**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:

>ACN15444.1 DhaA [Desulfobacterium autotrophicum HRM2]

MVTRDPAEQSRNIKSPGIRRKINGTMVGTKDFYEIYPFVPHFMTLDRHKLHYLDLGKGSPVVMVHGNPTWSFYFRRLARDLSVNHRVIVPDHMGCGLSDKPSTRDYDYTLASRVRDLDRLIQSLDLGKKITLVVHDWGGMIGCAWALRHLDRIDRIIITNTSGFHLPGAKRFPLRLWLIKYLPWFAIPGIQGLNLFARAALYMAPKQSLSTTVRQGLTAPYNSWKNRIATLKFVQDIPLSPRDKSYELVNWVDTHLEGLKTVPMMILWGRHDFVFDLSFLDEWNKRFPHAQTHIFEDAGHYLFEDKPDETSNLIKKFIEEY

>CP001087.1:2679075-2680040 Desulfobacterium autotrophicum HRM2, complete genome

ATGGTAACCAGGGATCCAGCGGAGCAAAGCAGAAACATCAAAAGTCCGGGCATCAGAAGAAAGATCAACGGCACCATGGTCGGCACCAAGGATTTTTATGAAATATATCCCTTTGTTCCCCATTTCATGACCCTGGACCGGCACAAACTCCACTACCTTGACCTGGGTAAGGGAAGTCCAGTTGTCATGGTCCACGGTAATCCCACCTGGTCGTTTTATTTTCGCAGGCTTGCCCGGGATCTTTCGGTGAACCACCGGGTCATTGTTCCCGACCACATGGGGTGCGGCCTGTCTGACAAGCCGTCCACCAGGGATTACGACTATACCCTTGCATCAAGGGTCCGGGACCTGGACCGTCTGATCCAGAGCCTTGACCTTGGAAAAAAGATCACCCTGGTCGTCCACGACTGGGGCGGTATGATCGGCTGCGCCTGGGCCCTTCGTCACCTGGACAGGATAGACAGGATCATCATCACCAACACCTCGGGGTTTCATCTTCCCGGGGCAAAACGATTTCCCCTGCGGCTTTGGCTGATCAAATACCTTCCCTGGTTTGCCATTCCAGGGATTCAGGGCCTGAATCTCTTTGCCAGGGCAGCCCTTTACATGGCTCCGAAACAATCACTTTCAACAACGGTCAGGCAGGGGCTCACGGCACCCTACAACTCGTGGAAAAACAGGATCGCCACCCTCAAATTTGTCCAGGACATTCCCCTTTCACCCAGGGACAAAAGCTACGAACTTGTCAACTGGGTGGACACCCACCTTGAAGGTCTTAAAACCGTTCCCATGATGATCCTATGGGGCAGACACGATTTTGTGTTTGATCTGTCGTTCCTTGACGAGTGGAACAAACGGTTTCCCCATGCCCAAACACATATTTTCGAGGATGCAGGCCATTATCTGTTTGAGGACAAACCCGATGAAACATCAAATCTTATCAAAAAATTCATAGAGGAGTACTAA

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:
3. Propose a cloning strategy – ligation-dependent versus ligation-independent cloning, selection of expression vector, affinity/solubility tags etc.? How will you check the error-free clones?
4. Briefly describe production process – how will you introduce a foreign gene into the host, from pre-culture to large-scale overproduction, inducible versus stable expression, cytotoxicity issue, timing, harvesting strategy etc.
5. How will you determine the quality and yield of the purified enzyme?
6. How will you determine oligomeric state of the DhaA enzyme?