

pET-30a-c(+) Vectors

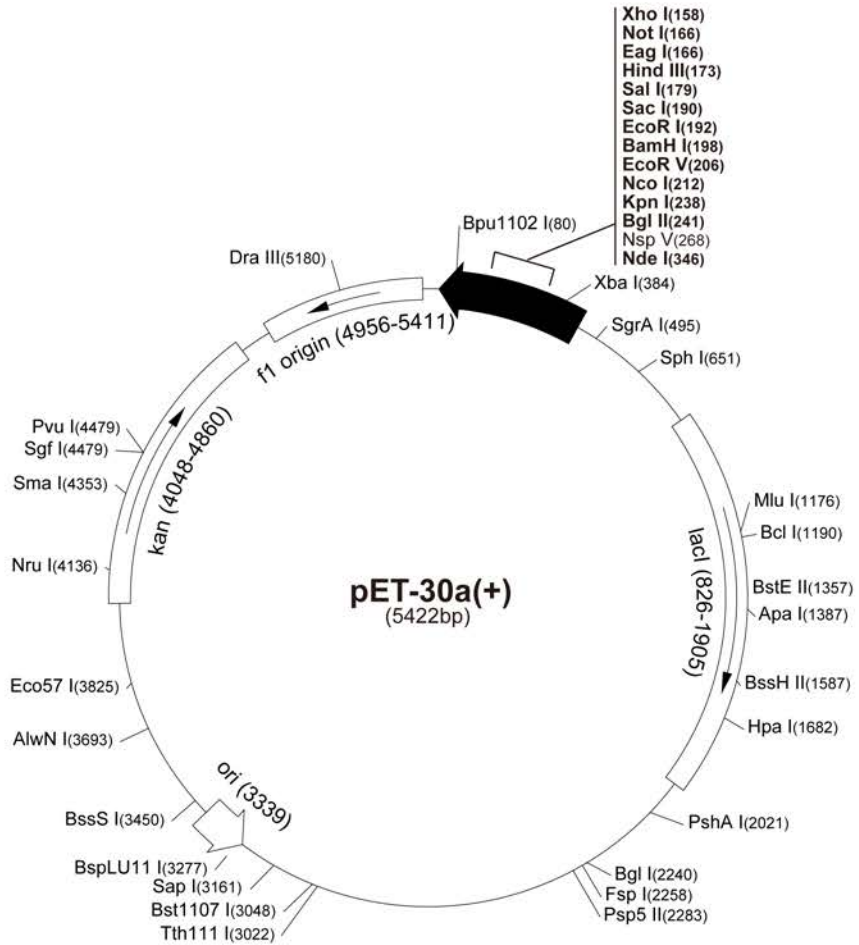
	Cat. No.
pET-30a DNA	69909-3
pET-30b DNA	69910-3
pET-30c DNA	69911-3

The pET-30a-c(+) vectors carry an N-terminal His•Tag®/thrombin/S•Tag™/enterokinase configuration plus an 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-30a(+) sequence landmarks

T7 promoter	419-435
T7 transcription start	418
His•Tag coding sequence	327-344
S•Tag coding sequence	249-293
Multiple cloning sites	
(<i>Nco</i> I - <i>Xho</i> I)	158-217
His•Tag coding sequence	140-157
T7 terminator	26-72
<i>lacI</i> coding sequence	826-1905
pBR322 origin	3339
Kan coding sequence	4048-4860
f1 origin	4956-5411

The maps for pET-30b(+) and pET-30c(+) are the same as pET-30a(+) (shown) with the following exceptions: pET-30b(+) is a 5421bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-30c(+) is a 5423bp plasmid; add 1bp to each site beyond *Bam*H I at 198.



pET-30a-c(+) cloning/expression region

T7 promoter primer #69348-3
pET upstream primer #69214-3

AGATCGATCTCGATCCCGCAAATTAATACGACTCACTATAGGGGAATGTGAGCGGATAACAATTCCTCCCTAGAAATAATTTGTTTAACTTTAAGAAGGAGA

lac operator *Xba*I **rbs**

*Nde*I **His•Tag** **S•Tag** *Nsp*V *Bgl*II

TATACATATGCACCATCATCATCTCTTCTGGTCTGGTGCCACGCGGTCTGGATGAAAGAACCGCTGCTGCTAAATTCGAACGCCAGCACATGGACGCCAGATGCTG
MethI sH i sH i sH i sH i sH i sH i sSerSerGlyLeuValProArgGlySerGlyMetLysGluThrAlaAlaAlaLysPheGluArgGlnHisMetAspSerProAspLeu

thrombin *Eag*I *Not*I *Xho*I **His•Tag**

GGTACCAGCAGCAGCAGCACAAGCCATGGCTGATATCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGGCCGCACTCGAGCACCACCACCACCCTGAGATCCGGCTGCTAA
GlyThrAspAspAspLysAlaMetAlaAspIleGlySerGluPheGluLeuArgArgIleAlaCysGlyArgThrArgAlaProProProProLeuArgSerGlyCysEnd

enterokinase

pET-30b(+)
...GCGATATCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGGCCGCACTCGAGCACCACCACCACCCTGA...
AlaIleSerAspProAsnSerSerSerValAspLysLeuAlaAlaAlaLeuGluHisHisHisHisHisEnd

pET-30c(+)
...GGATATCTGGATCCGAATTCGAGCTCCGTCGACAAGCTTGGCCGCACTCGAGCACCACCACCACCCTGAGATCCGGCTGCTAA...
...GlyTyrLeuTrpIleArgIleArgAlaProSerThrSerLeuArgProHisSerSerThrThrThrThrThrThrGluIleArgLeuLeu...

*Bpu*1102 I **T7 terminator**

CAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATACTAGCATAACCCCTTGGGGCTCTAAACGGGCTTGAGGGTTTTTTTGG

T7 terminator primer #69337-3

pET-30a(+) Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations
AccI	2	180 3047	Bst1107I	1	3048	NspI	4	651 2622 2914 3281
AccII	7	943 1671 2002 2786 2927 3229 5020	BstEII	1	1357	NspV	1	268
Acil	75		BstXI	3	978 1107 1230	PII1108I	1	2063
AflIII	2	1176 3277	BstYI	9	132 198 241 740 1952 2469 3918 3929 4728	PIIMI	3	260 758 4742
AluI	22		Cac8I	40		PleI	9	433 725 812 1608 3171 3656 4711 5115 5123
AlwI	13		CjeI	24		PshAI	1	2021
Alw21I	7	159 190 676 1160 2271 3095 3595	CjePI	18		Psp5II	1	2283
Alw44I	3	1156 3091 3591	ClaI	2	453 4170	Psp1406I	4	838 2206 2602 4965
AlwNI	1	3693	CviJI	85		PvuI	1	4479
ApaI	1	1387	CviRI	31		PvuII	3	1776 1869 2868
ApaBI	1	860	DdeI	11		RcaI	3	574 3997 4872
ApoI	7	192 270 1451 4092 4276 4982 4993	DpnI	23		RsaI	4	236 1323 3083 4314
AvaI	2	158 4351	DrallI	1	5180	SacI	1	190
AvaII	5	1728 2104 2192 2283 2562	DrdI	3	2970 3385 5135	SallI	1	179
BamHI	1	198	DrdII	2	899 5185	SapI	1	3161
BanI	10	234 310 498 519 633 1096 1815 1945 2071 5217	DsaI	3	212 613 2249	Sau96I	14	
BanII	6	190 560 574 1387 4134 5255	EaeI	4	166 484 616 1850	Sau3AI	23	
BbsI	4	1322 1661 2035 2395	EagI	1	166	ScrFI	21	
BbvI	25		EarI	3	794 3161 4292	SfaNI	23	
BccI	14		Ecil	3	953 3351 3497	SfiCI	4	418 3542 3733 5399
Bce83I	6	21 1990 2160 3368 3666 3907	Eco47III	3	581 2082 2531	SgfiI	1	4479
BceII	6	695 1036 1663 3779 4798 5206	Eco57I	1	3825	SgrAI	1	495
BcgI	8	160 194 1468 1502 2002 2036 2854 2888	EcoNI	2	711 4391	SmaI	1	4353
BclI	1	1190	EcoO109I	3	53 609 2283	SphI	1	651
Bfal	6	70 385 2291 3772 4079 5331	EcoRI	1	192	SspI	2	4404 4972
BglI	1	2240	EcoRII	9	899 1214 1754 1811 3303 3424 3437 4367 4724	StyI	2	57 212
BglII	1	241	EcoRV	1	206	TaqI	17	
BmgI	1	1385	FauI	17		TaqII	6	1084 1302 1975 3179 4733 5084
BpmI	4	1014 1503 2137 2804	FokI	9	1222 1231 2496 2558 2636 2822 2963 4117 4723	TfiI	9	1855 2157 2327 2831 3252 4390 4446 4618 4709
Bpu10I	2	2383 4496	FspI	1	2258	ThaI	36	
Bpu1102I	1	80	GdiII	4	166 484 616 1850	TseI	25	
BsaAI	2	3029 5180	HaeI	7	217 904 2225 3292 3303 3755 4566	Tsp45I	7	1357 2185 2716 2929 3024 4626 5353
BsaBI	3	449 459 2474	HaeII	14		Tsp509I	21	
BsaHI	5	499 520 634 1133 1816	HaeIII	24		Tth111I	1	3022
BsaJI	10	57 212 613 619 1811 2249 3437 4350 4351 4752	HgaI	11		Tth111III	8	1015 1708 2738 3867 3874 3906 4315 4442
BsaWI	7	2 1495 1998 2466 3483 3630 4614	HgiEII	2	774 3863	UbaII	18	
BsaXI	2	1835 5128	HhaI	46		VspI	5	433 1861 1920 4678 4867
Bsbl	2	2993 5087	Hin4I	4	203 1075 4165 4707	XbaI	1	384
BscGI	11		HinClI	2	181 1682	XcmI	3	1032 1548 1566
BsgI	3	1027 1227 2437	HindIII	1	173	XhoI	1	158
Bsil	1	3450	Hinfl	18		XmnI	2	2835 4868
BsIEI	5	169 1961 3193 3617 4479	HpaI	1	1682			
BsII	26		HphI	16				
BsmI	2	4363 4440	KpnI	1	238			
BsmAI	6	873 1278 1404 1791 2918 4495	MaeII	14				
BsmBI	3	1791 2918 4495	MaeIII	16				
BsmFI	4	637 2178 2548 5395	MbolI	13				
BsoFI	43		MluI	1	1176			
Bsp24I	10	466 498 1017 1049 1319 1351 3770 3802 3948 3980	MmeI	7	3492 3676 4121 4315 4677 4686 5157			
Bsp1286I	12		MnlI	25				
BspEI	2	2 2466	MseI	25				
BspGI	1	2803	MslI	6	1228 1516 1546 2264 2459 2850			
BspLU11I	1	3277	MspI	29				
BsrI	21		MspA1I	9	84 283 1206 1776 1869 2868 2987 3619 3864			
BsrBI	4	405 3210 4878 5324	MwoI	39				
BsrDI	2	1223 1589	NarI	4	499 520 634 1816			
BsrFI	7	486 495 862 2074 2234 4433 5281	NciI	12				
BssHII	1	1587	NcoI	1	212			
			NdeI	1	346			
			NgoAIV	4	486 2074 2234 5281			
			NlaIII	26				
			NlaIV	23				
			NottI	1	166			
			NruI	1	4136			
			Nsil	2	4329 4595			

Enzymes that do not cut pET-30a(+):				
AatII	AflIII	AgeI	AscI	AvrII
BaeI	BsaI	BseRI	BspMI	BsrGI
Bsu36I	DraI	Eam1105I	FseI	MscI
MunI	NheI	Pacl	PmeI	PmlI
PstI	RleAI	RsrII	SacII	Scal
SexAI	SfiI	SnaBI	SpeI	SrfI
Sse8387I	StuI	SunI	SwaI	