DNA Extraction

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Working with nucleic acids

- Extraction of DNA, RNA, and protein is the basic method used in molecular biology.
- In the past, complicated, time-consuming, labor-intensive, and limited in terms of overall throughput
- Currently, there are many specialized methods

Working with nucleic acids

- *Laboratory requirements!*

- **Gene manipulation can be carried out with relatively modest laboratory facilities**

Working with nucleic acids

- Every gene manipulation experiment requires a source of nucleic acid, in the form of either DNA or RNA.

- It is therefore important that reliable methods are available for isolating these components from cells.

Working with nucleic acids

There are three basic requirements:

- **Opening the cells**
- **Separation of the nucleic acids**
- **Recovery of the nucleic acid** in purified form.

Three basic requirements

- **Opening the cells** in the sample to expose the nucleic acids for further processing and to enable nucleic acids to be isolated.
- Should be done as gently as possible to avoid shearing large DNA molecules.

Three basic requirements

- **Separation of the nucleic acids** from other cell components
  - Cell preparations can be **Deproteinised**
  - Extractions using phenol or phenol/chloroform mixtures.
  - Formation of an emulsion and subsequent centrifugation to separate phases

Three basic requirements

- **Recovery of the nucleic acid** in purified form.
- nucleic acids purified by a range of techniques.
- Some applications require highly purified preparations
- Some require partially purified DNA or RNA

What are the Most Commonly used DNA Extraction Procedures

- Organic (Phenol-Chloroform) Extraction
- Non-Organic (Proteinase K and Salting out)
- Chelex (Ion Exchange Resin) Extraction
- FTA Paper (Collection, Storage, Isolation)
- Silica Based (Silica exchange resin- Qiagen)
- Magnetic Beads Based

The method utilized for DNA Extraction may be

- Sample dependent
- Technique dependent, or
- Analyst preference

1- ORGANIC EXTRACTION

- Cell Lysis Buffer –

  lyse cell membrane, nuclei are intact, pellet nuclei.

- Resuspend nuclei,

  add Sodium Dodecly Sulfate (SDS), Proteinase K. Lyse nuclear membrane and digest protein.

1- ORGANIC EXTRACTION

- DNA released into solution is extracted with phenol-chloroform to remove proteinaceous material.
- DNA is precipitated from the aqueous layer by the additional of ice cold 95% ethanol and salt.
- Precipitated DNA is washed with 70% ethanol, dried under vacuum and resuspended in TE buffer.

1- ORGANIC EXTRACTION

- Yields relatively pure, high molecular weight DNA
- DNA is double stranded - good for RFLP
- Time consuming
- Requires sample to be transferred to multiple tubes -risk of contamination
- Involves use of hazardous (and smelly!) chemicals

1- ORGANIC EXTRACTION
Differential Extraction

- Isolate the male and female DNA from a sexual assault evidentiary sample.

- a female fraction containing the DNA from the victim's epithelial cells, male fraction containing the DNA from the sperm are isolated.

1- ORGANIC EXTRACTION
Differential Extraction

- preferentially breaking open the female epithelial cells with incubation in a SDS/Proteinase K mixture.
- Sperm heads remain intact during this incubation.
- The sperm heads are pelleted and the supernatant containing the female fraction is collected and saved.

1- ORGANIC EXTRACTION
Differential Extraction

- The sperm pellet is washed several times to remove any residual DNA from the victim.
- The sperm are subsequently lysed by treatment with a SDS/proteinase K/ dithiothreitol (DTT) mixture.

1- ORGANIC EXTRACTION
Differential Extraction

- DTT is required to breakdown (reduce) the protein disulfide bridges in sperm head.(sperm resist lysis without the addition of the DTT.
- Both the male fraction and the female fraction are then extracted with phenol-chloroform, and the DNA precipitated with ethanol.

2- Non-Organic DNA Extraction (Proteinase K and Salting out)

- Does not use organic reagents such as phenol or chloroform.
- Digested proteins are removed by salting out with high concentrations of LiCl.
- if salts are not adequately removed, problems could occur.

2- Non-Organic DNA Extraction (Proteinase K and Salting out)

- 1.Cell Lysis Buffer - lyse cell membrane, nuclei are intact, pellet nuclei.
- 2.Resuspend nuclei in Protein Lysis Buffer containing a high concentration of Proteinase K. Lyse nuclear membrane and digest protein at 65 C for 2 hours. Temperature helps denature proteins, and Proteinase K auto digests itself.

2- Non-Organic DNA Extraction (Proteinase K and Salting out)

- 3.To remove proteinaceous material, LiCl is added to a final concentration of 2.5 M, and incubated on ice. Proteins precipitate out and are pelleted by centrifugation
- 4.DNA remains in solution. Transfer supernatant to a new tube, care must be taken not to take any of protein pellets.

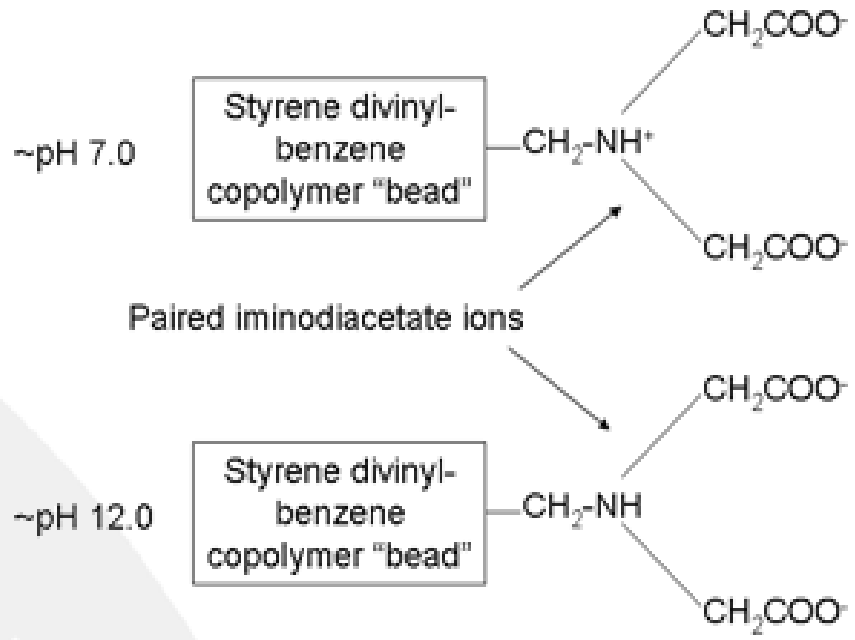
2- Non-Organic DNA Extraction (Proteinase K and Salting out)

- 5.DNA is precipitated by the addition of room temperature isopropanol. LiCl will not precipitate with DNA.
- 6.Precipitated DNA is washed with 70% ethanol, dried under vacuum and resuspended in TE buffer.

3- Chelex Extraction

- Chelex 100 is an ion exchange resin that is composed of chelating groups binding polyvalent metal ions such as magnesium (Mg^{2+}).
- By removing the Mg^{2+} from the reaction, nucleases are inactivated and the DNA is protected.

3- Chelex Extraction



Chelex® 100 resin is composed of styrene divinylbenzene copolymers with paired iminodiacetate ions. The iminodiacetate ions act as chelators for binding polyvalent metal ions.Chelex® 100 is very effective in binding metal contaminants with a high selectivity for divalent ions, without altering the concentration on non-metal ions.[06]

3- Chelex Extraction

- A 5% solution of Chelex is added to a blood stain or liquid blood and incubated at **56 C for 30 minutes.** lyse red cells and remove contaminants and inhibitors such as heme and other proteins.
- The sample is then **heated at 100°C for 8 minutes.** DNA denatured as well as disrupting membranes and destroying cellular proteins.

3- Chelex Extraction

- The tube is centrifuged, the Chelex is pelleted, the supernatant containing the DNA is removed.
- The Chelex extraction process denatures double stranded DNA and yields single stranded DNA..

3- Chelex Extraction

- Relatively fast
- Can extract directly from cloth (stain)
- Minimizes contamination -uses only a single tube
- Removes PCR inhibitors
- Results in single-stranded DNA

4- FTA PAPER

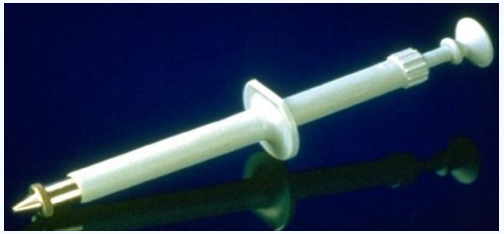
4- FTA PAPER

- A Unique Matrix For The Rapid Preparation And Ambient Storage Of DNA From Whole Blood And Other Biological Samples

4- FTA PAPER

- Is a mixture of strong buffers, protein denaturants, chelating agents, and a UV absorbing, free radical trap.
- The reagents are impregnated into a cellulose-based filter matrix such as Whatman

4- FTA PAPER

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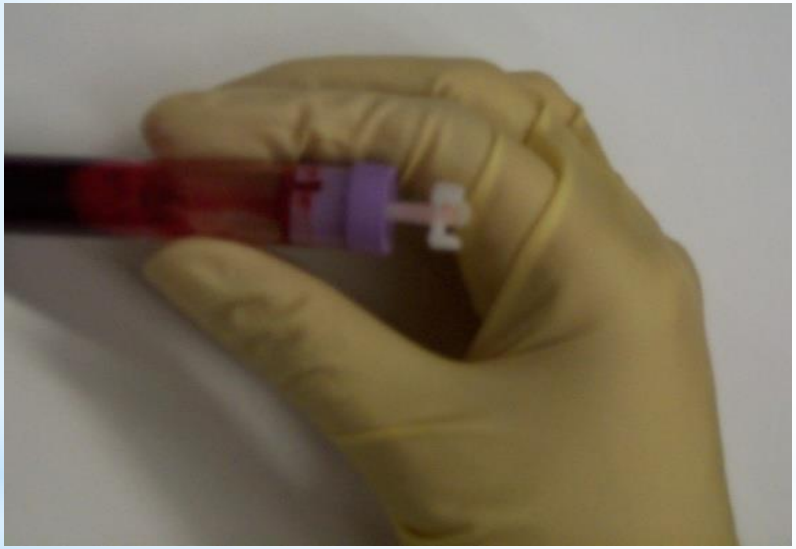
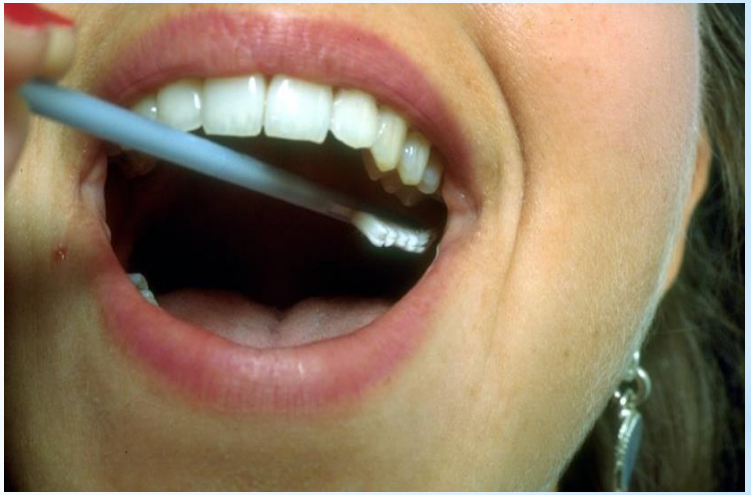
- Blood Samples Stored on FTA® Paper Either Dry or Wet for 6 Months in Barrier Pouch

Blood and Buccal Swab Collection and Direct Transfer to FTA® Paper

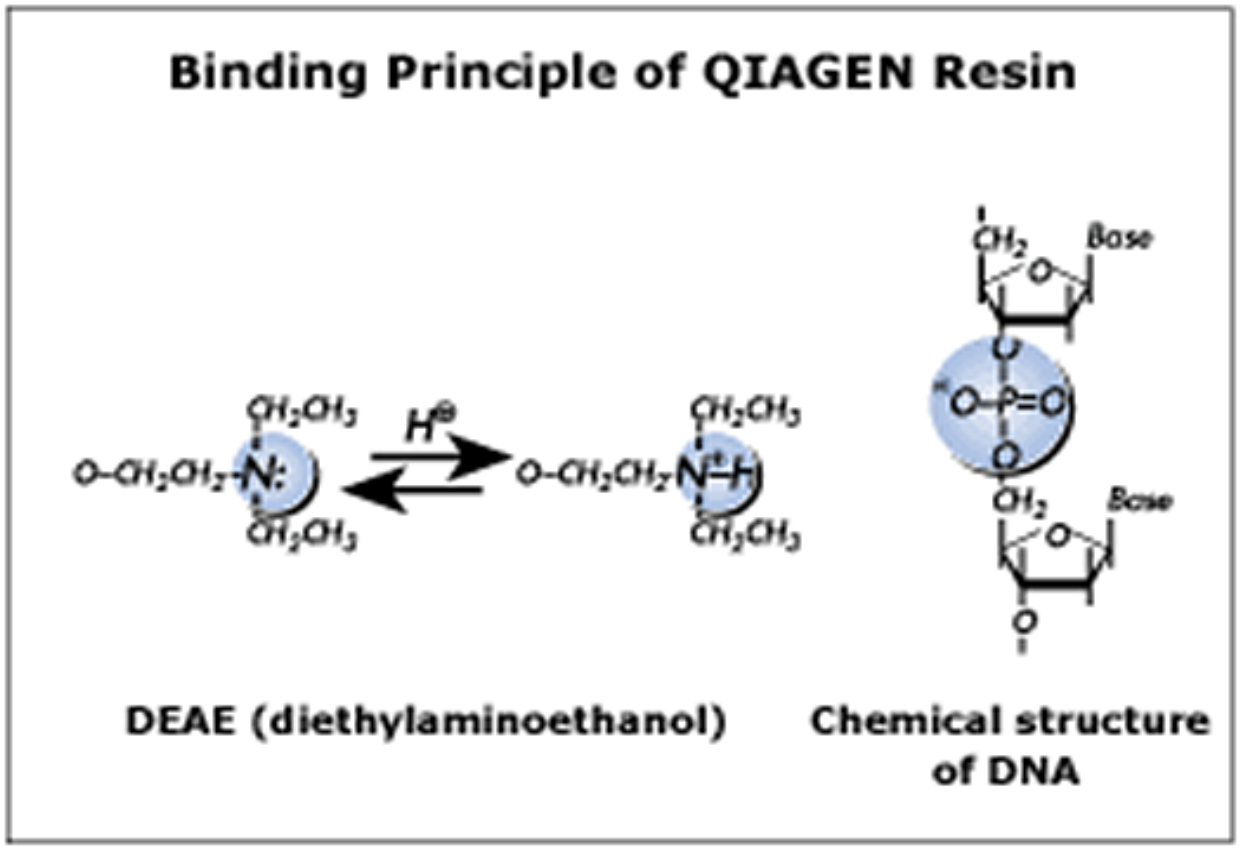




5- Silica-Based Extraction

- fast and cost-effective method to purify plasmid DNA without phenol/chloroform extraction.
- It is based on binding of DNA to silica-based membranes in a chaotropic salt.

5- Silica-Based Extraction



Chemical structure of positively charged DEAE groups of QIAGEN Resin

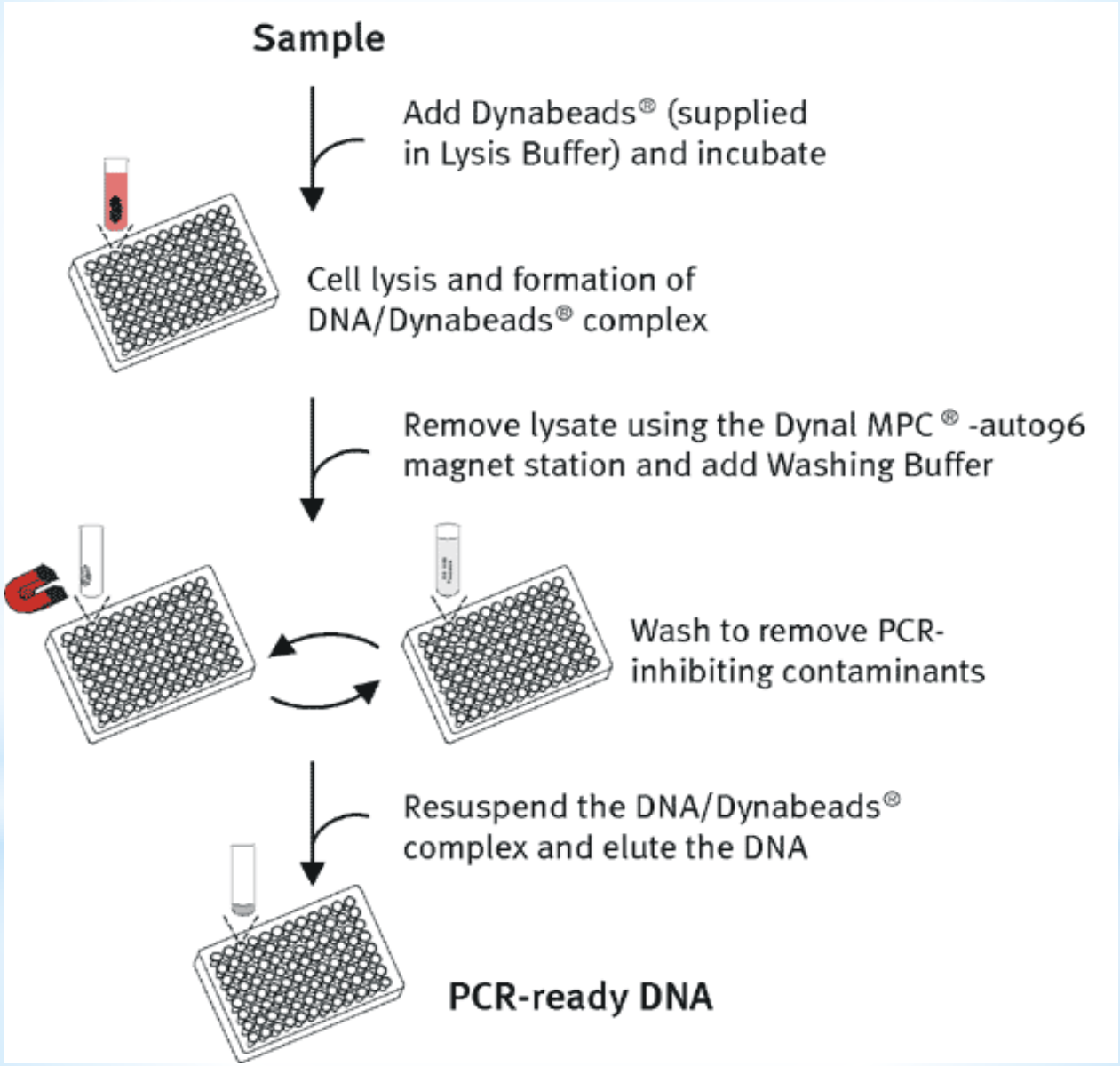
5- Silica-Based Extraction

- Selective adsorption to silica membranes
- Binding: high salt Elution: low salt
- no alcohol precipitation

6- Magnetic Beads -Based Extraction

Magnetic beads are coated with DNA antibodies to bind to DNA:

6- Magnetic Beads/Automated version



6- Magnetic Beads

- Very fast, may be automated
- Highly purified DNA
- Excellent for liquid blood
- Cannot be used directly on stain /i.e. need to remove cells from stain substrate (cloth, etc.)
- Very expensive

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