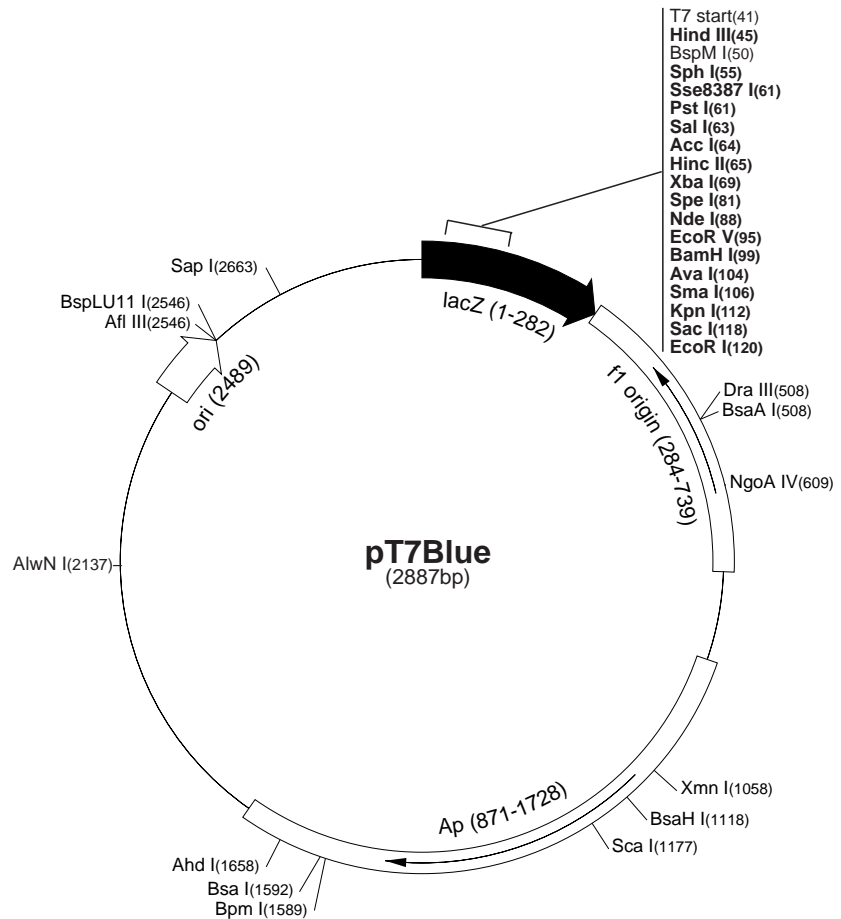


pT7Blue contains the pUC19 backbone (including high-copy number origin of replication and *lac* sequences), a T7 promoter, f1 origin of replication, and modified multiple cloning region. The multiple cloning region contains an *EcoR* V site used for T-cloning flanked by an *Nde* I site, which allows inserts to be conveniently subcloned into the *Nde* I site of many pET vectors. 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 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 pUC/M13 reverse primer (e.g. R-20mer, Cat. No. 69835-3).

pT7Blue sequence landmarks	
<i>lacZ</i> start codon	1
<i>lacZ</i> $\alpha$ -peptide	1-282
T7 promoter	24-40
T7 transcription start	41
multiple cloning region ( <i>Hind</i> III - <i>EcoR</i> I)	45-125
f1 origin*	284-739
<i>bla</i> coding sequence	871-1728
pUC origin	2489



R-20mer primer #69835-3 → T7 promoter primer #69348-3

1 lacZ start → T7 promoter →

ATGACCATGATTACGCCAAGCTCTAATACGACTCACTATAGGGAAAGCTTGCAATGCCCTGCAGGTCGACTCTAGAGGATCTACTAGTCATATGGATATCGGATCCCGGGTACCGAGCTCGAATTCA  
 MetThrMetIleThrProSerSerAsnThrThrHisTyrArgGluSerLeuHisAlaCysArgSerThrLeuGluAspLeuLeuValIleTrpIleSerAspProArgValIProSerSerAsnSer

CTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCTT  
 LeuAlaValValLeuGlnArgArgAspTrpGluAsnPro...

← U-19mer primer #69819-3

Blunt cloning site

Sma I  
Ava I

Spe I Nde I EcoR V BamH I Kpn I Sac I EcoR I

**pT7Blue cloning/expression region**

# pT7Blue Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations		
AccI	1	64	GdII	3	128 1265 2707	VspI	3	1483 2718 2777		
AccIII	3	348 1354 2594	HaeI	3	2072 2524 2535	XbaI	1	69		
AcII	32		HaeII	4	659 667 2306 2676	XmnI	1	1058		
AflIII	1	2546	HaeIII	12		Enzymes that do not cut pT7Blue:				
AluI	17		HgaI	4	725 1126 1856 2434	AatII	AflII	AgeI	ApaI	ApaBI
AlwI	11		HgiEI	1	1964	AscI	AvrII	BaeI	BbsI	BclI
Alw21I	4	118 990 1075 2236	HhaI	20		BglII	BmgI	Bpu10I	Bpu1102I	BsaBI
Alw44I	2	986 2232	Hin4I	3	97 1583 1657	BseRI	BsgI	BsmI	BsmBI	BsmFI
AlwNI	1	2137	HincII	1	65	BspEI	BspGI	BsrGI	BssHII	Bst1107I
ApoI	3	120 310 321	HindIII	1	45	BstEII	BstXI	Bsu36I	Clal	Dsal
AvaI	1	104	Hinfl	9	30 66 435 457 1659	EagI	Eco47III	EcoNI	EcoO109I	FseI
Avall	2	1294 1516	HphI	6	2176 2572 2647 2712	HpaI	MluI	MscI	MunI	NarI
BamHI	1	99			509 931 966 1172 1588	NcoI	NheI	NotI	NruI	NsiI
BanI	4	108 545 1705 2802			1815	NspV	Pacl	PfiMI	PmeI	PmlI
BanII	2	118 583	KpnI	1	112	PshAI	Psp5II	RleAI	RsrII	SacII
BbvI	12		Maell	9	144 293 452 464 507	SexAI	SfiI	Sgfi	SgrAI	SnaBI
BccI	5	498 515 1209 1496 1620			617 1056 1429 1845	SrfI	StuI	Styl	SunI	Swal
Bce83I	4	1054 1922 2163 2461	MaeIII	12		Tth111I	XcmI	XhoI		
Bcefl	3	116 534 2047	MbolI	8	238 648 876 985 1063					
Bcgl	2	1120 1154			1818 1889 2680					
Bfal	6	70 82 659 1465 1800	MmeI	3	485 2153 2337					
		2053	MnlI	12						
BglI	2	272 1540	MseI	18						
BpmI	1	1589	MslI	3	887 1246 1405					
BsaI	1	1592	MspI	12						
BsaAI	1	508	MspAII	5	212 1022 1963 2208 2726					
BsaHI	1	1118	Mwol	14						
BsaJI	5	103 104 162 2386 2807	NciI	5	105 106 1122 1473 2169					
BsaWI	3	1362 2193 2340	NdeI	1	88					
BsaXI	2	456 2692	NgoAIV	1	609					
Bsbl	1	415	NlaIII	9	9 55 822 1215 1251					
BscGI	6	602 1110 1632 1656 1877			1329 1339 1830 2550					
		2223	NlaIV	13						
Bsil	2	989 2373	NspI	2	55 2550					
BsiEI	5	243 1140 1289 2212 2636	Pfi1108I	1	1639					
BsII	7	404 730 2068 2347 2513	PleI	7	24 60 443 451 1667					
		2531 2705			2170 2655					
BsmAI	2	816 1592	Psp1406I	3	293 1056 1429					
BsoFI	20		PstI	1	61					
Bsp24I	4	1852 1884 2030 2062	PvuI	2	243 1289					
Bsp1286I	5	118 583 990 1075 2236	PvuII	2	212 2726					
BspLU11I	1	2546	RcaI	2	818 1826					
BspMI	1	50	RsaI	2	110 1177					
BsrI	12		SacI	1	118					
BsrBI	4	652 816 2617 2858	Sall	1	63					
BsrDI	2	1424 1598	SapI	1	2663					
BsrFI	2	609 1573	Sau96I	6	231 499 1294 1516 1533					
BstYI	8	75 99 1011 1028 1796			1612					
		1808 1894 1905	Sau3AI	12						
Cac8I	16		Scal	1	1177					
CjeI	12		ScrFI	10	105 106 164 1122 1473					
CjePI	10	1254 1287 1726 1759 1846			2169 2387 2400 2521 2809					
		1879 1992 2024 2025 2057	SfaNI	4	957 1206 1397 2449					
CviJI	46		Sfcl	6	36 57 727 1412 2090					
CviRI	11				2281					
Ddel	4	1157 1697 1863 2272	SmaI	1	106					
DpnI	16		SpeI	1	81					
DraI	3	1080 1772 1791	SphI	1	55					
DrallI	1	508	Sse8387I	1	61					
DrdI	2	463 2444	Sspl	2	300 853					
DrdII	1	513	TaqI	5	64 118 541 1004 2448					
EaeI	3	128 1265 2707	TaqII	6	412 956 973 1126 1311					
Eam1105I	1	1658			2650					
EarI	3	221 859 2663	TfiI	2	2572 2712					
Ecil	3	1502 2330 2476	Thal	11						
Eco57I	2	992 2004	Tsel	12						
EcoRI	1	120	Tsp45I	4	148 681 1185 1396					
EcoRII	5	162 2385 2398 2519 2807	Tsp509I	10	120 284 310 321 1225					
EcoRV	1	95			1480 1786 2774 2849 2866					
FauI	5	241 645 714 2706 2748	Tth1111I	3	1923 1955 1962					
FokI	4	182 1219 1506 1687	UbaJI	8	710 724 914 1143 1265					
Fspl	2	262 1435			2349 2504 2622					