

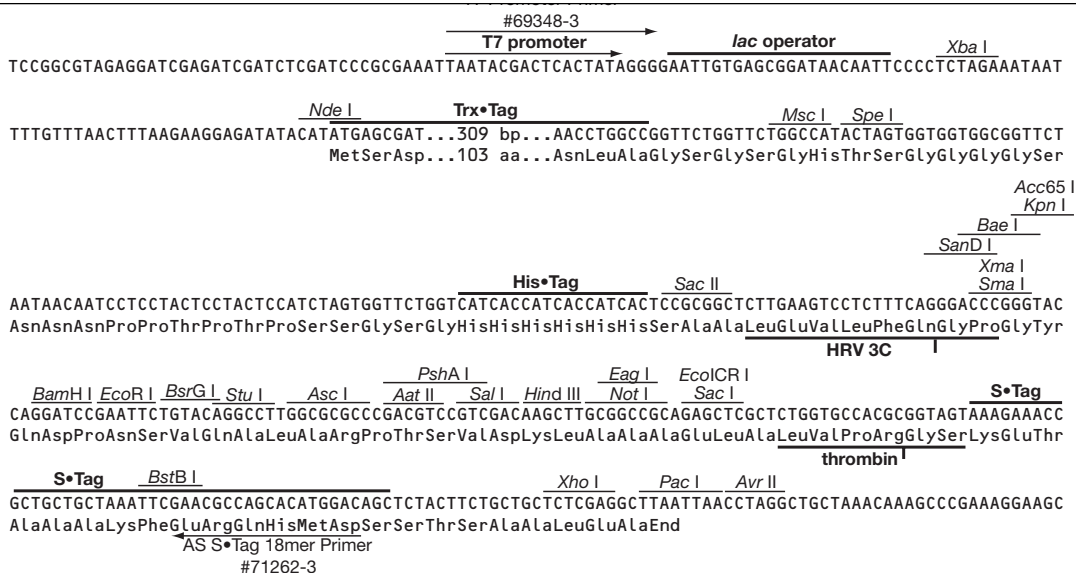
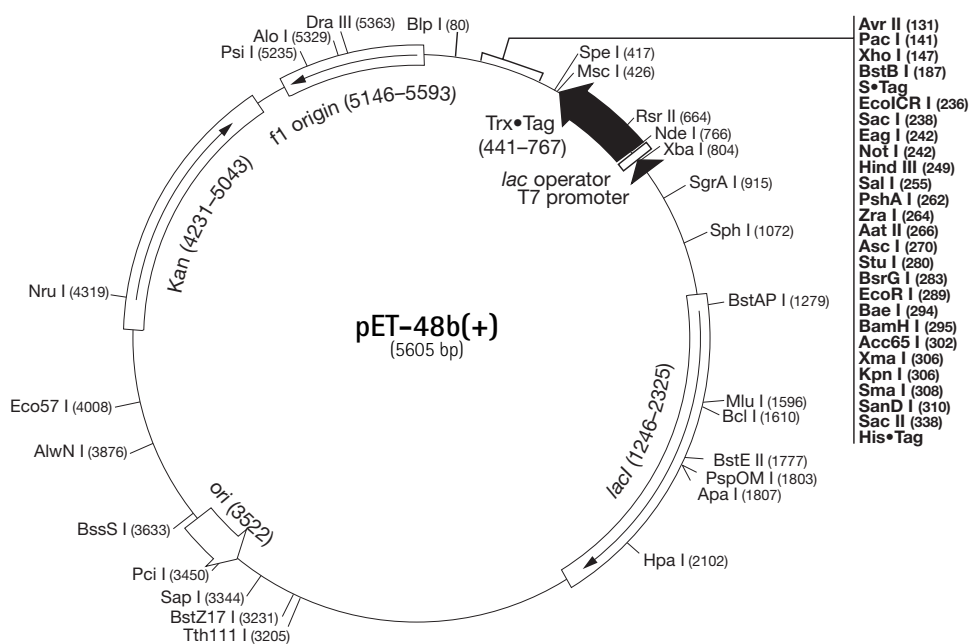
## pET-48b(+)<sup>+</sup> Vector

TB416 0804

	Cat. No.
pET-48b(+) <sup>+</sup> Cloning Kit	71462-3
<b>pET-48b(+)<sup>+</sup> sequence landmarks</b>	
T7 promoter	839-855
T7 transcription start	838
Trx•Tag coding sequence	441-767
His•Tag coding sequence	342-359
Multiple cloning sites ( <i>SanD</i> I – <i>Avr</i> II)	131-315
S•Tag coding sequence	168-212
T7 terminator	26-73
<i>lacI</i> coding sequence	1246-2325
pBR322 ori	3522
Kan coding sequence	4231-5043
f1 origin	5146-5593

The pET-48b(+)<sup>+</sup> vector carries N-terminal Trx•Tag™ and His•Tag® coding sequences followed by a recognition site for the human rhinovirus (HRV) 3C protease. This protease is highly specific for cleavage of the sequence LEVLFG↓GP (1), and is active at low temperatures (2). pET-48b(+)<sup>+</sup> also contains an optional C-terminal thrombin recognition site followed by an S•Tag™ coding sequence. Unique restriction sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circle map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with the helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the AS S•Tag 18mer Primer (Cat. No. 71262-3).

1. Cordingley, M.G., Register, R.B., Callahan, P.L., Garsky, V.M., and Colonno, R.J. (1989) *J. Virol.* 63, 5037-5045.
2. Wang, Q.M., Johnson, R.B., Cox, G.A., Villarreal, E.C., and Loncharich, R.J. (1997) *Anal. Biochem.* 252, 238-245..



pET-48b(+)<sup>+</sup> cloning/expression region

## pET-48b(+) Restriction Sites

TB416 0804

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations			
AatII	1	266	BtgI	3	335 544 1033	ZraI	1	264			
Acc65I	1	302	BtsI	5	572 1961 2329 4523 4610	Enzymes that do not cut pET-48b(+):					
AccI	2	256 3230	Clal	3	583 873 4353	AarI	Afill	AgeI	AhdI	AleI	AsiSI
AccII	3	1258 2785 5148	DrallI	1	5363	BbvCI	BgII	BgIII	BmgBI	BmtI	BpII
AfeI	2	1001 2714	DrdI	3	3153 3568 5318	BsaI	BseRI	BsiWI	BspMI	Bsu36I	BtrI
AflIII	2	1596 3460	EaeI	6	242 424 440 904 1036	Dral	EcoRV	FalI	FseI	FspAI	Fspl
Alol	1	5329	EagI	1	2270	MfeI	NcoI	NheI	PinAI	PmeI	PmlI
AlwNI	1	3876	EarI	3	1214 3344 4475	Psrl	PstI	PvuI	SbfI	Scal	SexAI
Apal	1	1807	Ecil	3	1387 3522 3668	SfiI	SnaBI	SrfI	Sse8387I	Swal	
ApaLI	4	485 1576 3274 3774	Eco57I	1	4008						
AscI	1	270	Eco57MI	4	1434 1923 2987 4008						
Asel	5	853 2281 2340 4861 5050	EcoCRI	1	236						
Aval	2	147 306	EcoNI	3	318 1131 4574						
AvrII	1	131	EcoO109I	4	53 310 1029 2466						
BaeI	1	294	EcoRI	1	289						
BamHI	1	295	HaeII	13							
BanI	9	223 302 918 939 1053	HincII	2	257 2102						
BanII	6	1516 2235 2365 5400	HindIII	1	249						
		238 980 994 1807 4317	HpaI	1	2102						
		5438	KasI	4	918 939 1053 2235						
BbeI	4	922 943 1057 2239	KpnI	1	306						
BbsI	3	1742 2081 2578	MluI	1	1596						
BceAI	6	1115 1455 2082 3962 4981	MscI	1	426						
		5388	MslI	7	420 1648 1936 1966 2447						
BcgI	5	236 516 673 1922 3037			2642 3033						
BciVI	4	533 2053 3663 5057	NaeI	2	908 5466						
BclI	1	1610	NarI	4	919 940 1054 2236						
BfrBI	2	4510 4776	NdeI	1	766						
BlpI	1	80	NgoMIV	2	906 5464						
Bme1580I	5	489 1580 1807 3278 3778	NotI	1	242						
BmrI	5	1125 1522 1759 2399 3199	NruI	1	4319						
BpmI	3	1434 1923 2987	NsiI	2	4512 4778						
Bpu10I	2	2566 4679	NspI	4	1071 2805 3097 3464						
BpuEI	5	21 2410 3551 3849 4090	NspV	1	187						
BsaAI	2	3212 5363	PacI	1	141						
BsaBI	3	869 879 2657	PciI	1	3460						
BsaHI	6	263 919 940 1054 1553	PfiMI	4	179 373 1178 4925						
		2236	PfoI	2	1163 3102						
BsaWI	7	2 1915 2418 2649 3666	PpiI	2	4175 5329						
		3813 4797	PpuMI	2	310 2466						
BsaXI	4	331 368 2271 5327	PshAI	1	262						
BseYI	3	1995 2130 3764	PsiI	1	5235						
BsgI	3	1447 1647 2620	PspOMI	1	1803						
BsiEI	4	245 2381 3376 3800	PvuII	3	2196 2289 3051						
BsiHKAI	7	238 489 1096 1580 2454	RsrII	1	664						
		3278 3778	SacI	1	238						
BsmAI	6	1293 1698 1824 2211 3101	SacII	1	338						
		4678	Sall	1	255						
BsmBI	3	2211 3101 4678	SanDI	1	310						
BsmFI	4	296 1057 2731 5578	SapI	1	3344						
BsmI	2	4546 4623	Sfcl	4	838 3725 3916 5582						
Bsp1286I	12		SfoI	4	920 941 1055 2237						
BspCNI	9	93 114 2181 2558 2720	SgrAI	1	915						
		3260 3748 4157 4692	Smal	1	308						
BspEI	2	2 2649	SmlI	6	36 147 2389 3566 3828						
BspHI	3	994 4180 5055			4105						
BspLU11I	1	3460	SpeI	1	417						
BsrBI	4	825 3393 5061 5507	SphI	1	1071						
BsrDI	2	1643 2009	Sspl	2	4587 5155						
BsrFI	6	438 906 915 1282 4616	StuI	1	280						
		5464	StyI	3	57 131 275						
BsrGI	1	283	TaqII	4	2395 3362 4916 5267						
BssHII	2	270 2007	TatI	3	283 715 3264						
BssSI	1	3633	TspGWI	8	274 561 677 711 2585						
Bst1107I	1	3231			2903 4471 4483						
BstAPI	1	1279	Tth111I	1	3205						
BstBI	1	187	XbaI	1	804						
BstEII	1	1777	XcmI	4	419 1452 1968 1986						
BstXI	4	422 1398 1527 1650	XhoI	1	147						
BstYI	7	295 1160 2372 2652 4101	XmaI	1	306						
		4112 4911	XmnI	3	463 3018 5051						
BstZ17I	1	3231									