

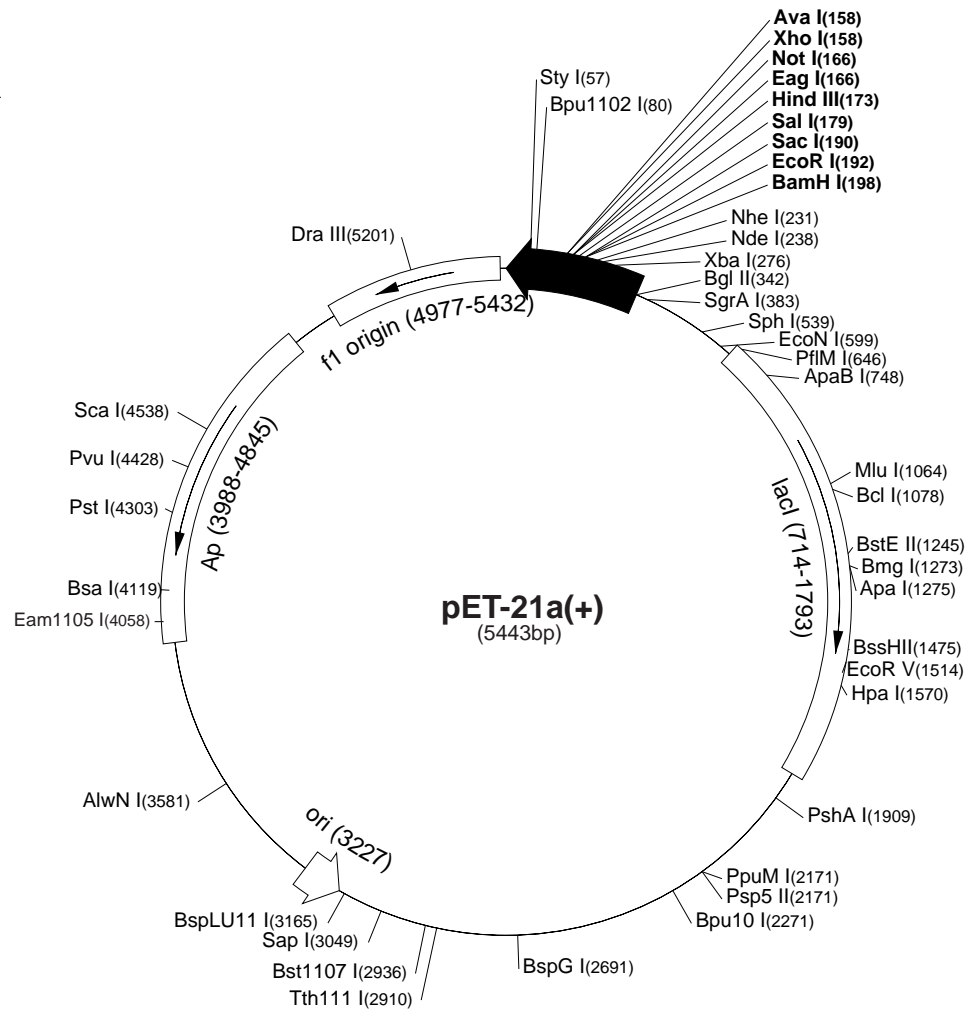
# pET-21a-d(+) Vectors

	Cat. No.
pET-21a DNA	69740-3
pET-21b DNA	69741-3
pET-21c DNA	69742-3
pET-21d DNA	69743-3

pET-21a(+) sequence landmarks	
T7 promoter	311-327
T7 transcription start	310
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
<i>lac</i> I coding sequence	714-1793
pBR322 origin	3227
<i>bla</i> coding sequence	3988-4845
f1 origin	4977-5432

The maps for pET-21b(+), pET-21c(+) and pET-21d(+) are the same as pET-21a(+) (shown) with the following exceptions: pET-21b(+) is a 5442bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-21c(+) is a 5441bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198. pET-21d(+) is a 5440bp plasmid; the *Bam*H I site is in the same reading frame as in pET-21c(+). An *Nco* I site is substituted for the *Nde* I site with a net 1bp deletion at position 238 of pET-21c(+). As a result, *Nco* I cuts pET21d(+) at 234, and *Nhe* I cuts at 229. For the rest of the sites, subtract 3bp from each site beyond position 239 in pET-21a(+). *Nde* I does not cut pET-21d(+). Note also that *Sty* I is not unique in pET-21d(+).

The pET-21a-d(+) vectors carry an N-terminal T7•Tag<sup>®</sup> sequence plus an optional C-terminal His•Tag<sup>®</sup> sequence. These vectors differ from pET-24a-d(+) only by their selectable marker (ampicillin vs. kanamycin resistance). 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).



T7 promoter primer #69348-3

*Bgl* II
T7 promoter
lac operator
*Xba* I
rbs

AGATCTCGATCCCGCAAATAAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCTCTAGAAATAATTTGTTAACCTTAAGAAGGAGA

*Nde* I
*Nhe* I
T7•Tag
pET-21a
*Bam* HI
*Eco* R I
*Sac* I
*Sal* I
*Hind* III
*Eag* I
*Ava* I
*Xho* I
His•Tag

TATACATATGGCTAGCATGACTGGTGGACAGCAAAATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGGCGCCGACTCGAGCACCACCACCACCCTGA

pET-21d <i>Nco</i> I ...TACCATGGCTAGC... MetAlaSer...	pET-21b ...GGTCGGGATCCGAATTCGAGCTCCGTCGACAAGCTTGGCGCCGACTCGAGCACCACCACCACCCTGA ...GlyArgAspProAsnSerSerSerValAspLysLeuAlaAlaAlaLeuGluuHisHisHisHisHisHisEnd	pET-21c,d ...GGTCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGGCGCCGACTCGAGCACCACCACCACCCTGA ...GlyArgIleArgIleArgAlaProSerThrSerLeuArgProHisSerSerThrThrThrThrThrThrGlu	
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*Bpu* 1102 I
T7 terminator

GATCCGGCTGCTAACAAAGCCGAAAGGAAGCTGAGTGGCTGCTGCCACCGCTGAGCAATACTAGCATAACCCCTTGGGGCCCTTAAACGGGCTTGGAGGGTTTTTTG

T7 terminator primer #69337-3

**pET-21a-d(+) cloning/expression region**

# pET-21a(+) Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations
AccI	2	180 2935	BstXI	3	866 995 1118	Psp1406I	6	726 2094 2490 4284 4657
AccIII	8	831 1559 1890 2674 2815	BstYI	12				4986
		3117 4357 5041	CacBI	39		PstI	1	4303
Acil	81		CjeI	26		PvuI	1	4428
AflIII	2	1064 3165	CjePI	20		PvuII	3	1664 1757 2756
AluI	25		CviJI	86		RcaI	3	462 3885 4893
AlwI	16		CviRI	24		RsaI	3	1211 2971 4538
Alw21I	9	159 190 564 1048 2159	DdeI	11		SacI	1	190
		2983 3483 4644 4729	DpnI	27		Sall	1	179
Alw44I	4	1044 2979 3479 4725	DraI	3	3924 3943 4635	SapI	1	3049
AlwNI	1	3581	DraIII	1	5201	Sau96I	18	
Apal	1	1275	DrdI	3	2858 3273 5156	Sau3AI	27	
ApaBI	1	748	DrdII	2	787 5206	Scal	1	4538
ApoI	4	192 1339 5003 5014	DsaI	2	501 2137	ScrFI	19	
AvaI	1	158	EaeI	5	166 372 504 1738 4446	SfaNI	20	
Avall	7	1616 1992 2080 2171 2450	EagI	1	166	Sfcl	5	310 3430 3621 4299 5420
		4196 4418	Eam1105I	1	4058	SgrAI	1	383
BamHI	1	198	EarI	3	682 3049 4853	SphI	1	539
BanI	9	386 407 521 984 1703	Ecil	4	841 3239 3385 4213	Sspl	2	4862 4993
		1833 1959 4006 5238	Eco47III	3	469 1970 2419	StyI	1	57
BanII	5	190 448 462 1275 5276	Eco57I	2	3713 4725	TaqI	13	
BbsI	4	1210 1549 1923 2283	EcoNI	1	599	TaqII	9	972 1190 1863 3067 4406
BbvI	26		EcoO109I	3	53 497 2171			4591 4744 4761 5105
BccI	21		EcoRI	1	192	TfiI	5	1743 2045 2215 2719 3140
Bce83I	7	21 1878 2048 3256 3554	EcoRII	7	787 1102 1642 1699 3191	Thal	35	
		3795 4663			3312 3325	Tsel	26	
Bcefl	5	583 924 1551 3667 5227	EcoRV	1	1514	Tsp45I	8	1245 2073 2604 2817 2912
Bcgl	11		FauI	17				4314 4525 5374
BclI	1	1078	FokI	10	1110 1119 2384 2446 2524	Tsp509I	16	
Bfal	8	70 232 277 2179 3660			2710 2851 4024 4205 4492	Tth111I	1	2910
		3913 4248 5352	FspI	2	2146 4280	Tth111II	6	903 1596 2626 3755 3762
BglI	2	2128 4178	GdIII	5	166 372 504 1738 4446			3794
BglII	1	342	HaeI	5	792 2113 3180 3191 3643	UbaII	20	
BmgI	1	1273	HaeII	14		VspI	4	325 1749 1808 4230
Bpml	5	902 1391 2025 2692 4128	HaeIII	24		XbaI	1	276
Bpu10I	1	2271	Hgal	12		XcmI	3	920 1436 1454
Bpu1102I	1	80	HgiEI	2	662 3751	XhoI	1	158
BsaI	1	4119	HhaI	45		XmnI	2	2723 4657
BsaAI	2	2917 5201	Hin4I	3	963 4057 4131			
BsaBI	3	341 347 2362	HincII	2	181 1570			
BsaHI	6	387 408 522 1021 1704	HindIII	1	173			
		4595	HinfI	14				
BsaJI	6	57 501 507 1699 2137	HpaI	1	1570			
		3325	HphI	16				
BsaWI	7	2 1383 1886 2354 3371	MaeII	15				
		3518 4349	MaeIII	18				
BsaXI	2	1723 5149	MbolI	14				
Bsbl	2	2881 5108	MluI	1	1064			
BscGI	13		MmeI	3	3380 3564 5178			
BsgI	3	915 1115 2325	MnlI	26				
Bsil	2	3338 4722	MseI	28				
BsiEI	6	169 1849 3081 3505 4428	MslI	9	1116 1404 1434 2152 2347			
		4577			2738 4310 4469 4828			
BsII	20		MspI	31				
BsmAI	7	761 1166 1292 1679 2806	MspA1I	9	84 1094 1664 1757 2756			
		4119 4895			2875 3507 3752 4693			
BsmBI	2	1679 2806	MwoI	38				
BsmFI	4	525 2066 2436 5416	NarI	4	387 408 522 1704			
BsoFI	46		NciI	12				
Bsp24I	12		NdeI	1	238			
Bsp1286I	13		NgoAIV	4	374 1962 2122 5302			
BspEI	2	2 2354	NheI	1	231			
BspGI	1	2691	NlaIII	25				
BspLU11I	1	3165	NlaIV	25				
BsrI	26		NottI	1	166			
BsrBI	4	297 3098 4899 5345	Nspl	4	539 2510 2802 3169			
BsrDI	4	1111 1477 4119 4293	Pfl1108I	2	1951 4076			
BsrFI	7	374 383 750 1962 2122	PfIMI	1	646			
		4138 5302	PleI	9	325 613 700 1496 3059			
BssHII	1	1475			3544 4047 5136 5144			
Bst1107I	1	2936	PshAI	1	1909			
BstEII	1	1245	Psp5II	1	2171			

Enzymes that do not cut pET-21a(+):

AatII	AflII	AgeI	AscI	AvrII
BaeI	BseRI	BsmI	BspMI	BsrGI
Bsu36I	Clal	FseI	KpnI	MscI
MunI	NcoI	NruI	NsiI	NspV
PacI	PmeI	PmlI	RleAI	RsrII
SacII	SexAI	SfiI	SgfI	SmaI
SnaBI	SpeI	SrfI	Sse8387I	StuI
SunI	Swal			