

# gSYNC Mini Kit

For research use only

<b>Sample</b>	: 50-200 µl whole blood, up to 200 µl fresh blood, cultured animal cells (up to $1 \times 10^7$ ), cultured bacterial cells (up to $1 \times 10^9$ ) fungus cells (up to $5 \times 10^7$ ), up to 25 mg tissue
<b>Operation</b>	: centrifuge
<b>Format</b>	: spin column
<b>Yield</b>	: up to 50 µg
<b>Operation time</b>	: < 30 minutes
<b>Elution volume</b>	: 50-200 µl

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## Introduction

The gSYNC Mini Kit provides an efficient method for purifying total DNA (including genomic, mitochondrial and viral DNA) from whole blood (fresh/frozen blood), cultured animal/bacterial cells, fungus and tissue. Chaotropic salt is used to lyse cells and degrade protein, allowing DNA to bind to the glass fiber matrix of the spin column (1). Contaminants are removed using a Wash Buffer (containing EtOH) and the purified genomic DNA is eluted by a low salt Elution Buffer or TE. The entire procedure can be completed in less than 30 minutes without phenol/chloroform extraction or alcohol precipitation, with an average DNA yield of 6 µg from 200 µl of whole human blood. Purified DNA, with approximately 20-30 Kb, is suitable for use in PCR or other enzymatic reactions.

## Quality Control

The quality of the gSYNC Mini Kit is tested on a lot-to-lot basis by isolating genomic DNA from 200 µl of whole human blood. The purified DNA (4-6 µg with an A260/A280 ratio of 1.6 - 1.8) is quantified with a spectrophotometer and checked by electrophoresis.

## Kit Contents

Name	GS004	GS100	GS300
RBC Lysis Buffer	6 ml	135 ml	135 ml x 2
Proteinase K* (Add ddH <sub>2</sub> O)	1 mg (0.1 ml)	11 mg x 2 (1.1 ml)	65 mg (6.5 ml)
GSB Buffer	4 ml	60 ml	155 ml
GST Buffer	3 ml	60 ml	155 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer** (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	30 ml	75 ml
GD Column	4 pcs	100 pcs	300 pcs
2 ml Collection Tube	8 pcs	200 pcs	600 pcs

## Order Information

Product Name	Package Size	Cat. No.
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Midi Kit (Blood/Cultured Cell)	25 preps	GDI25
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM10/25
gSYNC Mini Kit	100/300 preps	GS100/300
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM10/25
96-Well Genomic DNA Kit	2/4/10 x 96 Wells	GBP02/04/10
96-Well Genomic DNA Kit (Plant)	2/4/10 x 96 Wells	GPP02/04/10
Vacuum Manifold (Accessories)	1 set	ZFV04

\* Add ddH<sub>2</sub>O (see the bottle label for volume) to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4°C

\*\* Add absolute EtOH (see the bottle label for volume) to the Wash Buffer prior to initial use

## Caution

GSB Buffer contains guanidine hydrochloride which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

## References

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

## gSYNC Mini Kit (Whole Blood Protocol)

When the sample consists of nucleated blood cells, we recommend using the Cultured Cell Protocol to purify genomic DNA.

- Add ddH<sub>2</sub>O (see bottle label for volume) to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4°C
- Add absolute EtOH (see bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: microcentrifuge tubes, absolute EtOH, RNase A (50 mg/ml), phosphate-buffered saline (PBS)

Step 1 Cell Lysis	<p><b>Whole Blood</b></p> <ul style="list-style-type: none"> <li>● Collect whole blood in EDTA-NA<sub>2</sub> treated collection tubes (or other anticoagulant mixtures).</li> <li>● Transfer 50-100 µl of whole blood to a 1.5 ml microcentrifuge tube. Adjust volume to 200 µl with PBS.</li> <li>● Add <b>20 µl Proteinase K</b> to the sample and mix by pipetting.</li> <li>● Incubate the tube for 5 minutes at 55°C.</li> </ul> <ul style="list-style-type: none"> <li>● Add <b>200 µl of GSB Buffer</b> to the 1.5 ml microcentrifuge tube and mix by shaking vigorously.</li> <li>● Incubate at 55°C for 5 minutes. During incubation, invert the tube every 2 minutes (it is essential that the sample and GSB Buffer are mixed thoroughly to yield a homogeneous solution).</li> <li>● At this time, preheat the required <b>Elution Buffer</b> (100 µl per sample) in a 65°C water bath (for Step 4 DNA Elution).</li> </ul> <hr/> <p><b>Optional Step: RNA Degradation</b> (if RNA-free genomic DNA is required, perform this optional step)</p> <ul style="list-style-type: none"> <li>● Add 5 µl of RNase A (50 mg/ml) to the sample lysate and mix by vortex.</li> <li>● Incubate at room temperature for 3 minutes.</li> </ul>
Step 2 DNA Binding	<ul style="list-style-type: none"> <li>● Add 200 µl of absolute EtOH to the sample lysate and immediately mix by shaking vigorously for 10 seconds. If precipitate appears, break it up by pipetting (it is important that the sample and EtOH are mixed thoroughly to yield a homogeneous solution).</li> <li>● Place a <b>GD Column</b> in a <b>2 ml Collection Tube</b>.</li> <li>● Transfer all of the mixture (including any precipitate) to the <b>GD Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 1 minute (following centrifugation, if the mixture did not flow-through the GD Column membrane, increase the centrifuge time until it passes completely).</li> <li>● Discard the <b>2 ml Collection Tube</b> containing the flow-through and place the <b>GD Column</b> in a new <b>2 ml Collection Tube</b>.</li> </ul>
Step 3 Wash	<ul style="list-style-type: none"> <li>● Add <b>400 µl of W1 Buffer</b> to the <b>GD Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 30 seconds.</li> <li>● Discard the flow-through and place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li> <li>● Add <b>600 µl of Wash Buffer</b> (EtOH added) to the <b>GD Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 30 seconds.</li> <li>● Discard the flow-through and place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li> <li>● Centrifuge again for 2 minutes at 14-16,000 x g to dry the column matrix.</li> </ul>
Step 4 DNA Elution	<p>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 step to increase DNA recovery and the total elution volume to approximately 200 µl.</p> <ul style="list-style-type: none"> <li>● Transfer the dried <b>GD Column</b> to a clean 1.5 ml microcentrifuge tube.</li> <li>● Add <b>100 µl of preheated Elution Buffer</b> or TE to the center of the column matrix.</li> <li>● Let stand for at least 1 minute to ensure the <b>Elution Buffer</b> or TE is absorbed by the matrix.</li> <li>● Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.</li> </ul>

## gSYNC Mini Kit (Fresh Blood Protocol)

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

- Add absolute EtOH (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: microcentrifuge tubes, absolute EtOH, RNase A (50 mg/ml)

Step 1 RBC Lysis	<p><b>Fresh Blood</b></p> <ul style="list-style-type: none"> <li>● Collect fresh blood in EDTA-NA<sub>2</sub> treated collection tubes (or other anticoagulant mixtures).</li> <li>● Transfer up to 200 µl of fresh blood to a 1.5 ml microcentrifuge tube. If the blood sample is more than 200 µl, add the sample to a sterile 15 ml centrifuge tube.</li> <li>● Add 3X the sample volume of <b>RBC Lysis Buffer</b> and mix by inversion. <b>Do not vortex.</b></li> <li>● Incubate the tube for 10 minutes at room temperature.</li> <li>● Centrifuge for 5 minutes at 3,000 x g and remove the supernatant completely.</li> <li>● Add <b>100 µl of RBC Lysis Buffer</b> to resuspend the cell pellet.</li> </ul>
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<p>Step 2 Cell Lysis</p>	<ul style="list-style-type: none"> <li>● Add <b>200 µl of GSB Buffer</b> to the 1.5 ml microcentrifuge tube and mix by shaking vigorously.</li> <li>● Incubate at 65°C to 70°C for 10 minutes or until the sample lysate is clear. During incubation, invert the tube every 3 minutes.</li> <li>● At this time, preheat the required <b>Elution Buffer</b> (200 µl per sample) in a 70°C water bath (for Step 5 DNA Elution).</li> </ul> <hr/> <p><b>Optional Step: RNA Degradation</b> (if RNA-free genomic DNA is required, perform this optional step)</p> <ul style="list-style-type: none"> <li>● Add 5 µl of RNase A (50 mg/ml) to the sample lysate and mix by vortex.</li> <li>● Incubate at room temperature for 3 minutes.</li> </ul>
<p>Step 3 DNA Binding</p>	<ul style="list-style-type: none"> <li>● Add 200 µl of absolute EtOH to the sample lysate and immediately mix by shaking vigorously for 10 seconds. If precipitate appears, break it up by pipetting.</li> <li>● Place a <b>GD Column</b> in a <b>2 ml Collection Tube</b>.</li> <li>● Transfer all of the mixture (including any precipitate) to the <b>GD Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 2 minutes.</li> <li>● Discard the <b>2 ml Collection Tube</b> containing the flow-through and place the <b>GD Column</b> in a new <b>2 ml Collection Tube</b>.</li> </ul>
<p>Step 4 Wash</p>	<ul style="list-style-type: none"> <li>● Add <b>400 µl of W1 Buffer</b> to the <b>GD Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 30 seconds.</li> <li>● Discard the flow-through and place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li> <li>● Add <b>600 µl of Wash Buffer</b> (EtOH added) to the <b>GD Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 30 seconds.</li> <li>● Discard the flow-through and place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li> <li>● Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.</li> </ul>
<p>Step 5 DNA Elution</p>	<p>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 step to increase DNA recovery and the total elution volume to approximately 200 µl.</p> <ul style="list-style-type: none"> <li>● Transfer the dried <b>GD Column</b> to a clean 1.5 ml microcentrifuge tube.</li> <li>● Add <b>100 µl of preheated Elution Buffer</b> or TE to the center of the column matrix.</li> <li>● Let stand for at least 1 minute to ensure the <b>Elution Buffer</b> or TE is absorbed by the matrix.</li> <li>● Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.</li> </ul>

## gSYNC Mini Kit (Cultured Cell Protocol)

- Add absolute EtOH (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: microcentrifuge tubes, absolute EtOH, RNase A (50 mg/ml)

<p>Step 1 Sample Preparation</p>	<p><b>Cultured Animal Cells</b></p> <p>If using adherent cells, trypsinize the cells before harvesting.</p> <ul style="list-style-type: none"> <li>● Transfer cells (up to <math>1 \times 10^7</math>) to a 1.5 ml microcentrifuge tube and harvest with centrifugation for 20 seconds at 6,000 x g.</li> <li>● Discard the supernatant and resuspend the cells with <b>150 µl of RBC Lysis Buffer</b>.</li> </ul> <p><b>Fresh Blood (excluding human blood)</b></p> <p>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.</p> <ul style="list-style-type: none"> <li>● Add <b>150 µl of GST Buffer</b> to a 1.5 ml microcentrifuge tube along with the blood sample and mix by shaking vigorously.</li> </ul>
<p>Step 2 Lysis</p>	<ul style="list-style-type: none"> <li>● Add <b>200 µl of GSB Buffer</b> to the sample and mix by shaking vigorously for 5 seconds.</li> <li>● Incubate at 70°C for 10 minutes or until the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, incubate the required <b>Elution Buffer</b> (200 µl per sample) at 70°C (for Step 5 DNA Elution).</li> </ul> <hr/> <p><b>Optional Step: RNA Degradation</b> (if RNA-free genomic DNA is required, perform this optional step)</p> <ul style="list-style-type: none"> <li>● After 70°C incubation, add 5 µl of RNase A (50 mg/ml) to the sample lysate and mix by vortex.</li> <li>● Incubate at room temperature for 3 minutes.</li> </ul>
<p>Step 3 DNA Binding</p>	<ul style="list-style-type: none"> <li>● Add 200 µl of absolute EtOH to the sample lysate and immediately mix by shaking vigorously. If precipitate appears, break it up by pipetting.</li> <li>● Place a <b>GD Column</b> in a <b>2 ml Collection Tube</b>.</li> <li>● Transfer all of the mixture (including any precipitate) to the <b>GD Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 1 minute.</li> <li>● Discard the <b>2 ml Collection Tube</b> containing the flow-through and place the <b>GD Column</b> in a new <b>2 ml Collection Tube</b>.</li> </ul>

<p>Step 4 Wash</p>	<ul style="list-style-type: none"> <li>● Add <b>400 µl of W1 Buffer</b> to the <b>GD Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 30 seconds.</li> <li>● Discard the flow-through and place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li> <li>● Add <b>600 µl of Wash Buffer</b> (EtOH added) to the <b>GD Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 30 seconds.</li> <li>● Discard the flow-through and place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li> <li>● Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.</li> </ul>
<p>Step 5 DNA Elution</p>	<p>Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution Step to increase DNA recovery and the total elution volume to approximately 200 µl.</p> <ul style="list-style-type: none"> <li>● Transfer the dried <b>GD Column</b> to a clean 1.5 ml microcentrifuge tube.</li> <li>● Add <b>100 µl of preheated Elution Buffer</b> or TE to the center of the column matrix.</li> <li>● Let stand for at least 1 minute to ensure the <b>Elution Buffer</b> or TE is absorbed by the matrix.</li> <li>● Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.</li> </ul>

### gSYNC Mini Kit (Bacteria Protocol)

- Add absolute EtOH (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: microcentrifuge tube, absolute EtOH, for gram-positive bacteria: lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0, prepare fresh lysozyme buffer immediately prior to use)

<p>Step 1 Cell Harvesting/ Pre-lysis</p>	<p><b>Gram-Negative Bacteria</b></p> <ul style="list-style-type: none"> <li>● Transfer cultured bacterial cells (up to <math>1 \times 10^9</math>) to a 1.5 ml microcentrifuge tube.</li> <li>● Centrifuge for 1 minute at 14-16,000 x g and discard the supernatant.</li> <li>● Add <b>200 µl of GST Buffer</b> to the tube and re-suspend the cell pellet by shaking vigorously or pipetting.</li> <li>● Incubate at room temperature for 5 minutes.</li> <li>● Proceed with the Lysis Step of the Cultured Cell Protocol.</li> </ul> <p><b>Gram-Positive Bacteria</b></p> <ul style="list-style-type: none"> <li>● Transfer cultured bacterial cells (up to <math>1 \times 10^9</math>) to a 1.5 ml microcentrifuge tube.</li> <li>● Centrifuge for 1 minute at 14-16,000 x g and discard the supernatant.</li> <li>● Add 200 µl of lysozyme buffer to the tube and re-suspend the cell pellet by shaking vigorously or pipetting.</li> <li>● Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes.</li> <li>● Proceed with the Lysis Step of the Cultured Cell Protocol.</li> </ul>
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### gSYNC Mini Kit (Fungus Protocol)

- Add absolute EtOH (see the bottle label for volume) to Wash Buffer prior to initial use
- Additional requirements: lyticase or zymolase, sorbitol buffer (1.2 M sorbitol; 10 mM CaCl<sub>2</sub>; 0.1 M Tris-HCl pH 7.5; 35 mM mercaptoethanol)

<p>Step 1 Cell Harvesting/ Pre-Lysis</p>	<ul style="list-style-type: none"> <li>● Harvest fungus cells (up to <math>5 \times 10^7</math>) by centrifugation for 10 minutes at 5,000 x g.</li> <li>● Discard the supernatant and re-suspend the pellet in 600 µl of sorbitol buffer.</li> <li>● Add 200 U of lyticase or zymolase. Incubate at 30°C for 30 minutes.</li> <li>● Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast.</li> <li>● Remove the supernatant and add <b>200 µl of GST Buffer</b> to the tube and re-suspend the cell pellet by shaking vigorously or pipetting.</li> <li>● Incubate at room temperature for 5 minutes.</li> <li>● Proceed with the Lysis Step of the Cultured Cell Protocol.</li> </ul>
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## gSYNC Mini Kit (Tissue Protocol)

- Add ddH<sub>2</sub>O (see the bottle label for volume) to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4°C
- Add absolute EtOH (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: microcentrifuge tubes, absolute EtOH, RNase A (50 mg/ml), ddH<sub>2</sub>O

<p>Step 1 Tissue Dissociation and Lysis</p>	<ul style="list-style-type: none"> <li>● Cut up to 25 mg of animal tissue (or 0.5 cm of mouse tail) and transfer it to a 1.5 ml microcentrifuge tube. If the tissue has a higher number of cells (e.g. spleen or liver), reduce the starting material to 10 mg.</li> <li>● Add <b>200 µl of GST Buffer</b> to the tube.</li> <li>● Add <b>20 µl of Proteinase K</b> to the sample mixture and mix by vortex.</li> <li>● Incubate at 60°C overnight or until the sample lysate becomes clear.</li> </ul> <p>Note: Grinding the sample prior to incubation will increase yield.</p> <p>Note: Invert occasionally during incubation to disperse the sample or place in a thermomixer, shaking water bath, or on a rocking platform.</p> <ul style="list-style-type: none"> <li>● During this time, preheat the required <b>Elution Buffer</b> (200 µl per sample) in a 60°C water bath for Step 4 DNA Elution.</li> </ul> <hr/> <ul style="list-style-type: none"> <li>● Add <b>200 µl of GSB Buffer</b> and shaking vigorously for 10 seconds (it is essential that the sample and GSB Buffer are mixed thoroughly to yield a homogeneous solution).</li> </ul> <p>Note: If there is insoluble material present following incubation, centrifuge for 2 minutes at 14-16,000 x g and transfer the supernatant to a new 1.5 ml microcentrifuge tube.</p> <hr/> <p><b>Optional Step: RNA Degradation</b> (if RNA-free genomic DNA is required, perform this optional step)</p> <ul style="list-style-type: none"> <li>● Following GSB Buffer addition, add 5 µl of RNase A (50 mg/ml) to the sample lysate and mix by vortex.</li> <li>● Incubate at room temperature for 3 minutes.</li> </ul>
<p>Step 2 DNA Binding</p>	<ul style="list-style-type: none"> <li>● Add 200 µl of absolute EtOH to the sample lysate and vortex immediately for 10 seconds. If precipitate appears, break it up by pipetting.</li> <li>● Place a <b>GD Column</b> in a <b>2 ml Collection Tube</b>.</li> <li>● Transfer all of the mixture (including any precipitate) to the <b>GD Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 1 minute (following centrifugation, if the mixture did not flow through the GD Column membrane, increase the centrifuge time until it passes completely).</li> <li>● Discard the <b>2 ml Collection Tube</b> containing the flow-through and transfer the <b>GD Column</b> to a new <b>2 ml Collection Tube</b>.</li> </ul>
<p>Step 3 Wash</p>	<ul style="list-style-type: none"> <li>● Add <b>400 µl of W1 Buffer</b> to the <b>GD Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 30 seconds.</li> <li>● Discard the flow-through and place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li> <li>● Add <b>600 µl of Wash Buffer</b> (EtOH added) to the <b>GD Column</b>.</li> <li>● Centrifuge at 14-16,000 x g for 30 seconds.</li> <li>● Discard the flow-through and place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li> <li>● Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.</li> </ul>
<p>Step 4 DNA Elution</p>	<p>Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.</p> <ul style="list-style-type: none"> <li>● Transfer the dried <b>GD Column</b> to a clean 1.5 ml microcentrifuge tube.</li> <li>● Add <b>100 µl of preheated Elution Buffer</b> or TE Buffer to the center of the column matrix.</li> <li>● Let stand for at least 1 minute to ensure the <b>Elution Buffer</b> or TE is absorbed by the matrix.</li> <li>● Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.</li> </ul>

## gSYNC Mini Kit (Paraffin-Embedded Tissue Protocol)

- Additional Requirements: xylene, absolute EtOH, microcentrifuge tube

Step 1 Sample Preparation	<ul style="list-style-type: none"> <li>● Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube.</li> <li>● Add 1 ml of xylene to the tube. Vortex vigorously and incubate at room temperature for approximately 10 minutes. Vortex occasionally during incubation.</li> <li>● Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.</li> <li>● Add 1 ml of absolute EtOH to wash the sample pellet and mix by inverting.</li> <li>● Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.</li> <li>● Add 1 ml of absolute EtOH to wash the sample pellet again and mix by inverting.</li> <li>● Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.</li> <li>● Open the tube and Incubate at 37°C for 10-15 minutes to evaporate any EtOH residue.</li> <li>● Proceed with the Lysis Step (GSB Buffer addition) of the Tissue Protocol.</li> </ul>
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## Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	<p><b>Too much sample was used.</b></p> <ul style="list-style-type: none"> <li>● Reduce sample volume or separate into multiple tubes.</li> </ul>
Low Yield	<p><b>Precipitate was formed at DNA Binding Step</b></p> <ul style="list-style-type: none"> <li>● Reduce the sample material.</li> <li>● Prior to loading the column, break up precipitate in EtOH-added lysate.</li> </ul> <p><b>Incorrect DNA Elution Step</b></p> <ul style="list-style-type: none"> <li>● Ensure that the Elution Buffer or TE is added to the center of the GD Column matrix and is absorbed completely.</li> </ul>
Eluted DNA does not perform well in downstream applications	<p><b>Incomplete DNA Elution</b></p> <ul style="list-style-type: none"> <li>● Elute twice to increase yield.</li> </ul> <p><b>Residual EtOH contamination</b></p> <ul style="list-style-type: none"> <li>● Following the Wash Step, dry the GD Column with additional centrifugation at 14-16,000 x g for 5 minutes or incubate at 60°C for 5 minutes.</li> </ul> <p><b>Genomic DNA was degraded</b></p> <ul style="list-style-type: none"> <li>● Use fresh blood as long storage may result in fragmentation of genomic DNA.</li> </ul>