DNA extraction

Buffer solutions usage per sample:

Material	Quantity per sample	Note
DNA extraction buffer	400 μL	See the last page
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, because the eppendorf will explode when temperature raise.

1. Put two sterilized steel balls into each 1.5 mL eppendorf.
2. Add **400 μL DNA extraction buffer** into each eppendorf.
3. Directly submerge the samples into the DNA extraction buffer in each eppendorf.
4. Disrupt the tissue using Tissue Grinder (**30 hits / sec**, **10 mins**).

> - Place a thick layer of tissue paper between the tissue grinder adapter cover and the eppendorf tube caps to boost grinding efficiency and prevent the tube caps from popping open during high-speed reciprocal shaking.

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

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

Step 3: Collect DNA supernatant

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

Step 4: Precipitate DNA

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

Step 5: Wash

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

Step 6: Elute DNA

1. Dry bath at 60°C for around 10 mins, the ethanol should be totally gone;
2. 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;
3. 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.

Done !!!

DNA quality assurance

1. Check the DNA concentration and quality.

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

2. 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 solutions

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), otherwise the disodium salt of EDTA will not dissolve.
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.