Chapter 5
PCR Amplification for Low-Cost Mutation Discovery

Abstract PCR is used to amplify regions to be interrogated for the presence of mutations (SNP and small indel polymorphisms). While PCR is a common practice and many protocols exist, reaction conditions are provided here that are optimized for TILLING and Ecotilling assays utilizing native agarose gel electrophoresis.

5.1 Materials

Consumables and equipment for PCR amplification are listed in Table 5.1.

Table 5.1 Chemicals, enzymes, and equipment for PCR amplification

Material description	Examples of suppliers and specifications
Genomic DNA	Concentration 0.075 ng/µl for a 150-Mbp diploid genome. Scale accordingly and test different concentrations as necessary
TaKaRa HS Taq[a], 5 U/µl	ExTaq kit, TaKaRa, Japan
ExTaq PCR buffer	ExTaq kit, TaKaRa, Japan
dNTPs	ExTaq kit, TaKaRa, Japan
Forward and reverse primers	Tm 67–73 °C, designed to amplify a specific genomic region producing an amplicon between 800 and 1,600 bp. Primer design is aided with freely available software such as Primer3 (Rozen and Skaletsky 2000)
H_2O (distilled or deionized and autoclaved)	
DNA size ladder	Any standard ladder providing sizing standards between 100 bp and 2 kb, e.g., 1 kb Plus, Life Technologies 10787-018
0.2 ml tubes	Any general laboratory supplier
Thermocycler	Any standard thermocylcer, e.g., Biorad C1000 Thermal cycler
Microcentrifuge	Any standard microcentrifuge, e.g., Eppendorf Centrifuge 5415D
Agarose gel equipment	Horizontal electrophoresis from any general laboratory supplier

[a]While hot start Taq polymerases can offer improved results, lower cost polymerases can be used for PCR amplification

B.J. Till et al., *Low-Cost Methods for Molecular Characterization of Mutant Plants*,
DOI 10.1007/978-3-319-16259-1_5

5.2 Methods

1. Prepare a PCR master mix on ice by combining:

H_2O	82.5 µl
10× Ex Taq buffer	15 µl
2.5 mM dNTP mix	12 µl
10 µM L primer	1.5 µl
10 µM R primer	1.5 µl
TaKaRa HS taq (5 U/µl)	0.38 µl

2. Mix the PCR master mix by pipetting it up and down ten times followed by pulse centrifugation.
3. Combine 7.5 µl DNA at the appropriate concentration with 22.5 µl of PCR master mix. Mix by pipetting it up and down.
4. Incubate in a thermal cycler using the following parameters:
   95 °C for 2 min; loop 1 for 8 cycles (94 °C for 20 s, 73 °C for 30 s, reduce temperature 1 °C per cycle, ramp to 72 °C at 0.5 °C/s, 72 °C for 1 min); loop 2 for 45 cycles (94 °C for 20 s, 65 °C for 30 s, ramp to 72 °C at 0.5 °C/s, 72 °C for 1 min); 72 °C for 5 min; 99 °C for 10 min; loop 3 for 70 cycles (70 °C for 20 s, reduce temperature 0.3 °C per cycle); hold at 8 °C.
5. OPTIONAL: Check the yield of the PCR product by agarose gel electrophoresis. See Chap. 8 for example data. For the efficient discovery of nucleotide polymorphisms, PCR product yield should be approximately 10 ng/µl or higher in concentration. PCR product should be a single band. Co-amplification of multiple sequences can result in high error rates (Cooper et al. 2008).

References

Cooper JL, Till BJ, Laport RG, Darlow MC, Kleaffner JM et al (2008) TILLING to detect induced mutations in soybean. BMC Plant Biol 8:9

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Methods in molecular biology. Humana Press, Totowa, NJ, pp 365–386