Specimens in the Lab

	Adequate for PCR
- Whole Blood	- Dried Blood
- Bone Marrow	- Saliva
- Tissue/ Scrapings	- Bone, Teeth
- Bacteria	- Amniotic fluid
- Fungi/Yeasts	- Hair follicles/ Hair Shafts
- Buccal Cells	- Buccal Cells
- Hair	- Cerebrospinal fluid
- Urine	- Fixed tissue
- Stool	- Stool
	- Soil
	- Urine

DNA Extraction Methods

Organic

Uses organic chemical, phenol, choloroform

1. Lysis (NaOH, SDS)	2. Acidification (acetic acid, salt)
3 Extraction (phenol, chloro-form)	4. DNA precipitation (ethanol)

Inorganic

Uses inorganic chemicals, detergent, ethylenediamine tetraacetic acid (EDTA), acetic acid, salt (salting out, spooling)

1. Lysis (Tris, EDTA, SDS)	2. Protein precipitation (sodium acetate)
3. DNA precipitation (isopropanol)	

Solid phase

DNA is immobilized on a solid support, beads, or columns

1. Lysis (supplied reagents)	2. Acidification (supplied reagents)
3. Adsorption (low pH)	4. Wash (supplied buffer)
5. Elute DNA (low salt)	

DNA Extraction Methods (cont)

Solid-Phase Isolation	-Kits -Spin Columns

Nucleic acid binding to silica beads in the basis for many automated extraction systems

Limiting specimens (fixed tissue, dried, bone)

Rapid extration for routine testing

Crude lysis

DNA does NOT need to be pure	RE digestion, gel electrophoresis, screening large amounts of DNA, some PCR, challenging specimens
Proteolytic enzymes	Proteinase K (PK)
Chelating resins	Chelex

Chelex resin

Heat tissue (hair roots, saliva, etc.) in 300 uL 5%-20% Chelex 100 resin

Chelex removes multivalent cations

DNA is in supernatant

Fixed tissue

1. ~20 micron sections	Macrodissect or microdissect

2. Digest (proteinase K, Tris buffer)

DNA Isolation

DNA isolation, purification and quantitation	Free of proteins, lipids, other nucleic acids
Different methods depending on specimen type	??????????????????????????????????????
Different methods depending on type of DNA needed	??????????????????????????????????????

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DNA Quantitation

Spectrophotometry

Instrument that allows you to measure the amount of light that is absorbed in a sample or the amount of light transmitted based on the solute in the sample

Filters and/or prisms are used to filter the light to obtain a certain wavelength

A= absorbance
%T= transmittance

A260/A280 Ratio

1.65 <= A260/A280 <= 2.50

dsDNA concentration = 50 ug/mL x OD260 x dilution factor

Beer - Lambert Law

Absorbance is directly proportionate to the concentration of the solute (DNA)

Absorptivity constant - 50 for DNA

- Conversion factor from optical density unit (absorbance units) to concentration

- Residual phenol - bad!!

Calculations

Usually have to dilute samples from preps to begin

A DNA prep diluted 1:100 has an A260 reading of 0.200. Find the concentration in ug/mL

A DNA prep diluted 1:50 has an A260 reading of 0.307. Find the concentration in ug/mL.

Separating out the cells

Differetial Density gradient centrifugation

Breaking open the cells

Alkaline lysis

Differential lysis

Cell wall digestion

Lysozyme

Zymolyase

Detergents

Boiling

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