

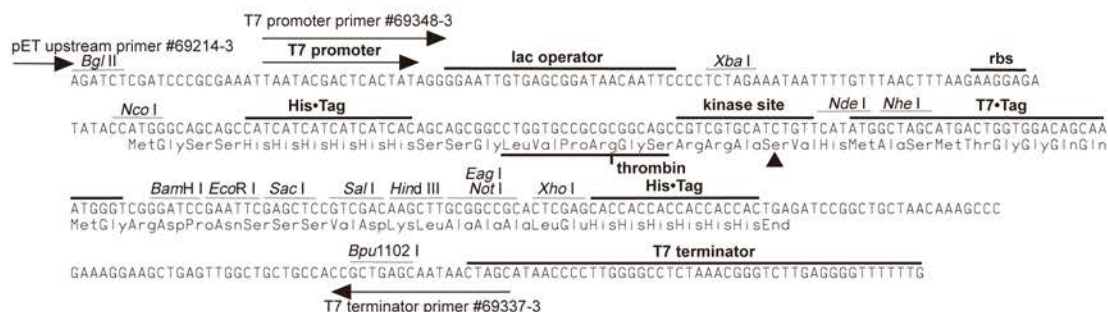
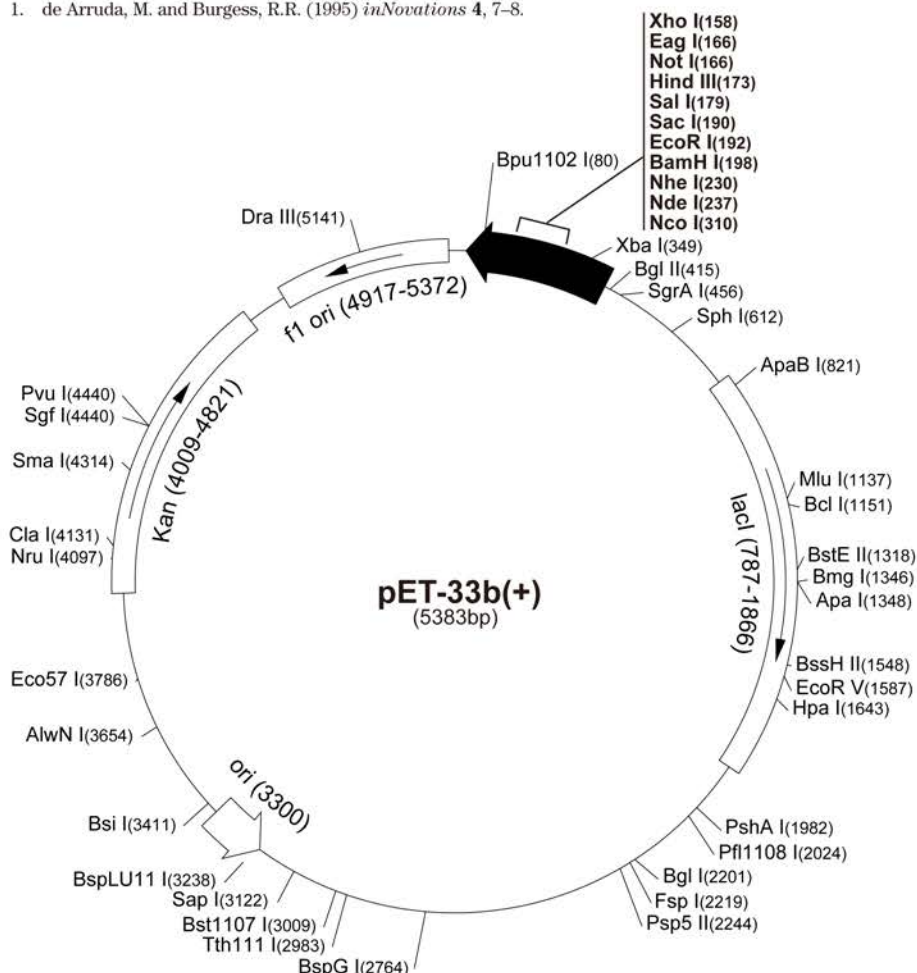
pET-33b(+) Vector

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 ³²P- or ³³P-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	
(<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	787-1866
pBR322 origin	3300
Kan coding sequence	4009-4821
f1 origin	4917-5372

1. de Arruda, M. and Burgess, R.R. (1995) *in* *Innovations* 4, 7-8.



pET-33b(+) cloning/ expression region

pET-33b(+) Restriction Sites

Enzyme	# Sites	Locations					Enzyme	# Sites	Locations					Enzyme	# Sites	Locations				
AccI	2	180	3008				BstEII	1	1318				PII1108I	1	2024					
AccIII	7	904	1632	1963	2747	2888	BstXI	3	939	1068	1191		PIIMI	2	719	4703				
		3190	4981				BstYI	9	132	198	415	701	1913	PleI	9	398	686	773	1569	3132
Acil	76								2430	3879	3890	4689			3617	4672	5076	5084		
AlIII	2	1137	3238				Cac8I	40						PshAI	1	1982				
AluI	22						CjeI	26						Psp5II	1	2244				
AlwI	13						CjePI	22						Psp1406I	4	799	2167	2563	4926	
Alw21I	7	159	190	637	1121	2232	Clal	1	4131					PvuI	1	4440				
		3056	3556				CviJI	86						PvuII	3	1737	1830	2829		
Alw44I	3	1117	3052	3552			CviRI	23						RcaI	3	535	3958	4833		
AlwNI	1	3654					DdeI	11						RsaI	3	1284	3044	4275		
Apal	1	1348					DpnI	21						SacI	1	190				
ApaBI	1	821					DraIII	1	5141					Sall	1	179				
ApoI	6	192	1412	4053	4237	4943	DrdI	3	2931	3346	5096			SapI	1	3122				
		4954					DrdII	2	860	5146				Sau96I	14					
Aval	2	158	4312				Dsal	3	310	574	2210			Sau3AI	21					
Avall	5	1689	2065	2153	2244	2523	EaeI	4	166	445	577	1811		ScrFI	22					
BamHI	1	198					EagI	1	166					SfaNI	24					
BanI	9	267	459	480	594	1057	EarI	3	755	3122	4253			StcI	4	383	3503	3694	5360	
		1776	1906	2032	5178		Ecil	3	914	3312	3458			SglI	1	4440				
BanII	6	190	521	535	1348	4095	Eco47III	3	542	2043	2492			SgrAI	1	456				
		5216					Eco57I	1	3786					SmaI	1	4314				
BbsI	4	1283	1622	1996	2356		EcoNI	2	672	4352				SphI	1	612				
BbvI	27						EcoO109I	3	53	570	2244			Sspl	2	4365	4933			
BccI	14						EcoRI	1	192					StyI	2	57	310			
Bce83I	6	21	1951	2121	3329	3627	EcoRII	10	270	860	1175	1715	1772	TaqI	15					
		3868							3264	3385	3398	4328	4685	TaqII	6	1045	1263	1936	3140	4694
Bcefl	7	270	656	997	1624	3740	EcoRV	1	1587						5045					
		4759	5167				FauI	17						TfiI	9	1816	2118	2288	2792	3213
Bcgl	10	160	193	194	227	1429	FokI	9	1183	1192	2457	2519	2597		4351	4407	4579	4670		
		1463	1963	1997	2815	2849			2783	2924	4078	4684								
BclI	1	1151					FspI	1	2219					ThaI	37					
Bfal	7	70	231	350	2252	3733	GdiII	4	166	445	577	1811		TseI	27					
		4040	5292				HaeI	6	865	2186	3253	3264	3716	Tsp45I	7	1318	2146	2677	2890	2985
BglI	1	2201							4527						4587	5314				
BglII	1	415					Haell	14						Tsp509I	20					
BmgI	1	1346					HaellI	24						Tth111I	1	2983				
BpmI	4	975	1464	2098	2765		Hgal	11						Tth111II	8	976	1669	2699	3828	3835
Bpu10I	2	2344	4457				HgiEII	2	735	3824					3867	4276	4403			
Bpu1102I	1	80					Hhal	47						UbaJI	21					
BsaAI	2	2990	5141				Hin4I	3	1036	4126	4668			VspI	5	398	1822	1881	4639	4828
BsaBI	3	414	420	2435			HincII	2	181	1643				XbaI	1	349				
BsaHI	5	460	481	595	1094	1777	HindIII	1	173					XcmI	3	993	1509	1527		
BsaJI	10	57	310	574	580	1772	HinfI	18						XhoI	1	158				
		2210	3398	4311	4312	4713	Hpal	1	1643					Xmnl	2	2796	4829			
BsaWI	7	2	1456	1959	2427	3444	HphI	16						Enzymes that do not cut pET-33b(+):						
		3591	4575				MaeII	14						AatII	AfilI	AgeI	AscI	AvrII		
BsaXI	2	1796	5089				MaeIII	16						BaeI	Bsal	BseRI	BspMI	BsrGI		
Bsbl	2	2954	5048				MbolI	12						Bsu36I	DraI	Eam1105I	FseI	KpnI		
BscGI	11						Mlul	1	1137					MscI	MunI	NspV	Pacl	PmeI		
BsgI	3	988	1188	2398			Mmel	7	3453	3637	4082	4276	4638	PmlI	PstI	RleAI	RsrII	SacII		
Bsil	1	3411							4647	5118				Scal	SexAI	Sfil	SnaBI	SpeI		
BsiEI	5	169	1922	3154	3578	4440	MnII	25						SrfI	Sse8387I	Stul	SunI	Swal		
BsII	23						MseI	25												
BsmI	2	4324	4401				MslI	6	1189	1477	1507	2225	2420							
BsmAI	6	834	1239	1365	1752	2879			2811											
		4456					Mspl	29												
BsmBI	3	1752	2879	4456			MspA1I	9	84	278	1167	1737	1830							
BsmFI	4	598	2139	2509	5356				2829	2948	3580	3825								
BsoFI	48						Mwol	39												
Bsp24I	12						NarI	4	460	481	595	1777								
Bsp1286I	12						Ncil	12												
BspEI	2	2	2427				NcoI	1	310											
BspGI	1	2764					NdeI	1	237											
BspLU11I	1	3238					NgoAIV	4	447	2035	2195	5242								
BsrI	22						NheI	1	230											
BsrBI	4	370	3171	4839	5285		NlaIII	26												
BsrDI	2	1184	1550				NlaIV	22												
BsrFI	7	447	456	823	2035	2195	NotI	1	166											
		4394	5242				NruI	1	4097											
BssHII	1	1548					Nsil	2	4290	4556										
Bst1107I	1	3009					NspI	4	612	2583	2875	3242								