
Isolation and sequencing of HRS60dim1, a dimeric member of the HRS60-family of a tobacco DNA repeat

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Recently, a BamHI family of a repetitive DNA sequence was found in the nuclear DNA of *N. tabacum*, comprising ca.2% of tobacco genome (1). This DNA repeat denoted as HRS60 consists of 182-184 bp units organized in tandems. After Southern blot hybridization of HRS60 with BamHI digest of tobacco nuclear DNA, a ladder of hybridization bands appeared, corresponding to multiples of the monomeric unit. The cause of this "ladder-like" hybridization pattern might be mutation or methylation or steric inhibition (2) of some BamHI restriction sites. A dimeric member of this family was analysed. Nuclear DNA of *N. tabacum* was BamHI digested and fractionally precipitated with PEG6000. Heavy DNA fragments were removed. RNA was removed by a LiCl precipitation. The remaining DNA was fractionated using a PAG (7%) electrophoresis. The isolated dimer band was cloned into pUC19. A clone homologous to HRS60 was sequenced (3) (Fig. 1), and denoted as HRS60dim1. The dimer consists of two homologous subunits (96.7% homology). The sequence connecting the two subunits is GGATCT. It differs from the GGATCC BamHI restriction site by one base pair. Thus, point mutations are responsible for the "ladder-like" pattern of hybridization at least in the present case. Comparisons of HRS60dim1 with the other already described members of the HRS60 family (1) show homology from 90.7 to 96.7%.

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GATCCATCCG GGCCCAAGGC GGAAGGCATG GGCTATAGCA CACAAAAAATT TGGGACTCGC GCGGATTTCG
AGTTTTATGG CTGTAAAATG CCAAAAAATA TAATGTGGTC ATTTTCGAGTG GGTGGAATTT CTTTCTTATG
CCGTATTTGA TGTCCGGGAC AAATATTAGG CGATTCCTCCG ACGGATCTAT CCGGGCCCAA GCGGGAAGGC
ATGGGCTATA GCACACACAA ATTTGGGACT CGCGCGGATT TGCAGTTTTA TGGCTGTAAA ATGCCAAAAA
ATATAATGTG GTCATTTAGA GTGGGTGGAA TTTCTTTCTT ATGTCGTATT TGATGTCCGG GACAAATATT
AGCGGATTCC ACGATG
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Fig.1 Nucleotide sequence of HRS60dim1

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GATCYATCCG GGCCCAAGGY GGAAGGCATG GGCTATAGCA CACAMAAATT TGGGACTCGC GCGGATTTCG
AGYTTTATGG CTRTAAAATK CCAMAAARAAT ATWATGTGGT CATTMGRGT GGGTGSAAWT TSTTTCTTAK
GYCGTATTTG ATGTCSGRKA CAAATATTAG GCGATTCCMC GAYG
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Fig.2 Consensus nucleotide sequence of 7 members of HRS60-family

References: 1) Koukalová B. et al. (1989) *Theor Appl Genet*, in press.
 2) Kato A. et al. (1984) *Nucl Acids Res* 12, 6415-6426. 3) Maxam A.M. and Gilbert W. (1980) in *Methods in Enzymology*, Vol. 65, pp. 499-560, Academic Press New York.