Figure 8. Map and restriction endonuclease sites for pFASTBAC HT expression vectors. The circle map of pFASTBAC HTb (4856 bp) is presented. A similar map can be drawn for pFASTBAC HTa (4855 bp) and pFASTBAC HTc (4857 bp) except that the sites after the *Bam*H I site are shifted by -1 and +1 bases respectively. Restriction endonucleases that cleave pFASTBAC HTb once are shown on the outer circle. The nucleotide position refers to the 5′ base of the recognition sequence.

Restriction endonucleases that do not cleave pFASTBAC HT DNAs:

Aat II	*Bpu*1102 I	*Bst*E II	*Eco*72 I	*Nhe* I	*Pfl*M I	*Pvu* II	*Sma* I	*Sun* II
Afl II	*Bsg* I	*Cla* I	*Eco*O109 I	*Nru* I	*Pin*A I	*Sex*A I	*Srf* I	*Swa* I
Apa I	*Bsp*M I	*Cvn* I	*Mlu* I	*Nsi* I	*Pme* I	*Sfi* I	*Sse* I	*Xcm* I
Asc I	*Bss*H II	*Eco*47 III	*Nde* I	*Pac* I	*Psp*5 II	*Sgr*A I	*Sse*8387 I	

Restriction endonucleases that cleave pFASTBAC HTa DNA twice:

Acc I	3882	4150	*Bsa*H I	835	4119	*Eco*R V	2823	4086
Afl III	2264	3246	*Bsm* I	4327	4426	*Rca* I	536	1544
Ban II	157	4156	*Bsm*F I	4015	4855	*Sca* I	893	4230
Bgl II	2547	3017	*Dra* III	230	3578	*Tfi* I	2290	4181
Bsa I	1304	3661	*Eam*1105 I	1371	4731	*Xmn* I	772	3797

Restriction endonucleases that cleave pFASTBAC HTb DNA twice:

Acc I	3882	4151	*Bsa*H I	835	4119	*Rca* I	536	1544
Afl III	2264	3246	*Bsm* I	4328	4427	*Sca* I	893	4231
Ban II	157	4157	*Bsm*F I	4015	4856	*Tfi* I	2290	4182
Bgl II	2547	3017	*Dra* III	230	3578	*Xmn* I	772	3797
Bsa I	1304	3661	*Eam*1105 I	1371	4732			

Restriction endonucleases that cleave pFASTBAC HTc DNA twice:

Acc I	3882	4152	*Bsm* I	4329	4428	*Rca* I	536	1544
Afl III	2264	3246	*Bsm*F I	4015	4857	*Sca* I	893	4232
Ban II	157	4158	*Dra* III	230	3578	*Tfi* I	2290	4183
Bsa I	1304	3661	*Eam*1105 I	1371	4733	*Xmn* I	772	3797
*Bsa*H I	835	4119	*Eco*R V	2823	4086			

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.

pFASTBAC HTa

Rsr II

CTCGGTCCGAAACC ATG TCG TAC TAC CAT CAC CAT CAC CAT CAC GAT TAC GAT ATC CCA ACG ACC GAA AAC
met ser tyr tyr his his his his his his asp tyr asp ile pro thr thr glu asn
6x histidine affinity tag   spacer region   rTEV

Ehe I   *Nco* I *Bam*H I *Eco*R I   *Stu* I   *Sal* I   *Sst* I   *Spe* I
CTG TAT TTT CAG** GGC GCC ATG GAT CCG GAA TTC AAA GGC CTA CGT CGA CGA GCT CAA CTA GTG CGG
leu tyr phe gln gly ala met asp pro glu phe lys gly leu arg arg arg ala glu leu val arg
protease cleavage site

Not I   *Nsp* V   *Xba* I   *Pxt* I   *Xho* I   *Sph* I   *Kpn* I   *Hin*d III
CCG CTT TCG AAT CTA GAG CCT GCA GTC TCG AGG CAT GCG GTA CCA AGC TTG TCG AGA AGT ACT AGA
pro leu ser asp leu glu pro ala val ser arg his ala val pro ser leu ser arg ser thr arg

GGA TCA *TAA* TCA GCC ATA
gly ser stop

pFASTBAC HTb

Rsr II

CTCGGTCCGAAACC ATG TCG TAC TAC CAT CAC CAT CAC CAT CAC GAT TAC GAT ATC CCA ACG ACC GAA AAC
met ser tyr tyr his his his his his his asp tyr asp ile pro thr thr glu asn
6x histidine affinity tag   spacer region   rTEV

Ehe I   *Nco* I *Bam*H I *Eco*R I   *Stu* I   *Sal* I   *Sst* I   *Spe* I
CTG TAT TTT CAG** GGC GCC ATG **G**GA TCC GGA ATT CAA AGG CCT ACG TCG ACG AGC TCA CTA GTC GCG
leu tyr phe gln gly ala met gly ser gly ile glu arg pro thr ser thr ser ser leu val ala
protease cleavage site

Not I   *Nsp* V   *Xba* I   *Pst* I   *Xho* I   *Sph* I   *Kpn* I   *Hin*d III
GCC GCT TTC GAA TCT AGA GCC TGC AGT CTC GAG GCA TGC GGT ACC AAG CTT GTC GAG AAG TAC *TAG*
ala ala phe glu ser arg ala cys ser leu glu ala cys gly thr lys leu val glu lys tyr stop

AGG ATC ATA ATC

pFASTBAC HTc

Rsr II

CTCGGTCCGAAACC ATG TCG TAC TAC CAT CAC CAT CAC CAT CAC GAT TAC GAT ATC CCA ACG ACC GAA AAC
met ser tyr tyr his his his his his his asp tyr asp ile pro thr thr glu asn
6x histidine affinity tag   spacer region   rTEV

Ehe I   *Nco* I *Bam*H I *Eco*R I   *Stu* I   *Sal* I   *Sst* I   *Spe* I
CTG TAT TTT CAG** GGC GCC ATG **GG**G ATC CGG AAT TCA AAG GCC TAC GTC GAC GAG CTC ACT AGT CGC
leu tyr phe gln gly ala met gly ile arg asn ser lys ala tyr leu asp glu leu thr ser arg
protease cleavage site

Not I   *Nsp* V   *Xba* I   *Pst* I   *Xho* I   *Sph* I   *Kpn* I   *Hin*d III
GGC CGC TTT CGA ATC *TAG* AGC CTG CAG TCT CGA GGC ATG CGG TAC CAA GCT TGT CGA GAA GTA CTA
gly arg phe arg ile stop

Figure 9. Multiple cloning site sequences of pFASTBAC HT expression vectors. The multiple cloning sites (MCS) for the three vectors are shown above. The sequence for the 6x histidine affinity tag, spacer region and rTEV protease cleavage site are underlined. The cleavage with rTEV protease occurs between the gln and gly and is signified by **. The shift in reading frame occurs at the *Bam*H I site in each vector. The added base(s) are shown in bold. The stop codon for each vector is underlined and italicized. In pFASTBAC HTc the stop codon is within the MCS at the *Xba* I site. The 5' end of a gene must be cloned upstream of the *Xba* I site in pFASTBAC HTc to be translated.