Prep Monday1 (pipetting and centrifuging practice)

(Amounts are per team unless otherwise noted)

1. For the class: make sure the benchtop mini-microfuges, the large-format centrifuge, and the high speed microfuges, and the analytical balances are out.
2. 100 ml beakers, 3 serological pipettes (2,5,10ml), 100 ml water, green pipette pump, Parafilm, weigh boats, micropipetter set, tips.
3. 4x 50-ml tubes, each containing 15 ml of a solution (these are A=water, B=soap, C=Corn syrup, and D=oil). Add food coloring so each is a different color.
4. 1 x 15-ml tube of isopropanol per team.
5. 1 x1.5 ml microfuge tube with 100ul of herring sperm DNA
6. 1 x 1.5 ml microfuge tube with 3M sodium acetate (NaOAC) per team.
7. 4 x 15 ml tubes – empty, for measuring mixtures of Solvents A,B,C,D
8. Place some of the autoclaved Nanodrop water (orange-cap reagent bottles) into 15 ml tubes – label as Molecular Biology grade water.

Prep Tuesday1 (Day 1 DNA purification)

1. Weller: bring coolers with crushed ice for ice buckets and dry ice for procedure. Bring the leaf samples collected this year (on dry ice).
2. Make sure the water bath is full and being heated.
3. Verify that Miracloth is at each bench, in a Baggie with a weigh boat.
4. Place an ice bucket, a mortar and a pestle at each bench, fill the ice bucket with ice and embed the mortar about half-way in it. Place the pestle on ice near it. Place a sturdy craft stick next to that (for scraping sample into tubes)
5. Place 3 x 15 ml Falcon tubes at each bench in a rack.
6. Make sure the box with 1.5 ml microfuge tubes has some in it.
7. 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).
8. Make sure there are 9 x 10ml serological pipettes and a green pipette pump in the chemical hood.
9. Place a 5 ml serological pipette and a green pipette pump at each station.
10. Aliquot 5 ml of Grinding buffer per station (use a 15 ml tube) – label it
11. Aliquot 5 ml of CTAB buffer per station in a 15 ml tube, label it.
12. Place 0.5 gm of PVP and 0.5gm of sorbitol in a labeled 1.5 ml microfuge tube and place at each station.
13. Measure 60ul of beta-mercaptoethanol into a labeled 1.5 ml microfuge tube and place at each station.
14. Measure 220ul of Tween 20 into a labeled 1.5ml microfuge tube and place at each station
15. Verify that there is a 1.5ml microfuge tube containing 3M sodium acetate, and a 15 ml tube of isopropanol at each station.

Prep Wed1. (Day 2 DNA purification)

1. There is a bottle of 1X TE in the hood. Aliquot 10 ml per team in 15ml Falcon tubes. If you run short, make up 100 ml of 1X TE from the 100X stock (1ml in a 100ml graduated cylinder, add 99 ml of molecular Biology grade water – mix thoroughly).
2. Set a heating block to 37C (or the water bath).
3. Place 12ul of RNAase A into a labeled 1.5ml microfuge tube and place at each station
4. Place 23ul of Proteinase K into a labeled 1.5 ml microfuge tubes and place at each station
5. Put 10ml of ethanol into a labeled 15 ml tube and place at each station
6. Make up 100ml of 70% ethanol by measuring 70ml of 100% ethanol in a graduated cylinder and adding 30ml of molecular biology grade water. Place 10ml of 70% ethanol in a labeled 15ml tube and place at each station.
7. Make sure there are Kimwipes at each station.
8. Make sure there is a 1.5ml tube with 3M sodium acetate at each station.
9. Place the phenol-chloroform bottle in the chemical hood, with a 1000 ul micropetter and the appropriate tips. Set the micropipetter to 500ul.
10. Make sure the chemical disposal bottle is labeled Organic Waste and the contaminated waste bag is present in the hood.
11. Make sure the high-speed microfuge is available (find the lid).

Prep Thursday1 (DNA quantification for concentration and size with electrophoresis)

1. Get out the spectrophotometer
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 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 Nanodrop 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. Make sure each bench still has a 1.5ml microfuge tube with DNA as a label.
11. Make sure each bench still has the 1X TE buffer tube.
12. Place a cuvette at each bench
13. Place control material used for dilution series, and buffer at each station (ask Ms. Smith for details on this)\*\*\*\*\*
14. Place 5 glass Pasteur pipettes at each bench and a green pipette pump.
15. For each group, measure out 20ul of Gel Loading buffer into a labeled 1.5 ml microfuge tube.
16. For each group, measure out 5ul of DNA ladder into a labeled 0.2ml tube (PCR tube).
17. Make up 1X FastBlue gel stain, and locate a staining tray and some plastic wrap to cover the gel over the weekend.

Prep Monday2 (PCR of chloroplast intergenic region and 3 RAPD PCR markers)

1. Weller bring crushed ice, 50mM MgSO4, and 4 tubes of PCR primers (10uM stocks of {CP-f + CP-rev}, OPC-10, OPJ-08, OPO-13), some holders for PCR tubes.
2. Get out the PCR machines and program as follows (and label so students know which is which):
   1. RAPD profile
      1. 98C 4 minutes
      2. 10 cycles of
         1. 98C 30sec
         2. 30C 30sec
         3. 68C 60sec
      3. 35 cycles of
         1. 98C 30sec
         2. 35C 30sec
         3. 68C 60sec
      4. 72C 7min
      5. 4C HOLD
   2. CP profile
      1. 95C 4 min
      2. 35 cycles of
         1. 92C 20sec
         2. 48C 20sec
         3. 72C 20sec
      3. 72C 7 min
      4. 4C HOLD
3. Check that the set of micropipetters, tips and the box of 1.5ml microfuge tubes are all well supplied at each station.
4. Place an ice bucket at each bench and fill with ice
5. Place a box with ~10 PCR tubes at each place
6. Make the following Master Reagent (MR) mix, then aliquot 105ul per labeled tube and place in ice bucket at each bench. Thaw each reagent (except the Taq), vortex and quick-spin, then store on ice. Add the reagents in the following order to a 1.5ml microfuge tube, mixing well after each addition, store on ice.
   1. 605ul MB-H2O
   2. 125ul 10X PCR buffer (no Mg)
   3. 100ul dNTP (10mM stock)
   4. 100ul MgSO4 (50mM stock)
   5. 50ul BSA (10mg/ml stock)
   6. 20ul Taq polymerase (5U/ul stock)
   7. Place 110ul of this mixture in 1.5ml microfuge tubes, label, place on ice at each station. Students will use 20ul of this per PCR tube, add 3ul of DNA (at 10ng/ul) and 2ul of primer (at 10uM primer) for a total of 25ul.
7. Aliquot 5 ul of each primer stock into a labeled PCR tube, place in ice bucket at each position.
8. Measure 1ml of Molecular Biology Grade water (MB-H2O) into a labeled 1.5ml microfuge tube and place in the ice bucket at each place.
9. Make sure the transilluminator/light box and gel documentation station are out, to take a picture of the gel from Friday.

Prep Tuesday2 (1.5% agarose gels of PCR products, more PCR reactions)

1. Check that students have agarose, 250 ml Erlenmeyer flasks, 1X TBE buffer, casting trays and combs, DNA ladder and gel loading dye.
2. Make sure the microwave is available.
3. Make up the same PCR MasterMix – this time students will do 4 microsatellite primers, supplied as pairs at 10uM: uS27, uS49, uS85, uS212.
4. Use the Chloroplast PCR profile PCR machine.
5. Make up 1X FastDNA stain and make sure staining trays and plastic wrap are available.
6. Make sure the transilluminator and gel documentation station are available.

Prep Wednesday2 (Agarose gels of second set of PCR products, analysis of data)

1. Check that students have agarose, 250 ml Erlenmeyer flasks, 1X TBE buffer, casting trays and combs, DNA ladder and gel loading dye.
2. Make sure the microwave is available
3. Make up 1X FastDNA stain and make sure staining trays and plastic wrap are available.

Prep Thursday 2 (set up stations for parents, make posters)

1. Weller bring wet ice, dry ice, some leaves – check if chloroform is needed.
2. Have a pre-stained gel to show.
3. Make sure the PCR products that worked are available – stain with EtBr for immediate show and tell.