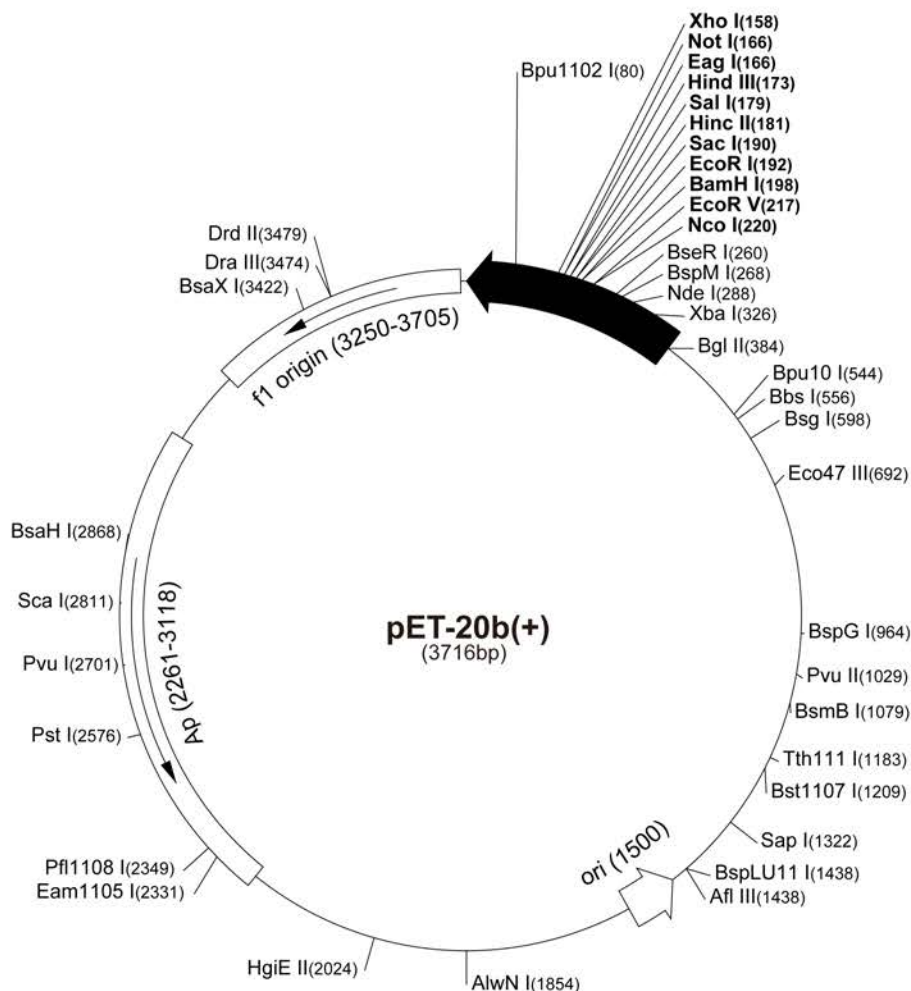


pET-20b(+) Vector

The pET-20b(+) vector (Cat. No. 69739-3) carries an N-terminal *pelB* signal sequence for potential periplasmic localization, plus optional C-terminal His•Tag® sequence. Unique 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 circular 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 helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the T7 terminator primer (Cat. No. 69337-3).

pET-20b(+) sequence landmarks

T7 promoter	353-369
T7 transcription start	352
<i>pelB</i> coding sequence	224-289
Multiple cloning sites	
(<i>Nco</i> I - <i>Xho</i> I)	158-225
His•Tag coding sequence	140-157
T7 terminator	26-72
pBR322 origin	1500
<i>bla</i> coding sequence	2261-3118
f1 origin	3250-3705



pET-20b(+) cloning/expression region

pET-20b(+) Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations
AccI	2	180 1208	DdeI	10	80 101 136 544 706	RsaI	2	1244 2811
AccII	5	947 1088 1390 2630 3314			1246 1713 2122 2288 2828	SacI	1	190
Acil	49		DpnI	19		Sall	1	179
AlfIII	1	1438	DraI	3	2197 2216 2908	SapI	1	1322
AluI	18		DraIII	1	3474	Sau96I	10	53 402 444 723 910
AlwI	13		DrdI	3	1131 1546 3429			2373 2452 2469 2691 3465
Alw21I	7	159 190 432 1256 1756	DrdII	1	3479	Sau3AI	19	
		2917 3002	Dsal	2	220 410	Scal	1	2811
Alw44I	3	1252 1752 2998	EaeI	4	166 223 407 2719	ScrFI	11	
AlwNI	1	1854	EagI	1	166	SfaNI	13	
ApoI	3	192 3276 3287	Eam1105I	1	2331	Sfcl	5	352 1703 1894 2572 3693
AvaI	2	158 388	EarI	2	1322 3126	SspI	2	3135 3266
Avall	5	402 444 723 2469 2691	Ecil	3	1512 1658 2486	Styl	7	159 180 190 381 1538
BamHI	1	198	Eco47III	1	692			2982 3507
BanI	2	2279 3511	Eco57I	2	1986 2998	TaqII	6	1340 2679 2864 3017 3034
BanII	2	190 3549	EcoO109I	3	53 402 444			3378
BbsI	1	556	EcoRI	1	192	TfiI	3	488 992 1413
BbvI	26		EcoRII	4	404 1464 1585 1598	Thal	16	
BccI	6	227 2368 2492 2779 3464	EcoRV	1	217	TseI	26	
		3481	FauI	8	368 453 734 920 1141	Tsp45I	6	877 1090 1185 2587 2798
Bce83I	5	21 1529 1827 2068 2936			1151 3611 3680			3647
BceII	2	1940 3500	FokI	8	657 719 797 983 1124	Tsp509I	11	
BcgI	6	160 194 1015 1049 2836			2297 2478 2765	Tth111I	1	1183
		2870	FspI	2	419 2553	Tth111II	4	899 2028 2035 2067
Bfal	7	70 327 452 1933 2186	GdIII	2	166 2719	UbaII	12	
		2521 3625	HaeI	5	225 409 1453 1464 1916	VspI	3	207 367 2503
BglI	2	237 2451	HaeII	6	611 694 1316 1686 3625	XbaI	1	326
BglII	1	384			3633	XhoI	1	158
BpmI	2	965 2401	HaeIII	14		XmnI	3	208 996 2930
Bpu10I	1	544	Hgal	6	974 1131 1549 2127 2857			
Bpu1102I	1	80			3691			
Bsal	2	351 2392	HgiEII	1	2024			
BsaAI	2	1190 3474	HhaI	23				
BsaBI	2	383 635	Hin4I	2	2330 2404			
BsaHI	1	2868	HincII	1	181			
BsaJI	4	57 220 410 1598	HindIII	1	173			
BsaWI	5	2 627 1644 1791 2622	HinfI	9	359 488 992 1338 1413			
BsaXI	1	3422			1809 2326 3401 3423			
Bsbl	2	1154 3381	HphI	9	483 1058 1067 2174 2401			
BscGI	10	44 414 799 1132 1765			2817 3023 3058 3475			
		2111 2332 2356 2878 3568	MaeII	12				
BseRI	1	260	MaeIII	15				
BsgI	1	598	MbolI	8	556 1309 2100 2171 2926			
Bsil	2	1611 2995			3004 3113 3614			
BsiEI	6	169 271 1354 1778 2701	MmeI	3	1653 1837 3451			
		2850	MnII	20				
BsII	12		MscI	2	225 409			
BsmAI	4	351 1079 2392 3168	MseI	23				
BsmBI	1	1079	MslI	6	425 620 1011 2583 2742			
BsmFI	2	709 3689			3101			
BsoFI	38		MspI	18				
Bsp24I	4	1931 1963 2109 2141	MspA1I	7	84 267 1029 1148 1780			
Bsp1286I	8	159 190 432 1256 1756			2025 2966			
		2917 3002 3549	MwoI	19				
BspEI	2	2 627	NciI	7	448 776 1082 1117 1818			
BspGI	1	964			2514 2865			
BspLU11I	1	1438	NcoI	1	220			
BspMI	1	268	NdeI	1	288			
BsrI	14		NgoAIV	2	231 3575			
BsrBI	3	1371 3172 3618	NlaIII	15				
BsrDI	2	2392 2566	NlaIV	15				
BsrFI	3	231 2411 3575	NotI	1	166			
Bst1107I	1	1209	NspI	3	783 1075 1442			
BstYI	10	132 198 384 630 2079	Pfi1108I	1	2349			
		2090 2176 2188 2956 2973	PleI	6	367 1332 1817 2320 3409			
Cac8I	16				3417			
CjeI	10	1451 1484 1925 1958 2003	Psp5II	2	402 444			
		2006 2036 2039 2103 2136	Psp1406I	4	763 2557 2930 3259			
CjePI	10	1925 1957 1958 1990 2103	PstI	1	2576			
		2136 2223 2256 2695 2728	PvuI	1	2701			
CviJI	57		PvuII	1	1029			
CviRI	13		RcaI	2	2158 3166			

Enzymes that do not cut pET-20b(+):

AatII	AflII	AgeI	Apal	ApaBI
AscI	AvrII	BaeI	BclI	BmgI
BsmI	BsrGI	BssHII	BstEII	BstXI
Bsu36I	ClaI	EcoNI	FseI	HpaI
KpnI	MluI	MunI	NarI	NheI
NruI	NsiI	NspV	PacI	PilMI
PmeI	PmlI	PshAI	RleAI	RsrII
SacII	SexAI	SfiI	SgfI	SgrAI
Smal	SnaBI	SpeI	SphI	SrfI
Sse8387I	StuI	SunI	Swal	XcmI