



TGEX-LC Expression Vector

INSTRUCTION MANUAL

TGEX-LC Transient Mammalian Expression Vector Catalog #: MX002 Version: A1.1 – December 2016

Table of Contents

Limited Use License for the TGEX Vector Series	
Description	5
Introduction	5
Content, Shipping & Storage	5
Limited Product Warranty	5
TGEX Vector Series	6
Vector Map	6
Cloning Site	7
Feature Table	7
Restriction Site Summary	8
Experimental Procedures	10
General Molecular Biology Techniques	10
Plasmid Maintenance	10
Cloning into TGEX-LC	10
Sequencing of Inserts	11
Antibody Expression	11
Appendix	12
MSDS Information	12
Quality Control	12
Technical Support	12
References	12

Limited Use License for the TGEX Vector Series

As a condition of sale of this product to you, and prior to using this product, you must agree to the terms and conditions of this license. Antibody Design Labs grants to the buyer with the sale of any of its **TGEX**[™]vectors (the "Product") a non-exclusive, non-transferable and limited license to use the Product in research only conducted by the buyer. Such license specifically excludes the right to sell or otherwise transfer the Product, its components or derivatives thereof to third parties. No modifications to the Product may be made without express written permission from Antibody Design Labs. The buyer is not granted a license to use the Product for human or animal therapeutic, diagnostic, or prophylactic purposes.

Antibody Design Labs does not warrant that the use or sale of the Product, the use thereof in combination with other products, or the use of the Product in the operation of any process will not infringe the claims of any United States or other patent(s).

If the buyer is not willing to accept the limitations of this license, without modification, buyer may refuse this license by returning the Product unopened and unused. By keeping or using the Product, buyer agrees to be bound by the terms of this license.

Entities wishing to use the Product for commercial purposes are required to obtain a license from Antibody Design Labs. Commercial purposes may include, but are not limited to: use of the Product in manufacturing, use of the Product to provide a service, use of the Product for therapeutic or diagnostic purposes, or resale of the Product, whether or not such Product is resold for use in research. For information on purchasing a commercial license to the Product, please contact a licensing representative by phone at (858) 480-6213 or by e-mail at licensing@abdesignlabs.com.

All trademarks are the property of their respective owners.

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.

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

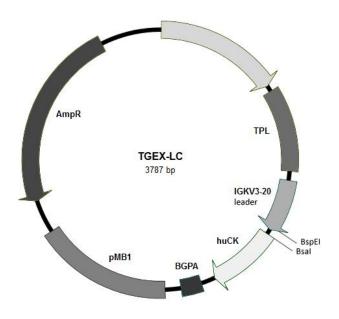
Combination of vectors to desired antibody format

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-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 METPAQLLFLLLWLPDTSG. SA/SD donor and acceptor sites.

	tgex-S3				EcoRI
971	GAAAGGCGTC	TAACCAGTCA	CAGTCGCAAG	TTTAAACGGA	I TCTCTAGCGA
	Start of IGK	V3-20 leader sequen	ice		
	I		1043		
			MetGluTh	rProAlaGln	LeuLeuPheL
1021	ATTCTAGGAC	CCAGAGGGAA	CCATGGAAAC	CCCAGCGCAG	CTTCTCTTCC
	euLeuleuLe	uTrpLeuPro	SD		
1071	TCCTGCTACT	CTGGCTCCCA	GGTGAGGGGA	ACATGGGATG	GTTTTGCATG
1121	TCAGTGAAAA	CCCTCTCAAG	TCCTGTTACC	TGGCAACTCT	GCTCAGTCAA
1171	TACAATAATT	AAAGCTCAAT	ATAAAGCAAT	AATTCTGGCT	CTTCTGGGAA
1221	GACAATGGGT	TTGATTTAGA	TTACATGGGT	GACTTTTCTG	TTTTATTTCC
		BspEl V	'L start	Bsal	huCK constant region
					Г
	SA	ThrSerGly			ArgThrValA
1271	AATCTCAGAT	ACCTCCGGAG	AACGTGAGTA	GACGGTCTCG	CGAACTGTGG
	laAlaProSe	rValPheIle	PheProProS	erAspGluGl	nLeuLysSer
1321	CTGCACCATC	TGTCTTCATC	TTCCCGCCAT	CTGATGAGCA	GTTGAAATCT

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 METPAQLLFLLLWLPDTSG; 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 <i>E. coli</i> 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

Ball TGG*CCA 1 3715 MLSI MUNI MOX20I MscI Msp20I BdyCI CCTCAAC(-5/-2) 1 1511 BogI CCTCAAC(-5/-2) 1 1529 BasXI (9/12) ACNINNINGTCC(12/10) 1 1759 BasXI (9/12) ACNINNINGTCC(10/7) 1 175 BagI CGCCAC(16/14) 1 1321 - BagI CGCCAC(16/1-4) 1 1374 - BagI TCGCCAC 1 1274 AccBSI MbII BagI CCCCTC(-3/-3) 1 3577 - AccBSI MbII BagI CCCCCC(-3/-3) 1 3577 - AccBSI MbII CCGCTC (-3/-3) 1 1277 AsarDseDI AccBSI MbII CCGCCC (-3/-7) 1 1273 AccBSI MbII BccDI CCGCT (-1/3) 1 1364 BccDI BasI Bst2I BCIXI EcoSZI Eagl (-1/3) GACMNNINCTC 1 1019 - BasI BasI Bst2I BCIXI EcoSZI Eagl (-1/3) GACMNINNCTC 1 10128	Enzyme	Site	Nb	Position	Strand	Isoschizomers
BbwC CCTCARC (-5/-2) 1 1511 BipI GCTTNAC 1 3234 BipI GCTTNAC 1 1529 ByRI GAGGAG (10/8) 1 715 BasRI GAGGAG (10/7) 1 1321 - BapEl TCCCACA (16/14) 1 1321 - BapEl TCCCACA (16/14) 1 1321 - BapEl TCCCACA (1) 3757 - AccIII BapEl CCCCTC (-3/-3) 1 3757 - AccBAI MolI CapCT (11/13) CAANINNINGTGG (12/10) 407 AccIII EcolII EcolII CapCT (-1/3) 1 1304 Beso31I BacI BapTNI EcolII EcolII EcolII 6707CO (1/5) 1 1304 Boo31I BacI BapTNI EcolII 6707CO (1/5) 1 1306 BapEI I BCUMI RCUI FajL 6707CO (1/5) 1 1308 BapEI I BCUMI RCUI FajL 6707CO (1/5) 1 1308 <td< td=""><td>BalI</td><td>TGG^CCA</td><td>1</td><td>3715</td><td></td><td>MlsI MluNI Mox20I MscI Msp20I</td></td<>	BalI	TGG^CCA	1	3715		MlsI MluNI Mox20I MscI Msp20I
BIDI GC*TNACC 1 1529 Bpull02I Bspl720I BasAXI GAGGAG(10/8) 1 715 BaspI GAGGAG(10/8) 1 1231 - BapDI TATACA 1 3749 - AccIII BapEI CCCGCC(-3/-3) 1 3557 - AccBXII Bspl3I Bspl3	BbvCI	CCTCAGC(-5/-2)	1	1511		-
BaskII (9/12) ACMINNUMPECT (10/7) 1078 BagT GGGGAG (10/8) 1 715 BagI OT CORG (16/14) 1 1321 - BapIADT T*CTACA 1 3749 BarGI EstAUI BapEI T*CCGGA 1 1284 Aor13HI BapIAT Kpn2I BseAI MroI AccIII BarBI CCGCTC (-3/-3) 1 3557 - AccBSI MbiI CGSCT (1/1/3) 1 3577 - AccBSI BstII EclXI Eco52I BagI GACINNNYMETC 1 2713 AhdI BmeRI DriI EcoRI G*ARTC 1 1019 Fail (8/13) AAGNINNINCT (13/8) 1 744 For *CAC 1 2938 Acc161 NsbI Gaul CTGGAC (13/14) 1 2838 Acc161 NsbI Gaul CCGCACC 1 1030 BapESI ELWI RruI Pfilm CCGCACC 1 1642 BpMAI Null TCGCAGC 1 1642 BpMAI PuuII CANTCT	BcgI	(10/12)CGANNNNNTGC(12/10)) 1	3234		
BesRI GAGGAG(10/8) 1 715 Bap14071 TCCCAG(16/4) 1 1321 - BapEI TCCCAG(-5/-4) 1 3749 BarGI BALAUT BapEI TCCCAG(-5/-4) 1 1284 AorIBHI BapI3I Kpn2I BacAI MroI AccIII BarGI GAGGAG(-5/-1) 1 1284 AorIBHI BapI3I Kpn2I BacAI MroI AccIII BarGI GACNNNMYNNGTC 1 1297 AasI DecDI EagI CCGCCC 1 1677 BacX3I BatZI EclXI EcoSI EagII CCGCCC 1 1677 BasCX3I BatZI EclXI EcoSI EagII CCGCCC 1 1670 BasCX3I BatZI EclXI EcoSI EagII GCTCT(1/5) 1 1304 BasIXI BatZI EclXI EcoSI EagII GCTCAG(15/1) 1 2803 - BpmI FalII (A/3)AASINNINTCT (13/8) 1 748 - FolII GCCCAG (21/19) 1 2803 - BpmI MakII CCGCAG (21/19) 1 1642 A	BlpI	GC^TNAGC	1	1529		Bpu1102I Bsp1720I
BesRI GAGGAG(10/8) 1 715 Bap14071 TCCCAG(16/4) 1 1321 - BapEI TCCCAG(-5/-4) 1 3749 BarGI BALAUT BapEI TCCCAG(-5/-4) 1 1284 AorIBHI BapI3I Kpn2I BacAI MroI AccIII BarGI GAGGAG(-5/-1) 1 1284 AorIBHI BapI3I Kpn2I BacAI MroI AccIII BarGI GACNNNMYNNGTC 1 1297 AasI DecDI EagI CCGCCC 1 1677 BacX3I BatZI EclXI EcoSI EagII CCGCCC 1 1677 BasCX3I BatZI EclXI EcoSI EagII CCGCCC 1 1670 BasCX3I BatZI EclXI EcoSI EagII GCTCT(1/5) 1 1304 BasIXI BatZI EclXI EcoSI EagII GCTCAG(15/1) 1 2803 - BpmI FalII (A/3)AASINNINTCT (13/8) 1 748 - FolII GCCCAG (21/19) 1 2803 - BpmI MakII CCGCAG (21/19) 1 1642 A	BsaXI	(9/12) ACNNNNNCTCC (10/7)	1	1078		
spil ""CCACA 1 3749 BarCI EstUI BspEI "CCCCA 1 1284 ArcIIII BsrEN CCCCTC (-3/-3) 1 1287 ArcEII BsrEN CCCCTC (-3/-3) 1 127 ArcESI Moi Drdi CACNNNNNNGTGG (12/10) 407 - ArcESI Moi Drdi CACNNNNNNTTC 1 127 ArcESI Berli ArceCSI Berli Eagl CCGCTC (1/5) 1 1304 BseX31 Berli EriXI EriXI EriXI EriXI Eco311 GCATTC (1/5) 1 1304 BseX31 Berli EriXI EriX	BseRI		1	715		
BageT T^CCGGA 1 1284 Accl3II Sep13I Kpn2I BasAI McoI AccIII BsrBI CCGCTC(-3/-3) 1 3557 - AccBGI MbiI CapCT (11/13) CAANNNNGTCG (12/10) 407 - Acal DeeDI EaqI CCGCGCG 1 1677 BasA3I BszIz EclXI Eco52I EaqI GCTCTC(1/5) 1 1304 Bso3II BsaI BasT Bso3I BsaI BasT EcoRI GCANNNYNNGTC 1 1019 - - Fall (8/13) AAGNNNNCTC (13/8) 784 - Bso3II BsaI BasT - GSUT CCGCAGC 1 2803 - Bpn1 - MindIII ACAGTT 1 1820 - - - MindIII CCGAGCGC 1 676 CcINT - </td <td>BsgI</td> <td>GTGCAG(16/14)</td> <td>1</td> <td>1321</td> <td>-</td> <td></td>	BsgI	GTGCAG(16/14)	1	1321	-	
Barblin AccIII AccIII Barblin CCCCCC (-3/-3) 1 3557 - AccBSI MbiI Drdi GACUNINN*NNGTC 1 1927 AasI DseDI Eagl C'SGCCG 1 1677 BseX3I BstZI EclXI Eco52I Eamlibis GACUNINN*NNGTC 1 2713 Ahdt BmeRI Dri Eco31I GACUNINNNNTTC 1 2713 Ahdt BmeRI Dri Eco31G GACUNINNNNTT(13/8) 1 784 Fall (K)13) AACMNNNNTT(13/8) 1 784 Fall (K)13/AACMNNNNTT(13/8) 1 2938 Acc161 Nabi Gout CTCGAG (16/14) 1 2833 - BpmI HindIII AACCTT 1 1820 - Neal - Neal GCCGAG (21/19) 1 2841 - - - Neal GCCGAG (21/19) 1 1060 MssI - - Phil CTCCACA 1 1062 BpmMI - <td>Bsp1407I</td> <td>T^GTACA</td> <td>1</td> <td>3749</td> <td></td> <td>BsrGI BstAUI</td>	Bsp1407I	T^GTACA	1	3749		BsrGI BstAUI
CspCI (11/13)CAANNENTGG(12/10)1 407 DrdI GACNNNYNNETC 1 1677 BexMI BetZI EclXI EcoS2I Eagl CrGGCG 1 1677 BexMI BetZI EclXI EcoS2I EaglI GACNNYNNETC 1 2713 AhdI BetZI EclXI EcoS2I Eco311 GACNTC 1 1019 Eco31 GACATTC 1 1019 Eco31 GACATTC 1 2938 Acc161 Nsb1 GsuI CTGGAC (16/14) 1 2803 - BpmI HindIII AAGCTT 1 1820 - - NeALI GCGGCGC 1 1676 CoNN - Nul CGCAGCGC 1 1676 CoNN - PfLI GCGAGCGCA 1 1000 Msg1 - Nul CGCACGCA 1 1000 Msg1 - PfLI CGCACGCA 1 1642 EspMAI - SacI CGCCCGC 1	BspEI	T^CCGGA	1	1284		
Dråt GACINNN'NNOTC 1 1927 Aast DeelI Eagl C^GGCCG 1 177 BseX31 BstZ1 EclX1 EclX1 EcoS21 Eaml1051 GACNNN'NNGTC 1 2713 AhdI EmeR1 Dr11 EcoRI GYATTC 1 100 BseX31 BstZ1 EclX1 EcoX1 EcoX1 EcoRI GYATTC 1 100 Fall 100 Fall (8/13) AAGNNNNTT (13/8) 784 Fall Acc161 Nsb1 GGCGCA 1 2838 Acc161 Nsb1 Asc GSUI CTGGAG(16/14) 1 2803 - BpmI MindII AccGCGC 1 1676 CciNI NruI TCGCCGA 1 1308 Bsp881 BtuMI RruI BstX1 NruI TCGCACGA 1 1642 BspMAT PrUI CGATCG 1 642 FalsystI Eco1051 SacII CCGCCGG 1 642 FalsystI Eco1051 SagI GCCTCTC(1/4) 1 1208 Bsp801 Eco11421	BsrBI	CCGCTC(-3/-3)	1	3557	-	AccBSI MbiI
Eagl CGGCG 1 1677 BekJI BatZI EckN EcoS2I Eaml1051 GACNNYNNGTC 1 2713 AhdI BmeRI Drif Eco311 GGTCTC (1/5) 1 1304 Bso31I BsaI BsyTN1 EcoRI G'AATTC 1 1019 Fall (8/13) AACNNNNCTT (13/8) 1 784 FspI TGGCGG (16/14) 1 2938 Acc161 NabI GSut CTGGAG (16/14) 1 2831 Acc161 NabI MindIII AcGCT 1 1820 NutI GCCGCGC 1 1676 Cc1NI NTUI TCGCAGA 1 1308 BspEI BtuMI RruI PfIMI CCANNNYNTGG 1 614 Acc871 VanJI PruI CGAT^CG 1 6162 BspMI PruI CGAT^CG 1 6162 BspMI PruI CGAT^CG 1 6162 BspMI SacII CCCTCGA 1 716 StrifisiaI	CspCI	(11/13) CAANNNNNGTGG (12/10)) 1	407		
Lami1051 GACUNN'NNETC 1 2713 AhdI EmeRI Drif Eco311 GGTCT (1/5) 1 1304 Beo311 Esal EspTNI EcoRI G'AATC 1019 Fall (8/13) AGNNNNCTT (13/8) 784 FspI TGC (2GA 2938 Accl6I NsbI GsuI CTGGAG (16/14) 2803 BpmI HindHI A'ACTT 1820 BpmI Nul CCGAG (21/19) 1 2841 NotI GO'GGCG 1 676 CciNT Nrul TGC (CGA 1 1308 Bsp681 BtuMI Rrul PfIM CCANNNN'NTGG 1 641 AccBT Van911 PwiI CGA'CG 1 642 BspMAI PvuII CAC'CTG 1 642 Bsp21 LguI PciSI SacII CCGC'CG 1 740 Sfr3031 KspI SgrBI Cfr42I SacII CCGC'CG 1 1641 ShI BouI Sse3871 CCTCCA'GG 1 1641 ShI BouI	DrdI	GACNNNN^NNGTC	1	1927		AasI DseDI
EcoRI Fall GCTCT(1/5) 1 1304 1019 Bso3ll Bsal BspTNI Fall 16/13) AGNNNNNCTT(13/8) 1 74 Fsp1 TGCCGCA 1 2938 Acd161 Nsb1 Gsu1 CTGCAG(16/14) 1 2938 Acd161 Nsb1 MindIII ACCTT 1 1820	EagI	C^GGCCG	1	1677		BseX3I BstZI EclXI Eco52I
ECORI G'AATTC 1 1019 CALL Fall (8/13) AGNNNNCTT (13/8) 1 784 Fsp1 TGC^GCA 1 2938 Acc16I NsbI GsuI CTGCAGC(16/14) 1 2803 - BpmI HindHI ACCAGA(12/19) 1 2841 - - NotI GCCAGCAG(21/19) 1 2841 - - NutI TCG^CGA 1 1076 CciNI - PfMI CCANNNN'NTGG 1 641 AccBI Van911 - Praid CTGCACA 1 1000 MssI - PstI CTGCACAG 1 1642 BspMAI - SacII CCGATCG 1 1642 - - SacII CCGATGG 1 1642 - - SacII CCGATGT 1 18 AhlI BcuI - SacII CCGATGT 1 1641 SbfI SdaI - <td>Eam1105I</td> <td>GACNNN^NNGTC</td> <td>1</td> <td>2713</td> <td></td> <td>AhdI BmeRI DriI</td>	Eam1105I	GACNNN^NNGTC	1	2713		AhdI BmeRI DriI
Fall (#13) AAGNNNNNCTT (13/8) 1 784 Fsp1 TGCAGA 1 2938 Acc161 Nsb1 Gsu1 CTGGAG (16/14) 1 2938 - Bpm1 HindIII A'AGCTT 1 1820 - - NmeANIII GCCGAG (21/19) 1 2841 - - Nuti GCCGAG (21/19) 1 2841 - - - Nuti GCCGAG (21/19) 1 1308 Bsp681 BtuMI RruI - - Nuti GCCACGGA 1 1000 Ms1 -<	Eco31I	GGTCTC(1/5)	1	1304		Bso31I BsaI BspTNI
Fsp1 TGCACA 1 293 Accl6I NsbI Gsu1 CTGGAG(16/14) 1 2803 - BpII MmaAIII GCGAG(21/19) 1 2841 - NmaLII GCCGAG(21/19) 1 2841 - Nu1 GCCGCGC 1 1676 CciNI NTU1 TGCAGGA 1 1308 Bsp681 BtuMI Rru1 PfIMI CCANNNN*NTGG 1 641 AccB71 Van91I PmeI GTTCAAG 1 1642 BspMAI Pvu1 CGAC*GG 1 642 - SapI GCTCTC (1/4) 1 208 Bsp01 LguI PcisI SapI GCTCTC (1/4) 1 208 Bsp01 LguI PcisI Sae83871 CCTGCA^GG 1 1641 Shf1 Sda1 Xhot CACGAG 2 2134 AhlI BcuI Sae83871 CAGCACAG 2 2164 Call PstNI Accl1 AA^CCTT 2 2364 AlwHU	EcoRI	G^AATTC	1	1019		
Gsn1 CGAG(16/14) 1 2803 - BpmI HindIII A'AGCTT 1 1820 -	FalI	(8/13) AAGNNNNNCTT (13/8)	1	784		
HindIII A^ACCT 1 1820 NmeAIII GCCGAC(21/19) 1 2841 NotI GCCGACCA 1 1676 CciNI Nrui TCG^CCA 1 1308 Bsp681 BtuMI RruI PfIMI CCANNN'NTGG 1 641 AccB7I Van91I PmeI CTGCA^G 1 1642 BspMAI PvuI CCAC^GG 1 642 SacII CCCC^GG 1 642 SacII CCGCAGG 1 740 Str303I KspI SgrBI Cfr42I SapI SacII CCGCAGG 1 740 SapI SgrBI Cfr42I SapI SapI GCCGCAGG 1 740 SapI SgrBI Cfr42I SapI SapI CCCCAGG 1 1641 ShI SaI SatII ScaII ShI TACGTA 1 1641 ShI ScaII SatII SapI CCCCCAGG 1 966 Sfr274I PacR7I SlaI SatII Acli AA^CGTC 2 2316	FspI	TGC^GCA	1	2938		Acc16I NsbI
NmeAIII GCCGAG (21/19) 1 2841 Noti GC^GGCGCG 1 1676 CiNI Nrui TCG^CGCA 1 308 Bsp681 EtuMI RruI PflMI CANNNN^MTGG 1 000 MsI PmeI GTTT^AAAC 1 1000 MsI PvuI CGA^G 1 1642 BspMAI PvuI CAA^CGG 1 1642 BspMAI PvuI CAA^CGG 1 3085 Ple191 PvuII CAA^CGG 1 740 Sfr3031 KspI SgrBI Cfr421 SapI GCTCTC (1/4) 1 1208 Bsp01 JguI PciS1 SnaBI TAC^AGTA 1 357 BstSNI Ecol051 SpeI A^CTAGT 1 1641 AblI BcuI Sse33871 CCTCCA/GG 1 1641 AblI BcuI Actio CAGGG 1 1641 AblI BcuI Actio CAGGG 1 1641 AblI BcuI Acti	GsuI	CTGGAG(16/14)	1	2803	-	BpmI
Noti GC^GGCCGC 1 1676 CciNI Nrui TCG^CGA 1 1308 Bsp681 BtuMI RruI PflMI CCANNNNTGG 1 641 AcCB71 Van91I PmeI GTT^AAAC 1 1000 MssI PstI CGACGAG 1 1642 BspMAI PvuI CGA^CGG 1 3085 Ple191 PvuII CGACGCGG 1 740 Sfr3031 KspI SgrBI Cfr421 SacII CCGCGG 1 740 Sfr3031 KspI SgrBI Cfr421 SapI GCTCTC(1/4) 1 1208 BspQI LguI PciS1 SnaBI TAC^GTA 1 357 BstSNI Ecol051 SpeI A^CCTGGT 1 1641 SbfI SdaI Xhoi CTGCACGG 1 966 Sfr2741 PaeR7I SlaI Acli AACGTT 2 2436 Psp1406I Arsi (8/13) GACNNNNNTTYG (11/6) 2 132 2 BssI GAGAC (2/6) 2 1332 <td>HindIII</td> <td>A^AGCTT</td> <td>1</td> <td>1820</td> <td></td> <td></td>	HindIII	A^AGCTT	1	1820		
Nrul TCG^CGA 1 1308 Bsp68I BtuMI Rrul PfMI CCANNINA^NTGG 1 641 AccB7I Van9II PmeI GTT^^AAAC 1 1000 MssI PstI CTGCA^G 1 1642 BspMAI PvuI CGA^^CG 1 3085 Ple191 PvuII CGC^CGG 1 740 Sfr303I KspI SgrBI Cfr42I SacII CCGC^GG 1 740 Sfr303I KspI SgrBI Cfr42I SapI GCTTCTC(1/4) 1 1208 BspQI LguI PciSI SnaBI TAC^GTA 1 357 BstSNI EcolO5I SpeI A^CTAGT 1 1641 SbfI SdaI Sse3871 CCTGCA^GG 1 1641 SbfI SdaI Acli AA^CGTT 2 2943 Psp1406I AlwNI CAGNN^CTG 2 3186 Cail PstNI Arsi (8/13) GACNNNNNTTYG (11/6) 2 851 E Bari GACAC (2/6) 2 13	NmeAIII	GCCGAG(21/19)	1	2841		
PflMI CCANNNN^NTGG 1 641 AccB71 Van911 PmeI GTTT'AAAC 1 1000 Mss1 PvuI CTGCA^G 1 1642 BspMAI PvuI CGA^CG 1 642 5 SacII CCGC^CG 1 642 5 SapI CCGCTCTC (1/4) 1 1208 BspD1 LguI PcilsI SapI ACCTGCAG 1 642 5 SapI CCGCCAG 1 740 Sfr303I KspI SgrBI Cfr42I SapI CCGCCAGG 1 740 Sfr303I KspI SgrBI Cfr42I SapI ACCTGAGA 1 764 Str303I KspI SgrBI Cfr42I SapI ACCTGAGA 1 641 SbfI SdaI ScaSa371 CCTGCAGG 1 1641 SbfI SdaI Acli AACT 2 243 Psp14061 Acli AACT 2 3316 Cali PstNI Arsi (8/13) GACNNNNNTTYG (11/6) 2 851 Sacii PstNI BapiI </td <td>NotI</td> <td>GC^GGCCGC</td> <td>1</td> <td>1676</td> <td></td> <td>CciNI</td>	NotI	GC^GGCCGC	1	1676		CciNI
PmeI GTT^^AAAC 1 1000 MssI PstI CTGCA^G 1 1642 BspMAI PvuI CGA^CGG 1 3085 Ple19I PvuII CAG^CTG 1 642 SacII CGCCGC^GG 1 740 Sfr303I KspI SgrBI Cfr42I SapI GCTCTTC1/4) 1 208 BspOI LguI PciSI SnaBI TAC^GTA 1 357 BstSNI Eco105I SpeI A^CTAGT 1 18 Ahll BcuI Sse83871 CCTGCA/GG 1 966 Sfr274I PaeR7I SlaI AclI A^CGTT 2 2943 Psp1406I AclI AA^CGTT 2 2136 Alw44I VneI ApaLI GAGACNNNNNTTYG(11/6) 2 2138 Alw44I VneI ArsI (8/13) GACNNNNNTTYG(11/6) 2 3385 1 Bri GAAGAC (2/6) 2 1332 1 Bri GAGAC (2/6) 2 361 1	NruI	TCG^CGA	1	1308		Bsp68I BtuMI RruI
PstI CTGCA^G 1 1642 BspMAI PvuI CGAT^CG 1 3085 Ple191 PvuI CGAT^CG 1 642 SacII CCGC^GG 1 740 Sfr3031 KspI SgrBI Cfr42I SapI GCTCTC(1/4) 1 1208 BspQI LguI PciSI SnaBI TAC^GTA 1 357 BstSNI Ecol05I SpeI A^CATG 1 1641 ShI Ecol05I SpeI A^CATG 1 1641 ShI SdaI StoI CAGNACG 1 966 Sfr3041 PaeR7I SlaI AclI AA^CGT 2 243 Psp1406I AclI AA^CGT 2 243 Psp1406I AlwNI CAGNNACTG 2 2136 Alw41 VneI ArsI G^AGAC 2 2139 Alw41 VneI Bs GAAGAC(2/6) 2 132 Psp1406I Br GAAGAC(2/6) 2 2132 Psp1406I Br GAAGAC(2/6) 2 132 Psp1406I Br GAAGAC	PflMI	CCANNNN^NTGG	1	641		AccB7I Van91I
PvuI CGAT^CG 1 3085 Ple191 PvuI CGA^CTG 1 642	PmeI	GTTT^AAAC	1	1000		MssI
PvuII CAG^CTG 1 642 SacII CCGC^GG 1 740 Sfr3031 KspI SgrBI Cfr42I SapI GCTCTC (1/4) 1 1208 BspQI LguI PciSI SnaBI TAC^GTA 1 357 BstSNI Ecol051 SpeI A^CTAGT 1 18 AhlI BouI Sse83871 CCTGCA^GG 1 1641 ShI SdaI XhoI C^TCGGAG 1 1641 ShI SdaI AclI AA^CGTT 2 2943 Psp1406I AclI AA^CGTT 2 2316 - AlwNI CAGNNN^CTG 2 2236 - ApaLI G^TGCAC 2 2139 Alw44I VneI 2 3385 - - - ArsI (8/13) GACNNNNNTTYG (11/6) 2 851 - 2 1221 - - - - BbsI GAAGAC (2/6) 2 1218 BpiI BstV2I - 2 361 - - - - 361	PstI	CTGCA^G	1	1642		BspMAI
SacII CCGC^GG 1 740 Sfr3031 KspI SgrBI Cfr421 SapI GCTCTT (1/4) 1 1208 BspQI LguI PciSI SnaBI TAC^GTA 1 357 BstSNI Eco105I SpeI A^CTAGT 1 1641 ShI BcuI Sse8387I CCGCAGG 1 1641 ShI SdaI Ahol CACTAGG 1 966 Sfr274I PaeR7I SlaI Acli AA^CGT 2 2943 Psp1406I Acli AA^CGT 2 236 AlWNI CAGNNN^CTG 2 1584 CaiI PstNI ApaLI G^TGCAC 2 236	PvuI	CGAT^CG	1	3085		Ple19I
Sapi GCTCTTC (1/4) 1 1208 BspQI LguI PciSI SnaBi TAC^GTA 1 357 BstSNI Ecol05I SpeI A^CTAGT 1 18 AhlI BcuI Sse83871 CCTGCA^GG 1 1641 SbfI SdaI Xhoi C^TCGAG 1 966 Sfr274I PaeR7I SlaI AclI AA^CGTT 2 2943 Psp1406I AclI AA^CGTC 2 2336 AlwNI CAGNNN^CTG 2 2336 ApaLI G^TGCAC 2 2335 ArsI (8/13) GACNNNNNTTYG (11/6) 2 851 2 1221 1332 BosI GAAGAC (2/6) 2 1332 BciVI GTATCC (6/5) 2 2034 Puil BsuI 2 363 1 2 363 BriI A^GATCT 2 3638 1 Bg1II A^GATCT 2 3638 1 BarrI ACTGGG (5/	PvuII	CAG^CTG	1	642		
SapI GCTCTTC (1/4) 1 1208 BspQI LguI PciSI SnaBI TAC^CTA 1 357 BstSNI Ecol051 SpeI A^CTAGT 1 18 AhlI BcuI Sse83871 CCTGCA^GG 1 1641 SbfI SdaI XhoI C^TCGAG 1 966 Sfr274I PaeR7I SlaI AclI AA^CGT 2 2943 Psp1406I AclI AA^CGTC 2 2336 AlwNI CAGNNN^CTG 2 236 ApaLI G^TGCAC 2 2385 ArsI (8/13) GACNNNNNTTYG (11/6) 2 851 2 1221 1322 1221 BbsI GAGAC (2/6) 2 1332 BciVI GTATCC (6/5) 2 2034 - 2 3561 2 3561 Bg1II A^GATCT 2 3638 BmrI ACTGGG (5/4) 2 313 - BseYI CCCAGC (-5/-1) <td< td=""><td>SacII</td><td>CCGC^GG</td><td>1</td><td>740</td><td></td><td>Sfr303I KspI SqrBI Cfr42I</td></td<>	SacII	CCGC^GG	1	740		Sfr303I KspI SqrBI Cfr42I
SnaBI TAC^GTA 1 357 BstSNI Ecol05I SpeI A^CTAGT 1 18 AhlI BcuI Sse83871 CCTGCA^GG 1 1641 SbfI SdaI Stol CCTGCAGG 1 966 Sfr274I PaeR7I SlaI AclI AA^CGTT 2 2943 Psp1406I AclI AA^CGTC 2 2138 Cail PstNI AlwNI CAGNNN^CTG 2 2139 Alw44I VneI 2 3385 - - - ApaLI G^TGCAC 2 2139 Alw44I VneI 2 3385 - - - Arsi (8/13) GACNNNNNTTYG (11/6) 2 851 - BbsI GAAGAC (2/6) 2 1218 BpiI BstV21 2 3636 - - - BruI ACTGGG (5/4) 2 1332 - BfuI BsuI BruI ACTGGG (5/4) 2 3638 - -	SapI	GCTCTTC(1/4)	1	1208		
See83871 CCTGCA^GG 1 1641 SbfI SdaI Xhoi C^TCGAG 1 966 Sfr274I PaeR7I SlaI Acli AA^CGTT 2 2943 Psp1406I Acli CAGNNN^CTG 2 3336	SnaBI	TAC^GTA	1	357		
Sse83871 CCTGCA^GG 1 1641 SbfI SdaI XhoI C^TCGAG 1 966 Sfr274I PaeR7I SlaI AclI A^CGTT 2 2943 Psp1406I AclI A^CGTT 2 2943 Psp1406I AlwNI CAGNNN^CTG 2 1584 Cail PstNI 2 236	SpeI	A^CTAGT	1	18		AhlI BcuI
AclI AA^CGTT 2 2943 Psp1406I AlwNI CAGNNN^CTG 3316 3316 1000000000000000000000000000000000000	-	CCTGCA^GG	1	1641		SbfI SdaI
AclI AA^CGTT 2 2943 Psp1406I AlwNI CAGNNN^CTG 3316 3316 1000000000000000000000000000000000000	XhoI		1			
AlwNI CAGNNN^CTG 2 3316 ApaLI G^TGCAC 2 2139 Alw44I VneI 2 3385 Alw44I VneI ArsI (8/13) GACNNNNNTTYG (11/6) 2 851 2 1221 BosI GAAGAC (2/6) 2 1218 BbsI GAAGAC (2/6) 2 1332 Bful Bsul BciVI GTATCC (6/5) 2 2034 - Bful Bsul BglII A^GATCT 2 3638 - Bful Bsul BseYI CCCAGC (-5/-1) 2 313 - Bmul BspHI T^CATGA 2 2129 - - BssSI CACGAG (-5/-1) 2 2129 - - BspHI T^CATGA 2 3553 - -						
AlwNI CAGNNN^CTG 2 1584 Cail PstNI 2 2236 2236 2236 ApaLI G^TGCAC 2 2139 Alw44I VneI 2 3385 3385 3385 3385 ArsI (8/13) GACNNNNNTTYG(11/6) 2 851 Dawn Dawn BbsI GAAGAC (2/6) 2 1221 Dawn Dawn Dawn BciVI GTATCC (6/5) 2 2034 - Bful Bsul BglII A^GATCT 2 3638 Dawn BgrII ACTGGG (5/4) 2 313 - BmuI BseYI CCCAGC (-5/-1) 2 2763 Dawn BspHI T^CATGA 2 2129 2129 BspHI CACGAG (-5/-1) 2 2545 Ccil PagI BssSI CACGAG (-5/-1) 2 1998 - Baul Bst2BI						
ApalI G^TGCAC 2 2139 Alw44I VneI 2 3385 3385 3385 ArsI (8/13) GACNNNNNTTYG (11/6) 2 851 2 BbsI GAGAC (2/6) 2 1221 2 BbsI GAAGAC (2/6) 2 1218 BpiI BstV2I BciVI GTATCC (6/5) 2 2034 - BfuI BsuI BglII A^GATCT 2 3638 - BruI BmrI ACTGGG (5/4) 2 313 - BmuI BseYI CCCAGC (-5/-1) 2 2763 - BmuI BspHI T^CATGA 2 2129 - - BssSI CACGAG (-5/-1) 2 2545 CciI PagI BssSI CACGAG (-5/-1) 2 1998 - BauI Bst2BI	AlwNI	CAGNNN^CTG				Cail PstNI
Apall G^TGCAC 2 2139 Alw441 Vne1 2 3385 3385 3385 ArsI (8/13) GACNNNNNTTYG(11/6) 2 851 Bpil BstV21 BbsI GAAGAC (2/6) 1218 Bpil BstV21 2 1332 1332 1332 BciVI GTATCC (6/5) 2 2034 - Bful Bsul 2 3561 2 363 - 1218 BglII A^GATCT 2 1312 - - BmrI ACTGGG (5/4) 2 3638 - - BseYI CCCAGC (-5/-1) 2 1051 - BmuI 2 1051 Gsal PspFI - - - BspHI T^CATGA 2 2545 Ccil PagI BssSI CACGAG (-5/-1) 2 1998 - Baul Bst2BI						
Arsi (8/13) GACNNNNNTTYG (11/6) 2 851 Bbsi GAAGAC (2/6) 2 121 Bbsi GAAGAC (2/6) 2 1218 Bpil BstV2I BciVI GTATCC (6/5) 2 2034 - Bful BsuI BglII A^GATCT 2 1814 - - BmrI ACTGGG (5/4) 2 313 - Bmul BseYI CCCAGC (-5/-1) 2 1051 Gsal PspFI BspHI T^CATGA 2 2129 - BssSI CACGAG (-5/-1) 2 2545 Ccil PagI BssSI CACGAG (-5/-1) 2 1998 - Baul Bst2BI	ApaLI	G^TGCAC				Alw44I VneI
Arsi (8/13) GACNNNNNTTYG (11/6) 2 851 2 1221 1221 Bbsi GAAGAC (2/6) 2 1218 Bpil BstV2I 2 1332 1332 1332 1332 BciVI GTATCC (6/5) 2 2034 - Bful Bsul BglII A^GATCT 2 1814 - 1814 2 3638 - - Bmul BmrI ACTGGG (5/4) 2 313 - Bmul 2 2763 - - - - BseYI CCCAGC (-5/-1) 2 1051 Gsal PspFI 2 2129 - - - - BspHI T^CATGA 2 2545 Ccil PagI 2 3553 - - - BssSI CACGAG (-5/-1) 2 1998 - Baul Bst2BI	TTP G TT T	0 100110				
BbsI GAAGAC (2/6) 2 1221 BciVI GTATCC (6/5) 2 2034 - Bful Bsul BglII A^GATCT 2 1814 - - BmrI ACTGGG (5/4) 2 313 - Bmul BseYI CCCAGC (-5/-1) 2 1051 Gsal PspFI 2 2129 2129 - - BssSI CACGAG (-5/-1) 2 2545 Ccil PagI BssSI CACGAG (-5/-1) 2 1998 - Baul Bst2BI	Arst	(8/13) GACNNNNNNTTYG $(11/6)$				
BbsI GAAGAC (2/6) 2 1218 Bpil BstV2I 2 1332 1332 1332 BciVI GTATCC (6/5) 2 2034 - Bful Bsul BglII A^GATCT 2 1814 - - BmrI ACTGGG (5/4) 2 313 - Bmul BseYI CCCAGC (-5/-1) 2 1051 Gsal PspFI 2 2129 2129 - - BspHI T^CATGA 2 2545 Ccil PagI BssSI CACGAG (-5/-1) 2 1998 - Baul Bst2BI	111.01	(0,10,000000000000000000000000000000000				
BciVI GTATCC (6/5) 2 2034 - Bful Bsul BglII A^GATCT 2 1814 - - BmrI ACTGGG (5/4) 2 313 - Bmul BseYI CCCAGC (-5/-1) 2 1051 Gsal PspFI 2 2129 - - - BspHI T^CATGA 2 2545 Ccil PagI 2 3553 - Baul Bst2BI -	BhsT	GAAGAC(2/6)				Bpil BstV21
BciVI GTATCC (6/5) 2 2034 - Bful Bsul 2 3561 - Bful Bsul BglII A^GATCT 2 1814 2 3638 - Bmul BmrI ACTGGG (5/4) 2 313 - Bmul BseYI CCCAGC (-5/-1) 2 1051 Gsal PspFI 2 2129 - - - BspHI T^CATGA 2 2545 Ccil PagI 2 3553 - Baul Bst2BI -	DDSI	GRAGAC (27 0)				DDII DSCASI
BglII A^GATCT 2 3561 BmrI ACTGGG (5/4) 2 3638 BmrI CCCAGC (-5/-1) 2 2763 BseYI CCCAGC (-5/-1) 2 1051 Gsal PspFI 2 2129 2129 2129 2129 BspHI T^CATGA 2 2545 Ccil PagI 2 3553 3553 3553	BOINT	CTATCC(6/5)			_	Rfut Reut
BglII A^GATCT 2 1814 2 3638 BmrI ACTGGG (5/4) 2 313 - BmuI 2 2763 2 2763 - - BseYI CCCAGC (-5/-1) 2 1051 Gsal PspFI - BspHI T^CATGA 2 2545 Ccil PagI - BssSI CACGAG (-5/-1) 2 1998 - Baul Bst2BI	DCIVI	GIAICC (0/ 5)				BIUI BSUI
2 3638 BmrI ACTGGG (5/4) 2 313 - BmuI 2 2763 2 263 BseYI CCCAGC (-5/-1) 2 1051 Gsal PspFI 2 2129 2 2 2129 BspHI T^CATGA 2 2545 Ccil PagI 2 3553 3553 Baul Bst2BI	Daltt					
BmrI ACTGGG(5/4) 2 313 - BmuI 2 2763 2763 2763 BseYI CCCAGC(-5/-1) 2 1051 Gsal PspFI 2 2129 2129 2129 BspHI T^CATGA 2 2545 Ccil PagI 2 3553 2 3553 BssSI CACGAG(-5/-1) 2 1998 - Baul Bst2BI	BYIII	A GAICI				
BseYI CCCAGC (-5/-1) 2 2763 BspHI T^CATGA 2 2129 BssSI CACGAG (-5/-1) 2 2545 Ccil PagI 2 3553 2553 2129	DmrT	$\lambda C = C C (5 / 1)$			_	DminT
BseYI CCCAGC (-5/-1) 2 1051 Gsal PspFI 2 2129 2129 BspHI T^CATGA 2 2545 Ccil PagI 2 3553 2553 Baul Bst2BI	BIILLT	ACIGGG(3/4)			-	DIIUT
BspHI T^CATGA 2 2129 BssSI CACGAG(-5/-1) 2 2545 Ccil PagI 2 3553 BssSI CACGAG(-5/-1) 2 1998 - Baul Bst2BI	D 117					
BspHI T^CATGA 2 2545 Ccil PagI 2 3553 BssSI CACGAG(-5/-1) 2 1998 - Baul Bst2BI	BSEYL	CCCAGC (-5/-1)				GSAI PSPEI
2 3553 BssSI CACGAG(-5/-1) 2 1998 - Baul Bst2BI	5					
BssSI CACGAG(-5/-1) 2 1998 - Baul Bst2BI	BSPHI	TYYCATGA				CCII Pagl
	BssSI	CACGAG(-5/-1)			-	BauI Bst2BI
2 3382			2	3382		

BtgZI	GCGATG(10/14)	2	368 -	
5		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, Bpul4I, 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, Eco065I, EcoRV, EcoT22I, EgeI, EheI, Esp3I, FbaI, FseI, FspAI, HpaI, I-CeuI, I-PpoI, I-SceI, KasI, KflI, KpnI, KroI, Ksp221, 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 37°C 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 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-LC** plasmid DNA can be isolated in large quantities.

Cloning into TGEX-LC

In Silico Clone Design

A complete IGKV3-20 leader sequence METPAQLLFLLLWLPDTSG 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 inframe 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 37°C, respectively. Please, consult the documentation of your restriction enzyme provider for buffer and optimal double digestion conditions.

Primer Design for Restriction Cloning with the BspEl Site

Oligo1 is an example of primer designed to amplify a VL domain sequence and clone it into the BspEI site. Six extranucleotides 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 NNNNNTCCGGA extension is added 5' to the VL domain primer.

Oligo1 5'-NNNNNTCCGGA-VL-start

Primer Design for Restriction Cloning with the Bsal Site

Oligo2 is an example of primer designed to amplify the VL domain from the end of the J region and includes a Bsal site compatible with **TGEX-LC** cloning site. Bsal is a type IIS restriction enzyme that cuts outside of its recognition site. Bsal 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 Bsal site situated symmetrically to the vector will generate a complementary overhang. After digestion and ligation, the two Bsal 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 Bsal site close to the end (source New Englands BioLabs).

Oligo2 5'-NNggtctcNTTCG-JH-end

Alternative to Bsal Sites

All restriction enzymes that generate 4-bases 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 VL domain contains another Bsal 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 assi	ance, call, write, fax, or email us at:
--	---

Antibody Design Labs 4901 Morena Blvd, Suite 203 San Diego, CA 92117 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)

References

- 1. LOGAN, J., & SHENK, T. (1984). ADENOVIRUS TRIPARTITE LEADER SEQUENCE ENHANCES TRANSLATION OF MRNAS LATE AFTER INFECTION. *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA*, *81*(12), 3655–9.
- MARIATI, HO, S. C. L., YAP, M. G. S., & YANG, Y. (2010). EVALUATING POST-TRANSCRIPTIONAL REGULATORY ELEMENTS FOR ENHANCING TRANSIENT GENE EXPRESSION LEVELS IN CHO K1 AND HEK293 CELLS. *PROTEIN EXPRESSION AND PURIFICATION*, 69(1), 9–15.
- 3. 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. 22:3385-93*.
- 4. SAMBROOK, J., FRITSCH, E.F., AND MANIATIS, T. (1989). IN MOLECULAR CLONING: A LABORATORY MANUAL. COLD SPRING HARBOR LABORATORY PRESS, NY, VOL. 1, 2, 3.
- 5. BACKLIWAL G, HILDINGER M, KUETTEL I, DELEGRANGE F, HACKER DL, WURM FM. (2008). VALPROIC ACID: A VIABLE ALTERNATIVE TO SODIUM BUTYRATE FOR ENHANCING PROTEIN EXPRESSION IN MAMMALIAN CELL CULTURES. BIOOTECHNOL BIOENG, 101(1):182-9.

This product is subject to Antibody Design Labs Terms & Conditions of Sales available online at http://www.abdesignlabs.com/terms/. © 2015 Antibody Design Labs. All rights reserved.