How to set up a PCR for microsatellites

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Before you begin

1. Print out the PCR sheets for the samples you plan to amplify. You should do four PCRs at once and also print out 4 different PCR sheets with the respective primers and their annealing temperatures.

2. We will do the PCRs in 96-well-plates. Thus, it is advisable to set up your DNA extractions in strip tubes with strip caps. That makes all the consecutive pipetting steps much easier as you will be able to use a multichannel pipet for loading the DNA extractions into the PCR plates.

3. UV four 96-well-plates and 4 silica mats (remember you can reuse your 96-well-plates and silica mats). I recommend doing 4 PCRs at once, because you will later combine 4 plates into one plate to run on the 3730 sequencer as up to four different microsatellite primers can be combined/multiplexed.

4. Make sure that four 1.5 ml tubes are also UVed. You will use these to prepare the master mix for your PCR reactions.

5. Sign-out 4 thermal cyclers/PCR machines that hold 96-well-plates. It will take you about an hour to set up the PCR so plan accordingly (30 min once you get more familiar with the procedure) and the thermal cycler program will take about 2 hours to complete. If the lab is too busy at that time, sign out for 1-2 and only perform 1-2 PCRs at the time. You will then store those PCRs in the fridge until you are able to do the others. PCR products will be fine for one week if wrapped in aluminum foil. Never load less than 4 PCRs onto one plate for the sequencer as running samples on the sequencer is the expensive part!

Getting started

1. Label your 4 PCR plates with the number of the specific PCR (as indicated on the PCR sheet) and date them. Always give your PCRs consecutive numbers.

Load the DNAs into the 4 PCR plates. For this, set up the 4 plates in the exact same orientation (well A1 in the upper left). You will use 1 µl of DNA extraction per reaction. Use the 8-channel multichannel pipet and set to 1 µl. Use the same set of pipet tips four times for aliquoting the same DNA into the same position on all four plates. Remove the strip caps from the DNA extractions (first row of 8), suck up 1 µl of DNA, then pipet that into the bottom of your first plate (first column A1 through H1). Then, go back to your plate with the DNA extractions, suck up 1 µl of DNA, pipet that into your second plate, continue for the third and fourth plate. Then, eject the pipet tips and get a new set of 8 tips for the second row of 8 samples, that will be pipetted into the wells A2 through H2. So on....

Place the silica mats on the PCR plates after you are done pipetting the DNAs to prevent evaporation. Label the silica mats, so that you always put them on in the same orientation and never mix the mats for the four different plates! Get some ice and keep your plates on ice.

Quickly spin the down the plates to insure that the DNA is at the bottom of each well.

2. Get the reagents for your PCRs out of the freezer. You will need the following (for each master mix, but keep in mind that you will be setting up 4 master mixes using 4 different primer pairs in each tube).

For each Master Mix you will need the following:

Reagent	For 1 reaction	For *100 reactions
H_2O	4.0	400
BSA	2	200
roche buffer (10x)	1.0	100
dNTPs	0.6	60
Forward primer*	0.4	40
Reverse primer*	0.4	40
free Taq	1.0	100
DNA	1.0	Not in Master Mix!
Master mix		9 µl in each well

Use new tip each time and rinse pipet tip with what's already in the vial. Be patient, Taq is thick...

Get this from the freezer: 2 tubes with H2O (1.6 ml total), 2 tubes with BSA (800 µl total).
* You will find on the PCR sheet, which primer to use! Each master mix will be prepared using a different pair of primers!

3. Store the Taq polymerase on ice as this enzyme is temperature sensitive. Defrost the other reagents. Label the four 1.5 ml tubes (with the exact same PCR number that you have on your PCR sheet and also on your PCR 96-well-plates). Then, pipet 4 different master mixes using 4 different sets of primers into 4 different 1.5 ml tubes (labeled). Make sure not to mix up your reagents during this step. No need to use filtered tips for microsatellite PCRs, without filters is fine and less expensive.

4. Once your master mixes are ready, get the electronic pipet (20-200 µl or 10-100 µl). Get a box of yellow tips without filters. Set the electric pipet to 9 µl (mode "multi"). Now, it is time to dispense the master mix into the plate that already contains the DNA. Make sure to always pipet the master mix on the side on one corner of the well. This will make it easier to keep an overview of which wells already have master mix in them. Add the master mix to all 96 wells of one plate and then put the septa back on. Quickly spin down in the plate centrifuge and then place in thermal cycler/PCR machine. This is the program for microsats:

Program			
Initial denaturation	95°C	4 min	
Denature	95°C	30 sec	35 x
Anneal	varies*	30 sec	
Extension	72°C	45 sec	
Final extension	72°C	7 min	

* the annealing temperature varies between 50 and 61ºC and depends on the primer pair you are using. The appropriate annealing temperature should be noted on your PCR sheet.

4. After your PCR is finished, take it out of the PCR machine, make sure the septa is on tight, and wrap it in aluminum foil (the labeled primers are light sensitive), and store in the fridge until you are ready to load on the 3730 sequencing machine (there are also instructions for this).