PCR – MicSat – used for agarose/acrylamide gel visualization

1. 1.85 µl ddH_2O
2. 0.35 µl 10mM $MgCl_2$
3. 2.0 µl Cresol Red dye
4. 0.8 µl 10mM dNTPs
5. 1.0 µl 10x PCR Buffer (with 15 mM $MgCl_2$)
6. 3.0 µl 2µM primer1+primer2

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. 68°C for 7 min. (final extend)
6. 4°C forever (hold)

Repeat steps 2 – 4 35 times. The last 68°C extension can also be left out. Annealing temperature depends on the primer used, and length of denaturation and annealing depends on the thermocycler. The addition of the Cresol Red dye into the sequencing reaction allows direct loading onto an agarose/acrylamide gel of the PCR product.