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A glutamate dehydrogenase gene sequence

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A Chlorella sorokiniana genomic DNA library was screened with a NADP-specific glutamate dehydrogenase (GDH) cDNA. Two putative GDH sequences were isolated. One sequence is typically eukaryotic in that its coding region is interrupted by many introns (1). Southern blots showed that C. sorokiniana DNA contains the eukaryotic GDH gene but the second sequence could not be detected. The second sequence has characteristics of a prokaryotic GDH gene. The gene was expressed in E. coli CLR207recA (gdhA'; 2) from a multicopy plasmid, giving GDH activity 54-fold higher than basal level in untransformed cells. The cloned gene is not positioned near a plasmid promoter, suggesting expression occurred from its own promoter. The proposed coding region is highly homologous to other cloned bacterial GDH genes, i.e., 81% identical (amino acid level) to the E. coli gdhA gene (3,4) but only 51% identical to the Neurospora crassa am gene (5). Codon usage (6) and the promoter sequences are those typical of a highly expressed bacterial gene (7). Nucleotides underlined in the sequence correspond, from 5′ to 3′, to putative -35 and -10 boxes, a consensus Shine-Dalgarno sequence, and initiation/termination codons, respectively. The positions of initiation/termination codons correspond exactly with those of the E. coli gdhA gene. Both strands were sequenced completely by the method of Sanger et al. (8).

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GGCCATCCGCGCCAGCCCGCCGACCTCGCTGAGGGGCACTGGAGCATCGGTTACGCCTCGCCCAAAACCGGCCGCGAATCCCCCTGGGAATACCTGACCGACGACGGC
CATACGCAACGCCTCGAACTGACCCAGCCGGGTGGTGGTCAACAACGCCGAAAGCTATATCGCCTGCTGCCGCGCCGGGCTGGGGCTGATGCAAATCCCGCGCTACGA
CGTGCAACACCTGCTCGACGCCGGTGAACTGGTGGAAGTCCTGCCCGGTTACCGCGCCGCCTCCATGCCGATCGCCCTGATCTATCCCCATCGCCGCCAGCGATCGCG
CCGCCTGGCGGTGTTTCATGAGTGGTTTGAAAGCCTGCTGCAGCCGCATCTGGAGCGCTGATTGCACATTTAGCGCTATACTTTGTTTAATAACGCTTATAATTCAAG
CGGTCAGACCGTTAAAGCCCGCATAACCCATAGGCTTTTACGCCGCGCGGGTGTACAACGAAAAGTATCGGGCGGAGAGATTTTCGATGCGGCGCAGTGTGATCGCCC
CAACATATCATTGCGTTATCCGAATGATACTATGGAACCAAAAGCGCCAGGCGGGTAAACGGAACGCCTGCTAACCCTTTTTAATCGGCATGTTTATGGAAGGAGTAT
TCTTATGGCTTACAAACACATACTGATCCCGGTGGACCTATCCCCGGAAAGCAAGATCCTGGTAGAAAAAGCCGTCTCGATGGCGCGGCCGTACAACGCTAAAGTTTC
TCTGATCCACGTCGATGTCAACTATACCGACCTCTATACTGGCCTGATCGACGTCAATCTCGGCGACATGCAAAAACGCATCTCTGAGGAAACCCATCAGGCGCTGAC
CGAGCTGTCGCAGAACGCCGGCTACCCGATCACCGAGACCCTGAGCGGCAGCGGCGATCTGGCGCAGGTATTGGTCGATGCCATCAAGAAATACGACATGGACCTGGT
ACTGTGCGGCCACCATCAGGACTTCTGGAGCAAGCTGATGTCTTCCGCTCGCCAGCTGATCAATACCGTGCACATCGATATGCTGATCTGCGCCGCTGCGCGACGAAG
AAGACGAATAAAGTAAGCGCTTGATAACTTAACTACCCAACGCCCGCGCCCTGCGGGCTTTTTCATGCCGCCACGCCGATTAATCGCTTGATAATCAACCGCCCTCCG
ATGATATAGTCGCTAAAGACACAACATCCTCCCCCGGGCATCATCGACACCCAAATCTATAAGAAATAGCGGGGGAAACTTAATGCGAAAAGGAGTTATCCATGGAAT
ATGCCGTATCGGTAGAGTCTTTCCTGTCCTCGTTACAACGCCATAACCCCCACCAGCCGGAATATCTACAGGCGGTGCGCGAAGTGTTCACCTCGCTGTGGCCGTTTA
TCGAGCGCAACCCGGCCTATCGCGAACAGGCGCTGCTGGAGCGCCTGGTGGAGCCCGAGCGTATCATTCAGTTCCGCGTCAGCTGGGTGGACGATCGCGGCCAGGTGC
AGGTCAATCGCGCCTTCCGCGTGCAGTTCAACTCGGCCATCGGCCCCTATAAGGGCGGCATGCGCTTCCACCCATCGGTAAACCTGTCGATCCTGAAGTTCCTCGGCT
TTGAGCAGACCTTCAAGAATGCGCTGACCACCCTGCCGATGGGCGGCGGCAAAGGCGGCTCCGATTTCGATCCGAAGGGCAAAAGCCAGGGGCGGATCATGCGCTTCT
GCCAGGCGCTGATGACCGAGCTGTACCGCCATCTGGGGCCGGACACCGACGTGCCGGCCGGCGACATCGGCGACGGCGGCCGTGAAGTCGGCTTTATGGCCGGCATGA
TGAAGAAACTGTCCAACAACACCGCCTGCGTATTTACCGGCAAAGGGCTCTCCTTCGGCGGCAGCCTGATCCGCCCCGAGGCGACCGGCTACGGCCTGGTGTACTTTA
CCGACGCCATGCTGCAGCGCCACGGCCTGGGCTTTGAGGGCATGCGGGTGGCGGTGTCCGGCTCCGGCAACGTGGCGCAGTACACCATCGAAAAAGCGCTGGAGCTGG
ACGCGCGCGTGATCACCGTATCGGATTCCGGCGGCACCCTGGTGGACGAAGACGGCTTCACTACCGAGAAGCTGGCACACCTGGCGGAGATCAAAAACCAGCGTTACG
GCCGGGTGGCGGACTACGCCGCCGAACGCGGCCTGACCTATCTGGCGGGCCAGCAGCCGTGGAACGTGCCGGTGGATATCGCCCTGCCGTGCGCCACCCAGAACGAAC
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AGGTCGCGGAGGCGATGCTGGCCCAAGGCGTGCTGTAATCCGTCGTAAAGGTGATCTTGTT
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