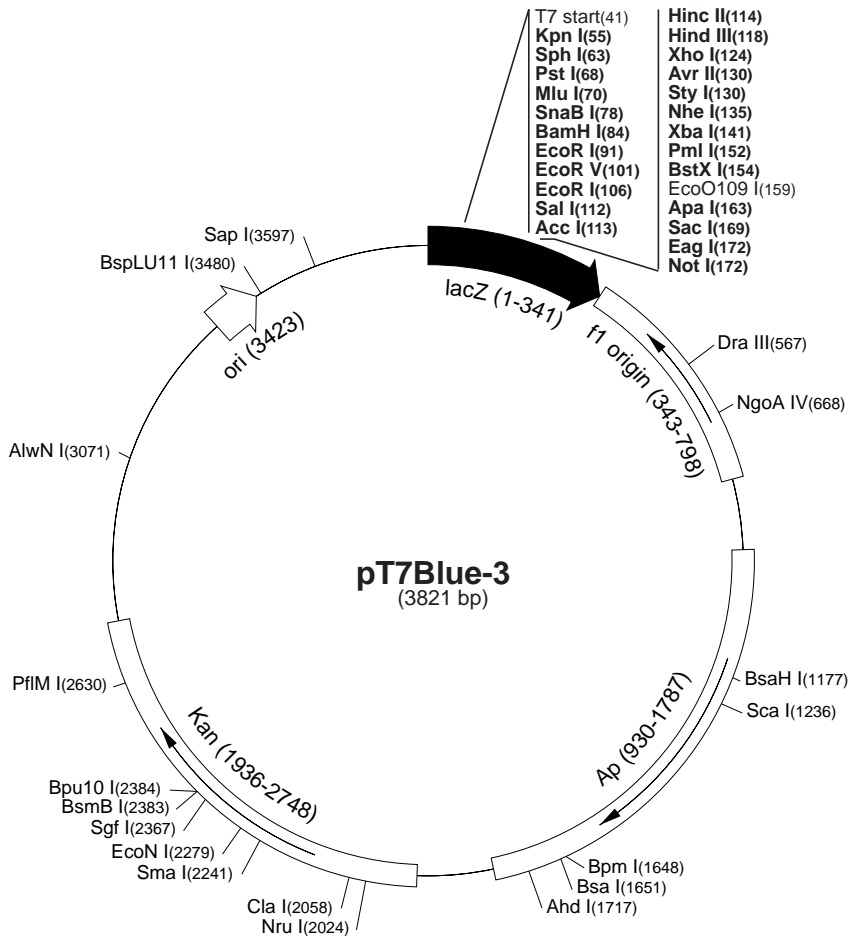


pT7Blue-3 is a multi-purpose cloning vector featuring a versatile multiple cloning region, blue/white screening, a T7 promoter and dual kanamycin/ampicillin resistance. Restriction sites producing 4-base 3' overhangs are conveniently positioned at each end of the polylinker to facilitate the generation of unidirectional deletions using exonuclease III. 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-3 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) or R-20mer primer (Cat. No. 69835-3).

### pT7Blue-3 sequence landmarks

<i>lacZ</i> start codon	1
<i>lacZ</i> $\alpha$ -peptide ORF	1-348
T7 promoter	24-40
T7 transcription start	41
multiple cloning region ( <i>Kpn</i> I - <i>Not</i> I)	51-178
f1 origin	343-798
<i>bla</i> coding sequence	938-1787
Kan coding sequence	1936-2748
pUC origin	3423



R-20mer primer #69835-3  
1

lacZ start

T7 promoter primer #69348-3  
T7 promoter

Blunt cloning site

*Kpn*I
*Sph*I
*Pst*I
*Mlu*I
*Sna*B I
*Bam*H I
*Eco*R I
*Eco*R V
*Eco*R I

ATGACCATGATTACGCCAAGCTCTAATACGACTACTATAGGGAAAGCTCGGTACCACGCGATGGTGCAGACGCGTTACGTATCGGATCCAGAATTCGTGATATCTGAATTC  
 Met Thr Met Ile Thr Pro Ser Ser Asn Thr Thr His Tyr Arg Glu Ser Ser Val Pro Arg Met Leu Gln Thr Arg Tyr Val Ser Asp Pro Glu Phe Val Ile Ser Glu Phe

*Acc*I
*Hinc* II
*Sal*I
*Hind* III
*Xho*I
*Avr* II
*Nhe*I
*Xba*I
*Pml*I
*Eco*O109 I
*Bst*X I
*Apa*I
*Sac*I
*Eag*I
*Not*I

GTCGACAAGCTTCTCGAGCCTAGGCTAGCTCTAGACCACACGTGTGGGGGCCGAGCTCGCGGCCGACAAATTCAGTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAAAC  
 Val Asp Lys Leu Leu Glu Pro Arg Leu Ala Leu Asp His Thr Cys Gly Gly Pro Ser Ser Arg Pro His Asn Ser Leu Ala Val Val Leu Gln Arg Asp Trp Glu Asn

U-19mer primer #69819-3

**pT7Blue-3 cloning/expression region**

# pT7Blue-3 Restriction Sites

Enzyme	# Sites	Locations				
AccI	1	113				
AcII	35					
AflIII	4	70	149	151	3480	
AhdI	1	1717				
AluI	19					
AlwI	12					
Alw26I	3	875	1651	2383		
AlwNI	1	3071				
ApaI	1	163				
ApaLI	2	1045	3166			
ApoI	6	91	106	369	380	1980
		2164				
AvaI	3	124	162	2239		
Avall	2	1353	1575			
AvrII	1	130				
BamHI	1	84				
BanI	4	51	604	1764	3736	
BanII	4	163	169	642	2022	
BbvI	13					
BcgI	1	1213				
BcgI'	1	1179				
Bfal	8	131	136	142	718	1524
		1859	1967	2987		
BglII	2	332	1599			
Bpml	1	1648				
Bpu10I	1	2384				
BsaI	1	1651				
BsaAI	3	78	152	567		
BsaHI	1	1177				
BsaJI	7	130	222	2238	2239	2640
		3320	3741			
BsaWI	4	1421	2502	3127	3274	
BsiEI	7	175	303	1199	1348	2367
		3146	3570			
BsiHKAI	4	169	1049	1134	3170	
BsII	10	463	789	2281	2613	2630
		3002	3281	3447	3465	3639
BsmI	2	2251	2328			
BsmBI	1	2383				
Bsp1286I	7	163	169	642	1049	1134
		2022	3170			
BspLU11I	1	3480				
BsrI	13					
BsrBI	4	711	875	3551	3792	
BsrDI	2	1483	1657			
BsrFI	3	668	1632	2321		
BssSI	2	1048	3307			
BstXI	1	154				
BstYI	8	84	1070	1087	1855	1867
		2616	2828	2839		
Cac8I	19					
Clal	1	2058				
CviJI	60					
Ddel	6	1216	1756	2384	2748	2797
		3206				
Dpnl	18					
DraI	3	1139	1831	1850		
DrallI	1	567				
DrdI	2	522	3378			
EaeI	4	172	188	1324	3641	
EagI	1	172				
EarI	4	281	918	2180	3597	
Eco57I	2	1051	2938			
EcoNI	1	2279				
EcoO109I	1	159				
EcoRI	2	91	106			
EcoRII	7	222	2255	2612	3319	3332
		3453	3741			
EcoRV	1	101				
FauI	5	301	704	773	3640	3682
Fnu4HI	24					

Enzyme	# Sites	Locations				
FokI	6	242	1278	1565	1746	2005
		2611				
FspI	2	322	1494			
HaeII	4	718	726	3240	3610	
HaeIII	16					
Hgal	5	78	784	1185	2790	3368
HhaI	24					
HincII	1	114				
HindIII	1	118				
Hinfl	13					
HphI	11					
KpnI	1	55				
MaellI	16					
MbolI	10	298	707	935	1044	1122
		1877	2167	2278	2823	3614
MluI	1	70				
MnlI	14					
MseI	22					
MslI	4	152	946	1305	1464	
MspI	14					
MspA1I	5	272	1081	2897	3142	3660
MwoI	19					
NciI	5	1181	1532	2240	2241	3103
NgoAIV	1	668				
NheI	1	135				
NlaIII	14					
NlaIV	15					
NotI	1	172				
NruI	1	2024				
NsiI	2	2217	2483			
NspI	2	63	3484			
PfIM1	1	2630				
PleI	7	24	502	510	1726	2599
		3104	3589			
PmlI	1	152				
Psp1406I	2	1115	1488			
PstI	1	68				
PvuI	3	303	1348	2367		
PvuII	2	272	3660			
RcaI	3	877	1885	2760		
RsaI	3	53	1236	2202		
SacI	1	169				
Sall	1	112				
SapI	1	3597				
Sau3AI	18					
Sau96I	8	159	160	291	558	1353
		1575	1592	1671		
Scal	1	1236				
ScrFI	12					
SfaNI	10	1016	1265	1456	1983	2067
		2202	2289	2409	2720	3383
Sfcl	6	36	64	786	1471	3024
		3215				
Sgfl	1	2367				
SmaI	1	2241				
SnaBI	1	78				
SphI	1	63				
Sspl	3	359	912	2292		
StyI	1	130				
TalI	11					
TaqI	8	113	125	600	1063	2058
		2332	2735	3382		
TfiI	6	2278	2334	2506	2597	3506
		3646				
Thal	16					
TseI	13					
Tsp45I	5	208	740	1244	1455	2514
Tsp509I	19					
TspRI	12					
VspI	5	1542	2566	2755	3652	3711
XbaI	1	141				

Enzyme	# Sites	Locations		
XhoI	1	124		
XmnI	2	1117	2756	

Enzymes that do not cut pT7Blue-3:

AatII	AflII	AscI	BbsI	BclI
BglII	Bpu1102I	BsaBI	BseRI	BsgI
BsmFI	BspEI	BspMI	BsrGI	BssIII
Bst1107I	BstEII	Bsu36I	DsaI	Eco47III
FseI	HpaI	MscI	MunI	NarI
NcoI	NdeI	NspV	Pacl	PinAI
PmeI	PshAI	Psp5II	RsrII	SacII
SanDI	SexAI	SfiI	SgrAI	SpeI
SrfI	Sse8387I	StuI	SunI	Swal
Tth111I	UbaEI	XcmI		