



TGEX-SCBlue-Zeo Expression Vector

INSTRUCTION MANUAL

TGEX-SCBlue-Zeo Mammalian Expression Vector Catalog #: MX024 Version: A1.2 – February 2024

Table of Contents

Limited Use License for TGEX Vector Series	
Description	5
Introduction	5
Content, Shipping & Storage	5
Limited Product Warranty	5
TGEX Vector Series	6
Vector Map	7
Cloning Site	7
Feature Table	8
Restriction Site Summary	9
Experimental Procedures	11
General Molecular Biology Techniques	11
Plasmid Maintenance	11
Cloning into TGEX-SCblue-Zeo	11
Sequencing of Inserts	11
Antibody Expression	12
Appendix	13
MSDS Information	13
Quality Control	13
Technical Support	13
References	13

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 implicitly 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 info@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 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™-SCblue-Zeo** 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-Zeo** 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 Sfil 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-Zeo	20 μl 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-SCBlue-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[™] vectors for desired isotypes

VECTOR	CATALOG	ISOTYPE	DESCRIPTION
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™-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[™] vectors 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

$\underline{\mathsf{TGEX}}^{\mathsf{m}}$ 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-SCBlue-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 5917 bp.



Cloning Site

Following is an illustration of **TGEX-SCBlue-Zeo** cloning site from the EcoRI site and onward. The scFv 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.

	tgex-S3				EcoRI
					I
971	GAAAGGCGTC	TAACCAGTCA	CAGTCGCAAG	TTTAAACGGA	TCTCTAGC <u>GA</u>
			pelBK leader seque	nce	
			MetGLuThrA	spThrLeuLe	uLeuTrpVal
1021	<u>ATTC</u> GGCTTG	GGCCGCCACC	ATGGAGACAG	ACACACTCCT	GCTATGGGTA
		Sfil Bgll		lacZ	
				Γ	
	LeuLeuLeuL	euAlaAlaGl	nProAlaMet	Ala	
1071	CTGCTGCTCT	TAGCGGCCCA	GCCGGCCATG	GCGCCCAATA	CGCAAACCGC
1121	CTCTCCCCGC	GCGTTGGCCG	ATTCATTAAT	GCAGCTGGCA	CGACAGGTTT
1171	CCCGACTGGA	AAGCGGGCAG	TGAGCGCAAC	GCAATTAATG	TGAGTTAGCT
1221	CACTCATTAG	GCACCCCAGG	CTTTACACTT	TATGCTTCCG	GCTCGTATGT
					Alpha-B-gal
					Metïn
1271	TGTGTGGGAAT	TGTGAGCGGA	TAACAATTTC	ACACAGGAAA	CAGCTATGAC

	rMetIleThr	AspSerLeuA	laValValLe	uGlnArgArg	AspTrpGluA
1321	CATGATTACG	GATTCACTGG	CCGTCGTTTT	ACAACGTCGT	GACTGGGAAA
	snProGlyVa	lThrGlnLeu	AsnArgLeuA	laAlaHisPr	oProPheAla
1371	ACCCTGGCGT	TACCCAACTT	AATCGCCTTG	CAGCACATCC	CCCTTTCGCC
	SerTrpArgA	snSerGluGl	uAlaArgThr	AspArgProS	erGlnGlnLe
1421	AGCTGGCGTA	ATAGCGAAGA	GGCCCGCACC	GATCGCCCTT	CCCAACAGTT
			Sfil Bgll	Linker	hulgG1-FC
			Sfil Bgll _Γ −−−- −−	Linker	hulgG1-FC
	uArgSerLeu	Asn	Sfil Bgll _「 - <i>G1yPr</i>	Linker OGlyGlyPro	hulgG1-FC
1471	uArgSerLeu GCGCAGCCTG	Asn AATTAAAATA	Sfii BgII _Γ −−- − <i>GlyPt</i> Gatag <u>GgcC</u> C	Linker oGlyGlyPro GGGA <u>GGCC</u> CC	hulgG1-FC GluProLysS GAGCCCAAAT
1471	uArgSerLeu GCGCAGCCTG	Asn AATTAAAATA	Sfil Bgli _Γ −−− − <i>GlyPr</i> Gatag <mark>ggcc</mark> c	Linker oGlyGlyPro GGGA <mark>GGCC</mark> CC	hulgG1-FC GluProLysS GAGCCCAAAT
1471	uArgSerLeu GCGCAGCCTG Pro -> Ser mut	Asn AATTAAAATA ation	Sfil Bgll r ── − GlyPr GATAG <mark>GGCC</mark> C	Linker oGlyGlyPro GGGA <u>GGCC</u> CC	hulgG1-FC GluProLysS GAGCCCAAAT
1471	uArgSerLeu GCGCAGCCTG Pro -> Ser mut erSerAspLy	Asn AATTAAAATA ation sThrHisThr	Sfil Bgll [G1yPr GATAGGGCCC CysProProC	Linker oGlyGlyPro GGGAGGCCCC ysProAlaPr	hulgG1-FC GluProLysS GAGCCCAAAT OGluLeu

Feature Table

The features of **TGEX-SCBlue-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.
lacZ	1104-1495	LacZ fragment with lac promoter.
Alpha-B-gal	1316-1486	B-gal alpha fragment.
Human IgG1-FC CDR	1511-2210	Sequence encoding the human IgG1 FC sequence comprising the CH2, and CH3 domains with the hinge region. The sequence is intronless; the C-terminal lysine residue is encoded.
IRES	2235-2808	Internal Ribosome Entry Site.
Zeo	2842-3216	Sh ble gene from <i>Streptoalloteichus hindustanus</i> conferring resistance to Zeocin.
WPRE	3225-3813	Woodchuck hepatitis virus post-transcriptional regulatory element.
BGpA	3829-3927	Rabbit beta-globin polyadenylation signal sequence.
pMB1 origin	4001 -4620	pBR322 origin for replication in <i>E. coli</i> with a temperature-sensitive high copy-number phenotype (Lin-Chao 1992).
TEM1 beta-lactamase	5635-4775	Ampicillin resistance for selection in <i>E. coli</i> .

Restriction Site Summary

AlfI (10/12) GCANNNNNTYG(11/6) 3179 ArsI (8/13) GCANNNNNTYG(11/6) 2386 AspA2I BlnI XmaJI BamHI C^CTAGO 1 2386 AspA2I BlnI XmaJI BamHI C^CCACCC 1 2386 AspA2I BlnI XmaJI BamHI C^CCACCC 1 2386 AspA2I BlnI XmaJI Borl G^CCCCC 1 2386 BasHII Paul Ptel CapCT (11/12) CGANNNNNTG(12/10) 407 607 EcoNI CCUNN*NNARGG 1 1189 BstENI XagI EcoNI GCUNN*NNARGG 1 1313 RigI GSUI CTGSA0 (16/14) 1 4333 - Kpai GGCCGCC 1 2476 Acc651 Asp7181 MauBI CCCCGCCC 1 213 AsuMHI BmtI Bsp01 Noti GC^CCCCCC 1 213 AsuMHI BmtI Bsp01 Noti GCCCGCC 1 2147 Bcollisli EcolCKI Ecol3kI Paci AccAct 1 2167 Paci Noti GCCCGCC 1 2286 Paci Pinic Sci	Enzyme	Site	Nb	Position	Strand	Isoschizomers
Arsi (8/13) GACINNENNTYG (11/6) 1 <t< td=""><td>AlfI</td><td>(10/12) GCANNNNNTGC $(12/10)$</td><td>1</td><td>3179</td><td></td><td></td></t<>	AlfI	(10/12) GCANNNNNTGC $(12/10)$	1	3179		
AVEIL C*CTAGC 1 2386 AspA21 Bin1 XmaJ1 BamHI GGATCC 1 2824 AspA21 Bin1 XmaJ1 BegI (10/12) CCANNUNUNTCC (12/10) 5564 BesHI Faul Ptel Speil GCCCCC 1 2878 BesHI Faul Ptel Capel (11/13) CANNUNUTCG (12/10) 407 BetENI XagI EcoNI CCTMA*CG (12/10) 407 BetENI XagI EcoNI GCCUM*NUNUTT (13/8) 1 748 Fail (8/13) AASUNUNUTT (13/8) 1 748 Fail GCCGCCC 1 3113 BigI GUIT GGAR(16/14) 1 4933 - BpmI MuBI GCCGCCC 1 210 PiuT SfoT SapT NarI GCCGCCC 1 2117 CaNH Noti GCCGCCCC 1 2127 Dail PiuT SfoT SapT Noti GCCGCCCC 1 2177 CaNH Noti GCCGCCCC 1 2167 Acot T Pail	Arst	(8/13) GACNNNNNTTYG $(11/6)$	1	851		
Bamil Greened 1 2824 Implified Similar Simil	AvrTT	(°, 1°, °, °, °, °, °, °, °, °, °, °, °, °, °	1	2386		AspA2T Bint Xma.IT
Each (10/12) CGANNINNINGT (12/10) 1 5364 BeyII CCTNAG(15/-2) 1 930 BesPII CCCCCC 1 2878 BasHII Paul PteI CapCI (11/13) CAANNINGT (12/10) 1 407 EcoNI CCTNNINGG (12/10) 1 407 EcoNI CCTNNINGG (12/10) 1 407 Fall (3/13) AACNNINGT (13/8) 1 784 FseI GCCCCC 1 1019 FseI GCCCCC 1 2877 NarI GCACCC 1 2676 Acc651 Asp7181 MauBI CCCCCCC 1 2877 NarI GCACCCC 1 2100 Dint Egel Eacl KasI Mly1131 PluTI SfoI SapD1 NotI CCCCCCCC 1 2100 Dint Egel Eacl KasI Mly1131 PluTI SfoI SapD1 NotI CCCCCCCC 1 2100 Dint Egel Eacl KasI Mly1131 PluTI SfoI SapD1 NotI CCCCCCCC 1 2100 Dint Egel Eacl KasI Mly1131 PluTI SfoI SapD1 NotI CCCCCCCC 1 2100 Dint Egel Eacl KasI Mly1131 PluTI SfoI SapD1 NotI CCCCCCCC 1 2100 Dint Egel Eacl KasI Mly1131 PluTI SfoI SapD1 NotI CCCCCCCC 1 2100 Dint Egel Eacl KasI Mly1131 PluTI SfoI SapD1 NotI CCCCCCCC 1 2100 Dint Egel Eacl KasI Mly1131 PluTI SfoI SapD1 NotI CCCCCCCC 1 2100 Dint Egel Eacl KasI Mly1131 PluTI SfoI SapD1 NotI CCCCCCCC 1 2100 Dint Egel Eacl KasI Mly1131 PluT SfoI SapD1 NotI CCCCCCCC 1 2213 AcuNNIN THE Fact I BapO1 NotI CCCCCCCC 1 225 PacI PmEI GTT"AAAC 1 1000 MasI SacI GACCT^C 1 2836 SapI GCCCTCC (1/4) 1 2179 - BapQ1 LguI PciSI SgrAI CCCCCCQVG 1 2856 SnaBI TACCTA 1 357 BatSNI EcolOSI SpeI A'CTAGT 1 357 BatSNI EcolOSI SpeI A'CTAGT 1 357 BatSNI EcolOSI SgrAI CCCCCCQVG 1 2656 AcaiI PatNI Acar CACCGC 2 1195 Bap120I PagONI Acar CACCGC (4/8) 2 2109 - PagCI Acar CACCGC (4/8) 2 2109 - PagCI AlwNI CACNNN'CTG 2 1554 CaiI PatNI Acar CACCGC (4/8) 2 2109 - PagCI Bap1407I TGTACA 2 2645 BagI GCCCC 2 1495 Bap1201 PagONI Acar CACCAG (16/14) 2 1790 - BasSI CACCAG (16/14) 2 1790 - BasSI CACCAG (16/14) 2 1790 - BasSI CACGAG (16/14) 2 1790 - 2 3645 BasII TGCAG (16/14) 2 1390 - 2 3645 BasII CCC'NNAGG 2 1634 EcoNII AxyI Bas21I BasII CCCNNYCTC 2 753 AdeT BasII CCCNNYCTC 2 753 AdeT	BamHT	C^CATCC	1	2824		MSPM21 DITT Mildol
Degli (100.127) CSAMANNA DEC (12710) 1 330 BsePI (20000) CCTRACC (12710) 1 407 EcoRI (11/13) CANNANCTG (12/10) 1 407 EcoRI (2000) CANNANCTT (13/8) 1 784 Fall (8/13) AAGNNNNCTT (13/8) 1 784 Fall (8/13) AAGNNNNCTT (13/8) 1 784 GSUI (2000) CTCGGA (16/14) 1 4933 - BpmI Kpni (3000) GCCC 1 2877 Nari (3000) GCCC 1 2877 Nari (3000) GCCC 1 2877 Nari (3000) GCCC 1 2100 Din (2000) CCCC 1 Nari (3000) GCCC 1 2107 Nari (3000) GCCC 1 2110 Din (3000) CCCCC 1 2107 Nari (3000) GCCC 1 2117 Nari (3000) GCCC 1 2117 Nari (3000) GCCC 1 2117 Nari (3000) GCCCC 1 2117 Nari (3000) GCCC 1 2117 Sci (3000) GCCC 1 2117 Sci (3000) GCCC 1 2147 Sci (3000) GCCC 1 2147 Sci (3000) GCCC 1 253 Sci (3000) GCCC 1 253		(10/12) CCANINININITICC $(12/10)$	⊥ .1	5264		
Bpuloi Clinko((5)/2) 1 330 BSEPI CCCCCC 1 2378 BSHII Paul Ptel CapCI (11/13) CAANNINGTGG (12/10) 1 407 407 EcoNI CCTNN'NNAGG 1 1019 BENEII (3/13) AACNNINNCTT (13/8) 1 784 Fasi GCCCCC 1 3113 RigI Guil CTGCAG (16/14) 1 4933 - BpmI Kpni GCCCCCC 1 2676 Acc651 Asp7181 AsuNHT BatI Bsp01 Nati GCCCCCC 1 210 Dint EgeT East KasI Miyl131 PuluT 5foI SapDI Noti GCCCCCC 1 217 CciNT SapDI CciNT Noti GCCACCC 1 2147 EcoRT21 Mph1031 Zsp21 Alei Noti GCCACCC 1 2147 EcoT21 Mph1031 Zsp21 Paic Oli CACNTOT 1 2259 Paic Paic Pamal GCCCCCT(1 2336 Scill Scill Scill Scill Sc	BCGI Devil 0 T	(10/12) CGANNNNN IGC $(12/10)$	1	020		
BBEP1 Ground Construction Description Description Description CCPCI (1/13) CAANININNEGTEG (12/10) 407 BatENI XagI EcoRI GrantC 1 1789 BatENI XagI Fall (8/13) AAGNININNGTG (12/16) 784	BPUIUI	CCTNAGC(-57-2)	1	930		Desuit Dest Dest
CSPC1 (11/13)CARMNNNGTG [12/10] 40/ ECONI (CTNN*NNAGG 1 17/10] 40/ ECONI (CTNN*NNAGG 1 1019 Fall (8/13)AAGNNNNTT(13/8) 784 Fsel GGCGGCC 1 3113 Nig1 GGU CTGGAG(16/14) 1 4933 - BpmI KpnI GGTAC^C 1 2676 Acc651 Asp7181 MauBI CCCGGCG 1 217 NarI GG^CGCCC 1 1100 Dinl Egel Ehel KasI Mly1131 PluTI SGI SspD1 Noti GC^GGCCC 1 213 AsUNHI Emtl BspO1 Noti GC^GGCCC 1 213 AsUNHI Emtl BspO1 Noti GCCGGCCC 1 2147 Eco7221 Mph1031 Zsp21 Oli1 CACNM*NNGTG 1 1642 Alei Pcil A^CATGT 1 2725 PscI PmacI CAC^GGG 1 2549 AcvI BbrPI Eco721 PmlI PspCI PmacI CAC^GGG 1 2549 AcvI BbrPI Eco721 PmlI PspCI SaeI GACCTC 1 583 Ecl13611 Eco1CRI Eco53kI Psp124BI SstI SalI C^TCGAC 1 2836 SapI GCTCTC (1/4) 1 2179 - BspQI LguI PciSI SpFAI CCACGTA 1 2830 Xhoi C^TCGAG 1 2830 Xhoi C^TCGAG 1 2830 Xhoi C^TCGAG 1 2830 Xhoi C^TCCAG 2 154 CaiI PstNI Xbai T^CTAGA 1 2830 Xhoi C^TCCAG 2 154 CaiI PstNI AcACCTG 2 4366 ApaI GGCCCC 2 1455 Bsp120I PspOMI CACNNACTG 2 2564 ApaI GGCCCC 2 2348 Ball TGC^CCA 2 2843 MlsI MluNI Mox20I MscI Msp20I ApaI GGGCCC 2 1495 Bsp120I PspOMI CACNNACTG 2 2348 Ball TGC^CCA 2 2348 Ball TGCCCA 2 2348 Ball TGCCCCA 2 2348 Ball TGCCCA 2 2348 Ball TGCCCA 2 2348 Ball TGCCCA 2 2348 Ball TGCCCA 2 2448 Ball TGCCCA 2 2448 Ball TGCCCA 2 2448 Ball TGCCCA 2 2448 Ball TGCCCA 2 2644 ApaI GGGCCC 2 1695 Bsp120I PspOMI 2 5645 Bsp120I PspOMI 22564 ApaI GGGCCC 2 1635 Bsp120I PspOMI 2 5645 Bsp120I PspOMI 2 2109 - FacCI 2 5645 Bsp120I PspOMI 2 2104 2 5645 Bsp120I PspOMI 2 2 2543 Bsp120I PspOMI 2 2004 2 5645 Bsp120I	BSEPI		1	28/8		BSSHII Paul Ptel
EcoNI CCTNN*NNNAGG 1 1789 BstENI XagI EcoNI CC*ANTC 1 1019 Fall (8/13) AAGNNNNTT(13/8) 1 784 Fall GCCG*CC 1 3113 RigI GSUI CTCGAG(16/14) 1 4933 - BpmT KpnI GCTCCC 1 2877 NarI GC*CCCC 1 2877 NarI GC*CCCC 1 2100 DinI EgeI EheI KasI Mly1131 MeI GC*CCCCC 1 2110 DinI EgeI EheI KasI Mly1131 NoLI GC*CCCCC 1 2213 AsuMH Emi BspOI NoLI GC*CCCCC 1 2217 CciNI NSI ATCCA^T 1 2147 EcoT21 Mph11031 Zsp21 Olif CACNN*NNGTG 1 1642 Alei PriI A*CATGT 1 2725 PscI PmacI CAC*GTG 1 2254 AcvI BbeFPI Eco721 PmlI PspCI PmacI CAC*GTG 1 2836 SacI GACC*C 1 583 Ecl1361 EcoTCRI Eco53kI Fsp124B1 SatI CC*CCGGVG 1 2355 SalI G*CCCAC 1 2856 SapI GC*CCTC 1/4) 1 2179 - BstSNI Eco1051 ShII CACCTGG 1 2856 SapI GC*CTCC 1/4) 1 2179 - BstSNI Eco1051 ShII CACCTGG 1 2856 SapI GC*CTCC 1/4) 1 2179 - BstSNI Eco1051 ShII CC*CCGGVG 1 2855 SnaBI TAC*CTA 1 357 BstSNI Eco1051 ShII CACCTGC 2 1554 CalI PstNI XbaI T*C*TAGA 1 2830 AcvI BbeFI Eco72 MscI MscI Stal AcvCTAGC 2 1554 CalI PstNI AarI CACCTGC 4/8) 2 2109 - PaqCI AlwNI CAGNN*CTG 2 1554 CalI PstNI ApaI GGCCCA 2 2483 MlsI MluNI Mox20I MscI Msp20I Ball TGC*CCA 2 2348 BalI TGC*CCA 2 1968 Bsp120I PspOMI 2 2564 AlwNI CAGNN*CTG 2 1554 CalI PstNI ApaI GGCCCC 2 1495 Bsp120I PspOMI 2 2348 BalI TGC*CCA 2 2483 MlsI MluNI Mox20I MscI Msp20I BalI TGC*CCA 2 1968 BsrGI BstAUI SasI CACCAG(16/14) 2 1790 - 2 2344 Bsp1407I T*GTACA 2 1908 BsrGI BstAUI SasI CACCAG(-5/-1) 2 4128 - BauI Bst2BI BssSI CACGAG(-5/-1) 2 4128 - BauI Bst2BI CaCNNNYCTG 2 7593 Ade	CspCl	(11/13) CAANNNNNGTGG (12/10)	1	407		
EcoRI G'AATC 1 1019 FalI (8/13)AAGNNNNTT(13/8) 1 784 FseI GGCCGCGC 2 1 3113 RigI GGTAC^C 1 2676 Acc651 Asp7181 MuBI CGCGCGC 1 2877 MaUBI CGCGCGC 1 2100 DinI EgeI EheI KasI Mly1131 PluTI SGT SspD1 NhEI GCCGCC 1 213 AsuNHI EmtI BspD1 NotI GCCGCCC 1 3217 CciNI NSII ATCCA'T 1 2147 Eco721 Mph1031 Zsp21 Alex Alex Alex Alex Factor 1 275 PscI PmaCI CAC^GTG 1 2549 AcvI EbrPI Eco721 PmlI PspCI PmaCI CAC^GTG 1 2549 AcvI EbrPI Eco721 PmlI PspCI PmaCI CAC^GTG 1 2549 AcvI EbrPI Eco721 PmlI PspCI SacI GACCTC 1 583 Eci13611 Eco1CRI Eco53kI SapI GCTCTC 1 2836 SapI GCTCTC 1 2836 SapI GCTCTC 1 2836 SapI ACTAGT 1 2836 SapI ACCTAGT 1 2836 Xhai C'TCGAG 1 2836 Xhai C'TCCAG 2 109 - PaqCI Xhai T'CTAGA 1 2830 Xhai C'TCCAG 2 1554 CaiI PstNI CACONN^CTG 2 1554 ApaI GGCCCC 2 1455 Bsp120I PspOMI CACONN^CTG 2 2348 Ball TGC^CCA 2 2443 MlsI MluNI Mox20I MscI Msp20I ApaI GGCCCC 2 1455 Bsp120I PspOMI ApaI GGCCCC 2 1455 Bsp120I PspOMI ApaI GGCCCC 2 1455 Bsp120I PspOMI Ball TGC^CCA 2 2443 MlsI MluNI Mox20I MscI Msp20I Ball TGCACG 1 2954 Ball GAATGC (1/-1) 2 2361 - Mva1269I PctI Bsp1407I T'GTACA 2 1908 BsrGI BstAUI Bsp1407I T'GTACA 2 1908 BsrGI BstAUI BssSI CACGAG(-5/-1) 2 4128 - BauI Bst2BI BssSI CACGAG(-5/-1) 2 4128 - BauI Bst2	EcoNI	CCTNN^NNNAGG	1	1789		BstENI XagI
Fali (8/13) AAGNNNNCTT (13/8) 1 784 Fsei GGCCGCCC 1 313 RigI Gsui CTGCAG(16/14) 1 4933 - April Kpni GGCCGCCC 1 2676 Acc651 Asp7181 MauBI CG^CGCCGC 1 2877 PluTISf01 Ssp01 Nati GC^CGCCC 1 213 AsuMNI EmtL Bsp01 Noti GC^CGCCC 1 213 AsuMNI EmtL Bsp01 Noti GCCGGCCC 1 2147 EccT221 Mph11031 Zsp21 Olii CACNN'NNGTG 1 2147 EccT221 Mph11031 Zsp21 Olii CACNN'NNGTG 1 2147 EccT221 Mph11031 Zsp21 Olii CACNN'NNGTG 1 2147 EccT212 Mph11031 Zsp21 Poli ArCATGT 1 2725 PscI PmeI GTTT'AAC 1 1000 MssI Sac GACCT'C 1 2836 AcvTaBcTi Ecc721 Pml1 PspCI Spita GACCTCC 1 2836 Spita Ecit31 Spita Ecit31 Spita	EcoRI	G^AATTC	1	1019		
Fase I GGCCGGCCC 1 3113 RigI GSull CTGGAG(16/14) 1 4933 - BpmI KpnI GGTACCC 1 2676 Acc65I Asp718I MauBI CGCCGCGC 1 2877 PluTI SfoI SspDI NheI GCCGCC 1 213 AsuMNI BmII BspOI Noti GCCGCCGC 1 3217 Ccini Noti GCCGCCGCC 1 213 AsuMNI BmII BspOI Noti GCCGCCGCC 1 2147 EcoT221 Mph103I Zsp2I Olii CACMTGT 1 1642 Alei Pradi CACATGT 1 275 PscI PmaCI CACATGT 1 275 PscI Saci GACCAC 1 280 Scl1361I EcoICRI Eco53kI Sapi GCTCAC 1 275 Scl Sali GATCACA 1 285 Scl336 Sapi GCTCATC (1/4) 1 275 Scl33 <td>FalI</td> <td>(8/13) AAGNNNNNCTT (13/8)</td> <td>1</td> <td>784</td> <td></td> <td></td>	FalI	(8/13) AAGNNNNNCTT (13/8)	1	784		
Gsui CTGGAC(16/14) 1 4933 - BpmI KpnI GGTAC'C 1 2676 Acc65I Asp718I MauBI CG^CGCCG 1 2877 DinI EgeI EheI KasI Mlyll3I Nari GG^CGCC 1 213 AsuMHI EmtL Bsp01 Noti GC^CGCCGC 1 213 AsuMHI EmtL Bsp01 Noti GC^CGCCGC 1 2147 CciNI Nsi ATGCA'T 1 2147 Ecol221 Mph1103I Zsp21 Oliii CACMT'NNOTG 1 2147 Ecol21 Mph103I Zsp21 PmeI GTCTAC 1 2725 PscI PmeI GTT'AAC 1 1000 MssI Sald GACGT'C 583 EclISCI EclISCI Sapi GCTCTC(1/4) 1 2179 - Bsp01 LguI PciSI SgrAI CACCTGC (4/8) 2 2169 StISI StaISI ShaI TACGRCA 1 2830 StISI StaISI	FseI	GGCCGG^CC	1	3113		RigI
KpnI GGTAC^C 1 2676 Asplasi MauBI GG^CGGCG 1 2877 Pullin Egel Ehel Kasl Mlyll3I NarI GG^CGGCG 1 1100 Dinl Egel Ehel Kasl Mlyll3I NhEI G^CGGCGC 1 3217 ColNI Noti GC^GGCGC 1 3217 ColNI Noti GCCACAT 1 2147 EcoT22I Mphl03I Zsp2I Olii CACNATGT 1 2152 PscI PmaCI CACCGTG 1 2549 AcvI BbrPI Eco72I Pml1 PspCI PmaCI CACCGTG 1 2563 Ecl136II EcoICRI Eco53kI SalI GATCAC 1 2036 Stall Stall SalI GATCAC 1 2836 Stall Stall SalI GATCAC 1 2836 Stall Stall EcoICSI SalI GATCAC 1 2836 Stall EcoIOSI Stall SalI GATCAC 1 2836 Stall EcoIOSI <t< td=""><td>GsuI</td><td>CTGGAG(16/14)</td><td>1</td><td>4933</td><td>-</td><td>BpmI</td></t<>	GsuI	CTGGAG(16/14)	1	4933	-	BpmI
MaBI CGCGCGCG 1 2877 Nari GG^CGC 1 1100 DinI Egel Bhel Kasi Mlyll31 PluTI Sfol SspD1 Nhei G^CTAGC 1 2213 AsUNH Bmll BspO1 Noti GC^GCGCC 1 3217 CciNI Nsii AGCGCGC 1 2213 AsUNH Bmll BspO1 Nait ACCATG 1 2147 EcoT22I Mphl103I Zsp2I Olii CACNN^NNGTG 1 1642 Alei Pcil A^CATGT 1 2725 PscI PmeI GTT^*AAC 1 1000 MssI SacI GAGCT^C 1 2836 Sapp1 Astri Eco1CRI Eco53kI Sapia GCTCTTC1(1/4) 1 2179 - BspQ1 Lgul PciSI Sapia GCTCTCTC(1/4) 1 2179 - BstSNI Eco105I Sapia T^CTAGA 1 280 AhlI EcuI Xbai T^CTAGA 1 280 AhlI EcuI Xbai T^CGAGA <t< td=""><td>KpnI</td><td>GGTAC^C</td><td>1</td><td>2676</td><td></td><td>Acc65I Asp718I</td></t<>	KpnI	GGTAC^C	1	2676		Acc65I Asp718I
Nari GG^CGCC 1 1100 Dini Egel Ehel Kasi Mly1131 Plutt Stol SspD1 Nhei G^CTAGC 1 2213 AsuNHI Emil BspO1 Noti GC^GGCGC 1 3217 CciNI Nsii ATGCA^T 1 2147 EcoT221 Mph1103I Zsp21 Nsii ArGCA^T 1 2147 EcoT221 Mph1103I Zsp21 Olii CACNN^NNGTG 1 1642 Alei Profil A^CATGT 1 2725 Psci PmaCI CAC^YCG 1 2739 AcvI BbrPI Eco721 Pml1 PspCI PmaCI GTTT^AAAC 1 1000 Mssi Saci GACCAC 1 2830 GCTCTC(14) 2179 Sali G^TCGAC 1 2830 Stol Stol Stol Sapi GCTCTC(1/4) 1 18 Ahli Eco1051 Spei A^CTAGT 1 8 Stol Asti CACCAGC (4/8) 2 2109 PaqCI Zatac 2	MauBI	CG^CGCGCG	1	2877		da.
NheI G^CTAGC 1 2213 AsuMH Emt. BspOI NotI GC^GCGCC 1 3217 CciNi NsiI ATGCA^T 1 2147 EcoT221 Mphl103I Zsp2I OliI CACMYNNOTG 1 1642 AleI PciI A^CATGT 1 2725 PscI PmeI GTC*AAC 1 1000 MssI SacI GAGC*C 1 2836 Ecl1361I EcoTCRI Eco53kI Sapi GCTCTC (1/4) 1 2179 - BspQI LguI PciSI Sapi GCTCTTC (1/4) 1 2179 - BspQI LguI PciSI SgrAi GC*CGCG 1 2836 - - Sapi GCTCTTC (1/4) 1 2179 - BspQI LguI PciSI SigrAi GC*CGCA 1 2836 - - Spei A^CTAGT 1 18 Ahl B EcuI Xbai T^C*CGAGA 1 2836 - Xhoi C*CCGC(4/8) 2 2109 - 2 <td>NarI</td> <td>GG^CGCC</td> <td>1</td> <td>1100</td> <td></td> <td>DinI Egel Ehel Kasl Mlv113I</td>	NarI	GG^CGCC	1	1100		DinI Egel Ehel Kasl Mlv113I
NheI G^CTAGC 1 2213 AsUNHT Emt I BapOI Noti GC^GGCCGC 1 3217 CCiNI Nsii ATGCAT 1 2147 EcoT221 Mph11031 Zsp21 Olii CACMMYNNOTG 1 1642 Alei Praci CACYGTG 1 2725 Psci PmaCI CACYGTG 1 2549 AcvI BbrPI Eco721 PmlI PspCI PmaCI CACYGTG 1 2549 AcvI BbrPI Eco721 PmlI PspCI Saci GAGCT^C 1 260 Sst Saci GACTCGCGC 1 281 Scilit Eco1CRI Eco53kI Sali G^TCGAC 1 286 Ssp1 Ssp1 Eco1051 Sapi GCTCTC (1/4) 1 2179 - BspQI LguI PciSI Systa T^CCAGAT 1 387 BstSNI Eco1051 384 Arcotage 1 386 Sfr2741 PaeR7I Slai 341 Arcotage 2 2109 - PaqCI Z			-	1100		PluTI Sfot SspDt
And Gorde Corde 2 2113 Main Larger Noli Gorde Corde 1 2117 Colini Nsii ArcCArT 1 2147 EcoT221 Mphl1031 Zsp21 Olii CACNA'NNOTG 1 1642 Alei Pcii ArCATGT 1 2725 Psci Pmaci CACA'CTG 1 2749 AcVI BbrPI Eco721 PmlI PspCI PmeI GTT'AAAC 1 1000 Mssi Saci GAGCT'C 1 2836 Ecl13611 EcoICRI Eco53kI Sapi GCTCTTC (1/4) 1 2179 - BspQI LguI PciSI SmaBi TAC'GTA 1 2836 Sapi GCTCTTA Sat 1 2836 Sapi GCTCTTA 1 18 AhlI EcuI Xbai Tr'CTAGA 1 2830 Xhoi CACGGYG 1 2830 Xhoi CACGGG 1 966 Sfr274I PaeR7I Slai Aari CACCTGC (4/8) 2 2109 - PaqCI 2 24366 AlwNI CAGNNN^CTG 2 1554 CaiI PstNI 2 2436 Bali TGG^CCA 2 2843 Misi MluNI Mox20I MscI Msp20I 2 2<	Nhot	C^CTACC	1	2213		AsuNHT BmtT BenOT
Noil of Geoded 1 3217 Column Noil ATGCATT 1 2147 Econ221 Mph11031 Zsp2I Olli CACNTT 1 275 PscI PmaCI CACAGTG 1 2549 AcvI BbrPI Eco721 PmlI PspCI PmeI GTTT^AAAC 1 1000 MssI Saci GAGCAC 1 2836 Ecl136II EcoICRI Eco53kI Sali GATCGAC 1 2955 Sali CCCCGYG BspQI LguI PciSI Sin ACCGGYG 1 2830 Sali Eco1051 Sali Eco1051 Spei A^CTAGA 1 357 BstSNI Eco1051 Sali Eco1051 Spei A^CTAGA 1 2830 Sali Eco1051 Sali Eco1051 Xbai T^CTAGA 2 2109 PaqCI Sali Eco1051 Aari CACTGCG (4/8) 2 2166 <	NotT	G CIAGE	1	2213		CoiNI
NS11 ATGCATT 1 2147 EC01221 Mpf11031 25p21 OllI CACNNYNNETG 1 1642 AleI PedI ACATEGT 1 2725 PscI PmaCI CACATEGT 1 2725 PscI PmaCI GACGTCG 1 2549 AcvI BbrPI Eco72I PmlI PspCI SacI GACCT^C 1 2836 Ecl136II EcoICRI Eco53kI SapI GCTCTTC (1/4) 1 2179 - BspQI LguI PciSI SgrAI CCYCGGGG 1 2855 - - SnaBI TAC^GTA 1 357 BstSNI Eco105I - SprI ACATEGT 1 18 Ahll BcuI - XbaI T^CTACA 1 2830 - - XhoI C^TCGAC 1 966 Sfr274I PaeR7I SlaI - AarI CACGTCC (4/8) 2 2109 - PaqCI 2 2436 - - - - ApaI GGGCC^C 2 1495 Bsp1201 Psp0MI <t< td=""><td>NOLI</td><td></td><td>1</td><td>3217</td><td></td><td>CCINI Reemoor Makilloor Reeoor</td></t<>	NOLI		1	3217		CCINI Reemoor Makilloor Reeoor
Olil CACNN'NNGTG 1 1642 Alei PciI A^CATGT 1 2725 PscI PmaCI CAC^GTG 1 2549 AcvI BbrPI Eco72I PmlI PspCI PmaCI GACGT^C 1 1000 MssI SacI GACGT^C 1 2836 Ecl1361I Eco1CRI Eco53kI SapI GCTCTC(1/4) 1 2179 - BspQI LguI PciSI SgrAI CR^CCGGYG 1 2955 - - SnBI TAC^GTA 1 357 BstSNI Eco105I - XbaI T^CTAGA 1 2830 - - PaqCI XbaI T^CTAGA 1 2830 - - PaqCI XbaI T^CTAGA 1 2830 - - PaqCI XhoI CACGAGC 1 966 Sfr274I PaeR7I SlaI - AarI CACGNN^CTG 2 1554 CaiI PstNI - ApaI GGGCC^C 2 1495 Bsp120I Psp0MI - BajI TGCAG(16/14) </td <td>NSII</td> <td>ATGCANT</td> <td>1</td> <td>2147</td> <td></td> <td>ECOTZZI MPNIIU3I ZSPZI</td>	NSII	ATGCANT	1	2147		ECOTZZI MPNIIU3I ZSPZI
Pci1 A^CATGT 1 2725 PscI PmaCI CAC^GTG 1 2549 AcvI BbrPI Eco72I PmlI PspCI PmeI GTT^^AAAC 1 1000 MssI SacI GAGCT^C 1 2836 Ecl1361I EcoICRI Eco53kI Psp124BI SstI SalI G^TCGAC 1 2836 Ecl1361I EcoICRI Eco53kI Psp124BI SstI SapI GCTCTTC (1/4) 1 2179 - BspQI LguI PciSI SgrAI CR^CGGGG 1 2955	Olil	CACNN^NNGTG	1	1642		Alel
PmaCI CACYGTG 1 2549 AcvI BbrPI Eco72I PmlI PspCI PmeI GTT^AAAC 1 1000 MssI SacI GAGCT^C 1 583 Ecl136II Eco1CRI Eco53kI SapI GCTCTTC (1/4) 1 2179 - BspQI LguI PciSI SgrAI CACCGGYG 1 2955 - - SnaBI TACCGTA 1 357 BstSNI Eco105I - SpeI A^CTAGT 1 18 Ahll BcuI - - XbaI T^CTAGA 1 2830 - - PaqCI Z 2 2109 - PaqCI -	PciI	A^CATGT	1	2725		PscI
PmeI GTT*^AAAC 1 1000 MssI SacI GAGCT^C 1 583 Ecl13611 Eco1CRI Eco53k1 Psp124BI SstI SalI GTCGAC 1 2836	PmaCI	CAC^GTG	1	2549		AcvI BbrPI Eco72I PmlI PspCI
SacI GAGCT^C 1 583 Ecl136II EcoICRI Eco53kI Ball SalI G^TCGAC 1 2836 SapI GCTCTTC (1/4) 1 2179 - BspQI LguI PciSI SgrAI CR^CCGGYG 1 2955 - BstSNI Eco105I SnaBI TAC^GTA 1 357 BstSNI Eco105I SpeI A^CTAGT 1 8 AhlI BcuI XbaI T^CTAGA 1 2830 - XhoI CACTGC (4/8) 2 2109 - PaqCI AarI CACGNN^CTG 2 2564 - - AlwNI CAGNN^CTG 2 1495 Bsp120I Psp0MI 2 2348 - - - BalI TGG^CCA 2 1495 Bsp120I Psp0MI 2 2348 - - - BayI GGGCC1(16/14) 2 7790 - 2 2159 - - - <	PmeI	GTTT^AAAC	1	1000		MssI
Sall G^TCGAC 1 2836 SapI GCTCTTC(1/4) 1 2179 - BspQI LguI PciSI SgrAI CR^CCGGYG 1 2955 - BstSNI Ecol051 SnBI TAC^GTA 1 357 BstSNI Ecol051 - SpeI A^CTAGT 1 2830 - - XbaI T^CTAGA 1 2830 - - XbaI CACCTGC (4/8) 2 2109 - PaqCI Aari CACCTGC (4/8) 2 2564 - - AlwNI CAGNNN^CTG 2 1554 CaiI PstNI - ApaI GGGCC^C 2 4366 - - BalI TGG^CCA 2 2843 MISI MUNI Mox20I MscI Msp20I BagII A^GATCT 2 3944 - - 2 5768 - - - - BagI GAATGC (1/-1) 2 2881 - <td>SacI</td> <td>GAGCT^C</td> <td>1</td> <td>583</td> <td></td> <td>Ecl136II EcoICRI Eco53kI</td>	SacI	GAGCT^C	1	583		Ecl136II EcoICRI Eco53kI
SalI G^TCGAC 1 2836 SapI GCTCTTC(1/4) 1 2179 - BspQI LguI PciSI SgrAI CR^CCGGYG 1 2955 - BspQI LguI PciSI SnaBI TAC^GTA 1 357 BstSNI Ecol05I - SpeI A^CTAGT 1 18 AhlI BcuI XbaI T^CTGGA 1 2830 - - XhoI C^TCGAG 1 966 Sfr274I PaeR7I SlaI AarI CACCTGC (4/8) 2 2109 - PaqCI 2 2564 CaiI PstNI - - - AarI CACCTGC (4/8) 2 2564 CaiI PstNI - ApaI GGGCC^C 2 1495 Bsp120I PspOMI - - BalI TGG^CCA 2 2483 MIsI MluNI Mox20I MscI Msp20I - - SgrI GGACC (16/14) 2 1790 - - - - SgrI GATGC (1/-1) 2 2381 - Mva1269I PctI -						Psp124BI SstI
SapI GCTCTTC(1/4) 1 2179 - BspQI LguI PciSI SgrAI CR^CCGGGG 1 2955 - BstSNI Ecol05I SnaBI TAC^GTA 1 357 BstSNI Ecol05I - SpeI A^CTAGT 1 18 AhlI BcuI XbaI T^CTAGA 1 2830 - XhoI C^TCGGGG 1 966 Sfr274I PaeR7I SlaI Aari CACCTGC (4/8) 2 2109 - PaqCI Aari CACCTGC (4/8) 2 2564 - - AlwNI CAGNNN^CTG 2 1554 Cail PstNI - ApaI GGGCC^C 2 1495 Bsp120I PspOMI - 2 2348 - - - - - Ball TGG^CCA 2 2843 Misl MluNI Mox20I MscI Msp20I - <t< td=""><td>SalI</td><td>G^TCGAC</td><td>1</td><td>2836</td><td></td><td>-</td></t<>	SalI	G^TCGAC	1	2836		-
SgrAI CR^CCGGYG 1 2955 Art of the second sec	SapI	GCTCTTC(1/4)	1	2179	_	BspOI LauI PciSI
SynaBI TAC^CTA 1 357 BstSNI Ecol05I SpeI A^CTAGT 1 18 AhlI BcuI XbaI T^CTAGA 1 2830 XhoI C^TCGAG 1 966 Sfr274I PaeR7I SlaI AarI CACTGC (4/8) 2 2109 - PaqCI ApaI GGGCC^C 2 1495 Bsp120I PspOMI 2 2348 MlsI MluNI Mox20I MscI Msp20I 2 SgII A^GATCT 2 3944 2 2 5768 2 2811 - Mva1269I PctI 2 2109 - 2 2109 - SgI GTGCAG (16/14) 2 1790 - 2 2115 SsmI GAATGC (1/-1) 2 2381 - Mva1269I PctI 2 21908 </td <td>SarAT</td> <td>CB^CCGGYG</td> <td>1</td> <td>2955</td> <td></td> <td>-1×)</td>	SarAT	CB^CCGGYG	1	2955		-1×)
Shell A°CTAGT 1 18 Ahll Bcul XbaI T°CTAGA 1 2830 XhoI C°TCGAG 1 966 Sfr274I PaeR7I SlaI AarI CACCTGC (4/8) 2 2109 - 2 2564 - - PaqCI AlwNI CAGNNN^CTG 2 1554 Cail PstNI 2 4366 - - - ApaI GGGCC^C 2 1495 Bsp120I PspOMI 2 2384 - - - Ball TGG^CCA 2 845 - BgIII A^GATCT 2 3944 - 2 5768 - - - BsgI GTGCAG (16/14) 2 1790 - 2 2159 - - - BsmI GAATGC (1/-1) 2 2381 - Mva12691 PctI 2 5879 - - - - - BssSI CACGAG (-5/-1) 2 1084 Eco811	SnaBI		1	357		BstSNI Ecol051
Spein A GTAGA 1 10 Anil bour Xhai TACTAGA 1 2830 Xhoi CACCTGC (4/8) 2 2109 - PaqCI 2 2564 - PaqCI - - Awii CAGNNN^CTG 2 1495 Bsp1201 Psp0MI 2 2348 - - - - Bali TGG^CCA 2 1495 Bsp1201 Psp0MI 2 2348 - - - - Bali TGG^CCA 2 2436 - - - Bali TGG^CCA 2 2443 MIsi MluNi Mox201 Msci Msp201 - 2 5845 -<	Spot		1	1.8		Ablt Rout
Abai 1 2630 XhoI C^TCGAG 1 966 Sfr274I PaeR7I SlaI AarI CACCTGC (4/8) 2 2109 - PaqCI AlwNI CAGNNN^CTG 2 1554 Cail PstNI ApaI GGGCC^C 2 1495 Bsp120I PspOMI Ball TGG^CCA 2 2843 MlsI MluNI Mox20I MscI Msp20I Bg1II A^GATCT 2 3944 - 2 5768 - - - BsgI GTGCAG (16/14) 2 1790 - 2 2811 - Mval269I PctI 2 2879 - - BsmI GATGC (1/-1) 2 281 - 2 2879 - - - BssSI CACGAG (-5/-1) 2 4128 - BauI Bst2BI 2 5512 - - - - - Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I - 2 1676 -	Spei		1	2020		AIIII DCUI
Xhoi CMTCGAG 1 966 SH2/41 PAER/I SIAI Aari CACCTGC (4/8) 2 2109 - PaqCI AlwNI CAGNNN^CTG 2 1554 Cail PstNI Apal GGGCC^C 2 1495 Bsp120I PspOMI Ball TGG^CCA 2 2843 MlsI MluNI Mox20I MscI Msp20I BglII A^GATCT 2 3944 - 2 2159 - - - BsgI GTGCAG (16/14) 2 1790 - - 2 2814 - Mva1269I PctI - - Bsp1407I T^GTACA 2 2879 - - - BssSI CACGAG (-5/-1) 2 4128 - BauI Bst2BI - - Su36I CC^TINAGG 2 1634 Eco81I AxyI Bse21I - - - - - DrallI CACNNN^GTG 2 2533 Adet - - - - - - - - - - - </td <td>ADAI</td> <td>I CIAGA</td> <td>1</td> <td>2030</td> <td></td> <td></td>	ADAI	I CIAGA	1	2030		
Aari CACCTGC (4/8) 2 2109 - PaqCI 2 2564 2564 Cail PstNI AlwNI CAGNNN^CTG 2 1554 Cail PstNI ApaI GGGCC^C 2 1495 Bspl201 PspOMI 2 2348 MlsI MluNI Mox20I MscI Msp20I 25845 Ball TGG^CCA 2 2843 MlsI MluNI Mox20I MscI Msp20I BgJII A^GATCT 2 3944 2 2 2159 2 2159 2 BsmI GAATGC (1/-1) 2 2381 - Mval269I PctI 2 2159 2 26879 - 2 BssSI CACGAG (-5/-1) 2 26879 - - Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I 2 5512 - - - - BraIII CACNNYGTG 2 2593 Adel	XNOL	CATCGAG	1	966		Sir2/41 PaeR/1 Slal
AlwNI CAGNNN^CTG 2 1554 Cail PstNI ApaI GGGCC^C 2 4366 Bsp120I Psp0MI ApaI TGG^CCA 2 2348 Bsp120I Psp0MI Ball TGG^CCA 2 2843 MlsI MluNI Mox20I MscI Msp20I BglII A^GATCT 2 3944 - 2 5768 - - - BsgI GTGCAG (16/14) 2 1790 - - BsmI GAATGC (1/-1) 2 2159 - - BssSI CACGAG (-5/-1) 2 1908 BsrGI BstAUI 2 5879 - - - - Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I 2 1676 2 1676 - -	Aarl	CACCTGC (4/8)	2	2109	-	PaqCI
AlwNI CAGNNN^CTG 2 1554 Cail PstNI ApaI GGGCC^C 2 4366 Bspl20I PspOMI 2 2348 Bspl20I PspOMI 2 2348 BalI TGG^CCA 2 2843 MlsI MluNI Mox20I MscI Msp20I 2 5845 2 5845 1 BglII A^GATCT 2 3944 2 2 5768 2 2159 1 BsmI GAATGC (1/-1) 2 2159 1 Bsp1407I T^GTACA 2 1908 BsrGI BstAUI 2 5879 1 1 1 Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I 2 1676 2 1676 1			2	2564		
ApaI GGGCC^C 2 1495 Bspl20I PspOMI 2 2348 MlsI MluNI Mox20I MscI Msp20I 2 2843 MlsI MluNI Mox20I MscI Msp20I 2 5845 2 BglII A^GATCT 2 3944 2 5768 2 BsgI GTGCAG (16/14) 2 1790 2 2159 2 BsmI GAATGC (1/-1) 2 2381 2 2414 2 Bsp1407I T^GTACA 2 1908 BssSI CACGAG (-5/-1) 2 4128 - Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I 2 1676 2 2593 AdeI	AlwNI	CAGNNN^CTG	2	1554		Cail PstNI
ApaIGGGCC^C21495Bsp120I PspOMI223482348MlsI MluNI Mox20I MscI Msp20IBalITGG^CCA22843MlsI MluNI Mox20I MscI Msp20I25845257682BsgIGTGCAG(16/14)21790-22159221592BsmIGAATGC(1/-1)22381-2241421908BsrGI BstAUI25879258792BssSICACGAG(-5/-1)24128-25879258792Bsu36ICC^TNAGG21634Eco81I AxyI Bse21I2167622593AdeI			2	4366		
BalI TGG^CCA 2 2348 BglII A^GATCT 2 2843 MlsI MluNI Mox20I MscI Msp20I BglII A^GATCT 2 3944 2 BsgI GTGCAG (16/14) 2 1790 - BsmI GAATGC (1/-1) 2 2381 - Bsp1407I T^GTACA 2 1908 BsrGI BstAUI BssSI CACGAG (-5/-1) 2 5879 BauI Bst2BI Bsu36I C^ TNAGG 2 1634 Eco81I AxyI Bse21I DrallI CACNNN^GTG 2 2593 Ade I	ApaI	GGGCC^C	2	1495		Bsp120I PspOMI
Ball TGG^CCA 2 2843 MlsI MluNI Mox20I MscI Msp20I BglII A^GATCT 2 3944 - 2 5768 - - BsgI GTGCAG (16/14) 2 1790 - 2 2159 - - - BsmI GAATGC (1/-1) 2 2381 - Mva1269I PctI Bsp1407I T^GTACA 2 1908 BsrGI BstAUI BssSI CACGAG (-5/-1) 2 4128 - BauI Bst2BI Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I DrallI CACNNN^GTG 2 2593 Adel			2	2348		
BglII A^GATCT 2 5845 BsgI GTGCAG(16/14) 2 5768 BsmI GAATGC(1/-1) 2 2159 BsmI GAATGC(1/-1) 2 2381 - Mval269I PctI Bsp1407I T^GTACA 2 1908 BsrGI BstAUI BssSI CACGAG(-5/-1) 2 5879 BauI Bst2BI Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I DrallI CACNNN^GTG 2 2593 Adel	BalI	TGG^CCA	2	2843		MlsI MluNI Mox20I MscI Msp20I
BglII A^GATCT 2 3944 2 5768 BsgI GTGCAG(16/14) 2 1790 2 2159 BsmI GAATGC(1/-1) 2 2381 - Bsp1407I T^GTACA 2 1908 BsrGI BstAUI SSSI CACGAG(-5/-1) 2 5879 - Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I DrallI CACNNN^GTG 2 2593 Adel			2	5845		-
BsgI GTGCAG(16/14) 2 5768 BsgI GTGCAG(16/14) 2 1790 - BsmI GAATGC(1/-1) 2 2381 - Mval269I PctI Bsp1407I T^GTACA 2 1908 BsrGI BstAUI BssSI CACGAG(-5/-1) 2 4128 - Baul Bst2BI Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I DrallI CACNNN^GTG 2 2593 Adel	BalII	A^GATCT	2	3944		
BsgI GTGCAG(16/14) 2 1790 - 2 2159 2159 BsmI GAATGC(1/-1) 2 2381 - Mval269I PctI 2 2414 2 1008 BsrGI BstAUI Bsp1407I T^GTACA 2 1908 BsrGI BstAUI BssSI CACGAG(-5/-1) 2 5879 Baul Bst2BI Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I DrallI CACNNN^GTG 2 2593 Adel	- 5		2	5768		
DSGI DIGCAG(10/14) 2 2159 BsmI GAATGC(1/-1) 2 2381 - Mval269I PctI 2 2414 2 2414 1908 BsrGI BstAUI Bsp1407I T^GTACA 2 1908 BsrGI BstAUI 2 5879 2 5879 BssSI CACGAG(-5/-1) 2 5512 Bsu36I CC^TNAGG 2 1676 DrallI CACNNN^GTG 2 2593 Adel	Beat	CTCCAC(16/14)	2	1790	_	
BsmI GAATGC (1/-1) 2 2381 - Mval269I PctI 2 2414 2 2414 - BsrGI BstAUI Bsp1407I T^GTACA 2 1908 BsrGI BstAUI 2 5879 - BauI Bst2BI 2 5512 - BauI Bst2BI Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I DrallI CACNNN^GTG 2 2593 Adel	DSYI	GIGCAG(10/14)	2	2150		
BSMI GAATGC (1/-1) 2 2381 - MVal2691 Pct1 2 2414 2 2414 Bsp1407I T^GTACA 2 1908 BsrGI BstAUI 2 5879 2 5879 BssSI CACGAG (-5/-1) 2 4128 - Baul Bst2BI 2 5512 2 5512 Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I DrallI CACNNN^GTG 2 2593 Adel	Daws	$O_{A} = O_{A} O_$	2	2159		Mar 1000T Dat T
Bsp1407I T^GTACA 2 1908 BsrGI BstAUI 2 5879 2 5879 BssSI CACGAG(-5/-1) 2 4128 - Baul Bst2BI 2 5512 2 5512 Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I DrallI CACNNN^GTG 2 2593 Adel	BSMI	GAATGC(1/-1)	2	2381	-	MVal2691 PCt1
Bsp1407I T^GTACA 2 1908 BsrGI BstAUI 2 5879 BssSI CACGAG(-5/-1) 2 4128 - Baul Bst2BI 2 5512 Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I 2 1676 DrallI CACNNN^GTG 2 2593 Adel			2	2414		
2 5879 BssSI CACGAG(-5/-1) 2 4128 - Baul Bst2BI 2 5512 2 5512 Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I 2 1676 2 2593 Adel	Bsp1407I	T^GTACA	2	1908		BsrGI BstAUI
BssSI CACGAG(-5/-1) 2 4128 - Baul Bst2BI 2 5512 5512 Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I 2 1676 2 2593 Adel			2	5879		
2 5512 Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I 2 1676 2 2593 Adel	BssSI	CACGAG(-5/-1)	2	4128	-	BauI Bst2BI
Bsu36I CC^TNAGG 2 1634 Eco81I AxyI Bse21I 2 1676 Dralli CACNNN^GTG 2 2593 Adel			2	5512		
2 1676 Dralli CACNNN^GTG 2 2593 Adel	Bsu36I	CC^TNAGG	2	1634		Eco81I AxyI Bse21I
Dralli CACNNN^GTG 2 2593 Adel			2	1676		<u> </u>
	DraIII	CACNNN^GTG	2	2593		AdeI

TGEX-SCBlue-Zeo Instruction Manual

	2	3190	
GACNNNN^NNGTC	2	1673	AasI DseDI
	2	4057	
C^GGCCG	2	3173	BseX3I BstZI EclXI Eco52I
	2	3218	
GACNNN^NNGTC	2	1575	AhdI BmeRI DriI
	2	4843	
TGC^GCA	2	1470	Acc16I NsbI
	2	5068	
A^AGCTT	2	2807	
	2	3950	
CA^TATG	2	252	FauNDI
	2	3906	
CGAT^CG	2	1450	Ple19I
	2	5215	
A^CCWGGT	2	1947	CsiI MabI
	2	3005	
GGCCNNNN^NGGCC	2	1085	
	2	1496	
	GACNNNN^NNGTC C^GGCCG GACNNN^NNGTC TGC^GCA A^AGCTT CA^TATG CGAT^CG A^CCWGGT GGCCNNNN^NGGCC	GACNNNN^NNGTC 2 C^GGCCG 2 GACNNN^NNGTC 2 TGC^GCA 2 A^AGCTT 2 CA^TATG 2 CGAT^CG 2 A^CCWGGT 2 GGCCNNNN^NGGCC 2 2	2 3190 GACNNNN^NNGTC 2 1673 2 4057 C^GGCCG 2 3173 2 3218 GACNNN^NNGTC 2 1575 2 4843 TGC^GCA 2 1470 2 5068 A^AGCTT 2 2807 2 3950 2 CA^TATG 2 252 2 3906 2 CGAT^CG 2 1450 2 5215 3005 GGCCNNNN^NGGCC 2 1085 2 1496 2

Absent Sites:

AanI, AbsI, AccIII, AfeI, AflII, AgeI, AjuI, AloI, Aorl3HI, Aor51HI, AscI,
AsiGI, AsiSI, AsuII, BaeI, BarI, BbvCI, BclI, BfrI, BlpI, BoxI, BplI, Bpul102I,
Bpul4I, Bsa29I, BsaBI, BsaI, Bse8I, BseAI, BseCI, BseJI, BshTI, BshVI, BsiWI,
BsmBI, Bso31I, Bspl19I, Bspl3I, Bspl720I, Bsp68I, BspDI, BspEI, BspMAI,
BspT104I, BspTI, BspTNI, BssNAI, Bst1107I, BstAFI, BstAPI, BstBI, BstEII,
BstPAI, BstYI, Eco32I, Eco47III, Eco91I, Eco065I, EcoRV, Esp3I, FbaI, FspAI,
HpaI, I-CeuI, I-PpoI, I-SceI, KflI, Kpn2I, Ksp22I, KspAI, MfeI, MluI, MreI,
MroI, MspCI, MunI, NruI, NspV, PI-PspI, PI-SceI, PacI, PaeI, PalAI, PasI, PceI,
Pfl23II, PflFI, PinAI, PshAI, PsiI, PspEI, PspLI, SgrDI, SgsI, SmiI, SphI,
SrfI, Sse8387I, SseBI, StuI, SwaI, Tth111I, Vha464I, 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 TEGX vectors is obtained on any *recA1, endA1 E. coli* strain using LB or 2xYT medium supplemented with ampicillin (100 μ g/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-SCBlue-Zeo

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 *Sfil* site to obtain a complete hybrid leader encoding sequence.

sfil alleuleule uleuAlaAla GlnProAlaM etAla [Xxx] GlyProGlyG lyPro 1069 TACTGCTGCT CTTAGCGGCC CAGCCGGCCA TGGCN [NNN] GGCCCGGGAG GCCCC

Transfer from PADL Phagemid Vectors

scFv fragments and VHH domains can be excised from PADL[™] phagemids by Sfil or alternatively by BgIl and cloned directly into TGEX-SCblue opened by Sfil. 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

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.

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 technica	l assistance, call,	write, 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) (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 organspecific 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.

This product is subject to Antibody Design Labs Terms & Conditions of Sales available online at http://www.abdesignlabs.com/terms/. © 2024 Antibody Design Labs. All rights reserved.