pET-28a-c(+) Vectors

TB074 12/98

	Cat. No.
pET-28a DNA	69864-3
pET-28b DNA	69865-3
pET-28c DNA	69866-3

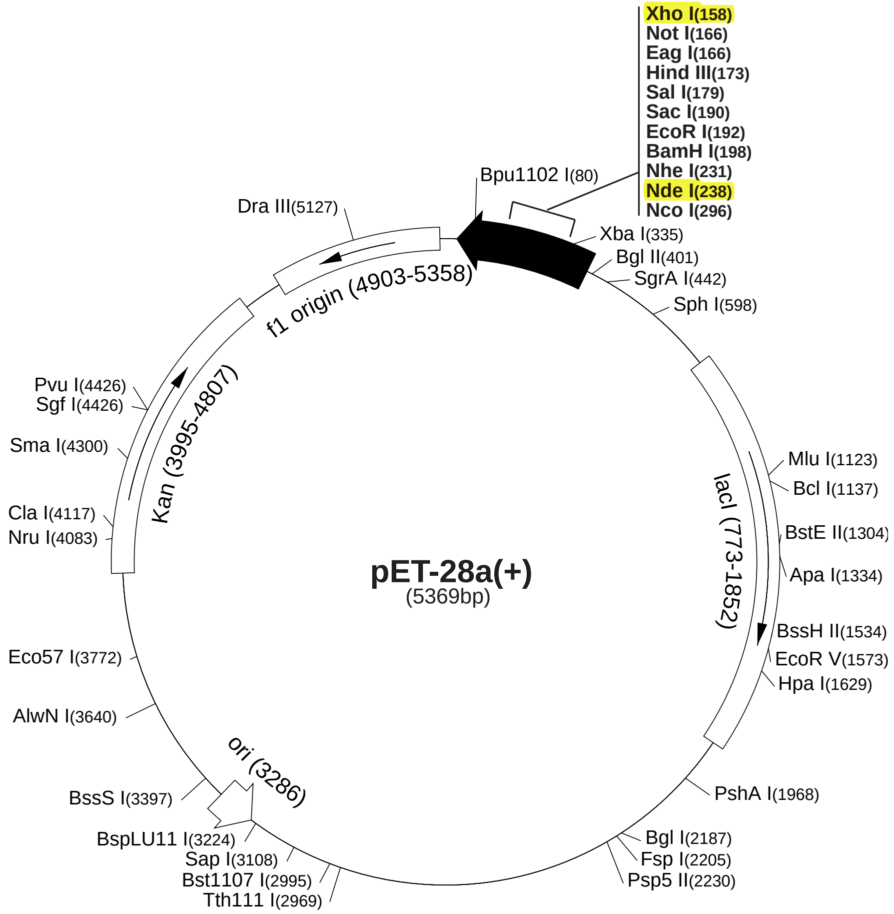
The pET-28a-c(+) vectors carry an N-terminal His•Tag®/thrombin/T7•Tag® configuration plus an optional C-terminal His•Tag sequence. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression 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).

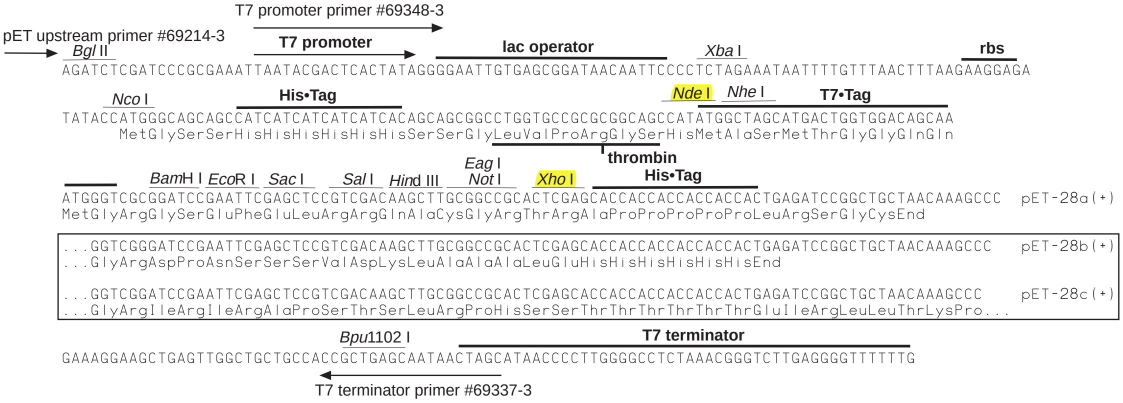
human DDX43 cDNA (full-length) was cloned into the NdeI and XhoI sites of a pET28a vector

pET-28a(+) sequence landmarks

T7 promoter	370-386
T7 transcription start	369
His•Tag coding sequence	270-287
T7•Tag coding sequence	207-239
Multiple cloning sites	
(*Bam*H I - *Xho* I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	773-1852
pBR322 origin	3286
Kan coding sequence	3995-4807
f1 origin	4903-5358

The maps for pET-28b(+) and pET-28c(+) are the same as pET-28a(+) (shown) with the following exceptions: pET-28b(+) is a 5368bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-28c(+) is a 5367bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198.





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AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA

TATACCATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAA
        MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerHisMetAlaSerMetThrGlyGlyGlnGln

ATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCC   pET-28a(+)
MetGlyArgGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProProLeuArgSerGlyCysEnd

...GGTCGGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCC   pET-28b(+)
...GlyArgAspProAsnSerSerSerValAspLysLeuAlaAlaAlaLeuGluHisHisHisHisHisHisEnd

...GGTCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCC    pET-28c(+)
...GlyArgIleArgIleArgAlaProSerThrSerLeuArgProHisSerSerThrThrThrThrThrThrGluIleArgLeuLeuThrLysPro...

GAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
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pET-28a-c(+) cloning/expression region

pET-28a(+) Restriction Sites

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Enzyme	# Sites	Locations				
AccI	2	180	2994			
AceIII	7	890	1618	1949	2733	2874
		3176	4967			
AciI	77					
AflIII	2	1123	3224			
AluI	22					
AlwI	13					
Alw21I	7	159	190	623	1107	2218
		3042	3542			
Alw44I	3	1103	3038	3538		
AlwNI	1	3640				
ApaI	1	1334				
ApaBI	1	807				
ApoI	6	192	1398	4039	4223	4929
		4940				
AvaI	2	158	4298			
AvaII	5	1675	2051	2139	2230	2509
BamHI	1	198				
BanI	9	253	445	466	580	1043
		1762	1892	2018	5164	
BanII	6	190	507	521	1334	4081
		5202				
BbsI	4	1269	1608	1982	2342	
BbvI	27					
BccI	14					
Bce83I	6	21	1937	2107	3315	3613
		3854				
BcefI	6	642	983	1610	3726	4745
		5153				
BcgI	9	160	194	228	1415	1449
		1949	1983	2801	2835	
BclI	1	1137				
BfaI	7	70	232	336	2238	3719
		4026	5278			
BglI	1	2187				
BglII	1	401				
BmgI	1	1332				
BpmI	4	961	1450	2084	2751	
Bpu10I	2	2330	4443			
Bpu1102I	1	80				
BsaAI	2	2976	5127			
BsaBI	3	400	406	2421		
BsaHI	5	446	467	581	1080	1763
BsaJI	10	57	296	560	566	1758
		2196	3384	4297	4298	4699
BsaWI	7	2	1442	1945	2413	3430
		3577	4561			
BsaXI	2	1782	5075			
BsbI	2	2940	5034			
BscGI	11					
BsgI	3	974	1174	2384		
BsiI	1	3397				
BsiEI	5	169	1908	3140	3564	4426
BslI	23					
BsmI	2	4310	4387			
BsmAI	6	820	1225	1351	1738	2865
		4442				
BsmBI	3	1738	2865	4442		
BsmFI	4	584	2125	2495	5342	
BsoFI	48					
Bsp24I	12					
Bsp1286I	12					
BspEI	2	2	2413			
BspGI	1	2750				
BspLU11I	1	3224				
BsrI	22					
BsrBI	4	356	3157	4825	5271	
BsrDI	2	1170	1536			
BsrFI	7	433	442	809	2021	2181
		4380	5228			
BssHII	1	1534				
Bst1107I	1	2995				

Enzyme	# Sites	Locations				
BstEII	1	1304				
BstXI	3	925	1054	1177		
BstYI	9	132	198	401	687	1899
		2416	3865	3876	4675	
Cac8I	40					
CjeI	26					
CjePI	30					
ClaI	1	4117				
CviJI	86					
CviRI	22					
DdeI	11					
DpnI	21					
DraIII	1	5127				
DrdI	3	2917	3332	5082		
DrdII	2	846	5132			
DsaI	3	296	560	2196		
EaeI	4	166	431	563	1797	
EagI	1	166				
EarI	3	741	3108	4239		
EciI	3	900	3298	3444		
Eco47III	3	528	2029	2478		
Eco57I	1	3772				
EcoNI	2	658	4338			
EcoO109I	3	53	556	2230		
EcoRI	1	192				
EcoRII	10	256	846	1161	1701	1758
		3250	3371	3384	4314	4671
EcoRV	1	1573				
FauI	17					
FokI	9	1169	1178	2443	2505	2583
		2769	2910	4064	4670	
FspI	1	2205				
GdiII	4	166	431	563	1797	
HaeI	6	851	2172	3239	3250	3702
		4513				
HaeII	14					
HaeIII	24					
HgaI	11					
HgiEII	2	721	3810			
HhaI	47					
Hin4I	3	1022	4112	4654		
HincII	2	181	1629			
HindIII	1	173				
HinfI	18					
HpaI	1	1629				
HphI	16					
MaeII	14					
MaeIII	16					
MboII	12					
MluI	1	1123				
MmeI	7	3439	3623	4068	4262	4624
		4633	5104			
MnlI	25					
MseI	25					
MslI	6	1175	1463	1493	2211	2406
		2797				
MspI	29					
MspA1I	9	84	264	1153	1723	1816
		2815	2934	3566	3811	
MwoI	39					
NarI	4	446	467	581	1763	
NciI	12					
NcoI	1	296				
NdeI	1	238				
NgoAIV	4	433	2021	2181	5228	
NheI	1	231				
NlaIII	26					
NlaIV	22					
NotI	1	166				
NruI	1	4083				
NsiI	2	4276	4542			
NspI	4	598	2569	2861	3228	

Enzyme	# Sites	Locations				
Pfl1108I	1	2010				
PflMI	2	705	4689			
PleI	9	384	672	759	1555	3118
		3603	4658	5062	5070	
PshAI	1	1968				
Psp5II	1	2230				
Psp1406I	4	785	2153	2549	4912	
PvuI	1	4426				
PvuII	3	1723	1816	2815		
RcaI	3	521	3944	4819		
RsaI	3	1270	3030	4261		
SacI	1	190				
SalI	1	179				
SapI	1	3108				
Sau96I	14					
Sau3AI	21					
ScrFI	22					
SfaNI	23					
SfcI	4	369	3489	3680	5346	
SgfI	1	4426				
SgrAI	1	442				
SmaI	1	4300				
SphI	1	598				
SspI	2	4351	4919			
StyI	2	57	296			
TaqI	15					
TaqII	6	1031	1249	1922	3126	4680
		5031				
TfiI	9	1802	2104	2274	2778	3199
		4337	4393	4565	4656	
ThaI	38					
TseI	27					
Tsp45I	7	1304	2132	2663	2876	2971
		4573	5300			
Tsp509I	20					
Tth111I	1	2969				
Tth111II	8	962	1655	2685	3814	3821
		3853	4262	4389		
UbaJI	21					
VspI	5	384	1808	1867	4625	4814
XbaI	1	335				
XcmI	3	979	1495	1513		
XhoI	1	158				
XmnI	2	2782	4815			

Enzymes that do not cut pET28a(+):

AatII AflII AgeI AscI AvrII
BaeI BsaI BseRI BspMI BsrGI
Bsu36I DraI Eam1105I FseI KpnI
MscI MunI NspV PacI PmeI
PmlI PstI RleAI RsrII SacII
ScaI SexAI SfiI SnaBI SpeI
SrfI Sse8387I StuI SunI SwaI