DNA extraction

Preparation:

Material	Quantity per sample	Note
DNA extraction buffer	400 µL	
Isopropanol (2-propanol)	300 µL	Around 70% of the supernatant
Ethanol (EtOH)	1000 µL	DNase-free ethanol
Tris-EDTA	100 µL	Commercial product

Procedure

Step 1: Cell lysis

> Caution:
> If directly powderized the sample with liquid nitrogen within the ependorf, after filling in the liquid nitrogen into the eppendorf, **DO NOT** close the lid, the eppendorf will explode as temperature raise.

1. Put two steel balls into each 1.5 mL eppendorf;

> - The steel balls must had been washed with 70% EtOH and autoclaved.

2. Add **400 µL DNA extraction buffer** into each eppendorf;
3. Directly submerge the samples into the DNA extraction buffer in each eppendorf;
4. Homogenize the samples and the buffer with a homogenizer;

> - **There are two containers for the homogenizer;**
> - each containers should be balanced (the amount and placement of the samples);
> - put a tissue on top of the eppendorfs' lid, this can prevent the solution seep out to the container bottom, and also eliminates the space between the container and the cover, improves grinding efficiency;
> - after mounted the containers on the machine, fasten tight the big roller, and stuck in the tenons.

5. Confirm the machine settings and start homogenizing;

> - Frequency : 30 hits / sec (maximum frequency)
> - Time : 2.0 mins × 3

6. Centrifuge at room temperature (> 15000 rpm, 20 mins) to bring down the bubbles;
7. Dry bath (60°C, > 1 hour);

Step 2: Disrupt protein—DNA interactions

8. Add **400 μL 5 M NaCl** (same volume with the extraction buffer, therefore the NaCl final concentration will be 2.5 M);
9. Centrifuge at room temperature (> 15000 rpm, 20 mins) to bring down the remaining tissues;

Step 3: Collect DNA supernatant

10. Carefully take the supernatant (around 90% of the supernatant volume) to a new 1.5 mL eppendorf;

Step 4: Precipitate DNA

11. Add **300 μL isopropanol** (around 70% of the supernatant volume) into the supernatant, leave overnight at -20°C;
12. Centrifuge at 4°C (> 15000 rpm, 20 mins) to bring down the DNA;
13. Discard the supernatant carefully. Do not touch the tube bottom, which sticking with the DNA palette;

Step 5: Wash

14. Add **200 μL 70% ethanol** and gently invert the tube;
15. Centrifuge at 4°C (> 15000 rpm, 20 mins) to bring down the DNA;
16. Discard the ethanol carefully with small pipette;

Step 6: Elute DNA

17. Dry bath at 60°C for around 10 mins, the ethanol should be totally gone;
18. Add **50 μL DNase-free water or TE buffer (pH 8.0)** and dry bath at 60°C for around 10 mins to dissolve the DNA palette;
19. After the DNA was fully dissolved, slightly centrifuge at room temperature to bring down all solution on the tube wall;

- Store the purified DNA at −20°C for up to 2 years. Avoid repeated freeze–thaw cycles and vigorous mixing.

Step 7: DNA quality assurance:

20. Check the DNA concentration and quality.

- Reference: https://ntuhmc.ntuh.gov.tw/epaper-57th.htm

21. Standardize the DNA concentration according to the lowest concentration of the samples.

- At least 10 ng / μL for PCR
- the diluted DNA should be used as soon as possible

Wavelength detection

Wavelength (nm)	Detected Compound
230	EtOH, EDTA, carbohydrates, phenol, Guanidone HCL (for DNA isolation)
260	RNA,ssDNA, dsDNA, guanidine isothiocyabate (for RNA isolation)
270	Phenolic solution (TRIzol for RNA isolation)
280	Protein, phenol, other contamination

Ideal wavelength ratio (quality indicator):

Nucleic acid	260 / 280 nm	260 / 230 nm	260 / 270 nm
DNA	~ 1.8	> 2.0	> 1.2
RNA	~ 2.0	> 2.0	> 1.2

Stock solution recipe

† DNA extraction buffer

Chemical	Concentration	Addition
1 M Tris-HCl (pH 7.5) ※	200 mM	20 mL
5 M NaCl	250 mM	5 mL
500 mM EDTA †	25 mM	5 mL
10% SDS §	0.5% (v/v)	5 mL
ddH_2O		65 mL
Total:		100 mL

Tris-HCl stock solution (1 M)

1. Dissolve 121.1 g of Tris base in 800 mL of ddH_2O;
2. Adjust the pH to the desired value by adding concentrated HCl;
3. Allow the solution to cool to room temperature before making final adjustments to the pH;
4. Adjust the volume of the solution to 1 L with ddH_2O. Dispense into aliquots and sterilize by autoclaving.

pH	12N HCl addition
7.4	70 mL
7.6	60 mL
8.0	42 mL

EDTA stock solution (0.5 M, pH 8.0)

> EDTA (Ethylenediamenetetraacetic acid)

1. Add 186.1 g of disodium EDTA • $2H_2O$ to 800 mL of ddH_2O;
2. Stir vigorously on a magnetic stirrer to dissolve the EDTA;
3. Adjust the pH to 8.0 with NaOH (~ 20 g of NaOH pellets);

> Note: The disodium salt of EDTA will not go into solution until the pH is adjusted to ~ 8.0 by the addition of NaOH.

4. Dispense into aliquots and sterilize by autoclaving.

SDS stock solution (10%)

> Sodium dodecyl sulfate, sodium lauryl sulfate

1. Dissolve 100 g of electropheresis-grade SDS in 900 mL of ddH_2O;
2. Heat to 68°C and stir with a magnetic stirrer to assist dissolution. If necessary, adjust the pH to 7.2 by adding a few drops of concentrated HCl;
3. Adjust the volume to 1 L with ddH_2O;
4. Store at room temperature. Sterilization is not necessary.
   DO NOT autoclave.
