

**MOLECULAR AND INFORMATIC TOOLS TO IDENTIFY
SPECIFIC RICE DELETION MUTANTS**

**May 27-August 1
(11 wk, May 19-August 11)**

Wednesday, May 28

Day 1: *Welcome, Introductions and Overview: Summer Projects, Training Course and Overview of biology & genetics (as related to summer projects)*
Jan Leach & Bill Hsu

1. Introductions
2. Summer projects and training course
3. Lecture
4. Lab safety video- 30min
5. Tour of Throckmorton and greenhouses (Marietta)

LUNCH 12:00-1:30

Laboratory #1: Project assignments and lab orientation
Marietta and Grisel

1. Assignment of lab partners and workbench, location in lab of equipment, handout materials
2. Demonstrations: pipetman use; sterile technique; handling toxic chemicals (EtBr, acrylamide); use of UV light box, etc
3. Lecture: How to keep a research notebook: Grisel

Thursday, 5/29

Day 2: *Developing deletion mutant collection*
Patty Manosalva (9:00-10:15am)

- Mutagenesis strategies (insertion vs deletion)
 - Advantages and disadvantages of deletion mutants vs insertion mutants
- Mutagens (DEB, FN, GR)
 - What sorts of mutations do they cause?
 - How do they cause mutations?
 - Sizes of deletions caused?
 - Why use all three?
- Advancing the lines
 - What is M1, M2, M3, M4?
 - How are lines harvested & stored?
 - Types of mutations found: lesion mimic mutants, etc

Laboratory #2 : Isolate genomic DNA from plant tissue
Marietta (Patty to help) (10:30-5:00)

1. Mini lecture: genomic DNA extraction protocol
2. Extraction of genomic DNA from rice cultivars IR64 or a d1 mutant
3. Stop at incubation step in 37°C and finish after lunch

LUNCH 12:00-1:30

4. Continue DNA extraction lab

Friday, 5/30

Day 3: *Database development for rice deletion collection: e.g., IRIS*
Bill Hsu and Youping Deng (9:00-12:00) :

LUNCH 12:00-1:30

**Laboratory #2 cont: Genomic DNA extraction
(1:30-5:00)**

1. Mini lecture: Review from day before
2. DNA electrophoresis and quantify DNA with prepared standards
3. **Summary presentation of genomic DNA extraction results by first group**

Monday, 6/2

Day 4: Polymerase Chain Reaction (PCR)-based DNA Pooling Strategy for detection of deletion mutants (9:00-10:15)

Marietta Ryba-White

- Principle of PCR
- Principles of DNA Pooling:
 - what is 3-D pooling; why aren't we using it?
- How do we apply PCR to detection of deletions in target genes?

Laboratory #3: PCR experiment

Patty (Marietta to help) (10:30-5:00)

1. Mini Lecture: PCR details: steps, ingredients and what each does
2. Set up PCR reaction with IR64 DNA (Patty's primers)

LUNCH 12:00-1:30

3. Run gel
4. **Summary presentation of results of PCR lab results by second group**

Tuesday 6/3

Day 5: Designing Primers:

Patty Manosalva & Bill Hsu (9:00-12:00)

- eukaryotic gene structure
 - Where are conserved and unique regions?
- Gene databases:
 - how do we find sequences of our target genes
- important features of primers:
- designing a good primer:
- designing gene-specific vs gene family specific primers

LUNCH 12:00-1:30

**Laboratory #4 Designing primers to target genes: computer lab
(1:30-3:30 PM)**

3:00-5:00 SUROP meeting; others continue primer design

Wednesday, 6/4

Day 6: Modeling: mathematical models

Bill Hsu and Lev Kapilanski (9:00-12:00)

LUNCH 12:00-1:30

**Laboratory #4 (cont): Designing primers for target genes
Patty (1:30-5:00)**

1. Continue primer design

2. *Summary presentation of Primer design by third group*

Thursday, 6/5

Day 7: *Lecture: Cloning PCR fragments & plasmid isolation*
Patty Manosalva (9:00-10:00)

Laboratory #5: Cloning PCR fragments
Patty (10:30-5:00)

1. ligate DNA into TOPO vector
2. transform DNA into competent bacterial cells by heat shock
3. express 1hr

LUNCH 12:00-1:30

Laboratory #5: cont.

1. plate transformants on Xgal/Cb plates
2. pick colonies for plasmid prep (Pre-plated cells)
3. miniprep with precultured bacteria

Friday, 6/6

Day 8: *Lecture: Principles of sequencing DNA- tour facility*
Steven Brooks (9:00-10:00)
-Principles of sequencing

10:30: Tour of sequencing facility

LUNCH 12:00-1:30

Laboratory #6: Gel electrophoresis of plasmid DNA, preparation of plasmids for sequencing
Patty (1:30-5:00)

Monday, 6/9

Day 9: *Sequence analysis*
Steven Brooks (9:00-10:00)
-How to use programs for sequence comparisons and searches

Laboratory #7: Analyze sequence data
Steve and Patty (10:30-12:30)

LUNCH 12:30-2:00

Computer lab:2:30-4:30

Tuesday, 6/10

Day 10: *Microarray demonstration*
Jianfa Bai (9:00-12:00)

LUNCH 12:00-1:30

Laboratory #7: Continue
Patty & Marietta (1:30-3:00)

1. Continue sequence analysis
2. *Presentations from Group 1 on sequencing results*

3:00-5:00 SUROP

Wednesday, 6/11

**Day 11: *Modeling gene expression profiling data*
 *Bill Hsu (9:00-10:30)***

***Review of projects for the rest of the summer*
*Jan Leach (11:00-12:00)***

Computing lab schedule: 213 Nichols, Bill Hsu **Bill: is this just the 3 CSI students?

Monday, 6/9

1:30-2:30 PM, , Bill Hsu, RobyJoehanes

Thursday, 6/12

2:30-4:30 PM, Bill Hsu, RobyJoehanes

Monday, 6/16

2:30-4:30 PM, Bill Hsu, RobyJoehanes

Thursday, 6/19

2:30-4:30 PM, Bill Hsu, RobyJoehanes

Monday, 6/23

2:30-4:30 PM, Bill Hsu, RobyJoehanes

Groups

Florence Loo and Ryan Williams

Brandon Robben and John Gu

Veronica Blackman and Alex Garrett

SUPPLIES:

Pipetmen: 3 of each P10, P20, P200, P1000

6 sharpies

6 blue notebooks

6 1" binders

plastic sheet covers

6 glue sticks

3 ice buckets

IR64 or d1 plant tissue

Solutions for genomic DNA extraction

Solutions for plasmid DNA extraction

Gels w/ EtBr

Loading buffer

Safety glasses

Topo vector

Chemically competent cells

42° C waterbath

37° C shaker

65° C shaker

Centrifuge

Rnase

Ethanol- 95% and 70%

TE

Chloroform/isoamyl alcohol 24:1

2x CTAB

Bmercaptoethanol

Solutions I,II,III for alkaline lysis

Pcr machine

Xgal/IPTG/CB agar plates

Pcr tubes, buffer, MgCl, dNTP's, primers, DNA, Taq, HPLC water

Sterile microfuge tubes

Tips

Sterile 10ml glass tubes with lids

Sterile mortars and pestles

Liquid nitrogen

Sterile 50ml tubes

Sterile centrifuge tubes

IR64 DNA

Designed primers for their projects

Oxalate oxidase

PLD

Chitinases

Dehydrins

14-3-3

Chalcone synthase

Assign 2 genes per group

Websites for references and bioinformatics

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