory strains such as XL1-blue or DH5α. The high copy number phenotype is temperature sensitive and requires incubation at 37ºC (Lin-Chao 1992). Some DNA stabilizing strains are known to produce smaller amounts of plasmid DNA. In case of issues, we recommend using XL10-Gold® from Agilent Technologies, Inc., on which **TGEX- SCblue** plasmid DNA can be isolated in large quantities.

Cloning into TGEX-SCblue

Primer Design and pelBK Leader Sequence

A complete hybrid leader sequence is necessary for secretion and proper removal of the leader peptide by host proteases. In the following schema, where [NNN] represents the insert sequence and [Xxx] the translated amino acid sequence, the short hexanucleotide ATGGCN must be appended immediately to the first *SfiI* site to obtain a complete hybrid leader encoding sequence.



Transfer from PADL Phagemid Vectors

scFv fragments and VHH domains can be excised from PADL™ phagemids by SfiI or alternatively by BglI and cloned directly into TGEX-SCblue opened by SfiI. Classical blue/white screen can be applied to detect insert-containing clones; IPTG is dispensable thanks to the very high copy number of TGEX-SCblue.

Sequencing of Inserts

The following primers give a strong PCR amplification of the TGEX vector series inserts between the EcoRI site and the NotI site. The primer tgex-S3 can be used to sequence the scFv fragment.

tgex-S3 5'- AGGCGTCTAACCAGTCACAGTC

tgex-R2 5'- CAAAAAATTCCAACACACTATTGC

Antibody Expression

Cell lines

Cell lines adapted for culture in suspension and serum-free conditions are recommended. HEK293 and CHO cells are often used for antibody expression by transient transfection; you can either adapt your own cell line or get it from a supplier (e.g. Life Technology). HEK293 cells are particularly well suited for expression using **TGEX™** vector series.

Transient Transfection

Many transfection reagents especially designed for transient transfection are commercially available from multiple providers (e.g. Life Technologies, Mirus Bio LLC). We recommend testing the transfection conditions with a reporter plasmid first to determine the percentage of cells effectively transfected and optimal transfection conditions; fluorescent reporters are often used with that purpose. Similarly, any condition known to boost expression should be carefully tested in your system before being scaled up. We did observe a strong increase in expression in 293 cells upon exposure to sodium valproate (Backliwal 2008).

Appendix

MSDS Information

MSDSs (Material Safety Data Sheets) are available on **Antibody Design Labs** website at the corresponding product page.

Quality Control

Specifications and quality control are detailed on the online product page. **Antibody Design Labs** certifies that the product will perform according to these specifications.

Technical Support

Visit **Antibody Design Labs** website at **www.abdesignlabs.com** for technical resources, including manuals, vector maps and sequences, application notes, FAQs, etc.

For more information or technical assistance, call, write, fax, or email us at:

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Phone: 1-877-223-3104 (Toll Free)
Fax: 1-858-272-6007 (24 hour)
(Monday – Friday 9:00 AM – 5:00 PM PST)

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