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## **Mutagenesis: A laboratory course module for site-directed mutagenesis and gene knockout technology**

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### **Abstract**

The techniques of introducing and confirming specific mutations on isolated cloned genes and knocking out targeted genes from an organism have many applications in biotechnology and gene therapy research. To teach these skills, a course was developed for undergraduate and graduate students in site-directed mutagenesis and gene knockout techniques, and offered in the Biotechnology Program at North Carolina State University. The combined lab/lecture course is a two-credit, half-semester module that was designed not only to teach the practical techniques of mutagenesis, but also to engage students in learning basic biological concepts such as homologous recombination, DNA replication and protein translation. The overall course design allows students to apply their newly acquired skills to design their own site-directed mutagenesis strategies using computer-based DNA analysis programs.

The wet-lab component is comprised of three on-going laboratories involving PCR technology and two easy-to-interpret results: whole organism color changes on solid media or altered restriction mapping patterns. Students learn biotechnology laboratory skills including 1) DNA purification and quantitation, 2) PCR and primer design, 3) agarose gel electrophoresis, 4) restriction mapping, and 5) growth and transformation of bacteria and yeast cultures. In addition to learning how to create point mutations versus complete gene deletions, students observe mutagenesis in both prokaryotic and eukaryotic systems. Finally, students have the opportunity to discuss ethics of these biotechnological procedures and the impact of biotechnology on society. Overall course evaluations indicate that students enjoyed the course and felt they learned the concepts and process of mutagenesis and experimental design.