Molecular Markers in Chestnut

RAPD markers

V_t = 25 ul
1uM primer (OPA 2,3,4,7,9,10) give 8-12 bands each
0.2mM each dNTP
1X RAPD PCR buffer.
50 ng DNA
1.5U Taq

Master Mix: if you are adding almost the same thing to every reaction, you can make one solution and subdivide it among the tubes, then add the last component. This limits the pipetting you have to do, and thus the errors and loss of precision from performing many steps. There are 6 different primers, but everything else is the same. Since there are errors associated with pipetting small volumes,, always make at least 10% more volume than you think you need. Here, the final volume per reaction is 25ul. For 6 reactions and a negative control, this is 175ul. I will make enough for 8 reactions, or 200ul.

Master Mix - 22ul (8 reactions) = 180 ul (leaving 2.5ul per reaction for primer)
Add in the given order, mix carefully, then place 22ul in each of 7 tubes

Component	Concentration	Vol to use (ul)
Water	NA	140
RAPD PCR buffer	10X	17.6
dNTP mix	2.5mM each	16
DNA	100ng/ul	4
Taq pol.	5U/ul	2.4

Label 7 PCR tubes and put them on ice. Put 22.5ul of the master mix in each.
Then add 2.5ul of a primer to a labeled tube, and add 2.5ul of water to the 7th tube (the primers are at 10 uM).

<u>PCR ThermoCycling Profile</u>
Melt
    94C for 5 min
Cycle 45 times
    94C 5 sec
    36C 30 sec
    72C 1 min
Finishing
    72C 5 min
4C hold

Place the tubes in the thermocycler and start the program. It will take several hours to run - by using a 4C hold you are able to let it sit overnight.
Remove 5ul of each PCR reaction from the tube, add 1.5ul of 4X loading dye.

Visualize on 1.5% agarose gels run in 1X TBE buffer, include EtBr in gel.

ISSR Markers

Vt = 20 ul
30 ng template
0.25mM each dNTP
1 U Taq
1uM primer [$(CA)_7$, $(TG)_7$, $(GT)_7$, $(CA)_8R$, $(AC)_8T$ where R is A or G)
1X ISSR PCR buffer

For ISSR reactions
Master Mix - 18ul * 7 reactions = 126 ul as a total volume (leave 2ul per reaction for primer)
Add in the order give, mix carefully, then place 18ul in each of 6 tubes (one is a negative control)

Component	Concentration	Vol to use (ul)
Water	NA	94.5
ISSR PCR buffer	10X	14
dNTP mix	2.5mM each	14
DNA	100ng/ul	2.1
Taq pol.	5U/ul	1.4

Label 6 PCR tubes and put them on ice.
Add 2ul of a primer to a labeled tube, and add 3ul of water to the 6th tube (the primers are at 10 uM).

PCR ThermoCycling Profile
Melt
94C 4min
Cycle x 27
94C 30 sec
52C 45 sec
72C 2 min
Finish
72C 7 min
4C Hold

Place the tubes in the thermocycler and start the program. It will take several hours to run - by using a 4C hold you are able to let it sit overnight.
Remove 3ul of each PCR reaction from the tube, add 1.0ul of 4X loading dye.
Visualize on 6% acrylamide /8M urea gels run in 1X TBE buffer.
Stain with SYBR Green I

How to make components

1X RAPD PCR buffer

50mM KCl
20mM Tris-HCl pH 8.2
2.5mM MgCl2
1mM DTT
0.5% glycerol
0.1% Tween20
0.1% NP40

RAPD primers are at 100uM. Dilute 3.33ul to 100ul in water.

1X ISSR PCR buffer

50mM KCl
1.5mM MgCl2
10mM Tris-HCl pH 9

ISSR primers are at 100uM. Dilute 10ul to 100ul in water.