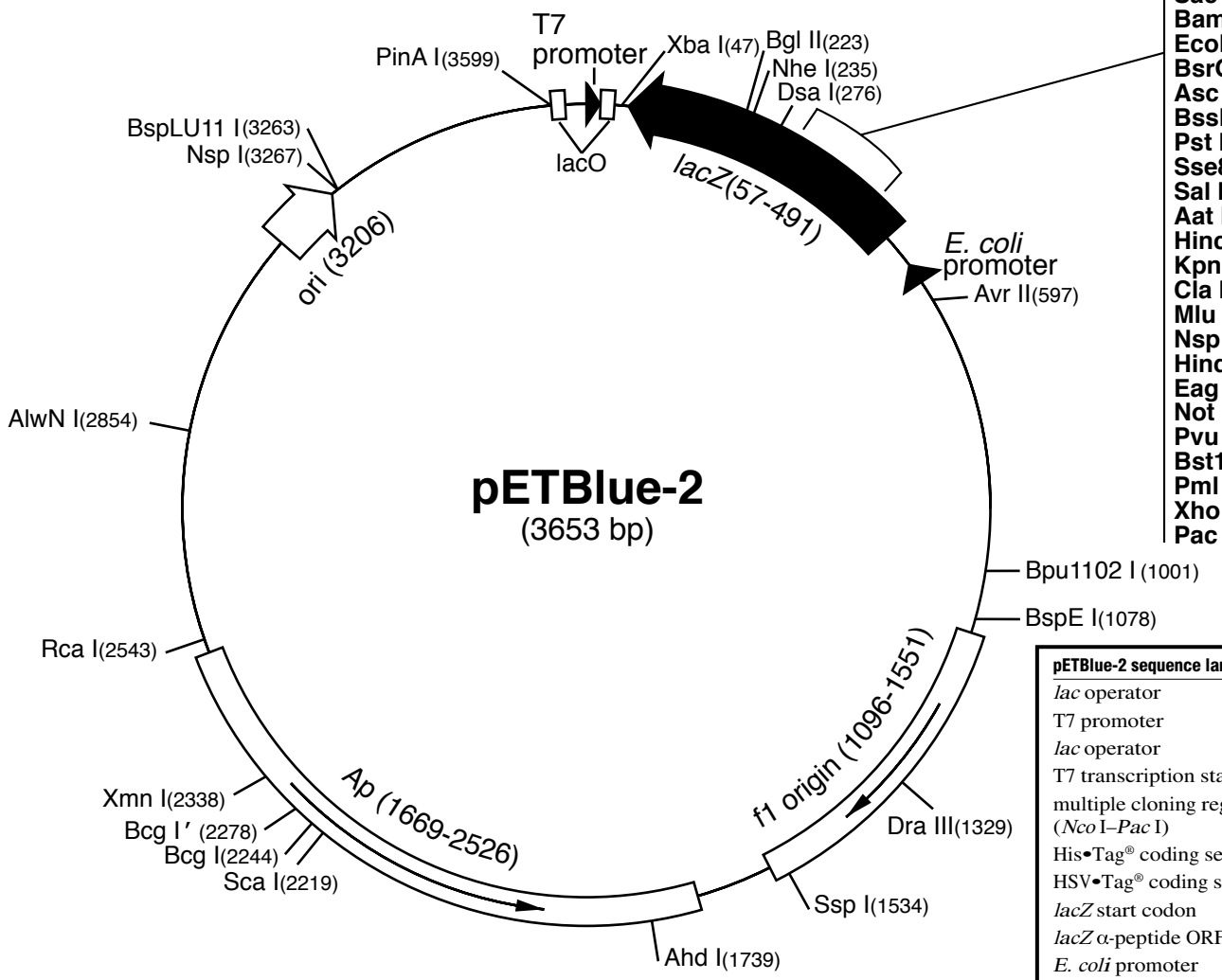
pETBlue-2 Vectors



Nco I (276)
EcoR V (285)
Sma I (290)
Ecl136 II (295)
3-ORF Site
Sac I (297)
BamH I (301)
EcoR I (307)
BsrG I (313)
Asc I (320)
BssH II (320)
Pst I (330)
Sse8387 I (330)
Sal I (335)
Aat II (336)
Hinc II (337)
Kpn I (345)
Cla I (348)
Mlu I (353)
Nsp V (359)
Hind III (362)
Eag I (369)
Not I (369)
Pvu II (379)
Bst1107 I (384)
Pml I (389)
3-ORF Site
Xho I (431)
Pac I (464)

pETBlue-2 sequence landmarks	
lac operator	3606–3625
T7 promoter	1–17
lac operator	22–42
T7 transcription start	18
multiple cloning region (*Nco* I–*Pac* I)	276–467
His•Tag® coding sequence	437–454
HSV•Tag® coding sequence	395–430
lacZ start codon	491
lacZ α-peptide ORF	57–491
E. coli promoter	541–569
f1 origin	1096–1551
bla coding sequence	1669–2526
pUC origin	3206

pETBlue-2 cloning/expression region

pETBlueT7UP primer #70725-3 — **T7 promoter** — **lac operator** — *Xba* I

```
TCATAACGTCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGACTTACAATTTCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTACGGGCCTCTTCGCTATTACGCCA
AGTATTGCAGGGCGCTTTAATTATGCTGAGTGATATCCCCTTAACACTCGCCTATTGTTAAGGGGAGATCTGAATGTTAAAGGTAAGCGGTAAGTCCGACGCGTTGACAACCCTTCCCGCTAGCCATGCCCGGAGAAGCGATAATGCGGT
```

lacZ α-peptide ORF

pETBlueUP primer #70604-3 — *Bgl* II — *Nhe* I — **RBS** — *Dsa* I — *Nco* I — Met

```
GCTTGCGAACGGTGGGTGCGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGATTCTCCCAGTCACGACGTTGTAAAACGACGGCCAGCGAGAGATCTTGATTGGCTAGCAGAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATG
CGAACGCTTGCCACCCACGCGACGTTCCGCTAATTCAACCCATTGCGGTCCTAAGAGGGTCAGTGCTGCAACATTTTGCTGCCGGTCGCTCTCTAGAACTAACCGATCGTCTTATTAAAACAAATTGAAATTCTTCCTCTATATGGTAC
```

lacZ α-peptide ORF

3-ORF site: *Eco*R V, *Sma* I, *Ecl*136 II — *Bam*H I, *Eco*R I, *Bsr*G I — *Asc* I, *Bss*H II, *Sse*8387 I, *Pst* I, *Aat* II, *Sal* I, *Hinc* II — *Kpn* I — *Cla* I — *Mlu* I, *Nsp* V, *Hind* III, *Eag* I, *Not* I — **3-ORF site**: *Pvu* II, *Bst*1107 I, *Pml* I — **HSV•Tag**

```
Ala Ile Ser Arg Glu Leu Val Asp Pro Asn Ser Val Gln Ala Arg Leu Gln Asp Val Asp Gly Thr Ile Asp Thr Arg Ser Lys Leu Ala Ala Ala Gln Leu Tyr Thr Arg Ala Ser Gln Pro Glu Leu Ala Pro Glu Asp Pro Glu Asp
GCGATATCCCGGGAGCTCGTGGATCCGAATTCTGTACAGGCGCGCCTGCAGGACGTCGACGGTACCATCGATACGCGTTCGAAGCTTGCGGCCGCACAGCTGTATACACGTGCAAGCCAGCCAGAACTCGCTCCTGAAGACCCAGAGGATC
CGCTATAGGGCCCTCGAGCACCTAGGCTTAAGACATGTCCGCGCGGACGTCCTGCAGCTGCCATGGTAGCTATGCGCAAGCTTCGAACGCCGGCGTGTCGACATATGTGCACGTTCGGTCGGTCTTGAGCGAGGACTTCTGGGTCTCCTAG
```

lacZ α-peptide ORF

Xho I — **His•Tag** — *Pac* I

```
Leu Glu His His His His His His End
TCGAGCACCACCACCACCACCACTAATGTTAATTAAGTTGGGCGTTGTAATCATAGTCATAATCAATACTCCTGACTGCGTTAGCAATTTAACTGTGATAAACTACCGCATTAAAGCTATTCGATGATAAGCTGTCAAACATGATAATTC
AGCTCGTGGTGGTGGTGGTGGTGATTACAATTAATTCAACCCGCAACATTAGTATCAGTATTAGTTATGAGGACTGACGCAATCGTTAAATTGACACTATTTGATGGCGTAATTTCGATAAGCTACTATTCGACAGTTTGTACTATTAAG
```

lacZ α-peptide ORF — **RBS** — pETBlueDOWN primer #70603-3 — ***E. coli* promoter**

HCAII gene sequence

5' C**ATG**GCCCATCACTGGGGGTACGGCA ... rest of *hca2*... CAGGCAAATCAAAGCTTCCTTCAAA**TAA** 3'

START — **STOP**