**Arabidopsis-based Genetics Project Lab.**

**Instructor Materials: Grading Rubrics and Instructions for Laboratory Preparation**

This document includes various materials developed by the instructor to aid in teaching the Arabidopsis-oriented genetics project lab, including grading rubrics for assignments and TA prep information. Some of this information is very specific to the particular research program on which the project lab was based.

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**TA Prep for Bio 224**

For the Arabidopsis lab the specific *axr4* enhancer lines used are not available, but many other mutant Arabidopsis lines are available from the Arabidopsis Biological Resource Center or from Lehle Seeds. See the 3rd section below, “What If the Instructor Doesn’t Have a Genetics Research Program?”, for additional information on possible Arabidopsis mutants that could be used.

Be sure genetic project lab room is clean & organized.

The following solutions will be used throughout the semester:

1. Growth medium for Arabidopsis seeds, without hormones: called “Ats” (Arabidopsis thaliana with sucrose). This medium contains 0.7% bacto-agar, 1.0 % sucrose, 0.05 mM Fe-EDTA, 5 mM KNO3, 2 mM Ca(NO3)2, 2.5 mM KPO4, 2 mM MgSO4, 70 μM H3BO3, 14 μM MnCl2, 0.5 μM CuSO4, 1 μM ZnSO4, 0.2 μM NaMoO4, 10 μM NaCl and 0.01 μM CoCl2

This medium is not commercially available. To make it, first prepare 6 stock solutions, as follows (recipes below are given for making 1 liter of each stock solution but 100 mL of each stock solution should be adequate for a semester-long course). Dissolve the indicated amounts of each chemical in about 800 mL of ddH2O first before bringing up to the final volume:

a. 20 mM Fe-EDTA (EDFS): Dissolve 7.34 g in ddH2O, bring final volume to 1 liter

b. 1 M KNO3 (MW 101.1): Dissolve 101 g in ddH2O, bring final volume to 1 liter.

c. 1 M Ca(NO3)2-4 H2O(MW 236): Dissolve 164.1 g in ddH2O, bring final volume to 1 liter.

d. 1 M KPO4:dissolve 123.8 g KH2PO4 and 15.79 g K2HPO4 in ddH2O, bring final volume to 1 liter.

e. 1 M MgSO4 (anhydrous, MW 120): Dissolve 120 g in ddH2O, bring final volume to 1 liter.

f. Micronutrients: Dissolve the following chemicals in ddH2O and bring the final volume to 1 liter.

70 mM H3BO3 (MW 61.8): 4.33 g

14 mM MnCl2-4H2O (MW 197.9): 2.77 g

0.5 mM CuSO4-5 H2O (MW 249.7): 0.125 g

1 mM ZnSO4-7H2O (MW 287.6): 0.288 g

0.2 mM NaMoO4-2 H2O (MW 241.98): 0.0484 g

10 mM NaCl (MW 58.44): 0.58 g

0.01 mM CoCl2-6H2O (MW 237.9): 0.0024 g

To make Ats, prepare 985 mL of ddH2O. Add 5 mL of 1 M KNO3, 2.5 mL of 20 mM Fe-EDTA, 2 mL of 1 M Ca(NO3)2, 2.5 mL of 1 M KPO4, 2 mL of 1 M MgSO4, and 1 mL of the micronutrients solution. Pour the solution into a 2 liter flask, and add 7 g of agar and 10 g of sucrose. Autoclave, cool, and pour about 25 mL per 100x15 mm plate (preferably in a laminar flow hood) to make about 40 plates per liter of solution.

As an alternative, one could use 1/2 X Murashige & Skoog (MS) salts. MS salts are commercially available (e.g. Sigma #M5524). After making the liquid solution according to the manufacturer’s directions, add the agar and sucrose as above and autoclave.

2. Growth medium for Arabidopsis seeds with inhibitory concentration of synthetic auxin: called “4D”

To Ats growth medium, after autoclaving, add 40 μL of 10 mM 2,4-dichlorophenoxyacetic acid (2,4-D) solution (see # 3 below) to bring to a final concentration of 4 x 10-7 M. Swirl gently to mix the 2,4-D into the Ats solution and then pour as above.

3. Stock auxin solutions:

2,4-D (MW 221): To make 5 mM (5 x 10-3 M) 2,4-D stock, dissolve 1.105 mg of 2,4-dichlorophenoxyacetic acid in 1.0 mL of 50% ethanol, shake or vortex until dissolved. 10 mM stock can similarly made at 2.21 mg/mL in 50% ethanol. Dilute with 50% ethanol as needed to make lower concentration.

1-naphthalene acetic acid (1-NAA) (MW 186.2): 5 mM (5 x 10-3 M) 1-NAA stock requires 0.93 mg 1-NAA per 1 mL of 50% EtOH.

indole -3-acetic acid (IAA) (MW 175.2): 50 mM (5 x 10-2 M) IAA stock requires 8.76 mg of IAA per 1 mL of 50% EtOH.

4. Sterilizing solution: 30% bleach, 0.002% Triton-X 100 in water

Make up 20% solution of Triton-X 100 in water (10 mL of TritonX-100 in 50 mL disposable tube, add 40 mL of sterile distilled water and let dissolve or rock gently until it dissolves). Add 30 mL of fresh bleach to 70 mL of distilled water, add 10 microliters of 20% TritonX-100 solution and mix.

5. Top agar: 100 mL ddH2O + 0.7 g agar

6. TBE buffer for running gels

**For 1st week (Jan. 25):**

Students will be sterilizing seeds and plating, and starting to make plates.

Need, in general for the labs:

Full boxes of 1 mL pipet tips, autoclaved

Micropipettors

2 x 150 mL beakers per table for waste solutions

rectangular plastic tub for waste tips, 1 per table

Sharpies

labeling tape,1 roll/table

Monday

6 x “4D” plates per pair of students = make 60 plates (1.5 liters of media)

9 bottles of top agar, each with 100 mL per bottle (can resterilize at end of lab) [melt the top agar before the lab and keep in water bath at 55 degrees C]

Sterilizing solution (make up fresh): 25 mL for each table in small flask

9 bottles of sterile water: about 75-100 mL per 125 mL bottle, autoclaved, 1 bottle per pair of students

2 x 150 mL beakers per table for waste solutions

micropore tape for wrapping plates

6 boxes of 5 mL pipets (autoclaved)

6 electronic PipetAids or manual green plastic Pi-pumps

The following seeds in labeled tubes, 1 set per pair of students:

Just a little of each of these 4 controls:

* wtWs
* wtLer
* *axr4-3 M30-3*
* enhancer

Somewhat more seed of F2 from enhancer x Ler, in 2 tubes

Wednesday Jan. 27

Re-autoclave:

* bottles of top agar
* bottles of sterile water
* cans of 5 mL pipets

Three ”ATS” plates per pair of students (total will need about 30 plates, = 0.75 liter of media)

1 bottle of top agar per pair of students

Sterilizing solution (make up fresh): 25 mL for each table in small flask

1 bottle of sterile water per pair of students

150 mL beaker for waste

Rectangular plastic tub for waste

Pipettors

Cans of 5 mL pipets

Micropore tape

The following seeds for each pair (10 sets):

wtCol

*axr4-3 M30-3*

enhancer

All available electronic balances capable of 0.01 g accuracy (ideally, 6)

For each table:

10 x 250 mL Erlenmeyer flasks and 2 x 500 mL Erlenmeyer flasks (empty)

Weigh paper

2 Spatulas

Agar (about 7 g, in a small clean beaker)

Sucrose (about 10 g, in a small clean beaker)

Small pieces of foil to cover flasks

Sharpies

Labeling tape

1000 mL Erlenmeyer flask filled with nanopure water

Stock solutions to make Ats: transfer needed amounts for 1 liter of media to small tubes, 1 set of 6 tubes per table; label tubes with ingredient

500 mL graduated cylinders, 1 per table (if available)

In front of lab: trays to put plates on

**For 2nd week (Feb. 1)**

**Students will make plates of different auxins at different concentrations, and transfer seedlings to these plates**

Monday Feb. 1

Use sterile hoods

Make sure autoclave is on and reserved for the class time

Stock solutions: Prepare the following stock solutions, all dissolved in 50% ethanol. Make up the first solution in each table, then make up the following by serial dilution as shown in the table using 1.5 mL microcentrifuge tubes. Then aliquot 100 µL into 0.5 mL microcentrifuge tubes so that each group will have a set of the tubes of each auxin that they will need.

(NAA and IAA made up fresh; protect IAA from light; concentrations 10,000X those given in the lab manual on pp. 32-33 and reproduced in tables below), and 1 mL of 50% ethanol as control (keep these by hoods)

|  |  |  |  |
| --- | --- | --- | --- |
| **1-NAA**  **Desired final concentrations** | **Required stock solution** | *Add* | *to* |
| 5 x 10-7 M 1-NAA | 5 x 10-3 M 1-NAA | See #3 at beginning of TA prep | 1.5 mL of 50% ethanol, adjust to give correct concentration |
| 3 x 10-7 M 1-NAA | 3 x 10-3 M 1-NAA | 0.84 mL of 5 x 10-3 M | 0.56 mL of 50% ethanol |
| 1.5 x 10-7 M 1-NAA | 1.5 x 10-3 M 1-NAA | 0.7 mL of 3 x 10-3 M | 0.7 mL of 50% ethanol |
| 0.5 x 10-7 M 1-NAA | 0.5 x 10-3 M 1-NAA | 0.47 mL of 1.5 x 10-3 M | 0.93 mL of 50% ethanol |
| 0 auxin | 50% ethanol | -- | -- |

|  |  |  |  |
| --- | --- | --- | --- |
| **2,4-D**  **Desired final concentrations** | **Required stock solution** | *Add* | *to* |
| 5 x 10-7 M 2,4-D | 5 x 10-3 M 2,4-D | See #3 at beginning of TA prep | 1.5 mL of 50% ethanol, adjust to give correct concentration |
| 3 x 10-7 M 2,4-D | 3 x 10-3 M 2,4-D | 0.84 mL of 5 x 10-3 M | 0.56 mL of 50% ethanol |
| 1.5 x 10-7 M 2,4-D | 1.5 x 10-3 M 2,4-D | 0.7 mL of 3 x 10-3 M | 0.7 mL of 50% ethanol |
| 0.5 x 10-7 M 2,4-D | 0.5 x 10-3 M 2,4-D | 0.47 mL of 1.5 x 10-3 M | 0.93 mL of 50% ethanol |
| 0 auxin | 50% ethanol | -- | -- |

|  |  |  |  |
| --- | --- | --- | --- |
| **IAA**  **Desired final concentrations** | **Required stock solution** | *Add* | *to* |
| 50 x 10-7 M IAA | 50 x 10-3 M IAA | See #3 at beginning of TA prep | 1.5 mL of 50% ethanol, adjust to give correct concentration |
| 25 x 10-7 M IAA | 25 x 10-3 M IAA | 0.7 mL of 50 x 10-3 M | 0.7 mL of 50% ethanol |
| 10 x 10-7 M IAA | 10 x 10-3 M IAA | 0.56 mL of 25 x 10-3 M | 0.84 mL of 50% ethanol |
| 5 x 10-7 M IAA | 5 x 10-3 M IAA | 0.7 mL of 5 x 10-3 M | 0.7 mL of 50% ethanol |
| 0 auxin | 50% ethanol | -- | -- |

100 mL graduated cylinders (at least 1 per table, 2 if available)

a few small pieces of foil for those who need them

bags of 100x15 mm Petri plates: will need 150 plates (6 bags); arrange these by the hoods

Also, bring to project lab room the trays of flasks the students made on Monday, from the refrigerators.

Wednesday Feb. 3

Set up by hoods:

Forceps in foil-wrapped packets, autoclaved (2 per packet; 1 packet per pair if possible)

In hood: small beakers (80-100 mL) half-filled with 95% ethanol; keep covered until before lab; put Kimwipe in bottom to protect tips

Gloves

Sharpies

Micropore tape

“Blank” plates, about 80 (just 0.7% agar + water)

Sharpies

Rulers, mm scale, 1/student

**3rd Week: The students will analyze the seedlings**

Monday, Feb. 8

Plates from growth chamber

Blank plates: need ~8 per pair (just water + 7 g agar/liter)

Forceps (don’t need to be sterilized)

Sharpies

Pots of dirt in green trays

Labeling stakes

Wednesday, Feb. 10

Large plates from growth chamber

Small rulers

Dissecting microscopes

Sharpies

Laptop computers

**4th Week (Feb. 15-17): Data analysis and literature search**

Need laptop computers

**5th Week (Feb. 22-24): Project planning and project plan presentations**

Need laptop computers

**6th Week through 10th Week (March 1-April 7): Independent projects**

Equipment and supplies needed will depend on student projects, but can anticipate that will need the following:

Seed sterilizing solution

Sterile water

Ats stock solutions

**11th Week (April 14-16): Plant DNA isolation & PCRs**

Mon.: The students will collect tissue and isolate DNA

Weds.: The students will set up PCRs

Mon.

We need the trays of plants that they put out two weeks ago from the plant room.

We also need:

* wooden applicator sticks (may be box in plant room), Sharpies, and tape for labeling the plants
* small scissors & forceps
* small beakers of ethanol
* gloves
* Kimwipes
* 0.65 mL microcentrifuge tubes (one 400-600 mL beaker’s worth per table, autoclaved)
* strip tubes (8 well) + caps: not the BioRad ones; 5 per group for Mon., 3 per group for Tues.
* QuickExtract DNA solution (this is an expensive solution; we will keep the main stock ourselves, & give it to students as they need it. Have 1.5 mL tubes ready for the aliquots. Each group should figure out how much they need—100 µL per plant—and receive a tube with this amount + 25 µL extra. Impress upon them to be careful in their pipetting!).
* micropipettors
* boxes of tips for 100 μL pipettors
* colored long skinny microcentrifuge tube racks, 1 per pair
* microcentrifuges with adapters for the small tubes placed in them
* ice buckets (Styrofoam boxes already in lab are fine)
* freezer boxes (square colored plastic boxes)
* PCR machines

You should prepare a large beaker of the 0.5 mL tubes, autoclaved, for the reagents.

We need to be able to spin strip tubes.

Please use a large black Sharpie to label the plastic freezer boxes “Bio 224”. Also, have several different colors of labeling tape on hand for the students to use in labeling their boxes.

Weds.

* all available PCR machines
* containers for ice (Styrofoam boxes), 1 per pair
* pipettors (if we can get more of the 10 µL pipettors that would be great)
* \*pipet tips: boxes for 100 and 10 µL pipettors, autoclaved and dried (ideally, two boxes per table)
* rectangular plastic tubs for pipet tip waste
* beakers of 0.5 mL PCR tubes, autoclaved, one per table
* Sharpies

Set up the microcentrifuges with inserts for spinning the small tubes

Tubes of reagents: keep these in the -20 freezer & only get them out as needed. When they are out, always keep them on ice. Tell the students to put their initials on the tubes once they take them and to keep them in their own freezer boxes.

Use the 0.5 mL tubes. We’ll need 1 tube of each per pair for the first class but it’s good to have backups. We will be using these reagents for the rest of the semester, so you could prepare lots of extra tubes and keep them in our –20 freezer (not in the lab!).

* water (sterile distilled nanopure), 400 µL
* 10 mM MgCl2, 60 µL (take a tube of 20 mM MgCl2 and dilute it 1:1 with ddH2O, or it may need to be made up).
* The following 3 reagents are in plastic packets from New England Biolabs,
* 10X PCR buffer (included with Taq polymerase, New England Biolabs #M0273), 120 µL
* 2 mM dNTPs, 120 µL
  + Stock tubes concentration is 10 mM (New England Biolabs #N0447). Thaw on ice, take 250 µL into each of two 1.5 mL microcentrifuge tubes on ice, add 1.0 mL (1000 µL) of sterile ddH2O to each tube. This gives you the desired final concentration of 2 mM. Aliquot; you should get 20 aliquots of 120 µL.
* Taq polymerase, 6 µL (5 U/µL) (New England Biolabs #M0273); try to get this to the bottom of the tubes.

Primers: For each primer pair to be tested, make one combined tube containing the two primers at 12.5 µM each in a total combined volume of 22 µL

The stocks are in the -80 degree Celsius freezer. There may be separate tubes for the two primers in a pair labeled 1 and 2, or they may have been combined already. If only the master stocks are left (the ones in the white capped larger tubes), they are at 100 µM.

Calculate how much room will be needed in the PCR machines to make sure the students’ reactions will fit.**12th Week (April 21-23)**

## The students will perform gel electrophoresis on the PCR samples that they prepared in the previous lab.

The students have already prepared the gels and poured the 1X TBE buffer on the gels.

## We need:

pipettors

pipet tips (10 µL)

racks

## 6X loading dye (Orange G): Dissolve 12 g of sucrose in 40 mL distilled water, add 60 mg of Orange G dye, bring to 50 mL with water. Aliquot 150 µL into each of 20 small tubes, labeled “6X LD”

MW marker (use the low molecular weight ladder from New England Biolabs, catalog #N3233): It needs to be thawed, diluted, and aliquoted. After thawing, keep on ice. Add 800 µL of TE buffer and 100 µL of 10X loading dye mix (blue). Mix and aliquot into 10 µL aliquots into individual small tubes, labeled “LMW”.

4 of the “Scooter 100” gel apparatuses

with lids and white plastic “gel scoop”

an extra flask to collect used 1X TBE buffer in

Power supplies, 4

4 Tupperware (plastic) boxes for staining the gels

waste containers: for pipet tips

ethidium bromide bottle (from our lab)--replenish the ethidium bromide

The students will also collect F2 tissue and prepare F2 DNA

Need the trays of plants from the plant room

Forceps & 0.5 mL tubes

**13th-14th Weeks (Rest of semester)**

Students will carry out more PCRs and run more gels. Prepare more primers, reagents for PCR, and gel reagents as for the weeks of April 14 and 21:

10X buffer: 25 µL

2 mM dNTPs: 25 µL

10 mM MgCl2: 25 µL

water: 250 µL

Taq: 2 µL

For the Arabidopsis lab the specific lines used are not available, but many other mutant Arabidopsis lines are available from the Arabidopsis Biological Resource Center or from Lehle Seeds

**Adapting the Genetics Project Lab to a Once-a-Week Schedule**

One unusual feature of the genetic project lab as it is currently offered is that it is scheduled to meet for 5 hours a week (twice a week for 2-1/2 hours each time). This schedule was adopted after a number of years in which the lab was scheduled more conventionally for once a week (also for 2-1/2 hours) (in both cases for a 15 week semester). When it was offered on a once a week schedule, students were asked to come in occasionally at other times during the week to carry out various tasks necessary to facilitate progress in the lab. In many cases these tasks were brief and simple ones such as moving plates from the refrigerator into a growth chamber, but even these arrangements were often difficult for our students due to scheduling conflicts with other classes and particularly with outside employment. Some of these tasks could have been carried out by the instructor or teaching assistant, but we wanted as much as possible for the students to carry out every step, rather than (as often happens with more traditional labs) having so much work taken care of for them “behind the scenes” that they lose a sense of the actual processes necessary for an experiment to be completed. Additionally, lab safety required either the teaching assistant or instructor to be present whenever students were in the genetics lab room, and this requirement also created scheduling difficulties. Scheduling was especially challenging during the portion of the semester when students worked on independent projects, as the students frequently needed individual assistance and the time demands often became very challenging for the instructor and teaching assistants. We have found that with the twice-a-week schedule, most of the students are able to carry out their independent projects primarily during the scheduled time. However, knowing that many instructors will need to teach within a once-a-week schedule, we present these additional resources:

1. Capsule descriptions and schedules of two alternative once-a-week lab courses, abstracted from earlier offerings of this course; and

2. An alternative once-a-week schedule for the lab course as given in the lab manual.

**1. Alternative once-a-week labs**

As mentioned, the specific experiments in the lab evolved over the years that the project lab was taught using Arabidopsis, in parallel with changes in the instructor’s (Hobbie’s) research. The two example lab courses given here both included a classical genetics project and a molecular genetics project. One semester the classical genetics project consisted of selection of mutants followed by a small amount of characterization (described in section A below), and in another semester the classical genetics project consisted of more extensive characterization of an already-isolated mutant (described in section B below). The molecular mapping project was similar in both semesters (described in section C below). The capsule descriptions are followed by week-by-week schedules.

A. Selection and characterization of mutants

i. At the first class meeting, students selected mutants from an M2 population (with wild-type controls). The seed had already been sterilized and grown on plates to the stage at which students could identify mutants based on root growth phenotype (in this case, resistance to auxin inhibition of root growth). However, a wide variety of types of mutations could potentially be selected for, depending on the interest of the instructor. For this lab the instructor generated the M2 seed, but mutagenized seed can also be purchased from Lehle Seeds.

ii. The mutants were placed into dirt and grown, along with wild-type controls.

iii. After a few weeks, students carried out crosses between their putative mutants and wild type (the instructor carried out crosses as backups, as student crosses were generally unsuccessful).

iv. About two months after the first class, students collected M3 and F1 seeds. Both F1 and M3 were analyzed for the originally-selected phenotypes, which allowed tentative determination of inheritance patterns.

v. M3 analysis also included a small independent project in which students analyzed another aspect of the phenotype, different from the originally-selected root phenotype and following procedures designed by the students with the instructor’s guidance.

B. Characterization of a previously-isolated mutant

i. Students characterized inheritance of the mutation by analyzing phenotypic ratios in an F2 population that had been generated by the instructor.

ii. Students developed, presented plans for, carried out, and then presented results from an independent project in which they characterized a novel aspect of the mutant phenotype.

C. Mapping a mutation

i. Early in the semester, students screened either an F2 or F3 mapping population (both generated by the instructor by crossing a recently-isolated mutant with the appropriate wild type of a different ecotype) for informative F2 or F3 plants.

ii. Students isolated DNA from the F2 or F3 seedlings and then carried out PCR mapping reactions using a genome-spanning panel of markers.

iii. If a rough location could be determined, then students could generate additional PCR markers in the targeted area and begin fine mapping.

We recognize that technological advances such as next-generation sequencing provide alternative approaches to mutation identification that may be preferable, if such methods are available for use in the lab.

Schedule of 1st example of project lab (starting with mutant isolation, parts A+C above)

[Items in brackets are suggestions of what to do outside of scheduled lab period.]

|  |  |  |
| --- | --- | --- |
| **Date** | Classical genetics project | **Molecular genetics project** |
| Week 1 | Select mutants, into dirt  [Finish mutant selection] |  |
| Week 2 | [Check plants; remove tray cover] | Sterilize F2 seed from mapping cross, spread on plates  [Staff will put plates at 4° & then into growth chamber] |
| Week 3 | [Check plants; water if needed] | DNA preps on control Col & Ler seedlings |
| Week 4 | Demonstration of crosses  [Check plants; crosses with mutants] | Score F2 seedlings for mapping; DNA preps on F2 mutant seedlings |
| Week 5 | [Crosses with mutants; check plants & crosses] | Set up PCRs |
| Week 6 | [Crosses with mutants; check plants & crosses] | Run gels on PCRs |
| Week 7 | [Check plants & crosses; stop watering plants if appropriate] | Discuss results, plan next experiments; set up PCRs |
| Week 8 | Presentations of projects [Collect crosses if ready] | [Continue mapping] |
| Week 9 | [Collect crosses if ready; harvest mutant seed if ready] | Discuss results, identify markers  [Continue mapping] |
| Week 10 | Sterilize, begin characterization of mutants | Continue mapping  1st lab report due |
| Weeks 11-13 | Characterization of mutants | Continue mapping |
| Week 14 | Poster presentation | Finish mapping & analyze results |
| Week 15 | Lab notebook due | Final lab report due |

Schedule of 2nd example of genetics project lab (starting with existing mutants, parts B+C above)

|  |  |  |
| --- | --- | --- |
| **Dates** | **Classical genetics project** | **Molecular genetics project** |
| Week 1 | Introduction  Review of micropipettor use  Sterilize control and F2 seeds for analysis of inheritance; spread on plates  Begin literature review |  |
| Week 2 | [One day before lab: Put plates into growth chamber]  Continue literature review | Introduction  Sterilize Col, Ler, mutant, and F3 seeds; spread on plates |
| Week 3 | Analyze inheritance using F2 seeds  Plant F2 seedlings  Continue literature review  Plan project: discuss with instructor | [One day before lab: Put plates into growth chamber]  Prepare Col and Ler DNA (from genetics project seedlings) |
| Week 4 | Revise project and prepare presentation | Analyze F3 seedlings  Prepare F3 DNA |
| Week 5 | Present project to class  Begin experiments | Set up 1st (control) PCR |
| Week 6 | Present project to class  Begin experiments | Gel electrophoresis of 1st PCR  Set up 2nd PCR (using F3 & control DNA) |
| Week 7 | Experiments | Mapping, continued: PCR and electrophoresis |
| Week 8 | Experiments | Mapping, continued: PCR and electrophoresis |
| Week 9 | Experiments  Discuss progress with instructor | 1st lab report due on mapping project  Choose additional markers if feasible |
| Week 10 | Experiments | Continue mapping |
| Week 11 | Experiments  Analysis of results  Prepare poster | Continue mapping |
| Week 12 | Analysis of results  Prepare poster | Continue mapping if necessary  Analysis of results |
| Week 13 |  | Analysis of results |
| Week 14 | Presentation of results (poster session) |  |
| Week 15 |  | Final lab reports due  Lab notebooks due |

**2. Adapting the given lab to a once-a-week schedule**

The suggested schedule below essentially follows the schedule of the second example above. Because it would be impossible to fit in all four of the originally-planned experiments into a once-a-week schedule, we suggest that the instructor should choose either to carry out the analysis of inheritance (experiment 1) or of an aspect of the phenotype (experiment 2). An alternative schedule that more closely follows the original schedule is to delay the start of the molecular genetics project until the independent project is finished or almost finished, to enable students to concentrate on their independent project.

|  |  |  |
| --- | --- | --- |
| **Dates** | **Classical genetics project (Expt 1 or 2, and Expt 3)** | **Molecular genetics project (Expt 4)** |
| Week 1 | Introduction  Review of micropipettor use  Sterilize control and experimental seeds for analysis of inheritance or phenotype; spread on plates  Begin literature review |  |
| Week 2 | [One day before lab: Put plates into growth chamber]  Continue literature review | Introduction  Sterilize Col, Ler, mutant, and F2 or F3 seeds; spread on plates |
| Week 3 | Analyze inheritance using F2 seeds or analyze phenotype  Continue literature review  Plan project: discuss with instructor | [One day before lab: Put plates into growth chamber]  Prepare Col and Ler DNA (from genetics project seedlings) |
| Week 4 | Revise project and prepare presentation | Analyze F2 or F3 seedlings  Prepare F2 or F3 DNA |
| Week 5 | Present project to class  Begin experiments | Set up 1st (control) PCR |
| Week 6 | Present project to class  Begin experiments | Gel electrophoresis of 1st PCR  Set up 2nd PCR (using F3 & control DNA) |
| Week 7 | Experiments | Mapping, continued: PCR and electrophoresis |
| Week 8 | Experiments | Mapping, continued: PCR and electrophoresis |
| Week 9 | Experiments  Discuss progress with instructor | 1st lab report due on mapping project  Choose additional markers if feasible |
| Week 10 | Experiments | Continue mapping |
| Week 11 | Experiments  Analysis of results  Prepare poster | Continue mapping |
| Week 12 | Analysis of results  Prepare poster | Continue mapping if necessary  Analysis of results |
| Week 13 |  | Analysis of results |
| Week 14 | Presentation of results (poster session) |  |
| Week 15 |  | Final lab reports due  Lab notebooks due |

**What if the Instructor Doesn’t Have a Genetics Research Program?**

For instructors who do not currently have a genetics research program, we present the following suggestions for a lab with a focus on auxin physiology and genetics that could be carried out using Arabidopsis materials that are available from the Arabidopsis Biological Resource Center. Note that the stock center has quite reasonable charges (currently $10/vial) for academic use, but for most stocks provides only small amounts of seed (about 30 seeds per vial), so that for use in a class, the instructor would need to be able to grow Arabidopsis to bulk up seed stocks. A number of websites and several laboratory handbooks give information on basic cultivation, crossing, and methods of analysis of Arabidopsis. These resources are listed at this site: https://www.arabidopsis.org/portals/education/aboutarabidopsis.jsp

We suggest that students could examine the auxin physiology of two different well-characterized auxin-resistant mutants, *axr1* and *aux1* (stock numbers are given at the end of this section). The *AXR1* gene is important for auxin perception (Leyser et al., 1993, Nature 364,161), with the result that mutants in *AXR1* are resistant to all types of auxin. The *AUX1* protein, on the other hand, is involved in auxin import into cells (Bennett et al., 1996, Science 273, 948), so that mutants in *AUX1* are resistant only to those types of auxins that enter cells through active import and show no change in their response to auxins that enter cells primarily by diffusion (Yamamoto & Yamamoto, 1998, Plant Cell Physiol. 39, 660; see lab manual, section on Experiment 2, for background and discussion). Students would carry out Experiment 2 using these mutants instead of the *axr4* and enhancer lines used in the original plan. The expected result is that seen in Yamamoto and Yamamoto (1998): *axr1* mutants will be resistant to IAA, 1-NAA, and 2,4-D, whereas *aux1* mutants will be resistant to IAA and 2,4-D but not to 1-NAA.

Additional characterization experiments with great visual appeal could be carried out with *aux1* and *axr1* lines that also contain the auxin-inducible DR5::GUS promoter. The DR5::GUS reporter construct gives a strong signal in regions of the plant with high auxin concentrations, and the GUS staining is relatively straightforward to carry out (e.g. Li 2011, Bioprotocol.org: e93). Notable differences will be seen between the mutants and wild-type plants. Both *aux1* and *axr1* also show defects in root gravitropism that can be assayed using square petri plates that permit growing seedlings to be turned 90 degrees.

Because of the limited commercial availability of F2 populations, especially mapping populations, it is more challenging to adapt the classical genetics experiment in which mode of inheritance is determined and the molecular genetics/ molecular mapping experiment. One possibility is to create a mapping population for either *aux1* or *axr1* by crossing the alleles above with the *Ler* line and allowing the F1 plants to self-fertilize (this would require enough familiarity with Arabidopsis to carry out crosses and, again, growing space suitable for growing up the plants). The resulting F2 seed could be screened by the students both to determine mode of inheritance of the mutations (recessive for both *aux1* and *axr1*) and for mapping, with the auxin-resistant F2 seedlings used as the mapping population. A second possibility would be to use the *aux1-100* allele, which results from insertion of a T-DNA into the *AUX1* gene (Bennett et al., 1996). Students could develop primers flanking the T-DNA insertion site and assay the *aux1-100* line and the parental *Ws-2* ecotype to detect the insertion.

These are the ABRC stock numbers of the relevant lines:

Col-0 (wild-type Columbia) stock CS76778

*aux1-7* stock CS3074 or CS16704 (contains DR5::GUS)

*axr1-3* (weak allele) stock CS3075 or CS16705 (contains DR5::GUS)

*axr1-12* (strong allele) stock CS3076

Landsberg *erecta* stock CS24596

*aux1-100* (T-DNA insertion line) stock CS9586

*Ws-2* parental line of *aux1-100*, stock CS2360

DR5::GUS line with wild-type like expression, stock CS16703

Genetics Project Lab

**Evaluation of Lab Report [same form used for 1st and 2nd reports]**

**Name:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Strong** | **Good** | **Acceptable** | **Needs more** |
| Introduction: clear description of goals & hypotheses? |  |  |  |  |
| Introduction: clear description of isolation of enhancer? Of approach? |  |  |  |  |
| Methods: clear & concise? Includes timing? |  |  |  |  |
| Results: clear & concise description? Accurate? |  |  |  |  |
| Results: figures clear, axes properly labeled? |  |  |  |  |
| Results: Statistical analysis properly carried out? |  |  |  |  |
| Discussion: accurate? Refers to hypotheses & statistical analysis? Compares to previous results? |  |  |  |  |
| Overall: clearly written? |  |  |  |  |
| Shows good understanding of project? |  |  |  |  |
| In own words? |  |  |  |  |
| References: proper format? |  |  |  |  |
| Mechanics (grammar, spelling) |  |  |  |  |

**Overall grade**

**Comments**

**Genetics Project Lab**

**Evaluation of Project Presentation [middle of semester]**

**Names:**

**Project Topic:**

**Date:**

|  |  |  |  |
| --- | --- | --- | --- |
| Feature | Pointspossible | Pointsreceived | Comments |
| Giving practice presentation on time to instructor | 10 |  |  |
| Choice and design of experiments and controls: good rationale for choice? Use of scientific literature? Clear statement of hypothesis? Well-designed controls? | 30 |  |  |
| Understanding and explaining background and related knowledge | 30 |  |  |
| Use of PowerPoint and manner of presentation | 20 |  |  |
| Written summary of plans handed in day of presentation | 10 |  |  |
| TOTAL | 100 |  |  |

**Comments:**

Lab Poster Presentation [end of semester]

**Name(s):**

**Topic:**

**Mutant**

|  |  |  |  |
| --- | --- | --- | --- |
| Feature | PointsPossible | PointsReceived | Comments |
| Abstract | 5 |  |  |
| Introduction:  Clear?  Good explanation of why the experiment was done?  Hypothesis? | 20 |  |  |
| Results:  Clearly presented?  Statistical analysis?  Correct controls? | 25 |  |  |
| Discussion:  Clear conclusion on hypothesis?  Good understanding of meaning of p value?  Correct interpretations of results? | 25 |  |  |
| Verbal presentation | 15 |  |  |
| Appearance of poster | 10 |  |  |
| Both partners contributed |  |  |  |
| If problems: sought help? |  |  |  |
| TOTAL | 100 |  |  |

Comments:

**Evaluation of Final Lab Report on Experiment 4**

**Name:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Strong** | **Good** | **Acceptable** | **Needs more** |
| Introduction: clear description of background of lab? What’s the enhancer, where does it come from, why are we studying it? |  |  |  |  |
| Introduction: clear description of goal? How does molecular mapping work, & why are we doing it? |  |  |  |  |
| Methods: clear & concise? Includes timing? |  |  |  |  |
| Results: clear & concise description of experiment tried and results obtained? Explanation of what’s visible on gel? |  |  |  |  |
| Discussion: addresses why PCRs didn’t work? What would be next step? |  |  |  |  |
| Discussion: what would results have been expected to look like for linkage, and for no linkage? |  |  |  |  |
| Overall: clearly written? |  |  |  |  |
| Shows good understanding of project? |  |  |  |  |
| In own words? |  |  |  |  |
| References: proper format? |  |  |  |  |
| Mechanics (grammar, spelling) |  |  |  |  |

**Overall grade**

**Comments**