Vectors for low copy transformation of *C.elegans*

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The amber suppressor gene *sup-7* has been used as a selectable marker to introduce cloned DNA at low copy number into *C. elegans* chromosomes (1). We describe structure and several characteristics of seven new *sup-7* containing transformation vectors.

All of the vectors were derived from plasmid pAST(1) and standard cloning vectors. A 366 base *sup-7* segment was inserted into the SspI sites of pUC18 and pUC19 (2) to generate pAST18a, 18b, 19a, and 19b. pPD26.14 is a pAST19b derivative which has a novel polylinker and lacks *lacα* sequences. pICT19h and 19r have *sup-7* in the midst of polylinker regions from pIC19h and pIC19r (3), allowing convenient excision of *sup-7* segments for incorporation into existing plasmids.

The *sup-7* segment in each vector is functional, as evidenced by transient suppression of *tra-3(e1107am)* following germ line injection (1). Derivatives of pAST19b, pAST18b and pICT19r have also been successfully used for integrative transformation [Ikue Mori, Dennis Dixon, A.F. and R.W., unpublished; 4].

Given the variability in expression patterns for genetically isolated amber suppressors (5), it might be expected that expression patterns of the reintroduced suppressor loci might differ both from the original *sup-7* locus and from each other. Such differences indeed occur and should be taken into account when designing experiments with *sup-7* vectors. **1)** As previously noted, *sup-7* expression levels vary between transformed lines made with any single *sup-7* plasmid (1). **2)** The immediate context of *sup-7* in plasmid constructs can have distinct effects on expression. The *sup-7* genomic segment used in the vectors extends only 22 bp upstream of the gene, with several different nematode and plasmid segments placed adjacent. We found one sequence (near the *myo-3* gene), which abolishes rescue of *tra-3(e1107am)* when placed at the HincII site upstream of *sup-7*. This sequence has no evident effect on *sup-7* function when placed elsewhere in the plasmid. More subtle context effects are suggested by the ranges of copy numbers and phenotypes seen in transformed lines obtained with different vectors. Copy numbers are presumably restricted by the need for *tra-3* rescue and by deleterious effects of high level *sup-7* expression. The majority of lines transformed with pAST19b derivatives have 1–2 copies of the injected plasmid, while pAST derived lines

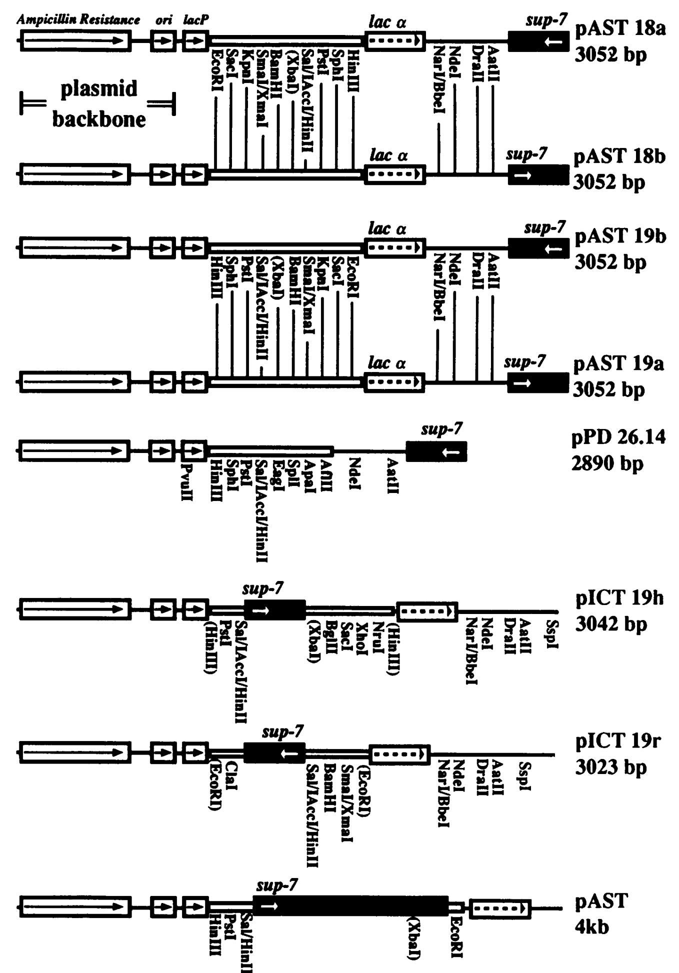

Figure 1. Structure of the new vectors (above) and original vector pAST (below). Restriction sites shown without parentheses are unique and can be used for fragment insertion. An XbaI site in the *sup-7* gene is methylated and hence resistant to cleavage after growth in Dam+ bacteria. Sequences were compiled with DNA Strider (6).

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generally have 3 − 10 copies. The different *sup-7* contexts in pAST and pAST19b also result in a phenotypic effect: strongly suppressing pAST19B derived loci cause animals to be unusually long, while similar pAST derived loci tend to shorten the animals. These observations suggest that the pAST and PAST19b context differ in both level and pattern of *sup-7* expression. **3)** The transgenic suppressor loci differ from the original *sup-7* locus in their ability to suppress different amber mutations. Three transgenic loci, derived from pAST and pAST19b and selected for suppression of *tra-3(e1107am)*, were unable to suppress *unc-13(e1091am)*. Both amber alleles are suppressed by the original chromosomal *sup-7* locus.

In order to minimize the types of context effects described we recommend use of a *tra-3(e1107am)* host and vectors such as pAST19b, in which polylinker inserts are well separated (920 bp) from *sup-7*.

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