



TGEX-HC-mG2a-Zeo Expression Vector

INSTRUCTION MANUAL

TGEX-HC-mG2a-Zeo Transient 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 cells in suspension culture. This new series, version 7, also allows the selection of stable transformants through the use of Zeocin®. This vector series features a cytomegalovirus (CMV) promoter, the adenovirus tripartite leader sequence (TPL) (Logan 1984, Mariati 2010), a composite leader sequence (hybrid between a mammalian kappa leader and the bacterial pelB leader (Valadon 2006)) followed by a universal double-inverted Bsal cloning site to insert antibody variable regions. The constant regions are derived from species-specific IgG, kappa and lambda sequences. The 3' end on the transcription unit is composed of an IRES element, the Zeocin-resistance gene (Sh ble from *Streptoalloteichus hindustanus*), a Woodchuck hepatitis virus post-transcriptional regulatory element, and the rabbit beta-globin polyadenylation signal. Transfection of the TGEX[™] expression vectors harboring antibody variable region inserts in widely available cell lines using large-scale transfection technologies (see experimental procedure) typically yields antibody titers between 50 and 250 mg/L in serum-free conditions in just a few days.

The **TGEX™-HC-mG2a-Zeo** vector is designed for the expression of a heavy chain variable region with the constant region of the murine IgG2a heavy chain. Expression of full-length antibody molecules is achieved by co-transfection with a light chain variable region cloned into one the **TGEX™** vectors, see below examples to achieve varied antibody formats.

Combination of vectors to desired antibody format (examples)

COMBINATION	FORMAT	PURIFICATION
TGEX™-HC-hG1-Zeo + TGEX™-LC-hK-Zeo	Full length human or chimeric human IgG1/K	Protein A or G
TGEX™-FH-hG1-Zeo + TGEX™-LC-hK-Zeo	Human or chimeric IgG1/K Fab fragment	Protein L, G, or IMAC
TGEX™-FC-hG1-Zeo	Human IgG1 Fc fusion	Protein A or G
TGEX™-SCblue-Zeo	Human IgG1 scFv-Fc fusion	Protein A or G

Content, Shipping & Storage

Content

VECTOR	COMPOSITION	AMOUNT
TGEX-HC-mG2a-Zeo	$20~\mu l$ at $0.5~\mu g/\mu l$ of DNA vector in DNA Conservation Buffer (Tris-HCL 5 mM, EDTA $0.1~mM,$ pH $8.5)$	10 μg

Shipping & Storage

TGEX-HC-mG2a-Zeo vector is shipped on wet ice. Upon receipt, store the vector at -20°C.

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

TGEX[™] vector series for desired isotypes

TGEX™-HC-hG1-Zeo MX026 Human IgG1 Heavy chain expression plasmid for human IgG1 TGEX™-FH-hG1-Zeo MX023 Human IgG1 CH1 For the expression of human IgG1 Fab fragments TGEX™-FC-hG1-Zeo MX025 Human IgG1 Fc For the expression of human IgG1 Fc fusions TGEX™-HC-hG2-Zeo MX027 Human IgG2 Heavy chain expression plasmid for human IgG2 TGEX™-HC-hG3-Zeo MX028 Human IgG3 Heavy chain expression plasmid for human IgG3 TGEX™-HC-hG4[S228P]-Zeo MX029 Human IgG4[S228P] Heavy chain expression plasmid for human IgG4 TGEX™-LC-hK-Zeo MX030 Human Kappa Light chain expression plasmid for human Kappa TGEX™-LC-hL2-Zeo MX031 Human Lambda 2 Light chain expression plasmid for human Lambda 2	
TGEX™-FC-hG1-Zeo MX025 Human IgG1 Fc For the expression of human IgG1 Fc fusions TGEX™-HC-hG2-Zeo MX027 Human IgG2 Heavy chain expression plasmid for human IgG2 TGEX™-HC-hG3-Zeo MX028 Human IgG3 Heavy chain expression plasmid for human IgG3 TGEX™-HC-hG4[S228P]-Zeo MX029 Human IgG4[S228P] Heavy chain expression plasmid for human IgG4 TGEX™-LC-hK-Zeo MX030 Human Kappa Light chain expression plasmid for human Kappa	
TGEX™-HC-hG2-Zeo MX027 Human IgG2 Heavy chain expression plasmid for human IgG2 TGEX™-HC-hG3-Zeo MX028 Human IgG3 Heavy chain expression plasmid for human IgG3 TGEX™-HC-hG4[S228P]-Zeo MX029 Human IgG4[S228P] Heavy chain expression plasmid for human IgG4 TGEX™-LC-hK-Zeo MX030 Human Kappa Light chain expression plasmid for human Kappa	
TGEX™-HC-hG3-Zeo MX028 Human IgG3 Heavy chain expression plasmid for human IgG3 TGEX™-HC-hG4[S228P]-Zeo MX029 Human IgG4[S228P] Heavy chain expression plasmid for human IgG4 TGEX™-LC-hK-Zeo MX030 Human Kappa Light chain expression plasmid for human Kappa	
TGEX™-HC-hG4[S228P]-Zeo MX029 Human IgG4[S228P] Heavy chain expression plasmid for human IgG4 TGEX™-LC-hK-Zeo MX030 Human Kappa Light chain expression plasmid for human Kappa	
TGEX™-LC-hK-Zeo MX030 Human Kappa Light chain expression plasmid for human Kappa	
TGEX™-LC-hL2-Zeo MX031 Human Lambda 2 Light chain expression plasmid for human Lambda 2	
TGEX™-HC-mG1-Zeo MX032 Murine IgG1 Heavy chain expression plasmid for murine IgG1	
TGEX™-HC-mG2a-Zeo MX033 Murine IgG2a Heavy chain expression plasmid for murine IgG2a	
TGEX™-HC-mG2b-Zeo MX034 Murine IgG2b Heavy chain expression plasmid for murine IgG2b	
TGEX™-HC-mG3-Zeo MX035 Murine IgG3 Heavy chain expression plasmid for murine IgG3	
TGEX™-LC-mK-Zeo MX036 Murine Kappa Light chain expression plasmid for murine Kappa	
TGEX™-LC-mL1-Zeo MX037 Murine Lambda 1 Light chain expression plasmid for murine Lambda 1	
TGEX™-LC-mL2-Zeo MX038 Murine Lambda 2 Light chain expression plasmid for murine Lambda 2	
TGEX™-HC-rbG-Zeo MX039 Rabbit IgG Heavy chain expression plasmid for rabbit IgG	
TGEX™-LC-rbKb4-Zeo MX040 Rabbit Kappa Light chain expression plasmid for rabbit Kappa	
TGEX™-HC-dG1-Zeo MX046 Dog IgG1 Heavy chain expression plasmid for dog IgG1	
TGEX™-HC-dG2-Zeo MX047 Dog IgG2 Heavy chain expression plasmid for dog IgG2	
TGEX™-HC-dG3-Zeo MX048 Dog IgG3 Heavy chain expression plasmid for dog IgG3	
TGEX™-HC-dG4-Zeo MX049 Dog IgG4 Heavy chain expression plasmid for dog IgG4	
TGEX™-LC-dK-Zeo MX050 Dog Kappa Light chain expression plasmid for dog Kappa	
TGEX™-LC-dL-Zeo MX051 Dog Lambda Light chain expression plasmid for dog Lambda	

TGEX™ vector series for Fc-engineered antibodies

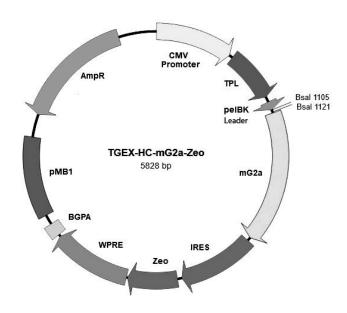
VECTOR	CATALOG	ISOTYPE	DESCRIPTION
TGEX™-HC-hG1[EA]-Zeo	MX041	Human IgG1	Human IgG1 heavy chain with increased ADCC/CDC in vitro
TGEX™-HC-hG1[NA]-Zeo	MX042	Human IgG1	Human IgG1 aglycosylated heavy chain
TGEX™-HC-hG1[LALA-PG]-Zeo	MX043	Human IgG1	Human IgG1 heavy chain with decreased ADCC/CDC in vitro
TGEX™-HC-hG1[YTE-KF]-Zeo	MX044	Human IgG1	Human IgG1 heavy chain with increased serum half-life
TGEX™-HC-hG4[SPLE-PG]-Zeo	MX045	Human IgG4[S228P]	Human IgG4 heavy chain with decreased ADCC/CDC in vitro

$\mathsf{TGEX}^\mathsf{TM}$ control vector, universal expression and Fc fusions

VECTOR	CATALOG	USE	DESCRIPTION
TGEX™-AC-Zeo	MX020	Any expressions	Universal expression vector
TGEX™-eGFP-Zeo	MX022	Transfection	Control plasmid for monitoring transient transfections
TGEX™-SCblue-Zeo	MX024	scFv cloning vector	For the transfer of scFv from any PADL phagemid vector and expression as an scFv-Fc fusion

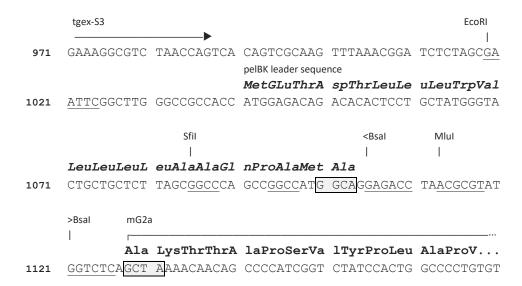
Vector Map

The figure below illustrates the main features of **TGEX-HC-mG2a-Zeo** 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 5828 bp.



Cloning Site

Following is an illustration of **TGEX-HC-mG2a-Zeo** cloning site from the EcoRI site and onward. The VH domain is inserted in a double inverted Bsal cloning site located between the pelBK leader sequence and the constant regions. The four base pair overhangs after a restriction digestion with Bsal are boxed and grayed.



Feature Table

The features of TGEX-HC-mG2a-Zeo 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	1041-1103	Hybrid kappa/pelB leader peptide sequence. The cleavage occurs on the C-terminal side of the terminal alanine.
Murine IgG2a CDS	1128-2120	Sequence encoding the murine IgG2a heavy chain sequence comprising the CH1, CH2, and CH3 domains with the hinge region. The sequence is intronless; the C-terminal lysine residue is encoded.
IRES	2146-2719	Internal Ribosome Entry Site.
Zeo	2753-3127	Sh ble gene from <i>Streptoalloteichus hindustanus</i> conferring resistance to Zeocin.
WPRE	3136-3724	Woodchuck hepatitis virus post-transcriptional regulatory element.
BGpA	3740-3838	Rabbit beta-globin polyadenylation signal sequence.
pMB1 origin	3912-4513	pBR322 origin for replication in <i>E. coli</i> with a temperature-sensitive high copy-number phenotype (Lin-Chao 1992).
TEM1 beta-lactamase	5546-4686	Ampicillin resistance for selection in <i>E. coli</i> .

Restriction Site Summary

Enzyme	Site	Nb	Position	Strand	Isoschizomers
AlfI	(10/12) GCANNNNNNTGC (12/10) 1	3090		
AloI	(7/12) GAACNNNNNNTCC (12/7)	1	1250		
AvrII	C^CTAGG	1	2297		AspA2I BlnI XmaJI
BbvCI	CCTCAGC(-5/-2)	1	1316		
BcgI	(10/12) CGANNNNNNTGC (12/10) 1	5275		
BsaXI	(9/12) ACNNNNNCTCC (10/7)	1	2989	_	
BsePI	G^CGCGC	1	2789		BssHII PauI PteI
BsgI	GTGCAG(16/14)	1	2070	_	
CspCI	(11/13) CAANNNNNGTGG (12/10) 1	407		
EcoNI	CCTNN^NNNAGG	1	1208		BstENI XagI
EcoRI	G^AATTC	1	1019		
FalI	(8/13) AAGNNNNNCTT (13/8)	1	784		
FseI	GGCCGG^CC	1	3024		RigI
FspI	TGC^GCA	1	4979		Acc16I NsbI
GsuI	CTGGAG (16/14)	1	4844	-	BpmI
KpnI	GGTAC^C	1	2587		Acc65I Asp718I
MauBI	CG^CGCGCG	1	2788		
MluI	A^CGCGT	1	1113		
NheI	G^CTAGC	1	2124		AsuNHI BmtI BspOI
NotI	GC^GGCCGC	1	3128		CciNI
PmaCI	CAC^GTG	1	2460		AcvI BbrPI Eco72I PmlI PspCI
PmeI	GTTT^AAAC	1	1000		MssI
PspXI	VC^TCGAGB	1	1339		
PstI	CTGCA^G	1	1296		BspMAI
PvuI	CGAT^CG	1	5126		Ple19I
SalI	G^TCGAC	1	2747		
SexAI	A^CCWGGT	1	2916		CsiI MabI
SfiI	GGCCNNNN^NGGCC	1	1085		

SgrAI CRCCGGYG 1 2866 SabaBit TACKGTA 1 357 BatSNI Ecol051 SpeI ACTAGT 1 18 Abli BcuI Abli					
Spel A CCTAGT 1 18 Ahll Boul Xbal T CCTAGA 1 2741 PaqCI AarI CACCTGC (4/8) 2 1367 PaqCI AlwNI CAGNNN^CTG 2 1277 Cail PstNI ApaI GGGC^C 2 1277 Bspl201 PspOMI Arsi (8/13)GACNNNNNNTTYG(11/6) 2 259 Bspl Ball TGG^CCA 2 2575 Bspl201 PspOMI Ball TGGCCA 2 2754 MlsI MluNI Mox20I MscI Msp20I Ball G^CATCC 2 2756 MlsI MluNI Mox20I MscI Msp20I Ball A^GATCT 2 2756 MlsI MluNI Mox20I MscI Msp20I Ball A^GATCT 2 2755 Mss21 Ball A^GATCT 2 2755 Mss21 Ball A^GATCC 2 2225 Mss21 Mss21 Mss21 Ball A^GATCC 2 2590 Mss21 Mss21 Mss21 Ball A^GATCC 2	SgrAI			2866	
	SnaBI	TAC^GTA	1	357	BstSNI Eco105I
AarI CACCTGC (4/8) 2 1367 PaqCI AlwNI CAGNNN^CTG 2 1327 Cail PstNI ApaI GGGCC^C 2 1428 Bsp1201 PapOMI ArsI (8/13) GACNNNNNTTYG (11/6) 2 259 Bs1 Ball TGG^CCA 2 2754 MlsI MluNI Mox20I MscI Msp20I BamHI G^GATCC 2 1257 MlsI MluNI Mox20I MscI Msp20I BgIII A^GATCT 2 2735 Ms1 BgIII A^GATCT 2 2735 Mval269I PctI BsmI QAATCC (1/-1) 2 2225 Mval269I PctI Bsp14071 T^GTACA 2 1993 BsrGI BstAUI Btr CACGTC (-3/-3) 2 2687 - AjiI BmgBI Btr CACGTC (-3/-3) 2 2687 - AjiI BmgBI Btr CACGTC (-3/-3) 2 2504 AdeI Btr CACGTC (-3/-3) 2 2504 AdeI Btr	SpeI	A^CTAGT	1	18	AhlI BcuI
AlwNI	XbaI	T^CTAGA	1	2741	
AlwNI	AarI	CACCTGC (4/8)	2	1367	PagCI
AlwNI	11011	01100100(1/0/			20402
ApaI	7. 7 2. T	CA CATAINIA CEIC			Coit DobNI
ApaI GGGC^C 2 1428 Bep120I PspCMI Ar8I (8/13) GACNNNNNNTTYG (11/6) 2 851 Ball TGG^CCA 2 2754 MISI MIUNI MOX20I MacI Map20I BamHI G^GATCC 2 2755 MISI MIUNI MOX20I MacI Map20I BgIII A^GATCT 2 2735 Wal269I PctI BsmI GAATCC (1/-1) 2 2292 - Mval269I PctI Bsp1407I T^GTACA 2 1993 BsrGI BstAUI BtrI CACGTC (-3/-3) 2 2895 AjiI BmgBI BtrI CACGTC (-3/-3) 2 2885 AjiI BmgBI BtrI CACNNA^GTG 2 2885 AdeI Drail CACNNA^GTG 2 2512 AdeI Drail CACNNA^NOGTC 2 3101 BseX31 BstZI EclXI Ecc52I Eagl C^GCCG 2 3084 BseX31 BstZI EclXI Ecc52I Ecc311 GACNNANNOGTC 2 1127 BscX1 Ecc311	ALWNI	CAGNINITOTIG			Call PSUNI
Arsi (8/13) GACNNNNNTTYG(11/6) 2 851 Ball TGG^CCA 2 1733 Ball TGG^CCA 2 2754 MlsI MluNI Mox20I MscI Msp20I BamHI G^GATCC 2 1257 Bg1II A^GATCC 2 1255 Bg1II A^GATCC 2 2735 BsmI GAATGC(1/-1) 2 2292 - Mval269I PctI 2 2325 BsmI GAATGC(1/-1) 2 2292 - Mval269I PctI 2 2325 Bsp1407I T^GTACA 2 1993 BsrGI BstAUI BtrI CACGTC(-3/-3) 2 2687 - AjiI BmgBI BtsI GCAGTG(2/0) 2 5152 BtsI GCAGTG(2/0) 2 5152 DraIII CACNNN^CTG 2 2554 AdeI DrdI GACNNNNNGTC 2 1584 AasI DseDI Eagl C^GGCCG 2 3084 BseX3I BstZI EclXI Eco52I Bamilo5i GACNNNNNNGTC 2 1886 AhdI BmeRI DriI Eco31I GGTCTC(1/5) 2 1105 - Bso31I BsaI BspTNI HindIII A^AGCTT 2 2718 Briani CACNNNNGTG 2 3861 NdeI CA^TATG 2 252 FauNDI CACNNNNNGTG 2 1886 DrdI CACNNNNNGTG 2 1986 SGCCC 2 3881 DrdI CACNNNNNGTG 2 1987 SGCCC 2 3881 SGCCC 3 3686 SGCCC 3 368					
Arsi	ApaI	GGGCC^C	2	1428	Bsp120I PspOMI
Ball TGG^CCA 2 2754 BamHI G^GATCC 2 1257 BamHI G^GATCC 2 1257 BgIII A^GATCC 2 1257 BgIII A^GATCC 2 3855 BsmI GAATGC 1/-1) 2 2292 - Mval2691 Pct1 2 2325 Bsp14071 T^GTACA 2 1993 BsrGI BstAUI 2 2790 BtrI CACGTC(-3/-3) 2 2687 - AjiI BmgBI BtsI GCAGTG(2/0) 2 5152 BrallI CACNNN^GTG 2 2554 BrallI CACNNN^GTG 2 2554 BamHI GANNNNNGTC 2 1884 AasI DseDI BtrI GANNNNNNGTC 2 1884 AasI DseDI BtrI GACNNNNNNGTC 2 1884 BseX3I BstZI EclXI Eco52I BtrI GACNNNNNNGTC 2 1884 AasI DseDI BtrI GACNNNNNNGTC 2 1884 BseX3I BstZI EclXI Eco52I BtrI GACNNNNNGTC 2 1884 AasI DseDI BtrI GACNNNNNNGTC 2 1884 BseX3I BstZI EclXI Eco52I BtrI GACNNNNNGTC 2 1884 BseX3I BstZI EclXI Eco52I BtrI GACNNNNNGTC 2 1886 BseX3I BstZI EclXI Eco52I BtrI GACNNNNNGTC 2 1886 AhdI BmeRI DriI BtrI GACNNC 2 1886 BseX3I BstZI EclXI Eco52I BtrI GGTCTC(1/5) 2 1105 - Bso3II BsaI BspTNI BtrI CACNN GGT 2 1884 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 AseI PshBI Cfr42I BtrI GACNN GGT 2 2718 AseI PshBI Cfr42I BtrI GACNN GGT 2 2714 PaeR7I SlaI BtrI GACNN GGT 2 3754 AseI PshBI AxeI PshBI Ax			2	2259	
Ball TGG^CCA 2 2754 BamHI G^GATCC 2 1257 BamHI G^GATCC 2 1257 BgIII A^GATCC 2 1257 BgIII A^GATCC 2 3855 BsmI GAATGC 1/-1) 2 2292 - Mval2691 Pct1 2 2325 Bsp14071 T^GTACA 2 1993 BsrGI BstAUI 2 2790 BtrI CACGTC(-3/-3) 2 2687 - AjiI BmgBI BtsI GCAGTG(2/0) 2 5152 BrallI CACNNN^GTG 2 2554 BrallI CACNNN^GTG 2 2554 BamHI GANNNNNGTC 2 1884 AasI DseDI BtrI GANNNNNNGTC 2 1884 AasI DseDI BtrI GACNNNNNNGTC 2 1884 BseX3I BstZI EclXI Eco52I BtrI GACNNNNNNGTC 2 1884 AasI DseDI BtrI GACNNNNNNGTC 2 1884 BseX3I BstZI EclXI Eco52I BtrI GACNNNNNGTC 2 1884 AasI DseDI BtrI GACNNNNNNGTC 2 1884 BseX3I BstZI EclXI Eco52I BtrI GACNNNNNGTC 2 1884 BseX3I BstZI EclXI Eco52I BtrI GACNNNNNGTC 2 1886 BseX3I BstZI EclXI Eco52I BtrI GACNNNNNGTC 2 1886 AhdI BmeRI DriI BtrI GACNNC 2 1886 BseX3I BstZI EclXI Eco52I BtrI GGTCTC(1/5) 2 1105 - Bso3II BsaI BspTNI BtrI CACNN GGT 2 1884 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 BseX3I BstZI EclXI Eco52I BtrI GACNN GGT 2 2718 AseI PshBI Cfr42I BtrI GACNN GGT 2 2718 AseI PshBI Cfr42I BtrI GACNN GGT 2 2714 PaeR7I SlaI BtrI GACNN GGT 2 3754 AseI PshBI AxeI PshBI Ax	ArsI	(8/13) GACNNNNNTTYG (11/6)	2	851	
Ball TGG^CCA 2 2754 MISI MluNI Mox20I MscI Msp20I BamHI G^GATCC 2 1257 BgIII A^GATCT 2 2735 BsmI GAATGC (1/-1) 2 2292 - Mval269I PctI BsmI GAATGC (1/-1) 2 2292 - Mval269I PctI Bsp1407I T^GTACA 2 1993 BsrGI BstAUI BtrI CACGTC (3/-3) 2 2687 - AjiI BmgBI BtrI CACGTG (2/0) 2 5152 - - BtrI CACNN^GTG 2 2504 AdeI - - Brail GACNNN^GTG 2 2504 AssI DseDI - <t< td=""><td></td><td></td><td></td><td></td><td></td></t<>					
BamHI G^GATCC 2 1257 2735 8gIII A^GATCT 2 27325 8gIII A^GATCA 2 1993 BsrGI BstAUI AgIII BmgBI	BalT	TGG^CCA			MlsT MluNT Moy20T MscT Msn20T
BamHI	Dali	100 001			misi midni monzoi msei mspzoi
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		C. A. W. A. W. ETT. C.			7007 1/ 1/7 7 7
2 5354	Xmnı	GAANN^NN'I'I'C			Asp/UU1 MroX1 PdmI
			2	5354	

Absent Sites:

Aanı, Absı, Accııı, Afei, Aflii, Agei, Ajui, Aoriihi, Aoriihi, Ascı, Asigi, Asisi, Asuii, Axyı, Baei, Bari, Bcli, Bfri, Blpi, Boxi, Bpli, Bpullozi, Bpulii, Bsa291, BsaBi, Bse211, Bse81, BseAi, BseCi, BseJi, BshTi, BshVi, BsiWi, BsmBi, Bsp1191, Bsp131, Bsp17201, Bsp681, BspDi, BspEi, BspQi, BspT1041, BspTi, BssNAi, Bst11071, BstAFi, BstAPi, BstBi, BstEII, BstPAI, BstPI, BstXi, BstZ171, Bsu151,

Bsu36I, BsuTUI, BtuMI, ClaI, CpoI, CspAI, CspI, DinI, Eco147I, Eco32I, Eco47III, Eco81I, Eco91I, Eco065I, EcoRV, EcoT22I, EgeI, EheI, Esp3I, FbaI, FspAI, HpaI, I-CeuI, I-PpoI, I-SceI, KasI, KflI, Kpn2I, Ksp22I, KspAI, LguI, MfeI, Mly113I, Mph1103I, MreI, MroI, MspCI, MunI, NarI, NruI, NsiI, NspV, PI-PspI, PI-SceI, PacI, PaeI, PalAI, PasI, PceI, PciSI, Pfl23II, PflFI, PinAI, PluTI, PshAI, PsiI, PspEI, PspLI, PsrI, PsyI, RgaI, RruI, Rsr2I, RsrII, SapI, SbfI, SdaI, SfaAI, SfoI, SfuI, SgfI, SgrDI, SgsI, SmiI, SphI, SrfI, Sse8387I, SseBI, SspDI, StuI, SwaI, Tth111I, Vha464I, XcmI, 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 TEGX vectors 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 37°C with agitation. TEGX vectors are derivatives 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 DH5 α . The high copy number phenotype is temperature-sensitive and requires incubation at 37°C (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 plasmid DNA can be isolated in large quantities.

Cloning into TGEX-HC-mG2a-Zeo

In Silico Design

A double inverted Bsal cloning site separates the pelBK leader peptide sequence from the beginning of the antibody constant region. After digestion with Bsal, the heavy chain variable domain is inserted in-frame between the two elements as illustrated below. During the cloning, the two Bsal sites are eliminated.

Vector Digestion

Bsal alone is sufficient to open the cloning site. Please, consult the documentation of your restriction enzyme provider for optimal conditions.

Cloning with FAST-Licase

Homologous recombination is the easiest and most efficient method to clone seamlessly antibodies into TGEX vectors. We recommend the **FAST-Licase™** (Antibody Design Labs cat# MB101S & MB101L) with the following overhangs. For synthetic dsDNA constructs, the overhangs are added on each side. For PCR-amplified constructs, primers should contain the overhang followed by the antibody priming area. The **FAST-Licase™** reaction contains the insert plus the purified vector digested with Bsal (see kit instructions).

pelBK overhang 5' - CGGCCCAGCCGGCCATGGCA

mG2a overhang 5' - GCTAAAACAACAGCCCCATC (reverse complement for PCR primers)

Primer Design for Restriction Cloning with the Bsal Sites

Oligo1 is an example of primer designed to amplify a VH domain sequence and clone it into the Bsal site situated next to the pelBK leader. A minimum of 2 nucleotides is recommended to cut Bsal site close to the end (source New Englands BioLabs); these 2 nucleotides are followed by the Bsal site GGTCTC and the last five nucleotides of the leader sequence; the resulting NNGGTCTCTGGCA extension is added 5' to the VH domain primer.

Oligo2 is an example of primer designed to amplify the VH domain from the end of the J region including a Bsal site compatible with **TGEX-HC-mG2a-Zeo** cloning site.

Bsal is a type IIS restriction enzyme that cuts outside of its recognition site. The second Bsal site of the cloning site will be cut immediately before the start of the murine IgG2a 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-base overhang GCAT on the sense strand. In Oligo2, a Bsal recognition site is situated symmetrically to the vector and will generate a complementary overhang.

After digestion and ligation, all the two Bsal sites, from both insert and vector, will be removed, resulting in a scarless insertion of the antibody domain.

Alternative to Bsal Sites

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

Sequencing of Inserts

The following primers give a strong PCR amplification of the TGEX vector series inserts and the antibody constant regions between the EcoRI site and the Nhel site. The primer tgex-S3 can be used to sequence the VH domain in full.

tgex-S3 5'- AGGCGTCTAACCAGTCACAGTC

ires-R 5'- GAATAAGGCCGGTGTGCGTT

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 obtain 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 different 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, e.g. TGEX™-eGFP-Zeo (Antibody Design Labs cat# MX022). Similarly, any condition known to boost expression should be carefully tested in your system before being scaled up. We did observe an increase in

expression in HEK293 cells upon exposure to sodium valproate (Backliwal 2008). Boosters and enhancers are often included in commercially available transfection kits.

Stable Cell Line Selection

Zeocin can be used very effectively to select stable cell lines secreting antibodies in a few weeks. The following protocol are suggestions that require adjustment to your particular constructs:

WEEK 1.

- Day 0: Transfect in duplicate cells in a 6-well plate containing 2 ml culture per well;
- Day 3-4: Expand each well in two wells with 2 ml culture per well.
- Day 6: Increase the volume to 4 ml per well and add Zeocin at 100 μg/ml.

WEEK 2-3

 Maintain a good cell density and the Zeocin concentration while replenishing the culture with fresh medium as needed.

WEEK 3-4

- Continue selection with possibly a higher Zeocin concentration (up to 1000 μg/ml).
- Proceed to single cloning and analyze stable transformants for expression.

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

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, or email us at:

Antibody Design Labs Email: support@abdesignlabs.com
4901 Morena Blvd, Suite 203 Phone: 1-877-223-3104 (Toll Free)

San Diego, CA 92117 (Monday – Friday 9:00 AM – 5:00 PM PST)

References

- 1. Logan J, & Shenk T. (1984). Adenovirus tripartite leader sequence enhances translation of mRNAs late after infection. *Proc Natl Acad Sci USA*, *81*(12):3655–9.
- 2. Mariati, Ho SCL, Yap MGS, & Yang Y. (2010). Evaluating post-transcriptional regulatory elements for enhancing transient gene expression levels in CHO K1 and HEK293 cells. *Protein Expr Purif*, 69(1):9–15.
- 3. Valadon P, Garnett JD, Testa JE, Bauerle M, Oh P, & Schnitzer JE. (2006). Screening phage display libraries for organ-specific vascular immunotargeting in vivo. *Proc Natl Acad Sci USA*, 103(2):407–12.
- 4. Lin-Chao S, Chen WT, Wong TT (1992). High copy number of the PUC plasmid results from a ROM/ROP-suppressible point mutation in RNA II. *Mol Microbiol*, 6(22):3385–93.
- 5. Sambrook J, Fritsch EF, & Maniatis T. (1989). In Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, NY, VOL. 1, 2, 3.
- 6. Backliwal G, Hildinger M, Kuettel I, Delegrange F, Hacker DI, Wurm FM. (2008). Valproic acid: A viable alternative to sodium butyrate for enhancing protein expression in mammalian cell cultures. *Biotechnol Bioeng*, 101(1):182–9.

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