Nucleotide sequence of ISH*11*, a new *Halobacterium halobium* insertion element isolated from the plasmid pGRB1

Mark P.Krebs[1,2]*, Uham L.RajBhandary[2] and Hobind G.Khorana[1,2]
Departments of [1]Biology and [2]Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Submitted October 15, 1990 EMBL accession no. X54752

We are developing vectors based on the halobacterial plasmid pGRB1 (1) for use in *H. halobium*. To identify a region that could serve as a site for cloning without affecting plasmid maintenance, 212 pGRB1 transformants of *H. halobium* R1 were screened for spontaneous insertion events in the plasmid. One derivative, pMPK29, was obtained, and the nucleotide sequence of the insert and target site was determined by dideoxy sequencing of both strands (Fig. 1). The 1068 bp insert has features typical of insertion elements, including a large open reading frame (334 aa) and a 15 bp inverted repeat at the ends of the element. A direct repeat of 7 bp flanks the element at the insertion site. No significant sequence homology was found between this and other halobacterial insertion elements, including ISH*1* (2), ISH*1.8* (3), ISH*2* (4), ISH*26* (5), ISH*50* (6), ISH*51* (7), and ISH *S1* (8). Furthermore, no correspondence was observed with the restriction enzyme maps of ISH*24* (9), ISH*27*, and ISH*28* (10) or with the limited terminal sequence available for ISH*24* (9) and ISH*27* (11). The insertion in pMPK29 therefore represents a new *H. halobium* insertion sequence, which we designate ISH*11*.

pGRB1 and related 1.8 kbp plasmids (12–15) contain an ≈ 1 kbp open reading frame and a region of four conserved hexanucleotide repeats 500–550 bp upstream. The insertion of ISH*11* in pMPK29 (position 189 of pGRB1, numbered as in (12)) does not interrupt the open reading frame or alter the spacing among the hexanucleotide repeats, consistent with a role of these regions in plasmid maintenance.

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AGCCAGTATTGGTCC  GAGGGTGTCATAGAACGAGTAGCACACCAAAGAGCAGGGTGAATGCTGAGGTGGATGAGTCCAGCGACCCTGCAGGATGA   80
TCCTTCGGTAGACTCGTTCTTCAATGTCGTGGAGACCGAGACGCTAGCGCTGTTCGAGCACCTCTCCTTCGAGTTTCTCGAAGAGTTCGACGTGTTCGCC  180
CCGGCGGAGACGGGGCGAACACGGGACCACGAACCACCAGAGCTGATGCGTGGGTTTCTCCACTGCTACTACAAGGACATCTACGGCATTCGTCCCGTTG  280
AACGAGAACTTCGGAACACGGTTGTCTGGCTGAGCTGTGGCTTCGATCGACCGCCGTCGAGAGACGCGGTCGATCGCTTCCTTACGGACCTCGAACACGT  380
CGTCAACAAGGTTTTCGACCACCTCGTTGAGCAGGCCGCCCTACGGGGCCTGCTCGATTTGACCTACTGCATTGATTCAACTGACGTGAGGGCGATGCCT  480
GCCGATCAAGATGCGTCGAAGTGCTACGATCCAACTGACGACGAGTACTACCACGGCTACGGCTGTACAATCGTCTCGACCGGGCAAAAGATCCCGATCG  580
CGGCGGAGTTCACAGAGAGTAAACAAGCGCCAGAGGAGACGGCGATGCGCGTCACGCGTGACGCGCTCGCCGTCGCCAAGCCGATCTGGATGGTCGGTGA  680
CAGCGCCTACGACACGCTTGACTGGCACGACCACCTGCTGGCCGCAGGGGTCGTGCCAGTCGCCCCATACAACGCGCGAAACACCGACGACCCGAAAGAC  780
ATCGAGTACAGGGTCGAAGACCGCATCGAACAACACAGCGAGGACGTTCAGCTGAAGCAATCCACGTTGGATGAGACGTACAACCGCCGTACTGGAGTCG  880
AACGAACCAATGAATCAGTGAAGGACTGCGGCCTCGGGCGAACGCATGCCCGAGGCCGCGTTCACGCACGAGCGCAGGTGTTCCTCGCTCTGTGCCTTCG  980
TCTCGTCGTCGCAATCACCAACTACGAACGCGGAGACAATCCGGGAAGCCCGATCATCACGGTGTGACAAGACTTCTATGACACCCTC    TTGGTCC
ATTATTGGTCCGGTATGTCA
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Figure 1. Complete nucleotide sequence of ISH*11* and the region of pGRB1 flanking the insertion site. The strand of pGRB1 shown is opposite that shown in reference (12). Gaps near the ends separate ISH*11* from pGRB1. The initiation and termination codons of the open reading frame of ISH*11* are marked by a small arrow and filled circles, respectively. The 15 bp inverted repeats at the ends of ISH*11* are marked with half-arrows. The 7 bp sequence duplicated at the insertion site is underlined.

* To whom correspondence should be addressed