

pET-5a-c Vectors

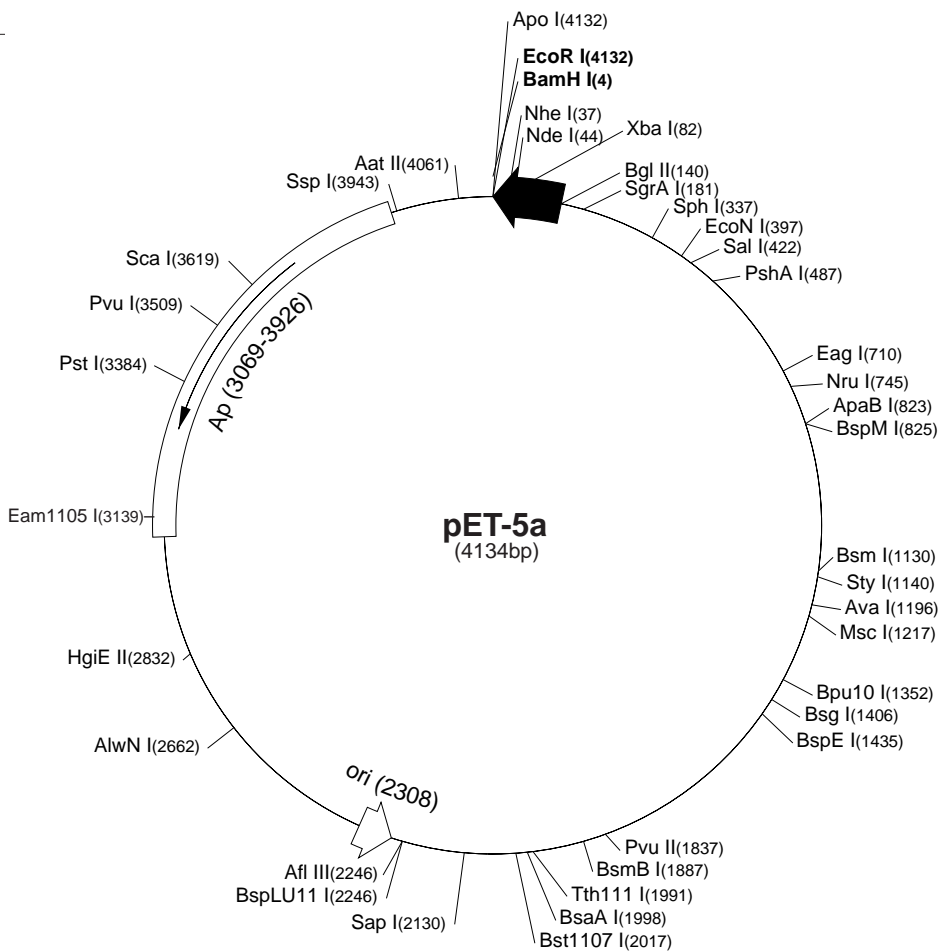
	Cat. No.
pET-5a DNA	69426-3
pET-5b DNA	69427-3
pET-5c DNA	69428-3

The pET-5a-c vectors carry an N-terminal T7•Tag[®] sequence and *BamH I*/*EcoR I* cloning sites. The T7 terminator present on many other pET vectors is missing in the pET-5a-c series; therefore, β-lactamase is generally produced in large amounts in pET-5 recombinants induced for T7 RNA polymerase expression. The pET-23a-d(+) series corresponds to pET-5a-c but incorporates several additional features, including the T7 terminator (Cat. No. 69337-3). 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.

pET-5a(+) sequence landmarks

T7 promoter	109-125
T7 transcription start	108
pBR322 origin	2308
<i>bla</i> coding sequence	3069-3926

The maps for pET-5b and pET-5c are the same as pET-5a (shown) with the following exceptions: pET-5b is a 4133bp plasmid; subtract 1bp from each site beyond *BamH I* at 4. pET-5c is a 4132bp plasmid; subtract 2bp from each site beyond *BamH I* at 4.



T7 promoter primer #69348-3

Bgl II
T7 promoter
Xba I
rbs

AGATCTCGATCCCGCAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTAGAAATAATTTGTTTAACTTTAAGAAGGAGA

Nde I *Nhe* I
T7•Tag
pET-5a
BamH I *EcoR* I

TATACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCCGGATCCGAATCTTGAAGACGAAAGGGCCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAAT
 MetAlaSerMetThrGlyGlyGlnGlnMetGlyArgGlySerGluPheLeuLysThrLysGlyProArgAspThrProIlePheIleGlyEnd

pBR322 *EcoR* I clockwise primer

pET-5b ...GGTCGGGATCCGAATCTTGAAGACGAAAGGGCCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAAT
...GlyArgAspProAsnSerEnd

pET-5c ...GGTCGGATCCGAATCTTGAAGACGAAAGGGCCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAAT
...GlyArgIleArgIleLeuGluAspGluArgAlaSerEnd

pET-5a-c cloning/expression region

pET-5a Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations		
AatII	1	4061	CacBI	29		PvuII	1	1837		
AccI	2	423 2016	CjeI	18		RcaI	4	260 2966 3974 4079		
AccIII	5	468 1755 1896 2198 3438	CjePI	16		RsaI	2	2052 3619		
Acil	64		CviJI	68		Sall	1	422		
AflIII	1	2246	CviRI	20		SapI	1	2130		
AluI	15		DdeI	8	1352 1514 2054 2521 2930	Sau96I	14			
AlwI	14				3096 3636 4062	Sau3AI	24			
Alw21I	7	362 949 1240 2064 2564	DpnI	24		Scal	1	3619		
		3725 3810	DraI	3	3005 3024 3716	ScrFI	14			
Alw44I	3	2060 2560 3806	DrdI	2	1939 2354	SfaNI	19			
AlwNI	1	2662	Dsal	2	299 1218	SfcI	4	108 2511 2702 3380		
ApaBI	1	823	EaeI	5	170 302 710 1215 3527	SgrAI	1	181		
ApoI	1	4132	EagI	1	710	SphI	1	337		
AvaI	1	1196	Eam1105I	1	3139	SspI	1	3943		
Avall	8	570 658 907 1210 1252	EarI	2	2130 3934	StyI	1	1140		
		1531 3277 3499	Ecil	4	1166 2320 2466 3294	TaqI	7	137 145 423 898 1039		
BamHI	1	4	Eco47III	3	267 548 1500			2346 3790		
BanI	7	184 205 319 537 976	Eco57I	2	2794 3806	TaqII	6	441 2148 3487 3672 3825		
		1060 3087	EcoNI	1	397			3842		
BanII	2	246 260	EcoO109I	4	295 1210 1252 4115	TfiI	6	623 777 1075 1296 1800		
BbsI	3	501 1364 4117	EcoRI	1	4132			2221		
BbvI	20		EcoRII	5	829 1212 2272 2393 2406	ThaI	24			
BccI	9	231 324 761 850 1157	FauI	10	124 467 669 816 1011	TseI	20			
		1169 3176 3300 3587			1261 1542 1728 1949 1959	Tsp45I	7	651 918 1685 1898 1993		
Bce83I	6	456 626 2337 2635 2876	FokI	10	771 816 1465 1527 1605			3395 3606		
		3744			1791 1932 3105 3286 3573	Tsp509I	8	74 124 1090 1104 3006		
Bcefl	3	381 938 2748	Fspl	3	1129 1227 3361			3312 3567 4132		
Bcgl	8	34 468 502 1823 1857	GdIII	4	170 302 710 3527	Tth111I	1	1991		
		3644 3678 4134	HaeI	7	691 763 820 1217 2261	Tth111II	4	1707 2836 2843 2875		
Bfal	6	38 83 1260 2741 2994			2272 2724	UbaII	19			
		3329	HaeII	10	188 209 269 323 550	VspI	2	123 3311		
BglI	3	706 940 3259			980 1419 1502 2124 2494	XbaI	1	82		
BglII	1	140	HaeIII	20		XmnI	2	1804 3738		
Bpml	4	603 1157 1773 3209	Hgal	11						
Bpu10I	1	1352	HgiEII	1	2832	Enzymes that do not cut pET-5a:				
BsaI	2	107 3200	Hhal	28		AflII	Agel	Apal	AscI	AvrII
BsaAI	1	1998	Hin4I	3	912 3138 3212	BaeI	BclI	BmgI	Bpu1102I	BsaXI
BsaBI	3	139 145 1443	HincII	2	424 3680	BseRI	BsrGI	BssHII	BstEII	BstXI
BsaHI	6	185 206 320 977 3676	Hinfl	11		Bsu36I	Clal	DrallI	DrdII	EcoRV
		4058	HphI	11		FseI	HindIII	HpaI	KpnI	MluI
BsaJI	6	299 305 938 1140 1218	MaeII	10	672 728 1317 1341 1571	MunI	NcoI	NotI	NsiI	NspV
		2406			1997 2949 3365 3738 4058	Pacl	PmeI	PmlI	RleAI	RsrII
BsaWI	5	464 1435 2452 2599 3430	MaeIII	15		SacI	SacII	SexAI	SfiI	Sgfl
Bsbl	2	1962 3682	MbolI	11		Smal	SnaBI	SpeI	SrfI	Sse8387I
BscGI	10	299 958 1222 1607 1940	Mmel	2	2461 2645	StuI	SunI	Swal	XcmI	XhoI
		2573 2919 3140 3164 3686	MnII	25						
BsgI	1	1406	MscI	1	1217					
Bsil	3	2419 3803 4110	MseI	16						
BsiEI	6	427 713 2162 2586 3509	MslI	7	802 1233 1428 1819 3391					
		3658			3550 3909					
BsII	17		MspI	24						
BsmI	1	1130	MspAII	6	912 1837 1956 2588 2833					
BsmAI	4	107 1887 3200 3976			3774					
BsmBI	1	1887	MwoI	27						
BsmFI	4	323 644 869 1517	NarI	4	185 206 320 977					
BsoFI	39		NciI	9	306 1030 1256 1584 1890					
Bsp24I	10	7 39 152 184 2739			1925 2626 3322 3673					
		2771 2917 2949 4043 4075	NdeI	1	44					
Bsp1286I	9	246 260 362 949 1240	NgoAIV	4	172 540 700 1054					
		2064 2564 3725 3810	NheI	1	37					
BspEI	1	1435	NlaIII	25						
BspGI	3	830 907 1772	NlaIV	21						
BspLU11I	1	2246	Nrul	1	745					
BspMI	1	825	Nspl	4	337 1591 1883 2250					
BsrI	18		Pfl1108I	2	529 3157					
BsrBI	2	2179 3980	PfIMI	2	1092 1141					
BsrDI	2	3200 3374	PleI	5	123 411 2140 2625 3128					
BsrFI	6	172 181 540 700 1054	PshAI	1	487					
		3219	Psp5II	2	1210 1252					
Bst1107I	1	2017	Psp1406I	4	672 1571 3365 3738					
BstYI	9	4 140 1438 2887 2898	PstI	1	3384					
		2984 2996 3764 3781	PvuI	1	3509					