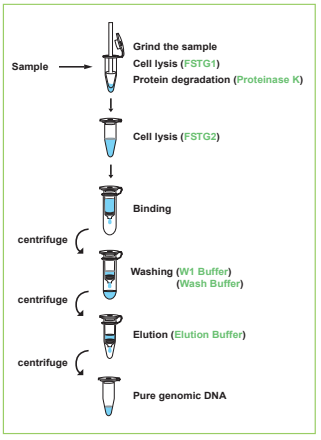
Tissue Genomic DNA Extraction Kit DE-012 & DE-013-200

Description

The Tissue Genomic DNA Extraction Kit is designed for rapid extraction of pure, small-scale genomic & mitochondrial DNA from several types of tissues), **fixed tissues (Fresh , Frozen ,Formalin, Paraffin) bacteria (gram-positive) ,water samples, yeast , blood ,(also dried spot), amniotic fluid sea urchins, marine mollusks, octopus and insects.**

Sampling

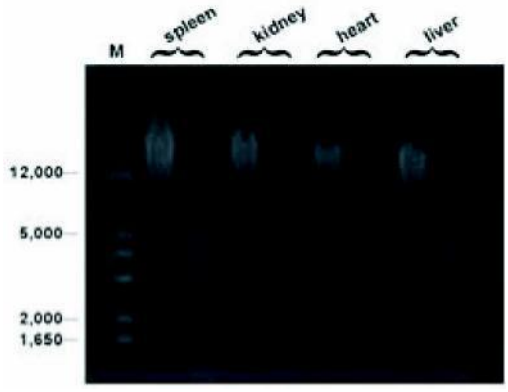
- **Principle:** mini spin column (silica matrix)
- **Operation time:** 30 ~ 60 minutes
- **Binding capacity:** up to 60 ug DNA/column
- **Typical yield**: 15 ~35 ug/ prep
- **Column applicability**: centrifugation and vacuum
- **Minimum elution volume**: 50 ul
- **Sample size**: < 25 mg animal tissue
  - 1.2 cm mouse tail
  - < 10^7 cultured cells



Rapid isolation cellular DNA from animal tissue (e.g. mouse tails), bacteria, yeast etc.
Purified DNA is ready for downstream application such as PCR, Southern blotting.
Centrifugation-based method.
Efficiently remove cellular inhibitors
No phenol/chloroform extraction and ethanol precipitation.

Applications

PCR
Southern Blotting
Forensic Analysis



The Quality of DNA after Purification

Genomic DNA Extracted from the indicated mouse tissue by the Tissue Genomic DNA Extraction Kit
For each tissue, the amount of undigested (left) and EcoRI digested (right) are equivalent
M1: 1Kb DNA Ladder (100bp-12,000bp)

Kit Components	DE-011s 4 preps	DE-012 100 preps	DE-013 200 preps
FSTG 1 Buffer	1,5 ml	30 ml	60 ml
FSTG 2 Buffer	1,5 ml	30 ml	60 ml
Proteinase K* (Lyophilized)	1 mg	11 mg x 2	11 mg x 4
W1 Buffer* (concentrated)	1,3 ml	44 ml	88 ml
Wash Buffer** (concentrated)	1 ml	20 ml	40 ml
Elution Buffer	1 ml	30 ml	60 ml
Collection Tube	4 pcs	200 pcs	400 pcs
Elution Tube	4 pcs	100 pcs	200 pcs
FSTG Mini Column	4 pcs	100 pcs	200 pcs
Micropestles	4 pcs	100 pcs	200 pcs
Preparation of Proteinase K solution (10 mg/ml) by adding ddH2O			
***ddH2O volume for Proteinase K**	**0.1ml**	**1.1 ml**	
Preparation of W 1 Buffer and Wash Buffer by adding ethanol (96 ~ 100%)			
**Ethanol volume for W 1 Buffer	**0,5 ml**	**8 ml**	**32 ml**
**Ethanol volume for Wash Buffer	**4 ml**	**40 ml**	**160 ml**

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Add 1.1 ml sterile ddH2O to Proteinase K tube to make a **10 mg/ml** stock solution. Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4 °C.**
3. Add ethanol (96- 100 %) to **W1 Buffer** and **Wash Buffer** when first open.
4. Prepare dry baths or water baths before the operation: one to 60 °C for point 4 and the other to 70 °C for point 7.
5. Preheat the Elution Buffer to 70 °C for point 13.
6. All centrifuge steps are done at full speed (~ 18,000 x g) in a microcentrifuge.

Storage Conditions

Stable for 1 year at room temperature 15 -25°C . The Proteinase K Powder store at -20 °C .

DNA extraction from Animal Tissues (Fresh/Frozen):

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Please Read Important Notes Before Starting The Following Steps.
For other special samples, please refer to Special Protocol and choose the appropriate one.

Hint: Prepare two dry baths or two water baths before the operation: One to 60°C for point 4 and other to 70°C for point 6 .

Step 1 **Tissue Dissociation**	**1.** **A.(For fresh sample)** Cut up to 25 mg tissue sample to a microcentrifuge tube (not provided). Use provided Micropestle to grind the tissue sample and break it into small pieces. Or you can grind the tissue sample in liquid nitrogen then transfer the powder to a new microcentrifuge tube. **B. (For frozen sample)** Weigh up to 25 mg tissue sample and grind the tissue sample in liquid nitrogen then transfer the powder to a microcentrifuge tube. If tissue sample has a high number of cells (e.g. Spleen), no more 10 mg should be used.
Step 2 Lysis	**2.**Add 200 ul FSTG1 Buffer and homogenize the tissue sample more completely with micropestle or pipette tip. **3.** Add 20ul Proteinase K(10mg/ml) to the sample mixture.Mix thoroughly by vortexing **4.** **Incubate at 60° C until the tissue is lysed completely** (usually in 1 hr, depends on the sample types). Vortex every 10-15 min during incubation. Briefly spin the tube to remove drops from the inside of the lid. **5.** (Optional) If RNA free genomic DNA is required, add 4ul of RNase A (100 mg/ml) incubate for 2 min at room temperature. **6.** Add 200ul FSTG2 Buffer to the sample mixture, mix thoroughly by pulse-vortexing and incubate at 70° C for 10 min. **7.** **Add 200ul ethanol (96-100%) to the sample mixture. Mix thoroughly by pulse vortexing** **8.** Briefly spin the tube to remove drops from the inside of the lid. If there is insoluble material present, centrifuge at full speed for 2 min and transfer the supernatant to a new microcentrifuge tube. (Not provided)
Step 3 **DNA Binding**	**9.** Place a FSTG Mini Column in a Collection Tube. Transfer the mixture (including precipitate) carefully to FSTG Column. Centrifuge at full speed (18,000X) and discard the flowthrough then place FSTG Column to a new Collection Tube.
Step 4 **Wash**	**10.** Add 400ul W1 Buffer to FSTG Column at full speed for 1 min then discard the flow through. --------Make sure that ethanol has been added into W1 Buffer when first open. **11.** Add 750ul Wash Buffer to the FSTG Column with by centrifuge for 1 min , ----- then discard the flow-through. -----Make sure that ethanol has been added into Wash Buffer when first open. **12.** Centrifuge for an additional 3 min to dry the column. Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
Step 5 **DNA Elution**	**13.** Add 100 ul preheated Elution Buffer or ddH O (pH 7.5 ~9.0) to the membrane center of FSTG Column. Stand FSTG Column for 3 min. ------**Important Step!** For effective elution, make surethat the elution solution is dispensed on the membrane center and is absorbed completely. ---- **SUGGESTION**: If less sample is loaded reduced the elution volume to 50 ul to increase DNA concentration and do not elute DNA using less than suggested volume (50 ul). It will lower the final yield **14**. Centrifuge for 2 min to elute total DNA. Store total DNA at 4°C or -20°C.

DNA extraction from Animal Tissues (Mouse Tail):

Mouse Tails - protocol

1.For mouse tail clippings, use 0.5–1.2cm of tail taken from the tip. Cut the tail into two equal parts, : The smaller the length of mouse tail, the more efficient the lysis will be. If processing more than 1cm of tail, cut it into smaller pieces with a razor blade or scalpel

2.Samples further from the tip of the tail contain more cartilaginous material that will clog the mini-column.

We recommend homogenizing or disrupting the tissue as much as possible (e.g., slicing the tissue with a razor blade or scalpel, grinding it in liquid nitrogen or using a homogenizer).

3. Transfer the disrupted samples into a 1.5ml tube , add FSTG1 Buffer 200 ul and provided and a FATG1 Buffer and Proteinase k solution 20 ul.

4. Incubate at 60℃ until the tissue is lysed completely (1~3 h). Vortex occasionally during incubation.

Note: Sample can be incubated overnight as well for complete lysis.

Use of a shaker/incubator will greatly help in releasing the DNA.

Follow the standard protocol 2. lysis

step. 6 OF THE paragraph “Add 200ul FSTG2 Buffer to the sample mixture” etc…..

DNA extraction from fixed Tissues:

(paraffin-formalin)

Additional equipment: • RNase A (optional),
96~100% ethanol
Xylene - for paraffin-embedded tissues -
Hint: Set dry or water baths: 60 °C and 70 °C

II. For paraffin-embedded tissues

1. Cut up to 25 mg paraffin-embedded tissue sample to a microcentrifuge tube (not provided).
2. Add 1 ml xylene, mix well and incubate at room temperature for 30 min.
3. Centrifuge at full speed for 5 min. Remove supernatant by pipetting.
4. Add 1 ml ethanol (96- 100 %) to the deparaffined tissue, mix gently by vortexing.
5. Centrifuge at full speed for 3 min. Remove supernatant by pipetting.
6. Repeat point 4 and 5.
7. Incubate at 37+°C for 10 ~15 min to evaporate ethanol residue completely.
8. Grind the tissue sample by micropestle or liquid nitrogen and follow the Animal Tissue Protocol starting from **Step 2**. **(Lysis)**

III. For formalin-fixed tissues

9. Wash 25 mg tissue sample twice with 1 ml PBS to remove formalin.
10. Grind the tissue sample by micropestle or liquid nitrogen and follow the Animal Tissue Protocol starting from **Step 2 (Lysis)**

Protocol: Isolation of genomic DNA from Animal Cultured Cells

Additional requirement:
RNase A (optional), 96~100% ethanol, trypsine or cell scraper (for monolayer cell), PBS

Hint: Set dry or water baths: 60 °C and 70 °C

1. Harvest cells

a. **Cells grown in suspension**

i. Transfer the appropriate number of cell (up to 1 x 10^7) to a microcentrifuge tube. ii. Centrifuge at 300 x g for 5 min. Discard supernatant carefully and completely.

b. **Cells grown in monolayer**

i. Detach cells from the dish or flask by trypsinization or using a cell scraper. Transfer the appropriate number of cell (up to 1 x 10^7) to a microcentrifuge tube.
ii. Centrifuge at 300 x g for 5 min. Discard supernatant carefully and completely.

c. Resuspend cell pellet in PBS to a final volume of 200 ul.
d. Follow the Animal Tissuel Protocol starting from **step 2. Lysis (add 200 ul of FSTG1 buffer)**

Protocol: Isolation of genomic DNA and Viral DNA from Blood

Additional requirment:
RNase A (optional), 96~100% ethanol, PBS

Hint: Set dry or water baths: 60 °C for point 3 and 70 °C for point 4.

1. Transfer up to 200 µl sample (whole blood, serum, plasma, body fluids, buffy coat) to a microcentrifuge tube.
--- If the sample volume is less than 200 µl , add the appropriate volume of PBS.
2. (Optional) If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A (not provided). Mixthoroughly by vortexing and incubate at room temperature for 2 min.
3. Add 20 µl Proteinase K to the sample, and then add 200 µl FSTG2 Buffer to the sample. Mix thoroughly by pulse-vortexing. Incubate at 60 °C for 30 min. Vrotex occasionally during incubation.
4. Incubate at 70 °C for 10 min.

2. Follow the Animal Tissuel Protocol starting from **Step 2**. **point 7**

Protocol: Isolation of DNA from Dried Blood Spot

Additional equipment: • RNase A (optional), 96~100% ethanol ,pbs

HINT: Set dry or water baths: one at 85 °C one at 60 °C and one at 70 °C

1. Cut the filter paper (e.g. S&S903) with dried blood spot into a microcentrifuge tube. Add 200 µl FSTG1 Buffer and incubate at 85 °C for 10 min.
2. Add 20 µl Proteinase K to the sample mixture. Mix thoroughly by vortexing. Incubate at 60 °C for 1 hr. Vortex occasionally during incubation.
3. Follow the Animal Tissue Protocol starting from **Step 2- Lysis - point 2**

Protocol: DNA Isolation from Bacteria (including water)

Additional Equipment : RNase A (optional) 96%/100% Ethanol

- For Gram-positive bacteria: lysozyme reaction solution (20 mg/ml lysozyme; 20 mM
- Tris-HCl, pH 8.0; 2mM EDTA; 1.2 % Triton)

Hint: **Set dry or water baths**: 60 °C for **point 4 STEP 2.** and 70 °C for **point 6 STEP 2.**

1) For bacterial cultures

1) Transfer 1 ml well-grown bacterial culture to a microcentrifuge tube (not provided).
2) Descend the cells by centrifuging at full speed for 2 min and discard supernatant completely.
3) Follow the Animal Tissue Protocol starting from **step 2**.**Lysis**

For bacterial in biological fluids

1) Collect cells by centrifuging biological fluids at 7,500 rpm (5,000 x g) for 10 min and discard supernatant completely.
2) Follow the Animal Tissue Protocol starting from **step 2.Lysis**

For bacteria from eye, nasal, pharyngeal, or other swabs

1) Soak the swabs in 2 ml PBS at room temperature for 2- 3 hr.
2) Collect cells by centrifuging at 7,500 rpm (5,000 x g) for 10 min and discard supernatant completely.
3) Follow the Animal Tissue Protocol starting from **Step 2.Lysis**

For Gram-positive bacteria

HINT: Set dry or water baths: one to 37 °C, another to 60 °C and the other to 95 °C.

a) Transfer 1 ml well-grown bacterial culture to a microcentrifuge tube (not provided).
b) Descend the cells by centrifuging at full speed for 2 min and discard supernatant completely.
c) Resuspend the cell pellet in **200 ul lysozyme reaction solution** (20 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0;2mM EDTA; 1.2 % Triton). Incubate at 37 °C for 30~60 min.
(*Optional*)*If RNA-free genomic DNA is required, add 4 ul of 100 mg/ml RNase A (not provided).*
Mix thoroughly by vortexing and incubate at room temperature for 2 min.
2) Add 20 µl Proteinase K to the sample, and then add **200 µl FSTG2 Buffer** to the sample. Mix thoroughly by pulse-vortexing. Incubate at 60 °C for 30 min and vortex occasionally during incubation.
d) Do a future incubation at 95 °C. for 15 min to.
e) Follow the Animal Tissue Protocol starting from **Step.2 Point 7. Add ethanol**

Protocol: Isolation of genomic DNA from Yeast

Additional equipment: • RNase A (optional), 96~100% ethanol

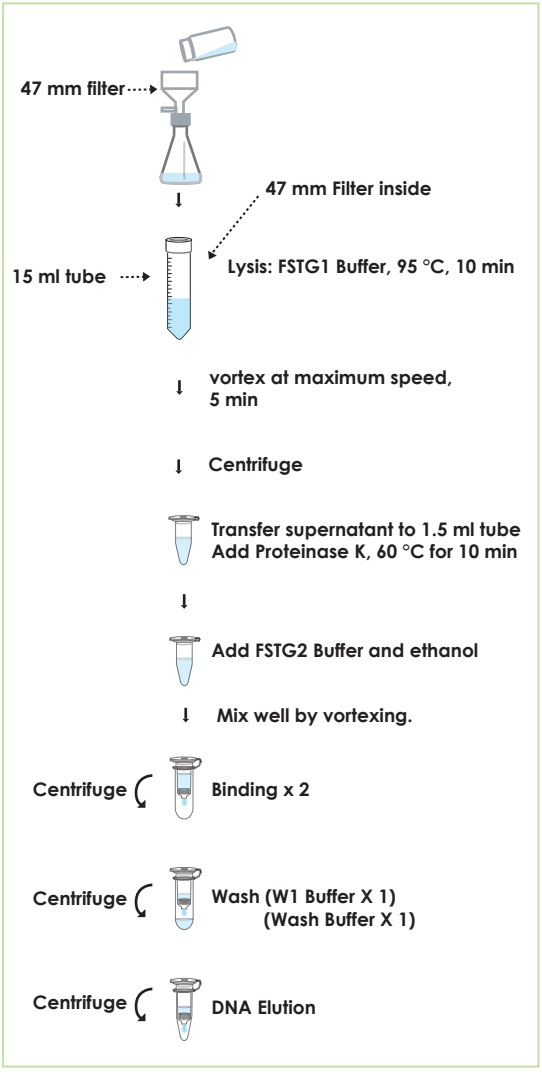
- Zymolyase - Lyticase 200 U for one preparation
- Sorbitol Buffer (1M sorbitol, 100 mM EDTA, 14 mM Beta- Mercaptoethanol)

HINT: Set dry or water baths: one to 30°C, another to 60°C and the other to 70°C.

1. Transfer 3 ml log-phase (OD600= 10) yeast culture to a microcentrifuge tube (not provided)
2. Descend the cells by centrifuginge at 7,500 rpm (5,000 x g) for 10 min. Discard supernatant completly
3. Resuspend the cell pellet in **600 ul sorbitol buffer** (1M sorbitol; 100 mM EDTA; 14 mM Beta-mercaptoethanol). Add 200 U zymolyase or lyticase and incubate at 30 °C for 30 min.
4. Centrifuge at 7,500 rpm (5,000 x g) for 5 min. Remove supernatant by pipetting
5. Follow the Animal Tissue Protocol starting from **Step. 2** / **add FATG1 Buffer 200 ul**

Special protocol for water DNA extraction by using Tissue Genomic DNA Mini Kit, DE-011

1. Use 47 mm of filter paper with pore size 0.22 μm (or 0.45 μm) and filter funnel (not provided) to filter the water samples.
2. Pick up the filter from funnel and roll the filter paper into a cylinder with the top side inward.
3. Insert the filter into a 15 ml tube. Add 1000 μl of FSTG1 Buffer. Vortex the tube at maximum speed for 5 min.
4. Incubate the sample at 95 °C for 10 min and vortex the sample twice during the incubation.
5. Centrifuge the tube at 2,500 x g for 3 min. Transfer 600 μl of the supernatant to a 1.5 ml centrifuge tube. Add 30 μl of Proteinase K and incubate at 60 °C for 10 min.
6. Add 300 μl of FSTG2 Buffer and 450 μl of ethanol. Mix well by vortexing.
7. Place a FSTG Column into a Collection Tube and transfer up to 700 μl of the sample mixture to the Column. Centrifuge at full speed (~18,000 x g) for 30 sec then discard the flow-through. Place the column back to the Collection Tube.
8. Repeat the step 7 for the rest of the sample mixture.
9. Add 400 μl of W1 Buffer (ethanol added) to the Column. Centrifuge at full speed for 30 sec then discard the flow -through. Place the column back to the Collection Tube.
10. Add 650 μl of Wash Buffer (ethanol added) to the Column. Centrifuge at full speed for 30 sec then discard the flow -through. Place the column back to the Collection Tube.
11. Centrifuge at full speed (~18,000 x g) for an additional 3 min to dry the column.
12. Place the column into a 1.5 ml microcentrifuge tube (not provided). Add 50 μl of preheated Elution Buffer or ddH2O to the membrane center of the column. Stand the column for 2 min at room temperature.
13. Centrifuge at full speed for 1 min to elute DNA.



Protocol: for extraction of DNA from insects

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Add 1.1 ml sterile ddH2O to Proteinase K tube to make a 10 mg/ml stock solution.
   Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4 °C.**
3. Add required volume of ethanol (96- 100 %) to W1 Buffer and Wash Buffer when first open.
4. Preheat a dry bath or water bath to 60 °C and 70 °C before the operation.
5. All centrifuge steps are done at full speed (14,000 rpm or 18,000 x g) in a microcentrifuge.

Please Read Important Notes Before Starting The Following Steps.

1. Transfer up to 100 µl of sample that disrupted by a tissue homogenizer to a microcentrifuge tube (not provided).
2. Add 100 µl of Elution Buffer to the sample. Mix well by slapping the tube.
3. Add 200 µl of FSTG1 Buffer and 20 µl of Proteinase K to the sample. **Mix thoroughly by pulse-vortexing.**
4. Incubate at 60 ºC for 10 min to lyse the sample. **During incubation, vortex the sample every 3-5 minutes.**
5. Centrifuge the sample mixture at 18,000 x g for 1 min to descent the debris.
6. Transfer the supernatant to a new 1.5 ml tube.
7. **(Optional)**: If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A (not provided)to the sample and incubate for 2 min at room temperature.
8. Add 200 µl of FSTG2 Buffer to the sample. **Mix thoroughly by pulse-vortexing.** Incubate at 70 ºC for 10 min.
9. Add 200 µl of ethanol (96- 100 %) to the sample. **Mix thoroughly by pulse-vortexing for 30 sec.**
10. Place a FSTG column to a Collection Tube. Transfer the mixture carefully to FSTG Column and centrifuge for 1 minute. **Place FSTG Column to a new Collection Tube.**
11. Wash the FSTG Column with 400 µl W1 Buffer by centrifuge for 1 minute then discard the flow-through.
12. Wash the FSTG Column with 650 µl Wash Buffer by centrifuge for 1 min then discard the flow-through.
    - Make sure that ethanol has been added into Wash Buffer when first open.
13. **Centrifuge for an additional 3 min to dry the column.**
    Important Step! The residual liquid can affect the quality of DNA and inhibit subsequent enzymatic reactions.
14. Place the FSTG Column to a Elution Tube.
15. Add 50 ~ 100 µl of Elution Buffer or ddH2O (pH 7.0) to the membrane of the FSTG Column.
    Stand the FSTG Column for 3 min.
    - **Important Step!** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
16. Centrifuge at 18,000 x g for 1 min to elute DNA.

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PROTOCOL: DNA ISOLATION FROM MARINE MOLLUSKS TISSUES & SEA URCHINS

PLEASE READ THE *IMPORTANT NOTES* BEFORE STARTING THESE STEPS

NOTES:

- Prepare dry baths to 60 °C for step 3, and to 70 °C for step 4.
- Preheat **Elution** Buffer to 70 °C for step 12 (elution step).

Sample lysis

1. Cut **up to 25 mg** of mollusk tissue sample to a microcentrifuge tube (not provided). Use a micro-pestle (provided) to grind the mollusk tissue.

2. Add 200 µl of **FSTG1** Buffer to the sample. Mix thoroughly bypipetting up and down to disrupt the sample mixture.

3. Add 20 µl of Proteinase K to the sample. Mix thoroughly by vortexing. Incubate the sample mixture at **60 ºC** until the sample is completely lysed (invisible) (15 min~ 3 hrs).

   OPTIONAL: If RNA-free DNA is required, add 8 µl of 100 mg/ml **RNase A** to the sample and incubate for 2 min at room temperature.

4. Add 200 µl of **FSTG2** Buffer to the sample. Mix thoroughly by vortexing. Incubate the sample mixture at **70 ºC** for 10 min.

5. Cool down the sample at room temperature for 1 min.
   Add 200 µl ethanol (96- 100 %) to the sample. Mix thoroughly by pulse-vortexing for 5 sec.

DNA binding

6. (Centrifuge) Place a **FSTG Column** in a Collection Tube,
   (Vacuum) or insert the **FSTG Column** tip into the vacuum adaptor (do notdiscard the Collection Tube).
7. Transfer the mixture to the **FSTG Column**
   (Centrifuge) Centrifuge at 18,000 x g for 30 sec. Discard the flow-through and return the **FSTG Column** back to the Collection Tube.
   (Vacuum) Apply a vacuum of -6 inches Hg until the column is empty. Switch off the vacuum and release vacuum from the manifold.

Wash the column's membrane

8. Add 500 µl of **W1** Buffer to the **FSTG Column**.
   (Centrifuge) Centrifuge at 18,000 x g for 30 sec. Discard the flow-through and return the **FSTG Column** back to the Collection Tube.
   (Vacuum) Apply vacuum of -6 inches Hg until the column is empty. Switch off the vacuum and release vacuum from themanifold.
9. Add 700 µl of **Wash** Buffer (ethanol added).
   (Centrifuge) Centrifuge at 18,000 x g for 30 sec. Discard the flow-through and return the **FSTG Column** back to the Collection Tube.
   (Vacuum) Apply vacuum of -6 inches Hg until the column is empty. Switch off the vacuum and release vacuum from themanifold. Place the **FSTG Column** to the Collection Tube.

Dry the column's membrane

10. Centrifuge at 18,000 x g for an additional 2 min to completely remove the residual liquid.
    Important Step! This step will avoid residual liquids to inhibitsubsequent enzymatic reactions.

Elution

11. Place the **FSTG Column** to a clean 1.5 ml tube (not provided)
12. Add 40 ~100 µl of **Elution** Buffer to the center of the column.Stand the column for 1 min.
    Important Step! For effective elution ensure the solution is dispensed onto the membrane center and is fully absorbed.
13. Centrifuge at 18,000 x g for 30 sec to elute DNA.

Troubleshooting

Problem/ Possible reasons	Solutions
• Low or no yield of genomic DNA	
Low amount of cells in the sample	Increase the sample size or concentrate a larger sample volume to 200 µl.
Too much amount of sample was used	Reduce the sample volume.
Poor cell lysis	
Poor cell lysis because of insufficient Proteinase K activity	Use a fresh or well-stored Proteinase K stock solution.
	Do not add Proteinase K into FATG2 Buffer directly.
Poor cell lysis because of insufficient mixing with FSTG2 buffer	Mix the sample and FSTG2 Buffer immediately and thoroughly by pulse -vortexing.
Poor cell lysis because of insufficient incubation time	Extend incubation time and make sure that no residual particle remain.
Insufficient binding of DNA to column's membrane	
Ethanol is not added into sample lysate before DNA binding	Make sure that the correct volumes of ethanol (96- 100 %) is added into the sample lysate before binding.
Ethanol and sample lysate did not mix well before DNA binding	Make sure that Ethanol and sample lysate have been mixed completely before DNA binding
Incorrect preparation of Wash Buffer	
The percentage of ethanol is not correct in Wash Buffer	Make sure that the correct volumes of ethanol (96- 100 %) is added into Wash Buffer when first open.
Elution of genomic DNA is not efficient	
pH of water (ddH2O) for elution is acidic	Make sure the pH of ddH2O is between 7.5-9.0.
	Use Elution Buffer (provided) for elution .
Elution Buffer or ddH2O is not completely absorbed by membrane	After Elution Buffer or ddH2O is added, stand the FSTG Column for 5 min before centrifugation.
Column is clogged	
Lysate contains insoluble residues	Remove insoluble residues (e.g. bone or hair) by centrifugation.
Sample is too viscous	Reduce the sample volume.
Insufficient activity of Proteinase K	Use a fresh or well-stored Proteinase K stock solution and do not add Proteinase K into FSTG2 Buffer directly.
Poor quality of genomic DNA	
A260/A280 ratio of eluted DNA is low	
Poor cell lysis because of insufficient Proteinase K activity	Use a fresh or well-stored Proteinase K stock solution.
	Do not add Proteinase K into FSTG2 Buffer directly.
Poor cell lysis because of insufficient mixing with FSTG2 buffer	Mix the sample and FSTG2 Buffer immediately and thoroughly by pulse vortexing.
Poor cell lysis because of insufficient incubation time	Extend the incubation time and make sure that no residual particulates remain.
A260/A280 ratio of eluted DNA is high	
A lot of residual RNA in eluted	Follow the Animal Tissue Protocol point 5 to remove RNA.
FSTG2 Buffer was added into sample lysate before added RNase	Make sure that RNase A has been added to the sample lysate before adding FSTG2 Buffer when using optional RNase A step.
Degradation of eluted DNA	
Sample is old	Always use fresh or well-stored sample for genomic DNA extraction. Genomic DNA extracted from paraffin-embedded tissue is usually degraded. It is still suitable for PCR reaction, but is not recommened for Southern blotting and restriction analysis.
Buffer for gel electrophoresis contaminated with DNase	Use fresh running buffer for gel electrophoresis.

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