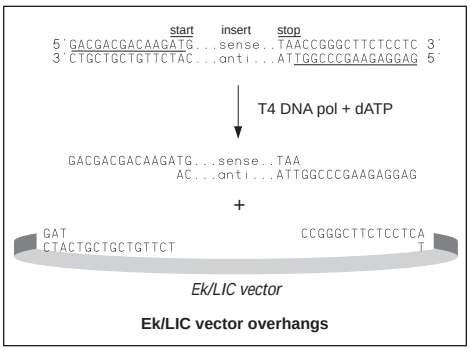
pT7Blue-2 Vector

pT7Blue-2 contains the pUC19 backbone (including high-copy number origin of replication and *lac* sequences), f1 origin of replication and modified multiple cloning region designed for optimal expression of cloned inserts by *in vitro* translation. The multiple cloning region contains a T7 promoter upstream of the *Xenopus* β-globin 5' UTR translation enhancer and S•Tag™ sequences, followed by an enterokinase cleavage site and extensive region of cloning sites. An SP6 promoter is placed within the multiple cloning sites to allow the synthesis of essentially unfused RNA from DNA inserted downstream. Unique sites are shown on the circle map. The coding strand for transcription from the T7 promoter is shown below. The f1 origin in pT7Blue-2 is oriented so that infection with helper phage will produce virions containing single stranded DNA that is complementary to the strand shown below. Therefore, single stranded sequencing should be performed using the T7 promoter primer (Cat. No. 69348-3), Blue2S•Tag primer (Cat. No. 69087-3), or SP6 promoter primer (Cat. No. 69349-3), depending on the location of the insert.

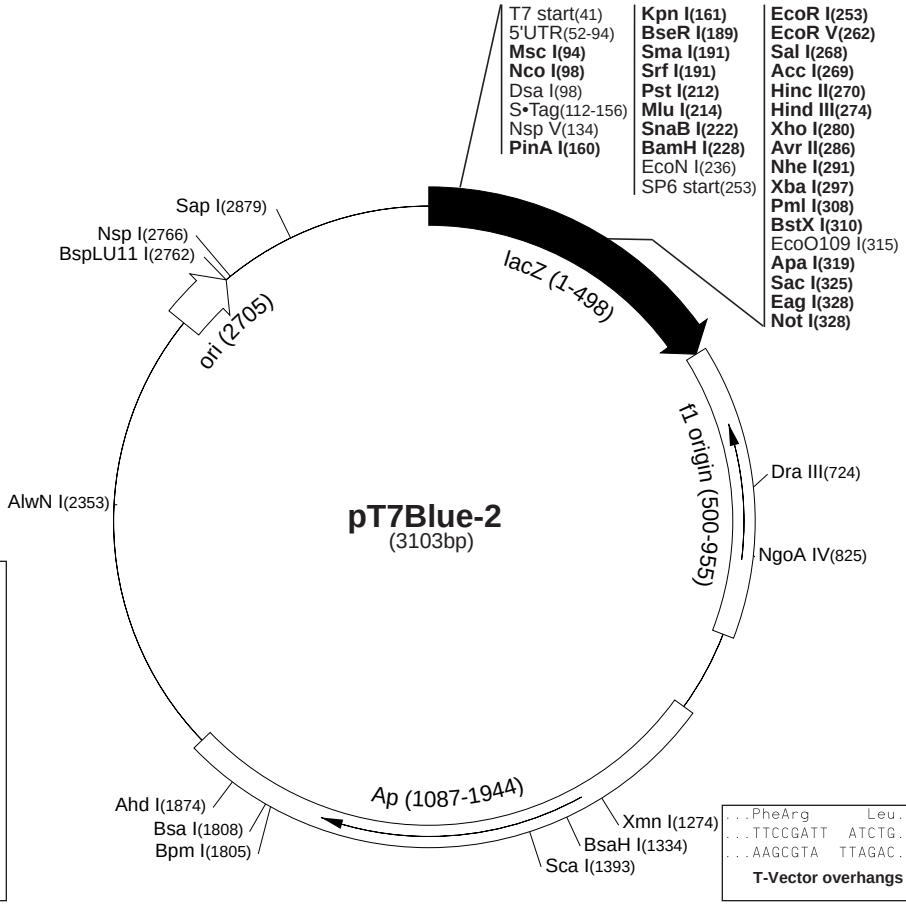
pT7Blue-2 sequence landmarks

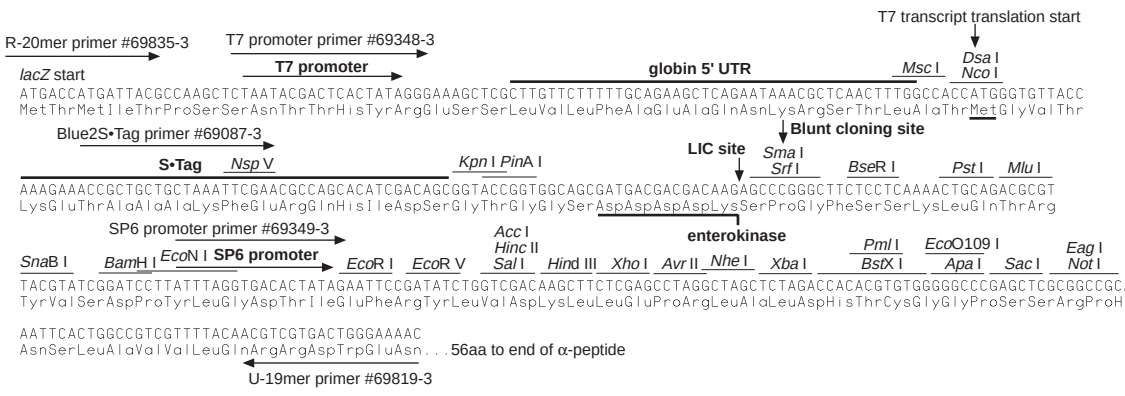
lacZ start codon	1
lacZ α-peptide	1-498
T7 promoter	24-40
T7 transcription start	41
Xenopus globin 5'UTR	52-94
S•Tag coding sequence	112-156
multiple cloning region (*Msc* I - *Not* I)	92-334
SP6 promoter	236-252
SP6 transcription start	253
f1 origin	500-955
bla coding sequence	1087-1944
pUC origin	2705

Notes: the *Srf* I and *Sma* I sites are destroyed during Ligation Independent Cloning.
Primer sequence extensions required for LIC compatibility are underlined in the diagram below.



Ek/LIC vector overhangs





pT7Blue-2 cloning/expression region

pT7Blue-2 Restriction Sites

Enzyme	# Sites	Locations
AccI	1	269
AceIII	3	564 1570 2810
AciI	36	
AflIII	4	214 305 307 2762
AgeI	1	160
AluI	20	
AlwI	10	223 236 1235 1239 1556
		2019 2020 2116 2118 2204
Alw21I	4	325 1206 1291 2452
Alw44I	2	1202 2448
AlwNI	1	2353
ApaI	1	319
ApoI	4	130 253 526 537
AvaI	3	189 280 318
AvaII	2	1510 1732
AvrII	1	286
BaeI	1	156
BamHI	1	228
BanI	4	157 761 1921 3018
BanII	4	190 319 325 799
BbvI	15	
BccI	5	714 731 1425 1712 1836
Bce83I	4	1270 2138 2379 2677
BcefI	3	332 750 2263
BcgI	2	1336 1370
BfaI	7	287 292 298 875 1681
		2016 2269
BglI	2	488 1756
BmgI	1	317
BpmI	1	1805
BsaI	1	1808
BsaAI	3	222 308 724
BsaHI	1	1334
BsaJI	6	98 189 286 378 2602
		3023
BsaWI	4	160 1578 2409 2556
BsaXI	2	672 2908
BsbI	1	631
BscGI	6	818 1326 1848 1872 2093
		2439
BseRI	1	189
BsiI	2	1205 2589
BsiEI	6	331 459 1356 1505 2428
		2852
BslI	9	238 264 620 946 2284
		2563 2729 2747 2921
BsmAI	2	1032 1808
BsoFI	25	
Bsp24I	4	2068 2100 2246 2278
Bsp1286I	7	190 319 325 799 1206
		1291 2452
BspLU11I	1	2762
BsrI	12	
BsrBI	4	868 1032 2833 3074
BsrDI	2	1640 1814
BsrFI	3	160 825 1789
BstXI	1	310
BstYI	7	228 1227 1244 2012 2024
		2110 2121
Cac8I	17	
CjeI	14	
CjePI	10	1470 1503 1942 1975 2062
		2095 2208 2240 2241 2273
CviJI	56	
CviRI	11	
DdeI	5	71 1373 1913 2079 2488
DpnI	15	
DraI	3	1296 1988 2007
DraIII	1	724
DrdI	2	679 2660
DrdII	1	729
DsaI	1	98

Enzyme	# Sites	Locations
EaeI	5	92 328 344 1481 2923
EagI	1	328
Eam1105I	1	1874
EarI	3	437 1075 2879
EciI	3	1718 2546 2692
Eco57I	2	1208 2220
EcoNI	1	236
EcoO109I	1	315
EcoRI	1	253
EcoRII	5	378 2601 2614 2735 3023
EcoRV	1	262
FauI	5	457 861 930 2922 2964
FokI	4	398 1435 1722 1903
FspI	2	478 1651
GdiII	4	328 344 1481 2923
HaeI	4	94 2288 2740 2751
HaeII	4	875 883 2522 2892
HaeIII	15	
HgaI	5	222 941 1342 2072 2650
HgiEII	1	2180
HhaI	20	
Hin4I	3	264 1799 1873
HincII	1	270
HindIII	1	274
HinfI	8	30 651 673 1875 2392
		2788 2863 2928
HphI	7	253 725 1147 1182 1388
		1804 2031
KpnI	1	161
MaeII	11	
MaeIII	15	
MboII	8	454 864 1092 1201 1279
		2034 2105 2896
MluI	1	214
MmeI	3	701 2369 2553
MnlI	12	
MscI	1	94
MseI	18	
MslI	4	308 1103 1462 1621
MspI	13	
MspA1I	7	121 155 428 1238 2179
		2424 2942
MwoI	15	
NciI	5	190 191 1338 1689 2385
NcoI	1	98
NgoAIV	1	825
NheI	1	291
NlaIII	9	9 102 1038 1431 1467
		1545 1555 2046 2766
NlaIV	15	
NotI	1	328
NspI	1	2766
NspV	1	134
Pfl1108I	1	1855
PleI	6	24 659 667 1883 2386
		2871
PmlI	1	308
Psp1406I	3	509 1272 1645
PstI	1	212
PvuI	2	459 1505
PvuII	2	428 2942
RcaI	2	1034 2042
RleAI	1	300
RsaI	2	159 1393
SacI	1	325
SalI	1	268
SapI	1	2879
Sau96I	8	315 316 447 715 1510
		1732 1749 1828
Sau3AI	15	
ScaI	1	1393

Enzyme	# Sites	Locations
ScrFI	10	190 191 380 1338 1689
		2385 2603 2616 2737 3025
SfaNI	4	1173 1422 1613 2665
SfcI	7	36 208 248 943 1628
		2306 2497
SmaI	1	191
SnaBI	1	222
SrfI	1	191
SspI	2	516 1069
StyI	2	98 286
TaqI	7	134 149 269 281 757
		1220 2664
TaqII	7	91 628 1172 1189 1342
		1527 2866
TfiI	2	2788 2928
ThaI	13	
TseI	15	
Tsp45I	5	241 364 897 1401 1612
Tsp509I	12	
Tth111II	3	2139 2171 2178
UbaJI	10	328 331 926 940 1130
		1359 1481 2565 2720 2838
VspI	3	1699 2934 2993
XbaI	1	297
XhoI	1	280
XmnI	1	1274

Enzymes that do not cut pT7Blue-2:

AatII	AflII	ApaBI	AscI	BbsI
BclI	BglII	Bpu10I	Bpu1102I	BsaBI
BsgI	BsmI	BsmBI	BsmFI	BspEI
BspGI	BspMI	BsrGI	BssHII	Bst1107I
BstEII	Bsu36I	ClaI	Eco47III	FseI
HpaI	MunI	NarI	NdeI	NruI
NsiI	PacI	PflMI	PmeI	PshAI
Psp5II	RsrII	SacII	SexAI	SfiI
SgfI	SgrAI	SpeI	SphI	Sse8387I
StuI	SunI	SwaI	Tth111I	XcmI