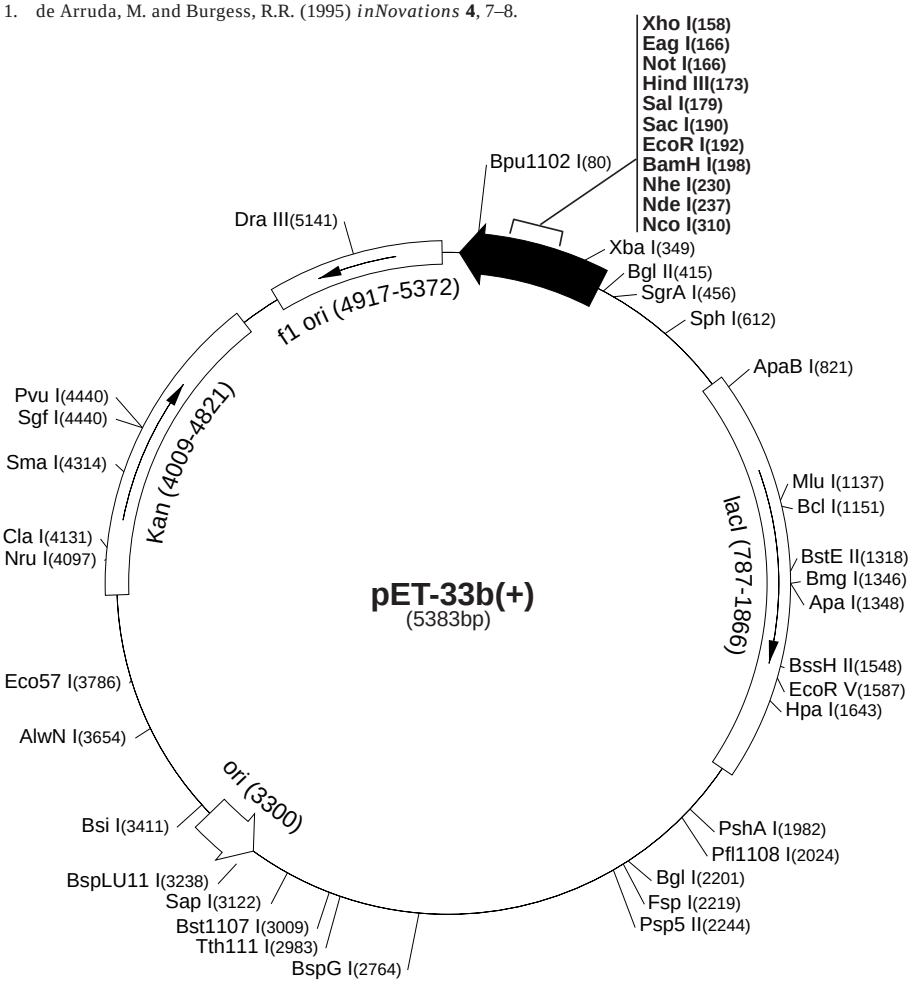
pET-33b(+) Vector

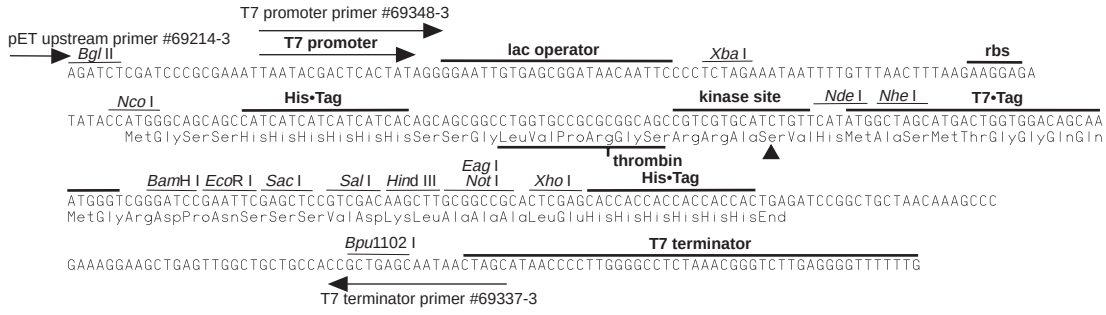
The pET-33b(+) vector (Cat. No. 69054-3) is derived from pET-28b(+) and carries a 15bp sequence encoding the protein kinase A (PKA) site RRASV, located between the thrombin cleavage and *Nde* I sites (1). Proteins expressed in pET-33b(+) can be easily purified by metal chelation chromatography (*via* either N- or C-terminal His•Tag® sequences) and efficiently labeled with ^{32}P- or ^{33}P-γATP and the catalytic subunit of cAMP-dependent protein kinase from heart muscle. Labeled proteins can be used as direct probes in protein-protein interaction studies. 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 circle 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-33b(+) sequence landmarks

T7 promoter	384-400
T7 transcription start	383
His•Tag coding sequence	284-301
PKA site coding sequence	242-256
T7•Tag® coding sequence	206-238
Multiple cloning sites	
(*Bam*H I - *Xho* I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	787-1866
pBR322 origin	3300
Kan coding sequence	4009-4821
f1 origin	4917-5372

1. de Arruda, M. and Burgess, R.R. (1995) *inNovations* **4**, 7–8.





pET-33b(+) cloning/expression region

pET-33b(+) Restriction Sites

TB147 12/98

Enzyme	# Sites	Locations				
AccI	2	180	3008			
AceIII	7	904	1632	1963	2747	2888
		3190	4981			
AciI	76					
AflIII	2	1137	3238			
AluI	22					
AlwI	13					
Alw21I	7	159	190	637	1121	2232
		3056	3556			
Alw44I	3	1117	3052	3552		
AlwNI	1	3654				
ApaI	1	1348				
ApaBI	1	821				
ApoI	6	192	1412	4053	4237	4943
		4954				
AvaI	2	158	4312			
AvaII	5	1689	2065	2153	2244	2523
BamHI	1	198				
BanI	9	267	459	480	594	1057
		1776	1906	2032	5178	
BanII	6	190	521	535	1348	4095
		5216				
BbsI	4	1283	1622	1996	2356	
BbvI	27					
BccI	14					
Bce83I	6	21	1951	2121	3329	3627
		3868				
BcefI	7	270	656	997	1624	3740
		4759	5167			
BcgI	10	160	193	194	227	1429
		1463	1963	1997	2815	2849
BclI	1	1151				
BfaI	7	70	231	350	2252	3733
		4040	5292			
BglI	1	2201				
BglII	1	415				
BmgI	1	1346				
BpmI	4	975	1464	2098	2765	
Bpu10I	2	2344	4457			
Bpu1102I	1	80				
BsaAI	2	2990	5141			
BsaBI	3	414	420	2435		
BsaHI	5	460	481	595	1094	1777
BsaJI	10	57	310	574	580	1772
		2210	3398	4311	4312	4713
BsaWI	7	2	1456	1959	2427	3444
		3591	4575			
BsaXI	2	1796	5089			
BsbI	2	2954	5048			
BscGI	11					
BsgI	3	988	1188	2398		
BsiI	1	3411				
BsiEI	5	169	1922	3154	3578	4440
BsII	23					
BsmI	2	4324	4401			
BsmAI	6	834	1239	1365	1752	2879
		4456				
BsmBI	3	1752	2879	4456		
BsmFI	4	598	2139	2509	5356	
BsoFI	48					
Bsp24I	12					
Bsp1286I	12					
BspEI	2	2	2427			
BspGI	1	2764				
BspLU11I	1	3238				
BsrI	22					
BsrBI	4	370	3171	4839	5285	
BsrDI	2	1184	1550			
BsrFI	7	447	456	823	2035	2195
		4394	5242			
BssHII	1	1548				
Bst1107I	1	3009				

Enzyme	# Sites	Locations				
BstEII	1	1318				
BstXI	3	939	1068	1191		
BstYI	9	132	198	415	701	1913
		2430	3879	3890	4689	
Cac8I	40					
CjeI	26					
CjePI	22					
ClaI	1	4131				
CviJI	86					
CviRI	23					
DdeI	11					
DpnI	21					
DraIII	1	5141				
DrdI	3	2931	3346	5096		
DrdII	2	860	5146			
DsaI	3	310	574	2210		
EaeI	4	166	445	577	1811	
EagI	1	166				
EarI	3	755	3122	4253		
EciI	3	914	3312	3458		
Eco47III	3	542	2043	2492		
Eco57I	1	3786				
EcoNI	2	672	4352			
EcoO109I	3	53	570	2244		
EcoRI	1	192				
EcoRII	10	270	860	1175	1715	1772
		3264	3385	3398	4328	4685
EcoRV	1	1587				
FauI	17					
FokI	9	1183	1192	2457	2519	2597
		2783	2924	4078	4684	
FspI	1	2219				
GdiII	4	166	445	577	1811	
HaeI	6	865	2186	3253	3264	3716
		4527				
HaeII	14					
HaeIII	24					
HgaI	11					
HgiEII	2	735	3824			
HhaI	47					
Hin4I	3	1036	4126	4668		
HincII	2	181	1643			
HindIII	1	173				
HinfI	18					
HpaI	1	1643				
HphI	16					
MaeII	14					
MaeIII	16					
MboII	12					
MluI	1	1137				
MmeI	7	3453	3637	4082	4276	4638
		4647	5118			
MnlI	25					
MseI	25					
MslI	6	1189	1477	1507	2225	2420
		2811				
MspI	29					
MspA1I	9	84	278	1167	1737	1830
		2829	2948	3580	3825	
MwoI	39					
NarI	4	460	481	595	1777	
NciI	12					
NcoI	1	310				
NdeI	1	237				
NgoAIV	4	447	2035	2195	5242	
NheI	1	230				
NlaIII	26					
NlaIV	22					
NotI	1	166				
NruI	1	4097				
NsiI	2	4290	4556			
NspI	4	612	2583	2875	3242	

Enzyme	# Sites	Locations				
Pfl1108I	1	2024				
PflMI	2	719	4703			
PleI	9	398	686	773	1569	3132
		3617	4672	5076	5084	
PshAI	1	1982				
Psp5II	1	2244				
Psp1406I	4	799	2167	2563	4926	
PvuI	1	4440				
PvuII	3	1737	1830	2829		
RcaI	3	535	3958	4833		
RsaI	3	1284	3044	4275		
SacI	1	190				
SalI	1	179				
SapI	1	3122				
Sau96I	14					
Sau3AI	21					
ScrFI	22					
SfaNI	24					
SfcI	4	383	3503	3694	5360	
SgfI	1	4440				
SgrAI	1	456				
SmaI	1	4314				
SphI	1	612				
SspI	2	4365	4933			
StyI	2	57	310			
TaqI	15					
TaqII	6	1045	1263	1936	3140	4694
		5045				
TfiI	9	1816	2118	2288	2792	3213
		4351	4407	4579	4670	
ThaI	37					
TseI	27					
Tsp45I	7	1318	2146	2677	2890	2985
		4587	5314			
Tsp509I	20					
Tth111I	1	2983				
Tth111II	8	976	1669	2699	3828	3835
		3867	4276	4403		
UbaJI	21					
VspI	5	398	1822	1881	4639	4828
XbaI	1	349				
XcmI	3	993	1509	1527		
XhoI	1	158				
XmnI	2	2796	4829			

Enzymes that do not cut pET-33b(+):

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