Cheatography

DNA Isolation and Quantitation Cheat Sheet by Morghay123 via cheatography.com/53154/cs/16995/

Specimens in the Lab		DNA
	Adequate for PCR	Solic
- Whole Blood	- Dried Blood	Isola
- Bone Marrow	- Saliva	Nucl
- Tissue/ Scrapings	- Bone, Teeth	exile
- Bacteria	- Amniotic fluid	LIIIII
- Fungi/Yeasts	- Hair follicles/ Hair Shafts	napi
- Buccal Cells	- Buccal Cells	
- Hair	- Cerebrospinal fluid	need
- Urine	- Fixed tissue	Prote
- Stool	- Stool	enzy
	- Soil	Chel
	- Urine	Che
		Heat
DNA Extraction Methods		Chel
Organic		DNA

Uses organic chemical, phenol, choloroform

 1. Lysis (NaOH, SDS)
 2. Acidification (acetic acid, salt)

 3 Extraction (phenol, chloroform)
 4. DNA precipitation (ethanol)

 Inorganic
 1. Lysis (NaOH, SDS)

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

1. Lysis (Tris, EDTA, SDS)

2. Protein precipitation (sodium acetate)

2. Acidification (supplied reagents)

4. Wash (supplied buffer)

3. DNA precipitation (isopropanol)

Solid phase

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

- 1. Lysis (supplied reagents)
- 3. Adsorption (low pH)
- 5. Elute DNA (low salt)

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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

С

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Breaking open the cells

Alkaline lysis

Differential lysis

Cell wall digestion

Lysozyme

Zymolyase

Detergents

Boiling

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