FISHER MOLECULAR BIOLOGY

BLOOD &TISSUE GENOMIC DNA EXTRACTION KIT DE 047/ DE 049

Whole Blood, Tissues (Fresh/Frozen-Fixed: Paraffin, Formalin), Bacteria, Yeast, dried blood spots, Amniotic fluid

Description

The Blood & Tissue Genomic DNA Extraction Kit is designed for rapid extraction of pure, small-scale genomic & mitochondrial DNA from several types of tissues (fresh or frozen), fixed tissues (Formalin, Paraffin) , Whole Blood , buffy coat , bacteria, yeast and ,blood spot , amniotic fluid, animal cells treated with ThinPrep solution, sea urchins, marine mollusks, octopus and insects..

Highlights:

- 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 debair and inhibitors
- No phenol/chloroform extraction and ethanol precipitation.

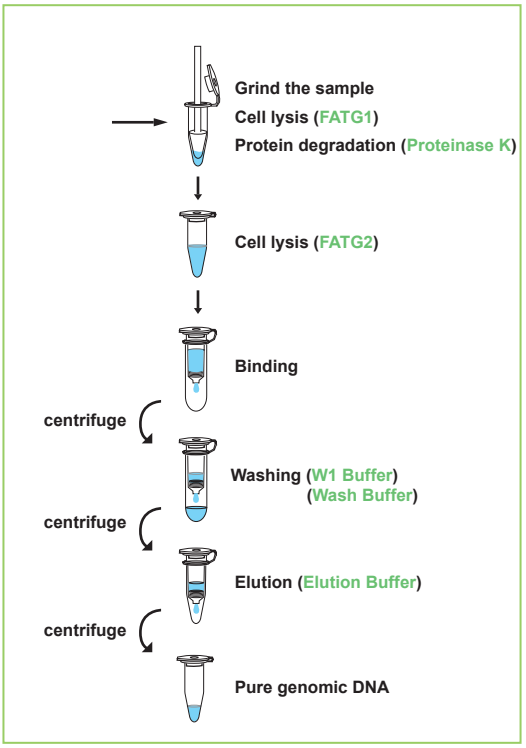
Applications

PCR
Southern Blotting
Medicolegal Analysis

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 vaccum
Minimum elution volume: 50 ul

Sample size:
Blood: 200 ul of blood
Tissue: < 25 mg animal tissue

1.2 cm mouse tail
Cell culture< 10^7 cultured cells
< 10^7 Bacterial cultured cells



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 ddH_2O 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 step 4 and the other to 70 °C for step 7.
5. Preheat the Elution Buffer to 70 °C for step 13.
6. All centrifuge steps are done at full speed (~ 18,000 x g) in a microcentrifuge.

Cat. No:	DE-047s (4preps_sample)	DE-047 (100 preps)	DE-049-200 (200 preps)
FSTG1Buffer	1.5 ml	30 ml	50 ml
FSTG2 Buffer	1.5 ml	30 ml	60 ml
Proteinase K (lyophilized)°	1 mg	11 mg x 2	11 mg x 4
W 1 Buffer * (contentrate)	1.3 ml	44 ml	88 ml
Wash Buffer **(concentrate)	1 ml	20 ml	40 ml
Elution Buffer	1 ml	30 ml	60 ml
FSTG Mini Column	4 pcs	100 pcs	200 pcs
Collection Tube	8 pcs	200 pcs	400 pcs
Elution Tube	4 pcs	100 pcs	200 pcs
Micropestle	4 pcs	100 pcs	200 pcs
User Manual	1	1	1

Preparation of Proteinase K solution (10 mg/ml) by adding ddH2O		
*ddH2O volume for Proteinase K	0.1 ml	1.1 ml

Preparation of W1 Buffer and Wash buffer by adding ethanol (96-100%)				
*Ethanol volume for W1 Buffer	0,5 ml	8 ml	16 ml	45 ml
** Ethanol volume for Wash Buffer	4 ml	40 ml	80 ml	220 ml

Tissue General Protocol (Fresh/Frozen):

Please Read Important Notes Before Starting The Following Steps.
For other special samples, please refer to special Protocol and choose the appropriate one.
Additional requirements: RNAse A (optional), 96-100% ethanol

Hint: Prepare two dry baths or two water baths before the operation:One to 60°C for step 4 and other to 70°C for step 7.

Step Tissue Dissociation	1. **(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. **(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. 2. Add 200 µl FSTG1 Buffer and homogenize the tissue sample more completely with micropestle.
Step Lysis	3. Add 20µl Proteinase K(10mg/ml) to the sample mixture. Mix thoroughly by vortexing. 4. **Incubate at 60°C until the tissue is lysed completely (1-3h).** Vortex occasionally during incubation. --- Sample can be incubated overnight as well as for complete lysis. 5. Briefly spin the tube to remove drops from the inside of the lid. 6. (Optional) If RNA free genomic DNA is required, add 4µl of RNase A (100 mg/ml) (not provided)incubate for 2 min at room temperature. 7. Add 200µl FSTG2 Buffer to the sample mixture, mix thoroughly by pulse-vortexing and incubate at 70° C for 10 min. 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 DNA Binding	9. Add 200 µl ethanol (96 ~ 100%) to the sample. Mix thoroughly by pulse-vortexing. 10. Briefly spin the tube to remove drops from the inside of the lid. 11. Place a FSTG Mini Column in a Collection Tube. Transfer the sample any precipitate) carefully to FSTG Column. Centrifuge for 1 min and discard the flowthrough then place FSTG Column to a new Collection Tube.
Step Wash	12. Add 400µl W1 Buffer to FSTG Columnby centrifuge for 1 min then discard the flow-through. • Make sure that ethanol has been added into W1 Buffer when first open. 13. Add 750µl Wash Buffer to FSTG Column by centrifuge for 1 min then discard the flow-through. 14. Centrifuge for an additional 3 min to dry the column. • Make sure that ethanol has been added into Wash Buffer when first open. • Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
Step DNA Elution	15.Add 100µl of pre-heated Elution Buffer or ddH2O(pH 7.5 -8.5)to the membrane center of FSTG Column. Stand FSTG Column for 3 min. • Important Step! For effective elution, make surethat the elution solution is dispen on the membrane center and is absorbed completely. • Standard volume for elution is 200µl. If tissue sample has a low number of cells, reduce the elution volume(50-150 µl) to increase DNA concentration. 16. Centrifuge at full speed for 2 min to elute total DNA. 17. Store total DNA at 4°C or -20°C.

Fixed Tissue (Paraffin & Formalin)

Additional requirements: RNAse A (optional), 96-100% ethanol
Xylene for paraffin embedded tissues -
Set 2 dry or water baths one to 60°C and the other to 70°C

- **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 the supernatant by pipetting.
4. Add 1 ml ethanol (96 ~ 100%) to the deparaffined tissue, mix gently by vortexing.
5. Centrigue at full speed for 5 min. Remove the supernatant by pipetting.
6. Add 1 ml ethanol (96 ~ 100%) to the deparaffined tissue, mix gently by vortexing.
7. Centrifuge at full speed for 5 min. Remove the supernatant by pipetting.
8. Incubate at 37° C for 10 min to evaporate ethanol residue.
9. Grind the tissue sample by micropestle or liquid nitrogen.

Step Lysis

10. Add 200µl FSTG1 Buffer to the sample mixture, mix well by Micropestle with pipette tips
11. Add 20µl Proteinase K(10mg/ml) to the sample mixture. Mix thoroughly by vortexing
12. Incubate at 60° C until the tissue is lysed completely (usually in 1 hr- 3 hr, depends on the Vortex every 10 minutes during incubation.
--Sample can be incubated overnight as well for complete lysis.
13. (Optional) If RNA free genomic DNA is required, add 4µl of RNase A (100 mg/ml) not provided . Mix thoroughly by vortexing and incubate for 2 min at room temperature.
14. 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.
15. Add 200µl FSTG2 Buffer to the sample mixture, mix thoroughly by pulse-vortexing and incubate at 70° C for 10 min

Step DNA Binding

16. Add 200 µl ethanol (96 ~ 100%) to the sample. Mix thoroughly by pulse-vortexing.
17. Briefly spin the tube to remove drops from the inside of the lid.
18. Place a FSTG Mini Column in a Collection Tube. Transfer the sample any precipitate) carefully to FSTG Column. Centri fuge for 1 min at full speed , discard the flowthrough, then place FSTG Column to a new Collection Tube.

Step Wash

19. Add 400 ul W1 Buffer to the FATG Mini Column ,centrifuge at full speed and discard the flowthrough.
- Make sure that ethanol has been added into W1 Buffer when first open.
20. Add 750 ul Wash Buffer to the FSTG Column by centrifuge for 1 min then discard the flow-through.
--Make sure that ethanol has been added into Wash Buffer when first open.
21. 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 DNA Elution

22. Add 100 ul of preheated Elution Buffer or ddH_2O (pH 7.5 ~ 9.0)to the membrane center of of FSTG Mini Column. Stand FSTG Mini Column for 3 min.
- Important Step! For effective elution, make surethat the elution solution is dispensed on the membrane center and is absorbed completely.
- Standard volume for elution is 100µl.If tissue sample has a low number of cells, reduce the elution volume to 50 µl to increase DNA concentration.
23. Centrifuge for 2 min to elute total DNA.
24. Store total DNA at 4°C or -20°C.

. ixed Tissues (Formalin)

1. Wash 25mg tissue sample twice with 1 ml PBS to remove formalin.
2. Grind the tissue sample by micropestle or liquid nitrogen .
3. Add 200µl FSTG1 Buffer to the sample mixture, mix well by Micropestle with pipette tips

Step Lysis

4. Add 20µl Proteinase K(10mg/ml) to the sample mixture. Mix thoroughly by
5. 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.
6. Briefly spin the tube to remove drops from the inside of the lid.
7. (Optional) If RNA free genomic DNA is required, add 4µl of RNase A (100 mg/ml) .
8. Add 200µl FSTG Buffer to the sample mixture, mix well by Micropestle with pipette tips and incubate at 70° C for 10 min.
9. 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 DNA Binding

- Add 200 µl ethanol (96 ~ 100%) to the sample. Mix thoroughly by pulse
- Briefly spin the tube to remove drops from the inside of the lid.
- Place a FSTG Mini Column in a Collection Tube. Transfer the sample any precipitate) carefully to FSTG Column. Centri fuge for 1 min at full speed , discard the flowthrough, then place FSTG Column to a new Collection Tube.

Step Wash

1 . Add 400 ul W1 Buffer to the FATG Mini Column ,centrifuge at full speed and discard the flowthrough.

- Make sure that ethanol has been added into W1 Buffer when first open.

. Add 750 ul Wash Buffer to the FSTG Column by centrifuge for 1 min then discard the flow-through.

--Make sure that ethanol has been added into Wash Buffer when first open.

1 . Centrifuge for an additional 3 min to dry the column.

- Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

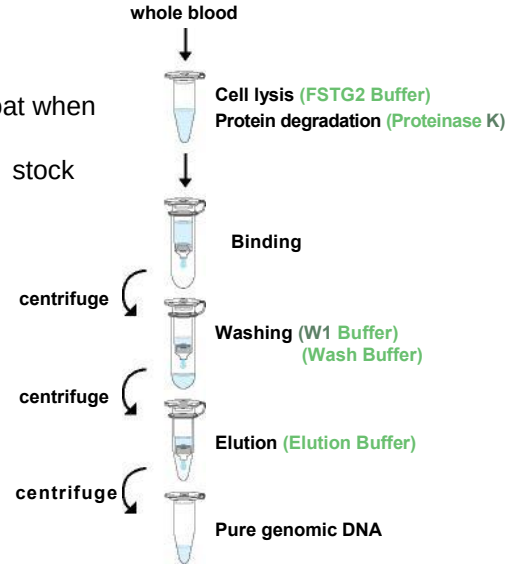
Step DNA Elution

. Add 100 ul of preheated Elution Buffer or ddH_2O (pH 7.5 ~ 9.0)to the membrane center of of FSTG Mini Column. Stand FSTG Mini Column for 3 min.

- Important Step! For effective elution, make surethat the elution solution is dispen sed on the membrane center and is absorbed completely.
- Standard volume for elution is 100µl.If tissue sample has a low number of cells, reduce the elution volume to 50 µl to increase DNA concentration.

. Centrifuge for 2 min to elute total DNA.
. Store total DNA at 4°C or -20°C.

Special protocol for extraction of Genomic DNA and Viral DNA from blood



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 before the operation.
5. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.

General Protocol for whole blood:

Please Read Important Notes Before Starting The Following Steps.

1. Transfer up to 200 µl sample (whole blood,serum, plasma, body fluids, buffy coat) to a microcentrifuge tube (not provided). - 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)to the sample and incubate for 2 min at room temperature.

3. Add 20 µl of Proteinase K and 200 µl of FSTG2 Buffer to the sample. **Mix thoroughly by pulse-vortexing.** -Do not add Proteinase K directly to FSTG2 Buffer.

4. Incubate at 70 °C for 10 minutes to lyse the sample. **During incubation, vortex the sample every 3-5 minutes.**

5. Briefly spin the tube to remove drops from the inside of the lid.

6. Add 200 µl of ethanol (96- 100 %) to the sample. **Mix thoroughly by pulse-vortexing for 30 sec.**

7. Briefly spin the tube to remove drops from the inside of the lid.

8. Place a FSTG column to a Collection Tube.
Transfer the mixture carefully to FSTG Column. Centrifuge for 1 minute **then place FSTG Column to a new Collection Tube.**

9. Add 400 µl W1 Buffer to the FSTG Column by centrifuge for 1 minute then discard the flow-through.

10. Add 750 µl 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.

11. **Centrifuge for an additional 3 minutes to dry the column.**
Important Step! The residual liquid can affect the quality of DNA and inhibit subsequent enzymatic reactions.

12. Place the FSTG Column to a Elution Tube.

13. Add 100 ~ 200 µl of Elution Buffer or ddH2O (pH 7.0) to the membrane of the FSTG Column.
Stand the FSTG Column for 3 minutes.
   - **Important Step!** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.

14. Centrifuge at 14,000 x g for 1 minute to elute total DNA.

15. Store total DNA at 4 °C or -20 °C.

Protocol: Isolation of DNA from Animal Cultured Cells

Additional requirment: 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.

2. Resuspend cell pellet in PBS to a final volume of 200 ul.

Cell Lysis

1. Add 200 µl of FATG2 Buffer to the sample and vortex for 5 secs.

2. Incubate for 10 mins at 70ºC or until the sample lysate is clear. During incubation, invert the tube every 3 mins.

3. Preheat required Elution Buffer (for Step DNA Elution) in a 70ºC water bath.

4. (Optional Step): If RNA-free genomic DNA is required, add 5 µl of 10 mg/ml RNase A to the sample and mix by vortexing. Incubate for 5 mins at room temperature.

5. Follow the General Protocol starting from Step.10

DNA Binding

Protocol for Animal Cells in culture – DNA Extraction from HU-Cells Treated with ThinPrep PreserveCyt solution

To extract DNA from ThinPrep PreservCyt solution, you first need to pellet the cells via centrifugation, then perform standard nucleic acid extraction with our kit (lysis, purification, washing, elution)

We suggest to pellet the cells via centrifugation 300 x g for 5 minutes

Sample Preparation

1. **Harvest cells**

a. Cells grown in suspension

i. Trypsinize the adherent cells before harvesting.

ii. Transfer the appropriate number of cells (up to 1×107) to a 1.5 ml microcentrifuge tube(not provided) and centrifuge at 6,000 xg for 20 secs. Discard the supernant

Cell Lysis

1. Add 200 µl of FATG2 Buffer to the sample and vortex for 5 secs.
2. Incubate for 10 mins at 70ºC or until the sample lysate is clear. During incubation, invert the tube every 3 mins.
3. Preheat required Elution Buffer (for Step DNA Elution) in a 70ºC water bath.
4. (Optional Step): If RNA-free genomic DNA is required, add 5 µl of 10 mg/ml RNase A to the sample and mix by vortexing. Incubate for 5 mins at room temperature.
5. Follow the General Protocol starting from Step.10

DNA Binding

Bacteria Protocol

Additional equipment: RNAse A (optional) -Ethanol 96-100%
For Gram-Positive bacteria: lysozyme reaction solution (20 mg/ml lysozyme: 20Mm TrisHCL, pH8.0,2mM EDTA 1.2% Triton)
Set: dry or water baths: 60°C ,another for step 4 and 70°C for step 6

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

- **II. For bacteria in biological fluids**
  1. Collect bacteria by centrifuging biological fluids at 7,500 rpm (5,000 xg) for 10 min.
  2. Follow the General Protocol starting from step 2.

- **III.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 bacteria by centrifuging at 7,500 rpm (5,000 xg) for 10 min.
  3. Follow the General Protocol starting from step 2.

- **IV. For Gram-positive bacteria**

Hint: Preheat two dry baths or two water baths before the operation: one to 60°C and the other to 95°C for step 7.

1. Transfer 1 ml well-grown bacterial culture to a microcentrifuge tube (not provided).
2. Descend the bacterial cells by centrifuging at full speed for 2 min and discard the supernatant completely.
3. Resuspend the cell pellet in 200 µl lysozyme reaction solution (20 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton).
4. Incubate at 37 °C for 30 min.
5. (Optional): If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate for 2 min at room temperature.
6. Add 20 µl Proteinase K to the sample, and then add 200 µl FSTG2 Buffer to the sample. Mix thoroughly by pulse-vortexing.
7. Incubate at 60 °C for 30 min and then for a further 15 min at 95°C .
8. Follow the General Protocol starting from step 9.

For Yeast

Additional equipment: RNAse A (optional) -Ethanol 96-100%
Zymolyase or Lyticase , 200U for one preparation
Sorbitol buffer (1M sorbitol: 100 mM EDTA, 14 mM Beta-mercaptoethanol
Set: dry or water baths: one to 30°C ,another 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 yeast cells by centrifuging at 7,500 rpm (5,000 x g) for 10 min and discard the supernatant completely.
3. Resuspend the cell pellet in 600 µl sorbitol buffer (1 M sorbitol; 100 mM EDTA; 14 mM ß-mercaptoethanol). Add 200 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 the supernatant by pipetting.
5. Follow the General Protocol starting from step 2.

For Dried Blood Spot

Additional equipment: RNAse A (optional) -Ethanol 96-100%
Hint: Preheat three dry baths or three water baths before the operation: one to 85 °C for step 2,
another to 60 °C for step 5 and the other to 70 °

1. Cut the filter paper (e.g. S&S 903) with dried blood spot into a microcentrifuge tube (not provided).
2. Add 200µl FSTG1 Buffer and incubate at 85 °C for 10 min.
3. Briefly spin the tube to remove drops from the inside of the lid.
4. Add 20µl Proteinase K to the sample mixture. Mix thoroughly by vortexing.
5. Incubate at 60 °C for 1 hr. Vortex every 10-15 min during incubation.
6. Follow the General Protocol starting from step 9.

Special protocol for extraction of Gram-positive bacterial DNA from Saliva

The additional requirement:

1. PBS
2. Lysozyme reaction solution: (20 mg/ml lysozyme; 20 mM Tris-HCI, pH 8.0; 2mM EDTA; 1.2 % Triton X-100) (200ul/preparation)
3. Incubator at 37 °C for step 4
4. Two incubators for step 6: one to 60 °C and the other to 95 °C
5. RNase A (50mg/ml) for Optional Step (step 7)
6. Absolute ethanol (96~100 %)

Protocol

1. Transfer 1 ml saliva sample to a 15 ml centrifuge tube. Add 4 ml PBS (not provided) and mix well.
2. Centrifuge the tube at 1,800 x g for 5 min.
3. Carefully discard the supernatant. Resuspend the pellet in 200 ul lysozyme reaction solution and transfer the sample mixture to a 1.5 ml microcentrifuge tube.
4. Incubate the sample mixture at 37 °C for 30 min.
5. Add 20 ul Proteinase K to the sample mixture and add 200 ul FSTG2 Buffer to the sample mixture. Mix thoroughly by pulse-vortexing.
6. Incubate at 60 °C for 30 min and then for a further 15 min at 95 °C.
7. (Optional): If RNA-free genomic DNA is required, add 6 ul of 50 mg/ml RNase A to the sample mixture and incubate at room temperature for 2 min .
8. Add 200 ul ethanol (96- 100 %) to the sample mixture. Mix thoroughly by pulse-vortexing for 10 sec.
9. Combind a FSTG Mini Column with a Collection Tube. Transfer the sample mixture (including any precipitate) carefully to the FSTG Mini Column. Centrifuge for 1 minute and place the FSTG Mini Column to a new Collection Tube.
10. Add 500 ul W1 Buffer to the FSTG Mini Column, centrifuge for 1 min, discard the flow-through and place the FSTG Mini Column back to the Collection Tube.
11. Add 750 ul W2 Buffer to the FSTG Mini Column, centrifuge for 1 min, discard the flow-through and place the FSTG Mini Column back to the Collection Tube.
12. Repeat step 11
13. Centrifuge for an additional 3 min to dry the FSTG Mini column.

Important Step! The residual liquid can affect the quality of DNA and inhibit subsequent enzymatic reactions.

14. Place the FSTG Mini Column to a Elution Tube.
15. Add 100 ~ 200 ul of Elution Buffer or ddH2O (pH 7.5- 9.0) to the membrane center of the FSTG Mini Column. Stand the FSTG Column for 1 minutes.
    - Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
    - Standard volume for elution is 200 ul. If less sample to be use, reduce the elution volume (50~150 Ul) to increase DNA concentration.
16. Centrifuge for 2 minutes to elute total DNA.

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Special Protocol for Extraction of DNA from Amniotic Fluid:

1. Transfer up to 1 ~ 3 ml amniotic fluid to a entrifuge tube (not provided). Centrifuge at 10,000 x g for 5 min then remove the supernatant.
2. Wash the cell pellet with 1 ml of PBS. Centrifuge at 10,000 x g for 3 min then remove the supernatant completely.
3. Add 200 µl of PBS and resuspend the cells by pipetting. Transfer the sample mixture to a 1.5 ml micro-centrifuge tube. (not provided)
4. **(Optional)**: If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A to the sample and incubate for 2 min at room temperature.
5. Add 20 µl Proteinase K and 200 µl FSTG2 Buffer to the sample. **Mix thoroughly by pulse-vortexing.** - Do not add Proteinase K directly to FSTG2 Buffer.
6. Incubate at 60 ^{o}C for 15 minutes to lyse the sample. **During incubation, vortex the sample every 3-5 minutes.**
7. Centrifuge the tube at 10,000 x g for 3 min and transfer the clarified supernatant to a new 1.5 ml microcentrifuge tube. (not provided)
8. Add 200 µl of ethanol (96- 100 %) to the sample mixture. **Mix thoroughly by pulse-vortexing for 30 sec.**
9. Briefly spin the tube to remove drops from the inside of the lid.
10. Place a FSTG Mini Column in a Collection Tube. Transfer the sample mixture (including any precipitate) carefully to the FSTG Mini Column. Centrifuge at 8,000 x g for 30 sec **then place the FSTG Mini Column to a new Collection Tube.**
11. Wash the FSTG Mini Column with 500 µl W1 Buffer by centrifuge at 8,000 x g for 30 sec then discard the flow-through.
12. Wash the FSTG Mini Column with 750 µl Wash Buffer by centrifuge at 8,000 x g for 30 sec then discard the flow -through. - Make sure that ethanol has been added into Wash Buffer when first open.
13. **Centrifuge the FSTG Mini Column at full speed (~18,000 x g) for an additional 3 minutes to dry the column.**
14. Place the FSTG Mini Column to Elution Tube.
15. Add 100 ~ 200 µl of Elution Buffer or ddH2O (pH 7.5- 9.0) to the membrane center of FSTG Mini Column. **Stand FSTG Column for 3 minutes.**
    - **Important Step!** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
16. Centrifuge at full speed (~18,000 x g) for 1 min to elute total DNA

Trouble Shooting

Low or no yield of genomic DNA	1. Low amount of cells in the sample • Concentrate a larger volume of a new sample to 200 µl. 2. Poor Cell Lysis-because of insufficient Proteinase K Activity • Repeat the extraction procedure with a new sample. Use a fresh or well-stored Proteinase K stock solution. 3.1. Poor Cell Lysis-because of insufficient mixing with FSTG2 buffer • Repeat the extraction procedure with a new sample. Mix the sample and FSTG2 Buffer immediately and thoroughly by pulse-vortexing. 3.2. Poor Cell Lysis-because of insufficient incubation time • Repeat the extraction procedure with a new sample. Extend the in cubation time and make sure that no residual particulates remain. 3.2. Ethanol is not added into the lysate before transferring into FSTG Mini Column • Repeat the extraction procedure with a new sample. 4.1. Incorrect preparation of wash buffer-Ethanol is not added into wash buffer when first open • Make sure that the correct volumes of ethanol (96-100%) is added into wash buffer when first open. Repeat the extraction procedure with a new sample. 4.2. Incorrect preparation of wash buffer-The volume or the percentage of ethanol is not correct before adding into wash buffer • Make sure that the correct volumes of ethanol (96-100%) is added into wash buffer when first open. Repeat the extraction procedure with a new sample. 5.1. Elution of genomic DNA is not efficient-pH of water (ddH2 O) for elution is acidic • Make sure the pH of ddH2O is between 7.5-9.0 or use Elution Buffer (provided) for elution. 5.2. Elution of genomic DNA is not efficient-Elution buffer or ddH2O is not completely absorbed by column membrane • After Elution buffer or ddH2O is added, stand the FSTG Mini column for 5 min before centrifugation.

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Brown residues remain on the column membrane after washing	1.1 Poor Cell Lysis-because of insufficient Proteinase K Activity • Repeat the extraction procedure with a new sample. Use a fresh or well store Proteinase K stock solution. • Don't add Proteinase K Directly to FSTG2 buffer. 1.2 Poor Cell Lysis-because of insufficient mixing with FSTG2 buffer • Repeat the extraction procedure with a new sample. Mix the sample and Buffer immediately and thoroughly by pulse-vortexing. 1.3 Poor Cell Lysis-because of insufficient incubation time • Repeat the extraction procedure with a new sample. • Extend the Incubation time and make sure that no residual particulates 2.1 Ethanol is not added into the lysate before transferring the sample mixture into FSTG Mini Column • Repeat the extraction procedure with a new sample 3.1 Incorrect preparation of Wash Buffer -Ethanol is not added into Wash Buffer when first open • Make sure that the correct volumes of ethanol (96-100%) is added into Wash Buffer when first open. • Repeat the extraction procedure with a new sample. 3.2 The volume or the percentage of ethanol is not correct before adding into Wash Buffer • Make sure that the correct volumes of ethanol (96-100%) is added into wash buffer when first open • Repeat the extraction procedure with a new sample
Column is clogged	1.1 Lysate contains insoluble residues • Remove insoluble residues (e.g. filter paper, bone or hari) before centrifuging. 1.2 Sample is too viscous • Reduce the sample volume 1.3 Insufficient activity of proteinase K • Use a fresh or well-stored Proteinase K stock solution. • Repeat the extraction procedure with a new sample. Do not add Proteinase K into FSTG2 buffer directly.

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Poor quality of genomic (DNA A_{260} /A_{280} ratio of eluted DNA is low)	1.1 Poor Cell Lysis-because of insufficient Proteinase K activity • Repeat the extraction procedure with a new sample. Use a fresh or well store Proteinase K stock solution. • Don't add Proteinase K Directly to FSTG2 buffer. 1.2 Poor Cell Lysis-because of insufficient mixing with FSTG2 buffer • Repeat the extraction procedure with a new sample. Mix the sample and FSTG2 Buffer immediately and thoroughly by pulse-vortexing. 1.3 Poor Cell Lysis-because of insufficient incubation time • Repeat the extraction procedure with a new sample. Extend the Incubation time and make sure that no residual particulates remain. 2.1 Ethanol is not added into the lysate before transferring the sample mixture into FSTG Mini Column • Repeat the extraction procedure with a new sample 3.1 Incorrect preparation of Wash Buffer -Ethanol is not added into Wash Buffer when first open • Make sure that the correct volumes of ethanol (96-100%) is added into Wash Buffer when first open. • Repeat the extraction procedure with a new sample. 3.2 The volume or the percentage of ethanol is not correct before adding into Wash Buffer • Make sure that the correct volumes of ethanol (96-100%) is added into wash buffer when first open • Repeat the extraction procedure with a new sample 3.3 Genomic DNA is contaminatd • Don't wet the rim of the column during sample and buffer lodading.
Poor quality of genomic (DNA-A /A ratio of eluted DNA is high)	1.1 A lot of residual RNA in eluted DNA • Follow the general protocol step 6 to remove RNA 1.2 FSTG2 Buffer added to the sample before adding RNase A • Make sure that RNase A has been added to the sample before adding FSTG2 Buffer when using optional RNase A step.
Degradation of eluted DNA	1.1 Sample is old • Always use fresh or well-stored sample for genomic DNA extraction 1.2 Buffer for gel electrophoreses contaminated with Dnase • Use fresh running buffer for gel electrophoresis 1.3 Buffer for gel electrophoresis contaminated with Dnase • Genomic DNA extracted from paraffin-embedded tissue is usually It is still suitable for PCR reaction, but is not recommended for Southern blotting and restriction analysis

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