Chapter 8
Example Data

Abstract Standard agarose gel electrophoresis is a quick method for the evaluation of the quality and quantity of DNA. This chapter provides examples of genomic DNA produced using the low-cost extraction protocol, PCR amplification using the extracted genomic DNA, and enzymatic mismatch cleavage of PCR products with crude celery juice extract and weed juice extract to detect mutations.

8.1 Quality of Genomic DNA Obtained by Silica Powder-Based DNA Extraction Method

While spectrophotometric approaches (e.g., Nanodrop) provide a quick and accurate measure of DNA concentration and protein contamination, and fluorometric methods (e.g., Qubit) provide high sensitivity, it is advisable that when optimizing the DNA extraction protocol, samples are also run on a traditional agarose gel. This allows an estimation of DNA concentration (relative to concentration standards such as lambda DNA, Till et al. 2007), the extent of RNA carryover, as well as an estimation of the extent of DNA degradation, something which cannot be easily determined from the other two techniques (Figs. 8.1 and 8.2). Furthermore, chaotropic salts can lower the accuracy of spectrophotometric methods. Reducing sample degradation may be a key optimization step for some species. Alternative buffers can be employed to limit degradation and the copurification of secondary metabolites that can inhibit downstream molecular assays (see Sect. 8.2).

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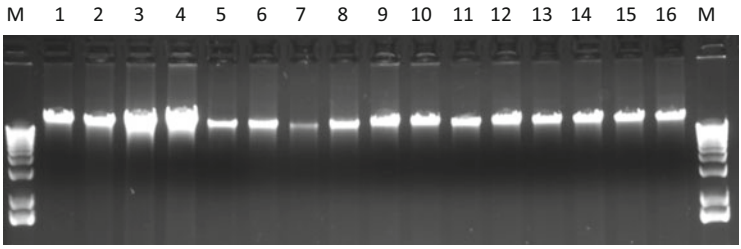

Fig. 8.1 Quality of barley genomic DNA extractions using combinations of self-made and commercial products. Eight microliters of each genomic DNA extraction was electrophoresed on a 0.7 % agarose gel. M = 1 kb Plus DNA ladder (Life Technologies). Lanes 1–4 are samples prepared with the DNeasy kit from Qiagen. Lanes 5–8 are samples prepared using DNeasy columns but with self-made 6M Guanidine thiocyanate buffer replacing commercial buffer AP3/E. Lanes 9–12 are samples prepared with self-made lysis buffer but with commercial DNA binding buffer AP3/E. Lanes 13–16 represent samples prepared using only self-made buffers described in Chap. 4

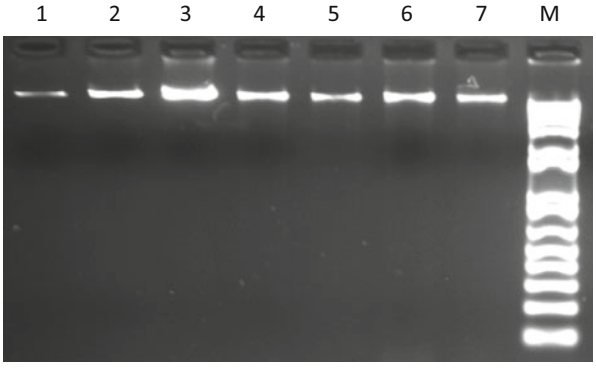

Fig. 8.2 Genomic DNA samples produced at the FAO/IAEA 2013 training course on "Plant Mutation Breeding: Mutation Induction, Mutation Detection, and Pre-Breeding." Lanes 1–3 represent lambda DNA concentration standards of 3, 10, and 30 ng/μL, respectively. Lanes 4–7 represent genomic DNA prepared by Ms. Sasanti Wisiarsih and Mr. Wijaya Murti Indriatama of Indonesia using the protocol described in Chap. 4 using a 6M KI buffer

8.2 Quality of Genomic DNA Obtained by Silica Powder-Based DNA Extraction Method Using Alternative Buffers

Alternative buffers can be used to extract DNA from tissues or species where the use of the standard buffer produces low-quality DNA. Tissue from *Sorghum bicolor* and grapevine (*Vitis vinifera)* were used to compare different buffer compositions (Tables 8.1 and 8.2).

Quality and quantity of genomic DNA using different buffers were assayed by native agarose gel electrophoresis (Fig. 8.3).

Table 8.1 Sorghum (*Sorghum bicolor*, Sb) genomic DNA extractions using the silica powder method with four different lysis buffers

Sample designation	Sb1a	Sb1b	Sb2a	Sb2b	Sb3a	Sb3b	Sb4a	Sb4b
Lysis buffer (LB)[a]	LB 1		LB 2		LB 3		LB 4	
Incubation temperature (for lysis)	RT	65 °C	RT	65 °C	RT	65 °C	RT	65 °C
DNA concentration (ng/μl)	11	15	2	3	6	5	7	7
Total yield (μg)	2.0	2.6	0.4	0.6	1.0	0.9	1.3	1.3

[a]See Table 4.3 for composition of buffers

Table 8.2 Grapevine (*Vitis vinifera*, Vv) genomic DNA extractions using the silica powder method with four different lysis buffers

Sample designation	Vv1a	Vv1b	Vv2a	Vv2b	Vv3a	Vv3b	Vv4a	Vv4b
Lysis buffer (LB)[a]	LB 1		LB 2		LB 3		LB 4	
Incubation temperature (for lysis)	RT	65 °C	RT	65 °C	RT	65 °C	RT	65 °C
DNA concentration (ng/μl)	22	32	7	3	11	6	4	9
Total yield (μg)	3.9	5.7	1.3	0.6	2.1	1.1	0.6	1.5

[a]See Table 4.3 for composition of buffers

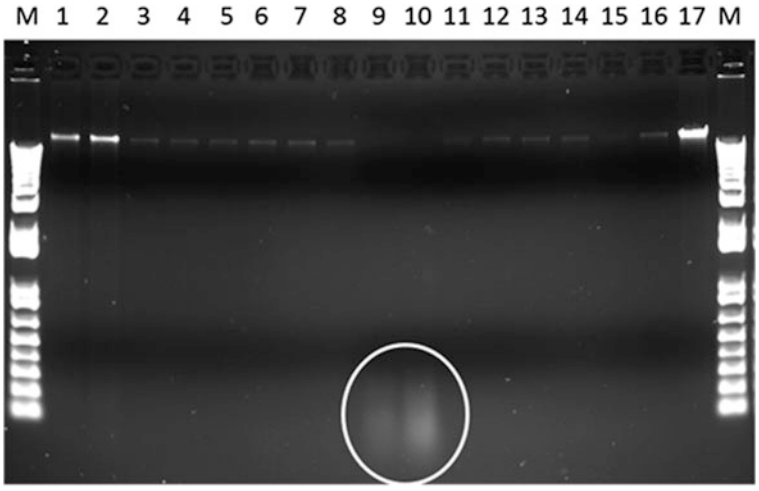

Fig. 8.3 Quality of genomic DNA extracted from sorghum and grapevine using the silica powder method with four different lysis buffers. Lanes 1–8 represent samples extracted from sorghum and lanes 9–16 from grapevine. Lanes 1, 2, 9, and 10 were prepared with lysis buffer 1 from Table 4.3. Lanes 3, 4, 11, and 12 with lysis buffer 2, lanes 5, 6, 13, and 14 with lysis buffer 3 and lanes 7, 8, 15, and 16 with lysis buffer 4. Lysis buffer 1 produced the highest yield with sorghum, but only degraded DNA with grapevine tissue (*circled*)

8.2.1 Summary

High yield and high quality genomic DNA can be recovered from sorghum using a simple lysis buffer. This buffer, however, is not suitable for grapevine DNA extraction and alternative buffers are required to recover high molecular weight DNA, albeit at a lower concentration than can be achieved from sorghum samples. This suggests further parameter changes can be made to increase yields.

8.3 Example of PCR Products Using TILLING Primers with Source Genomic DNA from a Commercial Kit and Low-Cost Silica Method

High quality and quantity of gene-specific PCR products are produced in reactions where source genomic DNA is extracted using either commercial kits or the low-cost silica method (Fig. 8.4).

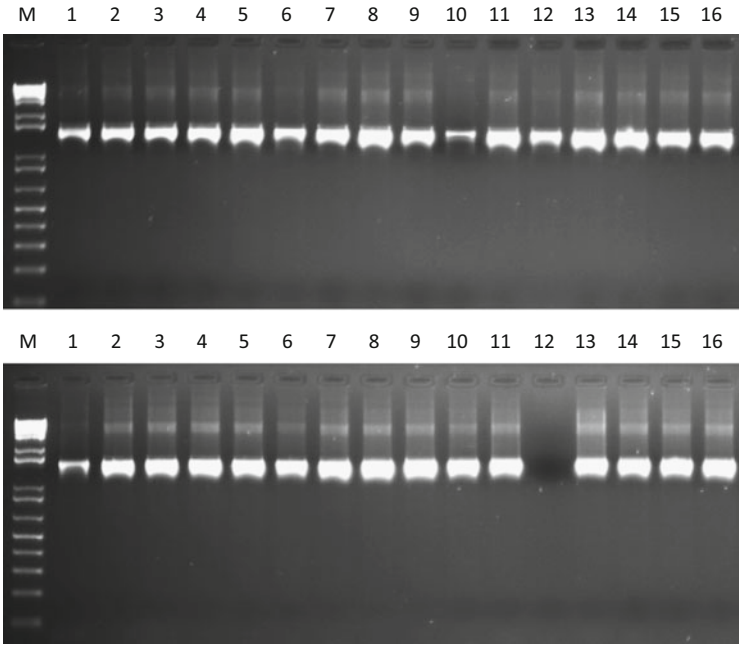

Fig. 8.4 PCR amplification of genomic DNAs described in Chap. 7 using primers for the barley nb2-rdg2a (*top panel*) and nbs3-rdg2a (*bottom panel*) gene targets as described in Hofinger et al. (2013). Samples are loaded in the same order as in Fig. 8.1

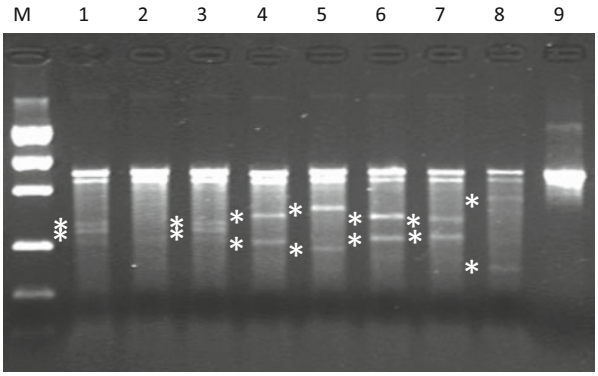

Fig. 8.5 Gel image of mutation discovery using crude celery juice extract for enzymatic mismatch cleavage. DNA from Arabidopsis plants with previously characterized induced point mutations in the *OXI1* gene was used (Till et al. 2004). Lane 2 represents a wild-type sample with no mutation. Lane 9 represents undigested PCR product. All other lanes contain samples with known mutations in the amplified region. Lower molecular weight bands representing cleavage products at the site of mutation are observable in all other lanes (marked by *asterisks*). This image was produced at the 2009 FAO/IAEA International Training Course on Novel Biotechnologies for Enhancing Mutation Induction Efficiency by Mr. Saad Alzahrani of Saudi Arabia, and Mr Azhar Bin Mohamad of Malaysia

8.4 Example of Low-Cost Agarose Gel-Based TILLING Assays for the Discovery of Induced Point Mutations

PCR products are the substrate for enzymatic mismatch cleavage assays for mutation discovery. Agarose gels provide a low-cost platform for mutation discovery using self-extracted enzymes (Fig. 8.5).

8.5 Example of Enzyme Activity Recovered from Weeds Compared to Crude Celery Juice Extract

The recovery of proteins from collected weeds versus celery is listed in Table 8.3. This was used to prepare reaction mixes (Table 8.4) to test for enzymatic activity. Samples were evaluated via standard agarose gel electrophoresis (Fig. 8.6).

8.5.1 Summary

Crude enzyme extracts of weeds show a similar activity to that of celery extract for the cleavage of single nucleotide polymorphisms. The per unit activity, however, was lower than for CEL I, likely owing to the co-precipitation of other plant

Table 8.3 Concentrations of protein extracts

Enzyme extract	Recovered volume	Concentration factor
Weed	~42 µl	11.9×
Celery	~33 µl	15.2×

Calculations of concentration factors after centrifugation with Amicon Ultra 10 kDa—starting volume: 500 µl ("Before" centrifugation is considered as 1× concentrated)

Table 8.4 Mismatch digestions using celery and weed enzyme extracts (prior and post centrifugation with Amicon Ultra 10 kDa filter devices

	Prior	Post-1	Post-2	Post-3
Enzyme	3.5 µl	0.5 µl	3 µl	6 µl
CEL I buffer	1.5 µl	1.5 µl	1.5 µl	1.5 µl
H_2O	5 µl	8.0 µl	5.5 µl	2.5 µl
Total volume	10 µl	10 µl	10 µl	10 µl

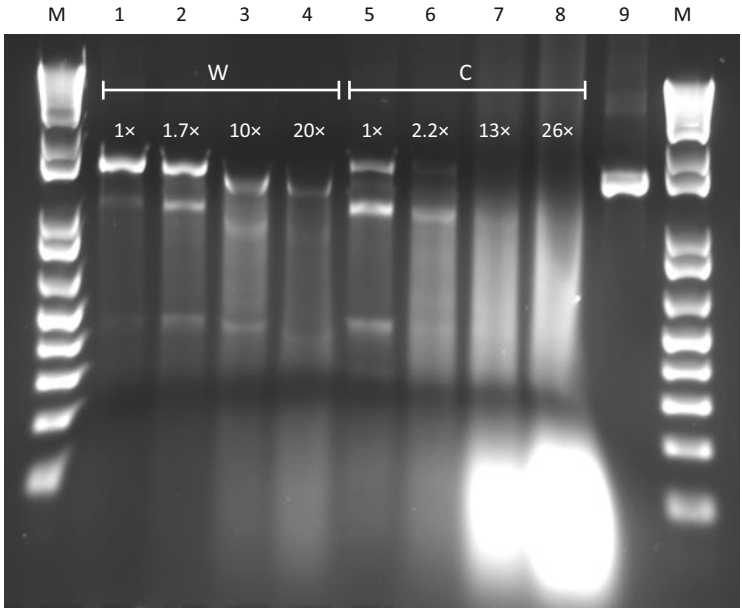

Fig. 8.6 Mismatch cleavage with crude enzyme extracts containing single-strand-specific nucleases prepared from weedy plants (W) or celery (C). PCR products of the target gene nb2-rdg2a (1,500-bp-PCR product) were produced from genomic DNA of barley containing a known SNP (Hofinger et al. 2013). The PCR products were digested with weed and celery enzyme extracts at different concentrations (listed above sample). Lower molecular weight bands are cleavage products. Cleavage activity from weed extract at 1.7× is similar to 1× activity observed from celery extracts. Undigested PCR product is loaded in lane 9

proteins in weeds, presumably including a larger amount of RuBisCO. This limitation can be overcome through the use of a simple centrifugation-based protein concentration step. Using this protocol, 150 ml of weed extract produces sufficient enzyme for approximately 2,000 reactions.

References

Hofinger BJ, Huynh OA, Jankowicz-Cieslak J, Müller A, Otto I, Kumlehn J, Till BJ (2013) Validation of doubled haploid plants by enzymatic mismatch cleavage. Plant Methods 9(1):43

Till BJ, Burtner C, Comai L, Henikoff S (2004) Mismatch cleavage by single-strand specific nucleases. Nucleic Acids Res 32:2632–2641

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