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Introduction, Purification, Enzymatic Activity, and Mutagenesis of Dihydrofolate Reductase

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Dihydrofolate reductase(DHFR) is a key enzyme in the metabolism of folate, catalyzing the reactions for purine synthesis, DNA synthesis, and certain amino acids. The objective of this project is to have students induce expression, purify, and enzymatically assay for DHFR activity. In addition, students conducted novel experiments by designing PCR primers for site-directed mutagenesis and examined specific activity of their mutant DHFR product and compared it to the wild-type DHFR gene. Furthermore, students also obtained protein concentrations of their purified DHFR protein product and performed Western Blot analysis to detect the glutathione S-transferase (GST) and Histidine (His) tags that were attached to the DHFR gene. In addition to standard biochemical and molecular techniques (e.g. protein purification, PCR amplification, transformation into competent expression cells, restriction enzyme digest, agarose gel electrophoresis, polyacrylamide gel electrophoresis, and Western blotting), students gained experience measuring specific enzyme activity. Also bioinformatics skills for primer design and sequence alignment were reinforced.

Mission, Review Process & Disclaimer

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