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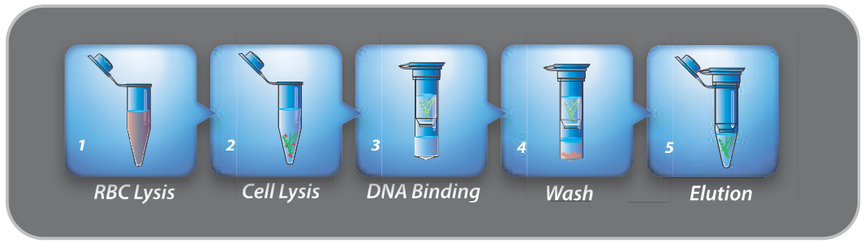
Real Genomics™

Store at room temperature 15°C~25°C



HiYield™ Genomic DNA Mini Kit (Blood / Bacteria / Cultured Cells) Protocol Book

Ready-to-use genomic DNA within 25 minutes

Cat. No. **YGB100 / YGB300**

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Real Genomics™

Index

HiYield™ Genomic DNA Mini Kit (Blood / Bacteria / Cultured Cells)

HiYield™ Genomic DNA Mini Kit (Blood/Bacteria/Cultured Cells)

Store at room temperature 15°C~25°C

Cat.No. **YGB100**
100 mini preps / kit
RBC Lysis Buffer: 135 ml
GB Buffer: 40 ml
GT Buffer: 30 ml
W1 Buffer: 45 ml
Wash Buffer (concentrated): 25 ml *
Elution Buffer: 30 ml
GB Column: 100 pcs
2 ml Collection Tube: 200 pcs

Cat.No. **YGB300**
300 mini preps / kit
RBC Lysis Buffer: 405 ml
GB Buffer: 100 ml
GT Buffer: 75 ml
W1 Buffer: 130 ml
Wash Buffer (concentrated): 50 ml *
Elution Buffer: 75 ml
GB Column: 300 pcs
2 ml Collection Tube: 600 pcs

Sample: Up to 300 µl of Whole Blood, 200 µl of Frozen Blood, 200 µl of Buffy Coat, 10^7 of Cultured Animal Cells, 10^9 of Cultured Bacterial Cells and 2 x 10^8 of Yeast / Fungus.
Yield: Up to 50 µg of genomic DNA (20-30 kb)
Format: Spin Column
Operation: Centrifuge or Vacuum
Operation Time: 25 Minutes
Elution Volume: 30 -200 µl

* Add 100 ml of Ethanol (96-100%) to Wash Buffer of YGB100 prior to initial use.
Add 200 ml of Ethanol (96-100%) to Wash Buffer of YGB300 prior to initial use.

Description

HiYield™ Genomic DNA Mini Kit (Blood/Bacteria/Cultured Cells) provides a fast and economical method for purification of total DNA (including genomic, mitochondrial and viral DNA) from fresh / frozen whole blood, buffy coat, cultured animal cells, cultured bacterial cells, yeast and fungus. The entire procedure can be completed in 25 minutes without phenol/chloroform extraction or alcohol precipitation, with an average DNA yield of 6 µg from 200 µl of whole human blood and up to 50 µg of DNA from 200 µl of buffy coat. Purified DNA, with approximately 20-30 kb, is suitable for direct use in PCR or other enzymatic reactions.

Features

Rapid and reliable purification of ready-to-use genomic DNA within 25 minutes.
Complete removal of all contaminants for reliable downstream applications.
No phenol, chloroform or alcohol.
Rapid and simple procedure.

Applications

Purified DNA is ready for direct use in PCR, Southern Blotting, Real-Time PCR, AFLP, RFLP, PADP.

Quality Control

The quality of HiYield™ Genomic DNA Mini Kit (Blood/Bacteria/Cultured Cells) is tested on a lot-to-lot basis by isolation of genomic DNA from 200 µl of human whole blood. Purified DNA is quantified with a spectrophotometer and the yield of genomic DNA is more than 5 µg with A_{260}/A_{280} ratio 1.8 - 2.0. The purified DNA is checked by electrophoresis.

Reference: Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Note: (1) For research use only. Not for use in diagnostic or therapeutic procedures. (2) GB Buffer contains guanidine hydrochloride which is harmful and irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Fresh Blood Protocol

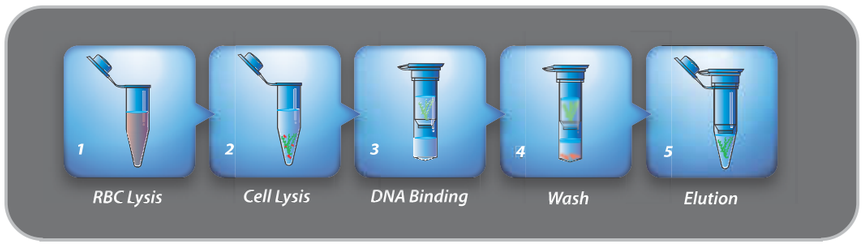
Additional requirements:

* 96% ~ 100% Ethanol.
* Sterile, DNase-free pipette tips and microcentrifuge tubes.
* Optional: RNase A (10 mg/ml).

Things to do before starting:

* Collect blood in EDTA-NA2 treated tubes (or other anticoagulant mixtures)
* Add 100 ml of Ethanol (96-100%) to Wash Buffer of YGB100 prior to initial use.
  Add 200 ml of Ethanol (96-100%) to Wash Buffer of YGB300 prior to initial use.

RBC Lysis Buffer is provided to remove non-nucleated red blood cells and reduce hemoglobin contamination. When the blood sample is less then 5 µl or the sample consists of nucleated blood cells, the Cultured Cells Protocol is recommended to purify genomic DNA.



RBC Lysis

1. Collect fresh blood in EDTA-Na_2-treated collection tubes (or other anticoagulant mixtures).
2. Add up to 300 µl of fresh blood to a 1.5 ml microcentrifuge tube.
3. Add 3 volumes of RBC Lysis Buffer to 1 volume of the sample and mix by inversion. (For example, add 900 µl of RBC Lysis Buffer to 300 µl of fresh blood) Do not vortex.
4. Incubate the tube at room temperature for 10 minutes.
5. Centrifuge at 3,000 xg for 5 minutes and then discard the supernatant completely. .
6. Add 100 µl of RBC Lysis Buffer to resuspend the cell pellet.

Cell Lysis

7. Add 200 µl of GB Buffer to the 1.5 ml microcentrifuge tube and then shake vigorously.
8. Incubate the mixture at 60°C for at least 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.
9. Preheat the required Elution Buffer (200 µl per sample) to 60°C (for DNA Elution).

Optional Step: RNA Degradation

(If RNA-free genomic DNA is required, perform this optional step)

a. Following 60°C incubation, add 5 µl of RNase A (10 mg/ml, not provided) to the clear lysate then mix by shaking vigorously.
b. Incubate at room temperature for 5 minutes.

DNA Binding

10. Add 200 µl of ethanol (96-100%) to the sample lysate and mix immediately by shaking vigorously for 10 seconds. If precipitate appears, break it up by pipetting.
11. Place a GB Column in the 2 ml Collection Tube.
12. Apply the total mixture (including any precipitate) from previous step to the GB Column.
13. Close the cap and centrifuge at 14,000 -16,000 x g for 5 minutes.
14. Discard the 2 ml Collection Tube containing the flow-through and place the GB Column in a new 2 ml Collection Tube.

Wash

15. Add 400 µl of W1 Buffer into the GB Column.
16 Centrifuge at 14,000-16,000 x g for 30-60 seconds.
17. Discard the flow-through and place the GB Column back in the 2 ml Collection Tube.
18. Add 600 µl of Wash Buffer (ethanol added) into the GB Column.
19. Centrifuge at 14,000-16,000 x g for 30-60 seconds.
20. Discard the flow-through and place the GB Column back in the 2 ml Collection Tube.
21. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.

DNA Elution

22. Transfer the dried GB Column into a clean 1.5 ml microcentrifuge tube.
23. Add 100 µl of preheated Elution Buffer or TE into the center of the column matrix.
    Standard elution volume is 100 µl. If less sample volume is used, reduce the elution volume (30~50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery. The total elution volume is approximately 200 µl.
24. Let stand at room temperature for at least 3 minutes (until Elution Buffer or TE is completely absorbed by the matrix).
25. Centrifuge at 14,000-16,000 x g for 30 seconds to elute purified DNA.

Frozen Blood Protocol

Additional requirements:

* PBS (phosphate-buffered saline).
* Proteinase K (10 mg/ml).
* 96% ~ 100% Ethanol.
* Sterile, DNase-free pipette tips and microcentrifuge tubes.
* Optional: RNase A (10 mg/ml).

Things to do before starting:

Add 100 ml of Ethanol (96-100%) to Wash Buffer of YGB100 prior to initial use.
Add 200 ml of Ethanol (96-100%) to Wash Buffer of YGB300 prior to initial use.

Cell Lysis

1. Apply 30 µl of Proteinase K (10 mg/ml) to a 1.5 ml microcentrifuge tube. Then add in up to 200 µl of frozen blood and mix briefly. (If the sample volume is less than 200 µl, add the appropriate volume of PBS.)
2. Incubate the mixture at 60°C for 15 minutes.
3. Add 200 µl of GB Buffer to the tube and mix by vortexing.
4. Incubate the mixture in a 70°C waterbath for 15 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.
5. Preheat the required Elution Buffer (200 µl per sample) in a 70°C waterbath (for DNA Elution).

Optional Step: RNA Degradation

If RNA-free genomic DNA is required, perform this optional step.

a. Following 60°C incubation, add 5 µl of RNase A (10 mg/ml, not provided) to the clear lysate then mix by shaking vigorously.
b. Incubate at room temperature for 5 minutes.

DNA Binding

6. Add 200 µl of ethanol (96-100%) to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
7. Place a GB Column in the 2 ml Collection Tube.
8. Apply the total mixture (including any precipitate) from previous step to the GB Column.
9. Close the cap and centrifuge at 14,000 -16,000 x g for 5 minutes.
10. Discard the 2 ml Collection Tube containing the flow-through and place the GB Column in a new 2 ml Collection Tube.

Wash

11. Add 400 µl of W1 Buffer into the GB Column.
12 Centrifuge at 14,000-16,000 x g for 30-60 seconds.
13. Discard the flow-through and place the GB Column back in the 2 ml Collection Tube.
14. Add 600 µl of Wash Buffer (ethanol added) into the GB Column.
15. Centrifuge at 14,000-16,000 x g for 30-60 seconds.
16. Discard the flow-through and place the GB Column back in the 2 ml Collection Tube.
17. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.

DNA Elution

18. Transfer the dried GB Column into a clean 1.5 ml microcentrifuge tube.
19. Add 100 µl of preheated Elution Buffer or TE into the center of the column matrix.
    Standard elution volume is 100 µl. If less sample volume is used, reduce the elution volume (30~50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery. The total elution volume is approximately 200 µl.
20. Let stand at room temperature for at least 3 minutes (until Elution Buffer or TE is completely absorbed by the matrix).
21. Centrifuge at 14,000-16,000 x g for 30 seconds to elute purified DNA.

Buffy Coat Protocol

Additional requirements:

* 96% ~ 100% Ethanol.
* Sterile, DNase-free pipette tips and microcentrifuge tubes.
* Optional: RNase A (10 mg/ml).

Things to do before starting:

Add 100 ml of Ethanol (96-100%) to Wash Buffer of YGB100 prior to initial use.
Add 200 ml of Ethanol (96-100%) to Wash Buffer of YGB300 prior to initial use.

RBC Lysis

1. Transfer up to 200 µl of buffy coat to a 1.5 ml microcentrifuge tube.
2. Add 3 volumes of RBC Lysis Buffer to 1 volume of the sample and mix by inversion. (For example, add 600 µl of RBC Lysis Buffer to 200 µl of buffy coat)
3. Incubate the tube at room temperature for 10 minutes . During incubation, invert the tube every 3 minutes.
4. Centrifuge at 14,000-16,000 x g for 1 minute and discard the supernatant completely.
5. Add 500 µl of RBC Lysis Buffer to resuspend the white pellet.
6. Centrifuge at 14,000-16,000 x g for 1 minute and discard the supernatant completely.
7. Add 200 µl of RBC Lysis Buffer to the tube and resuspend the white pellet completely (Mix the tube by vortexing only if the pellet is not resuspended completely and the column has become barred).

Cell Lysis

8. Add 250 µl of GB Buffer to the 1.5 ml microcentrifuge tube and mix by shake vigorously.
9. Incubate the mixture at 60°C for at least 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes.
10. Preheat the required Elution Buffer (200 µl per sample) to 60°C (for DNA Elution).

Optional Step: RNA Degradation

If RNA-free genomic DNA is required, perform this optional step.

a. Following 60°C incubation, add 5 µl of RNase A (10 mg/ml, not provided) to the clear lysate then mix by shaking vigorously.
b. Incubate at room temperature for 5 minutes.

DNA Binding

11. Add 250 µl of ethanol (96-100%) to the sample lysate and mix immediately by shaking vigorously for 10 seconds. If precipitate appears, break it up by pipetting.
12. Place a GB Column in the 2 ml Collection Tube.
13. Apply the total mixture (including any precipitate) from previous step to the GB Column.
14. Close the cap and centrifuge at 14,000-16,000 x g for 5 minutes.
15. Discard the 2 ml Collection Tube containing the flow-through and place the GB Column in a new 2 ml Collection Tube.

Wash

16. Add 400 µl of W1 Buffer into the GB Column.
17. Centrifuge at 14,000-16,000 x g for 30-60 seconds.
18. Discard the flow-through and place the GB Column back in the 2 ml Collection Tube.
19. Add 600 µl of Wash Buffer (ethanol added) into the GB Column.
20. Centrifuge at 14,000-16,000 x g for 30-60 seconds.
21. Discard the flow-through and place the GB Column back in the 2 ml Collection Tube.
22. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.

DNA Elution

23. Transfer the dried GB Column into a clean 1.5 ml microcentrifuge tube.
24. Add 100 µl of preheated Elution Buffer or TE into the center of the column matrix.
    Standard elution volume is 100 µl. If less sample volume is used, reduce the elution volume (30~50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery. The total elution volume is approximately 200 µl.
25. Let stand at room temperature for at least 10 minutes (until Elution Buffer or TE is absorbed by the matrix).
26. Centrifuge at 14,000-16,000 x g for 30 seconds to elute purified DNA.

Bacteria Protocol

Additional requirements:

- 96% ~ 100% Ethanol.
- Sterile, DNase-free pipette tips and microcentrifuge tubes.
- RNase A (10 mg/ml).
- Lysozyme Buffer (20 mg/ml lysozyme; 20 mM Tris-HCl, 2 mM EDTA, 1% Triton X-100, pH 8.0) for Gram-Positive Bacteria Sample. Prepare the lysozyme buffer fresh immediately prior to use.

Things to do before starting:

Add 100 ml of Ethanol (96-100%) to Wash Buffer of YGB100 prior to initial use.
Add 200 ml of Ethanol (96-100%) to Wash Buffer of YGB300 prior to initial use.

Cell Harvesting for Gram-Negative Bacteria

1. Transfer cultured bacterial cells (up to 1 x10^9) to a 1.5ml microcentrifuge tube.
2. Centrifuge at 14,000-16,000 x g for 1 minute and discard the supernatant.
3. Add 200 µl of GT Buffer to the tube and resuspend the cell pellet by vortexing or pipetting.
4. Incubate at room temperature for 5 minutes.

Proceed to Step 5 Cell Lysis Below.

Cell Harvesting for Gram-Positive Bacteria

1. Prepare the lysozyme buffer fresh immediately prior to use. Transfer cultured bacterial cells (up to 1 x 10^9) to a 1.5ml microcentrifuge tube.
2. Centrifuge at 14,000-16,000 x g for 1 minute and discard the supernatant.
3. Add 200 µl of Lysozyme Buffer to the tube and resuspend the cell pellet by vortexing or pipetting.
4. Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes.

Proceed to Step 5 Cell Lysis Below.

Cell Lysis

5. Add 200 µl of GB Buffer to the sample. Mix by vortexing for 5 seconds.
6. Incubate at 70°C waterbath for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes. Preheat the required Elution Buffer (200 µl per sample) at 70°C (for DNA Elution).

Optional Step: RNA Degradation

If RNA-free genomic DNA is required, perform this optional step.

a. Following 60°C incubation, add 5 µl of RNase A (10 mg/ml, not provided) to the clear lysate then mix by shaking vigorously.
b. Incubate at room temperature for 5 minutes.

DNA Binding

7. Add 200 µl of ethanol (96-100%) to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
8. Place a GB Column in a 2 ml Collection Tube.
9. Apply all the mixture (including any precipitate) from previous step to the GB Column.
10. Close the cap and centrifuge at 14,000-16,000 x g for 2 minutes.
11. Discard the 2 ml Collection Tube containing the flow-through and place the GB Column in a new 2 ml Collection Tube.

Wash

12. Add 400 µl of W1 Buffer in the GB Column.
13. Centrifuge at 14,000-16,000 x g for 30-60 seconds.
14. Discard the flow-through and place the GB Column back in the 2 ml Collection Tube.
15. Add 600 µl of Wash Buffer (ethanol added) in the GB Column and centrifuge at at 14,000-16,000 x g for 30-60 seconds.
16. Discard the flow-through and place the GB Column back in the 2 ml Collection Tube.
17. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.

DNA Elution

18. Transfer the dried GB Column into a clean 1.5 ml microcentrifuge tube.
19. Add 100 µl of preheated Elution Buffer or TE into the center of the column matrix.
    Standard elution volume is 100 µl. If less sample volume is used, reduce the elution volume (30~50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery. The total elution volume is approximately 200 µl.
20. Let stand at room temperature for at least 3 minutes (until Elution Buffer or TE is completely absorbed by the matrix).
21. Centrifuge at 14,000-16,000 x g for 30 seconds to elute purified DNA.

Cultured Cells Protocol

Additional requirements:

* 96% ~ 100% Ethanol.
* PBS (phosphate-buffered saline).
* 0.10-0.25% Trypsin.
* Sterile, DNase-free pipette tips, centrifuge tubes and microcentrifuge tubes.
* Optional: RNase A (10 mg/ml).

Things to do before starting:

Add 100 ml of Ethanol (96-100%) to Wash Buffer of YGB100 prior to initial use.
Add 200 ml of Ethanol (96-100%) to Wash Buffer of YGB300 prior to initial use.

Sample Preparation

Adherent Cultured Animal Cells: (trypsinize cells before harvesting)
1) Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS.
2) Once cells detach, add medium then transfer to a 15 ml centrifuge tube.
3) Proceed with Suspension Cultured Animal cells sample preparation steps.

Suspension Cultured Animal Cells:
1) Transfer cells (up to 1 x 10^7) to a 1.5 ml microcentrifuge tube then centrifuge at 300 x g for 5 minutes.
2) Discard the supernatant and resuspend the cells in 150 µl of RBC Lysis Buffer by pipette.

Mammalian Blood (except for human blood):
For mammalian blood (non-nucleated), the sample volume can be up to 50 µl.
For nucleated erythrocytes (e.g., bird or fish), the sample volume can be up to 10 µl.
1) Add 150 µl of GT Buffer and blood sample to a 1.5 ml microcentrifuge tube and then shake vigorously.

Cell Lysis

3. Add 200 µl of GB Buffer to the 1.5 ml microcentrifuge tube then shake vigorously.
4. Incubate at 60°C for at least 10 minutes (until the sample lysate is clear). During incubation, invert the tube every 3 minutes. Preheat the required Elution Buffer (200 µl per sample) to 60°C (for DNA Elution).

Optional Step: RNA Degradation
If RNA-free genomic DNA is required, perform this optional step.
a. Following 60°C incubation, add 5 µl of RNase A (10 mg/ml, not provided) to the clear lysate then mix by shaking vigorously.
b. Incubate at room temperature for 5 minutes.

DNA Binding

5. Add 200 µl of ethanol (96-100%) to the sample lysate and mix immediately by shaking vigorously for 10 seconds. If precipitate appears, break it up by pipetting.
6. Place a GB Column in a 2 ml Collection Tube.
7. Apply all the mixture (including any precipitate) from previous step to the GB Column.
8. Close the cap and centrifuge at 14,000-16,000 x g for 2 minutes.
9. Discard the 2 ml Collection Tube containing the flow-through and place the GB Column in a new 2 ml Collection Tube.

Wash

10. Add 400 µl of W1 Buffer into the GB Column.
11. Centrifuge at 14,000-16,000 x g for 30-60 seconds.
12. Discard the flow-through and place the GB Column back in the 2 ml Collection Tube.
13. Add 600 µl of Wash Buffer (ethanol addd) into the GB Column and centrifuge at 14,000-16,000 x g for 30 -60 seconds.
14. Discard the flow-through and place the GB Column back in the 2 ml Collection Tube.
15. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.

DNA Elution

16. Transfer the dried GB Column into a clean 1.5 ml microcentrifuge tube.
    Standard elution volume is 100 µl. If less sample volume is used, reduce the elution volume (30~50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery. The total elution volume is approximately 200 µl.
17. Add 100 µl of preheated Elution Buffer or TE into the center of the column matrix.
18. Let stand at room temperature for at least 3 minutes (until Elution Buffer or TE is completely absorbed by the matrix).
19. Centrifuge at 14,000-16,000 x g for 30 seconds to elute purified DNA.

Yeast / Fungus Protocol

Additional requirements:

* 96% ~ 100% Ethanol.
* Sterile, DNase-free pipette tips and microcentrifuge tubes.
* 50 mM EDTA (pH8.0).
* Lyticase or Zymolase.
* Optional: RNase A (10 mg/ml).

Things to do before starting:

Add 100 ml of Ethanol (96-100%) to Wash Buffer of YGB100 prior to initial use.
Add 200 ml of Ethanol (96-100%) to Wash Buffer of YGB300 prior to initial use.

Cell Harvesting

1. Transfer yeast / fungus cells (up to 2 x 10^8) to a 1.5 ml microcentrifuge tube and harvest cells by centrifugation at 5,000 xg for 10 minutes .
2. Discard the supernatant and resuspend the pellet in 600 µl of 50 mM EDTA (pH8.0).
3. Add 200 U of lyticase or zymolase. Incubate at 30°C for 30 minutes.
4. Centrifuge the mixture at 2,000 xg for 10 minutes to harvest the spheroplast.
5. Remove the supernatant and add 200 µl of GT Buffer to the tube. Resuspend the cell pellet by shaking vigorously or pipetting.
6. Incubate at room temperature for 5 minutes.

Cell Lysis

7. Add 200 µl of GB Buffer to the sample. Mix by shaking vigorously for 5 seconds.
8. Incubate at 60°C waterbath for 10 minutes to ensure the sample lysate is clear. During incubation, invert the tube every 3 minutes. Preheat the required Elution Buffer (200 µl per sample) to 60°C (for DNA Elution).

Optional Step: RNA Degradation

If RNA-free genomic DNA is required, perform this optional step.

a. Following 60°C incubation, add 5 µl of RNase A (10 mg/ml, not provided) to the clear lysate then mix by shaking vigorously.
b. Incubate at room temperature for 5 minutes.

DNA Binding

9. Add 200 µl of ethanol (96-100%) to the sample lysate and mix immediately by shaking vigorously for 10 seconds. If precipitate appears, break it up by pipetting.
10. Place a GB Column in a 2 ml Collection Tube.
11. Apply all the mixture (including any precipitate) from previous step to the GB Column.
12. Close the cap and centrifuge at full speed (approx. 13,000 rpm) for 2 minutes.
13. Discard the 2 ml Collection Tube containing the flow-through and place the GB Column in a new 2 ml Collection Tube.

Wash

14. Add 400 µl of W1 Buffer into the GB Column.
15. Centrifuge at 14,000-16,000 x g for 30 seconds.
16. Discard the flow-through and place the GB Column back in the 2 ml Collection Tube.
17. Add 600 µl of Wash Buffer (ethanol addd) into the GB Column and centrifuge at 14,000-16,000 x g for 30 seconds.
18. Discard the flow-through and place the GB Column back in the 2 ml Collection Tube.
19. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.

DNA Elution

20. Transfer the dried GB Column into a clean 1.5 ml microcentrifuge tube.
21. Add 100 µl of preheated Elution Buffer or TE into the center of the column matrix.
    Standard elution volume is 100 µl. If less sample volume is used, reduce the elution volume (30~50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery. The total elution volume is approximately 200 µl.
22. Let stand at room temperature for at least 3 minutes (until Elution Buffer or TE is completely absorbed by the matrix).
23. Centrifuge at 14,000-16,000 x g for 30 seconds to elute purified DNA.

Troubleshooting

Problem	Possible Reasons/ Solution
Column Clogged	**Overloaded Column with Sample** Reduce sample volume to recommended volume or separate sample into multiple tubes.
Low Yield	**Precipitate was Formed at DNA Binding Step** 1. Reduce the sample material. 2. Prior to loading into the column, break up precipitates in ethanol-added lysate.
	Incorrect DNA Elution Step Ensure that Elution Buffer or TE is added to the center of the GB Column and is absorbed completely.
	Incomplete DNA Elution Elute twice to increase yield.
Eluted DNA does not perform well in downstream applications	**Residual Ethanol Contamination** Following the wash step, dry GB Column with additional centrifugation 14,000-16,000 x g for 5 minutes or incubate at 60°C for 5 minutes.
	RNA Contamination Perform optional RNA degradation step.
	Genomic DNA was Degraded Use fresh blood. Prolonged storage may result in fragmentation of genomic DNA.
	Protein Contamination 1. Reduce the sample volume. 2. After DNA Binding Step, apply 200 ml of GB Buffer to wash GB Column and centrifuge at 6,000 xg for 30 seconds. Proceed with Wash Step of Wash Buffer.

Ordering Information

Genomic DNA Purification		Cat. No.	Size	Items	Contents
	Blood & Cultured Cells	QBT100	S	HiYield Plus™ Genomic DNA Mini Kit (Blood/Tissue/Cultured Cells)	100 preps/kit,(QGB, QGT, Wash, W1, Elution) Buffer, Proteinase K, GD Column, 2 ml Collection Tube.
		QBT300	S	HiYield Plus™ Genomic DNA Mini Kit (Blood/Tissue/Cultured Cells)	300 preps/kit,(QGB, QGT, Wash, W1, Elution) Buffer, Proteinase K, GD Column, 2 ml Collection Tube.
		YGBI25	M	HiYield™ Genomic DNA Midi Kit (Fresh Blood / Cultured Cells)	25 preps/kit,(RBC Lysis, GB, Wash, W1, Elution) Buffer, GBI Column.
		YGDI25	M	HiYield™ Genomic DNA Midi Kit (Frozen Blood / Cultured Cells)	25 preps/kit,(GB, Wash, W1, Elution) Buffer, Proteinase K, GDI Column.
		YGBM10	L	HiYield™ Genomic DNA Maxi Kit (Fresh Blood / Cultured Cells)	10 preps/kit,(RBC Lysis, GB, Wash, W1, Elution) Buffer, GBM Column.
		YGDM10	L	HiYield™ Genomic DNA Maxi Kit (Frozen Blood / Cultured Cells)	10 preps/kit,(GB, Wash, W1, Elution) Buffer, Proteinase K, GDM Column.
	Tissue	YGT50	S	HiYield™ Genomic DNA Mini Kit (Tissue)	50 preps/kit,(GT, GBT, Wash, W1, Elution) Buffer, Proteinase K, GT Column, 2 ml Collection Tube, Micropestle.
		YGT100	S	HiYield™ Genomic DNA Mini Kit (Tissue)	100 preps/kit,(GT, GBT, Wash, W1, Elution) Buffer, Proteinase K, GT Column, 2 ml Collection Tube, Micropestle.
		YGT300	S	HiYield™ Genomic DNA Mini Kit (Tissue)	300 preps/kit,(GT, GBT, Wash, W1, Elution) Buffer, Proteinase K, GT Column, 2 ml Collection Tube, Micropestle.
	Plant	YGP100	S	HiYield™ Genomic DNA Mini Kit (Plant)	100 preps/kit,(GP1, GPX1, GP2, GP3, Wash, W1, Elution) Buffer, RNase A, GP Column, 2 ml Collection Tube, Lysate Filter Column.
		YGPI25	M	HiYield™ Genomic DNA Midi Kit (Plant)	25 preps/kit,(GP1, GPX1, GP2, GP3, Wash, W1, Elution) Buffer, RNase A, GPI Column, Lysate Filter Column.
		YGPM10	L	HiYield™ Genomic DNA Maxi Kit (Plant)	10 preps/kit,(GP1, GPX1, GP2, GP3, Wash, W1, Elution) Buffer, RNase A, GPM Column, Lysate Filter Column.
	96-Well	YGB96B-2	-	HiYield™ 96-Well Genomic DNA Extraction Kit	2 preps/kit, (GB, GT, Wash, W1, Elution) Buffer, Proteinase K,Genomic DNA Binding Plate,350 µl Collection Plate...etc.
		YGB96B-4	-	HiYield™ 96-Well Genomic DNA Extraction Kit	4 preps/kit, (GB, GT, Wash, W1, Elution) Buffer, Proteinase K,Genomic DNA Binding Plate,350 µl Collection Plate...etc.
		YGB96B-10	-	HiYield™ 96-Well Genomic DNA Extraction Kit	10 preps/kit, (GB, GT, Wash, W1, Elution) Buffer, Proteinase K,Genomic DNA Binding Plate,350 µl Collection Plate...etc.
	Other	YVM96	-	Vacuum Manifold	Maximum Operating Vacuum: 28 in. Hg

Ordering Information

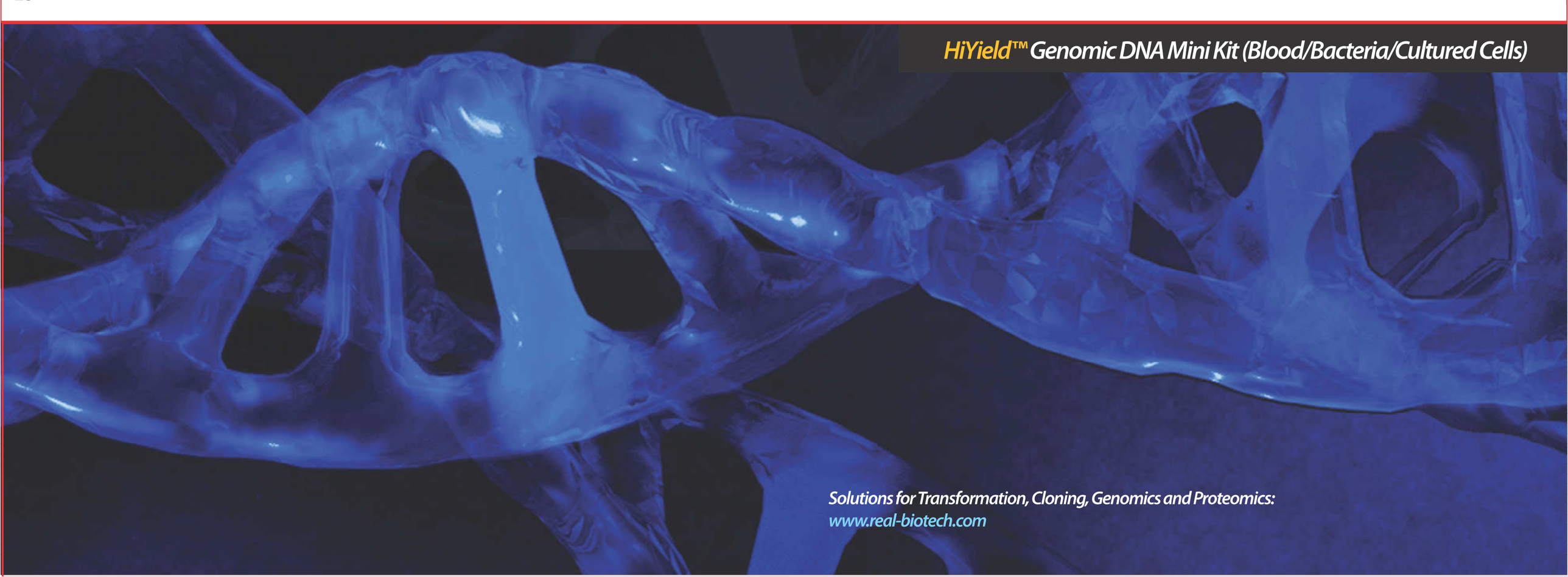
PCR Enzymes		
Standard PCR	RealTaq™ DNA Polymerase	RT001
	RealTaq™ DNA Polymerase(with $MgCl_2$ aside)	RT001C
	RealHi™ DNA Polymerase	RT003
	RealPfu™ DNA Polymerase	RT004
Highly Specific Hot-Start PCR	RealSens™ HotStart DNA Polymerase	RTH02
Ready-To-Use PCR Enzyme Mastermixes		
Standard PCR	RealTaq™ DNA Polymerase Mastermix	RT006
	RealTaq™ DNA Polymerase Mastermix (with loading dye)	RTD06
Highly Specific Hot-Start PCR	RealSens™ HotStart DNA Polymerase Mastermix	RT101
Ready-To-Use Real-Time PCR Enzyme Mastermixes		
Detection Using SYBR Green I	RealSens™ Real-Time PCR Mastermix(For SYBR Green System)	RT301
	RealSens™ Real-Time PCR Mastermix(For SYBR Green System W/ROX)	RT401
Detection Using Sequence-Specific Probes	RealSens™ Real-Time PCR Mastermix(For Probe System)	RT501
	RealSens™ Real-Time PCR Mastermix(For Probe System W/ROX)	RT601

Ordering Information

Ready-To-Use Real-Time PCR Enzyme Mastermixes (amplifying DNA template below 300 bp in a fast PCR mode)		
Detection Using SYBR Green I	PairFast™ Real-Time PCR Mastermix(For SYBR Green System W/ROX)	RT701
Detection Using Sequence-Specific Probes	PairFast™ Real-Time PCR Mastermix(For Probe System W/ROX)	RT801
PCR Reagent		
Deoxynucleotide	dNTP (10mM, 200 µl)	RT013
	dCTP (100mM, 100 µl)	RT014
	dATP (100mM, 100 µl)	RT015
	dGTP (100mM, 100 µl)	RT016
	dTTP (100mM, 100 µl)	RT017
Nucleic Acid Gel Electrophoresis		
Agarose Gel Preparation	RealPure™ Agarose (Low EEO)	RPA100
Nucleic Acid Detection in Agarose Gels	StaySafe™ Nucleic Acid Gel Stain	RSS01
DNA Size Identification	RealSharp™ 1 Kb DNA Marker	RD001
	RealSharp™ 100 bp DNA Marker	RD002
	RealSharp™ 50 bp DNA Marker	RD003

Notes

Notes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