

pET-23a-d(+) Vectors

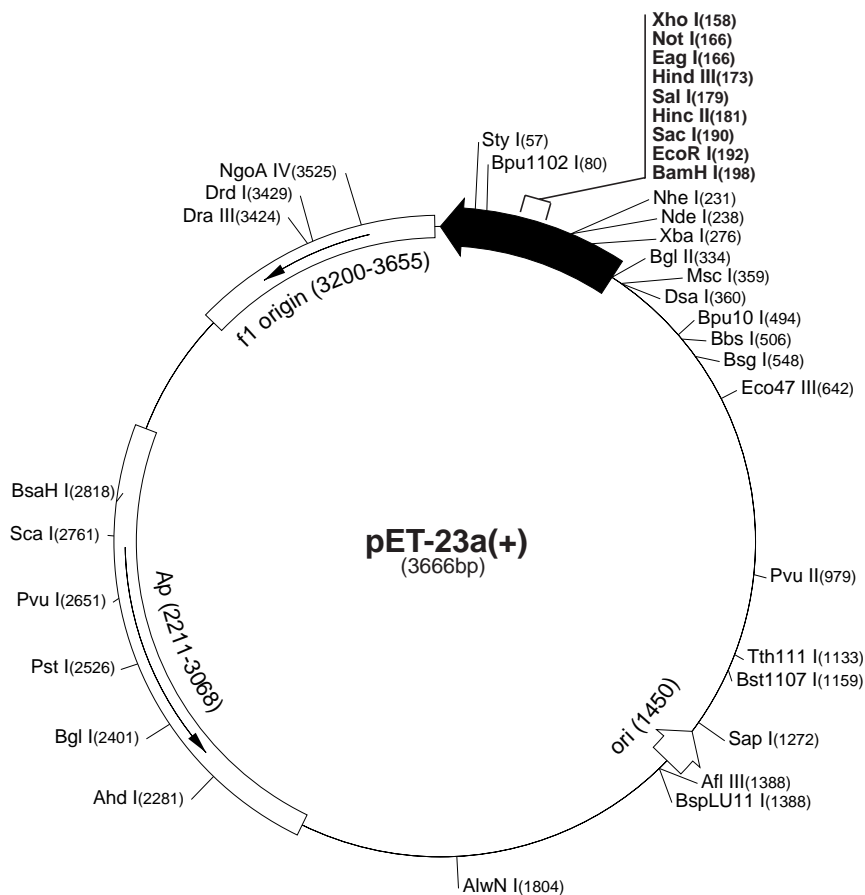
	Cat. No.
pET-23a DNA	69745-3
pET-23b DNA	69746-3
pET-23c DNA	69747-3
pET-23d DNA	69748-3

The pET-23a-d(+) vectors carry an N-terminal T7•Tag[®] sequence plus an optional C-terminal His•Tag[®] sequence. These vectors differ from pET-21a-d(+) by the "plain" T7 promoter instead of the T7lac promoter and by the absence of the lacI gene. 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-23a(+) sequence landmarks

T7 promoter	303-319
T7 transcription start	302
T7•Tag coding sequence	207-239
Multiple cloning sites (<i>Bam</i> H I - <i>Xho</i> I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
pBR322 origin	1450
<i>bla</i> coding sequence	2211-3068
f1 origin	3200-3655

The maps for pET-23b(+), pET-23c(+) and pET-23d(+) are the same as pET-23a(+) (shown) with the following exceptions:
 pET-23b(+) is a 3665bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198.
 pET-23c(+) is a 3664bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198.
 pET-23d(+) is a 3663bp plasmid; the *Bam*H I site is in the same reading frame as in pET-23c(+). An *Nco* I site is substituted for the *Nde* I site with a net 1bp deletion at position 238 of pET-23c(+). As a result, *Nco* I cuts pET-23d(+) at 234, and *Nhe* I cuts at 229. For the rest of the sites, subtract 3bp from each site beyond position 239 in pET-23a(+). *Nde* I does not cut pET-23d(+). Note also that *Sty* I is not unique in pET-23d(+).



T7 promoter primer #69348-3

Bgl II
T7 promoter
Xba I
rbs

AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCAACAACGGTTCCCTCTAGAATAATTTGTTTAACTTTAAGAAGGAGA

Nde I *Nhe* I
T7•Tag
pET-23α
*Bam*H I *Eco*R I *Sac* I
Hinc II *Sal* I *Hind* III
Eag I *Not* I
Xho I
His•Tag

TATACATATGGCTAGCATGACTGGTGGACAGCAATGGGTCGGGATCCGAATTCGAGCTCCGTCGACAAGCTTGGGCCGCACTCGAGCACCACCACCACCCTGA
 MetAlaSerMetThrGlyGlyGlnGlnMetGlyArgGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProLeu

pET-23d <i>Nco</i> I ...TACCATGGCTAGC... MetAlaSer...	pET-23b ...GGTCGGGATCCGAATTCGAGCTCCGTCGACAAGCTTGGGCCGCACTCGAGCACCACCACCACCCTGA ...GlyArgAspProAsnSerSerSerValAspLysLeuAlaAlaAlaLeuGluHisHisHisHisHisEnd	pET-23c.d ...GGTCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGGGCCGCACTCGAGCACCACCACCACCCTGA ...GlyArgIleArgIleArgAlaProSerThrSerLeuArgProHisSerSerThrThrThrThrThrGlu	
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*Bpu*1102 I
T7 terminator

GATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGCCCTTAAACGGGTCTTGAAGGGTTTTTTT

T7 terminator primer #69337-3

pET-23a-d(+) cloning/expression region

pET-23a(+) Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations		
AccI	2	180 1158	DrdI	3	1081 1496 3379	Sau3AI	19			
AccIII	5	897 1038 1340 2580 3264	DrdII	1	3429	Scal	1	2761		
Acil	49		Dsal	1	360	ScrFI	11			
AfilIII	1	1388	EaeI	3	166 357 2669	SfaNI	13			
AluI	18		EagI	1	166	SfiI	5	302 1653 1844 2522 3643		
AlwI	13		Eam1105I	1	2281	SspI	2	3085 3216		
Alw21I	7	159 190 382 1206 1706	EarI	2	1272 3076	StyI	1	57		
		2867 2952	Ecil	3	1462 1608 2436	TaqI	7	159 180 190 331 1488		
Alw44I	3	1202 1702 2948	Eco47III	1	642			2932 3457		
AlwNI	1	1804	Eco57I	2	1936 2948	TaqII	6	1290 2629 2814 2967 2984		
ApoI	3	192 3226 3237	EcoO109I	3	53 352 394			3328		
AvaI	2	158 338	EcoRI	1	192	TfiI	3	438 942 1363		
AvaII	5	352 394 673 2419 2641	EcoRII	4	354 1414 1535 1548	Thal	17			
BamHI	1	198	FauI	8	318 403 684 870 1091	Tsel	21			
BanI	2	2229 3461			1101 3561 3630	Tsp45I	6	827 1040 1135 2537 2748		
BanII	2	190 3499	FokI	8	607 669 747 933 1074			3597		
BbsI	1	506			2247 2428 2715	Tsp509I	9	192 268 318 2148 2454		
BbvI	21		FspI	2	369 2503			2709 3200 3226 3237		
BccI	5	2318 2442 2729 3414 3431	GdiII	2	166 2669	Tth111I	1	1133		
Bce83I	5	21 1479 1777 2018 2886	HaeI	4	359 1403 1414 1866	Tth111III	4	849 1978 1985 2017		
BceII	2	1890 3450	HaeII	6	561 644 1266 1636 3575	UbaII	12			
BcgI	7	160 194 228 965 999			3583	VspI	2	317 2453		
		2786 2820	HaeIII	13		XbaI	1	276		
Bfal	8	70 232 277 402 1883	HgaI	6	924 1081 1499 2077 2807	XhoI	1	158		
		2136 2471 3575			3641	XmnI	2	946 2880		
BglI	1	2401	HgiEI	1	1974					
BglII	1	334	HhaI	23		Enzymes that do not cut pET-23a(+):				
BpmI	2	915 2351	Hin4I	2	2280 2354	AatII	AfilI	AgeI	Apal	ApaBI
Bpu10I	1	494	HincII	1	181	AscI	AvrII	BaeI	BclI	BmgI
Bpu1102I	1	80	HindIII	1	173	BseRI	BsmI	BspMI	BsrGI	BssHII
BsaI	2	301 2342	Hinfl	9	309 438 942 1288 1363	BstEII	BstXI	Bsu36I	Clal	EcoNI
BsaAI	2	1140 3424			1759 2276 3351 3373	EcoRV	FseI	HpaI	KpnI	MluI
BsaBI	2	333 585	HphI	9	433 1008 1017 2124 2351	MunI	NarI	NcoI	NruI	NsiI
BsaHI	1	2818			2767 2973 3008 3425	NspV	Pacl	PfiMI	PmeI	PmlI
BsaJI	3	57 360 1548	Maell	12		PshAI	RleAI	RsrII	SacII	SexAI
BsaWI	5	2 577 1594 1741 2572	MaellI	15		SfiI	Sgfl	SgrAI	Smal	SnaBI
BsaXI	1	3372	MbolI	8	506 1259 2050 2121 2876	SpeI	SphI	SrfI	Sse8387I	StuI
Bsbl	2	1104 3331			2954 3063 3564	SunI	Swal	XcmI		
BscGI	10	44 364 749 1082 1715	MmeI	3	1603 1787 3401					
		2061 2282 2306 2828 3518	MnlI	19						
BsgI	1	548	MscI	1	359					
Bsil	2	1561 2945	Msel	22						
BsiEI	5	169 1304 1728 2651 2800	MslI	6	375 570 961 2533 2692					
BsII	12				3051					
BsmAI	4	301 1029 2342 3118	MspI	17						
BsmBI	1	1029	MspA1I	6	84 979 1098 1730 1975					
BsmFI	2	659 3639			2916					
BsoFI	33		MwoI	18						
Bsp24I	6	201 233 1881 1913 2059	NciI	7	398 726 1032 1067 1768					
		2091			2464 2815					
Bsp1286I	8	159 190 382 1206 1706	NdeI	1	238					
		2867 2952 3499	NgoAIV	1	3525					
BspEI	2	2 577	NheI	1	231					
BspGI	1	914	NlaIII	15						
BspLU11I	1	1388	NlaIV	15						
BsrI	15		NotI	1	166					
BsrBI	3	1321 3122 3568	NspI	3	733 1025 1392					
BsrDI	2	2342 2516	Pfi1108I	1	2299					
BsrFI	2	2361 3525	PleI	6	317 1282 1767 2270 3359					
Bst1107I	1	1159			3367					
BstYI	10	132 198 334 580 2029	Psp5II	2	352 394					
		2040 2126 2138 2906 2923	Psp1406I	4	713 2507 2880 3209					
Cac8I	16		PstI	1	2526					
CjeI	12		PvuI	1	2651					
CjePI	12		PvuII	1	979					
CviJI	56		RcaI	2	2108 3116					
CviRI	13		RsaI	2	1194 2761					
DdeI	10	80 101 136 494 656	SacI	1	190					
		1196 1663 2072 2238 2778	Sall	1	179					
DpnI	19		SapI	1	1272					
DraI	3	2147 2166 2858	Sau96I	10	53 352 394 673 860					
DrallI	1	3424			2323 2402 2419 2641 3415					