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
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**
---|---|---
Thu | 4/23/09 | 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

**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**
<table>
<thead>
<tr>
<th>DAY</th>
<th>DATE</th>
<th>EXPERIMENT</th>
<th>Intro:</th>
<th>Anhthu Bui</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tue</td>
<td>5/05/09</td>
<td>Experiment FIVE Continued - Amplification &amp; Cloning an Upstream Region</td>
<td><strong>B. Transformation of E. coli Cells</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transformation of <em>E. coli</em> Competent Cells with Ligation Mixtures</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Growing Transformed <em>E. coli</em> Cells in SOC Medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spreading Transformed <em>E. coli</em> Cells on LB + Antibiotic Plates</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Incubating Plates Overnight at 37°C</td>
<td></td>
</tr>
<tr>
<td>Wed</td>
<td>5/06/09</td>
<td>Experiment FIVE Continued - Amplification &amp; Cloning an Upstream Region</td>
<td><strong>B. Transformation of E. coli Cells</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Counting Bacterial Colonies</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. Isolation &amp; Verification of Recombinant Plasmid DNA</td>
<td>Inoculating of TB broth + Antibiotics with Selected Bacterial Colonies</td>
<td></td>
</tr>
<tr>
<td>Thu</td>
<td>5/07/09</td>
<td>Experiment FIVE Continued - Amplification &amp; Cloning a Promoter Region</td>
<td><strong>C. Isolation &amp; Verification of Recombinant Plasmid DNA</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Isolating Plasmid DNA from Four Colonies</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Determining Plasmid DNA Concentration</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Verification of Recombinant Plasmid via Restriction Enzyme Analysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gel Electrophoresis of Restriction Digested Plasmid DNA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>WEEK SEVEN</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mon</td>
<td>5/11/09</td>
<td><em>Ethical Research Case Discussion</em> – Professor Bob Goldberg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tue</td>
<td>5/12/09</td>
<td>Experiment SIX - Observation &amp; Characterization of Known &amp; Unknown Mutants</td>
<td><strong>A. Observation of Plant &amp; Seed Phenotypes</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Gene ONE)</td>
<td>Examine and Compare Wild Type and Mutant Plant Phenotypes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>If a Mutant Plant is Heterozygous → Open Silique &amp; Count White/Green Seeds</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fix Wild Type and Mutant Seeds in Fixative for Nomarski Optics Microscopy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Make Appointment to Use Nomarski Optics Microscope</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Appointments should be made from 5-12-09 to 5-19-09)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Experiment FIVE Continued - Amplification &amp; Cloning a Gene Promoter Region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>C. Isolation &amp; Verification of Recombinant Plasmid DNA</strong></td>
<td>Sequencing of Recombinant Plasmid DNA</td>
<td></td>
</tr>
</tbody>
</table>
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 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 5-26-09 to 5-29-09)
<table>
<thead>
<tr>
<th>DAY</th>
<th>DATE</th>
<th>EXPERIMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>WEEK NINE</strong></td>
</tr>
<tr>
<td>Mon</td>
<td>5/25/09</td>
<td><em>Memorial Day Holiday</em> – <em>No Class</em></td>
</tr>
<tr>
<td>Tue</td>
<td>5/26/09</td>
<td>Experiment NINE Continued - Observation &amp; Characterization of Known &amp; Unknown Mutants (GENE TWO)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EXPERIMENTS SIX AND SEVEN LAB REPORTS DUE</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>B. Characterization of Mutant Seeds Using Microscopy</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nomarski Optics Microscopy of Mutant Seeds</td>
</tr>
<tr>
<td></td>
<td></td>
<td>General Laboratory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Summarize Data, Prepare PowerPoint Presentation, &amp; Finish Experiments</td>
</tr>
<tr>
<td>Thu</td>
<td>5/28/09</td>
<td>General Laboratory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Summarize Data, Prepare PowerPoint Presentation, &amp; Finish Experiments</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>WEEK TEN</strong></td>
</tr>
<tr>
<td>Mon</td>
<td>6/01/09</td>
<td><em>Discussion of Data from All Experiments</em> – Professor Bob Goldberg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EXPERIMENTS EIGHT AND NINE LAB REPORTS DUE</td>
</tr>
<tr>
<td>Tue</td>
<td>6/02/09</td>
<td>Clean-Up Benches, Summarize Data, &amp; Organize Lab Notebook &amp; Webbook</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Organize &amp; Practice Group Research Talks</td>
</tr>
<tr>
<td>Wed</td>
<td>6/03/09</td>
<td>Exit Interviews With Professor Bob Goldberg</td>
</tr>
<tr>
<td>Thu</td>
<td>6/04/09</td>
<td>All Class Research Symposium and Oral Presentations of Research Results</td>
</tr>
<tr>
<td>Fri</td>
<td>6/05/09</td>
<td>Exit Interviews With Professor Bob Goldberg</td>
</tr>
</tbody>
</table>