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Universiti Malaysia PAHANG
Engineering • Technology • Creativity

BSB1163
CELL AND MOLECULAR BIOLOGY

Techniques in Molecular Biology

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Nucleic acid extraction

To obtain pure DNA for further investigations,
-PCR,
-sequencing,
-cloning
etc.

- Most DNA extraction protocols consist :

1. **Lyse the cells**
2. **Solubilize the DNA**
3. **Remove contaminating proteins, RNA, and macromolecules**
4. **Separation of the DNA molecules from the rest of the cells**

Four steps to remove and purify the DNA from other cells content

- Lysis
- Precipitation
- Wash
- Resuspension

LYSIS:

- The cell wall is disrupted
- Detergent breaks down the cell membranes
- The end result of LYSIS is that the contents of the plant cells are distributed in solution.

Washing:

The precipitated DNA is “washed” with a 70% ethanol solution to remove salts and other water soluble impurities

Resuspension:

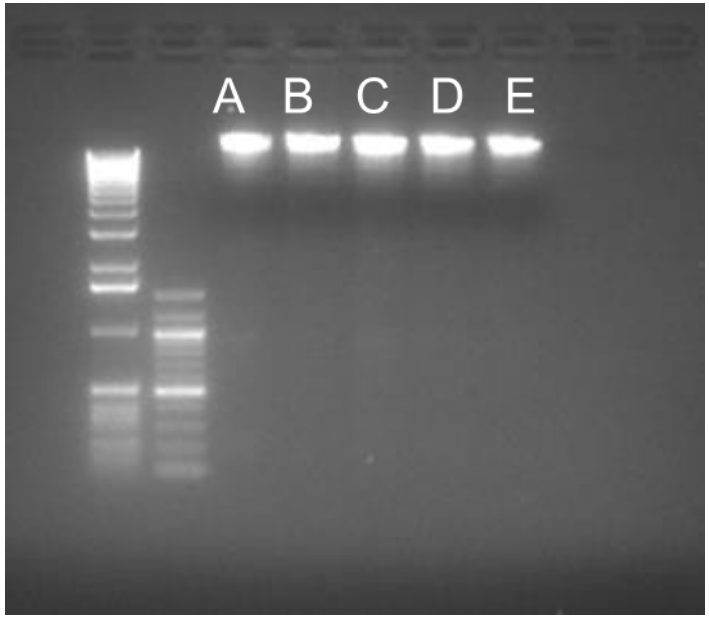
The clean DNA is resuspended in a buffer for stability and long term storage.

Checking the Quality of your DNA

- The product of your DNA extraction will be used in subsequent experiments
- Poor quality DNA will not perform well in PCR
- The quality of DNA can be checked using spectrophotometer and gel electrophoresis

Analyzing DNA Samples

If properly done, genomic extraction should result in bright bands in the very high base pair range of a gel electrophoresis.



Lane A: Barley

Lane B: Corn

Lane C: Oat

Lane D: Rice

Lane E: Wheat

- This genomic DNA preparation is not perfect but acceptable for further analysis

Lane A: Barley

Lane B: Corn

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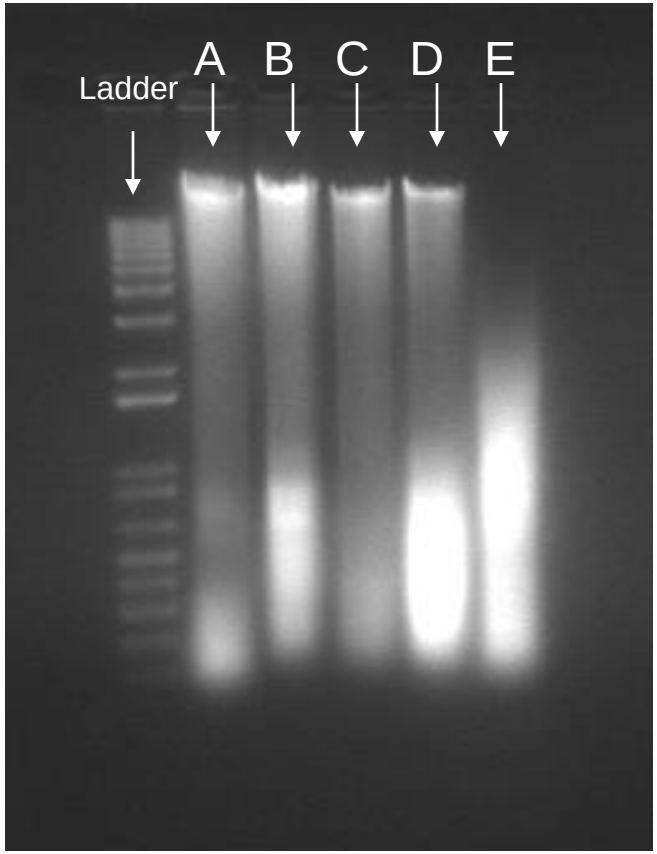

Plasmid Extraction

- Plasmid - small circular independent DNA molecules
- Advantage – the molecule much smaller, more compact

RNAses

- RNases degrade RNA
- Common laboratory contaminant
- Also released from cellular compartments
- Difficult to inactivate

Protecting Against RNAse

- Wear gloves at all times
- Use RNase-free tubes and pipet tips
- Use dedicated, RNase-free chemicals
- Pre-treat materials with:

a. extended heat (180°C for several hours),
b. wash with DEPC-treated water, NaOH or H_2O_2

- Supplement reactions with RNase inhibitors

Isolation of messenger RNA

- mRNA molecules have a tail of A's at the 3' end (polyA tail)
- Oligo(dT) probes can be used to purify mRNA from other RNAs
- mRNA can be eluted from oligo(dT) matrix using water or low-salt buffer