

How Can We Recover DNA From a Variety of Sources of Biological Evidence?

Blood

Semen

Saliva

Urine

Hair (w/Root & Shaft)

Teeth

Bone

Tissue

Cigarette Butts

Envelope &

Stamps

Fingernail

Clippings

Chewing Gum

Bite Marks

Feces

What are the essential components of 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 (RFLP or PCR)

How Much DNA Can We Recover

- A Diploid Cell contains approximately 6 pg of DNA
- Sperm contains approximately 3 pg of DNA
- The average WBC of an adult is $5 - 10 \times 10^6$ cells per ml of blood. Therefore, the theoretical recovery of DNA per μl of blood is 30 - 60 ng.

How Much DNA Do We Need?

- The PCR reactions call for on average 1 ng of DNA (single or double stranded).
- This is the equivalent of 1/20 of 1 ul of blood, or 350 sperm.
- Many of the commercially available kits are sensitive below 1 ng of DNA (100-250 pg).

How Much DNA Do We Need?

- The RFLP procedure on requires a minimum of 50 ng of high molecular weight double stranded DNA. This is the equivalent of approximately 2 ul of blood. The number of intact sperm (3 pg/sperm) is approximately 20,000.

What are the Most Commonly used DNA Extraction Procedures in Forensic Science?

- Organic (Phenol-Chloroform) Extraction
- Non-Organic (Proteinase K and Salting out)
- Chelex (Ion Exchange Resin) Extraction
- FTATM 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 forensic 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

ORGANIC EXTRACTION REAGENTS

- 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 Dnase nucleases. If the Mg^{2+} is bound up by EDTA, nucleases are inactivated.

ORGANIC EXTRACTION REAGENTS

- 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.

ORGANIC EXTRACTION REAGENTS

- 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

- Phenol - often means phenol equilibrated with buffer (such as TE) and containing 0.1% hydroxyquinoline and 0.2% b-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 ratio is 25:24:1

Concentrating DNA

Alcohol Precipitation

- The most widely used method for concentrating DNA is precipitation with ethanol.
- The precipitate of nucleic acid, forms in the presence of moderate concentrations of monovalent cations (Salt, such as Na^+), is recovered by centrifugation and re-dissolved in an appropriate buffer such as TE.
- The technique is rapid and is quantitative even with nanogram amounts of DNA.

Concentrating DNA

Alcohol Precipitation

- The four critical variables are the purity of the DNA, its molecular weight, its concentration, and the speed at which it is pelleted.
- DNA concentrations as low as 20 ng/ml will form a precipitate that can be quantitatively recovered.
- Typically 2 volumes of ice cold ethanol are added to precipitate the DNA.

Concentrating DNA

Alcohol Precipitation

- Very short DNA molecules (<200 bp) are precipitated inefficiently by ethanol.
- The optimum pelleting conditions depend on the DNA concentration. Relatively vigorous microcentrifuge steps such as 15 minutes at or below room temperature at 12,000 rpm are designed to minimize the loss of DNA from samples with yields in the range of a few micrograms or less.

Concentrating DNA

Alcohol Precipitation

- Solutes that may be trapped in the precipitate may be removed by washing the DNA pellet with a solution of 70% ethanol.
- To make certain that no DNA is lost during washing, add 70% ethanol until the tube is 2/3 full. Vortex briefly, and recentrifuge. After the 70% ethanol wash, the pellet does not adhere tightly to the wall of the tube, so great care must be taken when removing the supernatant.

Concentrating DNA

Alcohol Precipitation

- Isopropanol (1 volume) may be used in place of ethanol (2 volumes) to precipitate DNA. Precipitation with isopropanol has the advantage that the volume of liquid to be centrifuged is smaller.
- Isopropanol is less volatile than ethanol and it is more difficult to remove the last traces; moreover, solutes such sodium chloride are more easily coprecipitated with DNA when isopropanol is used.

Concentrating DNA

Microcon®100 Centrifugal Filter Unit



Concentrating DNA

Microcon®100 Centrifugal Filter Unit

- Excellent recovery of DNA samples with recoveries typically > 95%.
- Ideal for dilute (ng/mL to µg/mL range) of DNA solutions
- Concentrating and purifying proteins, antibodies and nucleic acids (alternative to EtOH precipitation)
- Desalting and buffer exchange
- Removal of primers, linkers and unincorporated label

Concentrating DNA

Microcon®100 Centrifugal Filter Unit

- Patented deadstop allows for reliable and reproducible concentrate volumes
- Inverted spin method of concentrate retrieval maximizes recovery
- Low-binding Ultracel-YM membrane with 100,000 NMWL (Nominal Molecular Weight Limit) cut-off
- Convenient sample storage of filtrate or concentrated sample in standard microfuge collection tube
- Use in standard 1.5 mL tube fixed-angle rotors

Resuspension and Storage of DNA

- TE Buffer - Tris-EDTA Buffer: 10 mM Tris-HCl pH 8.0, 1 mM EDTA, or TE-4 which is 10 mM Tris, 0.1 mM EDTA. DNA is resuspended and stored in TE buffer. DNA must be stored in a slightly basic buffer to prevent depurination, and the EDTA chelates any Mg^{2+} helping to inactivate DNases.
- DNA can be stored at 4°C for extended periods, however for long term storage, - 20°C is usually utilized.
- Avoid repetitive freeze thawing of DNA, since this can cause degradation.
- The storage of DNA at 4C is better than -20C and storage at room temp dried with stabilizer is even better (Lee et al. 2012)

Organic Extraction

Pros:

- yields relatively pure, high molecular weight DNA
- DNA is double stranded – good for RFLP

Cons:

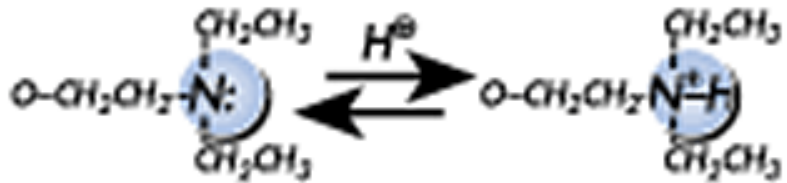
- Time consuming
- Requires sample to be transferred to multiple tubes – increases risk of contamination
- Involves use of hazardous (and smelly!) chemicals

What Does *Qiagen silica* Do?

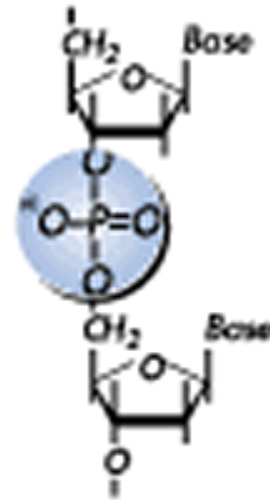
http://www.qiagen.com/resources/info/qiagen_purification_technologies_1.aspx

Greenspoon, S. A., M. A. Scarpetta, M. L. Drayton, and S. A. Turek. 1998 .
QIAamp spin columns as a method of DNA isolation for forensic casework. *J Forensic Sci* 43 (5): 1024–30.

Binding Principle of QIAGEN Resin



DEAE (diethylaminoethanol)



**Chemical structure
of DNA**

Silica-Based Extraction

Pro:

- Quick
- Highly purified DNA

Con:

- Multiple sample transfer
 - Increase risk of contamination

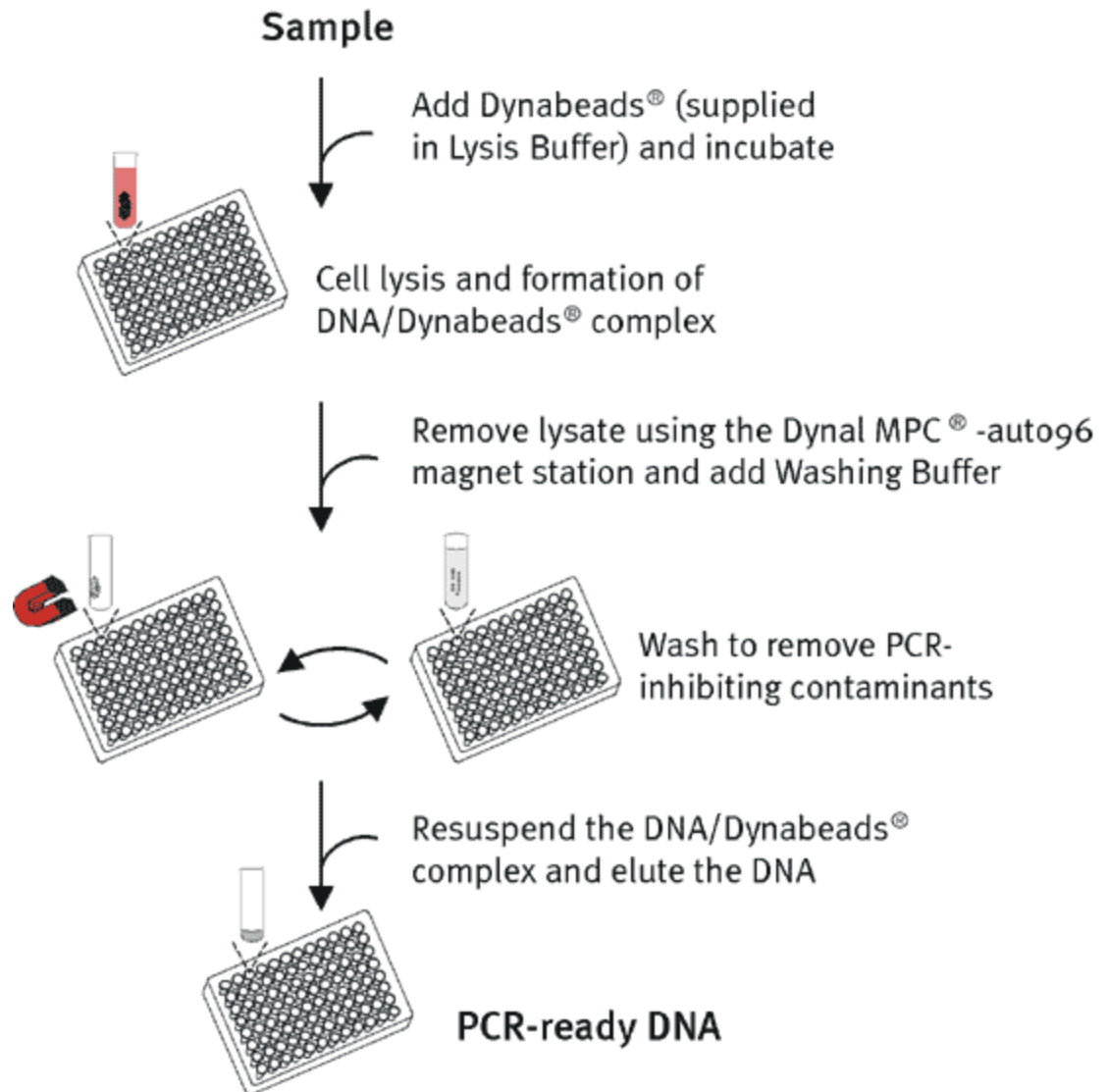
Magnetic Beads

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



Magnetic Beads

- Automated version:



Magnetic Beads

Pro:

- Very fast, may be automated
- Highly purified DNA
- Excellent for liquid blood

Con:

- Cannot be used directly on stain
 - i.e. need to remove cells from stain substrate (cloth, etc.)
- Very expensive

Non-Organic DNA Extraction

- Does not use organic reagents such as phenol or chloroform.
- Digested proteins are removed by salting out with high concentrations of LiCl.
- However, if salts are not adequately removed, problems could occur with the RFLP procedure due to alteration of DNA mobility (band shifting)

Non-Organic DNA Extraction Procedure

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
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.

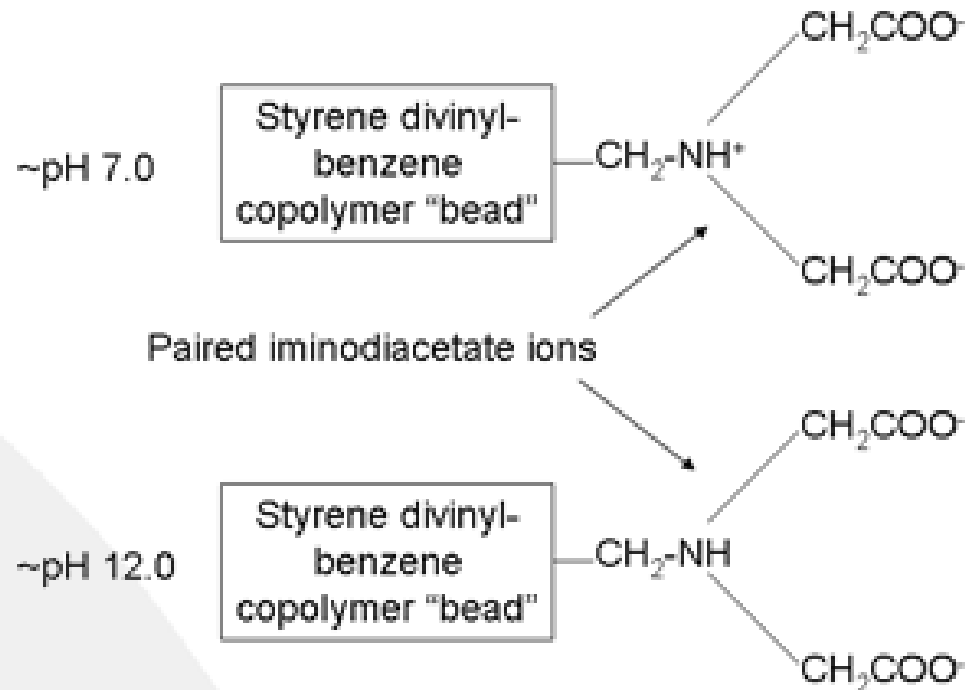
Non-Organic DNA Extraction Procedure

4. DNA remains in solution. Transfer supernatant to a new tube, care must be taken not to take any of protein pellet.
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.

Chelex Extraction

Chelex 100, Molecular Biology Grade resin from BioRad is a highly pure, nuclease and ligase inhibitor-free chelating resin, certified not to interfere with downstream PCR. Specifically designed to complement the inherent requirements of PCR, this pure, pipettable, small-scale resin is ready for downstream use. Ensuring the complete removal of PCR inhibitors, contaminating metal ions that catalyze the digestion of DNA

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

Chelex Extraction

- Chelex 100 is an ion exchange resin that is added as a 5% solution (wt/vol).
- Chelex is composed of styrene divinylbenzene copolymers containing paired iminodiacetate ions that act as chelating groups in 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.

Chelex Extraction

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Chelex Extraction

- A 5% solution of Chelex is added to a blood stain or liquid blood and incubated at 56C for 30 minutes. This step is used to lyse red cells and remove contaminants and inhibitors such as heme and other proteins.
- The sample is then heated at 100C for 8 minutes. This causes the DNA to be denatured as well as disrupting membranes and destroying cellular proteins.
- The tube containing the Chelex is centrifuged, the resin is pelleted, the supernatant containing the DNA is removed.

Chelex Extraction

- The Chelex extraction process denatures double stranded DNA and yields single stranded DNA, and thus cannot be used for the RFLP procedure.
- It is advantageous for PCR-based typing methods because it removes inhibitors of PCR and can be done in a single tube, which reduces the potential for laboratory-induced contamination and sample switching.
- Care should be taken not to have any residual Chelex with the DNA extract, since Mg^{2+} is required for the Taq Polymerase.

Chelex extraction

Pros:

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

Con:

- Results in single-stranded DNA – not useful for RFLP

FTA[®] PAPER

http://www.nfstc.org/pdi/Subject03/pdi_s03_m04_02_d.htm

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

FTA[®] Paper

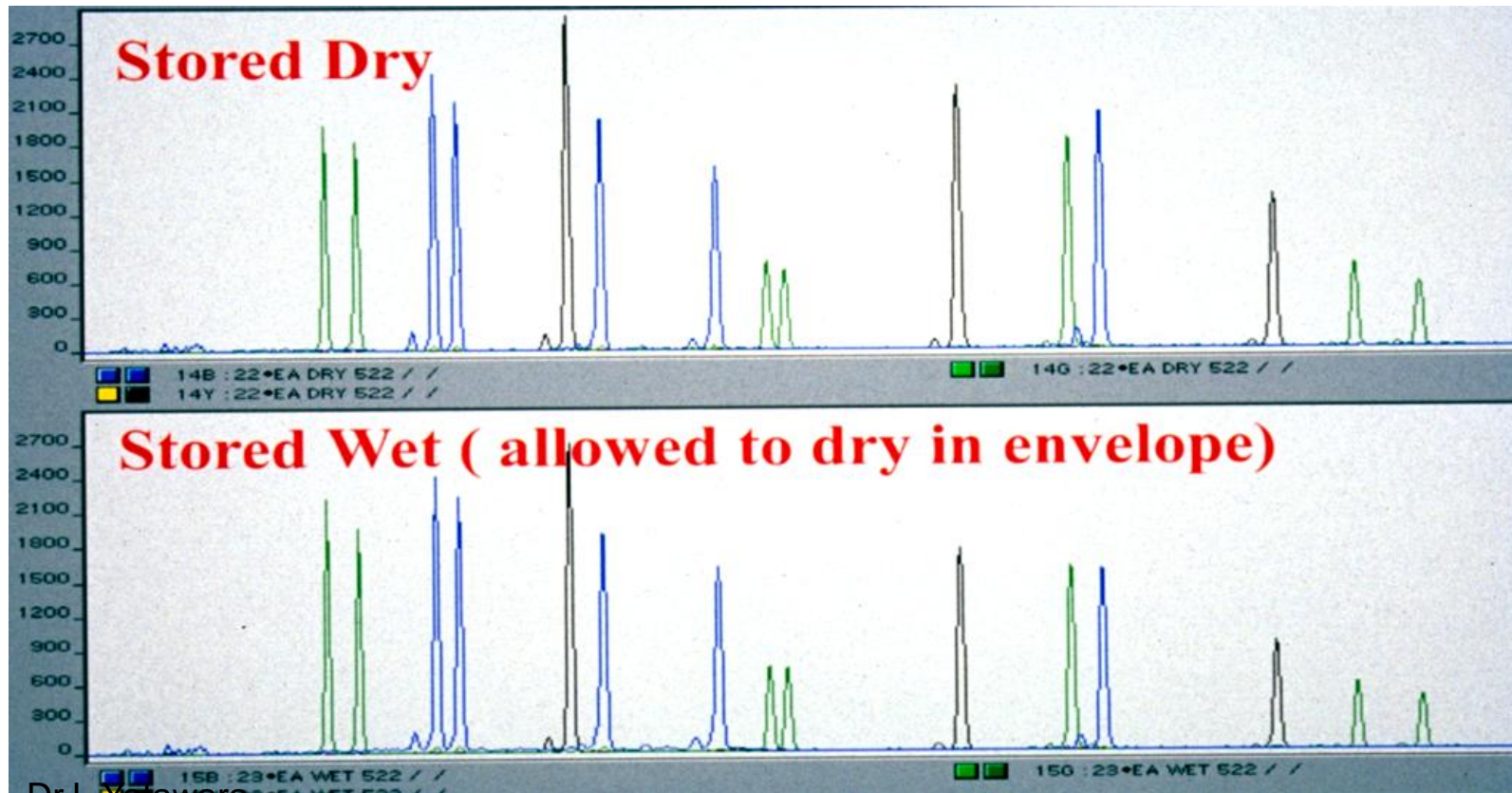


- Is a unique mixture of strong buffers, protein denaturants, chelating agents, and a UV absorbing, free radical trap.
- The reagents are impregnated into a cellulose-based filter matrix such as Whatman BFC180 or 31ET paper

What Does *FTA*[®] Paper Do?

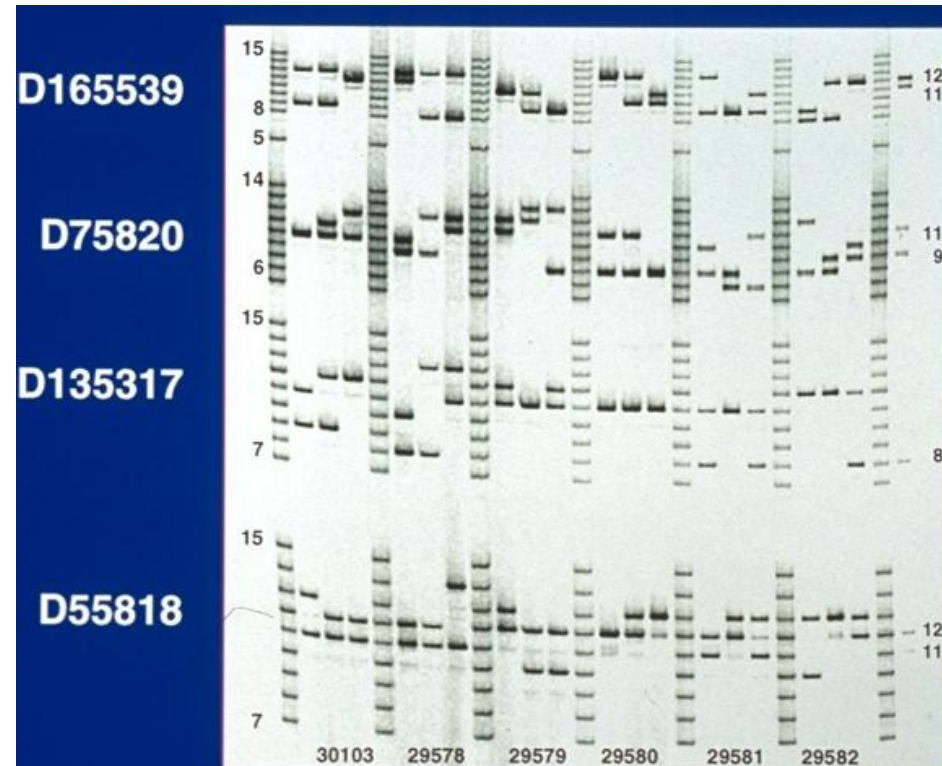
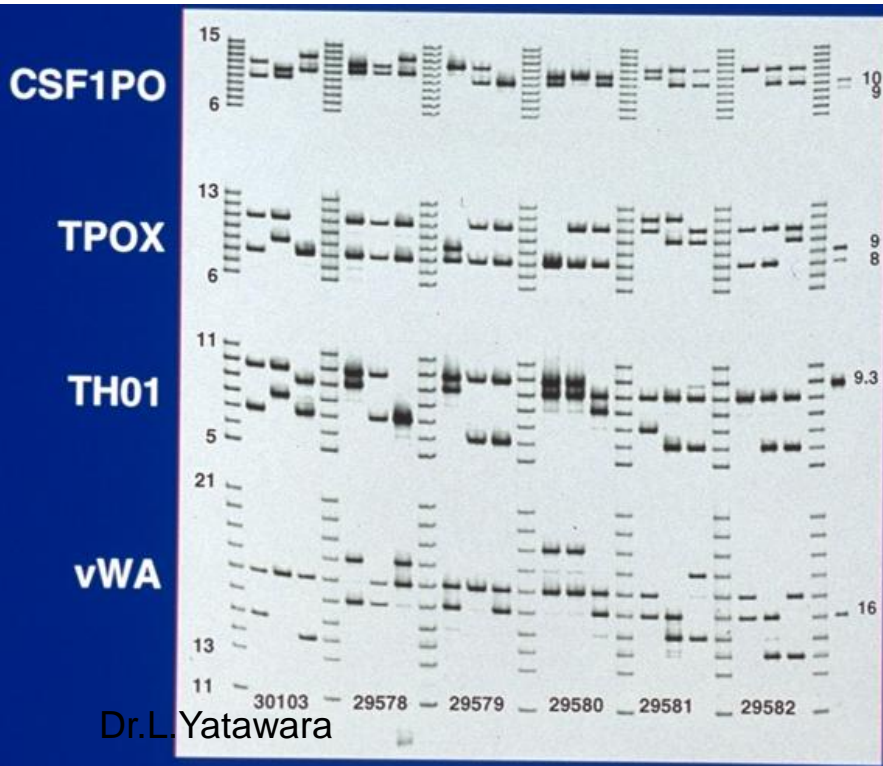
- kills blood borne pathogens on contact
- immobilizes DNA within the matrix
- protects DNA from degradation
- allows for long-term storage at room temp

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



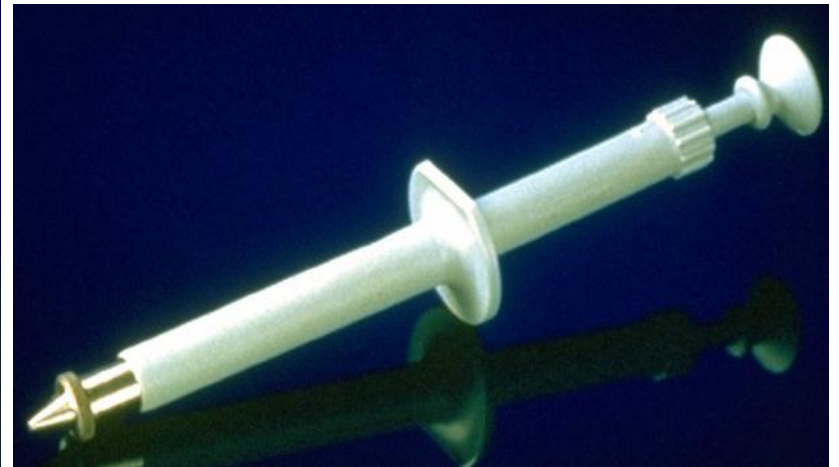
PCR Template Concentration Artifacts Minimized

No DNA Quantitation is Required



FTA[®] Gene Guard System

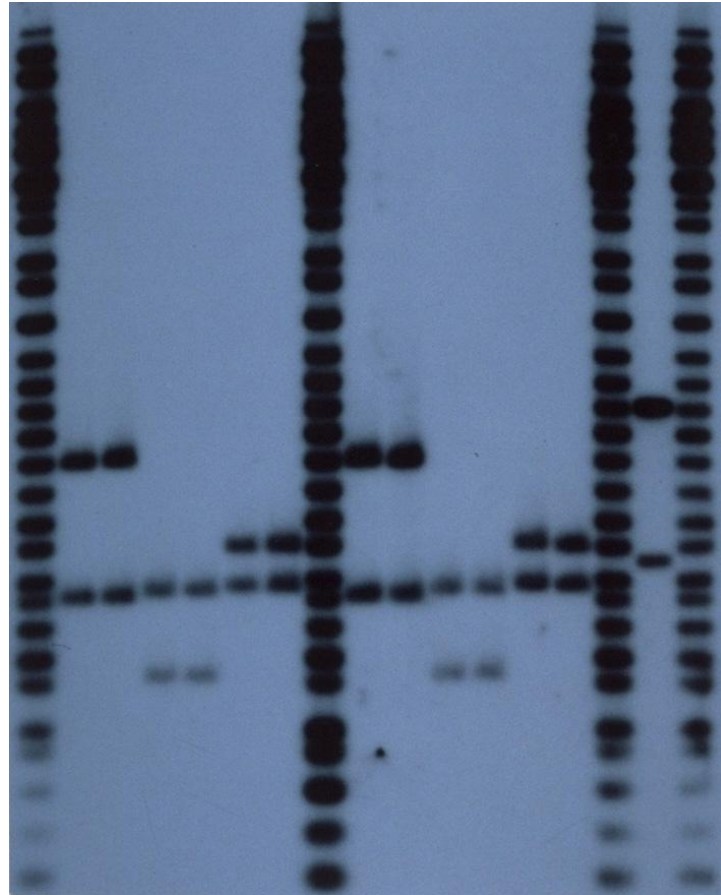
A novel system for the collection, transport, storage and purification of DNA



RFLP Analysis From Samples Stored on FTA[®] Paper

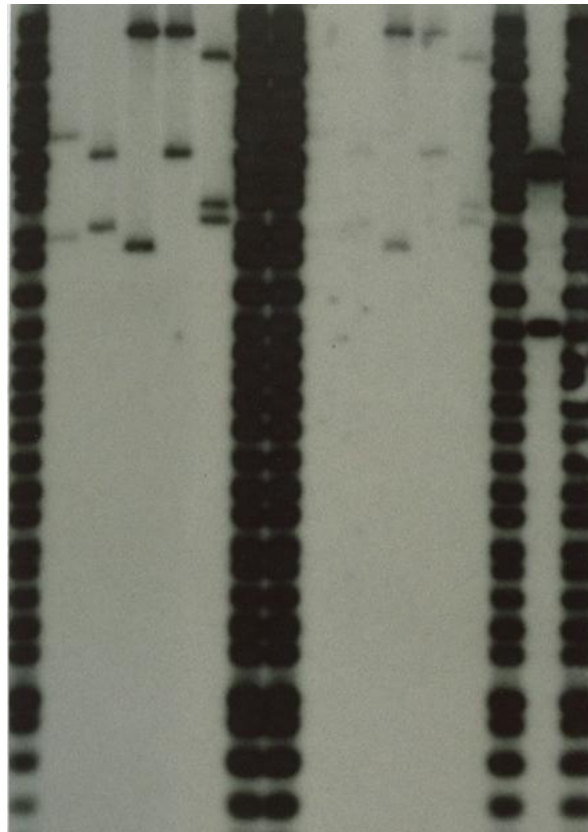


Quintix™ and
Plus CD-Dispenser
are used to see the paper

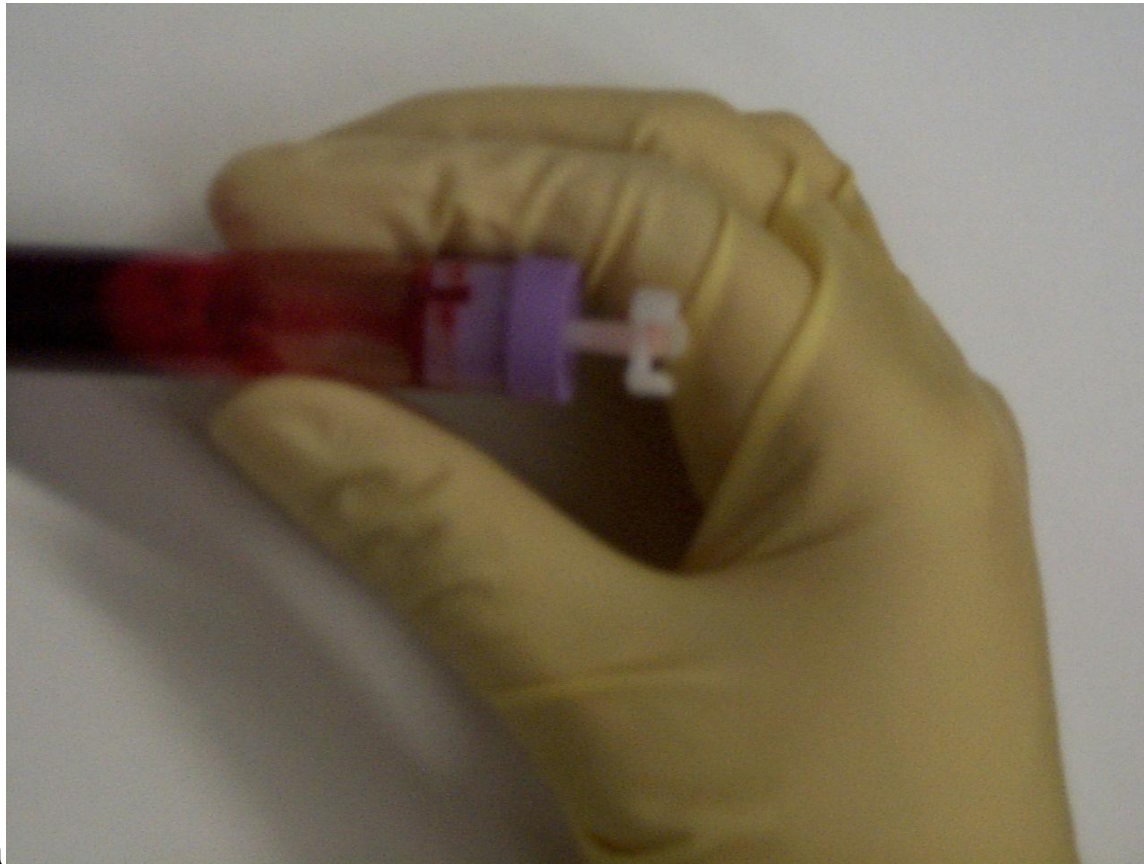


Samples Stored for 11 Months Prior to RFLP Analysis

FTA Untreated



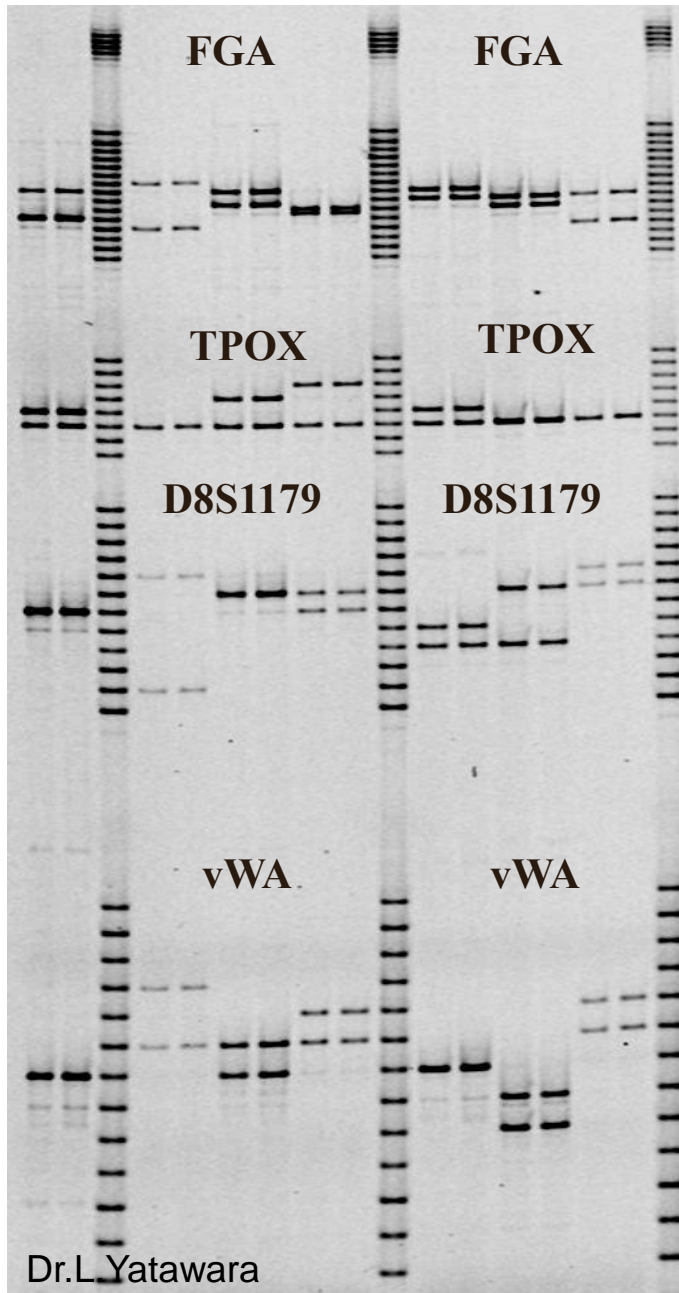
Simple Method For the Application of Blood onto FTA[®] Paper



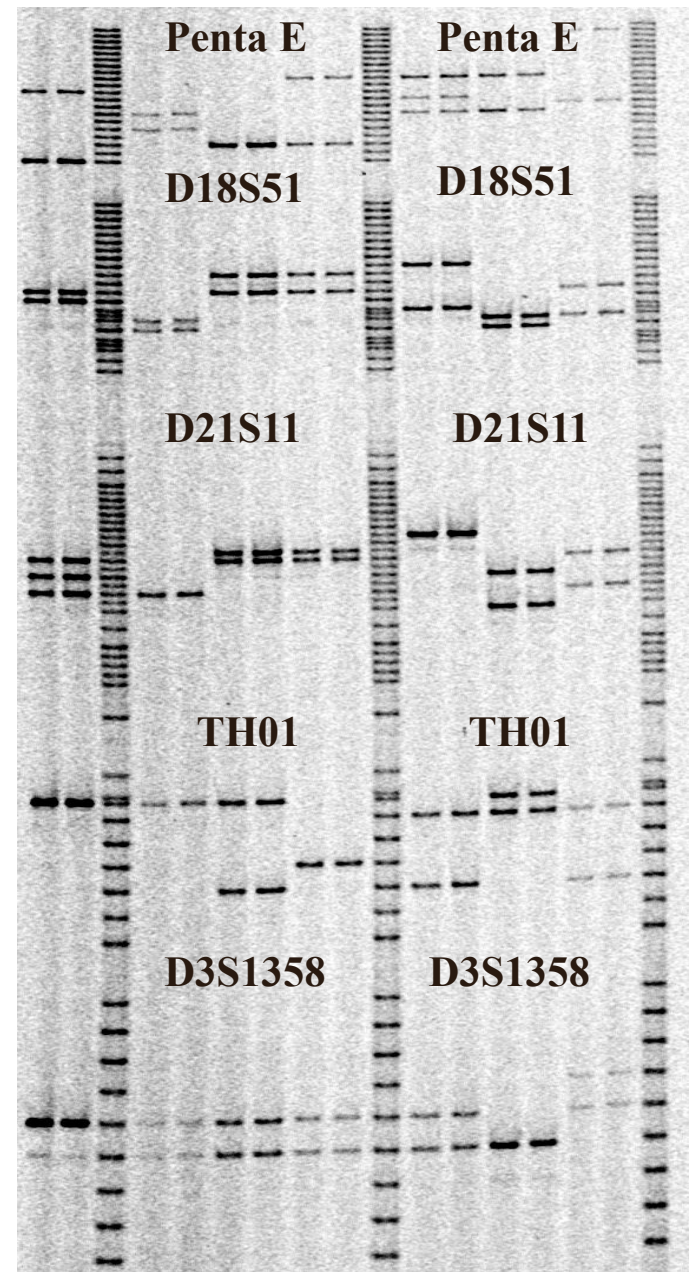
Buccal Swab Collection and Direct Transfer to FTA[®] Paper



25 ul Blood spotted on Elute Plate, DNA eluted in 200 ul, 10 ul PCR Rx with 2ul DNA



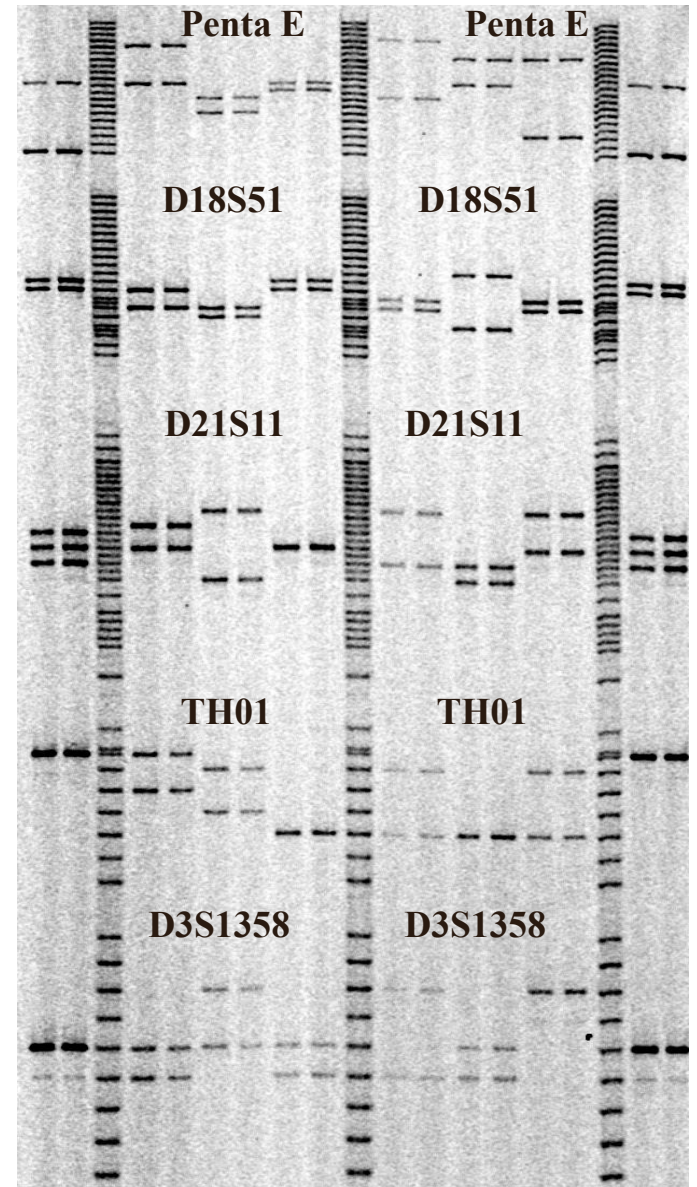
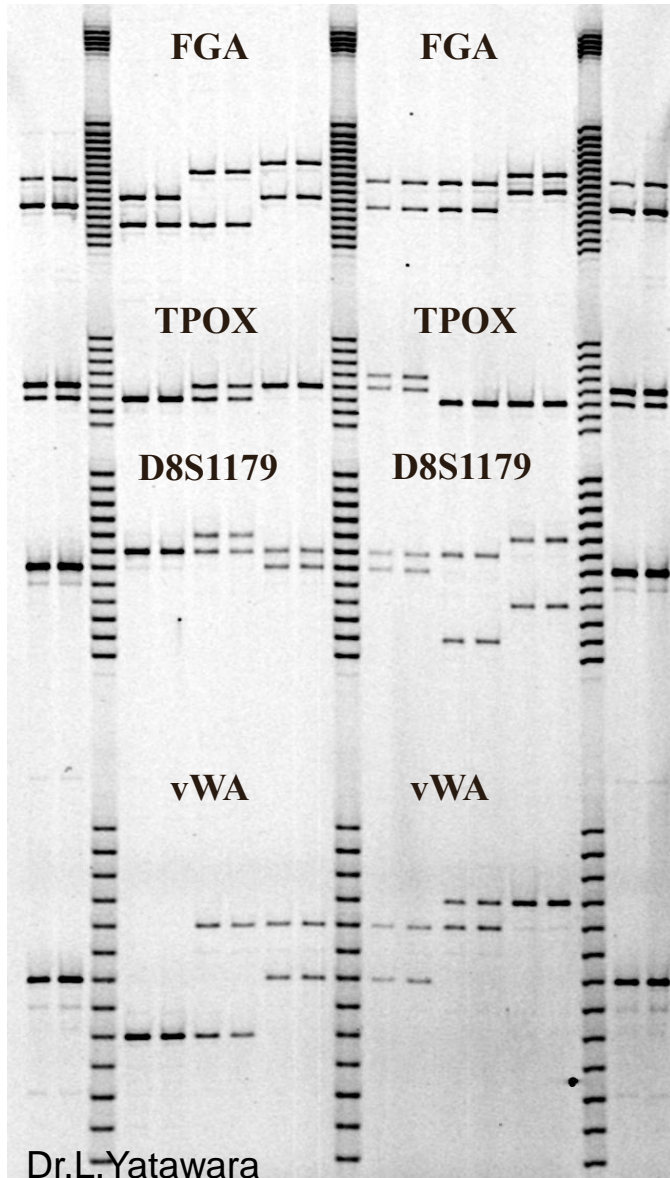
10 20ng [LL] [NN] [PP] [SS] [TT] [XX]
K562



10 20ng [LL] [NN] [PP] [SS] [TT] [XX]
K562

PowerPlex™ 2.1

DNA ISOLATED ON GENPLATE FTA-ELUTE FILTERPLATE FROM 25 ul BLOOD
2 ul DNA / 10 ul PCR Reaction



10 20ng A1 A2 C1 C2 D1 D2 H1 H2 J1 J2 K1 K2 10 20ng
K562 K562

10 20ng A1 A2 C1 C2 D1 D2 H1 H2 J1 J2 K1 K2 10 20ng
K562 K562

FTA™ Paper

Pros:

- Very quick
- Useful for both storage and extraction

Cons:

- Not useful for RFLP
- Paper punched “jump” because of static electricity – potential contamination

Differential Extraction

- Modified version of the organic extraction procedure. First described by Gill et al. 1985, and Guisti et al. 1986.
- Process to isolate the male and female DNA from a sexual assault evidentiary sample.
- From a single evidentiary sample, a female fraction containing the DNA from the victims epithelial cells, and a male fraction containing the DNA from the sperm of the assailant are isolated.

Differential Extraction

- The procedure involves preferentially breaking open the female epithelial cells with an 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.
- The sperm pellet is washed several times to remove any residual DNA from the victim.

Differential Extraction

- The sperm are subsequently lysed by treatment with a SDS/proteinase K/ dithiothreitol (DTT) mixture. The DTT is required to breakdown (reduce) the protein disulfide bridges that make up the sperm head. The sperm are impervious to 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.

DNA Quantitation

- Total DNA recovered
- Human Specific DNA recovered (DAB requirement for forensic casework samples)

DAB STANDARD 9.3

The laboratory shall have and follow a procedure for evaluating the quantity of the human DNA in the sample where possible.