Axen™ Genomic DNA Kit

Product description

Axen™ Genomic DNA kit provides a convenient method for the isolation of genomic DNA from blood cells, cultured cells, animal tissue, plant tissue, yeast, and bacteria without use of toxic chemical such as phenol or chloroform. This kit utilizes the specially formulated buffer systems so as to process the sample in various scale and obtain the almost intact size of genomic DNA. Extracted genomic DNA is pure enough to be applied directly to PCR, Southern blotting, restriction enzyme assay and other downstream applications.

Kit contents

Contents*1			**Storage conditions**
Cat. No.	**MG-P-011-100**	**MG-P-011-500**	
No. of preparation	100*2	500*2	
Buffer RL	100 mL	500 mL	Room Temperature
Buffer NL*3	50 mL	250 mL	
Buffer PP	25 mL	125 mL	
Buffer RE*4	50 mL	100 mL	
RNase A solution (20 mg/mL)*5	110 uL	550 uL	4˚C*5

*****1 All components of this kit except RNase A solution should be stored at room temperature (15~25˚C).

*****2 On the basis of 300 uL whole blood sample

*3 During shipment or storage under cold ambient condition, a precipitate can be formed in buffer NL. Heat the bottle at 20~40°C to dissolve completely in such a case.

*4 10 mM Tris-HCl, pH 8.0, 1 mM EDTA

*5 RNase A solution is delivered under ambient condition, but it should be stored at 2~8˚C on arrival for conservation of activity. Delivery time would not lead to noticeable loss of the enzyme's activity.

Product use limitations

Axen™ Genomic DNA Kit is intended for research use only. This kit is not intended for diagnosis or treatment for human. All due care and attention should be exercised in the handling of the products.

Safety information

Axen™ Genomic DNA Kit contains irritants which are harmful when in contact with skin or eyes, or inhaled or swallowed. Care should be taken when handling this product. Always wear gloves and eye protection, and follow standard safety precautions.

Examples of DNA yields from various samples

Materials	Species	Amount/p	Yields
Whole blood	Human	300 uL	5 - 15 ug
Cultured cells	Hela	2 x 10^6 cells	7 – 14 ug
	CHO	2 x 10^6 cells	6 – 8 ug
	NIH3T3	2 x 10^6 cells	10 – 20 ug
Animal tissue	Mouse tail	1 cm of tip	15 – 30 ug
	Rat liver	10 mg	16 – 22 ug
	Rat brain	10 mg	5 – 8 ug
Plant tissue	Potato leaf	50 mg	6 – 10 ug
	Chinese cabbage	100 mg	3 – 8 ug
Bacteria	*E.coli*	2 x 10^9 cells	20 ug
Yeast	*S. cerevisiae*	1.9 x 10^8 cells	4 – 7 ug

A. Procedure for 300 uL of mammalian whole blood

To check before start

- Prepare water bathes or heat blocks at 37°C and 65°C
- Required additional materials; 1.5 mL or 2 mL centrifuge tube,
  Isopropanol
  70% Ethanol

!!! If buffer NL has a precipitate in it, dissolve completely by heating before use.

1. **Transfer 900 uL of buffer RL to a fresh 1.5 mL or 2 mL microcentrifuge tube.**
2. **Add 300 uL of mammalian whole blood to the tube containing Buffer RL. Invert the tube 5-6 times to mix to get homogenate. Incubate the mixture for 10 min at room temperature.**
   - Invert 2-3 times during the incubation. The lysate should become translucent. If the lysate is opaque not translucent, it may be frozen or expired sample.
   - If fresh or well-stored sample is processed, it will take less times than 10 mins to acquire translucent mixtures.
3. **Centrifuge for 30 secs at 14,000 xg. Carefully remove the supernatant as much as possible without disturbing the visible white(or pink) pellet. Resuspend the pellet in residual supernatant by vigorous vortexing or flicking.**
   - A little residual liquid will remain. Steps 2-3 are critical steps for DNA recovery yield, so you have to check the translucent lysate and the white(or pink) pellet before processing next step.
4. **Add 300 uL of buffer NL and pipet 5 ~ 6 times to resuspend thoroughly. Incubate the lysate at 37°C until clumps of cells disappear.**

- Generally, cell lysis is completed in 5 mins. Complete resuspending is crucial for good yield. If the clumps are still visible after 1 hour, add an additional 100 uL of buffer NL and repeat incubation.

5. **(*Optional*) If RNA-free DNA is required, add 1 uL of RNase A solution (20 mg/mL) to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture for 15 mins at 37°C.**
6. **Cool the sample to room temperature. Apply 100 uL of buffer PP to the mixture and vortex vigorously for 15 secs. Centrifuge for 2 mins at 14,000 xg.**

   (*Optional*) Chill the sample on ice for 5 mins before centrifugation. This may slightly increase the quality of DNA.

   - A dark brown protein pellet should be visible.
7. **Carefully transfer the supernatant to a fresh 1.5 mL micro-centrifuge tube containing 300 uL of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**

   - Do not vortex after addition of isopropanol.
8. **Centrifuge at 14,000 xg for 1 min. Decant the supernatant and add 500 uL of 70% ethanol(room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**
9. **Centrifuge at 14,000 xg for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10 ~ 15 mins.**

   - The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.
   - Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.
10. **Add 100 uL of buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**

    - During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.

B. Procedure for 3 mL of mammalian whole blood

To check before start

- Prepare water bathes or heat blocks at 37°C and 65°C
- Required additional materials; 15 mL conical centrifuge tube,
  Isopropanol
  70% Ethanol

!!! If buffer NL has a precipitate in it, dissolve completely by heating before use.

1. **Transfer 9 mL of Buffer RL to a fresh 15 mL centrifuge tube.**
2. **Add 3 mL of whole blood to the tube containing buffer RL. Invert the tube 5~6 times to mix. Incubate the mixture for 10 mins at room temperature.**
   - Invert 2~3 times during the incubation. The lysate should become translucent. If the lysate is opaque not translucent, it may be frozen or expired sample, and you should resuspend the pellet and repeat step 2~3 with resuspended cells until lysate become translucent.
   - If fresh or well-stored sample is processed, it will take less times than 10 mins to acquire translucent mixtures. Do not incubate on ice or for more than 20 mins.
3. **Centrifuge for 3 mins at 2,000 xg. Carefully remove the supernatant as much as possible without disturbing the visible white(or pink) pellet. Resuspend the pellet in residual supernatant by vigorous vortexing or flicking.**
   - A little residual liquid will remain. Steps 2-3 are critical steps for DNA recovery yields, so you have to check the translucent lysate and the white(or pink) pellet before processing next steps.
   - Resuspending the cell pellet in residual supernatant will greatly accelerate the efficiency of cell lysis at next step.
4. **Add 3 mL of buffer NL and pipet 5 - 6 times to resuspend thoroughly. Incubate the lysate at 37°C until clumps of cells disappear.**
   - Generally, cell lysis is completed in 5 mins. Complete resuspending is crucial for good yield. If the clumps are still visible after 1 hour, add additional 1 mL of buffer NL and repeat incubation.

5. **(Optional) If RNA-free DNA is required, add 10 uL of RNase A solution (20 mg/mL) to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture for 15 min at 37°C.**
6. **Cool the sample to room temperature. Apply 1 mL of buffer PP to the mixture and vortex vigorously for 20 secs. Centrifuge at 2,000 xg for 5 mins.**

   (Optional) Chill the sample on ice for 5 mins before centrifugation. This may slightly increase the quality of DNA.

   - A dark brown protein pellet should be visible.
7. **Carefully transfer the supernatant to a fresh 15 mL centrifuge tube containing 3 mL of isopropanol(room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**

   - Do not vortex after addition of isopropanol.
8. **Centrifuge at 2,000 xg for 3 mins. Decant the supernatant carefully and add 3 mL of 70% ethanol(room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**
9. **Centrifuge at 2,000 xg for 2 mins. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10 ~ 15 mins.**

   - The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.
   - Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.
10. **Add 250 uL of buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**

    - During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.

C. Procedure for 10 mL of mammalian whole blood

To check before start

- Prepare water bathes or heat blocks at 37°C and 65°C
- Required additional materials; 50 mL conical centrifuge tube,
  Isopropanol
  70% Ethanol

!!! If buffer NL has a precipitate in it, dissolve completely by heating before use.

1. **Transfer 30 mL of buffer RL to a fresh 50 mL centrifuge tube.**
2. **Add 10 mL of whole blood to the tube containing buffer RL. Invert the tube 5~6 times to mix. Incubate the mixture for 10 mins at room temperature.**

   Invert 3~4 times during the incubation.

   - The lysate should become translucent. If the lysate is opaque not translucent, it may be frozen or expired sample, and you should resuspend the pellet and repeat step 2 ~ 3 with resuspended cells until lysate become translucent.
   - If fresh or well-stored sample is processed, it will take less times than 10 mins to acquire translucent mixtures. Do not incubate on ice or for more than 20 mins.
3. **Centrifuge for 5 mins at 2,000 xg. Carefully remove the supernatant as much as possible without disturbing the visible white(or pink) pellet. Resuspend the pellet in residual supernatant by vigorous vortexing or flicking.**
   - Approximately several hundreds microliter of residual liquid will remain. Steps 2-3 are critical steps for DNA recovery yields, so you have to check the translucent lysate and the white(or pink) pellet before processing next steps.
   - Resuspending the cell pellet in residual supernatant will greatly accelerate the efficiency of cell lysis at next step.
4. **Add 10 mL of buffer NL and pipet 5-6 times to resuspend thoroughly. Incubate the lysate at 37°C until clumps of cells disappear.**
   - Generally, cell lysis is completed in 5 mins. Complete resuspending is crucial for good yield. If the clumps are still visible after 1 hour, add additional 3 mL of buffer NL and repeat incubation.

5. **(Optional) If RNA-free DNA is required, add 33 uL of RNase A solution (20 mg/mL) to the lysate and mix the sample by inverting the tube 4 times. Incubate the mixture for 15 mins at 37°C.**
6. **Apply 3.3 mL of Buffer PP to the mixture and vortex vigorously for 15 secs. Centrifuge at 2,000 xg for 5 mins**

   (Optional) Chill the sample on ice for 5 mins before centrifugation. This may slightly increase the quality of DNA.

   - If additional buffer NL has been added at step 4, apply 4 mL of buffer PP instead of 3.3 mL.
   - A dark brown protein pellet should be visible.
7. **Carefully transfer the supernatant to a fresh 50 mL centrifuge tube containing 10 mL of isopropanol(room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**
   - Do not vortex after addition of isopropanol.
8. **Centrifuge at 2,000 xg for 3 mins. Decant the supernatant carefully and add 10 mL of 70% ethanol(room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**
   - DNA will be visible as a small white pellet.
9. **Centrifuge at 2,000 xg for 2 mins. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10 ~ 15 mins.**
   - The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.
   - Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.
10. **Add 800 uL of buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**
    - During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.

D. Procedure for buffy coat

To check before start

- Prepare water bathes or heat blocks at 37°C and 65°C
- Required additional materials; 15 mL conical centrifuge tube,
  Isopropanol
  70% Ethanol

!!! If buffer NL has a precipitate in it, dissolve completely by heating before use.

1. **Add 150~250 uL buffy coat to a 15 mL centrifuge tube containing 3 times of Buffer RL.**
   - For example, mix 250 uL buffy coat sample with 750 uL buffer RL.
   - Usually 150~250 uL of buffy coat will be prepared from 3 mL of whole blood.
2. **Invert the tube 5 ~ 6 times to mix. Incubate the mixture for 10 mins at room temperature.**
   - Invert 4 - 5 times during the incubation. If fresh or well-stored sample is processed, it will take less times than 10 mins to complete the lysis of residual RBC. Do not incubate sample mixture on ice or for more than 20 mins.
3. **Continue with step 3 of 3 mL of whole blood protocol. (Page 5)**

E. Procedure for cultured cells (2 x 10^6 cells)

To check before start

- Prepare water bathes or heat blocks at 37°C and 65°C
- Required additional materials; 1.5 mL or 2 mL centrifuge tube,
  Isopropanol
  70% Ethanol

!!! If buffer NL has a precipitate in it, dissolve completely by heating before use.

1. **Harvest up to 2 x 10^6 cells to a 1.5 mL fresh microcentrifuge tube by centrifugation at 14,000 xg for 10 secs. Discard the supernatant as much as possible.**
   - For adherent cells, treat trypsin-EDTA for detaching the cells before harvesting.
2. **Resuspend the cell pellet in residual supernatant by vigorous vortexing or flicking.**
   - Complete resuspending is crucial for efficient lysis of cells.
   - Certain cells, such as PC12, do not lyse well in buffer NL. For those cells, perform additional freeze-thaw step several times before proceeding to next step.
3. **Add 300 uL of buffer NL and pipet to lyse the cells until no visible cell clumps remain.**
   - Usually, no incubation time is required. But if the clumps are still visible after pipetting, incubate at 37°C until the mixture becomes homogeneous.
4. **If RNA-free DNA is required, add 1 uL of RNase A solution (20 mg/mL) to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture for 15 ~ 30 mins at 37°C.**
5. **Cool the sample to room temperature. Add 100 uL of buffer PP to the mixture and vortex vigorously for 20 secs. Chill the sample on ice for 5 mins.**
6. **Centrifuge at 14,000 xg for 1 min.**
   - A tight white protein pellet should be visible.

7. **Carefully transfer the supernatant to a fresh 1.5 mL micro centrifuge tube containing 300 uL of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**
   - If necessary, add glycogen or tRNA as nucleic acid carrier before addition of isopropanol.
   - Do not vortex after addition of isopropanol.
8. **Centrifuge at 14,000 xg for 1 min. Decant the supernatant and add 300 uL of 70% ethanol(room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**
9. **Centrifuge at 14,000 xg for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10 ~ 15 mins.**
   - The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.
   - Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.
10. **Add 50 uL of buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**
    - During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.

F. Procedure for cultured cells (2 x 10^7 cells)

To check before start

- Prepare water bathes or heat blocks at 37°C and 65°C
- Required additional materials; 15 mL conical centrifuge tube,
  Isopropanol
  70% Ethanol

!!! If buffer NL has a precipitate in it, dissolve completely by heating before use.

1. **Harvest up to 2 x 10^7 cells to a 15 mL fresh centrifuge tube by centrifugation at 1,000 xg for 2 mins. Discard the supernatant as much as possible.**
   - 100~200 uL of residual liquid will remain. For adherent cells, treat trypsin-EDTA for detaching the cells before harvesting.
2. **Resuspend the cell pellet in residual supernatant by vigorous vortexing or flicking.**
   - Complete resuspending is crucial for efficient lysis of cells.
   - Certain cells, such as PC12, do not lyse well in buffer NL. For those cells, perform additional freeze-thaw step several times before proceeding to next step.
3. **Add 3 mL of buffer NL and pipet to lyse the cells until no visible cell clumps remain.**
   - Usually no incubation time is required. But if the clumps are still visible after pipetting, incubate at 37°C until the mixture becomes homogeneous.
4. **If RNA-free DNA is required, add 10 uL of RNase A solution (20 mg/mL) to the lysate and mix the sample by inverting the tube 5 times.**

   Incubate the mixture for 15 ~ 30 mins at 37°C.
5. **Cool the sample to room temperature. Add 1 mL of buffer PP to the mixture and vortex vigorously for 20 secs. Chill the sample on ice for 5 min.**
6. **Centrifuge at 2,000 xg for 10 mins.**
   - A tight white protein pellet should be visible.

7. **Carefully transfer the supernatant to a fresh 15 mL centrifuge tube containing 3 mL of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**
   - Do not vortex after addition of isopropanol.
8. **Centrifuge at 2,000 xg for 3 mins. Decant the supernatant and add 3 mL of 70% ethanol(room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**
9. **Centrifuge at 2,000 xg for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10 ~ 15 mins.**
   - The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.
   - Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.
10. **Add 250 uL of buffer RE or distilled water and re-hydrate the DNA by incubating at 65°C for 1 hour.**
    - During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.

G. Procedure for animal tissue

To check before start

- Prepare water bathes or heat blocks at 37°C and 65°C
- Required additional materials; 1.5 mL or 2 mL centrifuge tube,
  Isopropanol
  70% Ethanol
  A device for homogenization

!!! If buffer NL has a precipitate in it, dissolve completely by heating before use.

1. **Homogenize up to 10 mg of tissue in 300 uL of buffer NL using small homogenizer. Transfer the lysate to a fresh 1.5 mL microcentrifuge tube. Proceed to step 2.**
   - Carefully homogenize the sample tissue not to foam if possible.

     Alternative 1: Grind sample tissue in liquid nitrogen with pre-chilled mortar and pestle. After grinding, let the liquid nitrogen evaporate and add up to 10 mg of tissue to 1.5 mL microcentrifuge tube containing 300 uL of buffer NL. Proceed to step 2.

     Alternative 2: Mince up to 10 mg of tissue sample as small as possible and put it into 1.5 mL microcentrifuge tube containing 300 uL of buffer NL. Incubate for 10 mins at 65°C. Homogenize flabby sample tissue with a small homogenizer.
2. **Incubate the lysate for 15 ~ 30 mins at 65°C.**
   - Mix the sample tube by vortexing or inverting periodically during incubation.
   - Do not incubate for more than 1 hour at 65°C.
   - If tissue particulates are still visible after lysis, the following can be tried for complete lysis; a. addition of 1.2 uL of Proteinase K solution (20 mg/mL, not provided), b. incubation for 3 hours or overnight at 55°C.
3. **Continue with step 4 of cultured cell protocol. (Page 10)**

H. Procedure for mouse tail

To check before start

- Prepare water bathes or heat blocks at 37°C and 65°C
- Required additional materials; 1.5 mL or 2 mL centrifuge tube,
  Isopropanol
  70% Ethanol
  A sterile sharp blade
  Proteinase K solution (20 mg/mL)

!!! If buffer NL has a precipitate in it, dissolve completely by heating before use.

1. **Mince 0.5 ~ 1 cm of mouse tail as small as possible. Transfer it to the 1.5 ~ 2 mL microcentrifuge tube containing 600 uL of buffer NL.**
2. **Add 3.6 uL of Proteinase K solution(20 mg/mL, not provided).**
3. **Incubate overnight at 56°C with gentle shaking.**
   - Alternatively, incubate for 3 hours at 56°C; vortex the sample once or twice per hour during 3-hours incubation. Make sure the tail is completely digested.
4. **(*Optional*) If RNA-free DNA is required, add 1 uL of RNase A solution (20 mg/mL) to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture for 15 ~ 30 mins at 37°C.**
5. **Cool the sample to room temperature. Add 200 uL of buffer PP to the mixture and vortex vigorously for 20 secs. Chill the sample on ice for 5 mins.**
6. **Centrifuge at 14,000 xg for 1 min.**
   - A tight white protein pellet should be visible.
7. **Carefully transfer the supernatant to a fresh 1.5 ~ 2 mL micro centrifuge tube containing 600 uL of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**
   - Do not vortex after addition of isopropanol.

8. **Centrifuge at 14,000 xg for 1 min. Decant the supernatant and add 600 uL of 70% ethanol(room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**
9. **Centrifuge at 14,000 xg for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10 - 15 mins.**
    - The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.
    - Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.
10. **Add 50 uL of buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**
    - During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.

I. Procedure for plant tissue

To check before start

- Prepare water bathes or heat blocks at 37°C and 65°C
- Required additional materials; 1.5 mL or 2 mL centrifuge tube,
  Isopropanol
  70% Ethanol
  Liquid nitrogen
  Mortar and pestle

!!! If buffer NL has a precipitate in it, dissolve completely by heating before use.

1. **Grind wet or dried plant tissue in liquid nitrogen with pre-chilled mortar and pestle.**

   After grinding, let the liquid nitrogen evaporate and add up to 30 mg of wet tissue or up to 10 mg of dried tissue to 1.5 mL micro centrifuge tube containing 300 uL of buffer NL.

   - It may be necessary to vary the amount of starting material depending on the species, age, tissue type and genome size.
2. **Vortex well to soak the sample in Buffer NL completely.**
3. **Incubate at 65°C for 60 mins.**
   - Invert the sample 5 ~ 10 times periodically during the incubation.
4. **Continue with step 4 of cultured cell protocol. (Page 10)**

J. Procedure for yeast cells

To check before start

- Prepare water bathes or heat blocks at 37°C and 65°C
- Required additional materials; 1.5 mL or 2 mL centrifuge tube,
  Isopropanol
  70% Ethanol
  50 mM EDTA solution
  Lyticase (20 mg/mL)

!!! If buffer NL has a precipitate in it, dissolve completely by heating before use.

1. **Add 1 mL of a culture grown for 20 hours in YPD broth to a 1.5 mL microcentrifuge tube.**
   - For *S. cerevisiae*, overnight culture contains 1-2 x 10^7 cells per milliliter approximately.
2. **Centrifuge at 14,000 xg for 30 secs to pellet the cells. Remove the supernatant.**
3. **Resuspend the cells thoroughly in 293 uL of 50 mM EDTA.**
4. **Add 7.5 uL of lyticase (20 mg/mL) and gently pipet 4 times to mix.**
5. **Incubate the sample at 37°C for 30 ~ 60 mins to digest the cell wall.**

   Cool to room temperature.
6. **Centrifuge at 14,000 xg for 1 min. Remove the supernatant.**
7. **Add 300 uL of buffer NL and gently pipet to lyse the cells.**
   - Incubation is not required.
8. **Add 100 uL of buffer PP and vortex vigorously for 20 secs. Incubate on ice for 5 mins.**
9. **Centrifuge at 14,000 xg for 3 mins.**

10. **Carefully transfer the supernatant to a fresh 1.5 mL micro centrifuge tube containing 300 uL of isopropanol(room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**
    - Do not vortex after addition of isopropanol.
11. **Centrifuge at 14,000 xg for 1 min. Decant the supernatant and add 500 uL of 70% ethanol(room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**
12. **Centrifuge at 14,000 xg for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10 - 15 mins.**
    - The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.
    - Ethanol should be completely removed, but over-dry will make the rehydration of DNA pellet difficult.
13. **Add 50 uL of buffer RE or distilled water.**
14. **(*Optional*) If RNA-free DNA is required, add 1 uL of RNase A soution (20 mg/mL) to the tube. Centrifuge briefly for 5 secs to collect the liquid and incubate at 37°C for 15 mins.**
15. **Rehydrate DNA by incubating at 65°C for 1 hour.**
    - During incubation, periodically mix the DNA solution by gently tapping the tube. DNA can be rehydrated alternatively by incubating the solution overnight at RT or 4°C.

K. Procedure for bacterial cells

To check before start

- Prepare water bathes or heat blocks at 37°C, 65°C and 80°C
- Required additional materials; 1.5 mL or 2 mL centrifuge tube,
  Isopropanol
  70% Ethanol
  50 mM EDTA solution
  Lysozyme (10 mg/mL)

!!! If buffer NL has a precipitate in it, dissolve completely by heating before use.

1. **Add up to 1 x 10^9 of bacterial cells to a 1.5 mL micro centrifuge tube.**
   - When OD_{600} =1, the cell density may be 1 x 10^9 cells per milliliter approximately.
2. **Centrifuge at 14,000 xg for 1 min to pellet the cells. Remove the supernatant. For Gram Negative bacteria, jump to the step 7.**
3. **Resuspend the cells thoroughly in 250 uL of 50 mM EDTA.**
4. **Add 50 uL of lysozyme (10 mg/mL) and gently pipet to mix.**
   - The purpose of this treatment is to weaken the cell wall so that efficient cell lysis can take place.
   - For certain species, such as *Staphylococcus*, addtional treatment of lysostaphin (1 mg/mL, working concentration) may be required for more efficient lysis.
   - However, lysozyme alone may be sufficient to lyse the cell wall for most Gram Positive bacterial strains.
5. **Incubate the sample at 37°C for 30 ~ 60 mins.**
6. **Centrifuge at 14,000 xg for 1 min. Remove the supernatant.**
7. **Add 300 uL of buffer NL and gently pipet until the cells are resuspended thoroughly.**
8. **Incubate at 80°C for 5 mins. Cool to room temperature.**
   - This step is especially necessary for pathogenic bacterial strains.

9. **Add 1 uL of RNase A solution(20 mg/mL). Invert the tube 2~5 times to mix. Incubate at 37°C for 15 - 60 mins.**
10. **Cool the sample to room temperature. Add 100 uL of buffer PP and vortex vigorously for 20 secs. Incubate on ice for 5 mins.**
11. **Centrifuge at 14,000 xg for 3 mins.**
12. **Carefully transfer the supernatant to a fresh 1.5 mL micro centrifuge tube containing 300 uL of isopropanol(room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.**
    - Do not vortex after addition of isopropanol.
13. **Centrifuge at 14,000 xg for 1 min. Decant the supernatant and add 300 uL of 70% ethanol(room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**
14. **Centrifuge at 14,000 xg for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air-dry the pellet for 10 ~ 15 mins.**
    - The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet.
    - Ethanol should be completely removed, but over-drying will make the rehydration of DNA pellet difficult.
15. **Add 100 uL of buffer RE or distilled water and rehydrate the DNA by incubating at 65°C for 1 hour.**
    - During incubation, periodically mix the DNA solution by gently tapping the tube. Alternatively, DNA can be rehydrated by incubating the solution overnight at RT or 4°C.

Trouble shooting guide

Facts	*Possible causes*	Recommendations
Low or no recovery	*Starting material is too old or mis-stored*	Best yield will be obtained from fresh sample. DNA yield is dependent on the type, size, age and storage of starting material. Lower yield will be obtained from material that has been inappropriately stored. For example, blood samples that have been stored at 4°C for more than 5 days may bring about reduced yield.
	Low cells in the sample	Some sample may contain low concentration of nucleated cells, and this may lead to poor yield. Increase the sample amount. If possible, harvest new sample and repeat the DNA purification with new sample.
	Insufficient lysis	Incomplete lysis can be due to too much starting material. Add more Buffer NL to completely lyse the cells. Start with proper amount of sample material. For cultured cells or bacteria, starting cell numbers should be determined with cell counter. When the sample is nucleated blood, use 1/10 volume of mammalian blood sample volume, and adjust to 1 volume with 1x PBS.
	White blood cell pellet was not resuspend thoroughly in step 3	The white blood cell pellet must be vortexed vigorously to resuspend the cells thoroughly.
	DNA pellet lost during isopropanol precipitation	Intensive care must be taken in removing the isopropanol or ethanol not to lose the pellet.
	DNA pellet is not completely rehydrated	Rehydrate DNA by incubating at 65°C for 1 hour. During incubation, periodically mix the DNA solution by gently tapping the tube. Alternatively, DNA can be rehydrated by incubating the solution overnight at RT or 4°C

Degraded DNA	*Starting material is too old or mis-placed*	Too old or mis-placed samples often yield degraded DNA. Use a fresh sample.
No protein pellet	*Lysate does not sufficiently cooled down*	To obtain a tight protein pellet, the sample should be cooled to room temperature or chilled on ice 5 mins before adding buffer PP. After addition of buffer PP, vortex vigorously to get homogenate.
DNA pellet difficult to dissolve	*Over-dried pellet*	DNA pellets should not be dried for longer than 15 mins at room temperature. Rehydrate DNA by incubating for 1 hour at 65°C and then leave the sample at room temperature or 4°C overnight. Do NOT leave DNA at 65°C overnight. This may degrade DNA.


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