

Abstract

We have reported the isolation of linking clones of HindIII and EcoRI fragments, altogether spanning a 230-kb continuous stretch of chromosome VI. The presence or absence of autonomously replicating sequence (ARS) activities in all of these fragments has been determined by using ARS searching vectors containing CEN4. Nine ARS fragments were identified, and their positions were mapped on the chromosome. Structures essential for and/or stimulative to ARS activity were determined for the ARS fragments by deletions and mutations. The organization of functional elements composed of core and stimulative sequences was found to be variable. Single core sequences were identified in eight of nine ARSs. The remaining ARS (ARS603) essential element is composed of two core-like sequences. The lengths of 3'- and 5'-flanking stimulative sequences required for the full activity of ARSs varied from ARS to ARS. Five ARSs required more than 100 bp of the 3'-flanking sequence as stimulative sequences, while not more than 79 bp of the 3' sequence was required by the other three ARSs. In addition, five ARSs had stimulative sequences varying from 127 to 312 bp in the 5'-flanking region of the core sequence. In general, these stimulative activities were correlated with low local ΔG s of unwinding, suggesting that the low local ΔG of an ARS is an important element for determining the efficiency of initiation of replication of ARS plasmids.