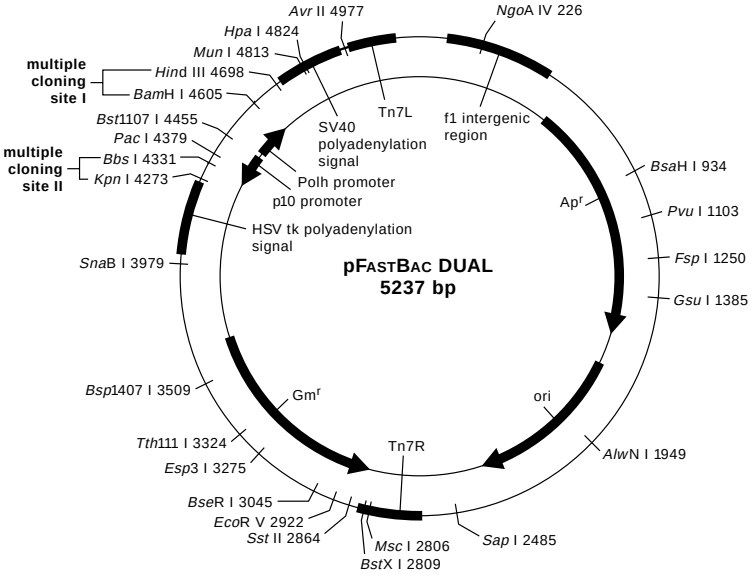

pFastBac DUAL polyhedrin promoter region and MCS I:

```
                                                  4515
                                                   |      +1
                                                   |     ►
5´ [polyhedrin promoter] - -AAATAAGTAT TTTACTGTTT TCGTAACAGT TTTGTAATAA AAAAACCTAT AAATATTCCG GATTATTCAT
```

```
                BamH I    Rsr II      BssH II     EcoR I      Stu I       Sal I     Sst I     Spe I      Not I          Nsp V   Xba I
                  |         |           |           |           |          |         |         |          |              |       |
ACCGTCCCAC CATCGGGCGC GGATCCCGGT CCGAAGCGCG CGGAATTCAA AGGCCTACGT CGACGAGCTC ACTAGTCGCG GCCGCTTTCG AATCTAGAGC
```

```
Pst I                Hind III
  |                     |
CTGCAGTCTC GACAAGCTTG TCGAGAAGTA C**TAG**AGGATC A**TAA**TC 3´
```

+1 corresponds to the transcriptional start for the polyhedrin promoter.
ATT corresponds to the original translational start codon. The ATG was mutated to an ATT.
An in-frame ATG codon must be provided by the cloned gene to initiate translation.
Stop codons are shown in bold.

pFastBac DUAL p10 promoter region and MCS II:

```
                                                  4417
                                                   |      +1
                                                   |     ►
5´ [p10 promoter] - AAA TAAGAATTAT TATCAAATCA TTTGTATATT AATTAAAATA CTATACTGTA AATTACATTT TATTTACAAT
```

```
          Bbs I          Sma I      Xho I      Nco I      Nhe I     Pvu II     Nsi I      Sph I     Kpn I
            |              |          |          |          |         |          |          |         |
CACTCGACGA AGACTTGATC ACCCGGGATC TCGAGCCATG GTGCTAGCAG CTGATGCATA GCATGCGGTA CCGGGAGATG GGGGAGGC**TA ACTGA**ACAC 3´
```

+1 corresponds to the transcriptional start for the p10 promoter and corresponds to position 4420 on the map.
Digestion at the *Bbs* I site generates a *Bam*H I compatible overhang. An in-frame ATG codon must be provided by the cloned gene to initiate translation when the *Bbs* I, *Sma* I or *Xho* I sites are used for cloning. When cloning into the *Nco* I site, or sites downstream of the *Nco* I site, make sure the reading frame of the cloned gene is in frame relative to the ATG sequence of the *Nco* I site.
Stop codons are shown in bold.

Restriction endonucleases that do not cleave pFastBac DUAL DNA.

Aat II	*Asc* I	*Bsp*M I	*Cvn* I	*Eco*O109 I	*Nde* I	*Pin*A I	*Sex*A I	*Srf* I
Afl II	*Bpu*1102 I	*Bst*E II	*Eco*72 I	*Mlu* I	*Nru* I	*Pme* I	*Sfi* I	*Sse*8387 I
Apa I	*Bsg* I	*Cla* I	*Eco*N I	*Nar* I	*Pfl*M I	*Psp*5 II	*Sgr*A I	*Sun* II

Restriction endonucleases that cleave pFastBac DUAL DNA twice:

Afl III	2363	3345	*Bsp*LU 11	2363	3345	*Tfi* I	2389	4674
Bcl I	4324	4962	*Bss*S I	806	2190	*Xmn* I	871	3896
Bgl II	2646	3116	*Dra* III	329	3677			
Bsm I	4808	4907	*Sca* I	992	4711			

Figure 10. Map and restriction sites for pFastBac DUAL expression vector. Restriction endonucleases that cleave pFastBac DUAL once are shown on the outer circle. The nucleotide position refers to the 5´ base of the recognition sequence.

The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector. The sequence and the location of sites for restriction endonucleases that cleave up to 10 times can be found in the Tech-OnLine℠ section of Life Technologies' web page, http://www.lifetech.com.