DNA, Blood, & Toxicology Unit

1. Understanding deoxyribonucleic acid (DNA)

↳ molecules carrying a body's genetic information. shaped in a double-helix

A. Human genome (DNA) - 1ST completely mapped in 1985.

- Alec Jeffries & colleagues @ Leicester University in England
- realized that portions of DNA structure contain genes that are unique to each individual
  - ↳ only identical twins have the same DNA
  - ↳ chimera - someone who absorbed their twin (not always identical) in utero & have 2 sets of dna in one body.
- dna fingerprinting (typing/profiling) - technique for isolating & reading those individual dna markers.
- dna can be obtained from biological evidence: blood, semen, hair (follicular tag), tissue, & bone.

B. What is dna?

(1) - human body has 60 trillion cells containing chromosomes - threadlike structures in the cell nucleus containing genes.

- genes - arranged on the thread like beads. Each chromosome has nearly 25,000 genes. fundamental unit of heredity - instructs body cells to make proteins that determine everything from hair color to susceptibility to diseases
- each gene is composed of dna designed to carry out a single body function

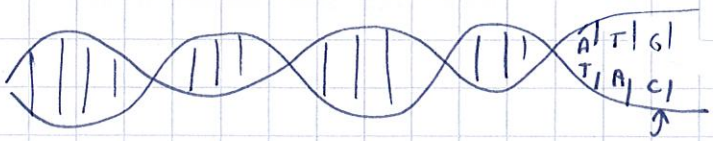
(2) structure of dna

- discovered by James Watson & Francis Crick in the 1950s.
- dna is a polymer - molecule made of a large # of atoms, usually arranged in repeating units called monomers. In dna, the monomers are called nucleotides.
  - ↳ repeating dna unit made of 4 bases attached to a phosphate-sugar group.
    - adenine pairs - cytosine
    - guanine - thymine

can be millions of bases long

see pic

(3) complementary base pairing
A & T go together as do C & G on the double helix to make the ladder



the number of different sequences combining is staggering since average chromosome contains 1 Million base pairs — human has dna that

- all the human chromosomes taken together contain ~3 billion base pairs.

(4) dna at work
- Inheritable traits are controlled by dna's ability to direct the production of proteins - made by linking a combo of amino acids (~20)

See pic 2

Ex) hemoglobin protein - (found in red blood cells, which carry O_2 to our cells & carry away CO_2, is made of 4 amino acid chains

Normal: valine - histidine - leucine - threonine - proline - glutamate - glutamate

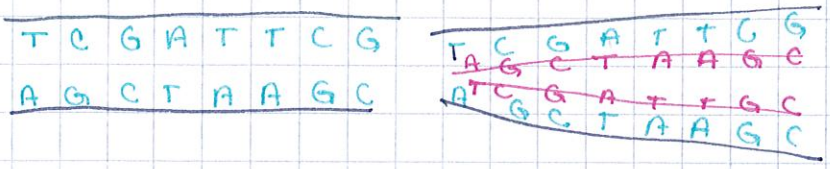
Sickle cell: valine - glutamate

- the genetic info that determins the amino acid sequence for every protein manufactured in the body is stored in dna (the A, T, G, & C).
  - the key for deciphering the genetic code for each amino acid is a sequence of 3 of those bases
    & alanine - C-G-T phenylalanine - A-A-A
    aspartate - C-T-A glutamate - G-A-G
    so looking at a dna segment
    (C-G-T)(C-T-A)(A-A-A)(C-G-T)
    alanine aspartate phenylalanine alanine

2. Replication of DNA

A. The Process of Replication (copying)

(1) dna replication - the synthesis of new dna from existing dna
- each strand is exposed to a collection of free nucleotides.
- Letter by letter, the double-helix is re-created as nucleotide are assembled in the proper order



See Pic 3

B. Polymerase Chain Reaction (PCR) - lab technique for replicating (copying) a dna strand outside the human body.

(1) small quantities of dna found at a crime scene can be replicated for testing so that the sample is not lost by the testing.

(2) done by the aid of a DNA polymerase in a DNA thermal cycler
- a. each cycle doubles the amount of dna. In a few hours 30 cycles can multiply dna by a billion
- b. now dna sample size is no longer a limitation in characterizing dna from a crime scene.
- c. process
  - i restriction enzymes cut dna molecules ~~from 2 different sources~~ → more than 20 commercially available)
  - ii insert dna into a foreign dna strand (usually bacterium) the bacteria multiply rapidly, passing on altered copies to descendants

3. DNA Typing

30% of human genome is made of repeating segments of DNA.
- acts as filler b/w coding regions of dna

RFLP - restriction fragment length polymorphisms
- typically 15-35 bases long that repeats many times

- fragments separated by electrophoresis
  - dna placed on a gel-coated plate
  - electrical charge ∴ dna migrates across the plate
  - smaller fragments move faster ∴ farther across the plate

1st scientifically accepted protocol for forensics but short lived (1985-mid 90's) (took too long)

used in Clinton-Lewinsky dress

see pic p 380

- transferred to a nylon membrane as is on gel, exposed to radioactive particles. X ray film is placed next to membrane ∴ then developed.

: PCR - Polymerase Chain Reaction
- used to duplicate a DNA strands millions of times
1st - short sequences of dna identified in pure form (primer)
2nd - add primers to separated dna strands ∴ allow them to combine w/ dna strands

primer

G-T-C-T-A-G-C-T-T-C-C-A-G
C-A-G-A-G-T-C-G-A-A-G-G-T-C

G-T-C-T-A-G-C-T-T-C-C-A-G
C-A-G-A

C-C-A-G
C-A-G-A-T-C-G-A-A-G-G-T-C

28-32 cycles are done yielding more than 1B copies

Each cycle takes 2 min.

3rd - add dna polymerase ∴ a mix of free nucleotides to separate strands ∴ molecules rebuild themselves.

- Advantages of PCR
  - RELP strands 1,000s bases long, PCR only 100's
  - shorter strands are more stable, less subject to degradation
  - use tiny amounts of original substance

STR - short tandem repeats
- most successful & widely used.
- repeating sequences of 3-7 basis, usually less than 450 basis long, found in great abundance.
- ideal for PCR, overcoming limited sample size
  1/1,000,000,000 g or less of dna required
  (1/50 - 1/100 the amount needed for RFLP)

Look @ a common STR known as TH01 - has repeating A-A-T-G
7 variants of TH01 are found in humans

TH01 is extracted & amplified by PCR, then separated by electrophoresis. By examining the distance the STR has migrated, you can determine # of A-A-T-G repeats in the STR.
- every person has 2 STR types for TH01 - one from each parent.
  so you might have a semen stain TH01 w/ 6 repeats & 8 repeats

Mutiplexing - detecting more than 1 dna marker in a single analysis.

see table 10 p388 In the US, the forensic sci community has standardized on 13 STRs for entry into CODIS.