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A unique type II restriction endonuclease from *Acinetobacter lwoffi* N

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A new type II restriction endonuclease, *Alw*N I, has been isolated from *Actnetobacter lwoffi* N (NEB 419). *Alw*N I recognizes the interrupted palindromic sequence 5' CAGNNNCTG 3' and cleaves between the 3' N and C to produce a 3 base 3' extension.

*Alw*N I cleaved pBR322 at one site, which was mapped to position 2900 with *EcoR* I, *Pvu* II and *Pst* I. Likewise the single M13mp18 site was mapped to position 2200 with *Bgl* II, *SnaB* I and *Bsm* I (fig.1). These positions are consistant with the sequence 5' CAGNNNCTG 3' which occurs at 2886 in pBR322 and 2187 in M13mp18. The predicted fragment sizes generated by cleavage of lambda, T7 and Adeno2 DNAs at the sequence 5' CAGNNNCTG 3' matches the observed fragment sizes from *Alw*N I digests of these DNAs (fig.1,lanes 2,3,4), from which we conclude that *Alw*N I recognizes the sequence 5' CAGNNNCTG 3'. *Alw*N I has the following number of sites in commonly used DNAs: Lambda,41; T7, 15; Adeno2, 25; M13mp18, 1; pBR322, 1; pUC19, 1; PhiX174, 0. The crude extract contained approximately 8000 units *Alw*N I per gram of cells.

The cleavage site was determined according to the method of Schildkraut, *et al.* (1) M13mp18 was the template and an appropriate primer upstream of the *Alw*N I site at 2187 was extended through the *Alw*N I site. *Alw*N I was added to the extension reaction and the cleaved product was run in the (-) lane, resulting in a single band which comigrates with the sixth base in the sequence 5' CAGTCTCTG 3'. The (+) lane indicates addition of Klenow subsequent to *Alw*NI digestion and contains a single band which comigrates with the 5' G in the sequence 5' CAGTCTCTG 3'.(fig.2) This result indicates that *Alw*NI cleaves 5' of the 3' C in the recognition sequence 5' CAGNNN / CTG 3' to produce a 3 base 3' extension.

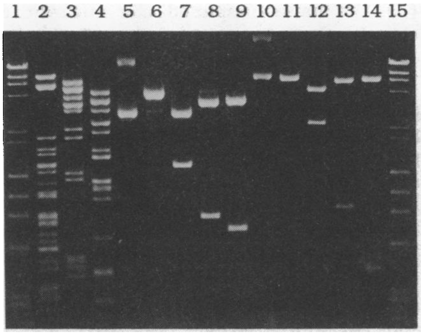

Figure 1. lane 2: lambda + AlwNI, 3: T7 + AlwNI, 4: Adeno2 + AlwNI, 5: pBR322, 6: pBR322 + AlwNI, 7: 6 + EcoRI, 8: 6 + PvuII, 9: 6 + PstI, 10: M13mp18, 11: M13mp18 + AlwNI, 12: 11 + Bgl II, 13: 11 + SnaBI, 14: 11 + BsmI, lanes 1&15: HindIII- lambda and HaeIII-PhiX174 marker.

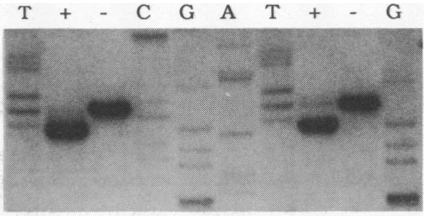

Figure 2

REFERENCES

1. Qiang, B. and Schildkraut, I. (1984) NAR 12 4507 - 4516.