

## **A New Mini-prep and Rapid DNA Extraction Method**

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### **Abstract**

DNA extraction is an important step in molecular assays and plays a vital role in obtaining high-resolution results in gel-based systems, particularly in the case of cereals with high content of interfering components in the early steps of DNA extraction. Here we report a new rapid mini-prep DNA extraction method, optimized for rice, which was achieved via creating some modifications in present DNA extraction methods, especially in first step of breaking down and lyses of cell wall, and the use of cheap and frequent chemicals, found in every lab, in the next steps. The normal quality and quantity was obtained by the method. The PCR based assays also revealed the efficiency of the method. The advantages of this method are: 1- it is applicable with both dry and fresh samples, 2- no need to large weight samples, 3- no need to liquid nitrogen and 4- easy, rapid and applicable in every laboratory.

### **Introduction**

High-quality DNA is required for molecular biological studies of plants. Several DNA extraction procedures for isolating genomic DNA from various plant sources have been described, including the salt extraction method and the cetyltrimethyl ammonium bromide (CTAB) method [1] and its modifications [2,3]. The need for a rapid and simple procedure is urgent, especially when hundreds of samples need to be analyzed [4].

In most protocols the use of liquid nitrogen [5] or freeze-drying (lyophilization) [6, 7] of tissue for the initial grinding is necessary, and these processes are unavailable in many regions of the world. After grinding the tissues in various extraction buffers, DNA is extracted with phenol-chloroform, or the extract is dialyzed against EDTA and a buffered Tris-HCl solution [8]. After extraction, the aqueous phase is concentrated, either by ethanol or isopropanol precipitation [9, 10], or with microconcentrators (*e.g.*, the Wizard genomic DNA purification system; Promega, USA). However, these methods are not time efficient for consistently obtaining PCR-quality DNA from cereal plants, since they require that the tissues be ground in liquid nitrogen, followed by precipitation of the DNA pellet in ethanol, washing and drying the pellet, etc.

However, in the protocols provided by Kang et al. (2004) the DNA concentrations from cereal crops (rice and maize) were relatively low. They suggested that this may be because homogenization using a hand-operated homogenizer with a plastic tip is incomplete, since the leaves of these plants are stronger than the leaves of tobacco, potato, cabbage, lettuce, and Siberian ginseng. For our purposes, we desire a simple and fast procedure for obtaining plant genomic DNA for PCR, and good-quality DNA for complete enzyme digestion. Therefore, we present a protocol for extracting genomic DNA from young or old, fresh or dry cereal plant leaves that is applicable to a variety of organisms, regardless of the complexity of their genomes. In addition, we present a rapid and reliable procedure for extracting genomic DNA for PCR from a small amount ( $\sim 0.5 \text{ cm}^2$ ) of leaf tissue.

## **Results and discussion**

Here is described a simple and reproducible procedure for PCR amplification of cereal plant genomes. Two different variations of the genomic DNA extraction protocol for PCR analysis were compared. Simple homogenization of fresh or dry plant leaf samples (gathered in 14 day-old or at flowering stage) was carried out with DNA extraction buffer using a hand-operated homogenizer. Then, genomic DNA was extracted with 0.7 volume of chloroform/isoamyl alcohol (24:1). After chloroform/isoamyl alcohol (24:1) extraction, the supernatant was transferred to a fresh tube and was extracted with 0.7 volume of cold isopropanol alcohol and dried in a heat vacuum and dissolved in 50  $\mu\text{l}$  of sterile dH<sub>2</sub>O containing 20  $\mu\text{g/ml}$  DNase-free RNase A (fig 1A, lanes 1-3). Alternatively, after isopropanol alcohol extraction, the supernatant was removed and washed with  $\sim 500 \mu\text{l}$  of 70% ethanol. After washing the DNA pellet with 70% ethanol, the DNA pellet was dried in a heat vacuum and dissolved in 50  $\mu\text{l}$  of sterile dH<sub>2</sub>O containing 20  $\mu\text{g/ml}$  DNase-free RNase A (Fig 1A, lanes 4-6).

Genomic DNA from different lines was electrophoresed on 1% agarose gels, and high-molecular-weight DNA was obtained (Figure 1A). When the genomic DNA was digested with *EcoRI*, the DNA was completely digested, and could be used for Southern blot analysis. Therefore, we concluded that the purity and quality of the genomic DNA was sufficient for enzyme digestion.

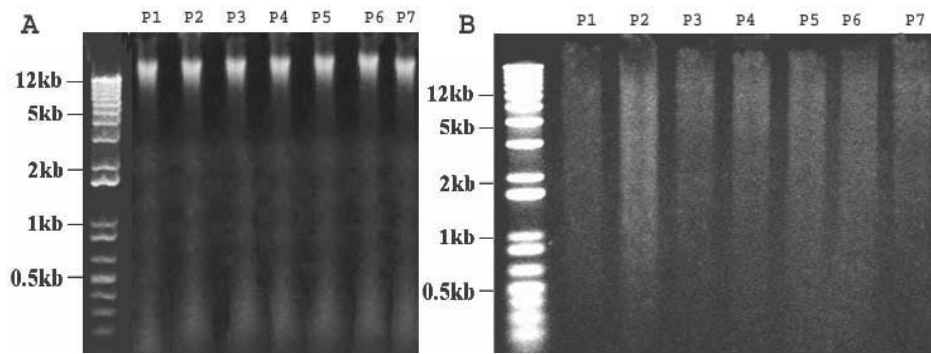


Fig 1. Total genomic DNA extracted by protocol 1 from seven rice lines (A) and then digested with *EcoRI* (B).

DNA samples prepared using the two different extraction procedures (lanes 1-7 in Fig 1A) were subjected to PCR amplification using different primers: RM171 on chromosome 10 and RM1 on chromosome 1 (both SSR rice markers). All the genomic DNA samples produced a clear, sharp, and reproducible PCR product when the primers were used for PCR amplification (Fig 2A). Although two variations of the DNA extraction procedure were used, there was no difference between two protocols. This result suggests that the pellet after the first chloroform treatment (protocol 1) was sufficiently pure to be used as the DNA template for PCR amplification. Therefore, PCR amplification with another primer, RG140, was performed using the DNA template extracted using the first protocol (Fig 2B). The PCR amplification was successful, and the same banding pattern was seen when we repeated the PCR amplification (production of a relatively large DNA fragment of 1304 bp). Therefore, we confirmed that the DNA template extracted using the first method was sufficient for amplification of relatively large DNA fragments, and it was used as the DNA template to amplify specific DNA from rice plants.

To examine the presence of *Rf1A* [11] in the genomic DNA, two different lines (one sterile line, Neda A, and a fertility Restorer line, Amol1) and some their sterile progenies were screened by PCR analysis (Fig 3). PCR amplification using primer combinations SPRF03-F/SPRF04-R resulted in ~940-, and ~890-bp fragments, respectively. The primer produced fragments containing *Rf1A* gene, which confirmed the occurrence of the gene in the lines used. A polymorphic product was produced using genomic DNA from sterile and fertility restorer plant with primer combination (Fig 3), demonstrating the some differences between CMS and fertile lines in the region. Therefore, we concluded that relatively large DNA fragments were amplified, although we did not use liquid nitrogen, but simply used a hand-operated homogenizer with a plastic tip.

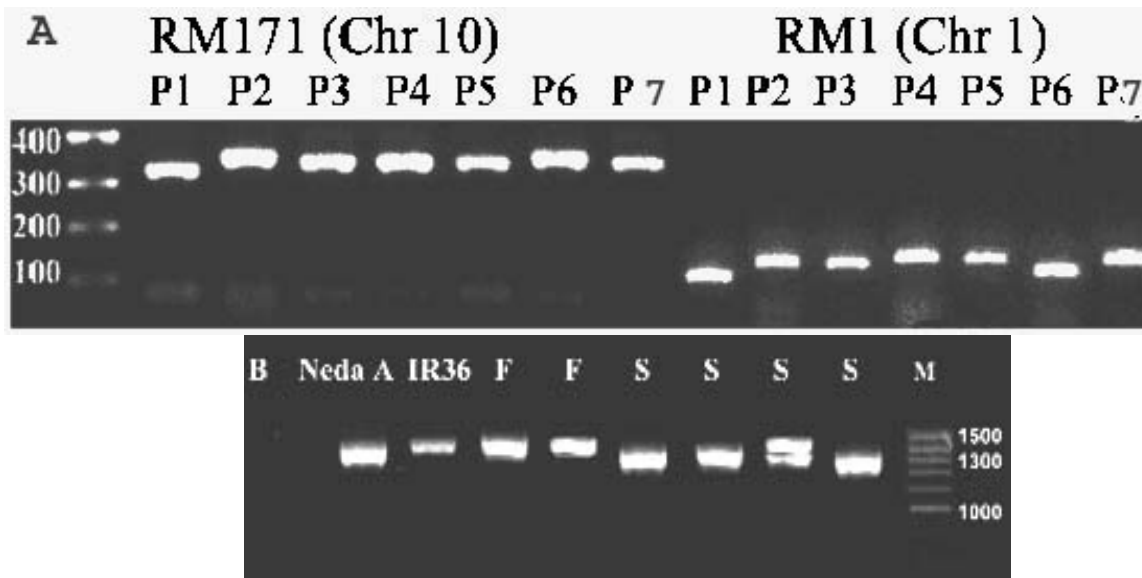


Fig 2. PCR amplification of total genomic DNA extracted by protocol 1 by (A) RM171 on Chr.10 and RM1 on Chr. 1, and (B) by RG140 on Chr. 1. F: fertile progeny, S: Sterile progeny of a cross between Neda A and IR36.

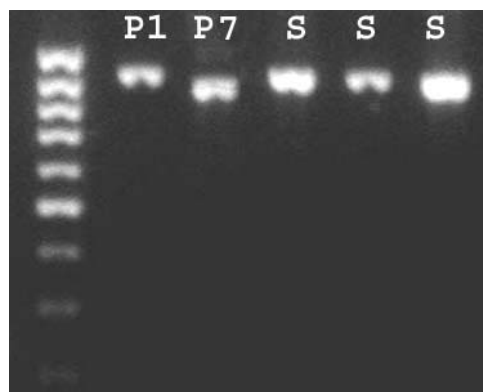


Fig 3. PCR products of primer combination SPRF03-F/SPRF04-R. S: F2 sterile progenies of a cross between Neda A and Am11.

There are many advantages in using our genomic DNA extraction method to obtain template for PCR amplification. Many different cereal plants could be amplified using the same DNA extraction method and the same PCR protocol. Using this protocol, we successfully amplified DNA repeatedly from all lines examined. Our procedure is not only very simple, but is also time and cost effective. After homogenization in DNA extraction buffer using a hand-operated homogenizer, the template DNA for PCR could be extracted by chloroform/isoamyl alcohol treatment followed by cold isopropanol extraction. Since this method does not require liquid nitrogen, expensive commercial DNA extraction kits, or ethanol precipitation to produce DNA template for PCR, we can save considerable time and expense. The time required for our DNA extraction method is less than 60 min, which is extraordinary compared with other

genomic DNA extraction methods. With our procedure, leaf tissue (~0.5 cm<sup>2</sup>) is put in a 1.5-ml microfuge tube and homogenized directly; consequently, a very small sample is required for DNA extraction. There is no sample waste with our method, whereas much larger samples are required when plant samples are ground in a mortar and pestle with liquid nitrogen and transferred to a tube. Previously reported techniques require several steps [12], use of expensive enzymes such as proteinase K [13], or beads and shakers [14]. Although the protocol for one-step plant DNA isolation was developed by Burr et al. [15], if plant material more than 1 mm<sup>2</sup> was used in the extraction, co-extracts (e.g., chlorophyll) were extracted alongside the DNA and inhibited the PCR. On the contrary, our protocol does not require appropriate sample size to extract DNA. Warner et al. [16] also reported a rapid DNA extraction method in barley, which requires NaOH. However, the extracted DNA samples were easily degraded. The DNA samples extracted by our protocol were very stable and could be stored in 4 ° C for two years without degradation.

## **Material and Methods**

### ***Plant material***

We examined plant material from rice (*Oryza sativa*) lines. The plants used for genomic DNA extraction were grown in a culture room or greenhouse. The seeds were grown in a controlled environment at 25°C on a 16-h continuous light and 8-h dark daily cycle or were grown in a greenhouse for genomic DNA extraction.

### ***DNA extraction***

We tested two different variations of the genomic DNA extraction procedure. About 30 mg of finely powdered leaf samples (for the case of dry samples) or ~0.5 cm<sup>2</sup> of culture room- or greenhouse-grown plant leaves were put in a 1.5-ml microfuge tube. The leaf tissue was homogenized in 100 µl DNA extraction buffer (for dry leaf samples: 2% CTAB, 1.5 M NaCl, 100 mM Tris-HCl pH 8., and 20 mM EDTA pH 8.0; for fresh leaf samples: 1% CTAB, 700 mM NaCl, 10 mM Tris-HCl pH 8., and 50 mM EDTA pH 8.0), using a hand-operated homogenizer with a plastic pestle, for 30~40 s. After an initial homogenization, another 350 µl of DNA extraction (pre-warmed up to 65 °C and addition of 38mg/ml sodium bi-sulphite just before the use) buffer were added and vortexed for ~60 s. The samples were incubated at 65°C for 20 min

for cell lysis. At this point, two different DNA extraction protocols were used for PCR amplification. Protocol 1: 0.7 volume of chloroform/isoamyl alcohol (24:1) was added to the samples, mixed by hand for 5 min, and then centrifuged at 14,000 g for 5 min at 4°C. The supernatant was transferred to a fresh tube and extracted one more time with 0.7 volume of cold isopropanol alcohol. The pellet was dried, and resuspended in sterile dH<sub>2</sub>O containing 20 µg/ml DNase-free RNase A. Protocol 2: ~500 µl of 70% ethanol was added to the pellet from protocol 1, microcentrifuged for 1 min., the pellet was dried, and resuspended in sterile dH<sub>2</sub>O containing 20 µg/ml DNase-free RNase A. The concentration and purity were determined on 1% agarose gels. Five micrograms of each genomic DNA sample were incubated at 37°C for 3 h for complete digestion with 20 U of *EcoRI* (Sibenzyme, Moscow) in a total volume of 50 µl and analyzed on 1.0% agarose gels using 10 µl aliquots of the reaction mixture.

Table 1. Primer sequences used in this study.

Name	F	R	Comments
RM1	gcgttggttgacctgac	gcgaaaacacaatgcaaaaa	SSR on Chr.1
RM171	acgagatacgtacgcctttg	aacgcgaggacacgtacttac	SSR on Chr.10
RG140	tccctagtttgctactcc	gtacatagtagcacctgctc	STS on Chr.1
SPRF03	gaattcaaatccatcaaacataggttct	gaattctattggtggaagcccaatgct	In A110443
SPRF04	gaattccgtataagacaaactgcgttgc	ggatccctcctctaataaggactgtaggaga	In AB110443

### ***PCR amplifications***

By using the genomic DNA isolated from the fresh young leaves (14-day old) or old fresh and dry leaves (at flowering stage) of rice lines (CMS Neda A (P1), IR24 (P2), IR28 (P3), Amol2 (P4), IR36 (P5), IR60966 (P6) and Amol1 (P7)) or F2 progenies from crosses of Neda A X IR36 and Neda A X Amol1, PCR amplifications were performed in a total volume of 20 µl containing 1 × PCR buffer, 0.2 mM dNTP, 10 pmol of each primer (Table 1), 50 ng template DNA from plants, and 0.25 U Taq DNA polymerase (Sibenzyme, Moscow) using the following profile: a 1-min denaturation at 94°C and 35 cycles of 1-min denaturation at 94°C, 1-min annealing at 55°C, and a 2-min extension at 72°C, followed by a final extension at 72°C for 7 min. The PCR products were resolved by electrophoresis in 1.5% agarose gels.

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