



pCJW93

pCJW93 was constructed in three stages but the overall effect was to take the majority of pIJ6021 from the *Ecl*136II site upstream of *t_o* to the *Sph*I site downstream of *ts^f*, the thiostrepton resistance gene and clone into pSET 152 cut with *Pvu*II and *Sph*I, thereby removing the *lacZ α* , integrase and *attP*.

The plasmid confers apramycin resistance on *E. coli* and *Streptomyces*.

The 6xHis tag is N-terminal; genes are cloned in *Nde*I to one of the unique sites: *Xba*I, *Hind*III, *Bam*HI, *Eco*RI and *Bgl*II.

We tested the plasmid by inserting the act KS+CLF (pCJW94); transforming *S. coelicolor* CH999 by conjugation with *E. coli* ET12567[pUB307][pCJW94]; overexpressing the two genes; and batch purification of the two proteins on nickel resin.

The problem is that any gene above ~3kb does not seem to insert. Genes below that size have worked fine so far.

The plasmid map is quite rough: don't take the numbers as gospel!

The His tag is the same as in pET28b(+). The end of *PtipA* now has the sequence:

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PtipA   TCA GAG AAG GGA GCG GAC CAT AC
ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC AGC GGC
M   G   S   S   H   H   H   H   H   H   S   S   G
CTG GTG CCG CGC GGC AGC CAT ATG ... polylinker
L   V   P   R   G   S   H   M
thrombin site                               NdeI
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