Protocol for PCR amplification of SSR marker

Basic PCR Protocol for single sample

1. Prepare ice bucket with crashed ice
2. Bring DNAs, Primers, PCR reagents (except for Taq DNA polymerase, see tips) as below from the stocked place (freezer), and place on ice.
3. Place PCR tubes on ice
4. Set up a 10 µL PCR reaction (Keep all your reagents on ice):

    7.15 µL of DW (autoclaved distilled water)

    1 µl of 10x Taq buffer

    0.8µl of dNTPs

    0.25 µL of Forward Primer (25 µM stock)

    0.25 µL of Reverse Primer (25 µM stock)

    0.5 µL of Template DNA (~20 ng/µL)

    0.05 µL of Taq DNA Polymerase (bring just before use from freezer)
5. Place reaction tubes in PCR thermal cycler and start program (see below).

Tips:

1. Taq DNA polymerase is a kind of enzyme and quite temperature sensitive. Taq DNA polymerase should be keep at freezer and bring just before use. After use, return to freezer immediately.

2. If you have several samples to test for a same SSR marker, you had better to make pre-mixture of PCR reaction (see below section for preparation for multiple samples, calculate for your sample number)

Prepare the pre-mixture for multiple samples

1. Multiply the volume of each reagent by the number of individual PCR reactions you wish to examine and add ~10% extra to account for pipetting error. In this example, you make 10 different PCR reactions (you have 10 samples to be examined), so we multiply each volume by 11 (=10+1).
2. In a single Eppendorf tube (1.5mL) combine the following:

    DW: 7.15µL x 11 samples = 78.65µL

    10x Taq buffer: 1µL x 11 samples = 11µL

dNTPs: 0.8μL x 11 samples = 8.8μL

Taq DNA polymerase: 0.05μl x 11 samples = 0.55μL (bring just before use from freezer)

3. Mix the above contents and keep tube on ice.
4. Transfer 9.5μL of pre-mixture into each PCR tube.
5. Add 0.5μL of template DNA into each sample tube.
6. Set the tubes on the PCR thermal cycler, and start program (see below)

PCR Program

Step1: 94°C for 3 min (Initial denaturation)

Step2: 94°C for 30 sec (Denaturation)

Step3: 55°C for 30 sec (Annealing)

Step4: 72°C for 1 min (Extension)

Step5: repeat Step2 to 4 for 34 times (=total 35 cycles)

Step6: 72°C for 10 min (Final extension)

Step8: 4°C forever (Storage temperature)

List of materials for PCR

- PCR tubes (0.2mL or 0.5mL, up to the product specifications of the heating block in your PCR thermal Cycler)
- Ice Bucket and crashed ice
- Extracted DNA (for PCR template)
- PCR reagent kit including;
  - 10x Taq buffer
  - dNTPs
  - Taq DNA Polymerase
- Forward Primer
- Reverse Primer
- DW (sterilized distilled water by autoclave)
- PCR Machine (thermal cycler)