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GENE PREP

Application Guide

DNA Tissue

I. **Purpose:** To extract genomic DNA from mouse tail and other types of animal tissues.

II. **Chemical Principal:** Proteinase K digestion & organic extraction method.

IIIA. Preparation of Tissue Digest (manual ProK digestion for mouse tail):

A. Cut 5 - 10 mm of tail (about 10-20 mg) and place it into AutoGen Tube Unit
B. Add 250 µl of a buffered Proteinase K solution (250µl of Reagent GPR-1 including 0.8mg/ml Proteinase K) and 250µl of Reagent GPR-2.
C. Incubate samples at 55-60°C overnight, and load tube unit onto GENE PREP

IIIB. Preparation of Tissue Digest (on-board ProK digestion for mouse tail):

A. Cut 5 - 8 mm of tail (about 10 mg) and place it into AutoGen tube unit
B. Load AutoGen Tube Unit onto the GENE PREP

IV. Protocol Parameters:

A. Sample Volume: 0.5 ml of digest solution.

B. Maximum Number of Samples: 48 samples per run
192 samples per run (with an optional Tube Unit Stacker)

C. Processing Time: 2.1hours for 48 samples (if digest is prepared manually)
5.5 hours for 48 samples (if digestion is done on GENE PREP)
(Includes 40 min drying and re-hydrating time).

D. Yield: 1 - 2 µg of DNA per 1mg of mouse tail (manual digestion).
0.5 - 1 µg of DNA per 1 mg of mouse tail (on-board digestion)

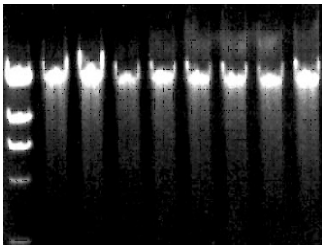
E. Quality: Typical OD260/280 values are >= 1.8*
The DNA can be used directly in downstream processes such as fluorescence DNA sequencing, PCR, Southern blotting* and restriction endonuclease digestions.
** If the tissue is digested on GENE PREP, the OD260/280 is lower (around 1.7) and the DNA may not be suitable for Southern blotting applications.*

V. Running the Protocol:

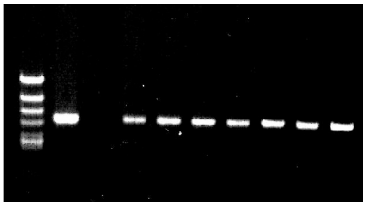
A. Load Reagents and Samples
B. Select a Protocol
C. Enter Number of Samples
D. Start the Run

VI. Example of Data:

Genomic DNA obtained from mouse tail
Stating Sample: 5-10 mm mouse tail (about 10-20 mg)
Digestion: 55°C for 14hrs manually
Gel Condition: 5 µl of 100ul resuspended DNA
1% agarose gel, 100V x 1hr



PCR of mouse tail DNA
Template: 200ng of the above DNA
Target Region: ICAM-1 (535bp)
PCR Reaction: 35cycles
Gel Condition: 10 µl of 25ul PCR reaction
1% agarose gel, 100V x 1hr
øX174 Hinc II size marker
C+/C-: positive and negative controls



VI. Extraction and Purification Process

Process Site	Purpose	System Process
1. Automated (or Manual)	Digest samples	Dissolve ProK in Reagent TD-M1, and Reagent TD-M2 to sample and incubate at 55-60C.
2. Automated	Precipitate protein	Add Reagent TD-R3, mix and centrifuge. Transfer supernatant to DNA tube.
3. Automated	Precipitate DNA	Add Reagent TD-R4, agitate, centrifuge and discard supernatant.
4. Automated	Wash DNA	Add Reagent TD-R5, mix, centrifuge and discard supernatant. Repeat a few times.
5. Automated	Dry DNA	Transfer DNA tube to agitation rack.
6. Automated	Resuspend DNA	Add Reagent TD-R6, mix and centrifuge.

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