

Digestion

Aims of the Experiment

To digest the impurity of the DNA/RNA solution produced from our homemade cell extract and visualize the nucleic acid from a Gel to confirm the presence and the type of the nucleic acid in the cell extract.

Material

- DNA/RNA samples produced from DNA Processing Protocol
- DNase I
- DNase I buffer
- RNase A (1 – 100µg/ml)
- Nuclease-free H₂O
- TE buffer at pH 7
- 200µl micro-centrifuge tubes

Procedure

1. Dilute all nucleic acid samples in 1:10 dilution in nuclease-free H₂O.
2. For RNA sample, mix the following reagents and incubate at 37°C for 30 minutes:

Volume (µl)	Reagents
80	1:10 diluted RNA
1	DNase I
10	DNase I buffer
9	Nuclease-free H ₂ O

3. For DNA samples, mix the following reagents and incubate at 37°C for 30 minutes:

Volume (µl)	Reagent
40	1:10 diluted DNA
0.1	RNase A
9.9	Nuclease-free H ₂ O or TE buffer

4. Carry out phenol-chloroform precipitation for all the samples in order to remove all the nucleases.
5. RNA denaturation before Gel loading:

Size of ribosomal RNA segments	
23S	2900 nt
5S	120 nt
16S	1500

6. All samples run on 1% Agarose Gel for 1.5 hour at 100V. Prepare separate gel for the RNA, DNA in Nuclease-free H₂O and DNA in TE buffer.