

Honors Collegium 70AL Gene Discovery Laboratory
Identifying Genes Important for Seed Development
Sponsored by NSF

Dr. Bob Goldberg
Spring 2011

OFFICE HOURS: Friday, 1-2 PM, Terasaki Life Sciences 4121, bobg@ucla.edu

INTRODUCTION TO LABORATORY RESEARCH: Monday 6-8 PM, Terasaki Life Sciences 4100

LABORATORY: Tuesday & Thursday 2-6 PM, Terasaki Life Sciences 4128

OPEN LABORATORY & RESEARCH CONSULTATION: Wednesday & Friday 2-6 PM, Terasaki Life Sciences 4128.

SEMINAR ROOMS: Terasaki 4100 (Monday & Tuesday), Terasaki 1000 (Thursday), Terasaki 1020 (Final Research Symposium).

ADMINISTRATIVE ASSISTANTS: Jennifer Kwan (kwanj@ucla.edu) , Terasaki Life Sciences 4125

TEACHING ASSISTANT & LAB COORDINATOR: Kelli Henry, Terasaki Life Sciences 4128 (kfhenry@ucla.edu)

BIOINFORMATICS COORDINATORS: Brandon Le (ble@ucla.edu) & Min Chen (minchen7@ucla.edu) , Terasaki Life Sciences 4128 (ble@ucla.edu)

POST-DOCTORAL FELLOW: Dr. Jungim Hur, Terasaki Life Sciences 4128 (jhur@ucla.edu)

UNDERGRADUATE TEAM LEADERS & LAB ASSISTANTS: Elaine Chiu (elainec90@ucla.edu) & Eden Maloney (eden.maloney@gmail.com), Terasaki Life Sciences 4128

UNDERGRADUATE TEAMS: Team One: Elaine Chieu (Leader) – Hanbee O, Mike Lyons, & Pauline Do. Team Two: Eden Maloney (Leader) – Reece Fanning, Lauren Daoust, and Krista Perry.

OPEN LABORATORY CONSULTANTS: Wednesday: Kelli Henry, Brandon Le, and Elaine Chiu. Friday: Jungim Hur, Min Chen, & Eden Maloney.

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 pdf file onto the Webbook and handed in by 6 PM on the Monday that they are due.

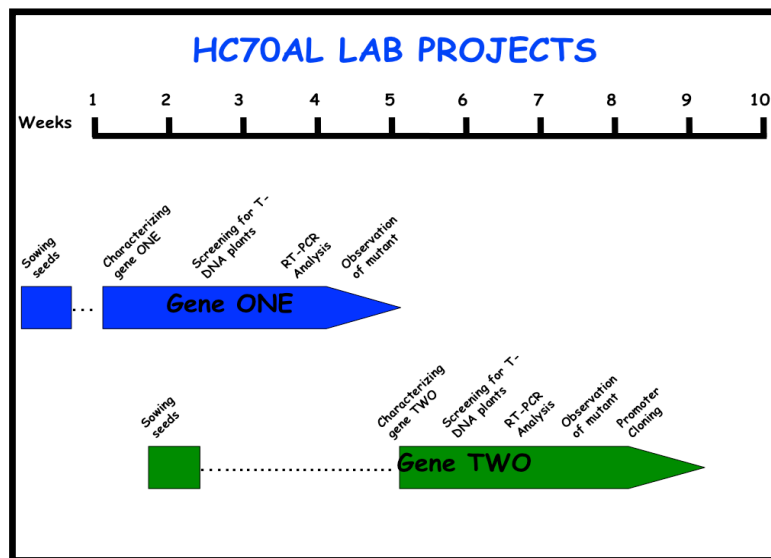
LAB WEBBOOK & BLUE BOOKS: Data generated for the week must be logged into the Lab Webbook – including all results, specific methods, and digital images. Protocols, written notes, data, and lab reports must be labeled and organized in your Bluebook Binder. Bluebook Binders with research data must be kept in 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 Bruin Online (bol) login, and the password is your 9-digit student identification number. Please report any problems, or suggestions, to Brandon Le (ble@ucla.edu).

GRADING: Grades will be based on (1) research results, (2) lab reports, (3) Monday evening discussion participation, (4) final oral presentation and (5) exit interview. Time and date of the exit interviews will be scheduled during the 9th week. The final oral presentations will be on Thursday, June 2 from 2 to 5 pm. Exit interviews will take place during finals week (11th week) at a date and time to be scheduled during the 9th week.

SUMMARY OF HC70AL EXPERIMENTS – SPRING 2011

Experiment 1	<i>Introduction To General Molecular Biology Techniques</i>
Experiment 2	<i>Screening Salk T-DNA Mutagenesis Lines (GENE ONE)</i>
Experiment 3	<i>RNA Isolation and RT-PCR Analysis (GENE ONE)</i>
Experiment 4	<i>Identifying Features of Mutant Seeds Using Nomarski Microscopy (GENE ONE)</i>
Experiment 5	<i>Screening Salk T-DNA Mutagenesis Lines (GENE TWO)</i>
Experiment 6	<i>RNA Isolation and RT-PCR Analysis (GENE TWO)</i>
Experiment 7	<i>Identifying Features of Mutant Seeds Using Nomarski Microscopy (GENE TWO)</i>
Experiment 8	<i>Amplifying and Cloning a Gene Upstream Region (GENE TWO)</i>

HC70AL SPRING 2011 – EXPERIMENTAL TIMELINES



WEEK ONE

- Mon 3/28/11** *Introduction to Seed Development & Research – Professor Bob Goldberg*
What Are We Going to Do This Quarter?
Data Recording & Organization - Introduction to the Webbook and Lab
Research Notebook – Brandon Le
- Tue 3/29/11** **EXPERIMENT ONE - Introduction to General Molecular Biology Techniques**
Introduction 1: Lab Orientation and Tour – Kelli Henry, Brandon Le, & Jungim Hur
Introduction 2: Lab Safety – Kelli Henry
Introduction 3: Proper Micropipetting Techniques – Jungim Hur
Introduction 4: Sizing DNA on Agarose Gels - Elaine Chiu
- A. Proper Micropipetting Techniques**
Accuracy / Precision Experiments
Gel Electrophoresis of Plasmid DNA
- EXPERIMENT FIVE - Screening Salk T-DNA Mutagenesis Lines (GENE TWO)**
Introduction 5: Plant Growth Center Tour – Kelli Henry, Brandon Le, & Jungim Hur
- Sowing *Arabidopsis* Seeds from Wild-Type (Ecotype Col-0) and Salk Lines
- Thu 3/31/11** **EXPERIMENT ONE - Introduction to General Molecular Biology Techniques**
Introduction 1: Polymerase Chain Reaction (PCR) - Eden Maloney
- B. Polymerase Chain Reaction (PCR) & Sequencing PCR Product**
Setting up a Gene-Specific Polymerase Chain Reaction
- EXPERIMENT TWO - Screening Salk T-DNA Mutagenesis Lines (GENE ONE)**
Introduction 2: Genomic DNA Isolation - Kelli Henry
- A1. Isolation of Genomic DNA - Set I**
Tissue Collection from Plants
Isolating Genomic DNA from Wild-Type and Salk Lines
-

WEEK TWO

- Mon 4/4/11** *Introduction to Knockout Screens and Genetics - Professor Bob Goldberg*
- Tue 4/5/11** **EXPERIMENT ONE - Introduction to General Molecular Biology Techniques**
Introduction 1: Introduction to Sanger DNA Sequencing - Professor Bob Goldberg
- B. Polymerase Chain Reaction (PCR) & Sequencing PCR Product**
Gel Electrophoresis of Gene-Specific Products from 3-31-11
Purifying PCR Products
Determining DNA Concentration Using Nanodrop Spectrophotometer

Sequencing of Gene-Specific Products

EXPERIMENT TWO - Screening Salk T-DNA Mutagenesis Lines (GENE ONE)

A1. Extraction of Genomic DNA - Set I

Determining DNA Concentration Using Nanodrop Spectrophotometer
Gel Electrophoresis of Genomic DNA

Thu 4/7/11 **EXPERIMENT ONE - Introduction to General Molecular Biology Techniques**
Introduction 1: Using Bioinformatics to Analyze DNA Sequences - Brandon Le

B. Polymerase Chain Reaction (PCR) & Sequencing

Retrieving and Analyzing DNA Sequences

EXPERIMENT TWO - Screening Salk T-DNA Mutagenesis Lines (GENE ONE)

Introduction 2: Plant Genotyping - Eden Maloney and Elaine Chiu

B1. Determination of Genotype - Set I

Determining Genotype of Salk Plants Using PCR

A2. Extraction of Genomic DNA - Set II

Tissue Collection from Plants
Isolating Genomic DNA from Wild Type and Salk Lines
Determining DNA Concentration Using Nanodrop spectrophotometer
Gel Electrophoresis of Genomic DNA

WEEK THREE

Mon 4/11/11 *Introduction to Bioinformatics - Brandon Le*
Discussion of Data from Experiment ONE – Professor Bob Goldberg
EXPERIMENT ONE LAB REPORT DUE

Tue 4/12/11 **EXPERIMENT TWO - Screening Salk T-DNA Mutagenesis Lines (GENE ONE)**
Introduction: Review of Plant Genotyping - Eden Maloney and Elaine Chiu

B1. Determination of Genotype - Set I

Gel Electrophoresis of PCR Product (From Part B1 on 4/7/11)

C1. Determination of T-DNA Insertion Site - Set I

Discussion of PCR Results
Purification of PCR Products
Determining DNA Concentration Using Nanodrop Spectrophotometer
Sequencing PCR Product with T-DNA and Gene-Specific Salk Primer

B2. Determination of Genotype - Set II (IF NECESSARY)

Determining Genotype of Salk Plants Using PCR

Thu 4/14/11 EXPERIMENT TWO - Screening Salk T-DNA Mutagenesis Lines (GENE ONE)

Introduction: Using Bioinformatics to Analyze DNA Sequences - Brandon Le

C1. Determination of T-DNA Insertion Site - Set I

Analysis of Sequenced PCR Product – *Brandon Le*

B2. Determination of Genotype - Set II (IF NECESSARY)

Gel Electrophoresis of PCR Product (From Part B2 on 4/12/11)

D. Observation of the Mature Plant Phenotype

WEEK FOUR

Mon 4/18/11 *Introduction to Gene Expression - RT-PCR, Microarrays and RNA-Seq - Professor Bob Goldberg*
Discussion of Data from Experiment TWO – Professor Bob Goldberg
EXPERIMENT TWO LAB REPORT DUE

Tue 4/19/11 EXPERIMENT THREE - RNA Isolation and RT-PCR Analysis (GENE ONE)
Introduction: RNA Isolation and Analysis – Jungim Hur

A. RNA Isolation

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

Thu 4/21/11 EXPERIMENT THREE - RNA Isolation and RT-PCR Analysis (GENE ONE)
Introduction 1: Discussion of Total RNA Quality – Jungim Hur
Introduction 2: Introduction to cDNA Synthesis & RT-PCR – Jungim Hur

B. cDNA Synthesis

Synthesizing cDNAs from Isolated Total RNA

C. RT-PCR

Amplification of cDNA Using PCR

WEEK FIVE

Mon 4/25/11 *Research Ethics Case Study Discussion - Professor Bob Goldberg*

Tue 4/26/11 **EXPERIMENT THREE - RNA Isolation and RT-PCR Analysis (GENE ONE)**

C. RT-PCR

Gel Electrophoresis of RT-PCR Products from 4-21-11

EXPERIMENT FOUR - Identifying Features of Mutant Seeds Using Nomarski Microscopy (GENE ONE)

Introduction: Observing Plants & Seeds for Mutant Phenotypes – Jungim Hur

A. Observation of Plant & Seed Phenotypes

Examine and Compare Wild Type and Mutant Plants

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 4-26-11 to 5-6-11)

Experiment FIVE - Screening Salk T-DNA Mutagenesis Lines (GENE TWO)

Introduction: Review of Knock-Out Screening - Eden Maloney and Elaine Chiu

A. Extraction of Genomic DNA

Tissue Collection from Plants

Isolating Genomic DNA from Wild Type and Salk Lines

Determining DNA Concentration Using Nanodrop Spectrophotometer

Gel Electrophoresis of Genomic DNA

Thu 4/28/11 **Experiment FIVE - Screening Salk T-DNA Mutagenesis Lines (GENE TWO)**

B. Determination of Genotype

Determining Genotype of Salk Plants Using PCR

Gel Electrophoresis of PCR Product

Experiment FOUR - Identifying Features of Mutant Seeds Using Nomarski Microscopy (GENE ONE)

WEEK SIX

Mon 5/2/11 *Discussion of Data from Experiment THREE – Kelli Henry & Brandon Le*
EXPERIMENT THREE LAB REPORT DUE

Tue 5/3/11 **Experiment FIVE - Screening Salk T-DNA Mutagenesis Lines (GENE TWO)**

C. Determination of T-DNA Insertion Site

Discussion of PCR Results

Purification of PCR Products

Determining DNA Concentration Using Nanodrop Spectrophotometer

Sequencing PCR Product with T-DNA and Gene-Specific Salk Primer

D. Observation of the Mature Plant Phenotype

Thu 5/5/11 Experiment FIVE - Screening Salk T-DNA Mutagenesis Lines (GENE TWO)

C. Determination of T-DNA Insertion Site

Analysis of Sequenced PCR Product – *Brandon Le*

Experiment SIX - RNA Isolation and RT-PCR Analysis (GENE TWO)

Introduction: Review of RNA Isolation and Analysis of RNA – Eden Maloney and Elaine Chiu

A. RNA Isolation

Preparation & Decontamination of Equipment for RNA Work
Isolating Total RNA from Wild type and Mutant Siliques
Removal of Genomic DNA from Isolated Total RNA with DNase I
Determining RNA Concentration Using Nanodrop Spectrophotometer
Capillary Gel Electrophoresis of Total RNA (Before and After DNase I Treatment)

WEEK SEVEN

**Mon 5/9/11 *How to Prepare and Present Research Data - Professor Bob Goldberg*
Discussion of Data from Experiment FOUR – Professor Bob Goldberg
EXPERIMENT FOUR LAB REPORT DUE**

Tue 5/10/11 Experiment SIX - RNA Isolation and RT-PCR Analysis (GENE TWO)
Introduction 1: Discussion of Total RNA Quality – Eden Maloney and Elaine Chiu
Introduction 2: Review of cDNA Synthesis & RT-PCR – Eden Maloney and Elaine Chiu

B. cDNA Synthesis

Synthesizing cDNAs from Isolated Total RNA

C. RT-PCR

Amplification of cDNA Using PCR

Thu 5/12/11 Experiment SIX - RNA Isolation and RT-PCR Analysis (GENE TWO)

C. RT-PCR

Gel Electrophoresis of RT-PCR Products from 5-10-11

Experiment SEVEN - Identifying Features of Mutant Seeds Using Nomarski Microscopy (GENE TWO)

A. Observation of Plant & Seed Phenotypes

Examine and Compare Wild Type and Mutant Plants

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 5-12-11 to 5-20-11)

WEEK EIGHT

- Mon 5/16/11** *Introduction to Cloning of Promoters - Kelli Henry*
Discussion of Data from Experiment FIVE- Professor Bob Goldberg
EXPERIMENT FIVE LAB REPORT DUE
- Tue 5/17/11** **Experiment EIGHT - Amplifying & Cloning a Gene Upstream Region (GENE TWO)**
Introduction: Amplification & Cloning of Upstream Regions - Kelli Henry
Demonstration: Observation of Promoter::GUS-GFP Lines - Kelli Henry
- A. Amplification of a Promoter Region**
Amplification of a Promoter Region Using PCR
Gel Electrophoresis of PCR Product
- Thu 5/19/11** **Experiment EIGHT - Amplifying & Cloning a Gene Upstream Region (GENE TWO)**
Introduction: Transformation & Bacterial Techniques – Kelli Henry
- A. Amplification of a Promoter Region**
Ligating PCR Product into a Plasmid (pENTR/D-TOPO Vector)
- B. Transformation of E. coli Cells**
Transformation of *E. coli* Competent Cells with Ligation Mixtures
Incubating Cells Overnight at 37°C
- Fri 5/20/11** *Note: TAs move plates to cold room*

WEEK NINE

- Mon 5/23/11** *Discussion - What Did I Learn in HC70A and HC70AL? – Professor Bob Goldberg*
EXPERIMENTS SIX AND SEVEN LAB REPORTS DUE
- Note: TAs Inoculate TB Broth + Antibiotics with Selected Bacterial Colonies*
- Tue 5/24/11** **Experiment EIGHT - Amplifying & Cloning a Gene Upstream Region (GENE TWO)**
Introduction 1: Plasmid DNA Preparation – Kelli Henry
Introduction 2: Restriction Enzyme Digestion – Kelli Henry
- C. Isolation & Verification of Recombinant Plasmid DNA**

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

Thu 5/26/11 Experiment EIGHT - Amplifying & Cloning a Gene Upstream Region (GENE TWO)

C. Isolation & Verification of Recombinant Plasmid DNA

Analyzing and Verifying Promoter DNA Sequence

General Laboratory

Finish Experiments, Summarize Data & Prepare PowerPoint Presentation

WEEK TEN

Mon 5/30/11 *Memorial Day – No Class*

Tue 5/31/11 *Discussion of Data From All Experiments - Professor Bob Goldberg*
EXPERIMENT EIGHT LAB REPORT DUE
Clean-Up Benches, Summarize Data, & Organize Lab Notebook & Webbook
Organize & Practice Research Talks

Wed 6/1/11 **Exit Interviews with Professor Bob Goldberg**

Thu 6/2/11 **All-Class Research Symposium**

11th Week Exit Interviews with Professor Bob Goldberg