PCR – MicSat – used for automatic sequencers

1. 3.65 µl ddH_2O
2. 0.35 µl 10mM $MgCl_2$
3. 0.8 µl 10mM dNTPs
4. 1.0 µl 10x PCR Buffer (with 15 mM $MgCl_2$)
5. 2.25 µl 2µM primer1+ primer2 with M13 addition on its 5' end
6. 0.75 µl 2µM M13 primer labeled with a fluorescent dye

Mix and add 9 µl of master mix to each reaction. Add 1 µl DNA (5-100 ng/µl).

1. 93°C for 120 sec. (hot start)
2. 93°C for 20 sec. (denature)
3. 58°C for 35 sec. (anneal – exact temperature depends on the primers)
4. 68°C for 60 sec. (extend)
5. 93°C for 20 sec. (denature)
6. 53°C for 35 sec. (anneal)
7. 68°C for 60 sec. (extend)
8. 68°C for 30 min. (final extend)
9. 4°C forever (hold)

Repeat steps 2 – 4 30 times (DNA product amplification), and repeat steps 5 – 7 10 times (labeling of product with fluorescent dye). The length of the last 68°C extension depends on the type of a microsatellite primer. It is there to make sure all primers have the terminal adenine added, and thus "microsat stutter" is reduced (see Brownstein et al. 1996). Annealing temperature depends on the primer used, and length of denaturation and annealing depends on the thermocycler. Lowering the annealing temperature to 53°C causes the M13 primer to start being incorporated, thus fluorescently labeling the micsat product. If the annealing temperature of your primers is low and similar to the M13 annealing temperature, the process will simultaneously amplify and label product, but appears to be slightly less efficient.

The PCR product will need to be diluted before it can be resolved on an automatic sequencer. Depending on the strength of the reaction, 1:10 to 1:50 dilution will work. For loading on an automatic sequencer, 2 µl of the diluted product are added to 0.2 µl of GENESCAN 400HD size standards from ABI or equivalent such as MegaBACE ET400, and 7.8 µl of Hi-Di Formamide or Tween20. The PCR product does not have to be purified, but purification increases signal quality on an automatic sequencer, and removes the initial dye peak.