FavorPrep™
Tissue Genomic
DNA Extraction Mini Kit

User Manual

Cat. No.: FATGK 001 (50 Preps)
FATGK 001-1 (100 Preps)
FATGK 001-2 (300 Preps)

For Research Use Only

Introduction

Tissue Genomic DNA Extraction Mini Kit is an excellent tool offering a speedy and economic method to purify total DNA from several types of animal tissue. Some specially modified protocols are developed for other samples, such as bacterial, fixed tissue, yeast etc. This technology first lyses cells and degrades protein by using a chaotropic salt and Proteinase K, then binds DNA to silicabased membranes, washes DNA with ethanol-contained wash buffer and then elutes purified DNA by low salt elution buffer or ddH_2O. Compare with other harmful and time-consuming procedures, this kit shortens the handling time within 1 hour (lysis time varies depending on the type of tissue processed.) The size of purified DNA is up to 50Kb (predominantly 20-30 Kb). DNA of this length performs the highest efficiency during PCR reaction. After using this kit, the high quality total DNA can be used directly for the down-stream applications.

Quality Control

The quality of Tissue Genomic DNA Extraction Mini Kit is tested on a lot-to-lot basis. The kits are tested by isolation of genomic DNA from 10 mg of mouse liver. Prufied DNA is quantified with a spectrophotometer. Genomic DNA yield is more than 10μg with A_{260}/A_{280} ration 1.7 to 1.9

Sampling: up to 25mg of fresh or frozen tissue
Protocols Included: Tissue, bacteria, yeast, fixed tissue, Buccal Swab
Yield: Up to 50μg of total DNA, depends on the samples types.
Formate: Spin column
Operation time: 30-60 min

Procedure

	FATGK001 (50preps)	FATGK001-1 (100preps)	FATGK001-2 (300preps)
FATG 1 Buffer	15 ml	30 ml	70 ml
FATG 2 Buffer	15 ml	30 ml	70 ml
W1 Buffer*	22 ml	44 ml	124 ml
Wash Buffer**	10 ml	20 ml	50 ml
Elution Buffer	15 ml	30 ml	30 mlx3
Proteines K***	11 mg	11 mgx2	11 mgx6
Collection Tube	100 pcs	200 pcs	600 pcs
Elution Tube	50 pcs	100 pcs	300 pcs
FATG Mini Colllumn	50 pcs	100 pcs	300 pcs
Micropestle	50 pcs	100 pcs	300 pcs

*Add 8 / 16 / 45 ml ethanol (96~100%) to W1 Buffer when first open.

**Add 40 /80 / 200 ml ethanol (96~100%) to Wash Buffer when first open.

***Add 1.1 ml sterile ddH_2O to each Proteinase K tube to make a 10mg/ml stock solution.

Caution

The component contains irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

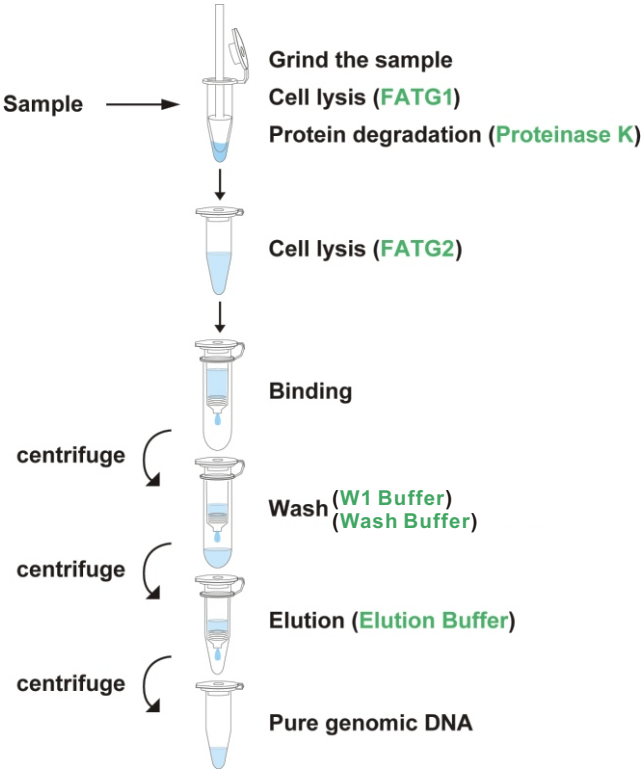
References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Important Notes

1. Buffers provided in this kit contain irritants. Wear glove and lab coat when handling these buffers.
2. Add 1.1 ml sterile ddH_2O to each 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℃.
3. For FATGK 001-1 (50 preps), add 8ml ethanol (96~100%) to W1 Buffer when first open. For FATGK 002-1 (100preps), add 16ml ethanol (96~100%) to W1 Buffer when first open.
4. For FATGK 001-1 (50 preps), add 40ml ethanol (96~100%) to Wash Buffer when first open. For FATGK 002-1 (100preps), add 80ml ethanol (96~100%) to Wash Buffer when first open.
5. Preheat two dry baths or two water baths before the operation one to 60℃ the other 70℃.
6. Equilibrate the sample to room temperature.
7. All centrifuge steps are done at full speed (14,000 rpm or 10,000 xg) in a microcentrifuge.

Prcedure



General Protocol

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℃ for step4 and other to 70℃ for step7.

Step 1 **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 FATG1 Buffer and homogenize the tissue sample more completely with micropestle.**
Step 2 **Lysis**	**3. Add 20 µl 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 1hr, depends on the sample types). **Vortex every 10-15 min during incubation.** **5. Briefly spin the tube to remove drops from the inside of the lid.** **6. (Optional)If RNA free genomic DNA is required, add 4 µ of RNase A (100 mg/ml) and incubate for 2 min at room temperature.** **7. Add 200 µl FATG2 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 3 **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 FATG Mini Column in a Collection Tube. Transfer the sample mixture (including any precipitate) carefully to FATG Column. Centrifuge for 1 min and discard the flow-through then place FATG Column to a new Collection Tube.**
Step 4 **Wash**	**12. Wash FATG Column with 500 µl W1 Buffer by centrifuge for 1 min then discard the flow-through.** ・Make sure that ethanol has been added into W1 Buffer when first open. **13. Wash FATG Column with 750 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. **14. 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**	**15. Place FATG Column to Elution Tube. Add 50~200 µl Elution Buffer or ddH_2O (pH 7.5 ~ 8.5) to the membrane center of FATG Column. Stand FATG 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 200µl. If tissue sample has a low number of cells, reduce the elution volume(50-150µl) to increase DNA concentration. **16. Centrifuge for 2 min to elute total DNA.** **17. Store total DNA at 4°C or -20°C.**

Spcial Protocol

●For bacteria

I. 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. Desend the bacteria cells from biological fluids by centrifuging 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. Desend the bacteria cells 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 and then 200 µl FATG2 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 8.

For fixed tissues

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. Centrifuge at full speed for 5 min. Remove the supernatant by pipetting. Add 1 ml ethanol (96 ~ 100%) to the deparaffined tissue, mix gently by vortexing.
3.Centrigue 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.Centrifuge at full speed for 5 min. Remove the supernatant by pipetting.
6.Incubate at 37°C for 10 min to evaporate ethanol residue.
7.Grind the tissue sample by micropestle or liquid nitrogen and follow the General Protocol starting from step 2.

For formalin-fixed tissues

1. Wash 25mg tissue sample twice with 1 ml PBS to remove formalin.
2. Grind the tissue sample by micropestle or liquid nitrogen and follow the General Protocol starting from step 2.

For yeast

1.Transfer 3 ml log-phase (OD_{600} =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 β-mercaptoethanol). Add 200 U zymolase 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

HINT: Preheat three dry baths or three water baths before the operation: one to 85℃ for step 2, another to 60℃ for step 5 and the other to 70℃ for General Protocol step 7.

1. Cut the filter paper (e.g. S&S 903) with dried blood spot into a microcentrifuge tube (not provided).
2. Add 200 µl FATG1 Buffer and incubate at 85℃ for 10 min.
3. Briefly spin the tube to remove drops from the inside of the lid.
4. Add 20 µl Proteinase K to the sample mixture. Mix thoroughly by vortexing.
5. Incubate at 60℃ for 1 hr. Vortex every 10-15 min during incubation.
6. Follow the General Protocol starting from step 5.

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 FATG2 buffer*** ・Repeat the extraction procedure with a new sample. Mix the sample and FATG2 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 FATG 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.Elutoin of genomic DNA is not efficient-pH of water (ddH_2O) 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. Elutoin of genomic DNA is not efficient-Elution buffer or ddH_2O is not completely absorbed by column membrane*** ・After Elution buffer or ddH_2O is added, stand the FATG Mini column for 5 min before centrifugation.

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 FATG2 buffer. ***1.2 Poor Cell Lysis-because of insufficient mixing with FATG2 buffer*** · Repeat the extraction procedure with a new sample. Mix the sample and FATG2 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 FATG 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. ***3.2The 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	***1.1Lysate contains insoluble residues*** · Remove insoluble residues (e.g. filter paper, bone or hari) before centrifuging. ***1.2Sample is too viscous*** · Reduce the sample volume ***1.3Insufficient 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 FATG2 buffer directly.

Poor quality of genomic (DNA A_{260}/A_{280} ratio of eluted DNA is low)	***1.1Poor 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 ・Don't add Proteinase K directly to FATG2 buffer ***1.2 Poor Cell Lysis-because of insufficient mixing with FATG2 buffer*** ・Repeat the extraction procedure with a new sample. Mix the sample and FATG2 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 FATG Mini Column*** ・Repeat the extraction procedure with a new sample ***3.1Incorrect 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. ***3.2Incorrect preparation of Wash Buffer-The volume or the percentage of ethanol is not correct before adding into washing 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. ***3.3Genomic DNA is contaminated*** ・Don't wet the rim of the column during sample and buffer lodading.
Poor quality of genomic (DNA-A_{260}/A_{280} 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 ***1.2FATG2 Buffer added to the sample before adding RNase A*** ・Make sure that RNase A has been added to the sample before adding FATG2 Buffer when using optional RNase A step.

Degradation of eluted DNA	***1.1Sample is old*** Always use fresh or well-stored sample for genomic DNA extraction ***1.2Buffer for gel electrophoreses contaminated with DNase*** Use fresh running buffer for gel electrophoresis ***1.3Buffer for gel electrophoresis contaminated with DNase*** Genomic DNA extracted from paraffin-embedded tissue is usually degraded. It is still suitable for PCR reaction, but is not recommended for Southern blotting and restriction *analysis**.***