DNA Extraction

Workshop in DNA Typing
FIU

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Sample Inputs

• DNA sample types
  – Blood/buccal
  – Semen/Rape kits
  – Tissue
  – Bone
  – Gum
  – Faeces
  – Cigarette butts/stamps
  – Clothing/fabric
  – Touch samples/guns
  – Plants/soil/Microbes

Manual Extractions

• Phenol/chloroform
• Chelex
• Guanadinium isothiocyanate
• FTA
• Minicolumns
• Laser Microdissection
• DNA IQ
Steps to DNA extraction

1. Breaking the **cells** open,
2. Removing membrane lipids by adding a **detergent**.
3. Removing **proteins** by adding a **protease** and extracting in PCIA
4. Adding a **chelating agent** (EDTA) to sequester divalent cations such as **Mg\(^{2+}\)** and **Ca\(^{2+}\)**. This stops **dnase** enzymes
5. Isolate DNA and resolubilize in TE or ultrapure water..

Example extraction

(1) Bloodstain 3mm*3mm is placed in a 2.2ml centrifuge tube
(a) stain extraction buffer is added - buffer, salt and detergent - 10mm tris, 100mm NaCl 39 mM dithiothreitol, 10mM EDTA, 2% SDS - sodium dodecyl sulfate
(b) salt and detergents helps solubilize cell components, Dtt reduces and breaks apart disulfide linkages (between cysteines in the protein)
(c) 2ul proteinase K is added - digests proteins
Isolation

phenol/chloroform/isoamyl alcohol is added (25/24/1) to form a milky emulsion
(a) then centrifuged till a bilayer is formed
(b) aqueous phase will contain the DNA - proteins, lipids and other hydrophobic crap will go in organic phase

Some proteins sit at interphase, uncertain as to which layer they belong

Aqueous phase is then removed and DNA isolated by microcon or ethanol precipitation and stored in TE or water

http://www.fabcousa.net/catalog/rapekit.htm

Sperm Cells and Non-Sperm Cells

Vaginal/Cervical Swab

http://www.fabcousa.net/catalog/rapekit.htm
(a) Swab material is removed from an applicator and placed in a tube
   (i) Salt, buffer, and EDTA added - a different detergent is used - Sarkosyl - N-lauryl sarcosine - CH₃NHCH₃COO⁻Na⁺
   (ii) 1ul proteinase K is added, incubate at 37°C for 2 hours
   (iii) These are milder conditions!
   (iv) Centrifuge with basket insert and remove swab material

(b) Carefully remove supernatant leaving any pelleted material in the tube -
   (i) this material is the female fraction
   (ii) The pellet remaining in the tube is the cell pellet
(c) Wash the cell pellet
   (i) resuspending it in sperm wash buffer - 10mM tris-HCl, EDT, 50mM NaCl 2% SDS
   (ii) vortex and spin again
   (iii) repeat several times
Sperm DNA is associated with protamines, proteins with a high cysteine content, however 4% histones are present, and these are associated with genes affecting embryo development.


Issues with PCIA/differential extraction

Requires good ventilation, components can be toxic

Procedure is technique dependant and time consuming, Results can vary between analysts. Aqueous layer must be isolated

pH is important – too low and the DNA will extract into the organic layer

Heme can coextract causing problems with inhibition, Phenol can be transferred causing similar problems.

For differential extractions, soluble DNA can be lost in the cell pellet, leading to incomplete isolation of sperm and non sperm fractions.
v) Chelex extraction
(a) Chelex is a cation exchange resin useful in PCR analysis. - In one test it was shown to have 6x higher yield than the alternative procedure- phenol-chloroform
(b) Note that in this procedure we are not digesting the protein and extracting the DNA - the efficiency of the PCR lets us get away with this.

Chelex resin is a styrene divinylbenzene copolymer containing paired iminodiacetate ions

It is regenerated in dilute acid and operates in solutions of pH 4 or higher

the selectivity for divalent cations is much higher than for monovalent cations (~5000 to 1).

A 5% suspension has a pH between 10 and 11.

http://structbio.vanderbilt.edu/chazin/wisdom/labpro/chelex.html

Advantages of Chelex
1. Simple 1 tube process
2. Combination of alkaline suspension and boiling disrupts cell membranes and releases DNA. Chelex also inhibits breakdown of DNA during this process producing ssDNA
3. Strongly chelates metals removing them from the cell suspension. This removes heme inhibitors and also other divalent cations, which might catalyze enzymatic breakdown.
### (2) Procedure:

(a) A small sample is suspended in 5% chelex and incubated for 30 min at 56°C
(b) The sample is then boiled for 8 minutes - this process lyses the cells and precipitates hemoglobin
(c) The sample is next centrifuged and a portion of the supernatant removed for PCR analysis
(i) Ion exchange beads, denatured protein and stuff will be in the bottom of the tube

<table>
<thead>
<tr>
<th>Chelex Suspension</th>
<th>Boiling the suspension</th>
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### FTA paper

#### Advantages and benefits

Samples can be collected, stored at room temperature:

- Rapidly inactivates organisms, including bloodborne pathogens
- Sample processing time to isolate DNA is 15 to 30 minutes
- Sample processing requires a simple hot water elution procedure to isolate DNA
- Sample volume requirements are minimal: 12 to 40 µl per collection area
- Hemoglobin, a known PCR inhibitor, is bound to the FTA Elute matrix

http://www.whatman.com/FTAElute.aspx
FTA is a great thing. Anyone can take a sample and send it off.

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**Sucrose Extraction** - c/o Conti and Buel Vermont Crime Lab

An application for stale donuts

1. **FTA Punch**
2. **50 µl Sucrose Solution**
3. **Store at 4°C**

Remove aliquot of Sucrose Solution (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 300 mM sucrose) from freezer to thaw.
2. Transfer sample to a sterile tube.
3. Add ~50µl of Sucrose Solution and vortex on setting #3 for thirty seconds. Centrifuge briefly.
4. Incubate at 100°C for ten minutes with shaking at 300 rpm. Centrifuge briefly.
5. Solid material may be removed from sample tube and discarded or retained in the extract if working with database samples.
6. Sample is recovered from sucrose via vacuum or spin filtration on membranes.
Solid Phase Extraction Methods

- Glass Beads or silica embedded membranes
  - Guanadine isothiocyanate, Cl⁻, NaI or NaClO₄
  - High concentrations are used under acidic conditions to disrupt the bonds between water molecules & DNA
  - DNA denatures and absorbs to glass or membrane.
  - Salt content is then lowered, conditions are made alkaline and DNA is released as glass becomes more negative

DNA IQ

The DNA IQ system uses silica coated magnetic beads to capture DNA from lysed cells. The beads saturate at about 100 ng of bound DNA. Excess DNA is removed by immobilizing the beads with a magnet and pipetting the excess DNA and cell debris away.

http://www.promega.com/profiles/501/ProfilesInDNA_501_03.pdf#search='DNA IQ magnetic'
DNA IQ

- Lysing of cells via chaotropic agent
- DNA binds to silica surface of magnetic particle
- Separation from lysate via magnet
- Washing
- Release of DNA from particles
- Elution

DNA IQ®: Maxwell™ 16
ChargeSwitch®

- Ionizable nucleic acid-binding ligand (magnetic beads)
- Charge is “switched” via pH of medium
- 3 step process: bind, wash, elute
- Water-based reagents

Different extraction methods

Ion Exchange

Silica Purification

ChargeSwitch™ Purification
Psychopath minds, give it up!

Even the vasectomy or lack of sperm cells won't save your butt from jail now ...  

A new DNA analysis technique will allow individual detection even in semen samples with no sperm cells, in cases of sexual assault. In fact, the main problem of forensics trying to fingerprint the DNA of rapists is that the quantity of male DNA in samples taken from the woman is often extremely low compared to the amount of her DNA. "The female DNA profile is so strong in the analyzed sample that the male DNA is swamped," says Andy Hopwood of the Forensic Science Service in Birmingham, UK.


**Laser micro-dissection schematic**

A. Sample is dried on a glass slide and coated w/ plastic laser cuts out plastic and sample drops into tube

B. Sperm in mixed cells are labeled using histological stains, immuno labeled antibodies and/or in-situ fluorescence hybridization

C. Cut out with a laser

D. Dropped into a collection tube.

E. Collected sample is then amplified.

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**Microfluidic Separation of Sperm Cells**

A

Epithelial cell

Sperm cell

Microchip

B

Large cells are filtered out

http://pubs.acs.org/cgi-bin/cjbenz?ancham/aspasp/html/ac0486239.html
Conclusions

• Extraction is performed by
  – Digestion of cellular material
  – Removal and Capture of DNA
    • PCIA liquid/liquid
    • FTA
    • Silica/guanidinium isothiocyanate (DNA IQ)
    • Charge Switch (ion exchange)
    • Laser Microdissection
    • Cell Sorting
  – The type of extraction affects the purity of DNA and its relative freedom from inhibition.