EasyPrep HY Genomic DNA Extraction Kit

For isolation of genomic DNA from blood, bacteria, cells, and animal tissues

Contents

Introduction

The EasyPrep HY Genomic DNA Extraction Kit is based on a special buffer system that is used for extraction of many types of gDNA from samples. Simple centrifugation completely removes contaminants and enzyme inhibitors such as proteins and divalent cations. The purified DNA is then rehydrated using DNA dissolving buffer that is ready for use in downstream applications.

DNA purified by using the EasyPrep HY Genomic DNA Extraction Kit is highly suitable for restriction analysis, polymerase chain reaction analysis, southern blotting, and cDNA library construction.

Sample (protocol included): 300 µL of whole blood (up to 1 mL), 10^7 cultured cells, 10^9 bacteria (Gram +/−), and 10^8 yeast cells.

Average yield: for 300 µL of whole blood, 6 µg; for 200 µL of buffy coat, 50 µg; and for 5×10^6 lymph./cult. cells, 50 µg

Kit Contents

Contents	TE-GD01 (500 preps, depends on sample type)
RBC Lysis Buffer	500 mL
Cell Lysis Buffer	200 mL
Protein Precipitation Buffer	100 mL
RNase A	1 mL
DNA Dissolving Buffer	100 mL

Storage

The EasyPrep HY Genomic DNA Extraction Kit can be stored dry at room temperature (15–25 °C) for up to 12 months without any reduction in performance or quality.

Protocols

A. Whole Blood (300 µL)

1. Pipette 900 µL of RBC lysis buffer into a sterile 1.5-mL microcentrifuge tube.
2. Transfer 300 µL of blood to the tube containing the RBC lysis buffer, and then invert the tube 5 or 6 times.
3. Incubate the mixture for 10 min at room temperature (invert 2 or 3 times during the incubation) to lyse the red blood cells. Centrifuge at 13,000–16,000 × g at room temperature for 20 s.
4. Discard as much supernatant as possible without disturbing the white pellet. Approximately 10–20 µL of residual liquid should remain.

Note: The tube may contain some red blood cells or debris and white blood cells. If the pellet appears to contain only red blood cells, add an additional 300 µL of RBC lysis buffer and repeat Steps 3 and 4.

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

Note: To obtain efficient cell lysis, it is essential that the white blood cells are completely resuspended.

6. Add 300 µL of cell lysis buffer to the tube containing the resuspended cells. Pipette the solution up and down 5 or 6 times to lyse the white blood cells. The solution should be viscous. If clumps of cells are visible after mixing, incubate the solution at 37 °C until the clumps have been disrupted. If the clumps remain visible after 1 h, add an additional 100 µL of cell lysis buffer and repeat the incubation.
7. OPTIONAL STEP**:** Add 1.5 µL of RNase solution to the nuclear lysate, and mix the sample by inverting the tube 25 times. Incubate the mixture at 37 ℃ for 15 min. Chill the sample to room temperature before vortexing for 10–20 s. Small protein clumps may be present after the vortexing.

8. Add 100 µL of protein precipitation buffer to the nuclear lysate, and vortex thoroughly for 10–20 s. Small protein clumps may be present after the mixing.

Note: If additional cell lysis buffer is added in Step 6, pipette 130 µL of protein precipitation buffer.

9. Centrifuge at 13,000–16,000 × g for 3 min at room temperature. A dark brown protein pellet should be visible. If no pellet is observed, refer to the Troubleshooting Section.
10. Transfer the supernatant to a clean 1.5-mL microcentrifuge tube containing 300 µL 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.

11. Gently mix the solution by inverting the tube until the white threadlike strands of DNA have formed a visible compact pellet.
12. Centrifuge for 1 min at 13,000–16,000 × g at room temperature. The DNA should form a small white pellet.
13. Discard the supernatant, and add 300 µL of room-temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and sides of the microcentrifuge tube. Centrifuge for 1 min at 13,000–16,000 × g at room temperature.
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. Avoid aspirating the pellet into the pipette. Invert the tube onto a clean piece of absorbent paper, and air-dry the pellet for 10–15 min.
15. Add 100 µL of DNA dissolving buffer (10 mM Tris-HCI/1mM EDTA, pH 7.4) to the tube, and rehydrate the DNA by incubating at 65 °C for 1 h. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature.
16. Store the DNA at 2–8 °C.

B. Whole Blood (3 mL)

1. Pipette 9 mL of RBC lysis buffer into a sterile 15-mL tube.
2. Transfer 3 mL of blood to the tube containing the RBC lysis buffer, and then invert the tube 5 or 6 times.
3. Incubate the mixture for 10 min at room temperature (invert 2 or 3 times during the incubation) to lyse the red blood cells. Centrifuge at 2,000 × g for 10 min at room temperature.
4. Discard as much supernatant as possible without disturbing the white pellet. Approximately 100–200 µL of residual liquid should remain.

Note: The tube may contain some red blood cells or debris and white blood cells. If the pellet appears to contain only red blood cells, add an additional 3 mL of RBC lysis buffer and repeat Steps 3 and 4.

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

Note: To obtain efficient cell lysis, it is essential that the white blood cells are completely

resuspended.

6. Add 3 mL of cell lysis buffer to the tube containing the resuspended cells. Pipette the solution up and down 5 or 6 times to lyse the white blood cells. The solution should be viscous. If clumps of cells are visible after mixing, incubate the solution at 37 °C until the clumps have been disrupted. If the clumps remain visible after 1 h, add an additional 1 mL of cell lysis buffer, and repeat the incubation process.
7. OPTIONAL STEP: Add 15 µL of RNase solution to the nuclear lysate and mix the sample by inverting the tube 25 times. Incubate the mixture at 37 °C for 15 min. Cool the sample to room temperature before vortexing for 10–20 s. Small protein clumps may be present after the vortexing.
8. Add 1 mL of protein precipitation buffer to the nuclear lysate, and vortex thoroughly for 10–20 s. Small protein clumps may be present after the mixing.

Note: If additional cell lysis buffer was added in Step 6, pipette 1.3 mL of protein precipitation buffer.

9. Centrifuge at 2,000 × g for 10 min at room temperature. A dark brown protein pellet should be visible. If no pellet is observed, refer to the Troubleshooting Section.
10. Transfer the supernatant to a clean 15-mL tube containing 3 mL 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.

11. Gently mix the solution by inverting the tube until the white threadlike strands of DNA have formed a visible compact pellet.
12. Centrifuge at 2,000 × g for 3 min at room temperature. The DNA should form a small white pellet.
13. Discard the supernatant, and add 3 mL of 70% ethanol at room temperature to the DNA. Gently invert the tube several times to wash the DNA pellet and sides of the microcentrifuge tube. Centrifuge at 2,000 × g for 1 min at room temperature.
14. Carefully aspirate the ethanol by using either a drawn Pasteur pipette or a sequencing pipette tip. The DNA pellet is very loose at this point. Therefore, avoid aspirating the pellet into the pipette. Invert the tube onto a clean piece of absorbent paper, and air-dry the pellet for 10–15 min.
15. Add 250 µL of DNA dissolving buffer (10 mM Tris-HCI/1mM EDTA, pH 7.4) to the tube, and rehydrate the DNA by incubating at 65 °C for 1 h. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature.
16. Store the DNA at 2–8 ℃.

C. Whole Blood (10 mL)

1. For a 10-mL blood sample, pipette 30 mL of RBC lysis buffer into a sterile 50-mL tube.
2. Transfer 10 mL of blood to the tube containing the RBC lysis buffer, and then invert the tube 5 or

6 times.

3. Incubate the mixture for 10 min at room temperature (invert 2 or 3 times during the incubation) to lyse the red blood cells. Centrifuge at 2,000 × g for 10 min at room temperature.
4. Discard as much supernatant as possible without disturbing the white pellet. Approximately 1.4 mL of residual liquid should remain.

Note: The tube may contain some red blood cells or debris and white blood cells. If the pellet appears to contain only red blood cells, add an additional 9–10 mL of RBC lysis buffer and repeat Steps 3 and 4.

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

Note: To obtain efficient cell lysis, it is essential that the white blood cells are completely resuspended.

6. Add 10 mL of cell lysis buffer to the tube containing the resuspended cells. Pipette the solution up and down 5 or 6 times to lyse the white blood cells. The solution should be viscous. If clumps of cells are visible after mixing, incubate the solution at 37 °C until the clumps have been disrupted. If the clumps remain visible after 1 h, add an additional 1 mL of cell lysis buffer, and repeat the incubation.
7. OPTIONAL **STEP:** Add 15 µL of the RNase solution to the nuclear lysate, and mix the sample by inverting the tube 25 times. Incubate the mixture at 37 °C for 15 min. Cool the sample to room temperature before vortexing for 10–20 s. Small protein clumps may be present after the vortexing.
8. Add 3.3 mL of protein precipitation buffer to the nuclear lysate, and vortex thoroughly for 10–20 s. Small protein clumps may be present after the mixing.

Note: If additional cell lysis buffer was added in Step 6, pipette 4 mL of protein precipitation buffer.

9. Centrifuge at 2,000 × g for 10 min at room temperature. A dark brown protein pellet should be visible. If no pellet is observed, refer to the Troubleshooting Section.
10. Transfer the supernatant to a clean 50-mL tube containing 10 mL 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.

11. Gently mix the solution by inverting the tube until the white threadlike strands of DNA have formed a visible compact pellet.
12. Centrifuge at 2,000 × g for 3 min at room temperature. The DNA should form a small white pellet.
13. Discard the supernatant, and add 10 mL of room-temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and sides of the microcentrifuge tube.

Centrifuge at 2,000 × g for 1 min at room temperature.

14. Carefully aspirate the ethanol by using either a drawn Pasteur pipette or a sequencing pipette tip. The DNA pellet is very loose at this point. Avoid aspirating the pellet into the pipette. Invert the tube on a clean piece of absorbent paper, and air-dry the pellet for 10–15 min.
15. Add 800 µL of DNA dissolving buffer (10 mM Tris-HCI/1mM EDTA, pH 7.4) to the tube, and rehydrate the DNA by incubating at 65 °C for 1 h. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature.
16. 16. Store the DNA at 2–8 °C.

D. Buffy Coat (from 3 mL of whole blood)

If the buffy coat contains red blood cells, follow Step 1. If the buffy coat preparation is clean and free of red blood cells, pipette 150–250 µL of the sample into a 1.5-mL microfuge tube containing 3 mL of cell lysis buffer as detailed in Step 4.

1. Pipette the buffy coat preparation (150–250 µL) obtained from a 3-mL whole blood sample into a 1.5-mL microcentrifuge tube containing 3 parts RBC lysis buffer (for example, mix 250 µL of buffy coat sample with 750 µL of RBC lysis buffer). Mix through inversion, and incubate for 5 min at room temperature. Invert 2 or 3 times during the incubation.
2. Centrifuge at 2,000 × g for 10 min. Discard as much supernatant as possible without disturbing the white pellet. Approximately 10–20 µL of residual liquid should remain.
3. Vortex the tube vigorously to resuspend the cells in the residual liquid to facilitate cell lysis in Step 4
4. Add the buffy coat preparation (150–250 µL) obtained from a 3-mL whole blood sample to a 15-mL microfuge tube on ice.
5. Add 300 µL of cell lysis buffer to the resuspended cells, and pipette up and down to lyse the cells. Generally, no incubation is required; however, if cell clumps are visible after mixing, incubate at 37 °C or room temperature until the solution has become homogeneous. Samples are stable in cell lysis buffer for at least 18 months at room temperature.

- **RNase treatment (optional)**

6. Add 1.5 µL of RNase A to the cell lysate.
7. Mix the sample by inverting the tube 25 times, and incubate at 37 °C for 15 min. Protein precipitation.
8. Cool the sample to room temperature.
9. Add 100 µL of protein precipitation buffer to the cell lysate.
10. Vortex vigorously at high speed for 20 s to mix the protein precipitation buffer uniformly with the cell lysate.
11. Centrifuge at 2,000 × g for 10 min. The precipitated proteins should form a tight white pellet. If the protein pellet is not tight, repeat Step 10, and then incubate on ice for 5 min before repeating

Step 11.

12. Pipette the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.50-mL tube containing 300 µL of 100% isopropanol.
13. Mix the sample by inverting gently 50 times.
14. Centrifuge at 2,000 × g for 3 min; the DNA should be visible as a small white pellet.
15. Discard the supernatant, and drain the tube onto a clean piece of absorbent paper. Add 300 µL of 70% ethanol, and invert the tube several times to wash the DNA pellet.
16. Centrifuge at 2,000 × g for 1 min. Carefully away off the ethanol. The pellet may be loose. Therefore, pour slowly and watch the pellet.
17. Invert and drain the tube onto a clean piece of absorbent paper, and allow the sample to air-dry for 10–15 min.
18. Add 250 µL of DNA dissolving buffer (250 µL will give a concentration of 400 µg/mL if the total yield is 100 µg DNA).
19. Incubate at 65 °C for 1 h to dissolve the DNA.
20. Incubate at room temperature overnight with gentle shaking. Ensure the tube cap is tightly closed to prevent leakage. The sample can then be centrifuged briefly and transferred to a storage tube. Store DNA at 2–8 °C.

E. Whole Blood (96-well plate)

1. Pipette 150 µL of RBC lysis buffer into each well.
2. Pipette 50 µL of fresh blood into each well, and pipette 2 or 3 times to mix.
3. Leave the plate at room temperature for 10 min, pipetteting the solution twice during the incubation to lyse the red blood cells.
4. Centrifuge at 8,000 × g in a tabletop centrifuge for 5 min to concentrate the cells.
5. Discard as much of the supernatant as possible using a micropipettete tip, leaving a small pellet of white cells and some red blood cells. An extended gel loading tip is recommended. Tilt the 96-well plate by 50°–80° (depending on the amount of liquid in the well) to thoroughly remove the liquid from the well.
6. Add 50 µL of cell lysis buffer to each well, and pipette 5 or 6 times to resuspend the pellet and lyse the white blood cells. The solution should become more viscous. To observe the DNA pellet, 2 µL per well of a carrier (e.g., polydactyl carrier) can be added in this step. DNA yields are not affected by the use of a carrier.
7. Add 16.5 µL of protein precipitation buffer per well, and pipette 5 or 6 times to mix.
8. Centrifuge at 14,000 × g for 10 min at room temperature. A brown protein pellet should appear.
9. Perform DNA precipitation and rehydration in the 96-well plate.
    a. Carefully transfer the supernatants to clean wells containing 50 µL per well of room-temperature isopropanol, and mix by pipetteting.
    b. Centrifuge at 14,000 × g for 10 min. Carefully remove the isopropanol by using a

micropipettete tip.

c. Add 100 µL of room-temperature 70% ethanol to each well.

d. Centrifuge at 14,000 × g for 10 min at room temperature.

e. Carefully aspirate the ethanol using either a drawn Pasteur pipettete or a sequencing pipettete tip. Avoid aspirating the DNA pellet. Air-dry at a 30°–45° angle for 10–15 min.

f. Add 25 µL of DNA dissolving buffer 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 prior to use.

F. Gram-Negative Bacteria

1. Pipette 1 mL of an overnight culture into a 1.5-mL microcentrifuge tube.
2. Centrifuge at 13,000–16,000 × g for 1 min to pellet the cells. Remove the supernatant.
3. Add 600 µL of cell lysis solution, and gently pipette up and down until the cells have resuspended.
4. Incubate the sample at 80 °C for 5 min to lyse the cells, and then cool to room temperature.
5. Add 3 µL of RNase solution to the cell lysate, and mix the sample by inverting the tube 25 times.
6. Incubate at 37 °C for 15–60 min. Cool the sample to room temperature.
7. Add 200 µL of the protein precipitation solution to the RNase-treated cell lysate. Vortex vigorously at high speed for 20 s to mix the protein precipitation buffer with the cell lysate.
8. Let the sample sit on ice for 5 min.
9. Centrifuge at 13,000–16,000 × g for 3 min.
10. Transfer the supernatant containing the DNA to a clean 1.5-mL microcentrifuge tube containing 600 µL of room-temperature isopropanol. Gently mix through inversion until the threadlike strands of DNA have formed a visible mass.
11. Centrifuge at 13,000–16,000 × g for 2 min.
12. Carefully pour away the supernatant, and drain the tube on paper tissue. Add 600 µL of room-temperature 70% ethanol, and invert the tube several times to obtain a clean DNA pellet.
13. Centrifuge at 13,000–16,000 × g for 2 min. Carefully pour away the ethanol.
14. Drain the tube on paper tissue, and allow the pellet to air-dry for 10–15 min.
15. Add 100 µL of DNA dissolving buffer, and rehydrate the DNA by incubating at 65 °C for 1 h. Periodically mix the solution by gently tapping the tube. Alternatively, incubate the solution overnight at room temperature.
16. Store at 4 °C.

G. Gram-Positive Bacteria

1. Pipette 1 mL of overnight culture into a 1.5-mL microcentrifuge tube.
2. Centrifuge at 13,000–16,000 × g for 2 min to pellet the cells. Remove the supernatant.
3. Resuspend the cells thoroughly in 480 µL of 50 mM EDTA.
4. Add 60 µL of 10 mg/mL lysozyme and 60 µL of 10 mg/mL lysostaphin, and gently pipette up and down.
5. Incubate the sample at 37 °C for 30–60 min.
6. Add 600 µL of cell lysis solution to the cell pellet, and gently pipette up and down.
7. Incubate the sample at 80 °C for 10 min to complete cell lysis.
8. Add 3 µL of RNase solution to the cell lysate, and mix the sample by inverting the tube 25 times. Incubate at 37 °C for 15–60 min, and then cool to room temperature.
9. Add 200 µL of protein precipitation buffer to the RNase-treated cell lysate. Vortex vigorously at high speed for 20 s to mix.
10. Let the sample sit on ice for 5 min.

11. Centrifuge at 13,000–16,000 × g for 3 min.
12. Transfer the supernatant containing the DNA to a clean 1.5-mL microcentrifuge tube containing 600 µL of room-temperature isopropanol. Gently mix through inversion until the threadlike strands of DNA have formed a visible mass.
13. Centrifuge at 13,000–16,000 × g for 2 min.
14. Carefully pour away the supernatant and drain the tube on paper tissue. Add 600 µL of room-temperature 70% ethanol and invert the tube several times to obtain a clean DNA pellet.
15. Centrifuge at 13,000–16,000 × g for 2 min. Carefully pour away the ethanol.
16. Drain the tube on paper tissue, and allow the pellet to air-dry for 10–15 min.
17. Add 100 µL of DNA dissolving buffer and rehydrate the DNA by incubating at 65 °C for 1 h. Mix the solution by gently tapping the tube several times. Alternatively, incubate the solution overnight at room temperature.
18. Store at 4 °C.

H. Animal Tissue

1. Pipette 600 µL of cell lysis buffer into a 15-mL centrifuge tube, and cool on ice.
2. Transfer 10–20 mg of fresh or thawed tissue to the chilled cell lysis buffer, and homogenize for 10 s using a small homogenizer. Transfer the lysate to a new 1.5-mL microcentrifuge tube.

Note: Alternatively, tissue frozen in liquid nitrogen may be ground in a mortar and pestle that has been prechilled in liquid nitrogen. After grinding, transfer approximately 10–20 mg of the ground tissue to 600 µL of cell lysis buffer in a 1.5-mL centrifuge tube, and proceed to Step 3.

3. Incubate the lysate at 65 °C for 15–30 min. To improve the DNA yield, 3 µL of proteinase K solution (20 mg/mL) can be added to the lysate. Mix by inverting 25 times, and incubate at 55 °C for 3 h to overnight until the tissue homogenates have dissolved. If possible, invert tube periodically during the incubation.
4. Proceed to Step 5 of the Tissue Culture Cells protocol.

I. Tissue Culture Cells

1. Harvest the cells, and transfer them to a 1.5-mL microcentrifuge tube (10^6–10^7 cells).
2. Centrifuge for 10 s at 13,000–16,000 × g to pellet the cells. For adherent cells, trypsinize the cells before harvesting.
3. Remove the supernatant, leaving behind the cell pellet plus 10–50 µL of residual liquid.
4. Pipette 600 µL of cell lysis buffer into a cell pellet, and pipette up and down several times. Mix well until no visible cell clumps remain.

Note: For cells that do not lyse well in this solution alone (i.e., PC12 cells), perform an additional freeze–thaw step as follows. Resuspend the cells as in Step 3, and then freeze them quickly in liquid nitrogen. Thaw the cells by heating at 95 °C. Repeat this procedure for a total of 4 cycles; then proceed to Step 4 and continue the isolation procedure.

5. Add 3 µL of the RNase solution to the nuclear lysate and mix the sample by inverting the tube 25 times. Incubate the mixture for 15–30 min at 37 °C. Allow the sample to cool to room temperature for 5 min before proceeding.
6. Add 200 µL of protein precipitation buffer to the RNase-treated cells, and vortex vigorously at high speed for 20 s.
7. Centrifuge for 3 min at 13,000–16,000 × g. The precipitated proteins form a tight white pellet.
8. Carefully remove the supernatant containing the DNA (leaving the protein pellet behind) and transfer it to a clean 1.5-mL microcentrifuge tube containing 600 µL 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.

9. Gently mix the solution through inversion until the white threadlike strands of DNA appear.
10. Centrifuge at 13,000–16,000 × g for 1 min at room temperature. A small white pellet of DNA should be visible in the tube. Carefully discard the supernatant.
11. Add 600 µL of 70% ethanol and invert the tube several times to wash the DNA.
12. Carefully aspirate the ethanol using a pipette tip. The DNA pellet is very loose at this point. Avoid aspirating the pellet into the pipette. Invert the tube onto a clean piece of absorbent paper, and air-dry the pellet for 10–15 min.
13. Add 100 µL of DNA dissolving buffer (10 mM Tris-HCI/1 mM EDTA, pH 7.4) to the tube, and rehydrate the DNA by incubating at 65 °C for 1 h. Mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature.
14. Store the DNA at 2–8 °C.

J. Mouse Tail Tissue

1. Cool a 1.5-mL tube containing 600 µL of cell lysis buffer on ice. The solution should turn cloudy.
2. Place 10 mm (10–20 mg) of fresh or frozen tail tissue (minced if possible) into the chilled cell lysis buffer.
3. Add 3 µL of proteinase K solution to the sample and mix by inverting 25 times. Incubate at 55 °C overnight or until the tissue has dissolved. If possible, invert the tube periodically during the incubation.
4. Add 3 µL of RNase A to the cell lysate.
5. Mix the sample by inverting the tube 25 times, and incubate at 37 °C for 15–60 min.
6. Cool the sample to room temperature.
7. Add 200 µL of protein precipitation buffer to the RNase-A-treated cell lysate.
8. Vortex at high speed for 20 s to mix the protein precipitation buffer uniformly with the cell lysate.
9. Centrifuge at 13,000–16,000 × g for 3 min. The precipitated proteins should form a tight pellet. If the protein pellet is not visible, repeat Step 3 and then incubate in an ice bath for 5–15 min; subsequently, repeat Step 4.

10. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a 1.5mL microcentrifuge tube containing 600 µL of 100% isopropanol (2-propanol).
11. Mix the sample by inverting gently 50 times.
12. Centrifuge at 13,000–16,000 × g for 1 min; the DNA should be visible as a small white pellet.
13. Discard the supernatant and drain the tube onto a clean piece of absorbent paper. Add 600 µL of 70% ethanol and invert the tube several times to wash the DNA pellet.
14. Centrifuge at 13,000–16,000 × g for 1 min. Carefully pour away the ethanol. The pellet may be loose, so pour slowly and watch the pellet.
15. Invert the tube onto a clean piece of absorbent paper, and air-dry the pellet for 10–15 min. Add 100 µL of DNA dissolving buffer (100 µL will give a concentration of 500 µg/mL if the total yield is 50 µg of DNA).
16. Rehydrate the DNA by incubating the sample for 1 h at 65 °C and/or overnight at room temperature. If possible, tap tube periodically to aid DNA dispersion.
17. Store the DNA at 4 °C. For long-term storage, store at −20 or −80 °C.

K. Plant Tissue

1. Add 10–20 mg of dried tissue (finely ground), 20–60 mg of frozen tissue (may be finely ground with a pestle and mortar in liquid nitrogen), or 20–60 mg of fresh leaf tissue (2–5 disks) to a 1.5-mL tube. A leaf disk (7-mm diameter) may be prepared by placing the leaf between the microfuge tube and its cap and then snapping the cap closed. Work quickly, and keep the tissue cold to minimize DNase activity. Note: it may be necessary to vary the amount of starting material depending upon the species, age, tissue preparation, and genome size.
2. Add 600 µL of cell lysis buffer to the leaf tissue. For dried tissue, vortex for 1–3 s to wet the tissue. For unground tissue, homogenize using 30–50 strokes with a microfuge tube pestle.
3. Incubate the cell lysate at 65 °C for 60 min. After 30 min and then after 60 min, invert the tube 10 times.
4. Add 3 µL of the RNase solution to the cell lysate and mix the sample by inverting the tube 25 times.
5. Incubate the mixture at 37 °C for 15 min. Allow the sample to cool to room temperature for 5 min before proceeding.
6. Add 200 µL of protein precipitation buffer, and vortex vigorously at high speed for 20 s.
7. Centrifuge for 3 min at 13,000–16,000 × g. The precipitated proteins form a tight pellet.
8. Carefully remove the supernatant containing the DNA (leaving the protein pellet behind) and transfer it to a clean 1.5-mL microcentrifuge tube containing 600 µL of room-temperature isopropanol.
9. Note: In Step 8, 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.

10. Gently mix the solution through inversion until the threadlike strands of DNA have formed a visible mass.
11. Centrifuge at 13,000–16,000 × g for 1 min at room temperature.
12. Carefully decant the supernatant. Add 600 µL 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 min at room temperature.
13. Carefully aspirate the ethanol using either a drawn Pasteur pipettete or a sequencing pipettete tip. The DNA pellet is very loose at this point. Therefore, avoid aspirating the pellet into the pipettete.
14. Invert the tube onto a clean piece of absorbent paper, and air-dry the pellet for 15 min.
15. Add 100 µL of DNA dissolving buffer, and rehydrate the DNA by incubating at 65 °C for 1 h. 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.

L. Yeast

1. Add 1 mL of a culture (e.g., overnight culture containing approximately 1–2 × 10^8 cells) grown for 20 h in a YPD broth to a 1.5-mL microcentrifuge tube.
2. Centrifuge at 13,000–16,000 × g for 2 min to pellet the cells. Remove the supernatant.
3. Resuspend the cells thoroughly in 293 µL of 50 mM EDTA.
4. Add 7.5 µL of 20 mg/mL lyticase, and gently pipette 4 times to mix.
5. Incubate the sample at 37 °C for 30–60 min to enable digestion of the cell wall. Cool to room temperature.
6. Centrifuge the sample at 13,000–16,000 × g for 2 min, and then remove the supernatant.
7. Add 300 µL of cell lysis buffer to the cell pellet, and gently pipette to mix.
8. Add 100 µL of protein precipitation buffer, and vortex vigorously at high speed for 20 s.
9. Let the sample sit on ice for 5 min.
10. Centrifuge at 13,000–16,000 × g for 3 min.
11. Transfer the supernatant containing the DNA to a clean 1.5-mL microcentrifuge tube containing 300 µL of room-temperature isopropanol.

Note: In Step 11, 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 through inversion until the threadlike strands of DNA have formed a visible mass.
13. Centrifuge at 13,000–16,000 × g for 2 min.
14. Carefully decant the supernatant, and drain the tube onto a clean piece of absorbent paper. Add 300 µL 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 min. Carefully aspirate all of the ethanol.

16. Drain the tube onto a clean piece of absorbent paper, and allow the pellet to air-dry for 10–15 min.
17. Add 50 µL of DNA dissolving buffer.
18. Add 1.5 µL of the RNase solution to the purified DNA sample. Vortex the sample for 1 s. Centrifuge briefly in a microcentrifuge for 5 s to collect the liquid, and incubate at 37 °C for 15 min.
19. Rehydrate the DNA by incubating at 65 °C for 1 h. 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.

M. Paraffin-Embedded Tissue

- Sample Deparaffinization

1. Place 0.5–2.0 mg (0.0005–0.002 g) of finely minced tissue into a 1.5-mL tube. Add 100 µL of xylene, and incubate for 5 min with constant mixing at room temperature.
2. Centrifuge at 13,000–16,000 × g for 1–3 min to pellet the tissue. Discard the xylene.
3. Repeat Steps 1 and 2 twice (for a total of 3 washes).
4. Add 100 µL of 100% ethanol to the tube, and incubate for 5 min with constant mixing at room temperature.
5. Centrifuge at 13,000–16,000 × g for 1–3 min to pellet the tissue. Discard the ethanol.
6. Repeat Steps 4 and 5 (for a total of 2 ethanol washes).

- Cell Lysis

7. Add 100 µL of cell lysis buffer, and homogenize using 30–50 strokes with a microfuge tube pestle.
8. Incubate the lysate at 65 °C for 15–60 min.
9. If the maximum yield is required, 0.5 µL of proteinase K solution (20 mg/mL) may be added to the lysate. Mix by inverting 25 times, and incubate at 55 °C until the tissue particulates have dissolved (3 h to overnight). If possible, invert the tube periodically during the incubation. The RNase Treatment is as follows:
   i. Add 0.5 µL of RNase A (4 mg/mL) to the cell lysate.
   ii. Mix the sample by inverting the tube 25 times, and incubate at 37 °C for 15–60 min.

- Protein Precipitation

10. Cool the sample to room temperature.
11. Add 33 µL of protein precipitation buffer to the RNase-A-treated cell lysate.
12. Vortex vigorously at high speed for 20 s to mix the protein precipitation buffer uniformly with the cell lysate. Place the sample on ice for 5 min.
13. Centrifuge at 13,000–16,000 × g for 3 min. The precipitated proteins form a tight pellet. If the protein pellet is not visible, repeat Step 12 and then incubate on ice for 5 min; subsequently, repeat Step 13

- DNA Precipitation

14. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean

1.5-mL centrifuge tube containing 100 µL of 100% isopropanol. If the DNA yield is expected to be low (<1 µg), add 0.5 µL of glycogen solution (20 mg/mL) to the isopropanol.

15. Mix the sample by inverting gently 50 times.
16. Centrifuge at 13,000–16,000 × g for 5 min.
17. Discard the supernatant, and drain the tube onto a clean piece of absorbent paper. Add 100 µL of 70% ethanol, and invert tube several times to wash the DNA pellet.
18. Centrifuge at 13,000–16,000 × g for 1 min. Carefully pour away the ethanol. The pellet may be loose, so pour slowly and watch the pellet.
19. Invert and drain the tube onto a clean piece of absorbent paper, and air-dry for 10–15 min.

- DNA Hydration

20. Add 20 µL of DNA dissolving buffer (20 µL should give a concentration of 50 ng/µL if the total yield is 1 µg of DNA).
21. Rehydrate the DNA by incubating the sample for 1 h at 65 °C and/or overnight at room temperature. If possible, tap the tube periodically to aid the DNA dispersion.
22. Store the DNA at 4 °C. For long-term storage, store at −20 or −80 °C.

N. Body Fluid (100 µL)

- Cell Lysis

1. Add 100 µL of a body fluid (e.g., cerebrospinal fluid, plasma, saliva, serum, sputum, synovial fluid, urine, whole blood, or milk) to a sterile 1.5-mL microfuge tube containing 500 µL of cell lysis buffer. Pipette up and down several times to mix thoroughly.

Note: If the sample has high protein content, 50 µL of body fluid may be added to 550 µL of cell lysis buffer.

2. Heat to 65 °C for 15 min to complete lysis. Alternatively, for maximum yield, add 3 µL of proteinase K (20 mg/mL), and incubate the lysate at 55 °C for 1 h to overnight.

- RNase Treatment (optional)

3. Add 3 µL of RNase A to the cell lysate.
4. Mix the sample by inverting the tube 25 times, and incubate at 37 °C for 15–60 min.

- Protein Precipitation

5. Cool the sample to room temperature.
6. Add 200 µL of protein precipitation buffer to the lysate.
7. Vortex the sample at high speed for 20 s to mix the protein precipitation buffer uniformly with the lysate.
8. Place the sample in an ice bath for 5–15 min.
9. Centrifuge at 13,000–16,000 × g for 3 min. The precipitated proteins should form a tight pellet.

Note: if the body fluid has high lipid content, particulates may stay near the top of tube; in this case,

refer to the alternative transfer method presented in Step 1 below.

- DNA Precipitation

10. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5-mL microfuge tube containing 600 µL of % isopropanol (2-propanol).
    Alternatively, if particulates are present, transfer the supernatant using a pipette so that the particulates are excluded. If the DNA yield is expected to be low (<20 µg), add a DNA carrier such as glycogen (1 µL of glycogen solution, 20 mg/mL, per 600 µL of isopropanol).
11. Mix the sample by inverting gently 50 times, and maintain the tube at room temperature for at least 5 min.
12. Centrifuge at 13,000–16,000 × g for 5 min. The DNA may or may not be visible as a small white pellet, depending on the yield.
13. Pour away the supernatant and drain the tube briefly onto a clean piece of absorbent paper. Add 600 µL of 70% ethanol and invert the tube several times to wash the DNA pellet.
14. Centrifuge at 13,000–16,000 × g for 1 min. Carefully pour away the ethanol. The pellet may be loose, so pour slowly and watch the pellet.
15. Invert and drain the tube onto a clean piece of absorbent paper, and air-dry for 10–15 min.

- DNA Hydration

16. Add 20 µL of DNA dissolving buffer (20 µL should give a concentration of 100 ng/µL if the yield is 2 µg of DNA).
17. Rehydrate the DNA by incubating at 65 °C for 1 h and/or overnight at room temperature. If possible, tap the tube periodically to aid DNA dispersion.
18. Store the DNA at 4 °C. For long-term storage, store at −20 or −80 °C.

Troubleshooting

Problem	Possible Causes	Suggestions
Blood clots are present in blood samples.	• Tube has been stored improperly, the blood has not been mixed thoroughly, or inappropriate tubes were used for drawing blood.	• Discard the clotted blood, and draw new • samples using EDTA-heparin- or citrate-anticoagulant tubes.
Poor DNA yield.	• The blood sample contained too few white blood • cells. • The white blood cell pellet was not resuspended thoroughly. • The blood sample was too old. • The DNA pellet was lost during • isopropanol precipitation.	• Draw new blood samples. • The white blood cell pellet should be vortexed vigorously to resuspend the cells. • The highest yields are obtained with fresh blood. Samples that have been stored at 2–5 °C for >5 days may result in reduced yields. • Extreme care must be used when removing the isopropanol to avoid losing the pellet.
Degraded DNA (<50 kb).	• Improper collection or storage of the blood sample.	• Obtain a new sample under the proper conditions.
Poor DNA yield when using the Gram-positive bacteria protocol.	• Cultures grown for an extended time may • contain a high proportion of cells that lyse easily upon exposure to lysostaphin • treatment.	• It is critical to start with a healthy cell culture.
No protein pellet	• The sample was not cooled to room temperature before adding the protein precipitation buffer. • The protein precipitation buffer was not thoroughly mixed with the nuclear lysate.	• Cool the sample to room temperature (for at least 5 min) or chill on ice for 5 min, vortex for 20 s, and centrifuge for 3 min at 13,000–16,000 × g • Always mix the nuclear lysate and protein precipitation buffer completely.
The DNA pellet was difficult to dissolve.	• The sample was overdried or not mixed properly during the rehydration step.	• Rehydrate the DNA by incubating • for 1 h at 65 °C, and then leave the sample at room temperature or at 4 °C • overnight. Remember to mix periodically during the rehydration step.