

## Interdisciplinary Training of STEM Students in Molecular Biotechnology at North Carolina

*Robert Kelly, Kathryn Auernik, Susan Carson, John Chisnell, Melissa Cox, Joanna Miller, and Scott Witherow*

**Biotechnology Program, North Carolina State University, Raleigh, NC 27696-7512**

### Abstract

At North Carolina State University, a centralized educational program has been developed for the training of undergraduate and graduate students in molecular biotechnology laboratory concepts and techniques. The NC State Biotechnology (BIT) Program operates a faculty-driven, campus-wide, core teaching facility aimed at educating and training students in laboratory aspects of molecular biotechnology ([www.ncsu.edu/biotechnology](http://www.ncsu.edu/biotechnology)). Since its creation in 1984, the BIT Program has enriched the laboratory-based training experience in molecular biotechnology of over 2500 students, postdocs and faculty coming primarily from several colleges at NC State: Agriculture and Life Sciences, Engineering, Veterinary Medicine, Physical and Mathematical Sciences, and, Natural Resources, in addition to part-time students currently employed in the biotechnology industry in the Research Triangle area.

The Program's overarching educational philosophy is that molecular biotechnology encompasses a spectrum of theoretical knowledge, skills and techniques needed for advances in modern life science research and technology, in disciplines ranging from biochemistry to botany to chemical engineering. The BIT Program has been central to life science-based biochemical/biomedical engineering education and research at NC State for over 20 years.

Presented here will be the academic structure of the NCSU BIT Program, information on the spectrum of laboratory courses offered, and specific educational aspects of selected laboratory modules. Furthermore, we will describe the integration of STEM graduate, postdoctoral and research faculty into the educational mission of the BIT Program to foster teaching capabilities of those within this group aspiring to academic careers.

### Introduction

Molecular Biotechnology comprises a set of universal laboratory skills that complement academic preparation in disciplines impacted by the life sciences.

Because of the broad applicability of Molecular Biotechnology expertise, operation of core teaching/laboratory facilities on a university campus is a strategic and efficient mechanism for educating undergraduate, graduate, and postdoctoral students, as well as faculty and staff, from a wide range of disciplines.

Undergraduate Minor	Graduate Minor	Graduate Certificate
Manipulation of Recomb. DNA General cloning techniques 2 advanced modules	Core technologies General cloning techniques 1 advanced module (2 recommended)	Core technologies General cloning techniques 2 advanced modules
Biotechnology Internship	This is an area of molecular biotechnology	Lecture elective (3cr)
Ethics course	Biotech faculty member on thesis committee	Issues in Biotechnology (1 cr)
	Research Ethics course (recommended)	

### Chronology



### Pre-doctoral Molecular Biotechnology Training Program

- BIT GRADUATE MINOR - MOLECULAR BIOTECHNOLOGY
- BIT B15K TRAINING IN RESEARCH ETHICS
- BIT B15D PROFESSIONAL DEVELOPMENT COURSE
- BIT B15N CAPSTONE BIOTECHNOLOGY COURSE
- INDUSTRIAL ROTATION or PREPARING THE PROFESSORATE
- SERVICE PROJECT
- ANNUAL RESEARCH SYMPOSIUM

### STEM Graduate, Postdoctoral and Research Faculty

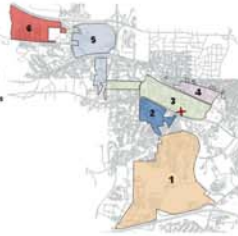
**GAANN Molecular Biotechnology Training Program Fellowships** funded by the U.S. Department of Education. Ninety-nine Fellows have been supported through nine 3 yr funded proposals since beginning in 1994. Eleven fellows participated in 07-08. Requirements of the training program include completing the BIT minor curriculum and a supervised teaching experience during their graduate program. Many Fellows serve as teaching assistants to the BIT module courses, giving lectures and developing laboratory exercises.

The BIT Program began a **Postdoctoral Teaching Associates** program in 2004. Completing a research postdoc is a prerequisite for these positions. During their tenure in the program the postdocs are mentored in teaching techniques, teach sections of the core technologies course and develop a complete new module course including lectures and laboratory exercises. Our first two postdocs have gone on to faculty positions at University of the Comberlands and Drew University.

Research faculty have developed and taught courses since the beginning of the program. Currently four research faculty and Ph. D. researchers teach module courses.

### Campus-wide Participation

Biochemistry  
Biological Sciences  
Biomedical Engineering  
Chemical Engineering  
Chemistry  
Civil Engineering  
Crop Science  
Entomology  
Fisheries and Wildlife Sciences  
Food Science  
Horticultural Sciences  
Immunology  
Microbiology  
Nutrition  
Plant Biology  
Plant Management  
Textiles  
Technology  
Veterinary Sciences  
Wood and Paper Science  
Zoology



### Manipulation of Recombinant DNA / Core Technologies in Molecular Biology

**Overview:**

- Techniques in DNA cloning and recombinant protein expression and purification.
- Introduction to advanced topics in molecular biology.

**Select Lecture Topics:**

- Plasmid cloning vectors and expression vectors
- Strategies of DNA cloning and manipulation
- Recombinant protein induction, expression, and affinity purification
- Polymerase chain reaction
- Screening recombinant clones
- Genomic and cDNA libraries
- Microarrays and qPCR
- SDS-PAGE and Western Blotting
- RNAi

**Select Lab Topics:**

1. Microplate Usage
2. Plasmid DNA purification and quantification
3. Restriction digestion, DNA gel electrophoresis, gel purification of DNA fragments
4. Ligation and transformation
5. Screening recombinant clones (PCR, DNA and antibody probes, restriction analysis)
6. SDS-PAGE and Western Blot
7. IPTG induction of protein expression
8. Affinity chromatography and protein concentration

### BIT Advanced Modules

- PCR
- RNA purification and analysis
- Plant tissue culture and transformation
- Animal cell culture
- Genome automation and instrumentation
- Genome mapping
- Protein purification
- Fermentation of cDNA microorganisms
- Microarrays
- Computer analysis of DNA sequences
- Real-time PCR
- Mutagenesis
- Computational biology
- RNA interference and model organisms
- Genetic engineering of eukaryotic microbes
- Analysis of mutant & transgenic plants
- New courses always under development:  
e.g., Protein-protein interactions (Spring 09)


### Protein-Protein Interactions

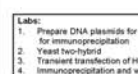
**Overview:**

- Introduce students to a variety of methods for studying protein-protein interactions, focusing on the advantages and limitations of each technique and how to apply the methods in a laboratory setting.
- Students perform a yeast two-hybrid experiment and a co-immunoprecipitation from proteins expressed in mammalian cell culture to confirm detected interactions.

**Lectures:**

1. Introduction to protein-protein interactions.
2. Yeast two-hybrid and its variants.
3. Antibody design, immunoprecipitation.
4. Pull-down assays; tandem affinity purification.
5. Live cell interactions using fluorescent techniques: FRET/BRET principles and applications.
6. Disruption of interactions using pharmacological inhibitors, mutagenesis, and genetic modification.
7. Bioinformatics

**Diagrams:** 

**Diagrams:** 

**Labs:**

1. Prepare DNA plasmids for yeast two-hybrid and cell transformation for immunoprecipitation	1 week
2. Yeast two-hybrid	5 weeks
3. Transient transfection of HEK293 cells for immunoprecipitation	1 week
4. Immunoprecipitation and western blot analysis	2 weeks

### Genetic Engineering of Eukaryotic Microbes

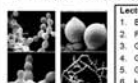
**Overview:**

Genetic manipulation of filamentous fungi and yeasts and their biotechnological applications

- In-vitro manipulation of filamentous fungi and yeasts
- Genetic transformation of filamentous fungi and molecular analysis of transformants
- Generation and analysis of yeast knockout strains

**Lectures:**

1. Biotechnological applications of eukaryotic microorganisms
2. Fungal growth and life cycles
3. Genetic transformation of yeasts and filamentous fungi
4. Generation of fungal mutant strains
5. Gene isolation and cloning strategies in fungi
6. Heterologous gene expression in fungi
7. Industrial exploitation of fungi

**Diagrams:** 

**Labs:**

1. 'In vitro' manipulation of eukaryotic microorganisms
2. Protoplast isolation from the filamentous fungi
3. Transformation of filamentous fungi and generation of yeast knockout constructs.
4. Electroporation of yeasts with knockout constructs
5. Molecular analysis of yeast knockouts
6. DNA isolation from transformed filamentous fungi and molecular analysis of transformants


### Animal Cell Culture Techniques

**Overview:**

- Acquire the necessary practical skills for the isolation of animal cells for in vitro studies, maintenance of animal cells in vitro and manipulation of animal cells in vitro.
- Prepare and use cells for the application of molecular techniques.

**Lectures:**

1. Introduction to cell culture
2. Design of the cell culture laboratory
3. Media preparation and formulations
4. Primary cell culture
5. Maintaining cells: Contamination and storage
6. Eukaryotic gene transfer
7. Immunocytochemistry

**Diagrams:** 

**Labs:**

1. Safety orientation and basic aseptic technique	1 week
2. Methods of media preparation and sterilization	1 week
3. Primary cell culture	2 weeks
4. Cell preservation and measures of cell viability	1 week
5. Eukaryotic gene transfer	1 week
6. Immunocytochemistry	1 week

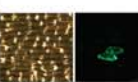
### Plant Transformation and Tissue Culture

**Overview:**

- Concepts and practices in plant transformation to further career skill set
- Discover the major techniques and basic skills necessary for plant transformation

**Lectures:**

1. Plant Tissue Culture: Concepts and Techniques
2. Constructing the Transgene
3. Plant Transformation and Analysis of Transgenic
4. Agrobacterium and Direct DNA Transformation
5. Achievements in Agricultural Biotechnology
6. Factors that Impact Transgene Expression
7. The Future of Ag Biotech

**Diagrams:** 

**Select Lab Topics:**

1. Effect of Media Composition on Cultured Tobacco Leaf Tissue
2. Use of Microscopy for Examination of Cell and Tissue Structure
3. Agrobacterium Transformation of Tobacco Leaf Discs
4. Reporter Genes as Model Transgenes
5. Microprojectile Bombardment Transient Transformation

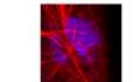
### Confocal Microscopy

**Overview:**

- Introduce students to the theory and practice of basic and advanced light microscopy techniques with emphasis on confocal laser scanning microscopy.
- Hands-on experience with research microscopes, a confocal microscope, and image analysis software.

**Lectures:**

1. Microscopy Basics
2. Geometrical Optics, Diffraction, Image formation
3. Contrast Generation: Darkfield, Phase, DIC
4. Widefield Fluorescence Microscopy
5. Confocal Laser Scanning Microscopy
6. Applications in Fluorescence Microscopy
7. Image Processing, 3D Image Reconstruction

**Diagrams:** 

**Labs:**

1. Microscope alignment, specimen preparation, imaging in bright field	2 weeks
2. Darkfield, phase contrast, differential interference contrast	3 weeks
3. Fluorescence Microscopy, immunofluorescence lab	2 weeks
4. Confocal microscopy, demos and student assignments	3 weeks
5. Confocal microscopy, student projects	2 weeks


### PCR & DNA Fingerprinting

**Overview:**

- Introduction of students to basic principles of the polymerase chain reaction (PCR) technology and some of the applications in science.
- Students will amplify a variety of DNA and cDNA templates that they prepare themselves along with a fun "forensic" DNA amplification.

**Lectures:**

1. Introduction to PCR
2. Primer Design and Carry-Over Prevention
3. Optimizing PCR Reactions
4. Asymmetric PCR and Cloning of PCR Products
5. Reverse Transcriptase PCR
6. Quantitative PCR Assays
7. qPCR Data Analysis

**Diagrams:** 

**Labs:**

1. Forensic PCR- DNA Fingerprinting and Circadian Rhythmic Gene Amplification
2. Recombinant PCR Laboratory- Parts 1 & 2- Optimizing PCR Reactions
3. PCR Sequencing
4. Reverse Transcriptase PCR
5. Real-time Quantitative PCR


### RNA Interference and Model Organisms

**Overview:**

- Introduce students to the history of RNA interference (RNAi) and its applications in common model organisms with a focus on the experimental design.
- Students perform RNAi experiments in *Nicotiana benthamiana* (tobacco plants), in *Caenorhabditis elegans* (C. elegans) and in mammalian cell culture (human embryonic kidney cell line, HEK293).

**Lectures:**

1. Introduction to RNAi
2. Post Transcriptional Gene Silencing in Plants
3. C. elegans: RNAi History and Experiments
4. Drosophila: RNAi Background and Experiments
5. RNAi and Mammalian Systems
6. MicroRNA, Antisense RNA and Other RNA Silencers
7. Therapeutic Uses of RNAi

**Diagrams:** 

**Labs:**

1. Preparation of DNA-Directed Silencing Constructs	1 week
2. In-vivo Induced Gene Silencing of Magnesium Chelatase, a Chlorophyll Biosynthesis Enzyme, in Tobacco Plants. Real-time RT-PCR Assay	5 weeks
3. C. elegans RNAi Experiment to Knockdown Transgenic GFP Expression via Feeding of Silencing Constructs. Qualitative Phenotypic Assessment	2 weeks
4. Transient Transfection Delivery of Short Hairpin RNA Expression Plasmids to Mammalian Cell Culture. Semi-quantitative Western Blot.	3 weeks

### Conclusion

NC State University has shown that a core teaching facility is effective for educating and training STEM students in molecular biotechnology laboratory skills. The BIT Program has been central to life science-based biochemical/biomedical engineering education and research at NC State for over 20 years.

**ACKNOWLEDGMENTS**  
Program funding is provided by:  
NCSU Provost  
NIH Pre-doctoral Training Grant GM006776  
DOE-GAANN Pre-doctoral Training Grant P200AOT0582  
NSF-REU Summers 2009-11  
Susan Carson PI

