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

Cat. No.:	QBT100	QBT300
Product Name:	HiYield Plus™ Genomic DNA Mini Kit (Blood/Tissue/Cultured Cells)	
Reactions:	100	300
Sample:	Whole blood (fresh or frozen), serum, plasma, buffy coat, body fluids, cultured cells, tissue, formalin-fixed paraffin-embedded tissue (FFPE), rodent tails, ear punches, insects, hair and amniotic fluid.	
Yield:	Up to 6 µg of genomic DNA from 200µl of fresh human whole blood samples	
Elution Volume:	30-100 µl	
Format:	Spin Column	
Operation:	Centrifuge	
Operation Time:	Within 20 Minutes	

Description

HiYield Plus™ Genomic DNA Mini Kit (Blood/Tissue/Cultured Cells) provides a fast and economical method for purification of total DNA (including genomic, mitochondrial and viral DNA) from whole blood (fresh or frozen), serum, plasma, buffy coat, body fluids, cultured cells, tissue, formalin-fixed paraffin-embedded tissue (FFPE), insects, hair and amniotic fluid. The entire procedure can be completed in 20 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

1. Reliable purification of ready-to-use genomic DNA within 20 minutes.
2. Complete removal of all contaminants for sensitive downstream applications.
3. No phenol, chloroform or alcohol.

Applications

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

Quality Control

The quality of HiYield Plus™ Genomic DNA Mini Kit (Blood/Tissue/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 ug with A260/A280 ratio 1.8 - 2.0. The purified DNA is checked by electrophoresis.

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Contents

Item	QBT100	QBT300
QGT Buffer	30 ml	75 ml
QGB Buffer	40 ml	75 ml
W1 Buffer	45 ml	130 ml
Wash Buffer (concentrated)*	25 ml	50 ml
Proteinase K**	22 ml	65 ml
Elution Buffer	30 ml	75 ml
GD Column	100 pcs	300 pcs
2 ml Collection Tube	200 pcs	600 pcs

* Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

**Add ddH$_2$0 to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle showing ddH$_2$0 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH$_2$0 and Proteinase K mixture at 4ºC.

Storage

HiYield Plus™ Genomic DNA Mini Kit (Blood/Tissue/Cultured Cells) shall be shipped and stored dry at room temperature (15-25℃). With proper storage, Hi Yield Plus™ Genomic DNA Mini Kit (Blood/Tissue/Cultured Cells) can be stored for up to 12 months without showing any deduction in performance and quality.

Important Notes

Please read the entire notes prior to starting any of the protocol procedures.

Caution:

During operation, always wear a lab coat, disposable gloves and protective goggles.

Preparation of Reagents:

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.
2. Add ddH$_2$0 to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle showing ddH$_2$0 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH$_2$0 and Proteinase K mixture at 4ºC.

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Reagents to Be Supplied by User:

For blood, serum, plasma, cultured cells samples, prepare Phosphate Buffered Saline (PBS, pH7.2).

For FFPE tissue samples, prepare Phosphate Buffered Saline (PBS, pH7.2) and Xylene.

Optional (if RNA-free DNA is required): prepare RNase A (50 mg/ml).

Amounts of Starting Material and the Protocol to be Followed:

Sample	Amount	Protocol	Page
Whole Blood (fresh or frozen), Serum, Plasma, Buffy Coat, Body Fluid	200 µl	Blood	3
Nucleated Blood (e.g. bird or fish)*	10 µl	Blood	3
Cultured Cells	1x 10^7	Cultured Cells	5
Tissue (fresh) and Insects**	25 mg	Tissue	7
Tissue (formalin-fixed paraffin-embedded)	25 mg	FFPE	9
Hair	0.5-1 cm	Hair	12
Amniotic Fluid	15 ml	Amniotic Fluid	14

* When isolating DNA from nucleated blood, use up to 10 µl then adjust the volume to 200 µl with PBS.

**When isolating DNA from tissue with higher number of cells (e.g. spleen or liver), use 10 mg of samples.

Yield and Quality of DNA:

Better: Using fresh samples or flash-frozen samples stored in constant -20ºC or -70ºC freezers.

Worse: Using formalin-fixed paraffin-embedded tissue (FFPE) or samples repeatedly frozen and thawed.

Increased storage length decreases DNA yield.

RNA Removal Options:

For RNA-free DNA, perform optional steps right after cells lysis listed in the protocol.

Blood Protocol

Please read the entire important notes prior to starting.

Things to Do before Starting:

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
2. Add ddH_20 to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle label showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC.

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Reagents to Be Supplied by User:

Prepare Phosphate Buffered Saline (PBS, pH7.2).

Optional (if RNA-free DNA is required): prepare RNase A (50 mg/ml).

Step 1 Sample Preparation	1. Transfer up to 200 µl of whole blood, serum, plasma, buffy coat or body fluids to a 1.5 ml microcentrifuge tube. Adjust the sample volume to 200 µl with PBS. 2. Add 20 µl of Proteinase K then mix by pipetting. 3. Incubate at 60ºC for 5 minutes. NOTE: Fresh blood sample is recommended. However, frozen or blood treated with anticoagulants (EDTA etc.) can also be used. Increased storage length decreases DNA yield. For nucleated blood (e.g. bird or fish), use up to 10 µl. Then adjust the sample volume to 200 µl with PBS.
Step 2 Cell Lysis	1. Add 200 µl of QGB Buffer then mix by shaking vigorously. Make sure the sample and QGB Buffer are mixed thoroughly to yield a homogenous solution. 2. Incubate at 60ºC for 5 minutes, inverting the tube every 2 minutes. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Incubating with a shaking incubator is another convenient option. 3. During incubation, transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution).
Optional Step: RNA Degradation	If RNA-free genomic DNA is required, perform this optional step. 1. Following QGB Buffer addition and 60ºC incubation, add 5 µl of RNase A (50 mg/ml) and mix by shaking vigorously. 2. Incubate at room temperature for 5 minutes to ensure efficient RNA degradation.
Step 3 DNA Binding	1. Add 200 µl of absolute ethanol to the sample lysate and mix IMMEDIATELY by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. 2. Place a GD Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the GD Column. Centrifuge at 14,000-16,000 x g for 1 minute. 3. Following centrifugation, if the mixture did not flow through the GD Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GD Column to a new 2 ml Collection Tube. NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.

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Step 4 Wash	1. Add 400 µl of W1 Buffer to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 2. Add 600 µl of Wash Buffer (absolute ethanol added) to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 3. Centrifuge again for 3 minutes at 14,000-16,000 x g to dry the column matrix. NOTE: Additional centrifugation at 14,000-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.
Step 5 DNA Elution	1. Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. 2. Add 100 µl[1] of pre-heated Elution Buffer[2], TE Buffer[3] or water[4] into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. 3. Centrifuge at 14,000-16,000 x g for 30 seconds to elute the purified DNA. NOTE: [1] Standard elution volume is 100 µl. To increase DNA concentration, reduce the elution volume to 30-50 µl. To increase DNA recovery, repeat the DNA Elution step. The total elution volume is approximately 200 µl. [2] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GD Column matrix and is completely absorbed. [3] Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GD Column matrix and is completely absorbed. [4] If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the GD Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20ºC to avoid degradation.

Cultured Cells Protocol

Please read the entire important notes prior to starting.

Reagents to Be Supplied by User:

Prepare Phosphate Buffered Saline (PBS, pH7.2).

Optional (if RNA-free DNA is required): prepare RNase A (50 mg/ml).

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Things to Do before Starting:

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
2. Add ddH_20 to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle label showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC.

Step 1 Sample Preparation	1. Trypsinize adherent cells prior to harvesting. Transfer cells (up to 1 x 10^7) to a 1.5 ml microcentrifuge tube then centrifuge for 5 minutes at 300 x g. 2. Discard the supernatant then resuspend cells in 200 µl of PBS by pipette. 3. Add 20 µl of Proteinase K then mix by pipetting. 4. Incubate at 60ºC for 5 minutes.
Step 2 Cell Lysis	1. Add 200 µl of QGB Buffer then mix by shaking vigorously. Make sure the sample and QGB Buffer are mixed thoroughly to yield a homogenous solution. 2. Incubate at 60ºC for 5 minutes, inverting the tube every 2 minutes. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Incubating with a shaking incubator is another convenient option. 3. During incubation, transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution).
Optional Step: RNA Degradation	If RNA-free genomic DNA is required, perform this optional step. 1. Following QGB Buffer addition and 60ºC incubation, add 5 µl of RNase A (50 mg/ml) and mix by shaking vigorously. 2. Incubate at room temperature for 5 minutes to ensure efficient RNA degradation.
Step 3 DNA Binding	1. Add 200 µl of absolute ethanol to the sample lysate and mix IMMEDIATELY by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. 2. Place a GD Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the GD Column. Centrifuge at 14,000-16,000 x g for 1 minute. 3. Following centrifugation, if the mixture did not flow through the GD Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GD Column to a new 2 ml Collection Tube. NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.

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Step 4 Wash	1. Add 400 µl of W1 Buffer to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 2. Add 600 µl of Wash Buffer (absolute ethanol added) to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 3. Centrifuge again for 3 minutes at 14,000-16,000 x g to dry the column matrix. NOTE: Additional centrifugation at 14,000-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.
Step 5 DNA Elution	1. Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. 2. Add 100 µl[1] of pre-heated Elution Buffer[2], TE Buffer[3] or water[4] into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. 3. Centrifuge at 14,000-16,000 x g for 30 seconds to elute the purified DNA. NOTE: [1] Standard elution volume is 100 µl. To increase DNA concentration, reduce the elution volume to 30-50 µl. To increase DNA recovery, repeat the DNA Elution step. The total elution volume is approximately 200 µl. [2] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GD Column matrix and is completely absorbed. [3] Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GD Column matrix and is completely absorbed. [4] If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the GD Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20ºC to avoid degradation.

Tissue Protocol

Please read the entire important notes prior to starting.

Things to Do before Starting:

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.

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2. Add ddH_20 to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle label showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC.

Step 1 Sample Preparation	1. Transfer up to 25 mg of fresh animal tissue (0.5 cm mouse tail x 2 or 0.5 cm rat tail x 1) to a 1.5 ml microcentrifuge tube. When isolating DNA from tissue with higher number of cells (e.g. spleen or liver), reduce the starting material to 10 mg. Weighing tissue is the most accurate way to determine the amount. 2. Add 200 µl of QGT Buffer and 20 µl of Proteinase K then vortex thoroughly. 3. Incubate at 60ºC overnight or until the sample lysate becomes clear. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Incubating with a shaking incubator is another convenient option. If not available, vortexing 2–3 times per hour during incubation is recommended. 4. During incubation, transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution). NOTE: Tissue homogenization prior to incubation will facilitate Proteinase K digestion, cell lysis, and subsequently increasing DNA yield.
Step 2 Cell Lysis	1. If insoluble material remains following incubation, centrifuge at 14,000-16,000 x g for 2 minutes then carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube. 2. Add 200 µl of QGB Buffer then shake vigorously for 10 seconds. Make sure the sample and QGB Buffer are mixed thoroughly to yield a homogenous solution.
Optional Step: RNA Degradation	If RNA-free genomic DNA is required, perform this optional step. 1. Following QGB Buffer addition and 60ºC incubation, add 5 µl of RNase A (50 mg/ml) and mix by shaking vigorously. 2. Incubate at room temperature for 5 minutes to ensure efficient RNA degradation.
Step 3 DNA Binding	1. Add 200 µl of absolute ethanol to the sample lysate and mix IMMEDIATELY by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. 2. Place a GD Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the GD Column. Centrifuge at 14,000-16,000 x g for 1 minute. 3. Following centrifugation, if the mixture did not flow through the GD Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GD Column to a new 2 ml Collection Tube. NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.

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Step 4 Wash	1. Add 400 µl of W1 Buffer to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 2. Add 600 µl of Wash Buffer (absolute ethanol added) to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 3. Centrifuge again for 3 minutes at 14,000-16,000 x g to dry the column matrix. NOTE: Additional centrifugation at 14,000-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.
Step 5 DNA Elution	1. Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. 2. Add 100 µl[1] of pre-heated Elution Buffer[2], TE Buffer[3] or water[4] into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. 3. Centrifuge at 14,000-16,000 x g for 30 seconds to elute the purified DNA. NOTE: [1] Standard elution volume is 100 µl. To increase DNA concentration, reduce the elution volume to 30-50 µl. To increase DNA recovery, repeat the DNA Elution step. The total elution volume is approximately 200 µl. [2] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GD Column matrix and is completely absorbed. [3] Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GD Column matrix and is completely absorbed. [4] If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the GD Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20ºC to avoid degradation.

FFPE Protocol

Please read the entire important notes prior to starting.

Reagents to Be Supplied by User:

Prepare Phosphate Buffered Saline (PBS, pH7.2) and Xylene.

Optional (if RNA-free DNA is required): prepare RNase A (50 mg/ml).

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Things to Do before Starting:

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
2. Add ddH_20 to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle label showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC.

Step 1 Sample Preparation	1. Cut up to 25 mg sections of FFPE and transfer to a 1.5 ml microcentrifuge tube. Using a sterile blade is recommended. Weighing tissue is the most accurate way to determine the amount. 2. Add 1 ml of xylene then mix by shaking vigorously. 3. Incubate at room temperature for approximately 10 minutes (shake occasionally during incubation). 4. Centrifuge at 14,000-16,000xg for 3 minutes then remove the supernatant. 5. Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting. 6. Centrifuge at 14,000-16,000 x g for 3 minutes then remove the supernatant. 7. Open the tube and incubate at 37ºC for 15 minutes to evaporate any ethanol residue. 8. Add 200 µl of QGT Buffer and 20 µl of Proteinase K then vortex thoroughly. 9. Incubate at 60ºC overnight or until the sample lysate becomes clear. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Incubating with a shaking incubator is another convenient option. If not available, vortexing 2–3 times per hour during incubation is recommended. 10. During incubation, transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution).
Step 2 Cell Lysis	1. If insoluble material remains following incubation, centrifuge at 14,000-16,000 x g for 2 minutes then carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube. 2. Add 200 µl of QGB Buffer then shake vigorously for 10 seconds. Make sure the sample and QGB Buffer are mixed thoroughly to yield a homogenous solution.
Optional Step: RNA Degradation	If RNA-free genomic DNA is required, perform this optional step. 1. Following QGB Buffer addition and 60ºC incubation, add 5 µl of RNase A (50 mg/ml) and mix by shaking vigorously. 2. Incubate at room temperature for 5 minutes to ensure efficient RNA degradation.

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Step 3 DNA Binding	1. Add 200 µl of absolute ethanol to the sample lysate and mix IMMEDIATELY by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. Make sure that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution. 2. Place a GD Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the GD Column. Centrifuge at 14,000-16,000 x g for 1 minute. 3. If the mixture did not flow through the GD Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GD Column to a new 2 ml Collection Tube.
Step 4 Wash	1. Add 400 µl of W1 Buffer to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 2. Add 600 µl of Wash Buffer (absolute ethanol added) to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 3. Centrifuge again for 3 minutes at 14,000-16,000 x g to dry the column matrix. NOTE: Additional centrifugation at 14,000-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.
Step 5 DNA Elution	1. Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. 2. Add 100 µl[1] of pre-heated Elution Buffer[2], TE Buffer[3] or water[4] into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. 3. Centrifuge at 14,000-16,000 x g for 30 seconds to elute the purified DNA. NOTE: [1] Standard elution volume is 100 µl. To increase DNA concentration, reduce the elution volume to 30-50 µl. To increase DNA recovery, repeat the DNA Elution step. The total elution volume is approximately 200 µl. [2] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GD Column matrix and is completely absorbed. [3] Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. [4] If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. DNA Eluted in water should be stored at -20ºC to avoid degradation.

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Hair Protocol

Please read the entire important notes prior to starting.

Reagents to Be Supplied by User:

Optional (if RNA-free DNA is required): prepare RNase A (50 mg/ml).

Things to Do before Starting:

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
2. Add ddH_20 to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle label showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC.

Step	Procedure
Step 1 Sample Preparation	1. Cut off a 0.5-1 cm piece from at least 10 hair bulbs, including follicle cells and transfer to a 1.5 ml microcentrifuge tube. 2. Add 200 µl of QGT Buffer and 20 µl of Proteinase K then vortex thoroughly. Make sure the hair is completely submerged. 3. Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, shake the tube every 5 minutes. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Incubating with a shaking incubator if possible. 4. During incubation, transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution).
Step 2 Cell Lysis	1. Add 200 µl of QGB Buffer and mix vigorously. 2. Incubate at 60ºC for 20 minutes. During incubation, shake the tube every 5 minutes. Make sure the sample and QGB Buffer are mixed thoroughly to yield a homogenous solution. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Incubating with a shaking incubator is another convenient option.
	Optional Step: RNA Degradation If RNA-free genomic DNA is required, perform this optional step. 1. Following QGB Buffer addition and 60ºC incubation, add 5 µl of RNase A (50 mg/ml) and mix by shaking vigorously. 2. Incubate at room temperature for 5 minutes to ensure efficient RNA degradation.
	3. Following incubation, centrifuge for 5 minutes at 3,000 x g. During centrifugation, place a GD Column in a 2 ml Collection Tube. 4. Following centrifugation, transfer the supernatant to a new 1.5 ml microcentrifuge tube.

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Step	Procedure
Step 3 DNA Binding	1. Add 200 µl of absolute ethanol to the sample lysate and mix IMMEDIATELY by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. 2. Place a GD Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the GD Column. Centrifuge at 14,000-16,000 x g for 1 minute. 3. If the mixture did not flow through the GD Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GD Column to a new 2 ml Collection Tube. NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.
Step 4 Wash	1. Add 400 µl of W1 Buffer to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 2. Add 600 µl of Wash Buffer (absolute ethanol added) to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 3. Centrifuge again for 3 minutes at 14,000-16,000 x g to dry the column matrix. NOTE: Additional centrifugation at 14,000-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.
Step 5 DNA Elution	1. Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. 2. Add 100 µl[1] of pre-heated Elution Buffer[2], TE Buffer[3] or water[4] into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. 3. Centrifuge at 14,000-16,000 x g for 30 seconds to elute the purified DNA. NOTE: [1] Standard elution volume is 100 µl. To increase DNA concentration, reduce the elution volume to 30-50 µl. To increase DNA recovery, repeat the DNA Elution step. The total elution volume is approximately 200 µl. [2] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GD Column matrix and is completely absorbed. [3] Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is good for long term storage of DNA. However, EDTA will affect PCR and other sensitive downstream applications. [4] If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. DNA Eluted in water should be stored at -20ºC to avoid degradation.

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Amniotic Fluid Protocol

Please read the entire important notes prior to starting.

Reagents to Be Supplied by User:

Optional (if RNA-free DNA is required): prepare RNase A (50 mg/ml).

Things to Do before Starting:

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
2. Add ddH_20 to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle label showing ddH_20 is added. Once it is dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC.

Step 1 Sample Preparation	1. Transfer up to 15 ml of amniotic fluid to a 15 ml centrifuge tube. Centrifuge for 3 minutes at 14,000-16,000 x g then discard the supernatant. 2. Add 200 µl of QGT Buffer to resuspend the pellet and transfer the mixture to a 1.5 ml microcentrifuge tube. 3. Add 20 µl of Proteinase K to the sample mixture and shake vigorously. 4. Incubate at 60ºC for 30 minutes. During incubation, invert the tube every 5 minutes. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis.
Step 2 Cell Lysis	1. Add 200 µl of QGB Buffer then mix by shaking vigorously for 5 seconds. 2. Incubate at 60ºC for at least 20 minutes to ensure the lysate is clear. During incubation, shake the tube every 5 minutes. Make sure the sample and QGB Buffer are mixed thoroughly to yield a homogenous solution. Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Incubating with a shaking incubator is another convenient option. 3. During incubation, transfer the required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60ºC (for Step 5 DNA Elution).
Optional Step: RNA Degradation	If RNA-free genomic DNA is required, perform this optional step. 1. Following QGB Buffer addition and 60ºC incubation, add 5 µl of RNase A (50 mg/ml) and mix by shaking vigorously. 2. Incubate at room temperature for 5 minutes to ensure efficient RNA degradation.

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Step 3 DNA Binding	1. Add 200 µl of absolute ethanol to the sample lysate and mix IMMEDIATELY by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. Make sure that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution. 2. Place a GD Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the GD Column. Centrifuge at 14,000-16,000 x g for 1 minute. 3. If the mixture did not flow through the GD Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GD Column to a new 2 ml Collection Tube.
Step 4 Wash	1. Add 400 µl of W1 Buffer to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 2. Add 600 µl of Wash Buffer (absolute ethanol added) to the GD Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the GD Column back in the 2 ml Collection Tube. 3. Centrifuge again for 3 minutes at 14,000-16,000 x g to dry the column matrix. NOTE: Additional centrifugation at 14,000-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.
Step 5 DNA Elution	1. Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. 2. Add 100 µl[1] of pre-heated Elution Buffer[2], TE Buffer[3] or water[4] into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. 3. Centrifuge at 14,000-16,000 x g for 30 seconds to elute the purified DNA. NOTE: [1] Standard elution volume is 100 µl. To increase DNA concentration, reduce the elution volume to 30-50 µl. To increase DNA recovery, repeat the DNA Elution step. The total elution volume is approximately 200 µl. [2] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GD Column matrix and is completely absorbed. [3] Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is good for long term storage of DNA. However, EDTA will affect PCR and other sensitive downstream applications. [4] If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. DNA Eluted in water should be stored at -20ºC to avoid degradation.

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Troubleshooting

Problem	Possible Reasons / Solution
Low Yield	**Improper sample homogenization** Yield and quality of DNA is better while using fresh samples or flash-frozen samples stored in constant -20ºC or -70ºC freezers. Yield and quality of DNA is worse while using formalin-fixed paraffin-embedded tissue (FFPE) or samples repeatedly frozen and thawed. Increased storage length decreases DNA yield. Fresh blood sample is recommended. However, frozen or blood treated with anticoagulants (EDTA etc.) can also be used. Increased storage length decreases DNA yield. For nucleated blood (e.g. bird or fish), use up to 10 µl. Then adjust the sample volume to 200 µl with PBS. Make sure the hair is completely submerged in QGT Buffer and Proteinase K. **Incomplete buffer preparation** Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation. Add ddH_20 to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle showing ddH_20 is added. Once it's dissolved completely, centrifuge for a few seconds to spin down the mixture. For extended periods, store the ddH_20 and Proteinase K mixture at 4ºC. **Clogged Column** Use the recommended amount of starting material or separate into multiple tubes. Add additional Proteinase K and extend the incubation time in the Lysis Step. Following the Lysis Step, centrifuge for 2 minutes at 14,000-16,000 x g to remove sample debris. Transfer the supernatant to a new microcentrifuge tube and proceed with the DNA Binding Step. If precipitate formed at the DNA Binding Step, reduce the sample material. Following ethanol addition, break up any precipitate as much as possible prior to loading GD Column.
Eluted DNA Does Not Perform Well In Downstream Applications	**Residual Ethanol Contamination** Following the wash step, dry the GD Column with additional centrifugation at 14,000-16,000 x g for 5 minutes to ensure the GD Column membrane is completely dry.

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