T-Pro Genomic DNA Mini Kit

Store at RT

(RB94-NGS100) 100 preps

T-Pro Biotechnology

This product is for laboratory research ONLY and not for diagnostic use.

Description	T-Pro Genomic DNA Mini Kit provides a fast and economical method for purification of total DNA (including genomic, mitochondrial and viral DNA) from whole blood (fresh), plasma, serum, buffy coat, tissue, other body fluids, lymphocytes, bacteria and cultured cells. There is no requirement for phenol/chloroform extraction or alcohol precipitation. Purified DNA is suitable for PCR or other enzymatic reactions.
Properties	- Complete removal of all contaminants for reliable downstream applications. - No phenol, chloroform or alcohol. - Rapid and simple procedure.
Applications	Purified DNA are ready for direct use in PCR, Southern Blotting, Real-Time PCR, AFLP, RFLP, PADP.
Quality Control	The quality of T-Pro Genomic DNA Mini Kit are tested on a lot–to-lot basis. The kit is tested 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 4-6 µg with A_{260}/A_{280} ratio 1.7 to 1.9.
Storage	T-Pro Plasmid Mini Kit is stable for RT

T-Pro Genomic DNA Mini Kit	
	RB94-NGS100
NT Buffer	45 ml
NB Buffer	45 ml
W1 Buffer	45 ml
W2 Buffer *	25 ml
Elution Buffer	30 ml
Proteinase K **	2 x 11 mg
GS Columns	100 pcs
Collection Tube	100 pcs

Sample size: 200 µl Whole Blood, 10^7 Cultured Cells, 10^9 Bacteria (Gram +/-), 10^7 Yeast, 25mg Tissue

Yield: Up to 60 µg

Format: Spin Columns

Operation: Centrifuge or Vacuum

Operation Time:20-30 Minutes

*Add 100 ml ethanol(96-100%) to W2 Buffer prior to the initial use.

**Add 1.1 ml ddH_2O to a Proteinase K tube, vortex to dissolve. Store prepared Proteinase K at 4℃

T-Pro Genomic DNA Mini Kit

Blood Protocol

When the blood sample is less then 50µl or sample consists of nucleated blood cells, the Cultured Cell Protocol is recommended to purify genomic DNA.

1. Add 100 ml ethanol (96-100%) to W2 Buffer prior to the initial use.
2. When the sample is low-copy(<10,000 copies), addition 1µl of aqueous solution Carrier DNA (10µg/µl) to 300µl NB Buffer before use.
3. Additional requirements:
   Microcentrifuge tube
   Ethanol (96-100%)
   RNase A (50 mg/ml)
   Carrier DNA (e.g., poly dA, poly dA:dT), concentration: 10µg/µl.

RBC Lysis	1	Apply 20µl Proteinase K (10mg/ml) to a microcentrifuge tube. Then add in up to 200µl of blood, plasma, serum or body fluids and mix by vortexing.
	2	Stand at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes.
	3	Proceed to step 4 Cell Lysis below.
Cell Lysis	4	Add 300µl NB Buffer to the tube and mix by vortexing.
	5	Incubate the mixture at 60℃ for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, preheat required Elution Buffer (100µl per sample) in a 60 ℃ waterbath (for DNA Elution).
	Optional Step: RNA degradation If RNA-free genomic DNA is required, perform this optional step. a. After step 4, add 2µl of RNase A (50mg/ml, not provided) to sample lysate and mix by vortexing. b. Proceed to Step 5.	
DNA Binding	6	Add 300µl of ethanol (96-100%) to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
	7	Place a GS Column in the Collection Tube.
	8	Apply the total mixture (including any precipitate) from previous step to the GS Column.
	9	Close the cap and centrifuge at full speed (approx. 13,000 rpm) for 5 minutes.
Wash	10	Add 400 µl of W1 Buffer into the GS Column.
	11	Centrifuge at full speed (approx.13,000 rpm) for 1 minute.
	12	Discard the flow-through and place the GS Column back in the Collection Tube.
	13	Add 600µl of W2 Buffer (ethanol added) in the GS Column.
	14	Centrifuge at full speed (approx.13,000 rpm) for 1 minute.
	15	Discard the flow-through and place the GS Column back in the Collection Tube.
	16	Centrifuge at full speed (approx.13,000 rpm) for 3 minutes to dry the column matrix.
DNA Elution	17	Transfer dried GS Column into a clean 1.5 ml microcentrifuge tube (not provided).
	18	Add 100µl of preheated Elution Buffer (60℃) into the center of the column matrix. Standard elution volume is 100µl. If less sample volume is used, reduce the elution volume (30~50µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery.
	19	Stand at room temperature for 3 minutes until Elution Buffer absorbed by the matrix.
	20	Centrifuge at full speed (approx.13,000 rpm) for 2 minutes to elute purified DNA.

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Cultured Cells Protocol

1. Add 100 ml ethanol (96-100%) to W2 Buffer prior to the initial use.
2. Additional requirements:
   Microcentrifuge tube
   Ethano (96-100%)
   RNase A (50mg/ml)
   PBS buffer

Sample Preparation	A	Cultured animal cells: If using adherent cells, trypsinize the cells before harvesting.
	1	Transfer 10^6 -10^7 of cells to a microcentrifuge tube (not provided) and harvest the cells with centrifugation at 3,000 rpm for micorocentrifuge 3 minutes.
	2	Discard the supernatant and add 100µl PBS Buffer and 20µl Proteinase K (10 mg/ml) to resuspend the cell by vortex and incubate at room temperature for 10 minutes.
	B	Mammalian Blood: For mammalian blood (non-nucleated), the sample volume is up to 50µl. For nucleated erythrocytes (e.g. bird or fish), the sample volume is up to 10µl.
	1	Add 100µl NT Buffer and 20µl Proteinase K (10mg/ml) to a microcentrifuge tube and apply blood sample to the tube.
	2	Mix by vortexing and incubate at room temperature for 10 minutes.
Lysis	3	Add 300µl NB Buffer to the sample. Mix by vortexing for 5 seconds.
	4	Incubate at 60℃ waterbath for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, incubate required Elution Buffer (100µl per sample) at 60℃.
	Optional Step: RNA degradation If RNA-free genomic DNA is required, perform this optional step. a. After step 3, add 2µl of RNase A (50 mg/ml, not provided) to sample lysate and mix by vortexing. b. Proceed to Step 4	
DNA Binding	5	Add 300 µl of ethanol (96-100%) to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
	6	Place a GS Column in the Collection Tube.
	7	Apply the total mixture (including any precipitate) from previous step to the GS Column.
	8	Close the cap and centrifuge at full speed (approx. 13,000 rpm) for 1 minute.
Wash	9	Add 400 µl of W1 Buffer in the GS Column.
	10	Centrifuge at full speed (approx.13,000 rpm) for 1 minute.
	11	Discard the flow-through and place the GS Column back in the Collection Tube.
	12	Add 600 µl of W2 Buffer (ethanol added) to wash again.
	13	Centrifuge at full speed (approx.13,000 rpm) for 1 minute.
	14	Centrifuge at full speed (approx.13,000 rpm) for 3 minutes to dry the column matrix.
DNA Elution	15	Transfer dried GS Column into a clean 1.5 ml microcentrifuge tube (not provided).
	16	Add 100µl of preheated Elution Buffer (60℃) into the center of the column matrix. Standard elution volume is 100µl. If less sample volume is used, reduce the elution volume (30~50µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery.
	17	Stand at room temperature for 3 minutes until Elution Buffer absorbed by the matrix.
	18	Centrifuge at full speed (approx.13,000 rpm) for 2 minutes to elute purified DNA.

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Bacterial Protocol

1. Add 100 ml ethanol (96-100%) to W2 Buffer prior to the initial use.
2. Additional requirements:
   Lysozyme Buffer (20mg/ml lysozyme; 20 mM Tris-HCl, 2mM EDTA, 1% Triton X-100, pH 8.0) for Gram-Positive Bacteria Sample. Prepare the lysozyme buffer fresh immediately prior to use.
   Microcentrifuge tube
   Ethano (96-100%)
   RNase A (50mg/ml)

Cell Harvesting / Prelysis	A. Cell Harvesting for Gram-Negative Bacteria	
	1a	Transfer bacterial culture (<10^9) to a microcentrifuge tube (not provided).
	2a	Centrifuge at 6,000 rpm for 3 minutes in a microcentrifuge and discard the supernatant.
	3a	Add 200µl of NT Buffer and 20µl Proteinase K (10mg/ml) to the tube and resuspend the cell pellet by vortexing or pipetting.
	4a	Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes.
	5a	Proceed to Step 6 Cell Lysis Below.
	B. Cell Harvesting for Gram-Positive Bacteria	
	1b	Prepare the lysozyme buffer fresh immediately prior to use. Transfer bacterial culture (<10^9) to a microcentrifuge tube (not provided).
	2b	Centrifuge at 6,000 rpm for 3 minutes and discard the supernatant.
	3b	Add 200µl of Lysozyme Buffer to the tube and resuspend the cell pellet by vortexing or pipetting.
	4b	Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes.
	5b	Proceed to Step 6 Cell Lysis Below.
Lysis	6	Add 300µl NB Buffer to the sample. Mix by vortexing for 5 seconds.
	7	Incubate at 60℃ waterbath for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, incubate required Elution Buffer (100 µl per sample) at 60℃.
	Optional Step: RNA degradation If RNA-free genomic DNA is required, perform this optional step. a. After step 6, add 2µl of RNase A (50mg/ml, not provided) to sample lysate and mix by vortexing. b. Proceed to Step 7.	
DNA Binding	8	Add 300µl of ethanol (96-100%) to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
	9	Place a GS Column in the Collection Tube.
	10	Apply the total mixture (including any precipitate) from previous step to the GS Column.
	11	Close the cap and centrifuge at full speed (approx. 13,000 rpm) for 1 minutes.
Wash	12	Add 400µl of W1 Buffer into the GS Column.
	13	Centrifuge at full speed (approx.13,000 rpm) for 1 minute.
	14	Discard the flow-through and place the GS Column back in the Collection Tube.
	15	Add 600µl of W2 Buffer (ethanol added) in the GS Column.
	16	Discard the flow-through and place the GS Column back in the Collection Tube.
	17	Centrifuge at full speed (approx.13,000 rpm) for 3 minutes to dry the column matrix.
DNA Elution	18	Transfer dried GS Column into a clean 1.5 ml microcentrifuge tube (not provided).
	19	Add 100µl of preheated Elution Buffer (60℃) into the center of the column matrix. Standard elution volume is 100µl. If less sample volume is used, reduce the elution volume (30~50µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery.
	20	Stand at room temperature for 3 minutes until Elution Buffer absorbed by the matrix.
	20	Centrifuge at full speed (approx.13,000 rpm) for 2 minutes to elute purified DNA.

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Yeast Protocol

1. Add 100 ml ethanol (96-100%) to W2 Buffer prior to the initial use.
2. Additional requirements:
   Sorbitol Buffer (1.2 M sorbitol; 10 mM CaCl2 0.1M Tris-Cl pH 7.5; 35mM mercaptoethanol)
   Lyticase or Zymolase
   Microcentrifuge tube
   Ethano (96-100%)
   RNase A (50mg/ml)

Cell Harvesting / Prelysis	1	Harvest yeast cells (up to 5x 10^7) by centrifugation for 10 minutes at 5,000g.
	2	Discard the supernatant and resuspend the pellet in 600µl sorbitol buffer.
	3	Add 200U lyticase or zemolase. Incubate at 30℃ for 30 minutes.
	4	Centrifuge the mixture for 10 minutes at 2,000g to harvest spheroplast.
	5	Remove supernatant and add 200µl of NT Buffer and 20µl Proteinase K (10mg/ml) to the tube to resuspend the cell pellet by vortexing or pipetting.
	6	Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes.
Lysis	7	Add 300µl NB Buffer to the sample. Mix by vortexing for 5 seconds.
	8	Incubate at 60℃ waterbath for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, incubate required Elution Buffer (100 µl per sample) at 60℃.
	Optional Step: RNA degradation If RNA-free genomic DNA is required, perform this optional step. a. After step 7, add 2µl of RNase A (50mg/ml, not provided) to sample lysate and mix by vortexing. b. Proceed to Step 8.	
DNA Binding	9	Add 300µl of ethanol (96-100%) to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
	10	Place a GS Column in the Collection Tube.
	11	Apply the total mixture (including any precipitate) from previous step to the GS Column.
	12	Close the cap and centrifuge at full speed (approx. 13,000 rpm) for 1 minutes.
Wash	13	Add 400µl of W1 Buffer into the GS Column.
	14	Centrifuge at full speed (approx.13,000 rpm) for 1 minute.
	15	Discard the flow-through and place the GS Column back in the Collection Tube.
	16	Add 600µl of W2 Buffer (ethanol added) in the GS Column.
	17	Discard the flow-through and place the GS Column back in the Collection Tube.
	18	Centrifuge at full speed (approx.13,000 rpm) for 3 minutes to dry the column matrix.
DNA Elution	19	Transfer dried GS Column into a clean 1.5 ml microcentrifuge tube (not provided).
	20	Add 100µl of preheated Elution Buffer (60℃) into the center of the column matrix. Standard elution volume is 100µl. If less sample volume is used, reduce the elution volume (30~50µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery.
	21	Stand at room temperature for 3 minutes until Elution Buffer absorbed by the matrix.
	22	Centrifuge at full speed (approx.13,000 rpm) for 2 minutes to elute purified DNA.

TROUBLESHOOTING (FOR Blood/Cultured Cells/Bacterial/Yeast)

Problem	Possible Cause and Solution
Column clogged	**Overloaded Column with Sample** * Reduce sample volume or separate sample into multiple tubes. Add extra 100 µl ethanol to lysate. **Precipitate was Formed at DNA Binding Step** 1. Reduce the sample material. 2. Prior to loading the column, break up precipitate in ethanol-added lysate.
Low yield	**Incorrect DNA Elution Step** * Ensure that Elution Buffer was added and absorbed to the center of GS Column matrix. **Incomplete DNA Elution Step** * Elute twice to increase yield.
Eluted DNA does not perform well in downstream applications	**Residual Ethanol Contamination** * Following the wash step, dry GS Column with additional centrifugation at full speed for 5 minutes or incubate at 60 ℃ for 5 minutes. **RNA Contamination** * Perform optional RNA degradation step. **Genomic DNA was Degraded** * Use fresh blood. Prolonged storage may result in fragmentation of genomic DNA. **Protein Contamination** 1. Reduce the sample amount. 2. After DNA Binding Step, apply 400 µl W1 Buffer to wash GS Column and centrifuge at 6,000 x g for 30 seconds. Proceed with Wash Step of W2 Buffer.

T-Pro Genomic DNA Mini Kit

Tissue Protocol

1. Add 1.1 ml ddH_2O to a Proteinase K tube (11mg), vortex to dissolve.Store prepared Proteinase K at 4℃.
2. Add 100 ml ethanol (96-100%) to W2 Buffer prior to the initial use.
3. Additional requirements:
   Microcentrifuge tube
   Ethano (96-100%)
   RNase A (50mg/ml)

Tissue Dissociation	1	Cut up to 25mg of animal tissue (or 0.5cm of mouse tail) and transfer into a microcentrifuge tube (not provided). If used tissue has a higher number of cells (e.g. spleen or liver), reduce the starting material to 10mg.
	2	Use Micropestle (not provided) to grind the tissue to a pulp.
	3	Add 200 µl NT Buffer into the tube and continually homogenize the sample tissue with grinding.
Lysis	4	Add 20µl Proteinase K (10mg/ml) to the sample mixture and mix by vortexing.
	5	Incubate at 60℃ for 30 minutes to lyse the sample. During incubation, invert the tube every 5 minutes.
	6	Add 300µl NB Buffer and mix by vortexing for 5 seconds.
	7	Incubate at 60℃ for 15 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, preheat required Elution Buffer (100µl per sample) at 60℃ for DNA elution step.
	8	Centrifuge for 3 minutes at full speed (13,000 rpm) and transfer the supernatant to a new microcentrifuge tube (not provided).
	Optional Step: RNA degradation If RNA-free genomic DNA is required, perform this optional step. a. After step 6, add 5µl of RNase A (50mg/ml, not provided) to sample lysate and mix by vortexing. b. Proceed to Step 7.	
DNA Binding	9	Add 300µl of ethanol (96-100%) to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
	10	Place a GS Column in the Collection Tube.
	11	Apply the total mixture (including any precipitate) from previous step to the GS Column.
	12	Close the cap and centrifuge at full speed (approx. 13,000 rpm) for 1 minutes.
Wash	13	Add 400µl of W1 Buffer into the GS Column.
	14	Centrifuge at full speed (approx.13,000 rpm) for 1 minute.
	15	Discard the flow-through and place the GS Column back in the Collection Tube.
	16	Add 600µl of W2 Buffer (ethanol added) in the GS Column.
	17	Discard the flow-through and place the GS Column back in the Collection Tube.
	18	Centrifuge at full speed (approx.13,000 rpm) for 3 minutes to dry the column matrix.
DNA Elution	19	Transfer dried GS Column into a clean 1.5 ml microcentrifuge tube (not provided).
	20	Add 100µl of preheated Elution Buffer (60℃) into the center of the column matrix. Standard elution volume is 100µl. If less sample volume is used, reduce the elution volume (30~50µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery.
	21	Stand at room temperature for 3 minutes until Elution Buffer absorbed by the matrix.
	22	Centrifuge at full speed (approx.13,000 rpm) for 2 minutes to elute purified DNA.

T-Pro Genomic DNA Mini Kit

Tissue Protocol (for paraffin-embedded tissue)

1. Add 1.1 ml ddH_2O to a Proteinase K tube (11mg), vortex to dissolve.Store prepared Proteinase K at 4℃.
2. Add 100 ml ethanol (96-100%) to W2 Buffer prior to the initial use.
3. Additional requirements:
   Microcentrifuge tube
   Ethano (96-100%)
   RNase A (50mg/ml)
   Xylene

Sample Preparation	1	Slice small sections (up to 25mg) from blocks of paraffin-embedded tissue and transfer to a microcentrifuge tube.
	2	Add 1ml xylene to each tube. Vortex vigorously and incubate at room temperature for about 10 minutes. Vortex occasionally during incubation step.
	3	Centrifuge at full speed for 3 minutes. Remove supernatant by pipetting.
	4	Add 1ml ethanol to wash sample pellet and mix by inverting.
	5	Centrifuge at full speed for 3 minutes. Remove supernatant by pipetting.
	6	Repeat the ethanol washing step.
	7	Open tube and Incubate at 37℃ for 15 minutes to evaporate the ethanol residue.
Lysis	8	Add 20µl Proteinase K (10mg/ml) to the sample mixture and mix by vortexing.
	9	Incubate at 60℃ for 30 minutes to lyse the sample. During incubation, invert the tube every 5 minutes.
	10	Add 200µl NB Buffer and mix by vortexing for 5 seconds.
	11	Incubate at 60℃ for 15 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, preheat required Elution Buffer (100µl per sample) at 60℃ for DNA elution step.
	12	Centrifuge for 3 minutes at full speed (13,000 rpm) and transfer the supernatant to a new microcentrifuge tube (not provided).
	Optional Step: RNA degradation If RNA-free genomic DNA is required, perform this optional step. a. After step 10, add 5µl of RNase A (50mg/ml, not provided) to sample lysate and mix by vortexing. b. Proceed to Step 11.	
DNA Binding	13	Add 200µl of ethanol (96-100%) to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
	14	Place a GS Column in the Collection Tube.
	15	Apply the total mixture (including any precipitate) from previous step to the GS Column.
	16	Close the cap and centrifuge at full speed (approx. 13,000 rpm) for 1 minutes.
Wash	17	Add 400µl of W1 Buffer into the GS Column.
	18	Centrifuge at full speed (approx.13,000 rpm) for 1 minute.
	19	Discard the flow-through and place the GS Column back in the Collection Tube.
	20	Add 600µl of W2 Buffer (ethanol added) in the GS Column.
	21	Discard the flow-through and place the GS Column back in the Collection Tube.
	22	Centrifuge at full speed (approx.13,000 rpm) for 3 minutes to dry the column matrix.
DNA Elution	23	Transfer dried GS Column into a clean 1.5 ml microcentrifuge tube (not provided).
	24	Add 100µl of preheated Elution Buffer (60℃) into the center of the column matrix. Standard elution volume is 100µl. If less sample volume is used, reduce the elution volume (30~50µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery.
	25	Stand at room temperature for 3 minutes until Elution Buffer absorbed by the matrix.
	26	Centrifuge at full speed (approx.13,000 rpm) for 2 minutes to elute purified DNA.

T-Pro Genomic DNA Mini Kit

Tissue Protocol (for buccal swab)

1. Add 1.1 ml ddH_2O to a Proteinase K tube (11mg), vortex to dissolve.Store prepared Proteinase K at 4℃.
2. Add 100 ml ethanol (96-100%) to W2 Buffer prior to the initial use.
3. Additional requirements:
   Microcentrifuge tube
   Ethano (96-100%)
   RNase A (50mg/ml)
   Swab: cotton, DACRON or C.E.P. swabs

Sample Preparation	1	Scrape the swab firmly against the inside of each cheek 6-7 times and air-dry the swab. (The person providing the sample should not eat or drink for at least 30 minutes prior to the sample collection.)
Lysis	2	Add 400µl of NT Buffer and 20µl Proteinase K (10mg/ml) into the tube into a 1.5ml microcentrifuge tube.
	3	Place the buccal swab into the tube and incubate at 60℃ for 10 minutes.
	4	Discard the swab and add 400µl NB Buffer into the lysate.
	5	Mix immediately by vortexing and incubate at 60℃ for 10 minutes. At this time, preheat required Elution Buffer (100µl per sample) in a 60℃ waterbath for DNA elution step.
DNA Binding	6	Add 400µl of ethanol to the sample lysate and mix immediately by vortexing.
	7	Place a GS Column in a Collection Tube.
	8	Add 700µl of the mixture from previous step to the GS Column.
	9	Incubate at 60℃ for 30 minutes to lyse the sample. During incubation, invert the tube every 5 minutes.
	10	Close the cap and centrifuge at full speed (approx. 13,000 rpm) for 1 minute.
	11	Repeat DNA Binding Step by apply the remaining mixture to GS Column.
Wash	12	Add 400µl of W1 Buffer into the GS Column.
	13	Centrifuge at full speed (approx.13,000 rpm) for 1 minute.
	14	Discard the flow-through and place the GS Column back in the Collection Tube.
	15	Add 600µl of W2 Buffer (ethanol added) in the GS Column.
	16	Discard the flow-through and place the GS Column back in the Collection Tube.
	17	Centrifuge at full speed (approx.13,000 rpm) for 3 minutes to dry the column matrix.
DNA Elution	18	Transfer dried GS Column into a clean 1.5 ml microcentrifuge tube (not provided).
	19	Add 100µl of preheated Elution Buffer (60℃) into the center of the column matrix. Standard elution volume is 100µl. If less sample volume is used, reduce the elution volume (30~50µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution steps to increase DNA recovery.
	20	Stand at room temperature for 3 minutes until Elution Buffer absorbed by the matrix.
	21	Centrifuge at full speed (approx.13,000 rpm) for 2 minutes to elute purified DNA.

TROUBLESHOOTING (FOR Tissue/Paraffin-embedded tissue/Buccal swab)

Problem	Possible Cause and Solution
Column clogged	Too much tissue was used. * Too much tissue was used. If using more than 25mg of tissue, separate into multiple tubes. Sample tissue was not lysed completely *Add additional Proteinase K and extend the incubation time in Lysis step. *After Lysis step, centrifuge for 2 minutes at full speed (13,000 rpm) to remove sample debris. Transfer the supernatant to a new microcentrifuge tube and proceed with DNA Binding Step. Precipitate was formed at DNA Binding Step *Reduce the sample material. *Before loading the column, break up the precipitate in ethanol-added lysate.
Low yield	Sample tissue was not lysed completely *Add additional Proteinase K and extend the incubation time in Lysis step. Column was clogged at DNA Binding Step *Following the Lysis Step, remove the insoluble debris by centrifugation. *Prior to loading the column, break up the precipitate in ethanol-added lysate. Incorrect DNA Elution Step *Ensure that Elution Buffer was added and absorbed to the center of GS Column matrix. Incomplete DNA Elution Step. *Elute twice to increase the DNA recovery.
Eluted DNA does not perform well in downstream applications	Residual Ethanol Contamination * Following the wash step, dry GS Column with additional centrifugation at full speed for 5 minutes or incubate at 60℃ for 5 minutes. RNA Contamination * Perform optional RNA degradation step. Genomic DNA was Degraded * Use fresh sample or freeze fresh sample in liquid nitrogen immediately and store at -80℃. Protein Contamination * Reduce the sample amount. * After DNA Binding Step, apply 400µl W1 Buffer to wash GS Column and centrifuge at 13,000 rpm for 1 minute. Proceed with Wash Step of W2 Buffer.