Product datasheet

pCANTAB 5 E

Catalog # MD020

Product information

The map of **pCANTAB 5 E**, presented below, shows the control regions and genealogy of the vector. The section above the vector map (V_H-Linker-V_L) depicts the orientation of a hypothetical ScFv fragment cloned into **pCANTAB 5 E**. Detailed sequence analysis of the vector follows. The sequence of **pCANTAB 5 E** was assembled from the sequences of its constituents. The restriction analysis was compiled using DNASIS ™ software. The enzymes chosen for the analysis are those which we believe to have been commercially available in June, 1992. **pCANTAB 5 E** has not been tested with all of these enzymes, and therefore the accuracy of the tables cannot be guaranteed. Please contact us if a discrepancy is identified.

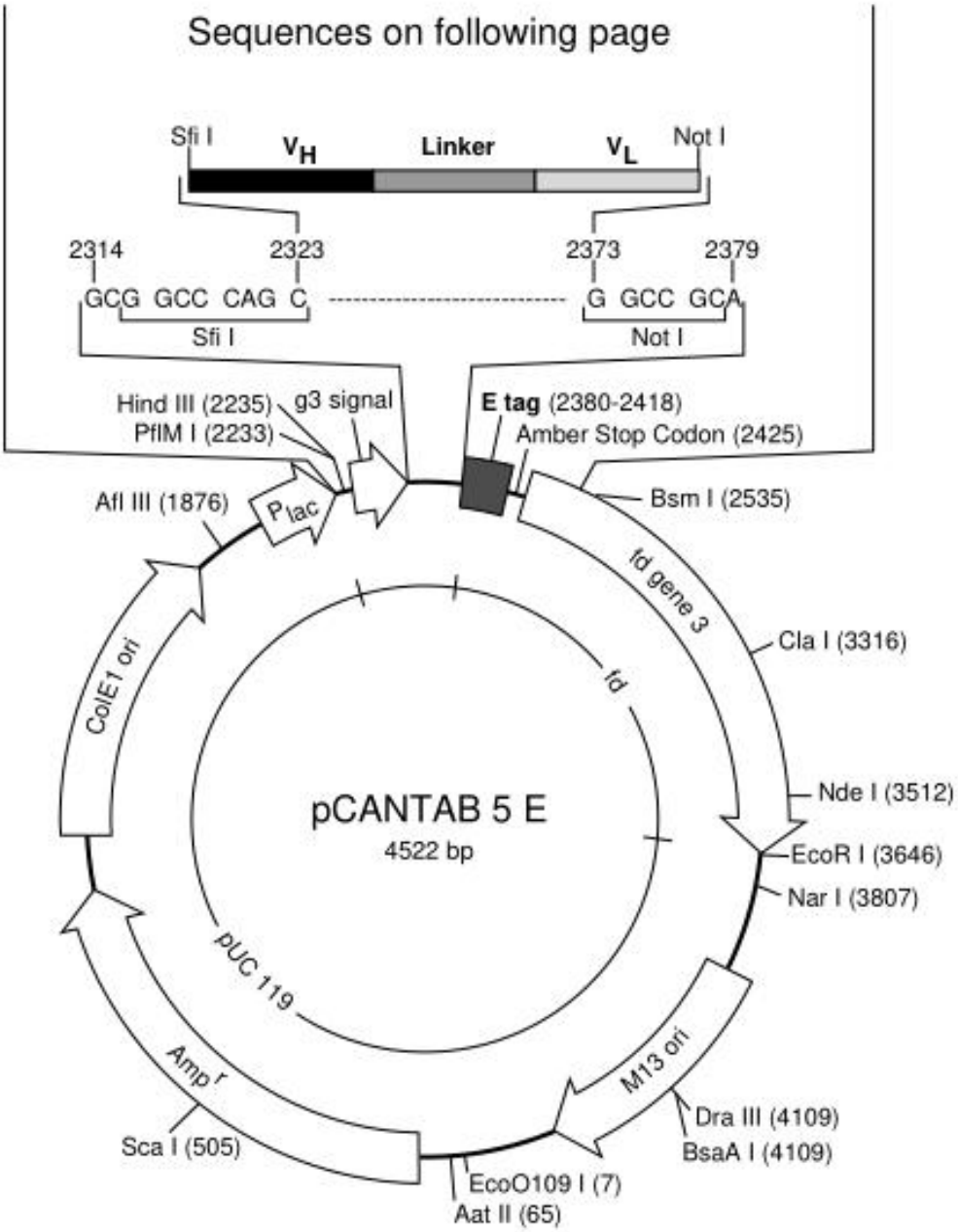
Control Regions

Expression control region: *lac* promoter: -35: 2144-2149; -10: 2168-2174;
Operator: 2180-2200

Gene 3 signal sequence:2269-2313; protein synthesis begins at the start of the gene 3 signal sequence (with GTG at position 2269)

Gene 3 protein:2428-3639 (two stop codons follow at bases 3640-3645)
M13 region:3863-4336

M13 origin of replication:4173 (base 4174 is the first base of the newly synthesized strand)


Sequences on following page
Sfi I
V_H
Linker
V_L
Not I
2314
2323
2373
2379
GCG GCC CAG C
G GCC GCA
Sfi I
Not I
Hind III (2235)
PflM I (2233)
g3 signal
E tag (2380-2418)
Amber Stop Codon (2425)
AfI III (1876)
P_{lac}
Bsm I (2535)
fd gene 3
Cla I (3316)
ColE1 ori
fd
pCANTAB 5 E
4522 bp
Nde I (3512)
EcoR I (3646)
Nar I (3807)
pUC 119
M13 ori
Amp r
Dra III (4109)
BsaA I (4109)
Sca I (505)
EcoO109 I (7)
Aat II (65)

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βlactamase gene region: Promoter: -35: 131-136; -10: 154-159; Start codon(ATG): 201; Stop codon (TAA): 1059

Plasmid origin of replication: 1819

Amber Stop Codon: 2425

E Tag: 2380-2418; **E Tag Sequence:** GGT GCG CCG GTG CCG TAT CCG GAT CCG CTG GAA CCG CGT

Restriction Enzyme Analysis

No sites: *Acc*65 I, *Afl* II, *Age* I, *Apa* I, *Apa*L I, *Asc* I, *Asu* II, Ava III, Avr II, Bal I, BbrP I, Bbs I, Bcl I, Bfr I, Bgl II, Bpu1102 I, BsaB I, Bsg I, BsiW I, BssH II, Bst1107 I, BstB I, BstE II, BstX I, Bsu36 I, Ecl136 II, Eco47 III, EcoN I, EcoR V, Esp I, Hpa I, Kpn I, Mam I, Mlu I, Msc I, Mun I, Nhe I, Nru I, Nsi I, Pac I, Pme I, Pml I, PpuM I, Rsr II, Sac I, Sac II, Sau I, Sce I, Sma I, SnaB I, Spe I, Sph I, Spl I, Spo I, Srf I, Sse8387 I, Stu I, Swa I, Tth111 I, Xba I, Xcm I, Xma I

One site: Aat II (65), Acc I (2350), Afl III (1876), Ban II (4036), Bcg I (461), Bpm I (898), Bsa I (916), BsaA I (4109), BseA I (2397), Bsm I (2535), BspD I (3316), BspE I (2397), BspM II (2397), Cla I (3316), Dra II (7), Dra III (4109), Eag I (2372), Eam1105 I (983), EcoO109 I (7), EcoR I (3646), Hinc II (2350), Hind II (2350), Hind III (2235), Kas I (3807), Nar I (3807), Nco I (2327), Nde I (3512), Not I (2371), PaeR7 I (2356), PflM I (2233), Pst I (2344), Sal I (2350), Sca I (505), Sfi I (2316), SgrA I (2384), Sty I (2327), Xho I (2356)

Two sites: AlwN I (1462, 2976), Ava I (2356, 4218), BamH I (2400, 3009), Dsa I (2327, 3552), Eco57 I (301, 1349), Esp3 I (4467, 4516), Fsp I (763, 3786), Nae I (2322, 4006), NgoM I (2322, 4006), Nsp I (1876, 4481), Pvu I (616, 3766), Pvu II (2054, 3736), Xmn I (384, 3435)

pCANTAB 5 E, as supplied, has a 49-bp region removed by Sfi I/Not I digestion. The following restriction sites are affected. The numbers in parentheses indicate the location of the site(s) remaining.

One-site enzymes eliminated: Acc I, Eag I, Hinc II, Hind II, Nco I, PaeR7 I, Pst I, Sal I, Sty I, Xho I

Two-site enzymes become one-site: Ava I (4218), Dsa I (3552), Nae I (4006), NgoM I (4006)

Three-site enzymes become two-site: Ava II (624, 846), Bgl I (864, 3792), BspM I (2369, 3047), Sin I (624, 846)

Additional Restriction Sites added by Linker Primers and 3'V_H Primer

The linker primers consist of two 93-base oligonucleotides which are complementary to each other and have homology with the 3'-end of the V_H gene and the 5'-end of the V_L gene. Twenty-four bases on either end of the linkers are complementary to the ends of the V_H and the V_L . The central 45 bases of the linkers encode the flexible (Gly 4 Ser) 3 linker which joins the V_H and the V_L to form an ScFv fragment. In addition to restriction sites encoded by the sequence of pCANTAB 5 Eitself, numerous restriction sites are encoded by the sequence of the 93-base linkers and an additional 8 bases upstream of the linker which are encoded by the 3' V_H primer.

Sites present (numbers in parentheses indicate number of recognition sites present): Aci I (4), Alu I (1), Alw I (1), Asu I (1), Ban I (1), Ban II (1), BsaJ I (2), BsmA I (2), Bsp1286 I (1), BspW I (2), BstE II (1), Bsu36 I (1), Dde I (2), Dpn I (1), Dpn II (1), Dsa I (1), Ecl136 II (1), Esp3 I (1), Hae III (1), HgiA I (1), Hph I (1), Mae III (1), Mbo I (1), Mnl I (3), Nla IV (2), Sac I (1), Sau I (1), Sau3A I (1), Sau96 I (1), Sdu I (1), Sec I (2), Sty I (1), Taq I (1)