

Honors Collegium 70AL Gene Discovery Laboratory
Identifying Genes Important for Seed Development
Sponsored by the National Science Foundation

Professor Bob Goldberg
Spring 2009

LABORATORY: Tuesday & Thursday 2-6 PM, Life Sciences 2822

OPEN LABORATORY: Wednesday & Friday 2-6 PM, Life Sciences 2822

SEMINAR AND RESEARCH DISCUSSIONS: Monday 6-8 PM, Life Sciences 2805

ADMINISTRATIVE ASSISTANT: Ingrid Nelson, Life Sciences 2835, (inelson@mcdb.ucla.edu)

SENIOR SCIENTIST & INSTRUCTOR: Dr. Anhthu Bui, Life Sciences 2836 (aqbui@ucla.edu)

LABORATORY ASSISTANTS: Kristin Gill (kgill36@ucla.edu)
Daisy Robinton (drobinto@ucla.edu)

RESEARCH CONCEPTS & ANALYSIS OFFICE HOURS: Wednesday & Friday 2-6 PM, LS 2822

LAB REPORTS: Lab reports are due Mondays at 6 PM on the dates indicated in this syllabus. Guidelines for the lab reports will be handed out during the lab sessions.

LAB WEBBOOK & BLUE BOOKS: Data generated for the week **MUST** be logged into the lab Webbook including gel images, gene annotation files, etc. Protocols, written notes, data, and lab reports must be labeled and organized in Blue Binders, which must be kept in the lab and cannot be taken out of the lab. The lab Webbook can be accessed at the following address: <http://estdb.biology.ucla.edu/webbook>. Access to the lab Webbook is password protected. The username is your email username, and the password is your 9-digit student identification number. Please report any problems or suggestions to Min Chen (m.chen@ucla.edu) or Brandon Le (ble@ucla.edu).

LAB REPORTS: Lab reports should be written in the form of a mini-journal article and documented with figures and/or tables from your experiments. The lab report should be modeled after an article published in Proceedings of the National Academy of Sciences (PNAS). A sample PNAS article will be handed out in a Monday evening session. PNAS can be accessed online at <http://www.pnas.org/>. Lab reports must be uploaded as a .doc or pdf file onto the Webbook and handed on the date that they are due.

GRADING: Grades will be based on (1) research results, (2) lab reports, (3) Monday evening discussion participation, (3) final oral presentation and (4) exit interview. Time and date of the exit interviews will be scheduled during the 9th week.

LABORATORY SCHEDULE:

DAY DATE EXPERIMENT

WEEK ONE

- Mon 3/30/09** *Introduction to Plants & Seed Development* -- Professor Bob Goldberg
What Will You Do This Quarter? – Professor Bob Goldberg
- Tue 3/31/09** **Experiment ONE - Introduction to General Molecular Biology Techniques**
Intro: *Lab Orientation and Tour* – Anhthu Bui
Intro: *Data Recording & Organization - Introduction to the Webbook and Lab Blue Books* – Anhthu Bui
Intro: *Introduction to Proper Micropipetting Techniques & Gel Loading* -- Anhthu Bui
- A. Proper Micropipetting Techniques**
Accuracy/Precision Experiments
Gel Electrophoresis of Plasmid DNA
Sowing Seeds from Wild Type (Ecotype Col-0) and SALK Lines
- Thu 4/02/09** **Experiment ONE Continued - Introduction to General Molecular Biology Techniques**
- Intro: *Introduction to DNA Sequencing* – Professor Bob Goldberg
Intro: *Introduction to Polymerase Chain Reaction (PCR)* -- Daisy Robinton
Intro: *Introduction to Sizing DNA on Agarose Gel* – Kristin Gill
- B. Polymerase Chain Reaction (PCR) & DNA Sequencing**
Setting up a Gene-Specific Polymerase Chain Reaction
Gel Electrophoresis of Gene-Specific PCR Products
Purification of PCR Products
Determining DNA Concentration Using a UV Spectrophotometer
Sequencing of Gene-Specific Products
-

WEEK TWO

- Mon 4/06/09** *Sequencing the Scarlet Runner Bean Genome* -- Professor Bob Goldberg
Introduction to Arabidopsis Knockout Screens and Genetics -- Professor Bob Goldberg
- Tue 4/07/09** **Experiment ONE Continued - Introduction to General Molecular Biology Techniques**
Intro: *Using the Computer to Analyze DNA Sequences* – Brandon Le & Min Chen
- C. Characterization of a Gene Being Studied**
Processing a DNA sequence from UCLA Sequencing Facility Server
Characterizing Gene Corresponding to the DNA Sequence
- Experiment TWO – Shotgun Sequencing of Scarlet Runner Bean Genome**
Intro: *Introduction to Genomic DNA Isolation: Part One* – Anhthu Bui
- Isolating Genomic DNA from Scarlet Runner Bean Leaves
Gel Electrophoresis of Isolated Genomic DNA

DAY DATE EXPERIMENT

Thu 4/09/09 Experiment THREE - Screening SALK T-DNA Mutagenesis Lines (GENE ONE)

Intro: *Introduction to Genomic DNA Extraction: Part Two* – Anhthu Bui

Intro: *Introduction to Plant Genotyping* – Kristin Gill & Daisy Robinton

A. Extraction of Genomic DNA

Leaf Collection from Wild Type and SALK Plants

Isolating Genomic DNA from Leaves of Wild Type and SALK Plants

Determining DNA Concentration Using a Fluorometer

Gel Electrophoresis of Isolated Genomic DNA

WEEK THREE

Mon 4/13/09 *Introduction to Bioinformatics – Annotating DNA Scaffolds* – Brandon Le & Min Chen

Discussion of Data From Experiment One – Anhthu Bui

EXPERIMENT ONE LAB REPORT DUE

Tue 4/14/09 Experiment TWO Continued – Shotgun Sequencing of Scarlet Runner Bean Genome

Intro: *Annotation of DNA Sequences: Part One* – Brandon Le & Min Chen

Annotating a Scarlet Runner Bean DNA Sequence Scaffold

Experiment THREE Continued - Screening SALK T-DNA Mutagenesis Lines (Gene ONE)

B. Determining Genotypes of Segregating Plant Population

Determining Genotypes of SALK Plants Using PCR

Thu 4/16/09 Experiment THREE Continued - Screening SALK T-DNA Mutagenesis Lines (GENE ONE)

C. Determination of T-DNA Insertion Site

Gel Electrophoresis of SALK Line PCR Products

Discussion of PCR Results

Purification of PCR Products

Determining DNA Concentration Using a UV Spectrophotometer

Sequencing PCR Products with a T-DNA Primer and a Gene-Specific Primer

WEEK FOUR

Mon 4/20/09 *Introduction to Gene Expression - RT-PCR and Microarrays* – Professor Bob Goldberg

Discussion of Data From Experiment TWO – Professor Bob Goldberg & Brandon Le

Tue 4/21/09 Experiment TWO Continued – Shotgun Sequencing of Scarlet Runner Bean Genome

Intro: *Annotation of DNA Sequences: Part Two* – Brandon Le & Min Chen

Annotating a Scarlet Runner Bean DNA Sequence Scaffold

Experiment THREE - Screening SALK T-DNA Mutagenesis Lines (GENE ONE)

Intro: *Review of Genetics and Genotyping* – Kristin Gill & Daisy Robinton

D. Determination of T-DNA Insertion Site

DAY DATE EXPERIMENT
Analysis of Sequenced PCR Products from SALK Line Screening

Thu 4/23/09 Experiment FOUR - RNA Isolation and RT-PCR Analysis
Intro: *Introduction to RNA Isolation and Analysis of RNA* – Chen Cheng

A. RNA Isolation

Preparation & Decontamination of Equipment for RNA Work
Isolating Total RNA from Wild Type Seeds and Leaves
Removal of Genomic DNA from Isolated Total RNA with DNase I
Determining RNA Concentration Using a UV Spectrophotometer
Gel Electrophoresis of Total RNA (Before and After DNase I Treatment)

WEEK FIVE

Mon 4/27/09 *Introduction to Cloning of Promoters* -- Kelli Henry
Discussion of Data from Experiments THREE & FOUR – Anhthu Bui & Brandon Le Anhthu

EXPERIMENTS TWO AND THREE LAB REPORTS DUE

Tue 4/28/09 Experiment FOUR Continued - RNA Isolation and RT-PCR Analysis
Intro: *Introduction to cDNA Synthesis & RT-PCR* – Anhthu Bui

B. cDNA Synthesis

Synthesizing cDNAs from Isolated Total RNA

C. RT-PCR-1

Amplification of cDNAs by PCR

Thu 4/30/09 Experiment FOUR Continued - RNA Isolation and RT-PCR Analysis

C. RT-PCR-2

Gel Electrophoresis of RT-PCR Products

Experiment FIVE - Amplification & Cloning an Upstream Region

Intro: *Introduction to Amplification & Cloning of Upstream Regions* -- Anhthu Bui

A. Amplification of an Upstream Region

Amplification of an Upstream Region Using PCR
Gel Electrophoresis of PCR Product
Ligating PCR Product into a Plasmid Vector pENTR/D-TOPO

WEEK SIX

Mon 5/04/09 *Research Paper Discussion* – Professor Bob Goldberg
Discussion of Data from Experiment FOUR – Professor Bob Goldberg
EXPERIMENT FOUR LAB REPORT DUE

DAY DATE EXPERIMENT

Tue 5/05/09 Experiment FIVE Continued - Amplification & Cloning an Upstream Region
Intro: *Introduction to Transformation & Bacterial Techniques -- Anhthu Bui*

B. Transformation of *E. coli* Cells

Transformation of *E. coli* Competent Cells with Ligation Mixtures
Growing Transformed *E. coli* Cells in SOC Medium
Spreading Transformed *E. coli* Cells on LB + Antibiotic Plates
Incubating Plates Overnight at 37°C

Wed 5/06/09 Experiment FIVE Continued - Amplification & Cloning an Upstream Region

B. Transformation of *E. coli* Cells

Counting Bacterial Colonies

C. Isolation & Verification of Recombinant Plasmid DNA

Inoculating of TB broth + Antibiotics with Selected Bacterial Colonies

Thu 5/07/09 Experiment FIVE Continued - Amplification & Cloning a Promoter Region

Intro: *Introduction to Plasmid DNA Preparation – Anhthu Bui*

C. Isolation & Verification of Recombinant Plasmid DNA

Isolating Plasmid DNA from Four Colonies
Determining Plasmid DNA Concentration
Verification of Recombinant Plasmid via Restriction Enzyme Analysis
Gel Electrophoresis of Restriction Digested Plasmid DNA

WEEK SEVEN

Mon 5/11/09 *Ethical Research Case Discussion* – Professor Bob Goldberg

Tue 5/12/09 Experiment SIX - Observation & Characterization of Known & Unknown Mutants (Gene ONE)

Intro: *Observing Plants & Seeds For Mutant Phenotypes -- Anhthu Bui*

A. Observation of Plant & Seed Phenotypes

Examine and Compare Wild Type and Mutant Plant Phenotypes
If a Mutant Plant is Homozygous → Open Silique & Observe Seeds
If a Mutant Plant is Heterozygous → Open Silique & Count White/Green Seeds

B. Characterization of Mutant Seeds Using Microscopy

Fix Wild Type and Mutant Seeds in Fixative for Nomarski Optics Microscopy
Make Appointment to Use Nomarski Optics Microscope
(Appointments should be made from 5-12-09 to 5-19-09)

Experiment FIVE Continued - Amplification & Cloning a Gene Promoter Region

C. Isolation & Verification of Recombinant Plasmid DNA

Sequencing of Recombinant Plasmid DNA

DAY DATE EXPERIMENT

Thu 5/15/09 **Experiment FIVE Continued - Amplification & Cloning a Gene Promoter Region**
Analyzing and Verifying a DNA Sequence of the Cloned Upstream Region

Experiment SEVEN - Screening SALK T-DNA Mutagenesis Lines (GENE TWO)
Intro: Review of Knock-Out Screening -- *Kristin Gill & Daisy Robinton*

A. Extraction of Genomic DNA

Tissue Collection from Plants
Isolating Genomic DNA from Wild Type and SALK Lines
Gel Electrophoresis of Genomic DNA

B. Determination of Genotype

Determining Genotype of SALK Plants Using PCR

WEEK EIGHT

Mon 5/18/09 *How to Give a Research Talk* – Professor Bob Goldberg
Discussion of Data from Experiment FIVE – Professor Bob Goldberg
EXPERIMENT FIVE LAB REPORT DUE

Tue 5/19/09 **Experiment SEVEN - Screening SALK T-DNA Mutagenesis Lines (GENE TWO)**

B. Determination of Genotype

Gel Electrophoresis of PCR Product from a SALK Line (From Part B on 5/15/09)

Experiment EIGHT - RT-PCR Analysis with Primers for Gene Two

Amplification of cDNAs (Generated in Week 5) Using PCR
Gel Electrophoresis of RT-PCR Products

Thu 5/21/09 **Experiment NINE - Observation & Characterization of Known & Unknown Mutants (GENE TWO)**

A. Observation of Plant & Seed Phenotypes

Examine and Compare Wild Type and Mutant Plant Phenotypes
If a Mutant Plant is Homozygous → Open Siliques & Observe Seeds
If a Mutant Plant is Heterozygous → Open Siliques & Count White/Green Seeds

B. Characterization of Mutant Seeds Using Microscopy

Fix Wild Type and Mutant Seeds in Fixative for Nomarski Optics Microscopy
Make Appointment to Use Nomarski Optics Microscope
(Appointments should be made from 5-26-09 to 5-29-09)

DAY DATE EXPERIMENT

WEEK NINE

Mon 5/25/09 *Memorial Day Holiday – No Class*

Tue 5/26/09 **Experiment NINE Continued - Observation & Characterization of Known & Unknown Mutants (GENE TWO)**
EXPERIMENTS SIX AND SEVEN LAB REPORTS DUE

B. Characterization of Mutant Seeds Using Microscopy
Nomarski Optics Microscopy of Mutant Seeds

General Laboratory
Summarize Data, Prepare PowerPoint Presentation, & Finish Experiments

Thu 5/28/09 **General Laboratory**
Summarize Data, Prepare PowerPoint Presentation, & Finish Experiments

WEEK TEN

Mon 6/01/09 *Discussion of Data from All Experiments* – Professor Bob Goldberg
EXPERIMENTS EIGHT AND NINE LAB REPORTS DUE

Tue 6/02/09 **Clean-Up Benches, Summarize Data, & Organize Lab Notebook & Webbook**
Organize & Practice Group Research Talks

Wed 6/03/09 **Exit Interviews With Professor Bob Goldberg**

Thu 6/04/09 **All Class Research Symposium and Oral Presentations of Research Results**

Fri 6/05/09 **Exit Interviews With Professor Bob Goldberg**