

Restriction fragment length polymorphism (RFLP) assay

PCR mix:

2.5	µl	10x Buffer (Mg ²⁺ free)
2	µl	dNTPs
1.5	µl	MgCl ₂ (25mM)
16.25	µl	ddH ₂ O
0.25	µl	Taq Polymerase (<i>TaKaRa</i> ; 5U/µl)
1	µl	primer A (10pmol/µl)
1	µl	primer B (10pmol/µl)
0.5	µl	genomic DNA (small-scale preparation)

25.0	µl	

10x Buffer:

- 100 mM HCl (pH8.3)
- 500 mM KCl

dNTPs mixture:

- 2.5 mM of each dNTP
- pH 7-8
- solved in water (sodium salts)

PCR program:

1. 5' 94°C

2. 30" 94°C

3. 30" 62°C

4. 2' 72°C

...39 cycles

6. 5' 72°C

7. ∞ 4°C

- Digest 5µl of PCR product with the appropriate restriction enzyme in a 20µl reaction.
- Separate on 1% agarose gels.