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Altered activity of restriction endonuclease Mnl-I cleavage of mouse satellite DNA

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Mnl-I is reported to recognise the sequence $CCTCN_7$, cleaving after the N_7 position to produce a blunt ended fragment (1). However, we have observed that following Mnl-I cleavage of mouse satellite DNA (2), the monomeric units that were subsequently cloned uniformly lacked the N_7 base. Mouse satellite DNA is composed of tandemly repeated 234bp monomeric units, most of which contain an Mnl-I site. As shown in Fig.1, direct cloning and sequencing of the Mnl-I monomeric units revealed that the N_7 base was consistently absent from the end of the clone with the Mnl-I site (Fig. 1C, D and G). These results suggest that cleavage of mouse satellite DNA by Mnl-I occurs after the N_6, in addition to after the N_7 position. It is not clear, however, if Mnl-I removes the N_7 base from both strands simultaneously, or, cleaves to leave a single-base 5' or 3' extension that is subsequently lost during the cloning procedure. This unusual activity of Mnl-I may be related to the high AT content or methylated nature of the mouse satellite DNA.

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                                          ▼
A  Mnl-I reconition sequence      C C T C N N N N N N N
B  Mouse satellite consensus   T T C C T C G C C A T A T T T C A C
C  24 clones                   T T C C T C G C C A T A · · · ←——
D  2 clones                    T T C C T C G T C A T A · · · ←——
E  26 clones                   ——→ · · · · · · · · · · T T C A C
F  1 clone                     ——→ · · · · · · · · · · T T C T C
G  15 clones                   —→ C C T C G C C A T A · · · · · ·
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Fig.1. Comparison between (A) Mnl-I recognition sequence, (B) mouse satellite DNA consensus sequence, (C-F) 53 independent satellite DNA clones of two different orientations and (G) the opposite end of 15 clones from E.

Satellite DNA was isolated from mouse LTK^- cells by CsCl gradient centrifugation (3) and digested by Mnl-I in buffer recommended by the manufacturer or in 50 mM NaCl, 10 mM Tris (7.5), 10 mM $MgCl_2$, 1 mM DTT. Monomers were isolated from an agarose gel, cloned into the phosphotased Sma-I site of M13MP8 and sequenced (4). Direction of sequencing is shown by arrows. Sequences shown are those at the junction between the M13 vector sequence (designated by .) and the satellite DNA sequence. ▼ indicates reported cleavage site of Mnl-I.

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