pYAC-4 Neo, a yeast artificial chromosome vector which codes for G418 resistance in mammalian cells

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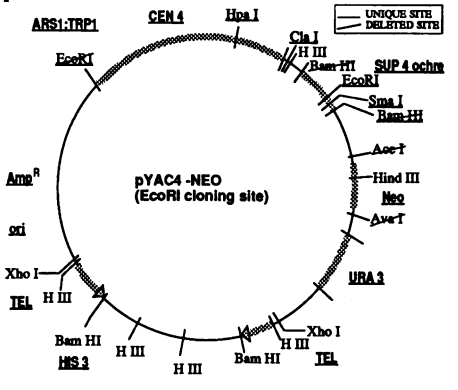
Submitted November 21, 1988

Yeast artificial chromosome vectors allow DNA molecules in the 100-1000kb range to be cloned and propagated as linear molecules in S. cerevisiae (1). The increase in the size of clonable fragments is of use in constructing physical maps of genomes and in correlating the physical and genetic maps (2). There will be many circumstances in which it is desirable to reintroduce such yeast artificial chromosomes into mammalian cells, particularly where a large functional unit is involved or suspected to exist. To facilitate the selection of cells which have taken up YACs we have constructed a yeast artificial chromosome vector which contains a neomycin resistance gene driven by SV40 72 base pair repeats.

pYAC-4-Neo is derived from pYAC-4 (1). As a plasmid in E. Coli it confers Kanamycin and Ampicillin resistance, it carries Tetrahymena telomeric sequences which can function in yeast, a yeast centromere and ARS and the yeast markers URA3 TRP1 and SUP4. In mammalian cells the pSV2Neo (3) derived neomycin gene is expressed and allows selection for G418 resistance. pYAC-4-Neo was constructed by filling in the Sal 1 site of the parent vector and ligating this to an Acc1- Aval fragment of pSV2Neo after filling in these sites. The orientation of the fragment from pSV2Neo in the final construct was determined by size measurements of double digestion products. Cloning sites which allow colour and positive selection of recombinants are Eco R1 and Sma 1. Yeast transformation frequencies are comparable to the parent vector.



We constructed this vector in order to be able to reintroduce YAC clones into mammalian cells for functional analysis of large fragments of DNA. Others of the YAC series of vectors can be simply adapted to give Neomycin resistance by the same approach or, in the case of YAC vectors in which the Sma 1 site is unaltered, by substitution of the Cla 1/Sma 1 fragment from such vectors into pYAC-4-Neo.

Acknowledgements

We should like to thank D.T.Burke, G.F. Carle and M.V. Olsen for YAC vectors and yeast strains.

References

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