

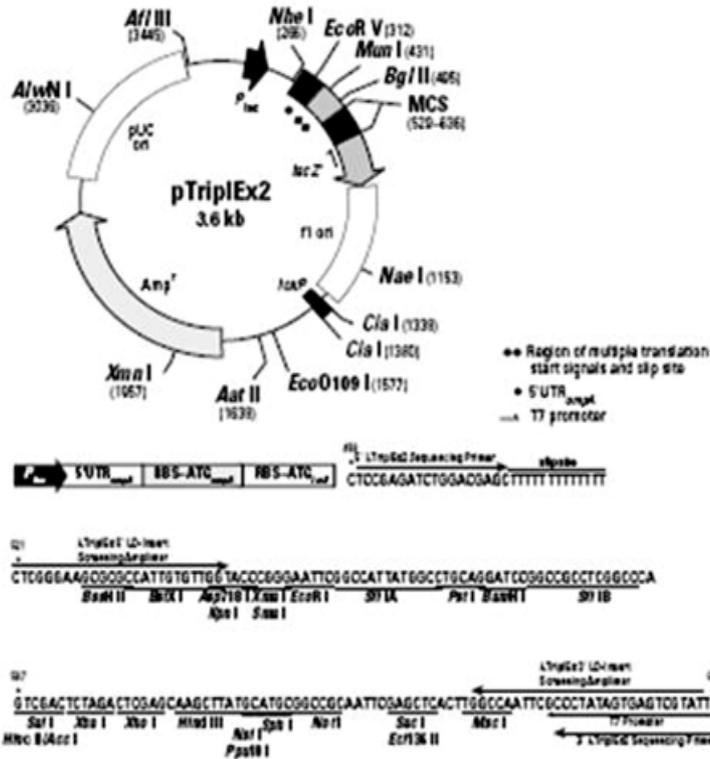
**APPENDIX B**

# 1. Vector

## 1.1. pTriplEx2 vector

pTriplEx2 Vector Information

PT3194-5



Restriction map and multiple cloning site (MCS) of pTriplEx2. Unique restriction sites are bold.

### Description

pTriplEx2 has the *E. coli* *lac* promoter and operator to provide regulated expression of inserts in *E. coli* hosts expressing the *lac* repressor (*lac*<sup>R</sup>). The 5' untranslated region (UTR) from the *E. coli* *ompA* gene stabilizes the mRNA, thereby increasing expression. pTriplEx2 incorporates a triple-reading-frame translation cassette consisting of translation initiation signals from the *E. coli* *ompA* and *lacZ* genes, in two different reading frames, followed by a transcription translation stop site. Downstream of this cassette is the pTriplEx2 MCS which is embedded within the *lacZ* α-peptide allowing clones with inserts to be identified by blue/white screening in an appropriate host strain. The T7 RNA polymerase promoter downstream of the MCS allows production of single-stranded RNA *in vitro* for use as a probe. In the presence of helper phage, the f1 origin in pTriplEx packages the noncoding strand of the *lacZ* gene into phage particles, and this single-stranded DNA can be used for sequencing or mutagenesis procedures. The ampicillin resistance gene and pUC origin of replication allow selection and propagation, respectively, of pTriplEx2 in *E. coli*.

## 1.2 pGEM<sup>®</sup>-T Easy vector

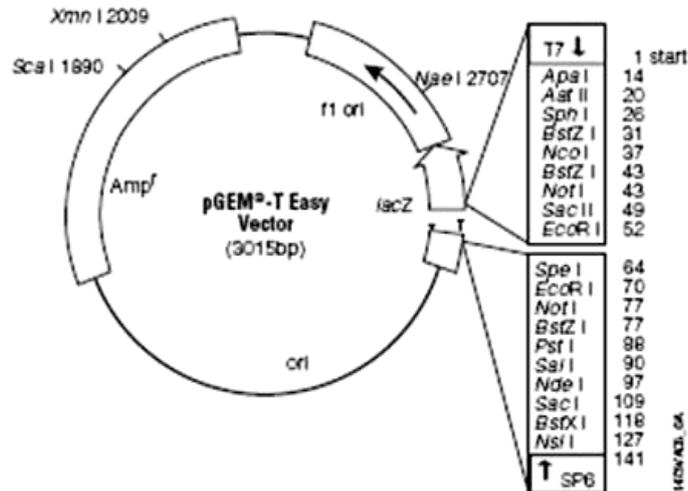


Figure 3. pGEM<sup>®</sup>-T Easy Vector circle map and sequence reference points.

### pGEM<sup>®</sup>-T Easy Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10-128
SP6 RNA polymerase promoter (-17 to +3)	139-158
SP6 RNA polymerase transcription initiation site	141
pUC/M13 Reverse Sequencing Primer binding site	176-197
<i>lacZ</i> start codon	180
<i>lac</i> operator	200-216
$\beta$ -lactamase coding region	1337-2197
phage <i>f1</i> region	2380-2835
<i>lac</i> operon sequences	2836-2996, 166-395
pUC/M13 Forward Sequencing Primer binding site	2949-2972
T7 RNA polymerase promoter (-17 to +3)	2999-3

Note: Inserts can be sequenced using the SP6 Promoter Primer (Cat.# Q5011), T7 Promoter Primer (Cat.# Q5021), pUC/M13 Forward Primer (Cat.# Q5601), or pUC/M13 Reverse Primer (Cat.# Q5421).



Note: A single digest with *BstZ* I (Cat.# R6881), *EcoR* I (Cat.# R6011) or *Not* I (Cat.# R6431) will release inserts cloned into the pGEM<sup>®</sup>-T Easy Vector. Double digests can also be used to release inserts.



## 2. Codon usage for amino acid

		Seconded Position								
		U		C		A		G		
		code	Amino Acid	code	Amino Acid	code	Amino Acid	code	Amino Acid	
First Position	U	UUU	phe	UCU	ser	UAU	tyr	UGU	cys	U
		UUC		UCC		UAC		UGC		C
		UUA	leu	UCA		UAA	STOP	UGA	STOP	A
		UUG		UCG		UAG	STOP	UGG	trp	G
	C	CUU	leu	CCU	pro	CAU	his	CGU	arg	U
		CUC		CCC		CAC		CGC		C
		CUA		CCA		CAA	gln	CGA		A
		CUG		CCG		CAG		CGG		G
	A	AUU	ile	ACU	thr	AAU	asn	AGU	ser	U
		AUC		ACC		AAC		AGC	C	
		AUA		ACA		AAA	lys	AGA	A	
		AUG	met	ACG		AAG		AGG	G	
	G	GUU	val	GCU	ala	GAU	asp	GGU	gly	U
		GUC		GCC		GAC		GGC		C
		GUA		GCA		GAA	glu	GGA		A
		GUG		GCG		GAG		GGG		G
		Third Position								

### 3. The amino acids preperities

Amino acids	3-code	1-code	properties
Alanine	Ala	A	hydrophobic
Arginine	Arg	R	free amino group makes it basic and hydrophilic
Asparagine	Asn	N	carbohydrate can be covalently linked ("N-linked") to its -NH
Aspartic acid	Asp	D	free carboxyl group makes it acidic and hydrophilic
Cysteine	Cys	C	oxidation of their sulfhydryl (-SH) groups link 2 Cys (S-S)
Glutamic acid	Glu	E	free carboxyl group makes it acidic and hydrophilic
Glutamine	Gln	Q	moderately hydrophilic
Glycine	Gly	G	so small it is amphiphilic (can exist in any surroundings)
Histidine	His	H	basic and hydrophilic
Isoleucine	Ile	I	hydrophobic
Leucine	Leu	L	hydrophobic
Lysine	Lys	K	strongly basic and hydrophilic
Methionine	Met	M	hydrophobic
Phenylalanine	Phe	F	very hydrophobic
Proline	Pro	P	causes kinks in the chain
Serine	Ser	S	carbohydrate can be covalently linked ("O-linked") to its -OH
Threonine	Thr	T	carbohydrate can be covalently linked ("O-linked") to its -OH
Tryptophan	Trp	W	scarce in most plant proteins
Tyrosine	Tyr	Y	a phosphate or sulfate group can be covalently attached to its -OH
Valine	Val	V	hydrophobic