Antibody Design LABORATORIES

TGEX-LC Expression Vector

INSTRUCTION MANUAL

TGEX-LC Transient Mammalian Expression Vector

Catalog #: MX002

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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™-LC** vector is designed for the expression of a light chain variable region with the constant region of the human kappa light chain. Expression of full length antibody molecules is achieved by co-transfection with a heavy chain variable region cloned into the vector **TGEX™-HC**.

Content, Shipping & Storage

Content

VECTOR	COMPOSITION	AMOUNT
TGEX-LC	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-LC vector is shipped on wet ice. Upon receipt, store the vector at -20ºC.

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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

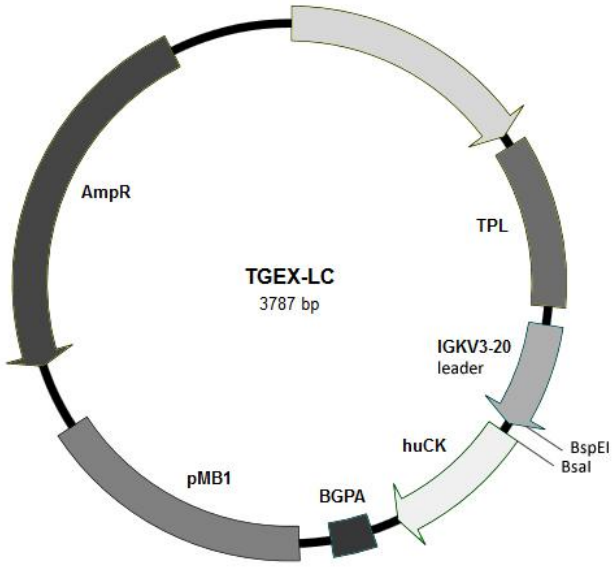
Combination of vectors to desired antibody format

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.

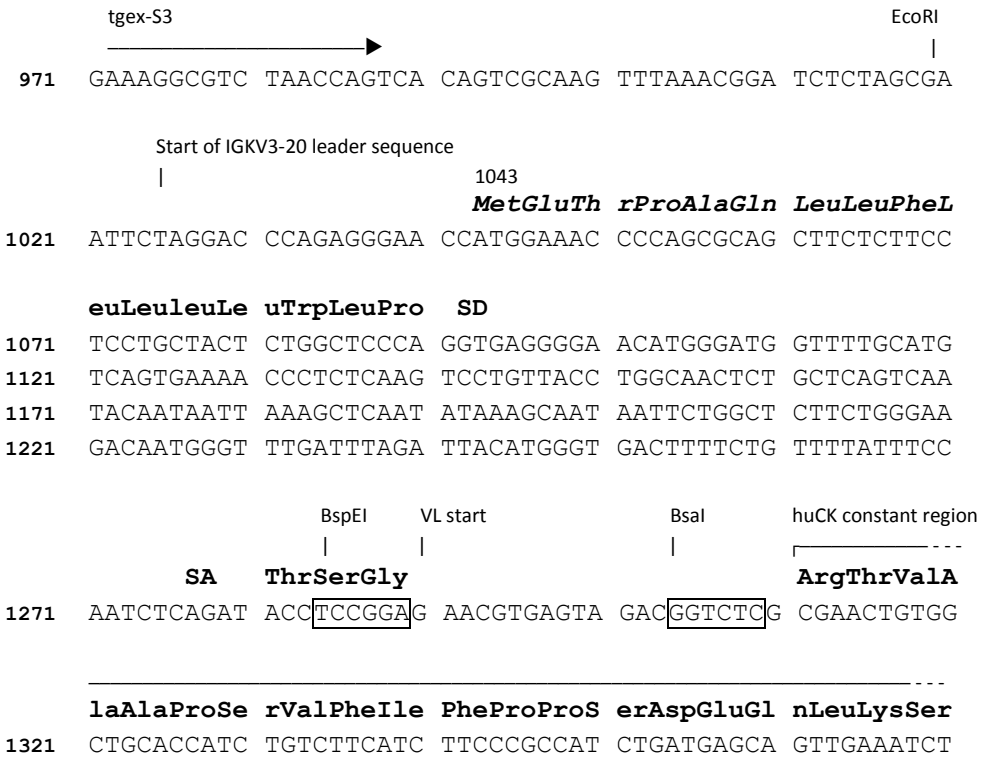
Vector Map

The figure below illustrates the main features of **TGEX-LC** 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 3787 bp.



Cloning Site

Following is an illustration of **TGEX-LC** cloning site from the EcoRI site and onward. The VL domain is inserted between the BspEI site and the BsaI site; after excision of the intron sequence, the IGKV3-20 sequence encodes the leader peptide METPAQLLFLLLLWLPDTSG. SA/SD donor and acceptor sites.



Feature Table

The features of **TGEX-LC** 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)
IGVK3-20 leader	1025-1289	Human IGVK3-20 leader sequence with intron. The mature RNA encodes the 20 a.a.–long signal sequence METPAQLLFLLLLWLPDTSG; cleavage occurs on the C-terminal side of the terminal glycine.
Human CK CDS	1311-1634	Sequence encoding the human kappa light chain constant region sequence.
BGpA	1699 - 1797	Rabbit beta-globin polyadenylation signal sequence.
pMB1 origin	1871 - 2490	pBR322 origin for replication in *E. coli* with a temperature-sensitive high copy-number phenotype (Lin-Chao 1992).
TEM1 beta-lactamase	2645 - 3505	Ampicillin resistance for selection in *E. coli*.

Restriction Site Summary

Enzyme	Site	Nb	Position	Strand	Isoschizomers
BalI	TGG^CCA	1	3715		MlsI MluNI Mox20I MscI Msp20I
BbvCI	CCTCAGC(-5/-2)	1	1511		
BcgI	(10/12)CGANNNNNNTGC(12/10)	1	3234		
BlpI	GC^TNAGC	1	1529		Bpu1102I Bsp1720I
BsaXI	(9/12)ACNNNNNCTCC(10/7)	1	1078		
BseRI	GAGGAG(10/8)	1	715		
BsgI	GTGCAG(16/14)	1	1321	-	
Bsp1407I	T^GTACA	1	3749		BsrGI BstAUI
BspEI	T^CCGGA	1	1284		Aor13HI Bsp13I Kpn2I BseAI MroI AccIII
BsrBI	CCGCTC(-3/-3)	1	3557	-	AccBSI MbiI
CspCI	(11/13)CAANNNNNGTGG(12/10)	1	407		
DrdI	GACNNNN^NNGTC	1	1927		AasI DseDI
EagI	C^GGCCG	1	1677		BseX3I BstZI EclXI Eco52I
Eam1105I	GACNNN^NNGTC	1	2713		AhdI BmeRI DriI
Eco31I	GGTCTC(1/5)	1	1304		Bso31I BsaI BspTNI
EcoRI	G^AATTC	1	1019		
FalI	(8/13)AAGNNNNNCTT(13/8)	1	784		
FspI	TGC^GCA	1	2938		Acc16I NsbI
GsuI	CTGGAG(16/14)	1	2803	-	BpmI
HindIII	A^AGCTT	1	1820		
NmeAIII	GCCGAG(21/19)	1	2841		
NotI	GC^GGCCGC	1	1676		CciNI
NruI	TCG^CGA	1	1308		Bsp68I BtuMI RruI
PflMI	CCANNNN^NTGG	1	641		AccB7I Van91I
PmeI	GTTT^AAAC	1	1000		MssI
PstI	CTGCA^G	1	1642		BspMAI
PvuI	CGAT^CG	1	3085		Ple19I
PvuII	CAG^CTG	1	642		
SacII	CCGC^GG	1	740		Sfr303I KspI SgrBI Cfr42I
SapI	GCTCTTC(1/4)	1	1208		BspQI LguI PciSI
SnaBI	TAC^GTA	1	357		BstSNI Eco105I
SpeI	A^CTAGT	1	18		AhlI BcuI
Sse8387I	CCTGCA^GG	1	1641		SbfI SdaI
XhoI	C^TCGAG	1	966		Sfr274I PaeR7I SlaI
AclI	AA^CGTT	2	2943		Psp1406I
		2	3316		
AlwNI	CAGNNN^CTG	2	1584		CaiI PstNI
		2	2236		
ApaLI	G^TGCAC	2	2139		Alw44I VneI
		2	3385		
ArsI	(8/13)GACNNNNNNTTYG(11/6)	2	851		
		2	1221		
BbsI	GAAGAC(2/6)	2	1218		BpiI BstV2I
		2	1332		
BciVI	GTATCC(6/5)	2	2034	-	BfuI BsuI
		2	3561		
BglII	A^GATCT	2	1814		
		2	3638		
BmrI	ACTGGG(5/4)	2	313	-	BmuI
		2	2763		
BseYI	CCCAGC(-5/-1)	2	1051		GsaI PspFI
		2	2129		
BspHI	T^CATGA	2	2545		CciI PagI
		2	3553		
BssSI	CACGAG(-5/-1)	2	1998	-	BauI Bst2BI
		2	3382		

BtgZI	GCGATG(10/14)	2	368	-	
		2	623		
BtsI	GCAGTG(2/0)	2	3111		
		2	3139		
NcoI	C^CATGG	2	379		Bsp19I
		2	1041		
NdeI	CA^TATG	2	252		FauNDI
		2	1776		
SacI	GAGCT^C	2	583		Ecl136II EcoICRI Eco53kI Psp124BI SstI
		2	1592		
TaqII	GACCGA(11/9)	2	3076	-	
		2	3235		
VspI	AT^TAAT	2	25		AseI PshBI
		2	2889		
XmnI	GAANN^NNTTC	2	1397		Asp700I MroXI PdmI
		2	3313		

Absent Sites:

AanI, AarI, AbsI, Acc36I, Acc65I, AcvI, AdeI, AfeI, AflII, AgeI, AjiI, AjuI, AleI, AlfI, AloI, Aor51HI, ApaI, AscI, AsiGI, AsiSI, Asp718I, AspA2I, AsuII, AsuNHI, AvrII, AxyI, BaeI, BamHI, BarI, BbrPI, BclI, BfrI, BfuAI, BlnI, BmgBI, BmtI, BoxI, BplI, Bpu14I, Bsa29I, BsaBI, Bse21I, Bse8I, BseCI, BseJI, BsePI, BshTI, BshVI, BsiWI, BsmBI, BsmI, Bsp119I, Bsp120I, BspDI, BspMI, BspOI, BspT104I, BspTI, BssHII, BssNAI, Bst1107I, BstAFI, BstAPI, BstBI, BstEII, BstENI, BstPAI, BstPI, BstXI, BstZ17I, Bsu15I, Bsu36I, BsuTUI, BtrI, BveI, Cfr9I, ClaI, CpoI, CsiI, CspAI, CspI, DinI, DraIII, Eco147I, Eco32I, Eco47III, Eco72I, Eco81I, Eco91I, EcoNI, EcoO65I, EcoRV, EcoT22I, EgeI, EheI, Esp3I, FbaI, FseI, FspAI, HpaI, I-CeuI, I-PpoI, I-SceI, KasI, KflI, KpnI, KroI, Ksp22I, KspAI, MabI, MauBI, MfeI, MluI, Mly113I, Mph1103I, MreI, MroNI, MspCI, MunI, Mva1269I, NaeI, NarI, NgoMIV, NheI, NsiI, NspV, OliI, PI-PspI, PI-SceI, PacI, PaeI, PalAI, PasI, PauI, PceI, PciI, PctI, PdiI, Pfl23II, PflFI, PfoI, PinAI, PluTI, PmaCI, PmlI, PscI, PshAI, PsiI, PspCI, PspEI, PspLI, PspOMI, PspXI, PsrI, PsyI, PteI, RgaI, RigI, Rsr2I, RsrII, SalI, SexAI, SfaAI, SfiI, SfoI, SfuI, SgfI, SgrAI, SgrDI, SgsI, SmaI, SmiI, SphI, SrfI, SseBI, SspDI, StuI, SwaI, TspMI, Tth111I, Vha464I, XagI, XbaI, XcmI, XmaI, XmaJI, Zsp2I.

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).

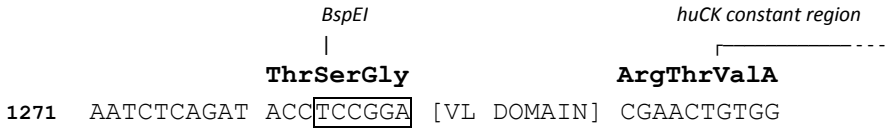
Plasmid Maintenance

Propagation and maintenance of **TGEX-LC** 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-LC** 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-LC** plasmid DNA can be isolated in large quantities.

Cloning into TGEX-LC

In Silico Clone Design

A complete IGKV3-20 leader sequence METPAQLLFLLLLWLPDTSG is necessary for secretion of the antibody in the culture supernatant and proper removal of the leader peptide by host proteases. In the following schema, after cutting by BspEI and BsaI, the end of the BspEI site 5'-CCGGA will be removed. The BspEI site must be restored in the final clone for proper protein maturation and secretion. The BsaI site will be eliminated during the cloning. The VL domain will be included in-frame between the last A of the leader peptide and the first C of the human CK constant region.



Vector Digestion

BspEI and BsaI restriction enzymes are fully active at 37ºC, respectively. Please, consult the documentation of your restriction enzyme provider for buffer and optimal double digestion conditions.

Primer Design for Restriction Cloning with the BspEI Site

Oligo1 is an example of primer designed to amplify a VL domain sequence and clone it into the BspEI site. Six extra-nucleotides were added before the BspEI site to ensure proper digestion close to the end; these 6 N are followed by the BspEI site TCCGGA; the resulting NNNNNNTCCGGA extension is added 5' to the VL domain primer.

Oligo1 5'-NNNNNNTCCGGA-VL-start

Primer Design for Restriction Cloning with the BsaI Site

Oligo2 is an example of primer designed to amplify the VL domain from the end of the J region and includes a BsaI site compatible with **TGEX-LC** cloning site. BsaI is a type IIS restriction enzyme that cuts outside of its recognition site. BsaI will cut immediately before the start of the human kappa constant region sequence, exactly 1 base after the end of the site and 5 bases further on the opposite strand, thus freeing a 5' 4-bases overhang CGAA on the sense strand. In Oligo2, a BsaI site situated symmetrically to the vector will generate a complementary overhang. After digestion and ligation, the two BsaI sites, the one in the vector and the one in Oligo2, will be removed. We added two nucleotides on the 5' end of the primer although a minimum of one nucleotide is recommended to cut BsaI site close to the end (source New Englands BioLabs).

Oligo2 5'-NNggtctcNTTCG-JH-end

Alternative to BsaI Sites

All restriction enzymes that generate 4-bases long 5' overhangs can be used in place of BsaI; this is the case for example of BsmBI (CGTCTC(1/5)) , another type IIS restriction enzyme. This option could come in handy when the VL domain contains another BsaI preventing cloning.

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 VH domain in full.

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).

LIGHT CHAIN TO HEAVY CHAIN RATIO

We recommend starting with a 1:1 light chain to heavy chain ratio during transfection. We observed many antibodies with a better expression at a 2:1 light chain to heavy chain ratio although each antibody requires fine tuning for optimal expression.

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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Email: support@abdesignlabs.com
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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