DNA FROM PLANTS

The protocol on pp. 24–25 is used to isolate DNA from the plant *Arabidopsis thaliana*. Although plants vary in their polysaccharide content, the protocol provided here is useful for most plant species as well as most plant tissues. For a discussion of the study of *Arabidopsis thaliana*, see Goodman et al. (1995).

PROTOCOL

DNA Isolation from *Arabidopsis thaliana*

This protocol is from V. Raz and J. Ecker (pers. comm.).

1. Place two or three *Arabidopsis* leaves in a 1.5-ml microcentrifuge tube containing approximately 20 glass beads (3-mm diameter) and freeze in **liquid nitrogen**.

   Note: This procedure should yield 100–500 µg of DNA per gram of tissue. The best yields are obtained from young, fresh tissues.

   liquid nitrogen (see Appendix for Caution)

2. Use a capmix mixer to grind the frozen leaves for 15 seconds.

   Note: A capmix mixer is sometimes called an amalgamator or triturator and is used in dental offices.

3. Add 0.5 ml of DNA extraction buffer to the powdered leaves and mix to homogenize the leaves in the buffer. Incubate the mixture at 65°C for 30 minutes.

   Note: The use of CTAB in the extraction buffer allows nucleic acids to be separated from polysaccharides and phenolic compounds.

   DNA extraction buffer

Component and final concentration	Amount to add per 0.5 liter
1.4 M NaCl	140 ml of 5 M
20 mM EDTA	20 ml of 0.5 M (pH 8.0)
100 mM Tris-Cl	50 ml of 1 M (pH 8.0 at 25°C)
3% (w/v) CTAB (e.g., Sigma H 6269)	15 g
H_2O	to make 0.5 liter
1% (v/v) **β-mercaptoethanol**	5 ml of 14.3 M

   Combine all of the components except the β-mercaptoethanol. Divide into 10-ml aliquots and store at room temperature for up to several years. Add the β-mercaptoethanol just before use.

   β-mercaptoethanol (see Appendix for Caution)

4. Extract with 1 volume of **chloroform**:isoamyl alcohol (24:1) (for extraction with organic compounds, see Appendix).

   Note: In this protocol, extraction with phenol is not necessary.

   chloroform (see Appendix for Caution)

5. Precipitate the DNA as follows:

a. Add 1 volume of isopropanol to the aqueous phase and mix by inverting the tube. Place at room temperature for at least 10 minutes.

b. Centrifuge in a microcentrifuge at 12,000*g* at room temperature for 10 minutes. Discard the supernatant.

c. Wash the pellet by adding 400 µl of 70% ethanol, recentrifuging for 5 minutes, and discarding the supernatant.

d. Air dry the DNA pellet.

6. Dissolve the DNA in 300 µl of TE (pH 7.4). Measure the DNA concentration (see Appendix) and adjust the final concentration to 100 µg/ml with TE (pH 7.4).

Note: Pure DNA samples that are free of contaminating nucleases can be stored at 4°C for several years. However, since slow degradation may occur at this temperature, −80°C is recommended for prolonged storage. If a nonfrost-free freezer is available, DNA can also be stored at −20°C.

DNA FROM YEAST

Baker's Yeast (*S. cerevisiae*)

In addition to its use in genetic and biochemical investigations, the powerful cloning methods made possible by YACs have brought the yeast *S. cerevisiae* widespread attention and usage by investigators unaccustomed to it. The presence of a cell wall that is resistant to many forms of chemical attack makes DNA isolation nontrivial. The protocol on pp. 27–29 uses Zymolyase to reduce the integrity of the cell wall and create spheroplasts, which are amenable to DNA isolation. For a discussion of the study of *S. cerevisiae*, see Tuite (1992) and Groffeau et al. (1996).

Fission Yeast (*S. pombe*)

A protocol for isolating DNA from the fission yeast *S. pombe* is provided on pp. 30–33. For a discussion of the study of *S. pombe*, see Zhao and Lieberman (1995).

PROTOCOL

DNA Isolation from *S. cerevisiae*

This protocol is from Mendez et al. (1993) and was adapted from Sherman et al. (1986).

1. Inoculate 5 ml of YPD medium in a 15-ml tube with a single freshly grown colony of the desired *S. cerevisiae* strain. Incubate at 30°C with agitation for aeration overnight.

   Notes: *S. cerevisiae* cells can be grown in either rich medium (YPD medium) or synthetic medium (e.g., SC medium) for DNA isolation. This protocol was designed for yeast grown in YPD medium but works well for yeast grown under other conditions.

   The incubation temperature must be adjusted for temperature-sensitive strains.

   This procedure should yield approximately 2 µg of DNA for each milliliter of haploid yeast culture grown to a density of 1×10^8 to 2×10^8 cells/ml. Diploid cultures should yield twice as much DNA.

2. Place 200 ml of YPD medium in a 2-liter flask and inoculate with the entire overnight culture from step 1. Incubate at 30°C with agitation at 250–350 rpm until the culture reaches late-log growth phase or early stationary growth phase.

   Notes: It takes approximately 10 hours (five generations) for the culture to reach late-log growth phase or early stationary growth phase. The yeast cell count should reach approximately 1×10^8 to 2×10^8 cells/ml.

   Cultures must be agitated to ensure good aeration. The capacity of the flask should be five to ten times the volume of the medium.

3. Place the cells on ice for 30 minutes.

4. Transfer the cells into appropriate sized centrifuge bottles or tubes. Centrifuge in a Sorvall RC-5B centrifuge (or equivalent) at 5000 rpm (2000*g*) at 4°C for 5 minutes to recover the cells. Discard the supernatant.

5. Resuspend the cell pellet in 1/12 volume of 1 M sorbitol/100 mM EDTA/100 mM β-mercaptoethanol.

   1 M sorbitol/100 mM EDTA/100 mM β-mercaptoethanol

Component and final concentration	Amount to add per 100 ml
1 M sorbitol	18.2 g
100 mM EDTA	20 ml of 0.5 M (pH 8.0)
H_2O	to make 100 ml
100 mM β-mercaptoethanol	0.7 ml of 14.3 M

   Combine all of the components except the β-mercaptoethanol. Store at 4°C for up to 6 months. Add the β-mercaptoethanol just before use.

   β-mercaptoethanol (see Appendix for Caution)

6. Add 10 µl of Zymolyase-60T (2.5 mg/ml) to the cells and mix by gently flicking the tube with a finger. Incubate at 37°C for 1 hour to allow spheroplasts to form.

   Note: This step reduces the integrity of the cell wall and creates spheroplasts, which are amenable to DNA isolation. There are several alternative enzyme preparations that can be used for this purpose including Zymolyase-100T and Zymolyase-20T (which are, respectively, more purified and less purified versions of the same enzyme as Zymolyase-60T), yeast lytic enzyme, and glusulase. All of these enzymes are β-glucanases, which digest, weaken, or remove the yeast cell wall. The reducing environment provided by the β-mercaptoethanol assists in this process.

   Zymolyase-60T (2.5 mg/ml)

   Suspend Zymolyase-60T (ICN) in 50% glycerol at a final concentration of 2.5 mg/ml. Divide into 100-µl aliquots and store at –20°C for up to 1 year.

7. Centrifuge in a Beckman GS-6R centrifuge (or equivalent centrifuge with swinging-bucket rotor) at 1500 rpm (450*g*) at 37°C for 5 minutes to recover the spheroplasts. Decant the supernatant carefully.

   Note: The pellet is very loose and soft.

8. Resuspend the pellet in 1/6 volume of 50 mM Tris-Cl/20 mM EDTA.

   50 mM Tris-Cl/20 mM EDTA

Component and final concentration	Amount to add per 1 liter
50 mM Tris-Cl	50 ml of 1 M (pH 7.4 at 25°C)
20 mM EDTA	40 ml of 0.5 M (pH 8.0)
H_2O	0.91 liter

   Store at room temperature indefinitely.

9. Add 1/60 volume of 10% **SDS** and mix. Incubate at 65°C for 30 minutes to lyse the spheroplasts.

   SDS (see Appendix for Caution)

10. Centrifuge the lysed spheroplasts in a Sorvall RC-5B centrifuge (or equivalent) at 5000 rpm (2000*g*) at room temperature for 1–2 minutes to remove residual cellular debris.

11. Use a pipette to transfer the supernatant into a new centrifuge tube. Discard the pellet.

12. Add 1/15 volume of 5 M potassium acetate to the supernatant. Place on ice for 1 hour.

    Note: This step precipitates the dodecyl sulfate detergent as the potassium salt.

13. Centrifuge in a Sorvall RC-5B centrifuge (or equivalent) at 3000 rpm (700*g*) at 4°C for 10 minutes.

14. Transfer the supernatant into a new centrifuge tube. Discard the pellet.

15. Precipitate the nucleic acids with 1 volume of isopropanol (see Appendix). Do not dry the DNA pellet.

    Note: The nucleic acids should be recovered by centrifugation.

16. Dissolve the nucleic acids in 300 µl of TE (pH 7.4). Add 15 µl of DNase-free RNase A (1 mg/ml) and mix by vortexing. Incubate at 37°C for 30 minutes.

    DNase-free pancreatic RNase A (1 mg/ml)

    Dissolve pancreatic RNase A in H_2O at a final concentration of 1 mg/ml. Boil for 10 minutes to inactivate any contaminating DNases. Divide into 400-µl aliquots and store at −20°C for up to 1 year.

17. Extract with 1 volume of **phenol** (see Appendix). Use phenol saturated with 1 M Tris-Cl (pH 8.0 at 25°C).

    phenol (see Appendix for Caution)

18. Precipitate the DNA in the aqueous phase with 1/3 volume of 7.5 M ammonium acetate (pH 7.4) and 2.5 volumes of absolute ethanol (see Appendix). Do not dry the DNA pellet.

19. Dissolve the DNA in 100 µl of TE (pH 7.4). Measure the DNA concentration (see Appendix) and adjust the final concentration to 200 µg/ml with TE (pH 7.4).

    Note: Pure DNA samples that are free of contaminating nucleases can be stored at 4°C for several years. However, since slow degradation may occur at this temperature, −80°C is recommended for prolonged storage. If a nonfrost-free freezer is available, DNA can also be stored at −20°C.

PROTOCOL

DNA Isolation from *S. pombe*

This protocol is from Gemmill et al. (1992) and was adapted from one by D. Schwartz (pers. comm.).

1. Inoculate 5 ml of YPD medium in a 15-ml tube with a single freshly grown colony of the desired *S. pombe* strain. Incubate at 30°C with vigorous agitation overnight.

   Note: The incubation temperature must be adjusted for temperature- sensitive strains.

2. Inoculate 200 ml of YPD medium in a 2-liter flask with the entire overnight culture from step 1. Incubate at 30°C with vigorous agitation (250–300 rpm) for 1 day.

3. After the 1-day incubation, measure the OD_{600} of the culture at several 1-hour intervals to determine whether or not the cells are in log growth phase. Confirm that the cells are in log growth phase by observing a logarithmic progression of OD_{600} readings and then proceed with step 4.

   Note: The cell density can also be determined by counting the cells in a 100-µl sample in a hemocytometer (see Appendix). The culture should reach a density of approximately 8×10^7 cells/ml. The OD_{600} of a tenfold dilution of this culture should be 0.6–0.8 (final culture). Each milliliter of culture should yield 0.5–2 µg of DNA.

4. Transfer the culture into a 225–500-ml centrifuge bottle. Centrifuge in a Sorvall GSA rotor at 3000 rpm (1000*g*) at 4°C for 5 minutes to recover the yeast cells. Decant the supernatant.

5. Add 0.5 volume of acidic washing buffer and resuspend the pellet by pipetting up and down and then gently vortexing.

   Acidic washing buffer

Component and final concentration	Amount to add per 1 liter
50 mM sodium citrate	50 ml of 1 M (pH 5.8)
50 mM sodium phosphate	50 ml of 1 M (pH 5.8)
40 mM EDTA	80 ml of 0.5 M (pH 8.0)
H_2O	0.82 liter

   Store at room temperature indefinitely.

1 M Sodium phosphate (pH 5.8)

Component and final concentration	Amount to add per 100 ml
Na_2HPO_4 (dibasic)	8 ml of 1 M
NaH_2PO_4 (monobasic)	92 ml of 1 M

Store at room temperature for up to 1 year. Check the pH before use. If the pH is not 5.8, prepare fresh solution, adjusting the ratio of dibasic to monobasic sodium phosphate to obtain the proper pH.

6. Centrifuge as described in step 4 and decant the supernatant.

7. Add 20 ml of acidic washing buffer and resuspend the pellet by pipetting up and down and then gently vortexing.

8. Transfer the resuspended pellet into a 50-ml conical tube. Rinse the bottle with 15 ml of acidic washing buffer and add to the tube.

9. Centrifuge in a Beckman GS-6R centrifuge (or equivalent centrifuge with swinging-bucket rotor) at 3000 rpm (1800*g*) at 4°C for 5 minutes. Decant the supernatant.

10. Add 10 ml of fission yeast spheroplasting buffer and resuspend the pellet by pipetting up and down and then gently vortexing. Incubate at 30°C until spheroplasts form and then place on ice.

Note: Monitor the culture for spheroplast formation at 15-minute intervals by examining an aliquot of cells with a phase-contrast microscope. As the Novozyme present in the spheroplasting buffer digests the yeast cell wall, the cells lose their rod-shaped appearance and begin to become round. This process is generally completed in approximately 1 hour. To confirm that the process is complete, mix a small drop of the incubation solution with a drop of 10% **SDS** and examine with a phase-contrast microscope. The presence of empty cell capsules indicates lysis of spheroplasts.

Fission yeast spheroplasting buffer

Component and final concentration	Amount to add per 100 ml
50 mM sodium citrate	5 ml of 1 M (pH 5.8)
50 mM sodium phosphate	5 ml of 1 M (pH 5.8)
40 mM EDTA	8 ml of 0.5 M (pH 8.0)
1.2 M sorbitol	60 ml of 2 M
H_2O	to make 100 ml
0.5 mg/ml Novozyme 234 (Novo BioLabs)	50 mg

Combine all of the components except the Novozyme. Store at 4°C for up to 1 year. Add the Novozyme (Novo BioLabs, 33 Turner Rd., Danbury, CT 06810-5101, 800-344-6686) just before use.

SDS (see Appendix for Caution)

11. Centrifuge in a Beckman GS-6R centrifuge at 1000 rpm (200*g*) at 4°C for 5 minutes. Use a pipette to carefully remove the supernatant.

Notes: The pellet is soft and easily disturbed.

Low-speed centrifugation is necessary at this point to avoid damaging the now-fragile spheroplasts.

12. Wash the pellet by adding 10 ml of fission yeast neutralization buffer, gently pipetting up and down, and repeating step 11.

Fission yeast neutralization buffer

Component and final concentration	Amount to add per 1 liter
20 mM Tris-Cl	20 ml of 1 M (pH 7.5 at 25°C)
1 mM EDTA	2 ml of 0.5 M (pH 8.0)
1.2 M sorbitol	0.6 liter of 2 M
H_2O	378 ml

Store at 4°C for up to 1 year.

13. Add 2 ml of fission yeast neutralization buffer and resuspend the spheroplasts by gently pipetting up and down.

14. Add 50 µl of 10% SDS and mix by pipetting up and down. Place on ice for 30 minutes to lyse the spheroplasts.

15. Centrifuge the lysate in a Sorvall RC-5B centrifuge (or equivalent) at 5000 rpm (2000*g*) at 4°C for 1–2 minutes to remove residual cellular debris.

16. Use a pipette to transfer the supernatant into a new centrifuge tube. Discard the pellet.

17. Add 1/15 volume of 5 M potassium acetate to the supernatant and mix by shaking the tube. Place on ice for 1 hour.

Note: This step precipitates the dodecyl sulfate detergent as the potassium salt.

18. Centrifuge in a Beckman GS-6R centrifuge (or equivalent centrifuge with swinging-bucket rotor) at 3000 rpm (1800*g*) at 4°C for 10 minutes.

19. Transfer the supernatant into a new centrifuge tube. Discard the pellet.

20. Precipitate the nucleic acids with 1 volume of isopropanol (see Appendix). Do not dry the DNA pellet.

Note: The nucleic acids should be recovered by centrifugation.

21. Dissolve the nucleic acids in 300 µl of TE (pH 7.4). Add 15 µl of DNase-free pancreatic RNase A (1 mg/ml in TE [pH 7.4]; for preparation, see also p. 29) and mix by pipetting up and down. Incubate at 37°C for 30 minutes.

22. Extract with 1 volume of **phenol** (see Appendix). Use phenol saturated with 1 M Tris-Cl (pH 8.0 at 25°C).

    phenol (see Appendix for Caution)

23. Precipitate the DNA in the aqueous phase with 1/3 volume of 7.5 M ammonium acetate (pH 7.4) and 2.5 volumes of absolute ethanol (see Appendix). Do not dry the DNA pellet.

24. Dissolve the DNA in 100 µl of TE (pH 7.4). Measure the DNA concentration (see Appendix) and adjust the final concentration to 1 mg/ml with TE (pH 7.4).

    Note: Pure DNA samples that are free of contaminating nucleases can be stored at 4°C for several years. However, since slow degradation may occur at this temperature, −80°C is recommended for prolonged storage. If a nonfrost-free freezer is available, DNA can also be stored at −20°C.

DNA FROM BACTERIA

Bacterial genomic DNA can be isolated relatively easily by lysing the cells with detergent and degrading the proteins with proteinase K. A critical aspect of the DNA isolation procedure is preventing DNA degradation by inhibiting DNases. The protocol on pp. 35–36 has been used for isolating DNA from *E. coli*.

PROTOCOL

DNA Isolation from *E. coli*

This protocol is from B. Gemmill (pers. comm.).

1. Inoculate 5 ml of LB medium in a 15-ml culture tube with a single freshly grown bacterial colony. Incubate at 37°C with vigorous agitation (300 rpm) overnight.

   Note: The precise growth conditions (medium, temperature, and oxygen content) should be modified for the specific strain being used.

2. Place 0.5 liter of LB medium in a 2-liter flask and inoculate with the entire overnight culture from step 1. Incubate at 37°C with vigorous agitation (300 rpm) until the culture reaches mid-log growth phase.

   Notes: It takes approximately 2–3 hours for an *E. coli* culture to reach mid-log growth phase (i.e., an OD_{600} of 0.7–0.8).

   A 0.5-liter culture should yield approximately 1 mg of DNA.

3. Place the cells on ice for 15 minutes.

4. Transfer the cells into a 0.5-liter centrifuge bottle. Centrifuge in a Sorvall RC-5B centrifuge in a GS-3 rotor at 8000 rpm (7000*g*) at 4°C for 10 minutes to recover the cells. Decant the supernatant.

5. To wash the cells, add 0.5 volume (50 ml per 100 ml of culture in step 2) of ice-cold minimal salts solution (isotonic) to the cell pellet, mix by vortexing, centrifuge as described in step 4, and decant the supernatant.

   Minimal salts solution

Component and final concentration	Amount to add per 1 liter
33 mM KH_2PO_4 (monobasic, anhydrous)	4.5 g
60 mM K_2HPO_4 (dibasic, anhydrous)	10.5 g
7.6 mM ammonium sulfate	1 g
3.3 mM sodium citrate (dihydrate)	0.97 g
4 mM $MgSO_4 \cdot 7H_2O$	1 g
H_2O	to make 1 liter

   Sterilize by autoclaving. Store at room temperature indefinitely.

6. Resuspend the cell pellet from step 5 in 1/50 volume (2 ml per 100 ml of culture in step 2) of ice-cold minimal salts solution. Transfer to a 50-ml Oak Ridge tube. Add a volume of bacterial cell lysis buffer equal to the volume

of the resuspended pellet, mix gently by inverting, and place on ice for 5 minutes.

Note: Vigorous mixing of the lysed bacterial suspension will shear the DNA and should therefore be minimized if large intact DNA is required. However, for most applications, the average size generated (50–100 kb) is not limiting. If larger DNA is necessary, a protocol for embedding the DNA in agarose is suggested (see Chapter 2).

Bacterial cell lysis buffer

Component and final concentration	Amount to add per 1 liter
10 mM Tris-Cl	10 ml of 1 M (pH 8.0 at 25°C)
100 mM EDTA	200 ml of 0.5 M (pH 8.0)
10 mM NaCl	2 ml of 5 M
0.5% **SDS**	50 ml of 10%
H_2O	0.738 liter

Store at 4°C indefinitely.

SDS (see Appendix for Caution)

7. Extract twice with 1 volume of **phenol** (see Appendix). Use phenol saturated with 1 M Tris-Cl (pH 8.0 at 25°C).

   phenol (see Appendix for Caution)

8. Precipitate the DNA in the aqueous phase with 1/3 volume of 7.5 M ammonium acetate (pH 7.4) and 2.5 volumes of absolute ethanol (see Appendix). Do not dry the DNA pellet.

9. Dissolve the DNA in TE (pH 7.4) at a final concentration of approximately 1 mg/ml. Measure the DNA concentration (see Appendix) and adjust the final concentration to 1 mg/ml with TE (pH 7.4).

   Note: Pure DNA samples that are free of contaminating nucleases can be stored at 4°C for several years. However, since slow degradation may occur at this temperature, −80°C is recommended for prolonged storage. If a nonfrost-free freezer is available, DNA can also be stored at −20°C.

Troubleshooting for Genomic DNA Isolation

The failure to recover pure HMW (>50 kb) DNA at approximately the expected concentration may be the result of one of a number of problems that may arise during the preparation of the DNA sample. These problems, along with approaches to solving them, are described below.

DEGRADATION AND FRAGMENTATION OF THE DNA

If the DNA is of the expected size (50–150 kb), little or no DNA will be visible below the size of limiting mobility on a conventional 0.7% agarose gel. A significant amount of DNA visible as a smear below this level indicates that the DNA has been degraded by enzymes or sheared by physical forces.

Degradation can occur during or after the isolation of DNA from the organism or tissue source. To minimize degradation during DNA preparation, it is particularly important to ensure that the source material is accessible to the lysis buffer (e.g., frozen tissue samples must be fully homogenized or yeast cell walls must be thoroughly removed). The proteinase K also needs to be fresh to ensure maximum activity and should therefore be added as a powder just before use. Including EDTA in the lysis buffer helps inhibit endogenous nucleases. Degradation after the DNA is isolated is caused by residual nucleases in the DNA solution. Proper treatment with proteinase K and extraction with phenol should prevent this problem.

Forces associated with shearing can cause fragmentation during the preparation of DNA. Mixing on a vortex mixer, vigorous pipetting, and pipetting through a small-bore opening can all shear the DNA and should therefore be avoided. Mixing by gently inverting the sample (particularly during the lysis step), mixing by flicking the tube with a finger, or gently pipetting through a wide-bore opening (~2 mm) all help minimize fragmentation.

POOR RECOVERY OF DNA

Poor recovery of DNA can result from a miscalculation of the number of cells processed, inadequate cell lysis, loss of material during processing, or inversion of aqueous and organic phases during extraction with phenol. Aliquots collected from each stage of the isolation procedure can be tested by electrophoresis (without digestion) to ensure that DNA is present and to determine where the DNA was lost or destroyed in the processing of the sample. If too few cells were processed in the original sample to obtain the desired amount of DNA, it may be necessary to start over with a larger cell or tissue sample. Determining the actual cell count instead of estimating the cell number on the basis of macroscopic inspection of a culture or the amount of time allotted for growth is always advisable.

The first indicator of low cell content may be low viscosity during lysis. If the low yield is caused by inadequate cell lysis, make sure the source material is accessible to the lysis buffer (see above).

Poor recovery of DNA should be evident when the DNA is first precipitated with isopropanol in the DNA isolation protocol. If the DNA does not precipitate from solution at this point, recover the DNA as described on p. 8. A high salt concentration in the DNA solution will lead to inverted organic/aqueous phases during extraction with phenol. If the phenol contains 8-hydroxyquinoline (see Appendix), the yellow color associated with this compound will help distinguish the two phases. If it is not clear which phase contains the phenol, place a small drop of the suspected organic phase on a piece of Styrofoam in a chemical fume hood. An organic solution will melt the Styrofoam. The amount of salt in the sample can be reduced by precipitating the DNA with ethanol (see Appendix) and then dissolving the DNA in TE (pH 7.4).

CONTAMINATION OF THE DNA WITH PROTEINS

An OD_{260}/OD_{280} ratio of less than 1.8 indicates that protein remains in the DNA preparation (see Appendix). Contaminating protein can inhibit many subsequent DNA manipulations (e.g., digestion with restriction enzymes). Since nucleases are almost always a component of the contaminating protein, DNA degradation can also occur. To remove protein contaminants, perform an additional extraction with phenol:chloroform:isoamyl alcohol (25:24:1) and then precipitate with ethanol again.

CONTAMINATION OF THE DNA WITH RNA

Contamination with RNA is visible on agarose gels as a bright ethidium-bromide-stained zone that migrates at the dye front. The amount of RNA can be reduced by recovering DNA precipitated with ethanol by the spooling method or by treating the DNA with DNase-free pancreatic RNase A.