from *E. coli* and the 3′ terminator region from *Agrobacterium tumefaciens* cloned in pBluescript® KS II (Stratagene, La Jolla, CA, USA). All loading buffers allowed the target amplification at 0.25 and 0.5× final concentration (Figure 2). However, at 0.5× final concentration, there was a decrease in the amplification that was more pronounced than that observed with the 359 bp target depicted in Figure 1.

After the 0.25× gel-loading buffers in the PCR mixture proved to be feasible even when the bacterial lysate was diluted 1000-fold, we simplified our protocol: we added the bacterial colony directly to the 10 μL of the PCR mixture distributed in the 0.2 mL tubes, according to Lee and Cooper (3). The cycling parameters were the same as already described except for the inclusion of one step at 94°C for 3 min before carrying out the 30 cycles. This protocol was used to amplify targets with intermediate sizes and to use other primers, as seen in Figure 3, using 0.25× buffer I (buffers II and III worked as well; results are not shown). Using *Taq* DNA polymerases from other brands or a PCR apparatus requiring mineral oil on the top of the reaction mixture was also successful (results not shown).

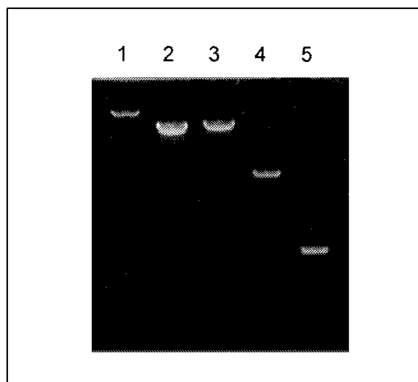

Figure 3. Colony PCR from plasmids containing inserts differing in size, source and using different primers. Amplifications were done directly from bacterial colonies coming from plasmids containing: lane 1, a 5046 bp insert corresponding to the pET3d entire sequence (New England Biolabs, Hitchin, England, UK) plus part of the coding region from the maize *Zmal1* gene (GenBank® Accession No. AF031083) using two gene-specific primers. Lane 2, a 3046 bp insert from p715 amplified with forward and reverse primers; lane 3, the same as in lane 2, using T3 (5′-AATTAACCCTCACTAAAGGG-3′) and T7 (5′-TACGACTCACTATAGGGCGA-3′) primers. Lane 4, a 2124 bp insert containing a plant virus promoter, the coding region from a *Panicum miliaceum* gene and the 3′ terminator from *Agrobacterium tumefaciens*, using forward and reverse primers. Lane 5, a 662 bp insert corresponding to a part of a plant virus promoter and the coding region of *Zmal1* gene amplified with gene-specific primers.

In conclusion, our results showed that the three gel-loading buffers can be added to the PCR mixture used to amplify target inserts from bacterial colonies, decreasing considerably the tedious handling of several small 0.2 mL tubes that are currently used in the most recent thermocyclers. We found that a 0.25× concentration of any of the three gel-loading buffers does not decrease target amplification, although a negative effect was observed at a 0.5× concentration. Finally, by decreasing the reaction volume to 10 μL, the cost of the assay is considerably lower.

REFERENCES

1. **Dallas-Yang, Q., G. Jiang and F.M. Sladek.** 1998. Avoiding false positives in colony PCR. BioTechniques *24*:580-582.
2. **Güssow, D. and T. Clackson.** 1989. Direct clone characterization from plaques and colonies by the polymerase chain reaction. Nucleic Acids Res. *17*:4000.
3. **Lee, A.B. and T.A. Cooper.** 1995. Improved direct PCR screen for bacterial colonies: wooden toothpicks inhibit PCR amplification. BioTechniques *18*:225-226.
4. **Menossi, M., J.A. Martínez-Izquierdo and P. Puigdomènech.** 1997. Tissue specific activity and hormone control of the promoter of the gene coding for a maize hydroxyproline-rich glycoprotein. Plant Sci. *125*:189-200.
5. **Sambrook, J., E.F. Fritsch and T. Maniatis.** 1989. Molecular Cloning: A Laboratory Manual. CSH Laboratory Press, Cold Spring Harbor, NY.
6. **Sandhu, G.S., J.W. Precup and B.C. Kline.** 1989. Rapid one-step characterization of recombinant vectors by direct analysis of transformed *Escherichia coli* colonies. BioTechniques *7*:689-690.
7. **Zon, L.I., D.M. Dorfman and S.H. Orkin.** 1989. The polymerase chain reaction colony miniprep. BioTechniques *7*:696-698.

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Heating Greatly Speeds Coomassie Blue Staining and Destaining

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SDS-PAGE is one of the most powerful and commonly used techniques in molecular biology. The most frequently used dye for visualizing proteins is Coomassie Brilliant Blue R-250 because of its relatively high sensitivity (3,6). However, its lengthy processing time is a major drawback of the standard Coomassie staining technique. Sambrook et al. (4) state that a staining time of at least 4 h is needed, with destaining overnight. This can be shortened to a minimum of 1 h staining time, but at least several hours of destaining are needed to start to visualize bands. In addition, the destaining process is often incomplete even after overnight destaining, leaving a light blue background on the gel. This makes photography and quantification of the proteins in the gel difficult and limits the sensitivity of the method.

We rediscovered a paper published in 1971 where Fairbanks et al. (1) proposed the use of three staining solutions, each containing 10% acetic acid and progressively lower concentrations

of Coomassie and isopropanol. This process successfully increases the sensitivity of the stain. However, the entire process as described requires up to two days to achieve a clear background of the gel.

Here, we describe a modification of the Fairbanks Coomassie staining technique, which involves heating the polyacrylamide gels in the staining solutions with a microwave. Only 11 min of processing time give a result better than can be achieved in 3 h with the standard Coomassie protocol. A total processing time of 20 min using the rapid Fairbanks method allows visualization of as little as 5 ng of protein. If desired, gels with a water-clear background can be obtained with an additional 15 min of destaining time giving a total processing time of 35 min. Water-clear backgrounds are usually unobtainable with the standard Coomassie protocol even after overnight destaining. Thus, this new method, at each point along the way, is at least 15-fold faster than the standard method (Figure 1).

In part because of the water-clear background, as little as 2.5 ng of protein can be observed using the rapid Fairbanks method. The limit of detection of standard Coomassie is generally quoted as 50–100 ng (2,3,6), but if sufficient destaining can be achieved, then as little as 10 ng can be detected. This suggests that the sensitivity of Coomassie is currently limited by inadequacies in the destaining protocols. That our new method is slightly more sensitive than the standard Coomassie protocol may be because of its ability to achieve water-clear backgrounds. Destaining the gel with sodium chloride solutions (5) does help speed up the destaining process, but it still requires 2–3 h for the gel background to become water-clear.

Various modifications to the Meyer and Lambert protocol (3), including heat treatment during the staining process (2) or the use of bleach and elevated temperature (7), have been proposed to speed up the staining and destaining process. We compared our rapid Fairbanks technique to these two published methods (Figure 2) and found that our method is superior, especially in sensitivity and in speed (Figure 2). The Kurien and Scofield method (2) involves a 5 min staining step at 70°C, followed by a 20 min destaining step at the same temperature and a rinsing step with distilled water at room temperature for a further 20 min, resulting in a total processing time of 45 min.

Although this method is fairly rapid, it pays a significant penalty in terms of sensitivity (Figure 2). Here, the sensitivity is at least 5–10 times lower than the sensitivity obtained using our rapid Fairbanks technique. Another technique, using 2.5% bleach at 55°C followed by rinsing of the gel with distilled water, has been proposed as a method of speeding up the destaining process (7). This method, which takes a total processing time of 90 min, is significantly slower than ours and takes large sacrifices in terms of sensitivity with its detection limit of approximately 100 ng protein (7) (Figure 2).

Our method is also faster and more sensitive than the colloidal blue method that has recently been commercially marketed by at least three companies. We tested our method side by side against the colloidal blue stain, GelCode Blue® (Pierce Chemical, Rockford, IL, USA). This method requires washing the gels with distilled water 3× for 5 min before staining to remove the SDS in the gel-running buffer, followed by a staining time of 1 h and then a destaining time of 2 h. Thus, our method is faster than the commercial product by at least 2.5 h. In addition, the GelCode Blue technique is about 3× more expensive and twofold less sensitive than ours. In summary, our modification of the Coomassie staining method of protein gels is faster and more sensitive than any other Coomassie method we are aware of.

To demonstrate the method, dilutions of a mixture of seven proteins ranging from 500–5 ng per band were run on 1 mm Novex® (San Diego, CA, USA) pre-cast 14% Tris-glycine minigels as described in the manufacturer's instructions. After electrophoresis, the gels were placed in a microwavable plastic box (e.g., Nalgene® reusable plastic utility box available from Fisher Scientific (Pittsburgh, PA, USA) or standard Tupperware® with a hole punched in the lid to vent gases, with approximately 100 mL of Fairbanks A staining solution (0.05% Coomassie, 25% isopropanol, 10% acetic acid).

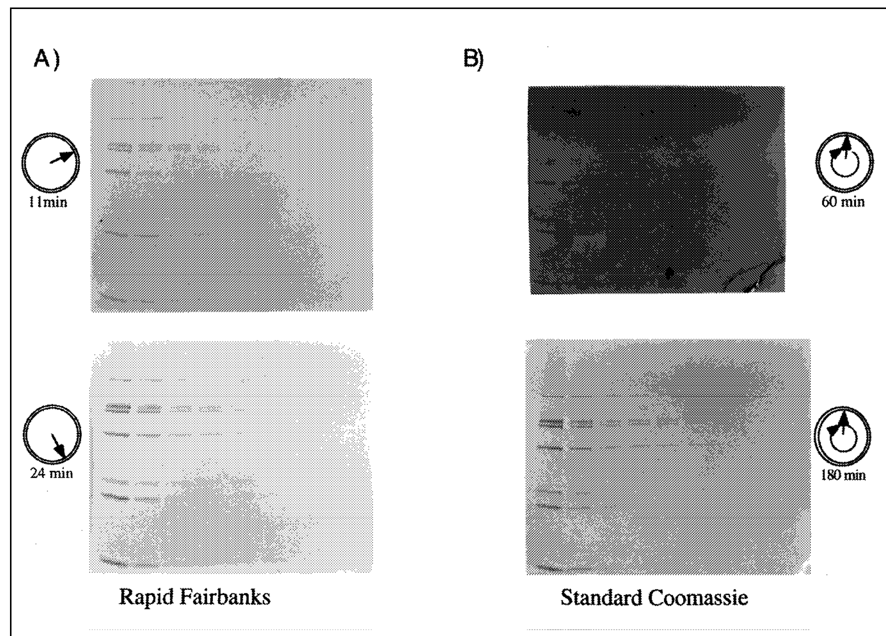

Figure 1. Comparison with the standard Coomassie Brilliant Blue staining method (8). Identical 14% Novex SDS-polyacrylamide gels were stained with the following methods: Panel A) rapid Fairbanks or Panel B) standard Coomassie Blue. The gels were photographed at the times indicated by the clocks, with transmitted white light using identical exposure times. The amount of protein per band from left to right on the gel is: 500, 250, 100, 75, 50, 25, 10, 7.5 and 5 ng. The proteins shown are: β-galactosidase (116 kDa), bovine serum albumin (70 kDa), GroEL (60 kDa), citrate synthase (50 kDa), carbonic anhydrase (31 kDa), FtsJ (25 kDa) and aprotinin (6.5 kDa).

The gel in staining solution was then heated in a conventional 1000 W output microwave oven on full power until the solution reached the boiling point (about 2 min). The gels were then cooled at room temperature for approximately 5 min with gentle shaking. After this step, bands with more than 100 ng of protein could be seen on the gel despite the blue background.

The Fairbanks A staining solution was discarded, and distilled water at room temperature was added for rinsing and then immediately discarded. Approximately 100 mL of Fairbanks B staining solution (0.005% Coomassie, 10% isopropanol, 10% acetic acid) were added to the gels and microwaved to the boiling point (approximately 1 min and 20 s). After this step, protein bands containing 50 ng or more could easily be observed.

The hot Fairbanks B solution was discarded, room temperature distilled water was added and immediately discarded. The gels were then transferred to about 100 mL of Fairbanks C staining solution (0.002% Coomassie, 10% acetic acid) and again microwaved to the boiling point (approximately 1 min and 20 s). At this time, protein bands containing more than 25 ng became visible.

The gels were again rinsed with distilled water at room temperature and placed in about 100 mL Fairbanks D destaining solution (10% acetic acid) and microwaved for 1 min 20 s. A piece of Kimwipe® was placed in the solution to absorb excess dye. The gels were then allowed to cool at room temperature for about 5 min with gentle shaking. At this point, protein bands containing 5 ng or more could be observed against the light blue background. Water-clear gel background could be achieved immediately if the Fairbanks D destaining step was repeated for two or more times or if the gels were left shaking in the destaining buffer for about 15 min more. As little as 2.5 ng of protein could then be observed against a clear background on the gels.

In conclusion, it takes about 16 min of processing time plus about 4 min to switch the gels between stains to visualize as little as 5 ng of protein against a light blue background of the gel. A water-clear background, which allows the visualization of 2.5 ng of protein, can be achieved in about 15 min more. We note that mild heating of the standard 0.25% Coomassie stain solution, though it has been found to be faster than the room temperature protocol (2), is not optimal. This solution contains more dye than the Fairbanks solutions, which results in about three-fold longer processing times than our method and is much less sensitive (Figure 2).

To further speed and simplify the

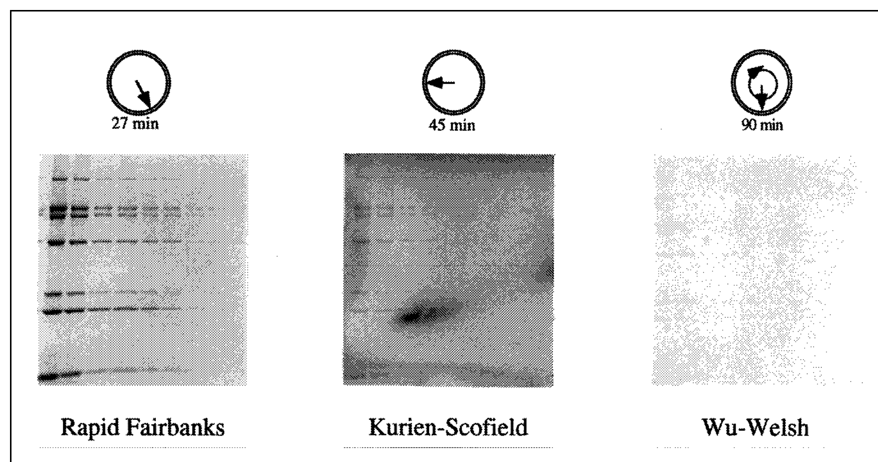

Figure 2. Comparison with Kurien and Scofield (2) and Wu and Welsh (7) staining method. Identical 14% Novex SDS-polyacrylamide gels were stained in parallel with either the rapid Fairbanks methods (left panel), Kurien and Scofield method (middle panel) or the Wu and Welsh method (right panel). The gels were photographed at the times indicated by the clocks with transmitted white light using identical exposure times. The amount of protein per band and the composition of the standard are the same as the one described in Figure 1.

protocol, we found that we could omit the use of Fairbanks B and Fairbanks C solutions. This elimination speeds up the processing time to about 15 min with only a twofold sacrifice of sensitivity. It also reduces the number of solutions that need to be prepared from four to only two. Bringing the solution containing the gel to the boiling point appears to be a prerequisite for the improved sensitivity and speed of our method. Our method is quite forgiving, however, and all steps can be performed for longer periods. As long as the gels are not boiled to near dryness, the actual boiling times are not important. Evaporation of the isopropanol presumably occurs early in the boiling process, so for consistent results, we avoid reusing the solutions. Though longer boiling or rinsing times do not reduce sensitivity, they also do not improve it. The times listed have been optimized for speed.

Silver staining is another commonly used technique with extremely high sensitivity. However, unlike our process, it involves the use of toxic chemicals, its process is lengthy, complicated and unforgiving. Moreover, it stains different proteins to different degrees, depending on the protein's silver-binding property, making silver less suited for protein quantification. Fluorescent reagents such as fluorescamine and *o*-phthalaldehyde are also sensitive. However, their fluorescent intensity diminishes with time, and UV radiation must be used to observe the staining pattern. Coomassie blue remains the most popular protein stain. In conclusion, our rapid Fairbanks method combines all the features of a useful protein staining technique: it is fast, highly sensitive and inexpensive.

REFERENCES

1. **Fairbanks, G., T.L. Steck and D.F.H. Wallach.** 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry *10*:2606-2617.
2. **Kurien, B.J. and R.H. Scofield.** 1998. Heat mediated quick Coomassie blue protein staining and destaining of SDS-PAGE gels. Indian J. Biochem. Biophys. *35*:385-389.
3. **Meyer, T. S. and B.L. Lambert.** 1965. Use of coomassie brilliant blue R250 for the electrophoresis of microgram quantities of parotid saliva proteins on acrylamide-gel strips. Biochim. Biophys. Acta *107*:144-145.
4. **Sambrook, J., E.F. Fritsch and T. Maniatis.** Molecular Cloning: A Laboratory Manual. (2nd ed.) CSH Laboratory Press, Cold Spring Harbor, NY.
5. **Sreeramulu, G. and N.K. Singh.** 1995. Destaining of Coomassie brilliant blue R-250-stained polyacrylamide gels with sodium chloride solutions. Electrophoresis *16*:362-365.
6. **Syrovy, I. and Z. Hodny.** 1991. Staining and quantification of proteins separated by polyacrylamide gel electrophoresis. J. Chromatog. *569*:175-196.
7. **Wu, W. and M.J. Welsh.** 1995. Rapid Coomassie blue staining and destaining of polyacrylamide gels. BioTechniques *20*:386-388.
8. **Merril, C.R.** 1990. Guide to Protein Purification. Methods Enzymol. *182*:477-488.

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Modified CTAB Protocol Using a Silica Matrix for Isolation of Plant Genomic DNA

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Of the DNA extraction methods described in the literature, the cetyltrimethylammonium bromide (CTAB) method (1) is widely used because it is fast, efficient and can yield high-quality DNA from a variety of starting materials. However, with this method, it is difficult to obtain high-quality DNA from many woody plant species that contain high levels of polysaccharides and/or secondary metabolites. Such polysaccharides have solubility properties similar to those of DNA and interfere with the isolation of pure DNA when ethanol or isopropanol is used to precipitate the DNA.

Complex or time-consuming procedures such as the cesium chloride density gradient technique are frequently used to surmount this problem. But this procedure is not suitable for genetic analyses in population studies, where large numbers of samples are often used. Glass or silica can specifically bind nucleic acids in the presence of a chaotropic salt (5). These materials may be alternatives to ethanol or isopropanol for DNA precipitation. They have been widely used for DNA fragment binding, as in cleaning up PCR products. The purity of the recovered DNA is high and adequate for most molecular analyses.

In this study, we describe a simple, efficient minipreparation procedure for genomic DNA extraction from recalcitrant plant species, such as some mangrove species and sweet potato. The procedure combines the advantages of the high DNA yield using the CTAB method with the high DNA purity from cleaning up silica. The plant species *Amaranthus tricolor* L. was used to optimize conditions for high quantitative yield of DNA. The recalcitrant mangrove plant *Bruguiera gymnorrhiza* (L.) Lamk. (Rhizophoraceae) and several other plant species, including sweet potato *Ipomoea batatas* (L.) Lam. (Convolvulaceae), *Sonneratia caseolaris* (L.) Engl. (Sonneratiaceae), *Sonneratia alba* J. Smith and *Alpinia formosana* K. Schum. (Zingiberaceae) were used to test the quality of extracted DNA. Reagents used in the procedure include the following: homogenization buffer (HB) containing 2% (wt/vol) CTAB, 100 mM Tris-HCl, pH 8.0, 1.4 M NaCl and 50 mM EDTA; extraction buffer (EB): chloroform: isoamyl alcohol (24:1, vol/vol); Sephaglas BP (Amersham Pharmacia Biotech, Piscataway, NJ, USA); 4 M guanidine thiocyanate (GuSCN); and TE buffer, pH 8.5.

PCR amplifications of inter-simple sequence repeat (ISSR), using UBC primer no. 807 (5′-AGAGAGAGAGA-