علوم الحياة / كورس الأول

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المادة: جزيئي عملي / 5

المرحلة: الرابعة

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Plasmid DNA extraction

A plasmid is a super coiled (covalently closed circular CCC) extra-chromosomal DNA molecule, it is capable of replicating independently from chromosomal DNA.

- There are many methods are used fro plasmid DNA isolation, such as:
  a) Alkaline lysis method
  b) Boiling lysis method
  c) Phenol method
  d) Ethidium bromide- Caesium chloride density gradient centrifugation method
  e) Mini- column

Alkaline lysis protocol for plasmid extraction:

- **Harvesting**
  1) Inoculate *E. coli* (RRI) carried PBR 322 plasmid into 2 ml of LB medium containing the appropriate **ampicillin 100 mg/ml** in 15 ml tube volume. Incubate the culture **overnight at 37C in shaking incubator**.
  2) Pour 1.5 ml of the culture into a microfuge tube. **Centrifuge for 30 sec. at 12,000 rpm** in a **microfuge at 4 C**.
  3) Remove the medium, leaving the bacterial pellet as dry as possible.

- **Lysis by alkali:**
  1) Resuspend the bacterial pellet (obtained from step 3 above) in **100 µl of ice- cold solution I** by vigorous avertexing.

Solution I
50 mM glucose
25 mM Tris –Hcl
10 mM EDTA

Solution I can be prepared in batches of approximately 100 ml, adjust the pH to 8 with HCl then autoclave for 15 min. at 10 1b/in 2 hr. and store at 4C.

2) Add 200 µl of freshly prepared **solution II**, close the tube tightly and mix the contents by inverting the tube gently **(Do not vortex).** and store the tube on ice.

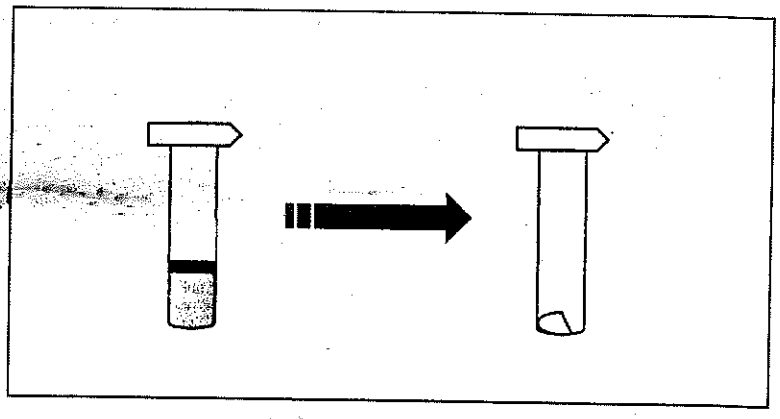
Solution II
0.2 N NaOH (freshly diluted from a 1 N stock)
1% SDS

3) Add 150 µl of **ice – cold solution III.** Close the tube and mix gently by inversion (6 times), then disperse solution III through the viscous bacterial lysate and store the tube on ice for 3-5 min.

Solution III	
5 M potassium acetate	60 ml
Glycial acetic acid	11.5 ml
H_2O	28.5 ml

4) Centrifuge milky suspension (emulsion) at 12000 rpm for 5 min. in a microfuge Eppendorf tube. Transfer **the supernatant** to a new tube.

5) Add **450 µl of phenol : chloroform (ratio 25/25).** Mix by vortexing, after centrifugation at 12000 rpm for 2 min. in a microcentrifuge, transfer the aqueous phase to a new tube.



6) Add **(0.7 volume) of ice- cold isopropanol 100%** to supernatant, mix by vortexing, centrifuge at 12000 rpm for 20 min. at 4 C.

7) Rinse the pellet of double strand DNA (super coiled) with **1 ml of 70% ethanol.** Remove the supernatant and allow the pellet of nucleic acid to dry in the air for 10 min.

8) Re-suspend the nucleic acids in **50 µl of TE (pH 8)** containing DNAse- free RNAse solution (20 µg/ml), vortex briefly and store the DNA at 4 C.

9) Test the size of plasmid and finding protocol using gel electrophoresis technique.

Extraction of RNA

Main types of RNA are:

1) **mRNA:** transfer the genetic information from DNA to protein synthesis place (ribosome) in cytoplasm.
2) **tRNA:** found in cytoplasm, carrying amino acids for arranging them on the strand of mRNA. There are more than 20 kinds of tRNA in each cell.
3) **rRNA:** it calls ribosomal RNA, because it builds the ribosomal structure with nucleoproteins, have a role in protein synthesis in the cytoplasm.

To isolate RNA molecules, we have to follow the steps below:

A) Lysis the cell

- Treat the suspension of *E. coli* (0.5 O.D at 600 nm) with equal volume of **Diethyl pyrocarbonate (DEPE)**, which work as **inhibiter to RNAse (exogenous RNAse).** Homogenized and incubate at 4 C.

- Add **(0.4 ml) of 5% SDS** to the cell suspention

  (SDS acts on lipid of membrane that allowed access of lysozyme to peptidoglycan, also SDS inhibits RNAse).

- Use (**2ml**) of **lysozyme (400 µg/ml)** containing buffer per (10 ml) of *E. coli*, homogenized and incubated at room temperature (37C) for 5-20 min.

B) Purification of RNA

- Centrifuge the suspension of lysis cells at 3000 rpm for 10 min.

- Suspend the pellet with equal volume of **guanidinium thiocyanate** which acts as **strong inhibitors of RNAse** and **denaturation of protein.** The pH should not be alkaline due to liability of rNA.

- DNA can be removed by using **DNAse I** to minimize genomic DNA contamination.

- The contaminant proteins are removed by adding a mixture of **phenol: chloroform: isoamyl alcohol (25:24:1)**, and repeat this step many times.
- Transfer the upper layer to another tube and add **1 volume of ice cold absolute isopropanol** to precipitate RNA.
- Centrifuge 1000 rpm for 20 min. at 4 C
- Discard supernatant and wash pellet with **70% ethanol**
- Centrifuge 1000 rpm for 5 min. at 4 C
- Re-suspend the RNA in **100 µl of TE buffer** and frozen

Calculate the Concentration of RNA:

Concentration of RNA (µg/ml) = O.D 260 nm x 40 x dilution factor

- 1 O.D 260 nm = 40 µg/ml of RNA

Calculate the Purity of RNA:

O.D 260 nm/ O.D 280 nm = should be 2