Instruction Manual for

LaboPass™ Genomic

Genomic DNA Isolation Kit

Product Description

Principle method

LaboPass ™ Genomic kit provides a method for the isolation of genomic DNA from animal and plant cells without using toxic chemical such as phenol, chloroform. Genomic DNA purification method of LaboPass ™ Genomic kit is consisted of four- step processes. The first step in these procedure lyses the cells and the nuclei. For isolation of DNA from white blood cell, this step involves lysis of the red blood cells in RBC Lysis Buffer (Buffer RL), followed by lysis of the white blood cells and their nuclei in Lysis Buffer (Buffer AL). RNA digestion step may be included at this time. But it is optional process for some application. The cellular proteins are removed by adding Precipitation Buffer (Buffer PP), which precipitate protein but leaves the genomic DNA in the supernatant. Finally genomic DNA is concentrated and desalted by alcohol precipitation.

Storage condition

All components in this kit should be stored at room temperature. Check Buffer AL, PP. These buffers may precipitate at cool ambient temperature. If that happens, warm the bottles at 50 ℃ with occasional mixing until completely dissolved. The LaboPass ™ Genomic kit is guaranteed for 1 year after delivered to an end user.

Kit Contents

Product	Size
LaboPass™ Genomic	200 preps from 300 ㎕ blood
- Buffer RL(RBC lysis buffer)	220 ml
- Buffer AL (Lysis buffer)	100 ml
- Buffer PP(Precipitation buffer)	30 ml
- Buffer AE	30 ml
- 1 protocol	
LaboPass™ Genomic	1000preps from 300 ㎕ blood
- Buffer RL(RBC lysis buffer)	1L
- Buffer AL (Lysis buffer)	500 ml
- Buffer PP(Precipitation buffer)	150 ml
- Buffer AE	60 ml x 3
- 1 protocol	

Additional equipment and material to be supplied by the user

-. Microcentrifuge for miniprep or Centrifuge capable of handling 15 ㎖ and 50 ㎖ centrifuge tube for Maxiprep.
-. 70 % ethanol, Isopropanol
-.Sterile microcentrifuge for 300 ㎕ blood sample
-. Sterile 15 ㎖ centrifuge tube for 3 ㎖ blood sample
-. Sterile 50 ㎖ centrifuge tube for 10 ㎖ blood sample
-. Water bath at 65 ℃
-. RNase solution (4㎎/㎖)

Chemical Hazard

Buffer AL, PP contains irritant which is harmful when in contact with skin, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves, and follow standard safety precautions.

Application

This kit can be used to isolate genomic DNA from animal, plant, yeast and bacterial cells with the modification of cell lysis step. Detailed methods other than that of blood are shown in our company's home page.

Procedure for Animal Blood

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

1. **For 300 ㎕ Sample Volume**: Add 900 ㎕ of Buffer RL to a sterile 1.5ml microcentrifuge tube. **For 3 ml Sample Volume**: Add 9.0 ml of Buffer RL to a sterile 15ml centrifuge tube.
2. Gently rock the tube of blood until thoroughly mixed; then transfer blood to the tube containing the Buffer RL. 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 x g for 20 seconds at room temperature for 300 ㎕ sample. Centrifuge at 2,000 x g for 10 minutes at room temperature for 3 ml sample.
4. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Approximately 10 - 20 ㎕ of residual liquid will remain in the 1.5 ml tube (300 ㎕ sample). Approximately 50 - 100 ㎕ of residual liquid will remain in the 15ml tube (3 ml 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.
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 Buffer AL (300 ㎕ for 300 ㎕ sample volume; 3.0 ml for 3 ml 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 ℃ until the clumps are disrupted. If the clumps are still visible after 1 hour, add additional Buffer AL (100 ㎕ for 300 ㎕ sample volume; 1.0 ml for 3 ml sample volume) and repeat the incubation.
7. Optional: Add RNase Solution (1.5 ㎕ for 300 ㎕ sample volume; 15㎕ for 3ml sample volume) to the nuclear lysate and mix the sample by inverting the tube 25 times. Incubate the mixture at 37 ℃ for 15 minutes, and then cool to room temperature.
8. Add Buffer PP (100 ㎕ for 300 ㎕ sample volume; 1.0 ml for 3 ml sample volume) to the nuclear lysate and vortex vigorously for 10 - 20 seconds. Small protein clumps may be visible after vortexing.
9. Centrifuge at 13,000 - 16,000 x g for 3 minutes at room temperature for 300 ㎕ sample volume. Centrifuge at 2,000 x g for 10 minutes at room temperature for 3 ml sample volume. A dark brown protein pellet should be

visible.

10. For 300 µl sample volume, transfer the supernatant to a clean 1.5 ml microcentrifuge tube containing 300 µl of room temperature isopropanol. For 3 ml sample volume, transfer the supernatant to a 15 ml centrifuge tube containing 3 ml room temperature isopropanol.
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 x g for 1 minute at room temperature for 300 µl sample. Centrifuge at 2,000 x g for 1 minute at room temperature for 3 ml 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 buffer AE (100 µl for 300 µl sample volume; 250 µl for 3 ml sample volume) to the tube and rehydrate the DNA by incubating at 65 ℃ 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℃.
16. Store the DNA at 2 - 8℃.

DNA Purification From 0.5 ml Gram-Negative Bacteria Culture Medium

1. Add 0.5 ml cell suspension (*e.g.*, overnight culture containing approximately 0.5-1.5 billion cells) to a 1.5 ml tube.
2. Centrifuge at 13,000-16,000 x g for 5 seconds to pellet cells. For some species centrifugation for up to 60 seconds may be required to obtain a tight cell pellet. Remove as much supernatant as possible using a pipet.
3. Add 300 µl Buffer **AL** and pipet up and down until cells are suspended.
4. Incubate sample at 80°C for 5 minutes to lyse cells.
5. Add 1.5 µl **RNase A Solution** (4 mg/ml) to the cell lysate.
6. Mix the sample by inverting 25 times and incubate at 37°C for 15-60 minutes.
7. Cool sample to room temperature.
8. Add 100 µl **Buffer PP** to the RNase A-treated cell lysate.
9. Vortex vigorously at high speed for 20 seconds to mix the **Buffer PP** uniformly with the cell lysate.
10. Centrifuge at 13,000-16,000 x g for 3 minutes. The precipitated proteins will form a tight pellet. If the protein pellet is not visible, repeat Step 9 followed by incubation on ice for 5 minutes, then repeat Step 10.
11. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml centrifuge tube containing 300 µl **100% Isopropanol** (2-propanol).
12. Mix the sample by inverting gently 50 times.
13. Centrifuge at 13,000-16,000 x g for 1 minute; a white DNA pellet may or may not be visible, depending on the yield. If the DNA yield is expected to be low (<1 µg), increase the centrifugation time to 5 minutes.
14. Pour off supernatant and drain tube on clean absorbent paper. Add 300 µl **70% Ethanol** and invert tube several times to wash the DNA pellet.
15. Centrifuge at13,000-16,000 x g for 1 minute. Carefully pour off the ethanol. *Pellet may be loose so pour slowly and watch pellet.*
16. Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes.
17. Add 50 µl **Buffer AE** (50 µl will give a concentration of 100 µg/ml if the total yield is 5 µg DNA).
18. Rehydrate DNA by incubating sample 1 hour at 65ºC and/or overnight at room temperature. If possible, tap tube periodically to aid in dispersing the DNA.
19. Store DNA at 4°C. For long-term storage, store at -20°C or -80°C.

DNA Purification From 0.5 ml Gram-positive Bacteria Culture Medium

1. Add 0.5 ml cell suspension (*e.g.*, overnight culture containing approximately 0.5-1.5 billion cells) to a 1.5 ml tube.
2. Centrifuge at 13,000-16,000 x g for 5 seconds to pellet cells. For some species centrifugation for up to 60 seconds may be required to obtain a tight cell pellet. Remove as much supernatant as possible using a pipet.
3. Add 300 µl Tris-EDTA buffer(50 mM Tris-Cl pH7.5, 10mM EDTA) to cell pellet and gently pipet up and down until cells are suspended.
4. Add 50 µl (25 µl of lysostaphin(10mg/ml) and 25µl of lysozyme(10mg/ml)) and invert tube 25 times to mix.
5. Incubate at 37°C for 30 minutes to digest cell walls. Invert sample occasionally during the incubation.
6. Centrifuge at 13,000-16,000 x g for 1 minute to pellet the cells. Remove supernatant.
7. Add 300 µl **Buffer AL** to the cell pellet and gently pipet up and down to lyse the cells.
8. For some species heating the sample to 80°C for five minutes may be required to complete cell lysis.
9. Add 1.5 µl **RNase A Solution** (4 mg/ml) to the cell lysate.
10. Mix the sample by inverting 25 times and incubate at 37°C for 15-60 minutes.
11. Cool sample to room temperature.
12. Add 100 µl **Buffer PP** to the RNase A-treated cell lysate.
13. Vortex vigorously at high speed for 20 seconds to mix the **Buffer PP** uniformly with the cell lysate.
14. Centrifuge at 13,000-16,000 x g for 3 minutes. The precipitated proteins will form a tight pellet. If the protein pellet is not visible, repeat Step 13 followed by incubation on ice for 5 minutes, then repeat Step 14.
15. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml centrifuge tube containing 300 µl **100% Isopropanol** (2-propanol).
16. Mix the sample by inverting gently 50 times.
17. Centrifuge at 13,000-16,000 x g for 1 minute; a white DNA pellet may or may not be visible, depending on the yield. If the DNA yield is expected to be low (<1 µg), increase the centrifugation time to 5 minutes.
18. Pour off supernatant and drain tube on clean absorbent paper. Add 300 µl **70% Ethanol** and invert tube several times to wash the DNA pellet.
19. Centrifuge at13,000-16,000 x g for 1 minute. Carefully pour off the ethanol. *Pellet may be loose so pour slowly*

and watch pellet.

20. Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes.
21. Add 50 µl **Buffer AE** (50 µl will give a concentration of 100 µg/ml if the total yield is 5 µg DNA).
22. Rehydrate DNA by incubating sample 1 hour at 65ºC and/or overnight at room temperature. If possible, tap tube periodically to aid in dispersing the DNA.
23. Store DNA at 4°C. For long-term storage, store at -20°C or -80°C.

DNA Purification From 5-10 mg Solid Tissue

1. Dissect tissue sample quickly and freeze in liquid nitrogen. Store at -70° to -80°C. Fresh tissue may also be used. Work very quickly and keep tissue on ice at all times including when tissue is weighed.
2. Add 5-10 mg (0.005-0.01 g) frozen ground tissue or fresh tissue to a 1.5 ml centrifuge tube containing 300 µl **Buffer AL**, remove from ice, and homogenize thoroughly using a microfuge tube pestle. Place sample back on ice until next step.
3. Incubate lysate at 65°C for 15-60 minutes. Alternatively, if maximum yield is required, 1.5 µl **Proteinase K Solution** (20 mg/ml) may be added to the lysate. Mix by inverting 25 times and incubate at 55°C for 3 hours to overnight, until tissue particulates have dissolved. If possible, invert tube periodically during the incubation.
4. Add 1.5 µl **RNase A Solution** (4 mg/ml) to the cell lysate.
5. Mix the sample by inverting 25 times and incubate at 37°C for 15-60 minutes.
6. Cool sample to room temperature.
7. Add 100 µl **Buffer PP** to the RNase A-treated cell lysate.
8. Vortex vigorously at high speed for 20 seconds to mix the **Buffer PP** uniformly with the cell lysate.
9. Centrifuge at 13,000-16,000 x g for 3 minutes. The precipitated proteins will form a tight pellet. If the protein pellet is not visible, repeat Step 8 followed by incubation on ice for 5 minutes, then repeat Step 9.
10. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml centrifuge tube containing 300 µl **100% Isopropanol** (2-propanol).
11. Mix the sample by inverting gently 50 times.
12. Centrifuge at 13,000-16,000 x g for 1 minute; a white DNA pellet may or may not be visible, depending on the yield. If the DNA yield is expected to be low (<1 µg), increase the centrifugation time to 5 minutes.
13. Pour off supernatant and drain tube on clean absorbent paper. Add 300 µl **70% Ethanol** and invert tube several times to wash the DNA pellet.
14. Centrifuge at13,000-16,000 x g for 1 minute. Carefully pour off the ethanol. *Pellet may be loose so pour slowly and watch pellet.*
15. Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes.
16. Add 50 µl **Buffer AE** (50 µl will give a concentration of 100 µg/ml if the total yield is 5 µg DNA).
17. Rehydrate DNA by incubating sample 1 hour at 65ºC and/or overnight at room temperature. If possible, tap tube periodically to aid in dispersing the DNA.
18. Store DNA at 4°C. For long-term storage, store at -20°C or -80°C.

DNA Purification Protocol For 1-2 x 10^6 Culture Cells

1. Add 1-2 x 10^6 culture cells in balanced salt solution or culture medium to a 1.5 ml tube.
2. Centrifuge at 13,000-16,000 x g for 5 seconds to pellet cells. Remove supernatant leaving behind 10-20 µl residual liquid.
3. Vortex the tube vigorously to resuspend the cells in the residual supernatant. This greatly facilitates cell lysis in Step 4 below.
4. Add 300 µl **Buffer AL** to the resuspended cells and pipet up and down to lyse the cells. Usually no incubation is required; however, if cell clumps are visible after mixing, incubate at 37°C until the solution is homogeneous. Samples are stable in **Buffer AL** for at least 2 years at room temperature.
5. Add 1.5 µl **RNase A Solution** (4 mg/ml) to the cell lysate.
6. Mix the sample by inverting 25 times and incubate at 37°C for 15-60 minutes.
7. Cool sample to room temperature.
8. Add 100 µl **Buffer PP** to the RNase A-treated cell lysate.
9. Vortex vigorously at high speed for 20 seconds to mix the **Buffer PP** uniformly with the cell lysate.
10. Centrifuge at 13,000-16,000 x g for 3 minutes. The precipitated proteins will form a tight pellet. If the protein pellet is not visible, repeat Step 9 followed by incubation on ice for 5 minutes, then repeat Step 10.
11. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml centrifuge tube containing 300 µl **100% Isopropanol** (2-propanol).
12. Mix the sample by inverting gently 50 times.
13. Centrifuge at 13,000-16,000 x g for 1 minute; a white DNA pellet may or may not be visible, depending on the yield. If the DNA yield is expected to be low (<1 µg), increase the centrifugation time to 5 minutes.
14. Pour off supernatant and drain tube on clean absorbent paper. Add 300 µl **70% Ethanol** and invert tube several times to wash the DNA pellet.
15. Centrifuge at13,000-16,000 x g for 1 minute. Carefully pour off the ethanol. *Pellet may be loose so pour slowly and watch pellet.*
16. Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes.
17. Add 50 µl **Buffer AE** (50 µl will give a concentration of 100 µg/ml if the total yield is 5 µg DNA).
18. Rehydrate DNA by incubating sample 1 hour at 65ºC and/or overnight at room temperature. If possible, tap tube periodically to aid in dispersing the DNA.
19. Store DNA at 4°C. For long-term storage, store at 20°C or -80°C.

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