Nucleic acid extraction and quality control – A primer

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Introduction

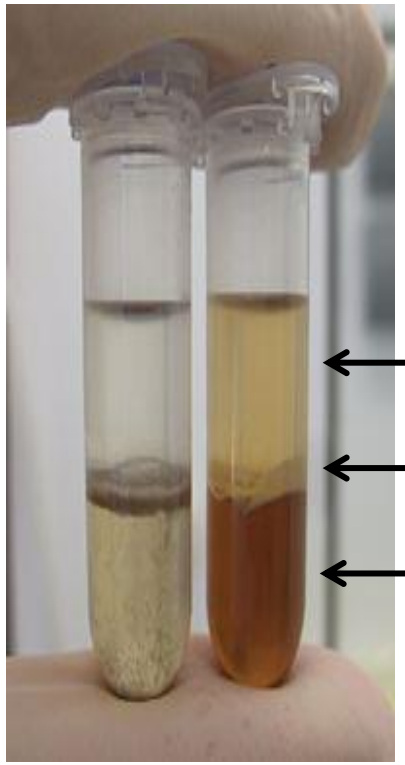
- Isolation and purification of nucleic acids is the first step before downstream processing (PCR, Cloning, Genome sequencing etc.)
- Nucleic acid extraction generally entails:
  - **Cellular disruption** by either physical or chemical means or combinations of both (Boiling, sonication, bead beating, detergents, lysozyme etc).
  - **Inhibition of degradative enzymes** such as DNAses or RNAses depending on the target of isolation.
  - **Purification** to remove contaminating agents such as salts, cellular debris, proteins etc.
  - **Recovery** of the final nucleic acid extract in water or buffer
- Depending on the desired purity, yield, simplicity, cost and convenience two main approaches are involved in nucleic acid extraction.
  - Liquid phase extraction methods
  - Solid phase extraction methods

Liquid phase methods

Phenol-Chloroform isoamyl alcohol extraction

- **Cell lysis:** physical or chemical means or combinations of both (Boiling, sonication, bead beating, detergents, lysozyme etc).
- **Phenol:** denature proteins rapidly. **Chloroform** supports phase separation.
- The upper phase which contains DNA is collected and DNA **precipitated** by **ethanol or isopropanol.** Salt is the common impurity in nucleic acid samples.
- DNA precipitate is collected by centrifugation, and excess salt is rinsed with **70% ethanol** and centrifuged to discard the ethanol supernatant.
- The DNA pellet is then dissolved with **TE buffer or sterile distilled water.**

Liquid phase methods

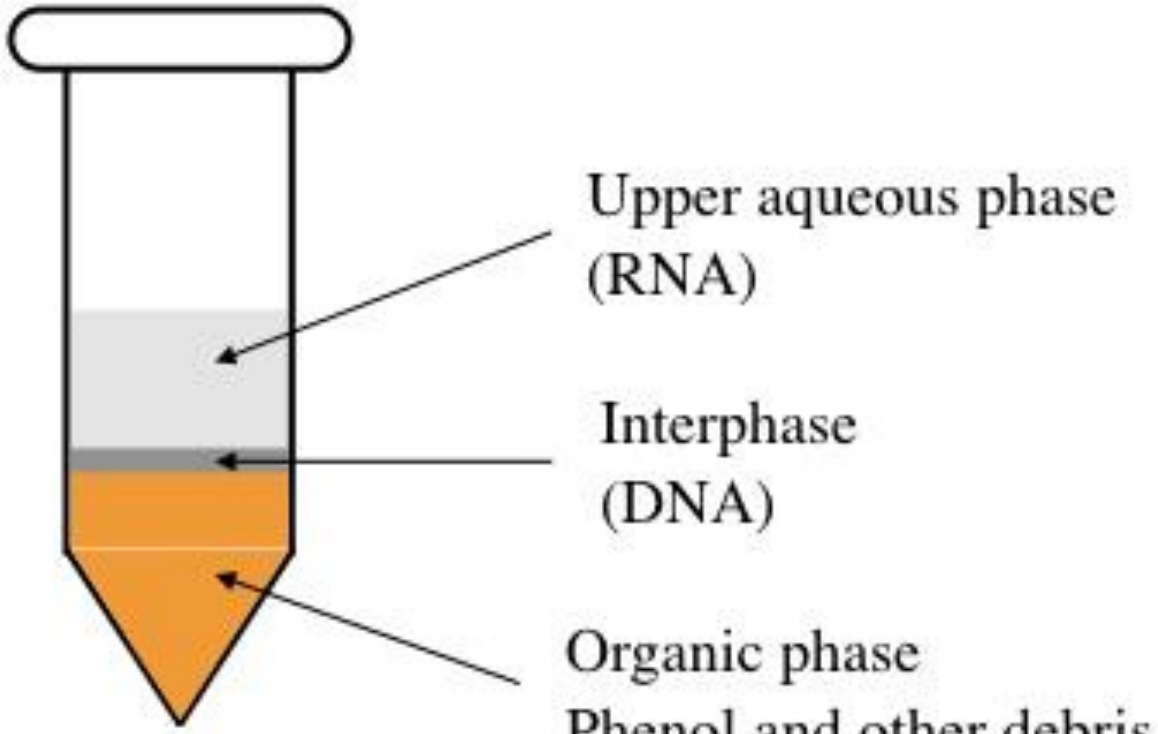


Aqueous phase:DNA

Interphase: proteins

Organic phase:RNA lipids

http://openwetware.org



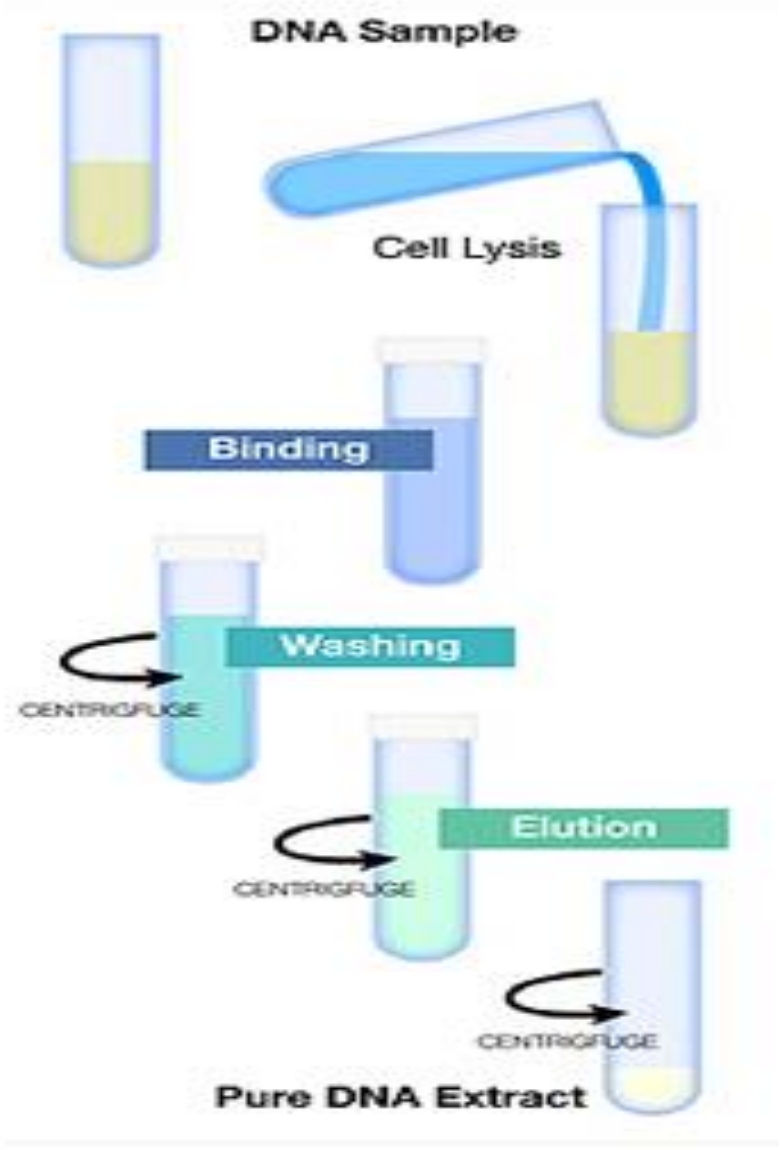
Challenges of solid phase extraction

- Long extraction times
- Poor yields
- Incomplete phase separation
- Tedious extraction process
- Poor reproducibility

Solid phase extraction

- **Cell lysis:** Physical, chemical or enzymatic approaches are used.
- **Column conditioning:** Buffers at a particular pH are used to convert the surface or functional groups on the solid into the desired chemical form.
- **Nucleic acid adsorption:** Adsorption of the target nucleic acid molecules to the purification columns.
- **Washing:** Specific wash buffers help remove contaminants that bind to columns with nucleic acids. Other systems use both pre-wash followed by a final wash buffer.
- **Elution:** Finally, nucleic acids are dislodged from the columns with an elution buffer or even sterile distilled water.

Solid phase extraction work flow



Advantages of solid phase extraction

- Higher reproducibility
- Higher yields
- Faster extraction
- Greater purity
- Simpler extraction process

Disadvantages??

Quality/ quantity assessment

- **Spectrophotometric** methods such as NanoDrop are used for quantification and quality assessment.
- Spectrophotometers measure absorbance of light by DNA at 260 nm.
- Other components such as proteins absorb light at 280nm.
- The A260/280 ratio is used to measure purity (A260/280 ratios >1.8 are desired.
- Spectrophotometric methods are not as sensitive so fluorometric methods are desired.

Quality/ quantity assessment

- **Fluorometric** methods use fluorescent dyes that are specific for a given nucleic acid (RNA, DNA etc.)
- Fluorometric assays are more sensitive but expensive.
- The do not give an idea of the sample purity
- Store samples at -20 degrees or -80 degrees

A few tips

- Ensure sterility during extraction procedure.
- If using pure cultures, ensure purity and adequacy of the starting biomass.
- Use extraction controls if possible.
- Use the right kit for the right sample for the right application.
- Take note of the elution volumes and desired concentrations for downstream processing.
- Pay particular attention to labeling and sample tracking for downstream processing.
- Reduce the number freeze-thaw cycles to preserve sample integrity.

Questions??

THANK YOU