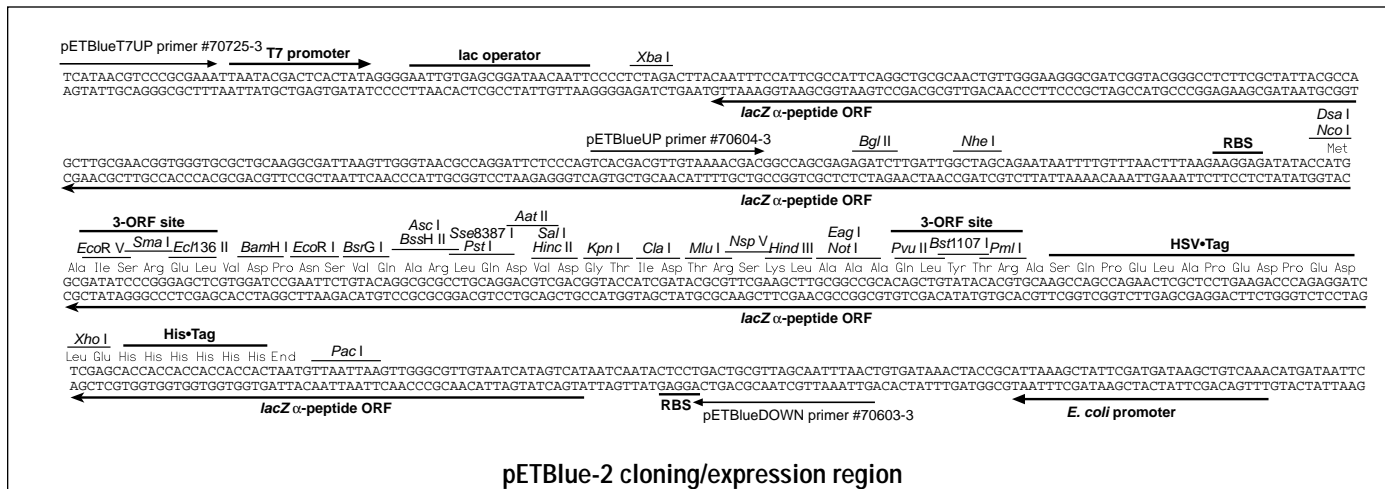
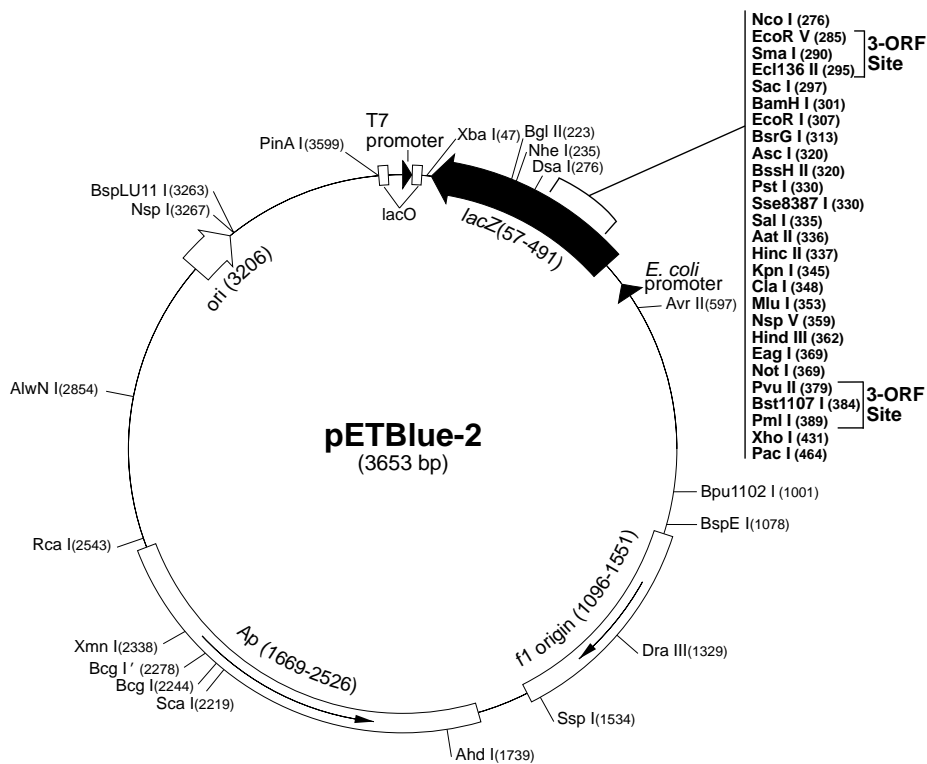


The pETBlue™ vectors are designed to identify recombinants by traditional blue/white screening while also allowing T7lac promoter based expression of target genes. Screening is independent of expression because the T7lac expression promoter is in an opposed orientation relative to the *E. coli* promoter that mediates blue/white screening. pETBlue-2 defines the open reading frame and inserts must be cloned in-frame if expression is desired. The vector features an expanded multiple cloning region (MCS) and optional C-terminal HSV•Tag® and His•Tag® sequences. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The sequence is numbered from the first base of the T7 promoter sequence. Unique sites are shown on the circle map. The f1 origin in pETBlue-2 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 pETBlueDOWN primer (Cat. No. 70603-3).

pETBlue-2 sequence landmarks

<i>lac</i> operator	3606–3625
T7 promoter	1–17
<i>lac</i> operator	22–42
T7 transcription start	18
multiple cloning region (<i>Nco</i> I– <i>Pac</i> I)	276–467
His•Tag® coding sequence	437–454
HSV•Tag® coding sequence	395–430
<i>lacZ</i> start codon	491
<i>lacZ</i> α-peptide ORF	57–491
<i>E. coli</i> promoter	541–569
f1 origin	1096–1551
<i>bla</i> coding sequence	1669–2526
pUC origin	3206



pETBlue-2 Restriction Sites

Enzyme # Sites Locations	Enzyme # Sites Locations	Enzyme # Sites Locations
AatII 1 336	EcoRII 7 177 620 730 898 3102	Tsp45I 6 191 1148 1995 2206 3430
AccI 2 336 383	3115 3236	3628
AcII 41	EcoRV 1 285	Tsp509I 18
AfIII 3 353 386 3263	FauI 6 866 893 1118 1187 3415	TspRI 9 1687 1792 2139 2166 2584
AhdI 1 1739	3652	2855 2868 3374 3435
AluI 16	Fnu4HI 25	VspI 1 1911
AlwI 11	FspI 2 82 1961	XbaI 1 47
Alw26I 2 709 1800	HaeII 6 688 1171 1179 3023 3540	XhoI 1 431
AlwNI 1 2854	3617	XmnI 1 2338
ApaI 2 2406 2949	HaeIII 20	
ApoI 4 307 614 1509 1520	HgaI 5 890 1104 2265 2573 3151	Enzyme that do not cut pETBlue-2:
AscI 1 320	HhaI 24	AfIII Apal BclI Bpu10I BsaBI BseRI
AvaI 2 288 431	HincII 1 337	BsgI BsmI BsmBI BspMI BstEII BstXI
Avall 5 641 1877 2099 3425 3513	HindIII 1 362	Bsu36I EcoNI FseI HpaI MscI MunI
AvrII 1 597	Hinfl 9 7 182 1374 1396 1734	NarI NdeI Nrul Nsil PfiI PmeI
BamHI 1 301	2893 3289 3364 3460	PshAI Psp5II RsrII SacII SanDI SapI
BanI 4 341 1285 1687 3545	HphI 8 810 1326 1582 1809 2225	SexAI SfiI SgfI SgrAI SnaBI SpeI
BanII 2 297 1255	2431 2466 3614	SphI SrfI StuI SunI SwaI Tth111I
BbsI 3 423 591 3581	KpnI 1 345	UbaEI XcmI
BbvI 12	MaeIII 14	
BcgI 1 2244	MbolI 10 107 428 596 1187 1579	
BcgI' 1 2278	2334 2412 2521 2606 3586	
Bfal 8 48 236 598 1012 1173	MluI 1 353	
1594 1929 2770	MnlI 18	
BglI 2 75 1859	MseI 23	
BgIII 1 223	MslI 3 1991 2150 2509	
Bpml 2 1809 3485	MspI 17	
Bpu1102I 1 1001	MspA1I 5 379 1000 2374 2680 2925	
Bsal 2 709 1800	MwoI 15	
BsaAI 2 389 1326	NciI 7 289 290 830 863 1922	
BsaHI 3 333 882 2276	2273 2886	
BsaJI 5 276 288 597 1023 3103	NcoI 1 276	
BsaWI 5 1078 2030 2910 3057 3599	NgoAIV 3 1221 3382 3542	
BsiEI 7 103 372 2109 2258 2929	NheI 1 235	
3353 3600	NlaIII 12	
BsiHKAII 5 297 438 2325 2410 2953	NlaIV 15	
BsII 8 869 1037 1107 1433 2785	NotI 1 369	
3064 3230 3248	NspI 1 3267	
BsmFI 4 627 1107 3438 3627	NspV 1 359	
Bsp1286I 6 297 438 1255 2325	Pacl 1 464	
2410 2953	PinAI 1 3599	
BspEI 1 1078	PleI 6 1 1382 1390 1728 2887	
BspLU11I 1 3263	3372	
BsrI 12	PmlI 1 389	
BsrBI 4 30 835 1182 3334	Psp1406I 5 845 1539 1965 2338	
BsrDI 2 1800 1974	3412	
BsrFI 5 1221 1819 3382 3542 3599	PstI 1 330	
BsrGI 1 313	PvuI 2 103 2109	
BssHII 1 320	PvuII 1 379	
BssSI 4 296 2403 3090 3626	RcaI 1 2543	
Bst1107I 1 384	RsaI 4 107 315 343 2219	
BstYI 9 223 301 427 1584 1596	SacI 1 297	
2364 2381 2611 2622	Sall 1 335	
Cac8I 24	Sau3AI 18	
Clal 1 348	Sau96I 13	
CviJI 58	Scal 1 2219	
Ddel 8 665 755 811 1001 1696	ScrFI 14	
2236 2580 2989	SfaNI 6 744 912 1995 2186 2435	
Dpnl 18	3166	
Dral 3 1605 1624 2316	Sfcl 6 13 326 1103 1980 2807	
DraIII 1 1329	2998	
DrdI 2 1373 3161	Smal 1 290	
Dsal 1 276	Sse8387I 1 330	
Ecl136II 1 295	Sspl 1 1534	
EaeI 3 212 369 2127	StyI 3 276 597 1023	
EagI 1 369	Tail 14	
EarI 2 120 2534	TaqI 9 336 348 359 432 552	
Eco47III 2 3538 3615	760 1291 2390 3165	
Eco57I 3 435 2406 2721	TfiI 3 182 3289 3460	
EcoO109I 2 594 1028	Thal 13	
EcoRI 1 307	Tsel 12	