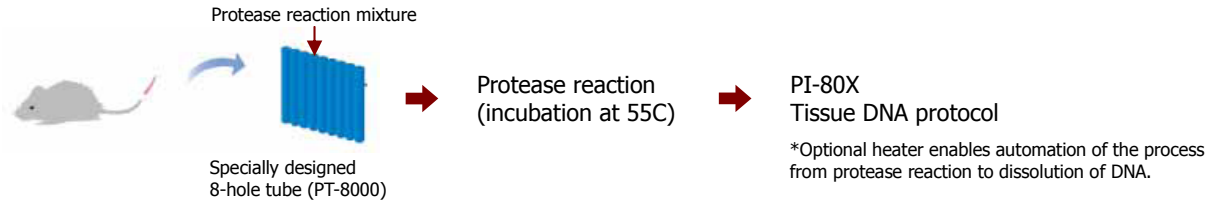
PI-80X Tissue DNA protocol

Genomic DNA isolation from Protease K digested mouse tissue

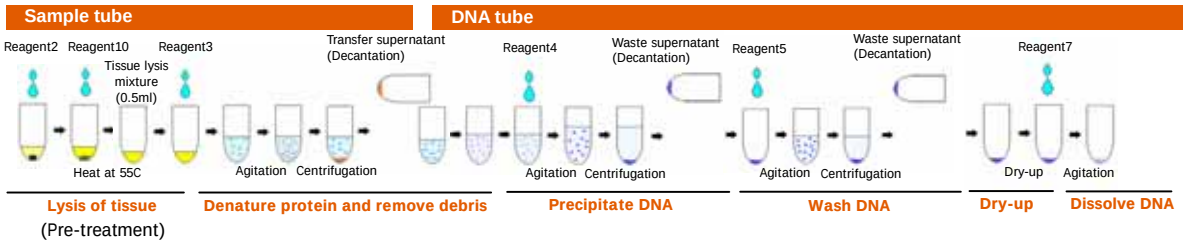
KURABO PI-80X is an automated DNA isolation system with throughput of 48 samples at a time. This data sheet shows an example of DNA isolation from protease digested mouse tissues. The specially designed 8-hole tube makes isolation process easy.



Experiment

Sample	Protease K digested mouse tail, brain, liver, kidney and heart.
Sample amount	Tail: 10mg The other tissues: 20mg
Isolation system and protocol	PI-80X Tissue DNA protocol
Reagents kit and consumables	NR-201 Sample tube, PT-8000; DNA tube, NT-8000
Chemical principle	Lysis of tissue: Protease K digestion (pre-treatment) Purification: Phenol extraction DNA isolation: Precipitation by alcohol
Process	**Pre-treatment:** Digest with 0.2mg/ml of protease K at 50-55C for 15hr. Reaction component is as follow; 0.25ml0.4mg/ml protease K in Protease dissolving reagent (No.2, NR2025) 0.25mlSuspending reagent (No. 10, NR10025) Total volume 0.5ml **DNA isolation with PI-80X:** Step1. Denature protein and remove debris Step2. Precipitate DNA Step3. Wash DNA Step4. Dry up Step5. Dissolve DNA in DNA dissolving reagent (KURABO, NR7025) Final volume 100ul

Workflow of Tissue DNA protocol



Processing time (PI-80X)
48 samples: 2.7hr
48 samples: 2.1hr with optional heater unit.

Analysis methods

Yield and purity calculation
Absorbance of 260nm was measured for each DNA solution by spectrometer.
DNA yield was calculated as follows:
A260 X 50 X dilution factor X final volume.
DNA purity was evaluated by the ratio of A260/A280.

Electrophoresis
Each 5µl of DNA solution was loaded on a 0.7% agarose gel.

Restriction enzyme digestion
Each 1ug of DNA isolated from mouse tail was digested with 10units of enzymes for 15hours at the optimal temperature, respectively.
Restriction enzyme: *Eco*RI, *Kpn*I, *Sac*I and *Bam*HI

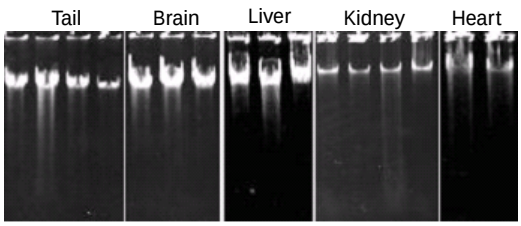
PCR amplification
Template: 200ng of isolated DNA from mouse tail
Target gene: Mouse ICAM 1, exon 6-7, amplicon size 535bp
Polymerase: AmpliTaq Gold® DNA Polymerase (0.6U)
PCR condition: 94C, 5min x 1 cycle
94C, 20sec/68C, 40sec/72C 1.5min x 35 cycles
72C, 7min x 1 cycle
Reaction volume: 25ul
Electrophoresis: 10ul of 25ul reaction mixture was loaded on a 1.8% agarose gel.

Results

Yield and purity

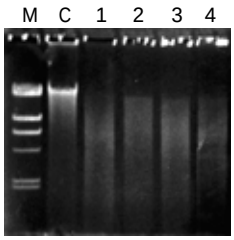
Sample	Tail	Brain	Liver	Kidney	Heart
Yield (ug)	20.1/10mg	30.8/20mg	22.7/20mg	21.5/20mg	17.5/20mg
Purity (A260/A280)	1.87	1.93	1.70	1.93	1.89

Electrophoresis



Each 5ul of 100ul DNAsolution isolated from mouse tissues.
The starting amount is;
tail: 10mg
the other tissues: 20mg

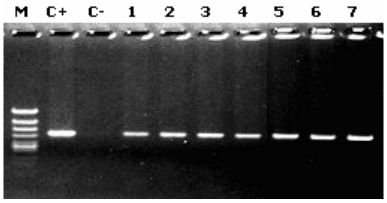
Restriction enzyme digestion



Each 1ug of DNA isolated from mouse tail was digested with restriction enzymes.

M：λ*Hin*dIII size maker
C：undigest DNA
1：*Eco*RI
2：*Kpn*I
3：*Sac* I
4：*Bam*HI

PCR amplification



M: ϕX174 HinII size marker
C+: Positive contol
C-: Negative contol
1-7: Amplicon of isolated DNA

Ordering information

DNA isolation system

PI-80X
Tissue DNA protocol

Reagents kit

Tissue DNA isolation reagents kit NR-201 (approx. for 650 preps)

Reagent name	Reagent No. (Code)	Content
Proteinase dissolving reagent	2 (NR2025)	1
Deproteinization reagnet	3 (NR3025)	1
Precipitating reagent	4 (NR4050)	1
Washing reagent	5 (NR5050)	3
DNA dissolving reagent	7 (NR7025)	1
Suspending reagent	10 (NR10025)	1

Processing tube

Sample tube: PT-8000 (8-hole, blue, 64pieces)

DNA tube: NT-8000 (8-hole, white, 64pieces)

KURABO INDUSTRIES LTD.
Bio-Medical Department
4-31, 2-chome, Kyutaro-machi, Chuo-ku
Osaka 541-8581, Japan
Phone: 81-6-6266-5010
Fax: 81-6-6266-5011
bio@ad.kurabo.co.jp