**071020 PCJ**

**General PCR procedure**

Do 2 separate PCRs for each genomic sample, using different sets of primers; see primer log for primer sequences. Choose 2 primer pairs from the available primer sets:

Long primer pair, UF15/ HR1654, expected size 1682 bp

Mid primer pair, Hemat18Sf #74/Hemat18Sr #75, expected size 451 bp

Short primer pair, HR1654/HF1487, expected size 187 bp

ITS1 primer pair, Hsp7F/Hsp9r expected size ~650 bp (varies with number of repetitive units in ITS1)

1. Remove 2 genomic DNA plates that were paired for extraction from the freezer to thaw. Using the paired plates together in PCR allows you to conserve extraction positive & negative control wells, giving you more flexibility for PCR control wells, re-runs, etc.
2. Remove PCR reagents\* from freezer to thaw.
3. Keep all PCR reagents & DNA on ice. There are blue ice trays for the plates & cocktail troughs (store upside down in freezer). Reagent & cocktail tubes can be kept in a bucket of ice.
4. On the Hemato PCR mixes excel sheet, in cell F2, enter the number of PCRs you wish to do; the sheet will update the reagent volumes. Include 10% extra reactions for the all-inclusive pipetting allowance. For a pair of plates, enter 212.
5. On spreadsheet, decide which wells will be used for extraction & PCR controls; list the sample numbers.
6. Label 4 new PCR plates (mark so can’t get plate turned upside down). On each plate include PCR date, PCR primers, genomic DNA plate name. Label all plates on 2 sides with all info to protect against accidental erasures. Tip: circle the control wells on the PCR plates with a Sharpie.
7. Label 2 cocktail tubes with primer names.
8. Use filter tips for everything.
9. For 212 reactions, mix the cocktails separately in two 5 ml tubes. Add all reagents, except the polymerase; vortex. Shake down tubes, return to ice.
10. Line up a rack of 96 filter tips with 1 extraction plate (makes it easier to keep track). Tip: to prevent unintended transfer of material from negative extraction control wells to PCR plate, remove the corresponding tips from the rack of tips (should also correspond to the wells circled on the PCR plates). Transfer 3.0 ul genomic DNA from each well in DNA extraction plates into a PCR plate for the first primer pair, except for some of the extraction negative control wells. If the paired extraction plates are PCR’d together, a total of only 1 positive & negative extraction control is needed. If 2 unpaired plates are PCR’d together, then 1 positive & 1 negative extraction control from each genomic plate should be used. PCR controls\*\* should be used in place of the other ‘negative’ controls from the extraction plate. Add PCR positive control template to appropriate well(s). Set aside PCR plate. Into a second PCR plate for the second primer pair, again transfer 3.0 ul genomic DNA to PCR wells, repeating the controls. Keep in mind that the 2 PCRs from each genomic extraction will be combined post-PCR to run on a gel, so the pattern of controls between the 2 primer sets for each plate of genomic samples must be the same. That is, if well A12 on the first primer plate (UF15/ HR1654) has positive control material, then A12 on the second PCR plate (Hemat18Sf #74/Hemat18Sr #75) for that same extraction should also have the same positive control material.
11. Repeat step 9 with second extraction plate.
12. Should now have 4 PCR plates with genomic templates, extraction positive & negative controls, & PCR positive controls.
13. To the 2 cocktails, add polymerase, gently invert tubes to mix if using NEB One Taq; Bioline Taq can be briefly vortexed. Shake down tubes. Pour 1 cocktail into trough.
14. Line up a rack of tips with each PCR plate. Pipet 22.0 ul cocktail into each of the 2 PCR plates labeled for that primer set. Pipet up & down gently twice to mix. Add PCR negative control water to designated wells. Seal plates, leave on blue ice.
15. Repeat step 14 with the second cocktail (use a new trough), & the 2 plates labeled for that primer set.
16. Spin down PCR plates before putting into thermal cyclers.
17. Use thermal cycling conditions\*\*\* on the spreadsheets.
18. After PCr, store amplified plates in freezer until ready to run products on gels.

\*NEB One Taq: New England Biolabs, OneTaq DNA Polymerase: M0480S, M0480L, M0480X.

Biolase DNA Polymerase: Bioline, BIO-21042, BIO-21043, BIO-21066.

dNTPs: Bioline, BIO-39028, BIO-39029

water: molecular grade

\*\*A note on positive controls: it is helpful to have as many positive controls on the gel as possible for comparing sizes of amplified bands. On large gels with few samples that are positive, it’s easiest with several controls. Having a different pattern of controls for the 2 extraction plates will help diagnose any errors made during set up of PCR (inverting, mixing up plates, etc).

\*\*Any change in thermal cycling conditions, or polymerase, often necessitates re-optimization of the assay, so proceed slowly if changes are made.