Prep Monday

Weller: Bring 3 microscale balances. Bring BSA. Bring food color if not found last week, and soap, oil, corn syrup. To put by the microscope: bring a tomato plant with flowers, also bring some of the scrapings of the fungus that were not frozen. Bring 10X F, 5X H and 50% G solutions. Bring gr-TE solution. Bring $CHCl_3$? Bring 1L each of solutions A,B,C,D. Check for herring sperm DNA. Bring the multi-dispenser pipetter and attachments.

1. Next to each balance place
    a. 100 ml Beaker of water
    b. 50ml tube of 50% glycerol
    c. Parafilm squares
    d. Small weigh boats
    e. Kimwipes.

2. Each bench (per team) should have as basic equipment/disposables
    a. 3 each of these serological pipettes (2,5,10)
    b. Green pipette pump
    c. Micropipetter set
    d. Micropipetter tips.
    e. Minifuge
    f. Vortexer
    g. Boxes with microfuge tubes
    h. Waste box for tips and tubes
    i. Kimwipes
    j. Racks for tubes
    k. Sharpie marking pens
3. Reagents <u>per bench</u> should include
    a. 5 x 15-ml tubes, each containing 15 ml of a solution labeled A=5M KCl, C=10% SDS (soap), B=Corn syrup, and D=oil, E = isopropanol. Color each to be a different color.
        i. Note: combining equal volumes of 3M KCl and 5% SDS should lead to a precipitate (10ul of each was added to a 100ul starting concentration so we might get away with less) .
    b. 4 x 15-ml Tubes, empty
    c. 1 x 15-ml tube of isopropanol per team.
    d. 3 x 1.7ml tubes labeled 10X F (0.5M DTT, 5% SDS), 5X H (1M KCl) and 50% G (glycerol), add 200ul of the provided stock solutions to each.
    e. 1 x 1.5ml 3M sodium acetate per team
    f. 1 x 1.5ml tube of chloroform (CHCl3)
    g. 1 x 15 ml tube of ethanol per team.
    h. 1 x1.5 ml microfuge tube labeled DNA with 100ul of herring sperm DNA and 20ul of 3M sodium acetate.
    i. 1 x 1.5 ml microfuge tubes labeled BSA with with 100ul of BSA + 250ul of gr-TE.

j. Place some of the autoclaved Nanodrop water (orange-cap reagent bottles) into 15 ml tubes – label as Molecular Biology grade water.

Prep Tuesday (Day 1 DNA purification)

Weller: Bring crushed ice for ice buckets and dry ice for procedure. Bring the leaf samples collected this year at Crowders (on dry ice). Bring leaves from 7 of the Chatahoochee offspring. Make 1L of grinding buffer and 1L of CTAB buffer to bring in. Aliquot the beta-mercaptoethanol, sorbitol, and PVPP. Bring mortars and pestles. Bring Chloroform. Bring Biohazard wates bag, bottle and secondary container. Bring Popsicle sticks for scraping. Bring another microfuge. Bring some squares of foil to set the mortars in ice in.

1. Make sure the large-format centrifuge is out and have a balance next to it with a beaker on it for balancing the tubes.
2. Make sure the water bath is full and being heated to 65C.
3. Make sure the minifuges and microfuges are set out.
4. Verify that Miracloth is at each bench, in a Baggie with a weigh boat (x2)
5. Place a Baggie with 3-4 Popsicle sticks in it at each bench.
6. Place an ice bucket, 2 mortars and 2 pestles are at each bench.
    a. Fill the ice bucket with wet ice and embed one of the mortars in it.
7. Place 4 x 15 ml Falcon tubes at each bench
8. Make sure the large-format centrifuge is plugged in, and that there is a balance next to it with a small beaker for placing tubes in (for adjusting balance).
9. Make sure there is a bag of 9 x 5 ml serological pipettes and a green pipette pump in the chemical hood.
10. Make sure the Biohazard waste containers are set up in the hood.
11. Place 2 x 5 ml serological pipettes and a green pipette pump at each station.
12. Aliquot 14 ml of Grinding buffer per station (use a 15 ml tube) – label it
13. Aliquot 14 ml of CTAB buffer per station
14. Make a 50-50 mixture of Grinding Buffer and CTAB buffer, then dispense 1.5ml per station in a 1.7ml microfuge tube.
15. Provide 1ml of 5M NaCl in a 1.7ml microfuge tube per station.
16. Place 0.5 gm of PVP and 0.5gm of sorbitol in a 1.5 ml microfuge tube and place at each station.
17. Measure 500ul of beta-mercaptoethanol into a 1.5 ml microfuge tube and place at each station.
18. Measure 500ul of Tween 20 into a 1.5ml microfuge tube and place at each station
19. Place 40 ml of isopropanol into a 50 ml tube and place at each station.
20. Place 40ml of 70% isopropanol in a 50 ml tube at each station.
21. Place 1.5ml of 70% ethanol in a 1.7ml microfuge tube per station
22. Place 1.5ml of 100% ethanol in a 1.7ml microfuge tube per station
23. Verify that there is a 1.5ml tube of 3M sodium acetate at each bench.

Prep Wed. (Day 2 DNA purification)

Weller: bring coolers with crushed ice for ice buckets

1. Make up 20 ml of 1X TE from the 100X stock. Aliquot 1 ml into a 1.5 ml microfuge tubes and place at each station.
2. Set a heating block to 37C (or the water bath)
3. Aliquot 25ul of RNAase A into a 0.5ml microfuge tube and place at each station
4. Place 50ul of Proteinase K into a 0.5 ml microfuge tubes and place at each station
5. Put 10ml of ethanol into a 15 ml tube and place at each station
6. Make sure there is a 1.5ml tube of 3M Sodium Acetate (NaOAc) at each station
7. Make up 100ml of 70% ethanol by measuring 70ml of 100% ethanol in a graduated cylinder and adding 30ml of molecular biology grade water.
    a. Place 10ml of 70% ethanol in a labeled 15ml tube and place at each station.
8. Make sure there are Kimwipes at each station.
9. Make sure there is a 1.5ml tube with 3M sodium acetate at each station.
10. Place the phenol-chloroform bottle in the chemical hood, with a 1000 ul micropipetter and the appropriate tips. Set the micropipetter to 500ul.
11. Make sure the chemical disposal bottle is labeled Organic Waste and the contaminated waste bag is present in the hood.
12. Make sure the high-speed microfuges are available .
13. Place the phenol-chloroform bottle in the chemical hood, with a 1000 ul micropetter and the appropriate tips.

Prep Thursday: DNA Quantification and Gel Electrophoresis

Weller: Bring a Nanodrop to use for DNA measurement. Set up the optical spectrophotometer in the back of the room with cuvettes and solutions in the right range for reading. Place control material used for dilution series, and buffer at each station (ask Ms. Smith for details on this)*****

1. Set up the Nanodrop – it needs water and TE, a 2ul pipetter and tips and Kimwipes.
2. Get out the microwave oven.
3. Get out heat-protective gloves for handling the hot solution.
4. Warm the water bath to 60C, so the agarose can be kept molten until students are ready to pour the gels.
5. Get out electrophoresis mini-gel casting trays and combs, tanks and power supplies – one per bench
6. Get out a 250ml Erlenmeyer flask for each station.
7. Make up 2L of 1X TBE buffer (use 200ml of 10X TBE buffer and 1600 ml of the Nanopure water.) Mix thoroughly and label. Dispense 400ml into a 5 stock reagent bottles and put one on the benches being used.
8. Weigh out 5.0g of Agarose into a labeled 15 ml tube, per bench.
9. Make up 5L of 0.5X TBE (250ml of 10X TBE buffer in 4.75 L of Nanopure water) buffer to use as Electrophoresis running buffer. Put a 1L reagent bottle of this on each bench.

10. Measure out 5ul of DNA ladder into 0.5ml tubes for each station.
11. Make sure each bench still has the 1X TE buffer tube.
12. Place 5 glass Pasteur pipettes at each bench and a green pipette pump. \
13. For each group, measure out 20ul of Gel Loading Buffer into a labeled 1.5 ml microfuge tube.

Prep Friday – Field Trip to Dr. Weller's Lab.

We will do restriction digestion of DNA followed by size selection using magnetic beads, ligate adaptors and do PCR. This is material that we will do sequencing on later in the year.