CHAPTER 2
Nucleic Acid Extraction, Purification, Reannealing, and Hybridization Methods

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Introduction

The study of nucleic acids is an important area of research in biology and particularly in genetics. DNA is "the molecule of heredity", and RNA is the keystone in transferring genetic information into cellular structure and function. The techniques developed to study nucleic acids are providing tools to understanding such questions as the organization of the eukaryotic genome, species taxonomic relationships, chromosome structure, and potential productivity. This chapter describes certain nucleic acid techniques that have been applied to forest genetics research.

The body of publications related to nucleic acid research is too voluminous for total inclusion in this chapter. However, the references in the citation section are both general and specific enough to give the reader a starting base in nucleic acid research.

Details on the preparation of the chemical reagents used in the various techniques are given at the end of the appropriate sections.

Material and Methods

DNA Extraction and Purification

Modifications and variations of extraction procedures to accommodate tissue consistency, pH, DNA content per cell and bacterial and/or fungal contamination must be devised in order to obtain a pure product (Stern, 1968; Pearson and Ingle, 1972). Basically, three general processes are followed in DNA extraction and subsequent purification: 1. the material (tissue, cells and organelles) must be physically disrupted without major DNA shearing. 2. The deoxyribonucleoprotein (DNP) is extracted from the broken cells by detergent solution. 3. Enzymatic degradation of the DNA by endogenous and exogenous nucleases must be prevented by the use of high salt concentrations, detergents, chelating agents, chemical denaturants (e.g., phenol, chloroform) and deoxyribonuclease free ribonuclease and pronase.

If the above general procedures are not followed, the quantity and quality of the DNA yield will not be high. Therefore, rectification of a poor product yield can be accomplished by checking disruption, solubilization of DNP and enzymatic digestion procedures.

DNA extraction procedures from conifer whole seeds (sphorophytic and gametophytic tissue), seedlings or needles (Fig. 1) and young dicotyledonous leaves (Fig. 2) are given below.
DNA Extraction

**Seeds**

- Grind w/5% SLS, 1.0 M NaCl, 0.05 M Tris, 0.05M NaCitrate, pH 7.6 60°C
- Incubate 240 min. at 60°C

**Seedlings or needles**

- Grind w/5% SLS, 0.05 M Tris, 0.05M NaCitrate, pH 7.6 60°C
- Incubate 20 min. at 60°C

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**Procedure**

1. **Grind** w/5% SLS, 0.05 M Tris, 0.05M NaCitrate, pH 7.6 60°C
2. **Incubate** 240 min. at 60°C
3. **Filter** solid filtrate
4. **Centrifuge** supernatant + 2 vol. cold 95% etoh
5. **Centrifuge** pellet containing DNA
6. Wash x 3 w/70% etoh
7. **Dissolve in 1x SSC + pronase** incubate 17 hrs at 37°C
8. **Centrifuge** supernatant
9. **Centrifuge** pellet
10. **Shake** 20 min. w/equal vol. Chlor.: n-Amyl
11. **Increase NaCl** to 2.5 M
12. **Hold in frig. overnight**
13. **Wash** w/70% etoh upper phase + 2 vol. 95% etoh
14. **Dissolve in 1x SSC**
15. **Shake** 10 min. w/equal vol. 88% phenol
16. **Repeat x 3**
17. **Wash** w/70% etoh upper phase + 2 vol. 95% etoh
18. **Dissolve in 1x SSC**
19. **Further purification**

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**Fig. 1.** DNA extraction and purification procedure for conifer seeds, seedlings and needles