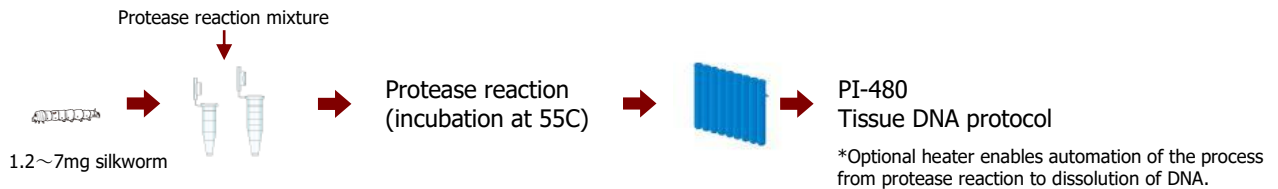


PI-480 Tissue DNA protocol

Genomic DNA isolation from protease K digested silkworm



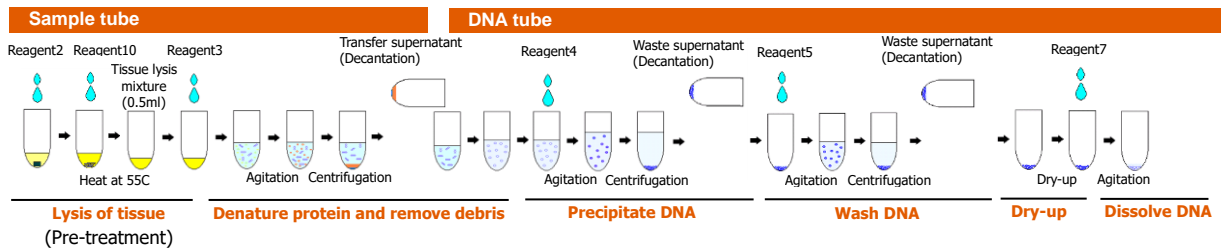
KURABO PI-480 is an automated DNA isolation system with throughput of 48 samples at a time. This data sheet shows an example of DNA isolation from protease digested silkworm. The specially designed 8-hole tube makes isolation process easy.



Experiment

Sample	Protease K digested silkworm.
Sample amount	Silkworm whole body : 1.2 ~ 7mg
Isolation system and protocol	PI-480 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	<p>Pre-treatment: Digest with 0.2mg/ml of protease K at 50-55C for 15hr. Reaction component is as follow;</p> <p>0.25ml 0.4mg/ml protease K in Protease dissolving reagent (No.2, NR-2025) 0.25ml Suspending reagent (No. 10, NR-10025)</p> <hr/> <p>Total volume 0.5ml</p> <p>DNA isolation with PI-480: Step1. Denature protein and remove debris Step2. Precipitate DNA Step3. Wash DNA Step4. Dry up Step5. Dissolve DNA in DNA dissolving reagent (KURABO, NR-7025) Final volume 100ul</p>

Workflow of Tissue DNA protocol



Processing time 48 samples: 2.7hr
(PI-480) 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:
 $A_{260} \times 50 \times \text{dilution factor} \times \text{final volume}$.
 DNA purity was evaluated by the ratio of A_{260}/A_{280} .

Electrophoresis Each 10 μ l of DNA solution was loaded on a 0.7% agarose gel.

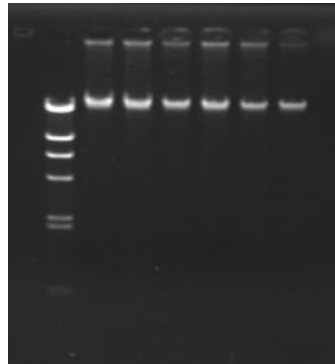
PCR amplification Template: ~100ng of isolated DNA from silkworm
 Target PCR region : R1F1—R1R1,
 Polymerase: Takara Ex Taq DNA Polymerase (0.5U)
 PCR condition: 95C, 5sec x 1 cycle
 95C, 10sec/55C, 15sec/72C 30sec x 40 cycles
 72C, 7min x 1 cycle
 Reaction volume: 10ul
 Electrophoresis: 10ul of 10ul reaction mixture was loaded on a 2% agarose gel.

Results

Yield and purity

Sample	No.1	No.2	No.3	No.4	No.5	No.6
Yield (ug)	18.7/6.2mg	21.4/7.0mg	17.2/6.7mg	17.7/6.1mg	14.0/2.5mg	5.0/1.2mg
Purity (A_{260}/A_{280})	2.1	2.1	2.2	2.0	2.1	2.2

Yield (ug) : DNA + RNA by spectrophotometer

Electrophoresis


Each 10ul of 100ul DNA solution isolated from silkworm.
The starting amount is;
Weight : 1.2 ~ 7mg

M : λ -HindIII size maker

PCR amplification


M: ϕ X174 HinII size marker

1-6: Amplicon of isolated DNA

Ordering information

DNA isolation system PI-480
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 (NR-2025)	1
Deproteinization reagent	3 (NR-3025)	1
Precipitating reagent	4 (NR-4050)	1
Washing reagent	5 (NR-5050)	3
DNA dissolving reagent	7 (NR-7025)	1
Suspending reagent	10 (NR-10025)	1

Processing tube Sample tube: PT-8000 (8-hole, blue, 64pieces)
DNA tube: NT-8000 (8-hole, white, 64pieces)

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