TECHNICAL MANUAL

# Wizard® Genomic DNA Purification Kit

Instructions for use of Product
**A1120 , A1123, A1125 AND A1620**


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# Wizard® Genomic DNA Purification Kit

**All technical literature is available on the Internet at: www.promega.com/tbs/ Please visit the web site to verify that you are using the most current version of this Technical Manual. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com.**



## 1. Description

The Wizard® Genomic DNA Purification Kit is designed for isolation of DNA from white blood cells (Sections 3.A, B and C), tissue culture cells and animal tissue (Section 3.D), plant tissue (Section 3.E), yeast (Section 3.F), and Gram positive and Gram negative bacteria (Section 3.G). Table 1 lists the typical yield for DNA purified from each of these sources.

The Wizard® Genomic DNA Purification Kit is based on a four-step process (1). The first step in the purification procedure lyses the cells and the nuclei. For isolation of DNA from white blood cells, this step involves lysis of the red blood cells in the Cell Lysis Solution, followed by lysis of the white blood cells and their nuclei in the Nuclei Lysis Solution. An RNase digestion step may be

included at this time; it is optional for some applications. The cellular proteins are then removed by a salt precipitation step, which precipitates the proteins but leaves the high molecular weight genomic DNA in solution. Finally, the genomic DNA is concentrated and desalted by isopropanol precipitation.

DNA purified with this system is suitable for a variety of applications, including amplification, digestion with restriction endonucleases and membrane hybridizations (e.g., Southern and dot/slot blots).

## 2. Product Components and Storage Conditions

### Small-Scale Isolation (minipreps)

| Product | Size | Cat.# |
|---|---|---|
| Wizard® Genomic DNA Purification Kit | 100 isolations | A1120 |

Each system contains sufficient reagents for 100 isolations of genomic DNA from **300µl** of whole blood samples. Includes:

- 100ml Cell Lysis Solution
- 50ml Nuclei Lysis Solution
- 25ml Protein Precipitation Solution
- 50ml DNA Rehydration Solution
- 250µl RNase Solution

| Product | Size | Cat.# |
|---|---|---|
| Wizard® Genomic DNA Purification Kit | 500 isolations | A1125 |

Each system contains sufficient reagents for 500 isolations of genomic DNA from **300µl** of whole blood samples. Includes:

- 500ml Cell Lysis Solution
- 250ml Nuclei Lysis Solution
- 125ml Protein Precipitation Solution
- 100ml DNA Rehydration Solution
- 1.25ml RNase Solution

**Large-Scale Isolation (maxiprep)**

| Product | Size | Cat.# |
|---|---|---|
| Wizard® Genomic DNA Purification Kit | 100 isolations | A1620 |

Each system contains sufficient reagents for 100 isolations of genomic DNA from **10ml** of whole blood samples. Includes:

- 3L Cell Lysis Solution
- 1L Nuclei Lysis Solution
- 350ml Protein Precipitation Solution
- 150ml DNA Rehydration Solution

**Note:** Cat.# A1620 does not include RNase Solution.

**Items Available Separately**

| Product | Size | Cat.# |
|---|---|---|
| Cell Lysis Solution | 1L | A7933 |
| Nuclei Lysis Solution | 1L | A7943 |
| Protein Precipitation Solution | 350ml | A7953 |
| DNA Rehydration Solution | 50ml | A7963 |
| RNase A (4mg/ml) | 1ml | A7973 |

**Storage Conditions:** Store the Wizard® Genomic DNA Purification Kit at room temperature (22–25°C). See product label for expiration date.

**Table 1. DNA Yields from Various Starting Materials.**

| Species and Material | Amount of Starting Material | Typical DNA Yield | RNase Treatment |
|---|---|---|---|
| **Human Whole Blood** | | | |
| (Yield depends on the quantity of white blood cells present) | 300µl | 5–15µg | Optional |
| | 1.0ml | 25–50µg | Optional |
| | 10.0ml | 250–500µg | Optional |
| 96-well plate (Process as little as 20µl/well; see Table 2.) | 50µl/well | 0.2–0.7µg | Optional |
| **Mouse Whole Blood** | | | |
| EDTA (4%) treated | 300µl | 6µg | Optional |
| Heparin (4%) treated | 300µl | 6–7µg | Optional |
| 96-well plate | 50µl/well | 0.2–0.7µg | Optional |
| **Cell Lines** | | | |
| K562 (human) | $3 \times 10^6$ cells | 15–30µg | Required |
| COS (African green monkey) | $1.5 \times 10^6$ cells | 10µg | Required |
| NIH3T3 (mouse) | $2.25 \times 10^6$ cells | 9.5–12.5µg | Required |
| PC12 (rat pheochromocytoma) | $8.25 \times 10^6$ cells | 6µg | Required |
| CHO (hamster) | $1–2 \times 10^6$ cells | 6–7µg | Required |
| **Animal Tissue** | | | |
| Mouse Liver | 11mg | 15–20µg | Required |
| Mouse Tail | 0.5–1.0cm of tail | 10–30µg | Optional |
| **Insects** | | | |
| Sf9 cells | $5 \times 10^6$ cells | 16µg | Required |
| **Plant Tissue** | | | |
| Tomato Leaf | 40mg | 7–12µg | Required |
| **Gram Negative Bacteria** | | | |
| *Escherichia coli* JM109 overnight culture, ~$2 \times 10^9$ cells/ml | 1ml | 20µg | Required |
| | 5ml | 75–100µg | Required |
| *Enterobacter cloacae* overnight culture, ~$6 \times 10^9$ cells/ml | 1ml | 20µg | Required |
| | 5ml | 75–100µg | Required |
| **Gram Positive Bacteria** | | | |
| *Staphylococcus epidermis* overnight culture, ~$3.5 \times 10^8$ cells/ml | 1ml | 6–13µg | Required |
| **Yeast** | | | |
| *Saccharomyces cerevisiae* overnight culture, ~$1.9 \times 10^8$ cells/ml | 1ml | 4.5–6.5µg | Required |

## 3. Protocols for Genomic DNA Isolation

We tested the purification of genomic DNA from fresh whole blood collected in EDTA, heparin and citrate anticoagulant tubes and detected no adverse effects upon subsequent manipulations of the DNA, including PCR (2). Anticoagulant blood samples may be stored at 2–8°C for up to two months, but DNA yield will be reduced with increasing length of storage.

The protocol in Section 3.A has been designed and tested for blood samples up to 3ml in volume. The protocol in Section 3.B has been designed and tested for blood samples up to 10ml in volume. The yield of genomic DNA will vary depending on the quantity of white blood cells present. Frozen blood may be used in the following protocols, but yield may be lower than that obtained using fresh blood, and additional Cell Lysis Solution may be required.

**Caution:** When handling blood samples (Sections 3.A, B and C), follow recommended procedures at your institution for biohazardous materials or see reference 3.

### 3.A. Isolating Genomic DNA from Whole Blood (300µl or 3ml Sample Volume)

**Materials to Be Supplied by the User**

- sterile 1.5ml microcentrifuge tubes (for 300µl blood samples)
- sterile 15ml centrifuge tubes (for 3ml blood samples)
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature
- water bath, 65°C (optional, for rapid DNA rehydration)

1. For 300µl Sample Volume: Add 900µl of Cell Lysis Solution to a sterile 1.5ml microcentrifuge tube.

   For 3ml Sample Volume: Add 9.0ml of Cell Lysis Solution to a sterile 15ml centrifuge tube.

   **Important:** Blood must be collected in EDTA, heparin or citrate anticoagulant tubes to prevent clotting.

2. Gently rock the tube of blood until thoroughly mixed; then transfer blood to the tube containing the Cell Lysis Solution. Invert the tube 5–6 times to mix.

3. Incubate the mixture for 10 minutes at room temperature (invert 2–3 times once during the incubation) to lyse the red blood cells. Centrifuge at 13,000–16,000 × *g* for 20 seconds at room temperature for 300µl sample. Centrifuge at 2,000 × *g* for 10 minutes at room temperature for 3ml sample.

4. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Approximately 10–20µl of residual liquid will remain in the 1.5ml tube (300µl sample). Approximately 50–100µl of residual liquid will remain in the 15ml tube (3ml sample).

If blood sample has been frozen, repeat Steps 1–4 until pellet is white. There may be some loss of DNA from frozen samples.

**Note:** Some red blood cells or cell debris may be visible along with the white blood cells. If the pellet appears to contain only red blood cells, add an additional aliquot of Cell Lysis Solution after removing the supernatant above the cell pellet, and then repeat **Steps 3–4.**

5. Vortex the tube vigorously until the white blood cells are resuspended (10–15 seconds).

**Completely resuspend the white blood cells to obtain efficient cell lysis.**

6. Add Nuclei Lysis Solution (300µl for 300µl sample volume; 3.0ml for 3ml sample volume) to the tube containing the resuspended cells. Pipet the solution 5–6 times to lyse the white blood cells. The solution should become very viscous. If clumps of cells are visible after mixing, incubate the solution at 37°C until the clumps are disrupted. If the clumps are still visible after 1 hour, add additional Nuclei Lysis Solution (100µl for 300µl sample volume; 1.0ml for 3ml sample volume) and repeat the incubation.

7. **Optional:** Add RNase Solution (1.5µl for 300µl sample volume; 15µl for 3ml sample volume) to the nuclear lysate, and mix the sample by inverting the tube 2–5 times. Incubate the mixture at 37°C for 15 minutes, and then cool to room temperature.

8. Add Protein Precipitation Solution (100µl for 300µl sample volume; 1.0ml for 3ml sample volume) to the nuclear lysate, and vortex vigorously for 10–20 seconds. Small protein clumps may be visible after vortexing.

   **Note:** If additional Nuclei Lysis Solution was added in **Step 6**, add a total of 130µl Protein Precipitation Solution for 300µl sample volume and 1.3ml Protein Precipitation Solution for 3ml sample volume.

9. Centrifuge at 13,000–16,000 × *g* for 3 minutes at room temperature for 300µl sample volume. Centrifuge at 2,000 × *g* for 10 minutes at room temperature for 3ml sample volume.

   A dark brown protein pellet should be visible. If no pellet is observed, refer to Section 4.

10. For 300µl sample volume, transfer the supernatant to a clean 1.5ml microcentrifuge tube containing 300µl of room-temperature isopropanol. For 3ml sample volume, transfer the supernatant to a 15ml centrifuge tube containing 3ml room-temperature isopropanol.

    **Note:** Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

11. Gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.

12. Centrifuge at 13,000–16,000 × *g* for 1 minute at room temperature for 300µl sample. Centrifuge at 2,000 × *g* for 1 minute at room temperature for 3ml sample. The DNA will be visible as a small white pellet.

13. Decant the supernatant, and add one sample volume of room temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and the sides of the microcentrifuge tube. Centrifuge as in Step 12.

14. Carefully aspirate the ethanol using either a drawn Pasteur pipette or a sequencing pipette tip. The DNA pellet is very loose at this point and care must be used to avoid aspirating the pellet into the pipette. Invert the tube on clean absorbent paper and air-dry the pellet for 10–15 minutes.

15. Add DNA Rehydration Solution (100µl for 300µl sample volume; 250µl for 3ml sample volume) to the tube and rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C.

16. Store the DNA at 2–8°C.

**3.B. Isolating Genomic DNA from Whole Blood** (10ml Sample Volume)

A large-scale kit is available for processing up to 1 liter of whole blood (Cat.# A1620). This kit does not include RNase Solution since the RNase digestion step is optional. RNase A solution (4mg/ml) is available as a separate item (Cat.# A7973). If it is needed, a total of 5ml of RNase A solution is required to process 1 liter of blood.

**Materials to Be Supplied by the User**

- sterile 50ml centrifuge tubes
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature
- water bath, 65°C (optional; for rapid DNA rehydration)

1. For 10ml whole blood samples: Add 30ml of Cell Lysis Solution to a sterile 50ml centrifuge tube.

**Important:** Blood must be collected in EDTA, heparin or citrate anticoagulant tubes to prevent clotting.

2. Gently rock the tube of blood until thoroughly mixed; then transfer 10ml of blood to the tube containing the Cell Lysis Solution. Invert the tube 5–6 times to mix.

3. Incubate the mixture for 10 minutes at room temperature (invert 2–3 times once during the incubation) to lyse the red blood cells. Centrifuge at 2,000 × *g* for 10 minutes at room temperature.

4. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Approximately 1.4ml of residual liquid will remain.

   If blood sample has been frozen, add an additional 30ml of Cell Lysis Solution, invert 5–6 times to mix, and repeat Steps 3–4 until pellet is nearly white. There may be some loss of DNA in frozen samples.

   **Note:** Some red blood cells or cell debris may be visible along with the white blood cells. If the pellet appears to contain only red blood cells, add an additional aliquot of Cell Lysis Solution after removing the supernatant above the cell pellet, and then repeat **Steps 3–4**.

5. Vortex the tube vigorously until the white blood cells are resuspended (10–15 seconds).

   **Completely resuspend the white blood cells to obtain efficient cell lysis.**

6. Add 10ml of Nuclei Lysis Solution to the tube containing the resuspended cells. Pipet the solution 5–6 times to lyse the white blood cells. The solution should become very viscous. If clumps of cells are visible after mixing, incubate the solution at 37°C until the clumps are disrupted. If the clumps are still visible after 1 hour, add 3ml of additional Nuclei Lysis Solution and repeat the incubation.

7. **Optional:** Add RNase A, to a final concentration of 20µg/ml, to the nuclear lysate and mix the sample by inverting the tube 2–5 times. Incubate the mixture at 37°C for 15 minutes, and then cool to room temperature.

8. Add 3.3ml of Protein Precipitation Solution to the nuclear lysate, and vortex vigorously for 10–20 seconds. Small protein clumps may be visible after vortexing.

   **Note:** If additional Nuclei Lysis Solution was added in **Step 6**, add 4ml of Protein Precipitation Solution (instead of 3.3ml).

9. Centrifuge at 2,000 × *g* for 10 minutes at room temperature.

   A dark brown protein pellet should be visible. If no pellet is observed, refer to Section 4.

10. Transfer the supernatant to a 50ml centrifuge tube containing 10ml of room temperature isopropanol.

    **Note:** Some supernatant may remain in the original tube containing the protein pellet. Leave the residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

11. Gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.

12. Centrifuge at 2,000 × *g* for 1 minute at room temperature. The DNA will be visible as a small white pellet.

13. Decant the supernatant and add 10ml of room temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and the sides of the centrifuge tube. Centrifuge as in Step 12.

14. Carefully aspirate the ethanol. The DNA pellet is very loose at this point and care must be used to avoid aspirating the pellet into the pipette. Air-dry the pellet for 10–15 minutes.

15. Add 800µl of DNA Rehydration Solution to the tube, and rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C.

16. Store the DNA at 2–8°C.

**3.C. Isolating Genomic DNA from Whole Blood** (96-well plate)

This protocol can be scaled to 20µl, 30µl or 40µl of blood. Table 2 outlines the various solution volumes used in each step. Fifty-microliter preps generally yield genomic DNA in the range of 0.2–0.7µg, depending upon the number of leukocytes in the blood sample.

**Table 2. Volumes of Reagents Required for Various Starting Amounts of Blood.**

| Sample | Cell Lysis Solution (RBC Lysis) | Nuclei Lysis Solution | Protein Precipitation Solution | Isopropanol | DNA Rehydration Solution |
|---|---|---|---|---|---|
| 20µl | 60µl | 20µl | 6.7µl | 20µl | 10µl |
| 30µl | 90µl | 30µl | 10µl | 30µl | 15µl |
| 40µl | 120µl | 40µl | 13.3µl | 40µl | 20µl |
| 50µl | 150µl | 50µl | 16.5µl | 50µl | 25µl |

**Materials to Be Supplied by the User**

- V-bottom 96-well plate(s) able to hold 300µl volume/well (Costar® Cat.# 3896)
- isopropanol, room temperature
- 70% ethanol, room temperature
- 96-well plate sealers (Costar® Cat.# 3095) (optional; for use with human blood)

1. Add 150µl Cell Lysis Solution to each well.

**Important:** Blood must be collected in EDTA, heparin or citrate anticoagulant tubes.

2. Add 50µl of fresh blood to each well and pipet 2–3 times to mix.

3. Leave the plate at room temperature for 10 minutes, pipetting the solution twice during the incubation to help lyse the red blood cells.

4. Centrifuge at 800 × *g* for 5 minutes in a tabletop centrifuge to concentrate the cells.

5. Carefully remove and discard as much of the supernatant as possible with a micropipette tip, leaving a small pellet of white cells and some red blood cells. The use of an extended pipette tip, such as a gel loading tip, is recommended. Tilting the 96-well plate 50–80° (depending on the amount of liquid present per well) allows more thorough removal of liquid from the well.

6. Add 50µl of Nuclei Lysis Solution to each well and pipet 5–6 times to resuspend the pellet and lyse the white blood cells. The solution should become more viscous. As an aid in DNA pellet visualization, 2µl per well of a carrier (e.g., Polyacryl Carrier [Molecular Research Center, Inc., Cat.# PC152]) can be added at this step. DNA yields are generally equivalent with or without carrier use.

7. Add 16.5µl of Protein Precipitation Solution per well and pipet 5–6 times to mix.

8. Centrifuge at 1,400 × *g* for 10 minutes at room temperature. A brown protein pellet should be visible. If no pellet is visible, refer to Section 4.

9. DNA Precipitation/Rehydration in 96-Well Plate

   a. Carefully transfer the supernatants to clean wells containing 50µl per well of room temperature isopropanol and mix by pipetting.

   **Note:** Some of supernatant may remain in the original well containing the protein pellet. Leave this residual liquid in the well to avoid contaminating the DNA solution with the precipitated protein. As in **Step 5**, tilting the plate will facilitate removal of liquid from the well. Using an extended pipette tip in this step does not allow easy sample mixing with isopropanol.

   b. Centrifuge at 1,400 × *g* for 10 minutes. Carefully remove the isopropanol with a micropipette tip.

   c. Add 100µl of room temperature 70% ethanol per well.

   d. Centrifuge at 1,400 × *g* for 10 minutes at room temperature.

   e. Carefully aspirate the ethanol using either a drawn Pasteur pipette or a sequencing pipette tip. Care must be taken to avoid aspirating the DNA pellet. Place the tray at a 30–45° angle and air-dry for 10–15 minutes.

   f. Add 25µl of DNA Rehydration Solution to each well. Allow the DNA to rehydrate overnight at room temperature or at 4°C.

   g. Store the DNA at 2–8°C.

   **Note:** Small volumes of DNA can be easily collected at the bottom of a V-well by briefly centrifuging the 96-well plate before use.

### 3.D. Isolating Genomic DNA from Tissue Culture Cells and Animal Tissue

**Materials to Be Supplied by the User**

- 1.5ml microcentrifuge tubes
- 15ml centrifuge tubes
- small homogenizer (Fisher Tissue Tearor, Cat.# 15-338-55, or equivalent) (for animal tissue)
- trypsin (for adherent tissue culture cells only)
- PBS
- liquid nitrogen (for mouse tail) (optional; for freeze-thaw, Step 1.d, and for tissue grinding, Step 2.b, in place of small homogenizer)
- mortar and pestle (optional; for tissue grinding, Step 2.b, in place of small homogenizer)
- 95°C water bath (optional; for freeze-thaw, Step 1.d)
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature
- water bath, 65°C (optional; for rapid DNA rehydration)
- 0.5M EDTA (pH 8.0) (for mouse tail)
- Proteinase K (20mg/ml in water; Cat.# V3021) (for mouse tail)

**1. Tissue Culture Cells**

a. Harvest the cells, and transfer them to a 1.5ml microcentrifuge tube. For adherent cells, trypsinize the cells before harvesting.

b. Centrifuge at 13,000–16,000 × *g* for 10 seconds to pellet the cells.

c. Remove the supernatant, leaving behind the cell pellet plus 10–50µl of residual liquid.

d. Add 200µl PBS to wash the cells. Centrifuge as in Step 1.b, and remove the PBS. Vortex vigorously to resuspend cells.

**Note:** For cells that do not lyse well in Nuclei Lysis Solution alone (e.g., PC12 cells), perform an additional freeze-thaw step as follows before proceeding to Step 1.e: Wash the cells as in Step 1.d; then freeze in liquid nitrogen. Thaw the cells by heating at 95°C. Repeat this procedure for a total of 4 cycles.

e. Add 600µl of Nuclei Lysis Solution, and pipet to lyse the cells. Pipet until no visible cell clumps remain.

f. Proceed to Section 3.D, Step 4.

**2. Animal Tissue (Mouse Liver and Brain)**

a. Add 600µl of Nuclei Lysis Solution to a 15ml centrifuge tube, and chill on ice.

b. Add 10–20mg of fresh or thawed tissue to the chilled Nuclei Lysis Solution and homogenize for 10 seconds using a small homogenizer. Transfer the lysate to a 1.5ml microcentrifuge tube. Alternatively, grind tissue in liquid nitrogen using a mortar and pestle that has been prechilled in liquid nitrogen. After grinding, allow the liquid nitrogen to evaporate and transfer approximately 10–20mg of the ground tissue to 600µl of Nuclei Lysis Solution in a 1.5ml microcentrifuge tube.

c. Incubate the lysate at 65°C for 15–30 minutes.

d. Proceed to Section 3.D, Step 4.

**3. Animal Tissue (Mouse Tail)**

a. For each sample to be processed, add 120µl of a 0.5M EDTA solution (pH 8.0) to 500µl of Nuclei Lysis Solution in a centrifuge tube. Chill on ice.

**Note:** The solution will turn cloudy when chilled.

b. Add 0.5–1cm of fresh or thawed mouse tail to a 1.5ml microcentrifuge tube.

**Note:** The tissue may be ground to a fine powder in liquid nitrogen using a mortar and pestle that has been prechilled in liquid nitrogen. Then transfer the powder to a 1.5ml microcentrifuge tube.

c. Add 600µl of EDTA/Nuclei Lysis Solution from Step 3.a to the tube.

d. Add 17.5µl of 20mg/ml Proteinase K.

e. Incubate overnight at 55°C with gentle shaking. Alternatively, perform a 3-hour 55°C incubation (with shaking); vortex the sample once per hour if performing a 3-hour incubation. Make sure the tail is completely digested.

4. **Optional for mouse tail:** Add 3µl of RNase Solution to the nuclear lysate and mix the sample by inverting the tube 2–5 times. Incubate the mixture for 15–30 minutes at 37°C. Allow the sample to cool to room temperature for 5 minutes before proceeding.

5. To the room temperature sample, add 200µl of Protein Precipitation Solution and vortex vigorously at high speed for 20 seconds. Chill sample on ice for 5 minutes.

6. Centrifuge for 4 minutes at 13,000–16,000 × *g*. The precipitated protein will form a tight white pellet.

7. Carefully remove the supernatant containing the DNA (leaving the protein pellet behind) and transfer it to a clean 1.5ml microcentrifuge tube containing 600µl of room temperature isopropanol.

**Note:** Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

8. Gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.

9. Centrifuge for 1 minute at 13,000–16,000 × *g* at room temperature. The DNA will be visible as a small white pellet. Carefully decant the supernatant.

10. Add 600µl of room temperature 70% ethanol, and gently invert the tube several times to wash the DNA. Centrifuge for 1 minute at 13,000–16,000 × *g* at room temperature.

11. Carefully aspirate the ethanol using either a drawn Pasteur pipette or a sequencing pipette tip. The DNA pellet is very loose at this point, and care must be used to avoid aspirating the pellet into the pipette.

12. Invert the tube on clean absorbent paper, and air-dry the pellet for 10–15 minutes.

13. Add 100µl of DNA Rehydration Solution, and rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C.

14. Store the DNA at 2–8°C.

### 3.E. Isolating Genomic DNA from Plant Tissue

**Materials to Be Supplied by the User**

- 1.5ml microcentrifuge tubes
- microcentrifuge tube pestle or mortar and pestle
- water bath, 65°C
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature

1. Leaf tissue can be processed by freezing with liquid nitrogen and grinding into a fine powder using a microcentrifuge tube pestle or a mortar and pestle. Add 40mg of this leaf powder to a 1.5ml microcentrifuge tube.

2. Add 600µl of Nuclei Lysis Solution, and vortex 1–3 seconds to wet the tissue.

3. Incubate at 65°C for 15 minutes.

4. Add 3µl of RNase Solution to the cell lysate, and mix the sample by inverting the tube 2–5 times. Incubate the mixture at 37°C for 15 minutes. Allow the sample to cool to room temperature for 5 minutes before proceeding.

5. Add 200µl of Protein Precipitation Solution, and vortex vigorously at high speed for 20 seconds.

6. Centrifuge for 3 minutes at 13,000–16,000 × *g*. The precipitated proteins will form a tight pellet.

7. Carefully remove the supernatant containing the DNA (leaving the protein pellet behind) and transfer it to a clean 1.5ml microcentrifuge tube containing 600µl of room temperature isopropanol.

   **Note:** Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

8. Gently mix the solution by inversion until thread-like strands of DNA form a visible mass.

9. Centrifuge at 13,000–16,000 × *g* for 1 minute at room temperature.

10. Carefully decant the supernatant. Add 600µl of room temperature 70% ethanol and gently invert the tube several times to wash the DNA. Centrifuge at 13,000–16,000 × *g* for 1 minute at room temperature.

11. Carefully aspirate the ethanol using either a drawn Pasteur pipette or a sequencing pipette tip. The DNA pellet is very loose at this point and care must be used to avoid aspirating the pellet into the pipette.

12. Invert the tube onto clean absorbent paper and air-dry the pellet for 15 minutes.

13. Add 100µl of DNA Rehydration Solution and rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C.

14. Store the DNA at 2–8°C.

### 3.F. Isolating Genomic DNA from Yeast

**Materials to Be Supplied by the User**

- 1.5ml microcentrifuge tubes
- YPD broth
- 50mM EDTA (pH 8.0)
- 20mg/ml lyticase (Sigma Cat.# L2524)
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature
- water bath, 65°C (optional; for rapid DNA rehydration)

1. Add 1ml of a culture grown for 20 hours in YPD broth to a 1.5ml microcentrifuge tube.

2. Centrifuge at 13,000–16,000 × *g* for 2 minutes to pellet the cells. Remove the supernatant.

3. Resuspend the cells thoroughly in 293µl of 50mM EDTA.

4. Add 7.5µl of 20mg/ml lyticase and gently pipet 4 times to mix.

5. Incubate the sample at 37°C for 30–60 minutes to digest the cell wall. Cool to room temperature.
6. Centrifuge the sample at 13,000–16,000 × *g* for 2 minutes and then remove the supernatant.
7. Add 300µl of Nuclei Lysis Solution to the cell pellet and gently pipet to mix.
8. Add 100µl of Protein Precipitation Solution and vortex vigorously at high speed for 20 seconds.
9. Let the sample sit on ice for 5 minutes.
10. Centrifuge at 13,000–16,000 × *g* for 3 minutes.
11. Transfer the supernatant containing the DNA to a clean 1.5ml microcentrifuge tube containing 300µl of room temperature isopropanol.

    **Note:** Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.
12. Gently mix by inversion until the thread-like strands of DNA form a visible mass.
13. Centrifuge at 13,000–16,000 × *g* for 2 minutes.
14. Carefully decant the supernatant and drain the tube on clean absorbent paper. Add 300µl of room temperature 70% ethanol and gently invert the tube several times to wash the DNA pellet.
15. Centrifuge at 13,000–16,000 × *g* for 2 minutes. Carefully aspirate all of the ethanol.
16. Drain the tube on clean absorbent paper and allow the pellet to air-dry for 10–15 minutes.
17. Add 50µl of DNA Rehydration Solution.
18. Add 1.5µl of RNase Solution to the purified DNA sample. Vortex the sample for 1 second. Centrifuge briefly in a microcentrifuge for 5 seconds to collect the liquid and incubate at 37°C for 15 minutes.
19. Rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C.
20. Store the DNA at 2–8°C.

### 3.G. Isolating Genomic DNA from Gram Positive and Gram Negative Bacteria

**Materials to Be Supplied by the User**

- 1.5ml microcentrifuge tubes
- water bath, 80°C
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature
- water bath, 65°C (optional; for rapid DNA rehydration)
- 50mM EDTA (pH 8.0) (for gram positive bacteria)
- 10mg/ml lysozyme (Sigma Cat.# L7651) (for gram positive bacteria)
- 10mg/ml lysostaphin (Sigma Cat.# L7386) (for gram positive bacteria)

1. Add 1ml of an overnight culture to a 1.5ml microcentrifuge tube.
2. Centrifuge at 13,000–16,000 × *g* for 2 minutes to pellet the cells. Remove the supernatant. For Gram Positive Bacteria, proceed to Step 3. **For Gram Negative Bacteria go directly to Step 6.**
3. Resuspend the cells thoroughly in 480µl of 50mM EDTA.
4. Add the appropriate lytic enzyme(s) to the resuspended cell pellet in a total volume of 120µl, and gently pipet to mix. The purpose of this pretreatment is to weaken the cell wall so that efficient cell lysis can take place.

   **Note:** For certain *Staphylococcus* species, a mixture of 60µl of 10mg/ml lysozyme and 60µl of 10mg/ml lysostaphin is required for efficient lysis. However, many Gram Positive Bacterial Strains (e.g., *Bacillus subtilis*, *Micrococcus luteus*, *Nocardia otitidiscaviarum*, *Rhodococcus rhodochrous*, and *Brevibacterium albidium*) lyse efficiently using lysozyme alone.
5. Incubate the sample at 37°C for 30–60 minutes. Centrifuge for 2 minutes at 13,000–16,000 × *g* and remove the supernatant.
6. Add 600µl of Nuclei Lysis Solution. Gently pipet until the cells are resuspended.
7. Incubate at 80°C for 5 minutes to lyse the cells; then cool to room temperature.
8. Add 3µl of RNase Solution to the cell lysate. Invert the tube 2–5 times to mix.
9. Incubate at 37°C for 15–60 minutes. Cool the sample to room temperature.
10. Add 200µl of Protein Precipitation Solution to the RNase-treated cell lysate. Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution with the cell lysate.
11. Incubate the sample on ice for 5 minutes.
12. Centrifuge at 13,000–16,000 × *g* for 3 minutes.

13. Transfer the supernatant containing the DNA to a clean 1.5ml microcentrifuge tube containing 600µl of room temperature isopropanol.

    **Note:** Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

14. Gently mix by inversion until the thread-like strands of DNA form a visible mass.

15. Centrifuge at 13,000–16,000 × *g* for 2 minutes.

16. Carefully pour off the supernatant and drain the tube on clean absorbent paper. Add 600µl of room temperature 70% ethanol and gently invert the tube several times to wash the DNA pellet.

17. Centrifuge at 13,000–16,000 × *g* for 2 minutes. Carefully aspirate the ethanol.

18. Drain the tube on clean absorbent paper and allow the pellet to air-dry for 10–15 minutes.

19. Add 100µl of DNA Rehydration Solution to the tube and rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C.

20. Store the DNA at 2–8°C.

## 4. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

| Symptoms | Comments |
|---|---|
| Blood clots present in blood samples | The tube may have been stored improperly; the blood was not thoroughly mixed, or inappropriate tubes were used for drawing blood. Discard the clotted blood and draw new samples using EDTA-, heparin- or citrate-treated anticoagulant tubes. |
| Poor DNA yield | The blood sample may contain too few white blood cells. Draw new blood samples. |
| | The white blood cell pellet was not resuspended thoroughly in Step 5 of Section 3.A or B. The white blood cell pellet must be vortexed vigorously to resuspend the cells. |
| | The blood sample was too old. Best yields are obtained with fresh blood. Samples that have been stored at 2–5°C for more than 5 days may give reduced yields. |

## 4. Troubleshooting (continued)

| Symptoms | Comments |
|---|---|
| Poor DNA yield (continued) | The DNA pellet was lost during isopropanol precipitation. Use extreme care when removing the isopropanol to avoid losing the pellet. |
| Degraded DNA (<50kb in size) | Improper collection or storage of the blood sample. Obtain a new sample under the proper conditions. |
| Poor DNA yield using Gram positive bacteria protocol | Cultures grown for an extended time contain a high proportion of cells that lyse easily upon exposure to lysostaphin treatment. Start purifications with a healthy culture. |
| No protein pellet | The sample was not cooled to room temperature before adding the Protein Precipitation Solution. Cool the sample to room temperature (at least 5 minutes) or chill on ice for 5 minutes, vortex 20 seconds, centrifuge for 3 minutes at 13,000–16,000 × *g* (10 minutes at 2,000 × *g* for 3ml sample volume) and proceed with the protocol. |
| | The Protein Precipitation Solution was not thoroughly mixed with the nuclear lysate. Always mix the nuclear lysate and Protein Precipitation Solution completely. |
| DNA pellet difficult to dissolve | Samples may have been overdried. Rehydrate DNA by incubating 1 hour at 65°C, and then leave the sample at room temperature or 4°C overnight. **Caution:** Do not leave the DNA at 65°C overnight. |
| | Samples were not mixed during the rehydration step. Remember to mix the samples periodically during the rehydration step. |

## 5. References


1. Miller, S.A., Dykes, D.D. and Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucl. Acids Res.* **16**, 1215.
2. Beutler, E., Gelbart, T. and Kuhl, W. (1990) Interference of heparin with the polymerase chain reaction. *BioTechniques* **9**, 166.
3. U.S. Department of Labor, Occupational Safety and Health Administration (1991) Occupational exposure to bloodborne pathogens, final rule. *Federal Register* **56**, 64175.

# 6. Appendix

## 6.A. Composition of Buffers and Solutions

**DNA Rehydration Solution (provided)**

10mM Tris-HCl (pH 7.4)
1mM EDTA (pH 8.0)

**RNase A**

Dissolve RNase A to 4mg/ml in DNA Rehydration Solution, boil 10 minutes to remove contaminating DNase and store in aliquots at –20°C. This solution is also available from Promega (Cat.# A7973).

## 6.B. Related Products

**DNA Purification Systems**

| Product | Size | Cat.# |
|---|---|---|
| ReadyAmp™ Genomic DNA Purification System | 100 reactions | A7710 |
| Wizard® *Plus* SV Minipreps DNA Purification System | 50 preps | A1330 |
| Wizard® *Plus* SV Minipreps DNA Purification System + Miniprep Vacuum Adapters | 50 preps | A1340 |
| Wizard® *Plus* SV Minipreps DNA Purification System | 250 preps | A1460 |
| Wizard® *Plus* SV Minipreps DNA Purification System + Miniprep Vacuum Adapters | 250 preps | A1470 |
| Miniprep Vacuum Adapters | 20 each | A1331 |

**DNA Amplification Systems**

| Product | Size | Cat.# |
|---|---|---|
| PCR Core System I | 200 reactions | M7660 |
| PCR Core System II | 200 reactions | M7665 |

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