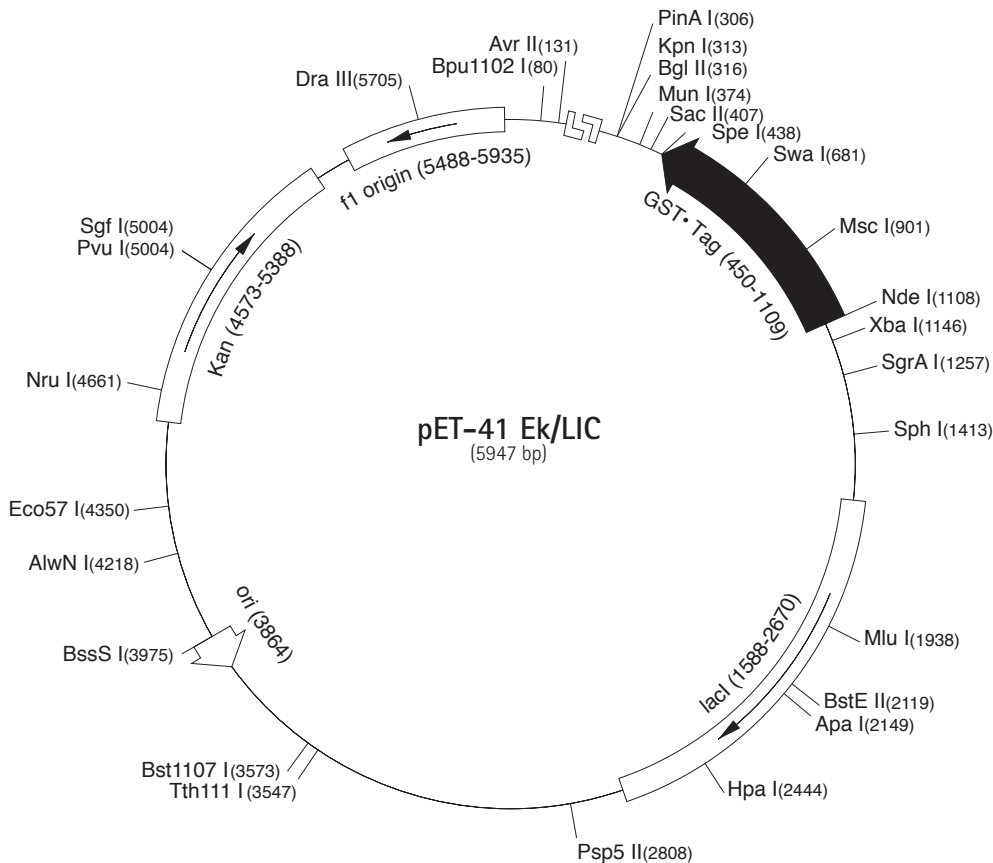
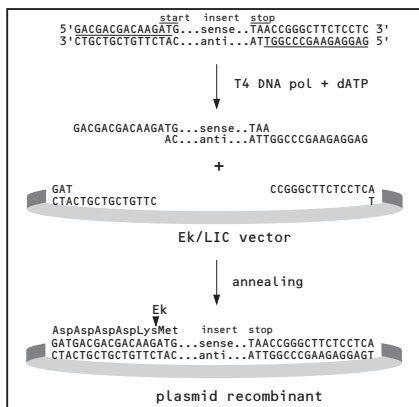


## pET-41 Ek/LIC Vector

TB317 0806

	Cat No.
pET-41 Ek/LIC Vector Kit	71071-3
<b>pET-41 Ek/LIC sequence landmarks</b>	
T7 promoter	1181-1197
T7 transcription start	1180
GST•Tag coding sequence	450-1109
His•Tag coding sequence	411-428
S•Tag coding sequence	324-368
Multiple cloning sites (BseR I-Xho I)	174-277
His•Tag coding sequence	150-173
T7 terminator	26-72
<i>lacI</i> coding sequence	1588-2670
pBR322 origin	3864
Kan coding sequence	4573-5388
f1 origin	5935-5488

The pET-41 Ek/LIC vector is prepared for rapid, directional cloning of PCR-amplified DNA for high-level expression of polypeptides fused with N-terminal GST•Tag™, His•Tag® and S•Tag™ sequences. Using specifically designed primers for amplification and the pET-41 Ek/LIC Vector Kit (Cat. No. 71071-3), inserts can be efficiently cloned without the need for restriction digestion or ligation. 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). Vector encoded sequence can be completely removed when cloning into the Ek/LIC site (as shown below left) by cleaving the fusion protein with enterokinase.



**T7 Promoter Primer #69348-3**

T7 promoter → lac operator Xba I rbs Nde I  
 AATTAATACGACTCACTATAGGGGAATTTGTAGCGGATAACAATTCGCCCTTGAAGAAATTTTGTAACTTAAAGAGGAGATATACATATGCCCCCT  
 TTAATTAATGCTGAGTGATATCCCTTAACACTCGCCTATTGTTAAGGGGAGATCTTTATTAAGCAAAATTTGAAATTCCTCTATATGTATACAGGGGG

**GST•Tag** Spe I His•Tag Sac II thrombin I  
 ILeLeuGlyTyr...209aa...AspHisProProLysSerAspGlySerThrSerGlySerGlyHisHisHisHisHisSerAlaGlyLeuValProArgGlySer  
 ATACTAGGTTAT...627bp...GACCATCTCCAATCGGATGGTCAACTAGTGGTCTGGTCATCACCATCACCATCACTCCGCGGGTCTGGTGCCACCGGTAGT  
 TATGATCCAATA CTGGTAGGAGGTTTTAGCTACCAAGTTGATCACCAAGACAGTAGTGGTAGTGAGGGCGCCAGACCACGGTGGCGCATCA

**S•Tag** S•Tag Primer #69945-3 Bgl II PinA I Ek/LIC Cloning Site  
 ThrAlaIleGlyMetLysGluThrAlaAlaLysPheGluArgGlnHisMetAspSerProAspLeuGlyThrGlyGlySerGlyAspAspAspLys  
 ACTGCAATTTGGTATGAAGAAACCGCTGCTGCTAAATTCGAACGCCAGACATGGACAGCCAGATCTGGGTACC6GTGGTGGCTCCGGTGT  
 TGACGTTAACCATACTTTCTTTGGCGACGACGATTAAAGCTTGGCGTGTGACTGTGGGCTTAGAACCCATGGCCACCAGGCCACTACTGCTGCTGTTCT

**Ek/LIC Cloning Site** Pst I Eag I  
 BseR I NcoI EcoR V BamH I EcoR I BsrG I Stu I Asc I Sse8387 I Sac I Sal I Hind III Not I Xho I His•Tag  
 ProGlyPheSerSerThrMetAspIleGlyAspProAsnSerValGlnAlaLeuAlaArgLeuGlnAlaSerSerValAspLysLeuAlaAlaLeuGluHisHisHis  
 CCGGGCTTCTCTCAACCATGGATATCGGGGATCCGAATTCGTACAGGCCCTGGCGGCTGCAAGGCGAGCTCCGTCGACAAGCTTGGCGCCGACTCGAGCCACCAC  
 TTGGTACCTATAGCCCTTAGGCTTAAAGACATGTCCGGAACCGCGCGGAGCTCGCTCGAGGCGAGCTGTTGAACGCGCGGCTGGAGCTGCTGGTGGT

**His•Tag** Avr II Bpu1102 I T7 terminator  
 HisHisHisHisHisHisEnd  
 CACCACCAACCACTAATTTGATTAATAACTAGGCTGAACTAACAAAGCCGAAGGAAAGCTGAGTTGGCTGCCACCGCTGAGCAATAACTAGCATAAACCCCTTGGGGC  
 GTGGTGGTGGTGGTATTAACTAATTTGGATCCGACGATTTGTTTGGGCTTCTCTCGACTCAACCGACAGCGTGGCGACTCGTTATTGATCGTTATGGGGAACCCGC  
 T7 Terminator Primer #69337-3

pET-41 Ek/LIC cloning/expression regions

## pET-41 Ek/LIC Restriction Sites

TB317 0806

Enzyme	# Sites	Locations	Enzyme	# Sites	Locations	Enzyme	# Sites	Locations
AccI	2	196 3572	EagI	1	182	SgfI	1	5004
AcII	73		EarI	4	1026 1556 3686 4817	SgrAI	1	1257
AflIII	3	866 1938 3802	Ecl136II	1	204	Smal	2	273 4878
AluI	25		Eco47III	2	1343 3056	SpeI	1	438
Alw26I	6	1635 2040 2166 2553 3443	Eco57I	1	4350	SphI	1	1413
		5020	EcoNI	3	1097 1473 4916	SrfI	1	273
AlwI	12		EcoO109I	4	53 1073 1371 2808	Sse8387I	1	214
AlwNI	1	4218	EcoRI	1	235	Sspl	2	4929 5497
ApaI	1	2149	EcoRII	10	598 1661 1976 2516 2573	StuI	1	226
ApaLI	3	1918 3616 4116			3828 3949 3962 4892 5249	StyI	4	57 131 221 254
ApoI	7	235 345 2213 4617 4801	EcoRV	1	251	Swal	1	681
		5507 5518	EheI	4	1262 1283 1397 2579	Tail	16	
AscI	1	216	FauI	17		TaqI	19	
AvaI	3	174 271 4876	Fnu4HI	40		TfiI	8	2617 2852 3356 3777 4915
Avall	4	611 2490 2808 3087	FokI	13				4971 5143 5234
AvrII	1	131	Haell	13		Thal	38	
BamHI	1	241	HaellI	24		TseI	24	
BanI	9	309 394 1260 1281 1395	Hgal	12		Tsp45I	6	2119 3241 3454 3549 5151
		1858 2577 2707 5742	Hhal	46				5878
BanII	7	206 278 1322 1336 2149	HincII	2	197 2444	Tsp509I	29	
		4659 5780	HindIII	1	189	TspRI	11	
BbsI	3	2084 2423 2920	HinfI	17		Tth111I	1	3547
BbvI	24		HpaI	1	2444	VspI	6	139 1195 2623 2682 5203
BcgI	4	176 1080 2264 3379	HphI	22				5392
BclI	2	670 1952	KpnI	1	313	XbaI	1	1146
Bfal	10	70 132 439 1094 1147	MaellI	16		XcmI	3	1794 2310 2328
		2781 2816 4297 4604 5856	MbolI	15		XhoI	1	174
BglII	1	316	MluI	1	1938	XmnI	3	715 3360 5393
BpmI	3	1776 2265 3329	MnlI	25				
Bpu10I	2	2908 5021	MscI	1	901			
Bpu1102I	1	80	MseI	31				
BsaAI	2	3554 5705	MslI	7	1014 1990 2278 2308 2789			
BsaBI	3	1211 1221 2999			2984 3375			
BsaHI	5	1261 1282 1396 1895 2578	MspA1I	10	84 358 406 1968 2538			
BsaJI	13				2631 3393 3512 4144 4389			
BsaWI	8	2 306 2257 2760 2991	MspI	28				
		4008 4155 5139	MunI	1	374			
BseRI	1	277	MwoI	36				
BsgI	4	824 1789 1989 2962	NarI	4	1261 1282 1396 2578			
BsiEI	5	185 2723 3718 4142 5004	NciI	14				
BsiHKAI	7	175 206 1438 1922 2796	NcoI	1	254			
		3620 4120	NdeI	1	1108			
BslI	28		NgoAIV	2	1248 5806			
BsmBI	3	2553 3443 5020	NlaIII	29				
BsmFI	4	1117 1399 3073 5920	NlaIV	21				
BsmI	2	4888 4965	NotI	1	182			
Bsp1286I	13		NruI	1	4661			
BspEI	2	2 2991	NsiI	2	4854 5120			
BspLU11I	2	866 3802	NspI	5	870 1413 3147 3439 3806			
BsrBI	5	817 1167 3735 5403 5849	NspV	2	343 709			
BsrDI	2	1985 2351	PfiMI	3	335 1520 5267			
BsrFI	6	306 1248 1257 1624 4958	PinAI	1	306			
		5806	PleI	9	1195 1487 1574 2370 3696			
BsrGI	1	229			4181 5236 5640 5648			
BsrI	20		Psp1406I	3	1600 3127 5490			
BssHII	2	216 2349	Psp5II	1	2808			
BssSI	1	3975	PstI	1	214			
Bst1107I	1	3573	PvuI	1	5004			
BstEII	1	2119	PvuII	3	2538 2631 3393			
BstXI	3	1740 1869 1992	RcaI	3	1336 4522 5397			
BstYI	8	241 316 1502 2714 2994	RsaI	7	231 311 383 535 2085			
		4443 4454 5253			3608 4839			
Cac8I	43		SacI	1	206			
Clal	2	1215 4695	SacII	1	407			
CviJI	90		Sall	1	195			
Ddel	11		SapI	2	1026 3686			
Dpnl	23		Sau3AI	23				
DraI	2	572 681	Sau96I	13				
DraIII	1	5705	Scal	2	383 535			
DrdI	3	3495 3910 5660	ScrFI	24				
Dsal	3	254 404 1375	SfaNI	24				
EaeI	5	182 899 1246 1378 2612	Sfcl	5	210 1180 4067 4258 5924			

Enzymes that do not cut pET41 Ek/LIC:

AatII	AflII	AhdI	BglI	BsaI	BspMI
Bsu36I	FseI	FspI	NheI	PacI	PmeI
PmlI	PshAI	RsrII	SanDI	SexAI	SfiI
SnaBI	SunI				