

Two sequence-tagged sites defining the ends of a 380 kb YAC clone from 19q13

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The genetic markers D19S51 (1) and ERCC-1 (2) flank the myotonic dystrophy locus on chromosome 19q13 and define a region of approximately 1 Mb in the human genome within which the gene responsible for this autosomal dominant disease resides. Partial sequencing of the D19S51 locus has allowed screening of a gridded yeast artificial chromosome (YAC) library (3) with a view to assembling a YAC contig for this region. Sequencing of both ends of the insert in a YAC (8IC8) identified in this manner (4) has generated two sequence-tagged sites for D19S51 separated by 380 Kb in the human genome (Figure 1a, 1b; sequences of PCR primers are underlined). The right hand end probe defines a single 400 bp EcoRI-AluI fragment. The left hand end probe defines a single 700bp EcoRI-PvuII genomic fragment.

PCR Primers:

Right hand end forward 5'd CAGTAGAGATAGGGTTTCACCATGTTGG
 Right hand end reverse 5'd TTGCCTATAATTTCIGCACTTGGGAGG
 Left hand end forward 5'd AACTTCTCTGAATCTCAGTTT
 Left hand end reverse 5'd ATCTCTAACAGGTACAAGGA

PCR Reactions: 50 ng human genomic DNA, 70 pM each primer, 100 μM dNTPs, 2 U Taq polymerase (Perkin Elmer Cetus) in 100 μl of 50 mM KCl, 10 mM Tris-HCl, pH 8.3 (at room temperature), 1.5 mM MgCl₂, 0.1% (v/v) gelatin with 50 μl mineral oil overlay.

PCR Profile: 92°C for 2 minutes; 60°C for 2 minutes; 72°C for 3 minutes for 35 cycles.

(a) YAC 8IC8 Right Hand End.

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  1 CAGTAGAGAT AGGGTTTCAC CATGTTGGCC AGGCTGGTCA AGAACTCCTG
  51 ACCTTAGTG ATCCACCCAC CTCGACCTCC CAAAGTGCNG AAATTATAGG
  101 CAA

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(b) YAC 8IC8 Left hand End.

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  1 AACTTCTCTG AATTCAGTT TAAAAATCTG TAAAATACAG ATGATGATAC
  51 TTACAATGGA GTTCTTAGAA TANATGATAG TATGTAGGT GCATGCCATA
  101 TTTCGAAGTG TTCAACNAAC AATCATAATA CTAGAACTTT CTTTTATT_
  151 CCTTGACCT GTTGAGAGAT AAGGCCATAA GG

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Figure 1. (a) YAC 8IC8 Right Hand End. (b) TAC 8IC8 Left Hand End

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