Sequence analysis of a DNA fragment with yeast autonomously replicating sequence activity from the extrachromosomal ribosomal DNA circle of *Entamoeba histolytica*

V. Mittal, S. Ramachandran¹, D. Sehgal, A. Bhattacharya and S. Bhattacharya¹
School of Life Sciences and ¹School of Environmental Sciences, Jawaharlal Nehru University, New Delhi 110067, India

Submitted April 15, 1991

The 4.4 kb EcoRI fragment of the 25 kb extrachromosomal ribosomal DNA circle of *Entamoeba histolytica* (1, 2) contains a stretch of tandem DraI repeats (2). We have demonstrated autonomously replicating sequence (ARS) activity in this 4.4 kb EcoRI fragment by cloning it in the yeast shuttle vector, YIP5, which lacks an ARS. The resulting construct (pYHM-d) could transform *ura3 his5* *Saccharomyces cerevisiae* at high frequency (596 transformants/µg DNA and 104 transformants/µg DNA for two independent clones with the 4.4 kb insert) as against the negligible frequency (0 transformant/µg DNA) with YIP5 plasmid alone and 474 transformants/µg DNA with pl75B7, a plasmid containing yeast ARS1 and CEN4. The *Ura*⁺ yeast transformants arising from pYHM-d were not due to homologous recombination since the *Ura*⁺ phenotype was lost upon repeated subculturing (five cycles) in nonselective medium.

The sequence of one complete DraI repeat unit is presented (Fig. 1). It is 170 bp long and contains three, 12-nucleotide sequences which are completely homologous with the 11-nucleotide yeast ARS consensus sequence (A/TTTTATA/TA/G/T) except for the presence of an extra nucleotide (Fig. 2) in each. The extra nucleotide is located at a position shown to be less critical for ARS activity in yeast (3). It remains to be determined whether the DraI repeat unit does indeed serve as ARS in the extrachromosomal rDNA of *E. histolytica*.

**ACKNOWLEDGEMENTS**

This work was funded by grants from Department of Science and Technology and Council of Scientific and Industrial Research, and research fellowships from U.G.C. (V.M. and S.R.) and C.S.I.R. (D.S.).

**REFERENCES**


**Figure 1.** A single DraI repeat unit. The yeast consensus ARS sequences are underlined, with arrows to denote orientation.

**Figure 2.** The three ARS consensus sequences in the DraI repeat. For comparison with the yeast sequence, the 'extra' nucleotide in the *E. histolytica* sequence is shown above.