

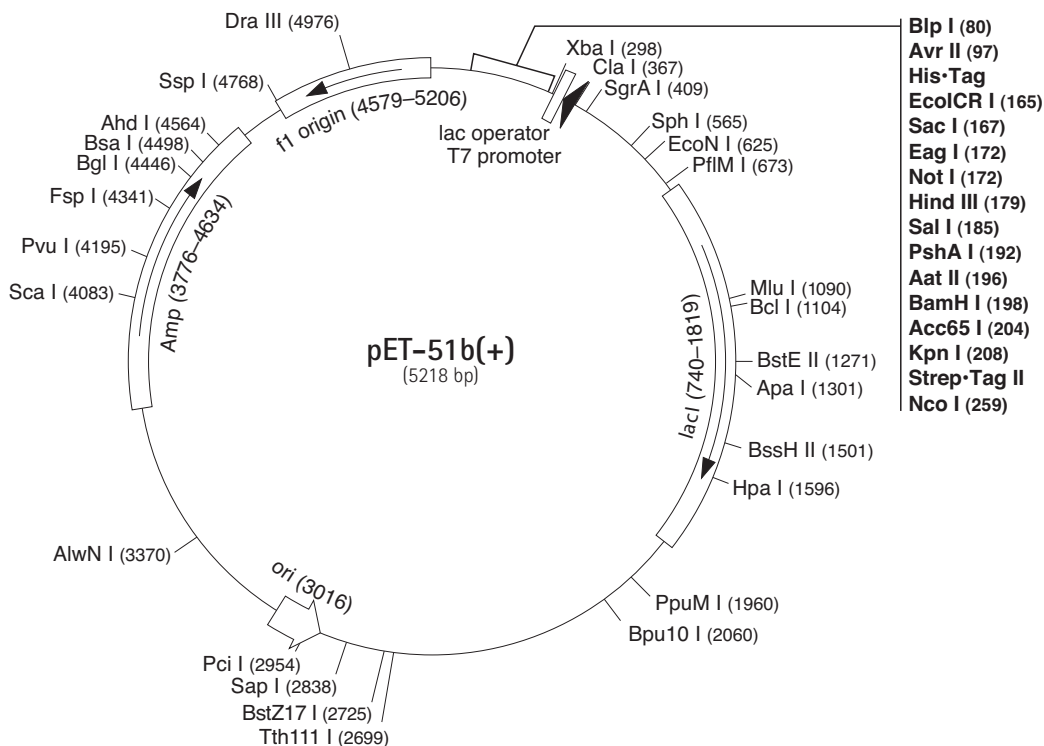
pET-51b(+) Vector

TB429 0905

	Cat No.
pET-51b(+) DNA	71553-3
pET-51b(+) sequence landmarks	
T7 promoter	333-349
T7 transcription start	332
Strep•Tag II coding sequence	230-253
Multiple cloning sites	97-209
(<i>Kpn</i> I – <i>Avr</i> II)	
His•Tag coding sequence	109-139
T7 terminator	26-73
<i>lac</i> I coding sequence	740-1819
pBR322 ori	3016
<i>bla</i> (Amp ^R)	3777-4634
f1 origin	4579-5206

The pET-51b(+) vector carries an N-terminal Strep•Tag® II coding sequence (1) followed by a recognition site for enterokinase, and an optional 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 (2). 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.
- Fiedler, M., Horn, C., Bandtlow, C., Schwab, M.E. (2002) *Protein Eng.* 15, 931-941.



pET Upstream Primer #69214-3

T7 Promoter Primer #69348-3

SGR A I

TG G C C C G G T G A T G C C G G C C A G A T G C G T C C G G C T A G A G G A T C G A G A T C G A T C T C G A T C C C G C G A A A T T A A T A C G A C T C A C T A T A G G G G A A T T G T G A G C

lac operator

Xba I

rbs

Nco I

Strep•Tag II

Met Ala Ser Trp Ser His Pro Gln Phe Glu Lys Gly Ala Asp Asp enterokinase

Acc65 I

PshA I

Eag I

EcoICR I

C G A C G A C A A G G T A C C G G A T C C G A C G T C G G T C G A C A A G C T T G C G G C C G C A G G A G C T C C G G C T T C T C C T C A A T T T C C G C T C A T C A C C A C C A T C A C C A T

Kpn I

BamH I

Aat II

Sal I

Hind III

Not I

Sac I

BseR I

His•Tag

Asp Asp Lys Val Pro Asp Pro Thr Ser Val Asp Lys Leu Ala La Ala La Gly Ala Pro Gly Phe Ser Ser Ile Ser Ala His His His His His His His enterokinase

His•Tag

Avr II

Blp I

T7 terminator

C A C C A C C A C T A A T T A A C C T A G G C T G C T G C C A C C G C T G A G C A A T A A C T A G C A T A A C C C T T G G G C C T C T A A C G G G T C T T G A G G G T T T T T T G C T G A A A G

His His His End

T7 Terminator Primer #69337-3

pET-51b(+) cloning/expression region

pET-51b(+) Restriction Sites

TB429 0905

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations			
AatII	1	196	DrallI	1	4976			
Acc65I	1	204	DrdI	3	2647 3062 4931			
AccI	2	186 2724	EaeI	5	172 398 530 1764 4171			
AccI	5	752 2279 3962 4335 4761	EagI	1	172			
AfeI	2	495 2208	EarI	3	708 2838 3765			
AfilIII	2	1090 2954	Ecil	4	881 3016 3162 4422			
AhdI	1	4564	Eco57I	2	3502 3898			
Alol	1	4942	Eco57MI	7	234 928 1417 2481 3502			
AlwNI	1	3370			3898 4495			
Apal	1	1301	EcoCRI	1	165			
ApaLI	4	1070 2768 3268 3892	EcoNI	1	625			
Asel	4	347 1775 1834 4389	EcoO109I	3	53 523 1960			
AvrII	1	97	FspI	1	4341			
BamHI	1	198	HaeII	13				
BanI	9	204 412 433 547 1010	HincII	2	187 1596			
		1729 1859 4611 5013	HindIII	1	179			
BanII	5	167 474 488 1301 5051	HpaI	1	1596			
Bbel	4	416 437 551 1733	KasI	4	412 433 547 1729			
BbsI	3	1236 1575 2072	KpnI	1	208			
BceAI	5	609 949 1576 3456 5001	MluI	1	1090			
Bcgl	5	166 214 1416 2531 4026	MslI	9	1142 1430 1460 1941 2136			
BciVI	3	1547 3157 3726			2527 3793 4152 4311			
BclI	1	1104	NaeI	2	402 5079			
BglI	1	4446	NarI	4	413 434 548 1730			
BlpI	1	80	NcoI	1	259			
Bme1580I	5	1074 1301 2772 3272 3896	NgoMIV	2	400 5077			
BmrI	6	619 1016 1253 1893 2693	NotI	1	172			
		4524	NspI	4	565 2299 2591 2958			
BpmI	5	234 928 1417 2481 4495	PciI	1	2954			
Bpu10I	1	2060	PfiMI	1	672			
BpuEI	6	21 1904 3045 3343 3584	PfoI	2	657 2596			
		3960	PpiI	3	3669 3936 4942			
BsaAI	2	2706 4976	PpuMI	1	1960			
BsaBI	3	363 373 2151	PshAI	1	192			
BsaHI	7	193 413 434 548 1047	PsiI	1	4848			
		1730 4024	PspOMI	1	1297			
Bsal	1	4498	PvuI	1	4195			
BsaWI	8	2 201 1409 1912 2143	PvuII	3	1690 1783 2545			
		3160 3307 4268	SacI	1	167			
BsaXI	2	1765 4940	SalI	1	185			
BseRI	1	165	SapI	1	2838			
BseYI	3	1489 1624 3258	Scal	1	4083			
BsgI	4	208 941 1141 2114	Sfcl	5	332 3219 3410 4318 5195			
BsiEI	7	175 190 1875 2870 3294	Sfol	4	414 435 549 1731			
		4046 4195	SgrAI	1	409			
BsiHKAI	8	167 590 1074 1948 2772	SmlI	6	36 1883 3060 3322 3599			
		3272 3896 3981			3939			
BsmAI	7	787 1192 1318 1705 2595	SphI	1	565			
		3721 4498	SspI	1	4768			
BsmBI	2	1705 2595	StyI	3	57 97 259			
BsmFI	3	551 2225 5191	TaqII	6	204 1889 2856 4032 4217			
Bsp1286I	12				4880			
BspCNI	9	93 1675 2052 2214 2754	TatI	2	2758 4081			
		3242 3651 4076 4595	TspGWI	4	2079 2397 3783 4125			
BspEI	2	2 2143	Tth111I	1	2699			
BspHI	4	488 3674 3723 3755	XbaI	1	298			
BsrBI	5	141 319 2887 3721 5120	XcmI	3	946 1462 1480			
BsrDI	4	1137 1503 4330 4504	XmnI	2	2512 3964			
BsrFI	5	400 409 776 4479 5077	ZraI	1	194			
BssHII	1	1501						
BssSI	2	3127 3895	Enzymes that do not cut pET-51b(+):					
BstAPI	1	773	AarI,	AfilI,	Agel,	AleI,	Ascl,	AsiSI,
BstBI	1	234	AvaI,	Bael,	BbvCI,	BfrBI,	BglII,	BmgBI,
BstEII	1	1271	BmtI,	BpII,	BsiWI,	BsmI,	BspMI,	BsrGI,
BstXI	3	892 1021 1144	Bsu36I,	EcoRI,	EcoRV,	FalI,	FseI,	FspAI,
BstYI	10	198 654 1866 2146 3595	MfeI,	MscI,	NdeI,	NheI,	NruI,	NsiI,
		3606 3917 3934 4702 4714	Pacl,	PmeI,	PmlI,	Psrl,	PstI,	RsrII,
BstZ17I	1	2725	SacII,	SanDI,	SbfI,	SexAI,	SfiI,	SmaI,
Btgl	2	259 527	SnaBI,	SpeI,	SrfI,	StuI,	Swal,	XhoI,
BtsI	4	1455 1823 4145 4165	XmaI					
Clal	1	367						
DraI	3	3986 4678 4697						