DNA extraction protocols

A. DNA extraction from tissue using non-organic solvents:

Buffers Preparation :

Sucrose buffer:

57 gm Sucrose
3.1 gm MgC12.6H2O
0.6 gm Tris. Hcl
500 ml Autoclaved d.d. H2O
adjust pH 7.5 with 0.1 N HCl. The solution is stable if stored at refrigerator for 1 week.

EDTA:

0.72 gm disodium EDTA
250 ml Autoclaved d.d. H20
adjust pH at 7.5 with 0.1 N Na0H
Store at room temperature
EDTA inhibits the action of Dnase enzymes, makes the lysis of nuclear membrane easier.

SDS:

25 gm sodium dodecyl sulfate (SDS)
250 ml d.d. Autoclaved H2O
dissolve 2 gm SDS in 20 ml H2O
Store at room temperature
SDS emulsifies the plasma and nuclear membrane.

2 M NaCl

29.2 gm Nacl
250 ml Autoclaved d.d. H20
Store at room temperature.
Nacl increases ion concentration, which disrupt ionic bonds between DNA and Protein.

Protocol:

1) Dice a piece of fresh tissue " 1 gm liver, 150 mg spleen".

2) Immerse diced tissue in isotonic sucrose buffer (10 ml for liver, 5 ml for spleen) in a blender and homogenize completely.

3) Strain homogenate through cheesecloth to remove clumps of gristle.

4) Pour homogenate equally in centrifuge tubes, spin for 10 min. at 8000 rpm.

5) Pour off suspension, resuspend the pellet in buffered sucrose (1.5 ml).

6) Transfer 1.5 ml well mixed suspended nuclei to small test tube.

7) Add 1 ml 28% EDTA, mix gently, let stand for 5 min.

8) Add 0.5 ml SDS, mix very gently and let stand for 2 min. Be careful at the following steps.

9) Add 300 µl prot. K incubate at 50^{o}C for 30 min.

10) Slowly add 0.3 ml of 2 M Nacl Pause after each drop and mix gently.

11) Draw 2 ml of 95% chilled ethanol, eject it slowly down the side of test tube.

12) Using a long stirring rod, make a slow stirring motion and spool the protein free DNA.

13) DNA may be saved in ethanol resuspended in one ml TE buffer.

B. Extraction of Chromosomal bacterial DNA by diatoms (Total or genomic bacterial DNA):

A) Preparation of diatoms suspension:

1) Add 50 ml H_2O and 500 µl of 35% Hcl to 10 g celite or diatoms.
2) Divide the suspension into aliquots into small bottles which are tightly closed and autoclave, keep in room temperature in the dark.

B) Buffers:

Lysis buffer (L6)

Dissolve 30 gm GuScN into 25 ml of 0.1 M Tris Hcl (pH 6.4) + 5.5 ml of 0.2 M EDTA. Adjusted with NaOH to pH 8.0 + 0.65 ml Triton X-100. Homogenize the solution in shaking water bath at 60°C (stable at room temp. in the dark for at least 3 weeks).

Washing buffer (L2)

Dissolve 30 gm of GuscN in 25 of 0.1 M Tris Hcl (pH 6.4) by heating in shaking water bath at 60 - 65°C. (stable at room temp. in the dark for at least 3 weeks).

Elution buffer (TE)

- 10 mM Tris-Hcl
- 1 mM EDTA pH 8
- 100 X concentrated stock solution must diluted to 1 X working solution.
- These buffers prepared under fume hood.

Reaction vessels

Add 900 µl of lysis buffer (L6) and 40 µl of diatoms suspension (shake well before use) to 1.5 ml ep-tube homogenize the solution by vortexing.
Reaction vessels can be successfully used up to 1 week after assembly.

C) Method:

1) Vortex the reaction vessel.

2) Vortex the overnight bacterial broth culture and take 1 ml in eppindorf tube or 50µl sample (urine or serum).

3) Centrifuge at 13000 for 15 min, then bacterial pellet is suspended in 50 µl autoclaved distilled water or TE buffer, vortex.

4) Add the suspended pellet to the reaction vessel.

5) Leave at room temperature for 10 min. vortex several times during the incubation time.

6) Centrifuge for 2 min. at 13000 rpm.

7) Dispose the supernatant.

8) Diatom NA pellet is washed twice with washing buffer **L2** then twice. with ethanol 70% and once with acetone. Vortex then centrifuge for one minute each time.

9) Dry the vessel at 56°C with open lid ep-tube (use the thermal cycler **or** eppendorf heat block) for 10 min.

10) Add elution buffer TE with or without Rnase then vortex briefly and incubate for 10 min at 56°C (100-75 µl TE).

11) Vortex again and centrifuge at 13000 for 3 min.

12) Use the supernatant containing DNA for further experiment.

C. Extraction of Plasmid DNA (Alkaline lysis method):

Preparation of media & solutions

* **L B Broth** (pH 7.0)

- Tryptone	10 gm
- Yeast extracte	5 gm
- Nacl	5 gm
- 4 N NaoH	1-2 ml
- D.W.	1000 ml

*** Sol.1**

- 50% glucose solution	1.8 ml
- 250 mM EDTA – pH8	4.0 ml
- 1 M tris pH 8.1	2.5 ml
- D.W.	60 ml

*** Sol. II**

a) 10% SDS	store	-20 in aliquots
b) 2 N NaoH	store	-20 in aliquots

Just before use, mix 1 ml of (a) **+** ml of (b) **+** 8 ml D. Water (the total volume is 10 ml).

Electrophoresis

- 0.7% agarose
- 10 ml of DNA + 5 µl dye
- 1µl marker + 7µl TE+ 5µl dye

Method:

1) Inoculate single colony in 5 ml LB Broth.

2) Incubate at 37°C for 10 hour (over night) in shaking bath.

3) Take 2 ml of the culture in eppendorf tube centrifuge for 1 min at 15,000 rpm at 4°C.

4) Aspirate supernatant and take the pellet.

5) Add 100 µl of solution (I) and vigorous vortexing till the pellet disappear.

6) Add 200 µl of solution 11 without vortex or shaking, just gentle inversions. keep on ice bath for 5 min.

7) Add 150 µl of 3 M sodium acetate (pH 4.8), gentle inversion and incubate on ice for 5 min.

8) Centrifuge 5 min at 15000 rpm at 4°C .

9) Transfer clear lysate to ep-tube.

10) Add double volume of cold ethanol with several inversions, incubate at-20 for 10 min.

11) Centrifuge for 5 min at 15,000 at 4°C.

12) Dry the pellet for 5-10 min.

13) Resuspend in 40 µl of TE pH 8.

14) Store at -20.

D- DNA extraction from blood:-

Procedure:

1) Add 1 ml of the DNA extraction buffer (provided with the kit), mix well till complete dissolve of the pellet and incubate 30 min in water bath at 65°C.

2) Spin down for 15 min (13000 xg) and transfer the upper 500 µl of the supernatant to a new tube containing 500 µl ice-cold isopropanol.

3) Mix well and wait 5-25 min at RT (you can wait longer).

4) Spin down for 20 min (13000 xg).

5) Gently, pour off the supernatant.

6) Wash pellets two times with 500 µl 70% EtOH for 5 min and air dry.

7) According to the size of the resulted pellet, resuspend pellets in 50-100 µl preheated (55°C) sterile water or 1x TE buffer {10 mM Tris, 1 mM Na2 EDTA (pH 8)} and then load 5-10 µl on Agarose gel to check.

8) Store at -20°C until use.