Prep Monday – PCR of 4 RAPD Markers

Weller: bring crushed ice, 50mM MgSO4, 4 tubes of 10uM stocks of RAPD primers, 100ul each. Bring 2 PCR machines. Check the Nanopure water carboy and refill as needed.

1. Get out the PCR machines and program as follows (and label so students know which is which):
    a. RAPD profile
        i. 98C 4 minutes
        ii. 10 cycles of
            1. 98C 30sec
            2. 30C 30sec
            3. 68C 60sec
        iii. 35 cycles of
            1. 98C 30sec
            2. 35C 30sec
            3. 68C 60sec
        iv. 72C 7min
        v. 4C HOLD
    b. CP –Microsatellite profile
        i. 95C 4 min
        ii. 35 cycles of
            1. 92C 20sec
            2. 48C 20sec
            3. 72C 20sec
        iii. 72C 7 min
        iv. 4C HOLD
2. Check that the set of micropipetters, tips and the box of 1.5ml microfuge tubes are all well supplied at each station.
3. Place an ice bucket at each bench and fill with ice
4. Make sure there is a box of PCR tubes at each station
5. Make two batches of the following Master Reagent Mix (MRM), then aliquot 110 ul into two labeled tubes (one for each sample) 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 2.0ml microfuge tube, mixing well after each addition, store on ice.
    a. 605ul MB-H2O
    b. 125ul 10X PCR buffer (no Mg)
    c. 100ul dNTP (10mM stock)
    d. 100ul MgSO4 (50mM stock)
    e. 50ul BSA (10mg/ml stock)
    f. 20ul Taq polymerase (5U/ul stock)
    g. Place 110ul of this mixture in each of two 0.5ml microfuge tubes, label, place on ice at each station. Students will use 20ul of this per PCR tube, add 3ul of DNA (at 50ng/ul) and 2ul of primer (at 10uM primer) for a total of 50ul.
6. Aliquot 5 ul of each primer stock into a labeled PCR tube, place in ice bucket at each position.

7. Measure 1ml of Molecular Biology Grade water (MB-H2O) into a labeled 1.5ml microfuge tube and place in the ice bucket at each station.
8. Make sure the transilluminator/light box and gel documentation station are out, to take a picture of the gels.
9. Verify that students have agarose and 10X TBE available, a 250ml flask, a 100ml graduated cylinder, gel casting tray, end gaskets and comb for pouring a gel.
10. Place 10ul of DNA ladder in a 0.5ul tube at each station.
    Place 100ul of 4X Gel Loading Buffer at each station.
11. Set up an electrophoresis station, with power supplies and electrophoresis tanks, and a carboy containing ~5L of 1X TBE buffer.

Prep Tuesday - agarose gels of PCR products, more PCR reactions

Weller: Bring crushed ice, 4 tubes combining the f/r microsatellite primers at 10uM, 200ul each.

1. Make sure the PCR protocol has been programmed correctly
2. Check that students have ~2g agarose, 250 ml Erlenmeyer flasks, 100ml 1X TBE buffer, mini-gel casting trays and combs, a 100ml graduated cylinder.
3. Make sure the N
4. Set out 10ul of DNA ladder per station
5. Make sure there is ~100ul of 4X Gel Loading Dye available.
6. Make sure the microwave is available.
7. Make up the same PCR MasterMix as yesterday – this time students will do 4 microsatellite primers, supplied as pairs at 10uM: uS27, uS49, uS85, uS212.
8. Use CP-Microsatellite profile on the PCR machine.
9. Make sure the transilluminator and gel documentation station are available.
10. Set up an electrophoresis station, with power supplies and electrophoresis tanks, and a carboy containing ~5L of 1X TBE buffer.

Prep Wed. – agarose gels of second set of PCR products, analysis of data

Weller: bring ?

1. Check that students required materials to run gels, if we still have reactions to visualize.

Prep Thursday: Make posters and prepare materials for the demonstrations for parents.

Weller: bring dry ice and crushed ice for the grinding demonstration. Print out gel pictures and whatever other images students need for their posters.