

Name:

UČO:

V. HOMEWORK

Your goal is to recombinantly produce of 2 g of highly pure haloalkane dehalogenase DhaA enzyme from *Desulfobacterium autotrophicum*. See amino acid and corresponding nucleotide sequences below:

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>ACN15444.1 DhaA [Desulfobacterium autotrophicum HRM2]
MVTTRDPAEQSRNIKSPGIRRKINGTMVGTKDFYEIYPFVPHFMTLDRHKLHYLDLGKGSFVVMVHGNPTWSFYFRRLARDLSVN
HRVIVPDHMGCGLSDKPSTRDYDYTLASRVRDLDRLIQSLDLGKKITLVVHDWGGMIGCAWALRHLDRIDRIIITNTSGFHLPG
AKRFPLRLWLIIKYLWFAIPGIQGLNLFARAALYMAPKQSLSTTVRQGLTAPYNSWKNRIATLKFVQDIPLSPRKSYELVNWV
DTHLEGLKTVPMMLIWGRHDFVFDLSFLDEWNRFPHAQTHIFEDAGHYLFEDKPDETSNIKKFIEEY
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>CP001087.1:2679075-2680040 Desulfobacterium autotrophicum HRM2, complete genome
ATGGTAACCAGGGATCCAGCGGAGCAAAGCAGAAACATCAAAAAGTCCGGGCATCAGAAGAAAGATCAACGGCACCATGGTCGGC
ACCAAGGATTTTTATGAAATATATCCCTTTGTTCCCATTTTCATGACCCTGGACCGGCACAAACTCCACTACCTTGACCTGGGT
AAGGGAAGTCCAGTTGTCATGGTCCACGGTAATCCCACCTGGTCGTTTTATTTTCGCAGGCTTGCCCGGGATCTTTCGGTGAAC
CACCGGGTCATTGTTCCCGACCACATGGGGTGGCGCCTGTCTGACAAGCCGTCCACCAGGGATTACGACTATACCCTTGCATCA
AGGGTCCGGGACCTGGACCGTCTGATCCAGAGCCTTGACCTTGGAAAAAAGATCACCTGGTCGTCACGACTGGGGCGGTATG
ATCGGCTGCGCCTGGGCCCTTCGTCACCTGGACAGGATAGACAGGATCATCATCACCAACACCTCGGGGTTTCATCTTCCCGGG
GCAAACGATTTCCCTGCGGCTTTGGCTGATCAAATACCTTCCCTGGTTTGCCATTCAGGGATTCAGGGCCTGAATCTCTTT
GCCAGGGCAGCCCTTTACATGGCTCCGAAACAATCACTTTCAACAACGGTCAGGCAGGGGCTCACGGCACCCCTACAACCTCGTGG
AAAAACAGGATCGCCACCCTCAAATTTGTCCAGGACATTTCCCTTTACCCAGGGACAAAAGCTACGAACTTGTCAACTGGGTG
GACACCCACCTTGAAGGTCTTAAAACCGTTCCCATGATGATCCTATGGGGCAGACACGATTTTGTGTTTGATCTGTCTTCCTT
GACGAGTGGAACAAACGGTTTCCCATGCCCCAAACACATATTTTCGAGGATGCAGGCCATTATCTGTTTGAGGACAAACCCGAT
GAAACATCAAATCTTATCAAAAAATTCATAGAGGAGTACTAA
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1. Select a suitable expression host (heterologous system) for the DhaA enzyme overproduction and explain why the selected host is the best choice:

2. Propose and design a strategy for the DNA template synthesis, including primer design:

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5. How will you determine the quality and yield of the purified enzyme?

6. How will you determine oligomeric state of the DhaA enzyme?