RmaI, a type II restriction endonuclease from *Rhodothermus marinus* which recognizes 5' CTAG 3'

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RmaI, an isoschizomer of *MaeI* (1) has been isolated from *Rhodothermus marinus* (2). *RmaI* recognizes the palindromic sequence 5'-CTAG-3'. Like its isoschizomer *RmaI* cleaves the sequence, C/TAG, generating 5'-protruding TA-dinucleotides.

The recognition sequence of *RmaI* was determined using double digestions on pBR322- and phiX174 DNAs (figure 1, lanes 2–6 and 8–12). The cleavage patterns obtained were compared with computer-derived mapping data (3). The data predicts the sequence 5'-CTAG-3'. The sequence was also tested by digesting Lambda- and M13mp18 DNAs with *RmaI* (figure 1, lanes 13 and 14). The fragments obtained matched the computer predicted fragments that would be produced when cleaving at 5'-CTAG-3'. *RmaI* has the following number of recognition sites on these commonly used DNAs: pUC19 (4), pBR322 (5), phiX174 (3), SV40 (12), M13mp18 (5), T7 (60), Lambda (13) and Adeno2 (54).

The cleavage site of *RmaI* was determined by cleavage of primed synthesis reaction (4). M13mp19 DNA containing the recognition site of *RmaI* was used as a template. Using a M13 sequencing primer the DNA was sequenced according to Sanger *et al.* (5). In addition to the four standard reactions a fifth reaction was performed which was extended through the *RmaI* recognition site. The reaction was terminated by heat treatment and the product was cleaved with *RmaI*. The cleaved product was divided in two. The addition of Klenow to one part resulted in a band migrating with the A-band (figure 2, lane +). The cleaved product (figure 2, lane –) migrated with the C-band. Thus, the cleavage site for *RmaI* is:

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Figure 1. *RmaI* digests of DNA. Lane 2, PBR322 + *RmaI*; lane 3, (2) + *EcoRI*; lane 4, (2) + *NruI*; lane 5, (2) + *PvuII*; lane 6, (2) + *PstI*; lane 8, phiX174 + *RmaI*; lane 9, (8) + *PstI*; lane 10, (8) + *SspI*; lane 11, (8) + *NciI*; lane 12, (8) + *StuI*; lane 13, Lambda + *RmaI*; lane 14, M13mp18 + *RmaI*. Markers: Lane 1, *HindIII*-Lambda and *HaeIII*-phiX174; lane 7, *BstEII*-Lambda and *MspI*-pBR322.

Figure 2. Determination of *RmaI* cleavage positions.