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JetFlex™ Genomic DNA Purification Kit

USER GUIDE

For purification of genomic DNA from blood, mammalian cells, tissue, buffy coat, and all types of body fluids

Catalog Numbers A30700 and A30701

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A.0	25 April 2016	Updated to current branding guidelines and standards
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Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288

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Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The JetFlex™ Genomic DNA Purification Kit is designed for rapid and efficient purification of genomic DNA (gDNA) from blood, body fluids (amniotic fluid, saliva, sperm, lymph...), mammalian cells and tissues, plant tissues, bacteria, and yeast. Its single-tube technology allows for isolation of high quality, high molecular weight, RNA-free gDNA without the use of spin columns of a vacuum manifold.

Contents and storage

Table 1 JetFlex™ Genomic DNA Purification Kit (Cat. Nos. A30700 and A30701)

Contents	Cat. No. A30700 (100 preps)	Cat. No. A30701 (500 preps)	Storage
Red Blood Cell (RBC) Lysis Buffer	33 mL	165 mL	15 to 30°C
Cell Lysis Buffer (CLB)	33 mL	165 mL	
Protein Precipitation (PPT) Buffer	17 mL	83 mL	
Pellet Compactor	8 mL	30 mL	
TE Buffer	22 mL	110 mL	
Proteinase K (lyophilized powder)	2 × 21 mg	10 × 21 mg	2 to 8°C
RNase A (4 mg/ml)	1.1 mL	5 × 1.1 mL	2 to 8°C[1]

[1] For long-term storage, store in single-use aliquots at –25°C to –15°C.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Item	Source
Equipment	
Benchtop microcentrifuge	MLS
Centrifuge capable of reaching >12,000 × *g*	MLS
Laboratory mixer (Vortex or equivalent)	MLS
Water baths or heat blocks at 37°C, 55°C, and 58°C	MLS
Mortar and pestle (for tissue samples only)	MLS
(Optional) Tube pestle (for tissue samples only)	MLS
Tubes, plates, and accessories	
Sterile, DNase-free microcentrifuge tubes, 1.5 mL	MLS
Sterile, DNase-free tubes, 50 mL	MLS
Sterile, DNase-free microcentrifuge tubes, 2 mL (for swab samples only)	MLS
Automatic pipetors	MLS
Aerosol-resistant pipette tips	MLS
Reagents	
Ethanol, 70%	MLS
Isopropanol	MLS
Phosphate Buffered Saline (PBS), 1X (for mammalian cells only)	10010023
Liquid nitrogen (for tissue samples only)	MLS
Lysozyme, 2 mg/ml, in 50 mM Tris-HCl, pH 8.0, 10 mM EDTA buffer (for gram-positive bacteria only)	MLS
Lysozyme, 2 mg/ml, and Lysostaphin, 1 mg/ml, in 50 mM Tris-HCl, pH 8.0, 10 mM EDTA buffer (for *staphylococcus* only)	MLS
Sorbitol (for yeast only)	MLS
EDTA, pH 7.5 (for yeast only)	MLS
ß-Mercaptoethanol (for yeast only)	MLS
Zymolase (for yeast only)	MLS

Methods

Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Use disposable, individually wrapped, sterile plasticware.
- Use only sterile, new pipette tips and microcentrifuge tubes.
- Maintain a sterile environment when handling DNA to avoid any contamination from DNases.
- Incubate samples overnight with TE Buffer.
- To minimize DNA degradation, perfom lysate preparation steps quickly and avoid repeated freezing and thawing of the samples.
- Wear a laboratory coat, disposable gloves, and eye protection when handling blood samples.

Before you begin

- Resuspend Proteinase K in double-distilled water to a final concentration of 20 mg/mL. Store in single-use aliquots at –20°C.
- **For yeast samples only:** Prepare Buffer YS1 (0.9 M sorbitol; 0.1 M EDTA, pH 7.5; 14 mM β-mercaptoethanol).
- **For yeast samples only:** Dilute Zymolase in distilled water to a final concentration of 1000 U/mL. Store in single-use aliquots at –20°C.

Isolate gDNA from blood samples

IMPORTANT! We recommend using fresh blood whenever possible. Using frozen blood results in decreased DNA yield.

Lyse the blood samples

1. Add fresh or frozen blood or buffy coat to an equal volume of RBC Lysis Buffer.
2. Homogenize the mixture by inverting the tube.
3. *(Optional)* If the blood sample was drawn within an hour, incubate for 1 minute to ensure complete lysis of the erythrocytes.

4. Centrifuge according to the following table.

Starting sample	Centrifugation conditions
300 µL	30 seconds at 12,000 × *g*
⩾ 2 mL	10 minutes at 2000 × *g*

5. Remove the red supernatant.
   The pellet includes the DNA-containing white blood cells.

6. Vortex by pulses the pellet to loosen the DNA-containing white blood cells from the wall of the tube.

7. Add the volume of CLB as the starting sample volume to the disrupted pellet.

8. Pipet up and down to lyse the cells.
   If cell clumps are visble, incubate at room temperature or at 37°C until the lysate is homogenous and clear.
   The samples are stable in CLB for several months at room temperature,

9. *(Optional)* Add 20 µL of Proteinase K for every 300 µL of starting sample volume, then incubate at 58°C for 1 hour to overnight until the lysis is complete and the mixture is clear.

   Note: The Proteinae K digestion is necessary if the blood sample is partially or completely blotted.

10. *(Optional)* Add 1.5 µL of RNase A for every 300 µL of starting sample volume, then incubate at 37°C for 5 minutes.

11. Allow the lysate to cool down to room temperature, then distribute 300 µL of lysate in clean, sterile microcentrifuge tubes.

Precipitate the gDNA

1. Add 150 µL of PPT to 300 µL of sample lysate.

2. Homogenize the suspension by vortexing for 20 seconds.

3. Centrifuge for 3 minutes at 12,000 × *g*.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or difficult to see, proceed to Appendix C, “Compact the protein precipitation pellets“.

4. Transfer the supernatant to a clean, sterile microcentrifuge tube.

5. Add an equal volume of isopropanol.

6. Homogenize the mixture by inverting the tube.
   Do not vortex.

7. Centrifuge for 3 minutes at 12,000 × *g*.

The precipitated DNA is visible as a white pellet.

Wash the gDNA

1. Remove the supernatant.
2. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.
3. Add 1 mL of 70% ethanol.
4. Invert the tube several times to wash the DNA pellet.
5. Centrifuge for 1 minute at 12,000 × *g*.
6. Remove carefully the supernatant.

   Note: Be careful not to discard the DNA pellet as it may be loosely adherent to the tube.
7. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.

Resuspend the gDNA

1. Incubate the sample at 50–55°C for 10 minutes to allow the residual ethanol to evaporate.

   Note: You may incubate the sample at room temperature if you increase the incubation time.
2. Resuspend the DNA pellet in a suitable volume of TE Buffer.
3. Incubate the sample at room temperature overnight or at 65°C for up to an hour to completely resuspend the DNA.

The DNA should be completely dissolved as a clear, colorless solution.

If particles are present, the A_{260}/A_{280} ratio is <1.7, or the DNA is contaminated with buffer, protein, or RNA, repurify the DNA sample (see Appendix D, "Purify impure gDNA").

Store the purified DNA:

- at 4°C for immediate use.
- at –20°C in aliquots for longer-term storage.

Isolate gDNA from mammalian cells

Lyse the cells

1. Collect 1–2 × 10^6 to 1–2 × 10^7 cells in PBS.
2. Centrifuge according to the following table.

Starting cell count	Centrifugation conditions
1–2 × 10^6	15 seconds at 12,000 × *g*
1–2 × 10^7	2 minutes at 2000 × *g*

3. Remove the supernatant.

4. Add CLB according to the following table.

Starting cell count	CLB
1–2 × 10^6	300 µL
1–2 × 10^7	3 mL

5. Pipet up and down to lyse the cells.

   If cell clumps are visble, incubate at 58°C until the lysate is homogenous and clear.

   The samples are stable in CLB for several months at room temperature,

6. *(Optional)* Add Proteinase K according to the following table, then incubate at 58°C for 1 hour to overnight until the lysis is complete and the mixture is clear.

Starting cell count	Proteinase K
1–2 × 10^6	20 µL
1–2 × 10^7	200 µL

7. Add RNase A according to the following table, then incubate at 37°C for 5 minutes.

Starting cell count	RNase A
1–2 × 10^6	10 µL
1–2 × 10^7	100 µL

8. Allow the lysate to cool down to room temperature, then distribute 300 µL of lysate in clean, sterile microcentrifuge tubes.

Precipitate the gDNA

1. Add 150 µL of PPT to 300 µL of sample lysate.
2. Homogenize the suspension by vortexing for 20 seconds.
3. Centrifuge for 3 minutes at 12,000 × *g*.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or difficult to see, proceed to Appendix C, “Compact the protein precipitation pellets“.

4. Transfer the supernatant to a clean, sterile microcentrifuge tube.
5. Add an equal volume of isopropanol.
6. Homogenize the mixture by inverting the tube.

   Do not vortex.

7. Centrifuge for 3 minutes at 12,000 × *g*.

The precipitated DNA is visible as a white pellet.

Wash the gDNA

1. Remove the supernatant.
2. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.
3. Add 1 mL of 70% ethanol.
4. Invert the tube several times to wash the DNA pellet.
5. Centrifuge for 1 minute at 12,000 × *g*.
6. Remove carefully the supernatant.

   Note: Be careful not to discard the DNA pellet as it may be loosely adherent to the tube.
7. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.

Resuspend the gDNA

1. Incubate the sample at 50–55°C for 10 minutes to allow the residual ethanol to evaporate.

   Note: You may incubate the sample at room temperature if you increase the incubation time.
2. Resuspend the DNA pellet in a suitable volume of TE Buffer.
3. Incubate the sample at room temperature overnight or at 65°C for up to an hour to completely resuspend the DNA.

The DNA should be completely dissolved as a clear, colorless solution.

If particles are present, the A_{260}/A_{280} ratio is <1.7, or the DNA is contaminated with buffer, protein, or RNA, repurify the DNA sample (see Appendix D, "Purify impure gDNA").

Store the purified DNA:

- at 4°C for immediate use.
- at –20°C in aliquots for longer-term storage.

Isolate gDNA from tissues

Lyse the tissues

1. Grind up to 10 mg of fresh, forzen, or paraffin-embedded tissue sample in liquid nitrogen with a mortar and pestle.

   Keep the sample on ice to minimize DNase activity.
2. Transfer the tissue sample to clean, sterile microcentrifuge tube.
3. Add 300 µL of CLB to the tissue sample.
4. *(Optional)* Homogenize the sample thoroughly using 30–35 strokes with a tube pestle.

5. Add 20 µL of Proteinase K, then incubate at 58°C for 1 hour to overnight until the lysis is complete and the mixture is clear.
6. Add 10 µL of RNase A, then incubate at 37°C for 5 minutes.
7. Allow the lysate to cool down to room temperature, then distribute 300 µL of lysate in clean, sterile microcentrifuge tubes.

Precipitate the gDNA

1. Add 150 µL of PPT to 300 µL of sample lysate.
2. Homogenize the suspension by vortexing for 20 seconds.
3. Centrifuge for 3 minutes at 12,000 × *g*.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or difficult to see, proceed to Appendix C, "Compact the protein precipitation pellets".
4. Transfer the supernatant to a clean, sterile microcentrifuge tube.
5. Add an equal volume of isopropanol.
6. Homogenize the mixture by inverting the tube.

   Do not vortex.
7. Centrifuge for 3 minutes at 12,000 × *g*.

The precipitated DNA is visible as a white pellet.

Wash the gDNA

1. Remove the supernatant.
2. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.
3. Add 1 mL of 70% ethanol.
4. Invert the tube several times to wash the DNA pellet.
5. Centrifuge for 1 minute at 12,000 × *g*.
6. Remove carefully the supernatant.

   Note: Be careful not to discard the DNA pellet as it may be loosely adherent to the tube.
7. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.

Resuspend the gDNA

1. Incubate the sample at 50–55°C for 10 minutes to allow the residual ethanol to evaporate.

   Note: You may incubate the sample at room temperature if you increase the incubation time.

2. Resuspend the DNA pellet in a suitable volume of TE Buffer.

3. Incubate the sample at room temperature overnight or at 65°C for up to an hour to completely resuspend the DNA.

The DNA should be completely dissolved as a clear, colorless solution.

If particles are present, the A_{260}/A_{280} ratio is <1.7, or the DNA is contaminated with buffer, protein, or RNA, repurify the DNA sample (see Appendix D, "Purify impure gDNA").

Store the purified DNA:

- at 4°C for immediate use.
- at –20°C in aliquots for longer-term storage.

Isolate gDNA from body fluids

Concentrate the samples

Follow this procedure to concentrate the sample if it is diluted or has few DNA-containing cells. Otherwise, proceed directly to "Lyse the samples" on page 13.

1. Centrifuge the sample according to the following table.

Starting sample	Centrifugation conditions
⩽ 1.5 mL	30 seconds at 12,000 × *g*
⩾ 1.5 mL	2 minutes at 5000 × *g*

2. Discard the supernatant and resuspend the pellet in PBS according to the following table.

Starting sample	PBS
⩽ 1.5 mL	200 µL
⩾ 1.5 mL	3–10 mL

Lyse the samples

1. Add 5 volumes of CLB to 1 volume of body fluid.
   For example, add 250 µL of CLB to 50 µL of body fluid.
   Reagents are included in the kit for 100 or 500 50–µL preparations.

2. Pipet up and down to lyse the cells.

3. Incubate at 58°C until the lysis is complete and the mixture is clear.

4. *(Optional)* Add 1 µL of Proteinase K for every 2.5 µL of starting sample volume, then incubate at 58°C for 1 hour to overnight until the lysis is complete and the mixture is clear.

   Note: If cellular debris or particles are still visible after lysis, centrifuge the sample and transfer the clear supernatant to a clean, sterile microcentrifuge tube.

5. *(Optional)* Add 1 µL of RNase A for every 5 µL of starting sample volume, then incubate at 37°C for 5 minutes.
6. Allow the lysate to cool down to room temperature, then distribute 300 µL of lysate in clean, sterile microcentrifuge tubes.

Precipitate the gDNA

1. Add 150 µL of PPT to 300 µL of sample lysate.
2. Homogenize the suspension by vortexing for 20 seconds.
3. Centrifuge for 3 minutes at 12,000 × *g*.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or difficult to see, proceed to Appendix C, "Compact the protein precipitation pellets".
4. Transfer the supernatant to a clean, sterile microcentrifuge tube.
5. Add an equal volume of isopropanol.
6. Homogenize the mixture by inverting the tube.

   Do not vortex.
7. Centrifuge for 3 minutes at 12,000 × *g*.

The precipitated DNA is visible as a white pellet.

Wash the gDNA

1. Remove the supernatant.
2. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.
3. Add 1 mL of 70% ethanol.
4. Invert the tube several times to wash the DNA pellet.
5. Centrifuge for 1 minute at 12,000 × *g*.
6. Remove carefully the supernatant.

   Note: Be careful not to discard the DNA pellet as it may be loosely adherent to the tube.
7. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.

Resuspend the gDNA

1. Incubate the sample at 50–55°C for 10 minutes to allow the residual ethanol to evaporate.

   Note: You may incubate the sample at room temperature if you increase the incubation time.

2. Resuspend the DNA pellet in a suitable volume of TE Buffer.
3. Incubate the sample at room temperature overnight or at 65°C for up to an hour to completely resuspend the DNA.

The DNA should be completely dissolved as a clear, colorless solution.

If particles are present, the A_{260}/A_{280} ratio is <1.7, or the DNA is contaminated with buffer, protein, or RNA, repurify the DNA sample (see Appendix D, "Purify impure gDNA").

Store the purified DNA:

- at 4°C for immediate use.
- at –20°C in aliquots for longer-term storage.

Isolate gDNA from swabs

Lyse the samples

1. Collect the swab (buccal, nasal, pharyngeal, or vaginal) using T-swab Kit, Dacron swab, C.E.P. Omni swab, or Cotton swab according to standard collection procedures.
2. Place the swab in a capped 2-mL microcentrifuge tube.
   - **For C.E.P. Omni swabs:** Press the stem end towards the swab to eject it into the tube.
   - **For other swabs:** snap or cut the swab at the break point.

   Make sure that the swab fit entirely inside the tube and the cap can close.
3. Distribute 300 µL of CLB in a 1.5-mL microcentrifuge tube.
4. Dip the swab in the CLB, rotate the swab 10–20 times to release as many cells as possible, then squeeze the swab against the wall of the tube.
5. Discard the swab.
6. Incubate the mixture of CLB and cells at 58°C until the lysis is complete and the mixture is clear.
7. *(Optional)* Add 10 µL of Proteinase K, then incubate at 58°C for 30 minutes to overnight until the lysis is complete and the mixture is clear.
8. *(Optional)* Add 10 µL of RNase A, then incubate at 37°C for 5 minutes.
9. Allow the lysate to cool down to room temperature, then distribute 300 µL of lysate in clean, sterile microcentrifuge tubes.

Precipitate the gDNA

1. Add 150 µL of PPT to 300 µL of sample lysate.
2. Homogenize the suspension by vortexing for 20 seconds.

3. Centrifuge for 3 minutes at 12,000 × *g*.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or difficult to see, proceed to Appendix C, "Compact the protein precipitation pellets".

4. Transfer the supernatant to a clean, sterile microcentrifuge tube.

5. Add an equal volume of isopropanol.

6. Homogenize the mixture by inverting the tube.

   Do not vortex.

7. Centrifuge for 3 minutes at 12,000 × *g*.

The precipitated DNA is visible as a white pellet.

Wash the gDNA

1. Remove the supernatant.

2. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.

3. Add 1 mL of 70% ethanol.

4. Invert the tube several times to wash the DNA pellet.

5. Centrifuge for 1 minute at 12,000 × *g*.

6. Remove carefully the supernatant.

   Note: Be careful not to discard the DNA pellet as it may be loosely adherent to the tube.

7. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.

Resuspend the gDNA

1. Incubate the sample at 50–55°C for 10 minutes to allow the residual ethanol to evaporate.

   Note: You may incubate the sample at room temperature if you increase the incubation time.

2. Resuspend the DNA pellet in a suitable volume of TE Buffer.

3. Incubate the sample at room temperature overnight or at 65°C for up to an hour to completely resuspend the DNA.

The DNA should be completely dissolved as a clear, colorless solution.

If particles are present, the A_{260}/A_{280} ratio is <1.7, or the DNA is contaminated with buffer, protein, or RNA, repurify the DNA sample (see Appendix D, "Purify impure gDNA").

Store the purified DNA:

- at 4°C for immediate use.
- at –20°C in aliquots for longer-term storage.

Isolate gDNA from bacteria

Lyse the bacteria

1. Collect 500 µL of overnight bacterial culture in a 1.5-mL microcentrifuge tube.
2. Centrifuge for 1 minute at 12,000 × *g*.
3. Discard the supernatant.
4. **For gram-positive bacteria:** disrupt cell walls with enzymatic pretreatment.
   a. Resuspend the cells in 300 µL of 50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 2 mg/mL lysozyme.

      Note: For Staphylococcus, add 1 mg/mL lysostaphin to the 50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 2 mg/mL lysozyme buffer.

   b. Mix thoroughly, then incubate for 30 minutes at 37°C.
   c. Centrifuge for 1 minute at 12,000 × *g*.
   d. Discard the supernatant.

   For gram-negative bacteria: proceed directly to next step.
5. Add 300 µL of CLB to the bacterial pellet.
6. Pipet up and down to lyse the cells.
   If cell clumps are visble, incubate at 58°C until the lysate is homogenous and clear.
7. *(Optional)* Add 10 µL of Proteinase K, then incubate at 58°C for 1 hour to overnight until the lysis is complete and the mixture is clear.
8. Add 10 µL of RNase A, then incubate at 37°C for 5 minutes.
9. Allow the lysate to cool down to room temperature, then distribute 300 µL of lysate in clean, sterile microcentrifuge tubes.

Precipitate the gDNA

1. Add 150 µL of PPT to 300 µL of sample lysate.
2. Homogenize the suspension by vortexing for 20 seconds.
3. Centrifuge for 3 minutes at 12,000 × *g*.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or difficult to see, proceed to Appendix C, "Compact the protein precipitation pellets".
4. Transfer the supernatant to a clean, sterile microcentrifuge tube.

5. Add an equal volume of isopropanol.
6. Homogenize the mixture by inverting the tube.
   Do not vortex.
7. Centrifuge for 3 minutes at 12,000 × *g*.

The precipitated DNA is visible as a white pellet.

Wash the gDNA

1. Remove the supernatant.
2. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.
3. Add 1 mL of 70% ethanol.
4. Invert the tube several times to wash the DNA pellet.
5. Centrifuge for 1 minute at 12,000 × *g*.
6. Remove carefully the supernatant.

   Note: Be careful not to discard the DNA pellet as it may be loosely adherent to the tube.
7. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.

Resuspend the gDNA

1. Incubate the sample at 50–55°C for 10 minutes to allow the residual ethanol to evaporate.

   Note: You may incubate the sample at room temperature if you increase the incubation time.
2. Resuspend the DNA pellet in a suitable volume of TE Buffer.
3. Incubate the sample at room temperature overnight or at 65°C for up to an hour to completely resuspend the DNA.

The DNA should be completely dissolved as a clear, colorless solution.

If particles are present, the A_{260}/A_{280} ratio is <1.7, or the DNA is contaminated with buffer, protein, or RNA, repurify the DNA sample (see Appendix D, "Purify impure gDNA").

Store the purified DNA:

- at 4°C for immediate use.
- at –20°C in aliquots for longer-term storage.

Isolate gDNA from yeast

Lyse the yeast cells

1. Grow the yeast culture to saturation in YPD or YEPD to a final concentration of 1–2 × 10^8 cells/mL.
2. Centrifuge 1 mL of the yeast culture for 1 minute at 12,000 × *g*.
3. Discard the supernatant.
4. Add 300 µL of Buffer YS1 to the pellet.
5. Centrifuge for 1 minute at 12,000 × *g*.
6. Discard the supernatant.
7. Resuspend the pellet in 1 mL of Buffer YS1.
8. Add 10 µL of Zymolase (1000 U/mL), then incubate for 30 minutes at 37°C.

   Note: Monitor the formation of spheroplasts by examining detergent sensitivity: dilute a small sample of cells in 1% SDS. Spheroplasting is sufficient when greater than 90% of the cells burst when examined under a microscope.
9. Centrifuge for 1 minute at 12,000 × *g*.
10. Discard the supernatant.
11. Add 300 µL of CLB to the yeast pellet.
12. Pipet up and down to lyse the cells.

    If cell clumps are visble, incubate at 58°C until the lysate is homogenous and clear.
13. Add 10 µL of RNase A, then incubate at 37°C for 5 minutes.
14. Allow the lysate to cool down to room temperature, then distribute 300 µL of lysate in clean, sterile microcentrifuge tubes.

Precipitate the gDNA

1. Add 150 µL of PPT to 300 µL of sample lysate.
2. Homogenize the suspension by vortexing for 20 seconds.
3. Centrifuge for 3 minutes at 12,000 × *g*.

   Note: The precipitated proteins form a compact pellet and the supernatant should be clear. If the protein pellet is loose or difficult to see, proceed to Appendix C, "Compact the protein precipitation pellets".
4. Transfer the supernatant to a clean, sterile microcentrifuge tube.
5. Add an equal volume of isopropanol.

6. Homogenize the mixture by inverting the tube.
   Do not vortex.
7. Centrifuge for 3 minutes at 12,000 × *g*.

The precipitated DNA is visible as a white pellet.

Wash the gDNA

1. Remove the supernatant.
2. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.
3. Add 1 mL of 70% ethanol.
4. Invert the tube several times to wash the DNA pellet.
5. Centrifuge for 1 minute at 12,000 × *g*.
6. Remove carefully the supernatant.

   Note: Be careful not to discard the DNA pellet as it may be loosely adherent to the tube.
7. Turn the tube over a sheet of absorbent paper towel for a few minutes to allow the residual liquid to drain.

Resuspend the gDNA

1. Incubate the sample at 50–55°C for 10 minutes to allow the residual ethanol to evaporate.

   Note: You may incubate the sample at room temperature if you increase the incubation time.
2. Resuspend the DNA pellet in a suitable volume of TE Buffer.
3. Incubate the sample at room temperature overnight or at 65°C for up to an hour to completely resuspend the DNA.

The DNA should be completely dissolved as a clear, colorless solution.

If particles are present, the A_{260}/A_{280} ratio is <1.7, or the DNA is contaminated with buffer, protein, or RNA, repurify the DNA sample (see Appendix D, "Purify impure gDNA").

Store the purified DNA:

- at 4°C for immediate use.
- at –20°C in aliquots for longer-term storage.

A Troubleshooting

Observation	Possible cause	Recommended action
The DNA yield is low	DNA is sheared or degraded.	Avoid repeated freezing and thawing of samples.
		Maintain a sterile environment to avoid DNases contamination.
The eluate is dark in color	Pigments from tissues or heme from blood co-precipitate with DNA.	Use RBC Buffer at room temperature. Chilled RBC Buffer may prevent lysis.
		Repurify the DNA as described in Appendix D, "Purify impure gDNA".
The blood sample is coagulated or contains clots	Frozen blood samples were thawed too slowly.	Mince the sample with a pestle.
		Thaw frozen blood samples quickly at 37°C to minimize clotting, white blood cell lysis, and DNase activity.
		Purify DNA from the unclotted portion of the sample.
The DNA is impure $A_{260}/A_{280} < 1.7$ or insoluble particles are present in resuspended DNA.	There is too much starting material.	Repurify the DNA as described in Appendix D, "Purify impure gDNA".

B

Estimate DNA yield

Estimate DNA yield using UV absorbance

Alternatively, Quant-iT™ DNA Assay Kit, High Sensitivity (Cat. No. Q33120) or Quant-iT™ DNA Assay Kit, Broad-Range (Cat. No. Q33130) can be used for DNA quantitation.

1. Dilute the DNA solution in 10 mM Tris-HCl, pH 7.5 and mix well.

2. Measure the absorbance of the dilution at 260 nm (A_{260}) in a spectophotometer (using a cuvette with an optical path length of 1 cm) blanked against 10 mM Tris-HCl, pH 7.5.

3. Calculate the DNA concentration as follow:

   DNA (µg/mL) = A_{260} × 50 × dilution factor

   For DNA< A_{260} = 1 for a 50 µg/mL solution measured in a cuvette with an optical path length of 1 cm.

Compact the protein precipitation pellets

Compact the protein precipitation pellet

Compact the protein precipitate using one of both of the following methods.

- Vortex the protein mix, chill the mixture on ice for 5 minutes, centrifuge for 3 minutes at 12,000 × *g*, then resume the purification procedure.
- Add 50 µL of Pellet Compactor to 450 µL of protein precipitation mix, vortex until mixture is homogenous, centrifuge for 3 minutes at 12,000 × *g*, then resume the purification procedure.

Purify impure gDNA

Repurify the gDNA

1. Add 500 µL of Cell Lysis Buffer to 100 µL purified DNA sample.
2. Mix thoroughly by inverting the tube or by pipetting up and down.

   Note: If DNA samples contains particles, incubate at 37°C until the particles are dissolved. Cool the sample down to room temperature before proceeding.
3. Add 300 µL of Protein Precipitation Buffer.
4. Homogenize by vortexing vigorously for 20 seconds.
5. Centrifuge for 3 minutes at 12,000 × *g* to pellet any precipitated impurities.

   Note: If a pellet is not visible, proceed to Appendix C, "Compact the protein precipitation pellets".

Resume the purification procedure.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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