

GENOMIC DNA EXTRACTION

BecA-ILRI Hub, Nairobi
20th September 2016

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What are the Key Expectations from a DNA Extraction Procedure?

1. Maximize DNA recovery
2. Remove inhibitors
3. Remove or inhibit nucleases
4. Maximize the quality of DNA
5. Double strand vs. Single strand
(Application e.g.: RFLP or PCR)

How Much DNA Can We Recover?

- A Diploid Cell contains approximately 6 pg of DNA
- The average WBC of an adult is 5 - 10 X 10^6 cells per ml of blood. Therefore, the theoretical recovery of DNA per ul of blood is 30 - 60 ug.
- NB: **QIAsafe DNA Blood Tubes:** stabilization of DNA at room Temp for over 30 years.

How Much DNA Do We Need?

- The **RFLP** procedure requires a minimum of 50 ng of high molecular weight double stranded DNA. This is the equivalent of approximately 2 ul of blood.
- The **PCR** reactions call for an average 1 ng of DNA (single or double stranded). This is the equivalent of 1/25 of 1 ul of blood.
- Many of the commercially available kits are sensitive below 1 ng of DNA (100-250 pg).

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, and Isolation)
- Silica Based (Silica exchange resin- Qiagen)

The method utilized may be sample-dependant, technique dependant, or analyst preference

ORGANIC EXTRACTION

- Perhaps the most basic of all procedures in molecular biology is the purification of DNA.
- The key step, the removal of proteins, can often be carried out simply by extracting aqueous solutions of nucleic acids with phenol and/or chloroform.

ORGANIC EXTRACTION PROCEDURE

- Cell Lysis Buffer - lyse cell membrane, nuclei are intact, pellet nuclei.
- Resuspend nuclei, add Sodium Dodecyl Sulfate (SDS), Proteinase K. Lyse nuclear membrane and digest protein.
- 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.

ORGANIC EXTRACTION

REAGENTS 1

- **Cell Lysis Buffer** - Non-ionic detergent, Salt, Buffer, EDTA designed to lyse outer cell membrane of blood and epithelial cells, but will not break down nuclear membrane.
- **EDTA** (Ethylenediaminetetraacetic disodium salt) is a chelating agent of divalent cations such as Mg^{2+} . Mg^{2+} is a cofactor for DNAses. If the Mg^{2+} is bound up by EDTA, nucleases are inactivated.

ORGANIC EXTRACTION REAGENTS 2

- **Proteinase K** - it is usual to remove most of the protein by digesting with proteolytic enzymes such as Pronase or proteinase K, which are active against a broad spectrum of native proteins, before extracting with organic solvents.
- Proteinase K is approximately 10 fold more active on denatured protein. Proteins can be denatured by SDS or by heat.

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REAGENTS 3

- **Phenol/Chloroform** - The standard way to remove proteins from nucleic acids solutions is to extract once with phenol, once with a 1:1 mixture of phenol and chloroform, and once with chloroform. This procedure takes advantage of the fact that deproteinization is more efficient when two different organic solvents are used instead of one.
- Also, the final extraction with chloroform removes any lingering traces of phenol from the nucleic acid preparation.
- Phenol is highly corrosive and can cause severe burns.

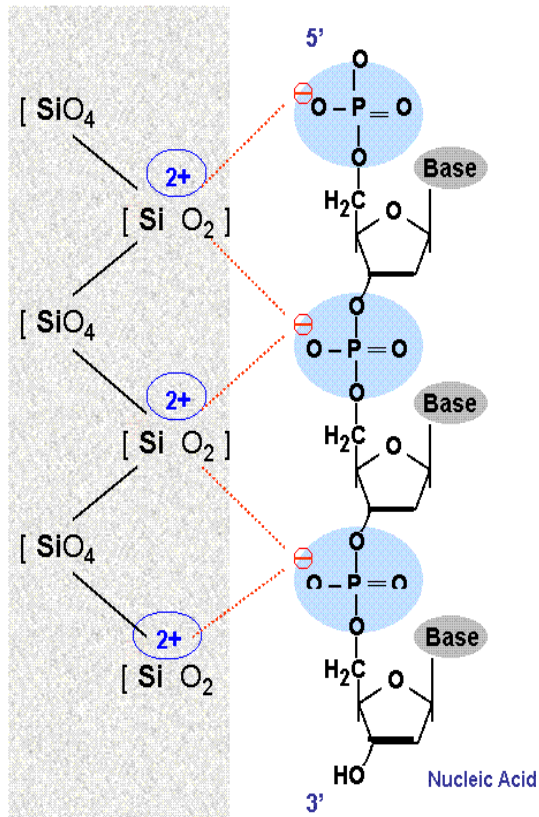
ORGANIC EXTRACTION

REAGENTS 4

- **Phenol** - often means phenol equilibrated with buffer (such as TE) and containing 0.1% **hydroxyquinoline** and 0.2% **β -mercaptoethanol** (added as antioxidants). The hydroxyquinoline also gives the phenol a yellow color, making it easier to identify the phases (layers).
- **Chloroform** - often means a 24:1 (v/v) mixture of chloroform and isoamyl alcohol. The isoamyl alcohol is added to help prevent foaming.
- The **Phenol/Chloroform/IsoAmyl Alcohol (IAA)** ratio is 25:24:1

Silica-Membrane Technology

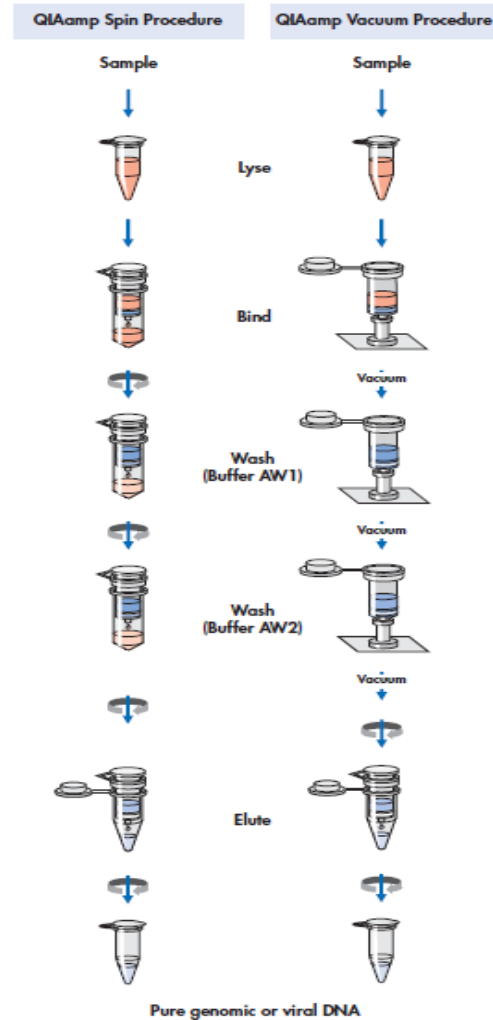
DNA binding principle of the silica membrane technology



The **negative net charges** of the **phosphate groups** of the DNA are absorbed to **positive charges** of the **silica (SiO_4) gel** membranes of the spin columns in the presence of high concentrations of chaotropic salts and low pH values, $< \text{pH}7.5$

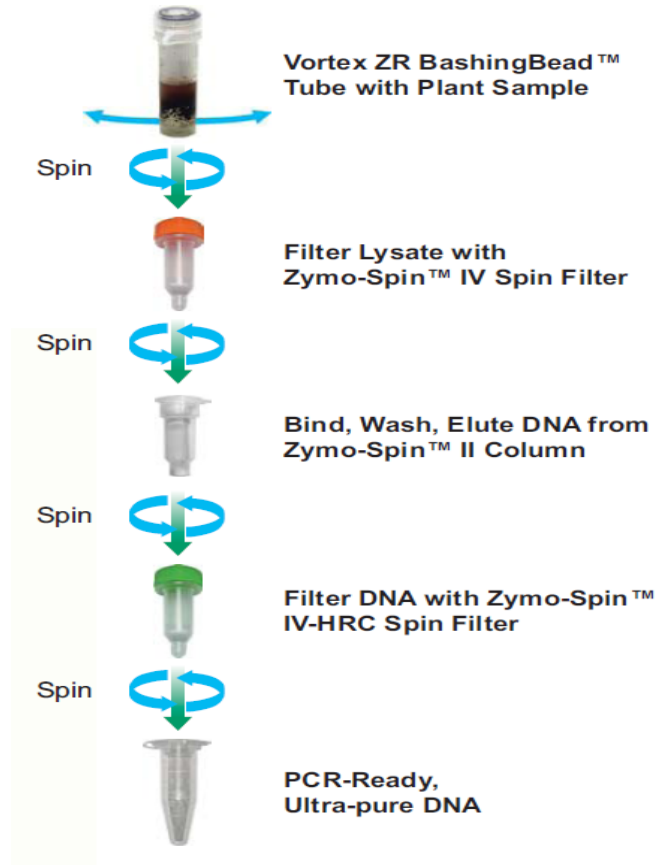
DNA extraction and Purification using mini kits: Qiagen

1. Lysis of cells
2. Spin column: DNA adsorption to silica gel membrane
3. Two-different wash steps: removal of contaminants
4. Elution: pure and concentrated DNA



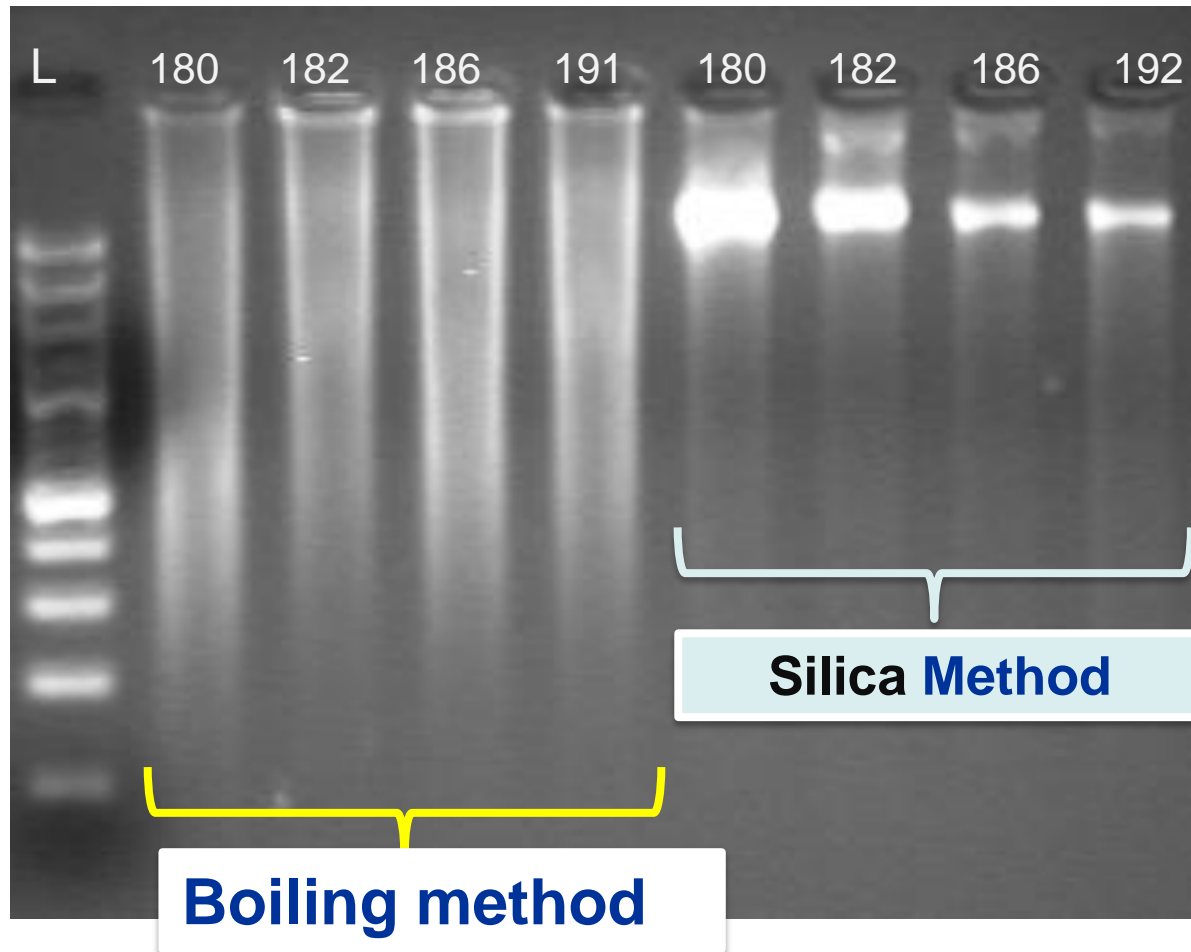
The QIAamp DNA Mini Kit

Quick-DNA™ Universal Kit : ZYMO RESEARCH



ZR Plant/Seed DNA Kit

Quality / Integrity of DNA



Thank you