

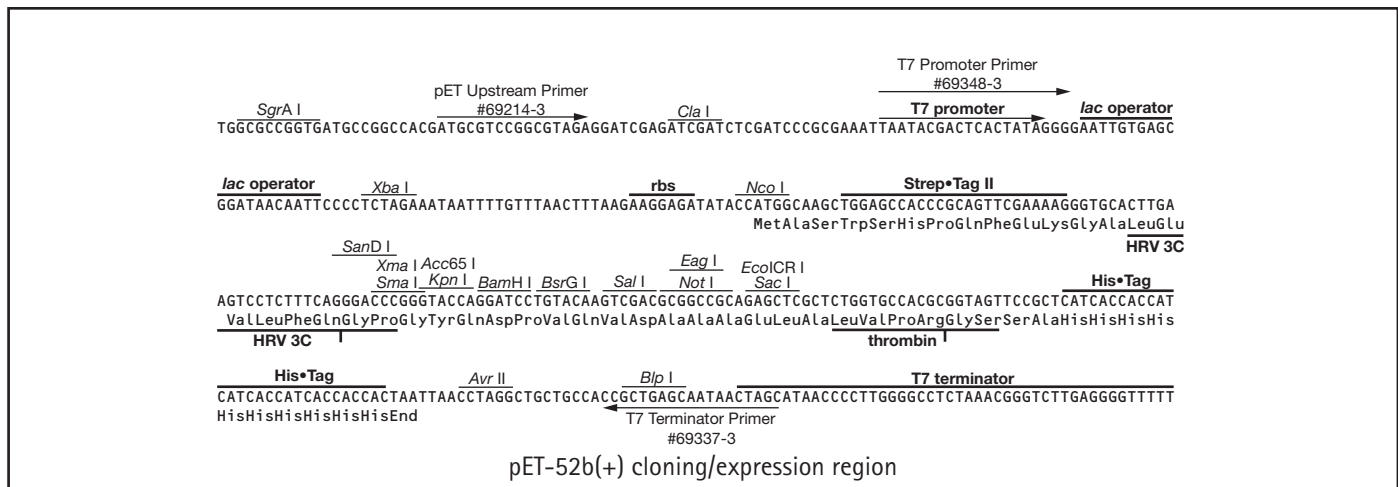
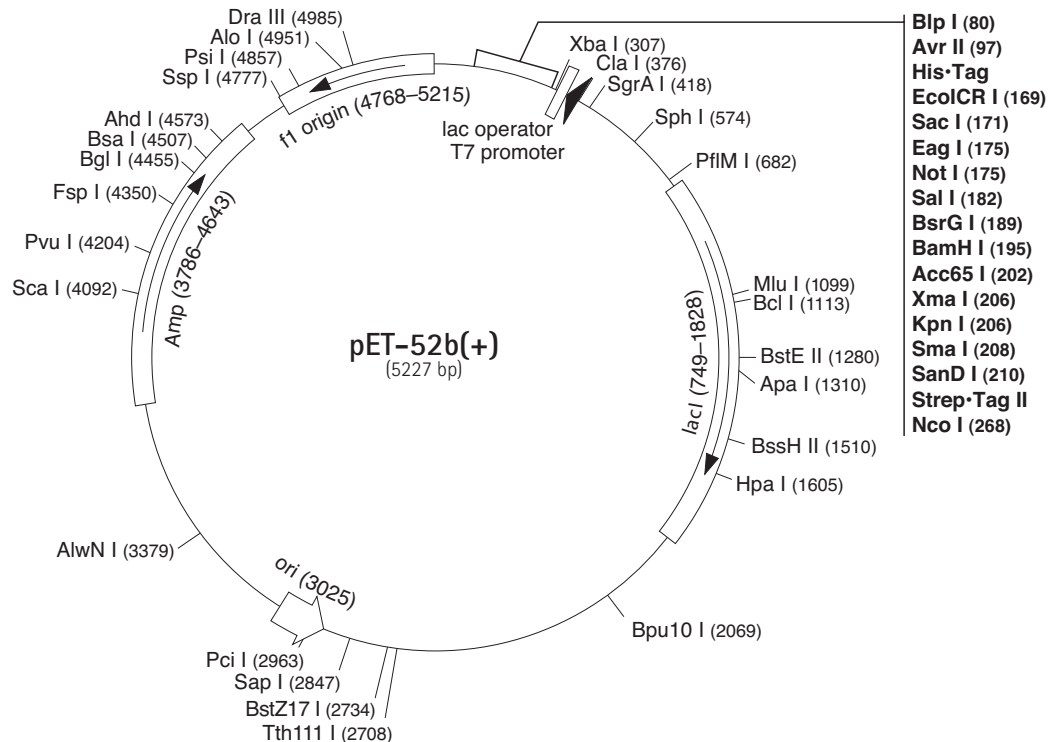
pET-52b(+)⁺ Vector

TB430 0905

	Cat No.
pET-52b(+) ⁺ DNA	71554-3
pET-52b(+)⁺ sequence landmarks	
T7 promoter	342-358
T7 transcription start	341
Strep•Tag II coding sequence	239-262
Multiple cloning sites (<i>SanD</i> I - <i>Avr</i> II)	97-215
His•Tag coding sequence	109-139
T7 terminator	26-73
<i>lacI</i> coding sequence	749-1828
pBR322 ori	3025
<i>bla</i> (Amp ^R)	3786-4643
f1 origin	4768-5215

The pET-52b(+)⁺ vector carries an N-terminal Strep•Tag[®] II coding sequence (1) followed by a recognition site for the human rhinovirus (HRV) 3C protease. This protease is highly specific for cleavage of the sequence LEVLFQ↓GP (2), and is active at low temperatures (3). The multiple cloning region is followed by an optional thrombin recognition site and a C-terminal His•Tag[®] coding sequence. The presence of two “gentle elution” tags at both the N-terminus and C-terminus is ideal for dual purification strategies designed to isolate full-length fusion proteins (4). 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 circle map. 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.

- Skerra, A., Schmidt, T.G.M. (2000) *Meth. Enzymol.* 326, 271-304.
- Cordingley, M.G., Register, R.B., Callahan, P.L., Garsky, V.M., and Colonna, R.J. (1989) *J. Virol.* 63, 5037-5045.
- Wang, Q.M., Johnson, R.B., Cox, G.A., Villarreal, E.C., and Loncharich, R.J. (1997) *Anal. Biochem.* 252, 238-245.
- Fiedler, M., Horn, C., Bandtlow, C., Schwab, M.E. (2002) *Protein Eng.* 15, 931-941.



pET-52b(+) Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations			
Acc65I	1	202	Clal	1	376			
AccI	2	183 2733	Dral	3	3995 4687 4706			
AccI	5	761 2288 3971 4344 4770	DraIII	1	4985			
AfeI	2	504 2217	DrdI	3	2656 3071 4940			
AflIII	2	1099 2963	EaeI	5	175 407 539 1773 4180			
AhdI	1	4573	EagI	1	175			
Alol	1	4951	EarI	3	717 2847 3774			
AlwNI	1	3379	Ecil	4	890 3025 3171 4431			
Apal	1	1310	Eco57I	2	3511 3907			
ApaLI	5	232 1079 2777 3277 3901	Eco57MI	7	243 937 1426 2490 3511			
AseI	4	356 1784 1843 4398			3907 4504			
AvaI	1	206	EcoCRI	1	169			
AvrII	1	97	EcoNI	2	218 634			
BaeI	1	194	EcoO109I	4	53 210 532 1969			
BamHI	1	195	FspI	1	4350			
BanI	10	156 202 421 442 556	HaeII	13				
		1019 1738 1868 4620 5022	HincII	2	184 1605			
BanII	5	171 483 497 1310 5060	HpaI	1	1605			
BbeI	4	425 446 560 1742	KasI	4	421 442 556 1738			
BbsI	3	1245 1584 2081	KpnI	1	206			
BceAI	5	618 958 1585 3465 5010	MluI	1	1099			
BcgI	4	223 1425 2540 4035	MsiI	9	1151 1439 1469 1950 2145			
BciVI	3	1556 3166 3735			2536 3802 4161 4320			
BclI	1	1113	NaeI	2	411 5088			
BglI	1	4455	NarI	4	422 443 557 1739			
BlpI	1	80	NcoI	1	268			
Bme1580I	6	236 1083 1310 2781 3281	NgoMIV	2	409 5086			
		3905	NotI	1	175			
Bmri	6	628 1025 1262 1902 2702	NspI	4	574 2308 2600 2967			
		4533	PciI	1	2963			
Bpml	5	243 937 1426 2490 4504	PfiMI	1	681			
Bpu10I	1	2069	PfoI	2	666 2605			
BpuEI	6	21 1913 3054 3352 3593	PpiI	3	3678 3945 4951			
		3969	PpuMI	2	210 1969			
BsaAI	2	2715 4985	PsiI	1	4857			
BsaBI	3	372 382 2160	PspOMI	1	1306			
BsaHI	6	422 443 557 1056 1739	PvuI	1	4204			
		4033	PvuII	3	1699 1792 2554			
BsaI	1	4507	SacI	1	171			
BsaWI	7	2 1418 1921 2152 3169	Sall	1	182			
		3316 4277	SanDI	1	210			
BsaXI	2	1774 4949	SapI	1	2847			
BseYI	3	1498 1633 3267	Scal	1	4092			
BsgI	3	950 1150 2123	Sfcl	5	341 3228 3419 4327 5204			
BsiEI	6	178 1884 2879 3303 4055	Sfol	4	423 444 558 1740			
		4204	SgrAI	1	418			
BsiHKA1	9	171 236 599 1083 1957	SmaI	1	208			
		2781 3281 3905 3990	SmlI	6	36 1892 3069 3331 3608			
BsmAI	7	796 1201 1327 1714 2604			3948			
		3730 4507	SphI	1	574			
BsmBI	2	1714 2604	Sspl	1	4777			
BsmFI	4	196 560 2234 5200	StyI	3	57 97 268			
Bsp1286I	13		TaqII	5	1898 2865 4041 4226 4889			
BspCNI	9	93 1684 2061 2223 2763	TatI	3	189 2767 4090			
		3251 3660 4085 4604	TspGWI	4	2088 2406 3792 4134			
BspEI	2	2 2152	Tth111I	1	2708			
BspHI	4	497 3683 3732 3764	XbaI	1	307			
BsrBI	5	141 328 2896 3730 5129	XcmI	3	955 1471 1489			
BsrDI	4	1146 1512 4339 4513	XmaI	1	206			
BsrFI	5	409 418 785 4488 5086	XmnI	2	2521 3973			
BsrGI	1	189						
BssHII	1	1510	Enzymes that do not cut pET-52b(+):					
BssSI	2	3136 3904	AarI,	AatII,	AfilI,	Agel,	AleI,	AscI,
BstAPI	1	782	AsiSI,	BbvCI,	BfrBI,	BglII,	BmgBI,	BmtI,
BstBI	1	243	BpII,	BseRI,	BsiWI,	Bsml,	BspMI,	Bsu36I,
BstEII	1	1280	EcoRI,	EcoRV,	FalI,	FseI,	FspAI,	HindIII,
BstXI	3	901 1030 1153	MfeI,	MscI,	NdeI,	NheI,	NruI,	NsiI,
BstYI	10	195 663 1875 2155 3604	Pacl,	PmeI,	PmlI,	PshAI,	Psrl,	PstI,
		3615 3926 3943 4711 4723	RsrII,	SacII,	SbfI,	SexAI,	Sfil,	SnaBI,
BstZ17I	1	2734	SpeI,	SrfI,	StuI,	Swal,	XhoI,	ZraI
BtgI	2	268 536						
BtsI	4	1464 1832 4154 4174						