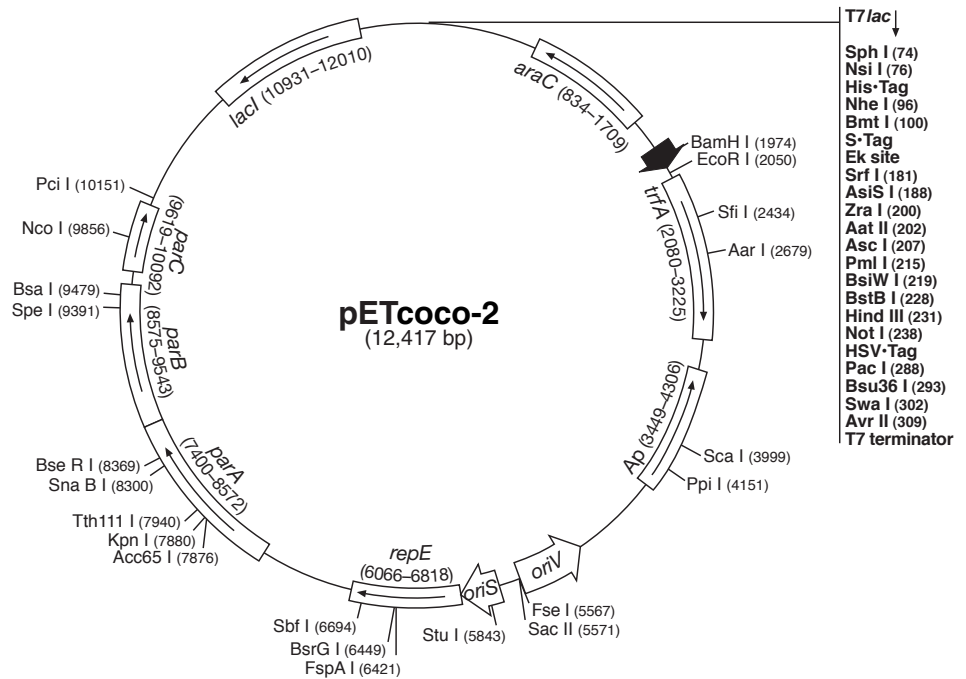


pETcoco-2 Vector

	Cat. No.
pETcoco-2 DNA	71148-3
pETcoco-2 sequence landmarks	
T7 promoter	12401-12417
<i>lac</i> operator	5-25
T7 transcription start	1
Multiple cloning sites (<i>Sph</i> I- <i>Avr</i> II)	74-309
His•Tag® coding sequence	75-92
S•Tag™ coding sequence	102-146
HSV•Tag® coding sequence	246-281
T7 terminator	410-457
<i>araC</i> coding sequence	834-1709
<i>trfA</i> coding sequence	2080-3225
Ap (<i>bla</i>) coding sequence	3449-4306
<i>oriV</i>	5040-5565
<i>oriS</i>	5756-5972
<i>repE</i> coding sequence	6066-6818
<i>parA</i> coding sequence	7400-8572
<i>parB</i> coding sequence	8575-9543
<i>parC</i> region	9619-10092
<i>lacI</i> coding sequence	10931-12010

The pETcoco™ expression vectors are designed to allow “on command” amplification of vector copy number from single copy (1–2 plasmid copies per cell) to medium copy (20– 50 per cell). The vector is maintained in the single copy state by utilizing the *oriS* and *repE* elements of the F episome together with the *parABC* partition determinants. The medium copy state is obtained by employing the RK-2 derived *trfA* replicator acting at *oriV*. The switch from single copy to medium copy status is achieved by inducing the expression of the *trfA* gene with arabinose. The dual replicon nature of these vectors promotes enhanced stability of recombinant plasmids and extremely low basal expression levels in the single copy state, while allowing robust target protein expression typical of the pET vectors. Unique restriction sites are shown on the circle map (below). The sequence is numbered relative to the start of T7 transcription at +1.



T7 transcription start

GGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTAACTTTAAGAAGGAGATATAAGCATGCATCACCATCACCATCACGGTGCAGC
 MetHisHisHisHisHisHisGlyAlaSer

S•Tag 18mer primer
S•Tag #70828-3

AAAGAAACCGCTGCTGCGAAATTTGAACGCCAGCACATGGACTCGCCACCGCCAACTGGTGATGACGACGACAAGAGCCGGCGATCGCATCGATGAC
 LysGluThrAlaAlaLysPheGluArgGlnHisMetAspSerProProProThrGlyAspAspAspAspLysSerProGlyAspArgIleAspAsp

enterokinase

HSV•Tag

GTCCAGGCGCGCCACGTGCGTACGGTTTCGAAGCTTGCGGCCGCAAGCCAGCCAGAAGCTCGCTCCGGAAGATCCCGAGGATCTTTAATTAACCTCAGGAT
 ValGlnAlaArgHisValArgThrValSerLysLeuAlaAlaAlaSerGlnProGluLeuAlaProGluAspProGluAspLeuEnd

T7 terminator primer #69337-3

TTAATAACCTAGCGCGCACTCGAGCACCACCACCACCACCAGGATCCGGCTGCTAACAAAGCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCAGCT

pETcoco-2 cloning/expression region

pETcoco-2 Restriction Sites

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations			
AarI	1	2679	BsmI	2	1832 2812	NruI	3	923 1443 10343			
AatII	1	202	Bsp1286I	22		NsiI	1	76			
Acc65I	1	7876	BspCNI	26		NspI	4	74 1103 10155 12188			
AccI	4	3277 4464 9563 10332	BspEI	4	263 476 1450 8436	NspV	1	228			
AcII	4	3745 4118 8268 11995	BspHI	6	4354 4756 7450 8104 9300	Pacl	1	288			
AfeI	2	7235 12254			12257	PciI	1	10151			
AfIII	3	1036 9277 9517	BspLU11I	1	10151	PfiI	6	137 1655 2763 4895 7968			
AfIII	5	1870 7642 7816 10151 11655	BspMI	6	2320 2679 3129 5135 6683			12080			
AgeI	4	1809 6496 7726 8619			10450	PfoI	3	2900 9420 12087			
AhdI	3	2844 3519 10155	BsrBI	6	13 580 2546 3072 4360	PinAI	4	1809 6496 7726 8619			
AleI	2	3118 9212			4844	PmlI	1	215			
Alol	2	4733 8370	BsrDI	12		Ppil	1	4151			
AlwNI	7	2763 2937 5010 7778 7798	BsrFI	19		PpuMI	4	2403 2760 2934 10527			
		8820 10682	BsrGI	1	6449	PshAI	9	4441 9686 9729 9772 9815			
Apal	2	9641 11452	BssHII	5	207 1405 8133 8677 11244			9858 9901 9987 10073			
ApalI	2	4186 11675	BssSI	4	4183 7826 9476 10039	Psil	2	5126 5791			
AscI	1	207	BstAPI	4	738 4507 10314 11979	PspOMI	2	9637 11448			
Asel	8	3691 4711 7668 8160 10431	BstBI	1	228	PstI	3	2690 6694 8235			
		10913 10972 12400	BstEII	3	1812 10273 11473	PvuI	3	188 3889 8542			
AsiSI	1	188	BstXI	4	7754 11609 11732 11861	PvuII	4	6644 8650 10966 11059			
Aval	7	179 273 320 889 4954	BstYI	16		SacI	3	10341 10349 10759			
		6160 8786	Bsu36I	1	293	SacII	1	5571			
AvrII	1	309	BtgI	18		Sall	2	3276 10331			
BaeI	3	1006 8164 10430	BtrI	6	2616 2628 2846 5428 7706	SapI	3	1798 7272 8482			
BamHI	1	1974			10466	SbfI	1	6694			
BanI	15		BtsI	6	1773 3919 3939 8228 10928	Scal	1	3999			
BanII	9	180 653 9641 10341 10349			11296	SexAI	2	3206 10269			
		10759 11452 12265 12279	Clal	2	193 12380	SfcI	9	501 2686 2821 3760 6222			
Bbel	4	11020 12202 12316 12337	DraI	6	302 914 4096 5139 7302			6690 8231 10166 12413			
BbsI	5	7719 7908 8785 11170 11509			10647	Sfil	1	2434			
BceAI	28		DraIII	2	4507 10492	SfoI	4	11018 12200 12314 12335			
Bcgl	6	866 1233 2700 4058 10306	DrdI	4	1769 5923 6818 8114	SgrAI	6	2395 2644 5556 7726 8883			
		11335	EaeI	21				12336			
BciVI	4	1682 4356 5556 11203	EagI	9	238 724 2288 4992 5037	Smal	2	181 6162			
BclI	2	8467 11641			5434 5561 5565 5626	SmlI	14				
BfrBI	1	74	EarI	9	1798 3152 4314 6318 6358	SnaBI	1	8300			
BglI	4	2434 3639 5840 10289			7272 8482 8892 12038	SpeI	1	9391			
BglII	2	5815 7882	Ecil	10	1077 1205 2127 3079 3662	SphI	1	74			
BlpI	2	399 7148			5197 5494 6084 6666 11870	SrfI	1	181			
Bme1580I	8	2864 3374 4190 5065 5341	Ecl136II	3	10339 10347 10757	Sse8387I	1	6694			
		9641 11452 11679	Eco57I	9	1685 4186 4783 6382 6895	Sspl	7	1371 4323 5131 6274 9108			
BmgBI	6	2616 2628 2846 5428 7706			7731 7826 9356 10416			9135 9627			
		10466	Eco57MI	16		StuI	1	5843			
Bmrl	7	3559 9687 9816 10857 11497	EcoCRI	3	10339 10347 10757	StyI	6	309 421 4574 5755 6402			
		11734 12131	EcoNI	2	6138 12123			9856			
BmtI	1	100	EcoO109I	7	426 2403 2760 2934 3228	Swal	1	302			
BpII	2	8517 8799			10527 12223	TaqII	17				
Bpml	7	3589 7133 7546 8512 9443	EcoRI	1	2050	TatI	3	1154 3997 6449			
		11334 11823	EcoRV	4	1121 6797 7026 11209	TspGWI	12				
Bpu10I	2	6596 7791	FseI	1	5567			7940			
BpuEI	9	463 1224 3058 4124 5257	FspAI	1	6421	XbaI	2	30 5861			
		5303 5475 8246 10847	FspI	3	3741 6421 10247	XcmI	4	5366 11270 11288 11804			
BsaAI	5	215 7817 8300 8405 9142	HaeII	14		XhoI	2	320 4954			
BsaBI	6	976 2350 10423 10507 12376	HincII	9	2619 3278 5464 5499 6194	XmaI	2	179 6160			
		12386			8875 10298 10333 11153	Xmnl	5	4118 6327 6397 9108 10592			
BsaHI	9	199 1928 2648 4056 11017	HindIII	1	231	ZraI	1	200			
		11700 12199 12313 12334	HpaI	2	10298 11153						
Bsal	1	9479	KasI	4	11016 12198 12312 12333	Enzymes that do not cut pETcoco-2:					
BsaWI	14		KpnI	1	7880	BbvCI	Bst1107I	BstZ17I	FalI	PmeI	Psrl
BsaXI	7	2028 4712 7519 8237 8922	MfeI	4	809 1233 1743 7656	RsrII	SanDI				
		9416 10987	MluI	2	1870 11655						
BseRI	1	8369	MscI	2	5418 8087						
BseYI	7	1588 3250 5636 8559 9316	MslI	17							
		11121 11256	NaeI	8	621 2397 5434 5558 5565						
BsgI	6	2869 4529 7991 8936 11610			6374 10290 12347						
		11810	NarI	4	11017 12199 12313 12334						
BsiEI	21		NcoI	1	9856						
BsiHKAI	12		NdeI	2	2992 7674						
BsiWI	1	219	NgoMIV	8	619 2395 5432 5556 5563						
BsmAI	11				6372 10288 12345						
BsmBI	3	2317 6611 11040	NheI	1	96						
BsmFI	25		NotI	1	238						