

Introduction to Molecular Testing

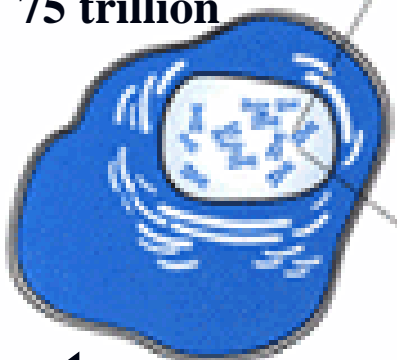
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Laboratory Services Section

Outline

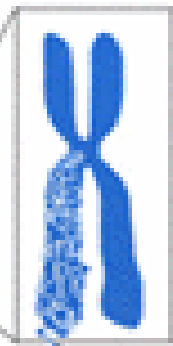
- **Basic genetics**
 - DNA
 - Genes & Gene Structure
 - Mutations
- **Basic molecular techniques**
 - Nucleic Acid Extraction
 - Electrophoresis
 - Hybridization
 - Restriction Digestion
 - Polymerase Chain Reaction
 - DNA sequencing



75 trillion



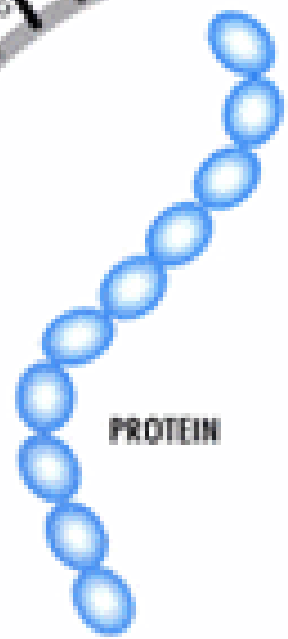
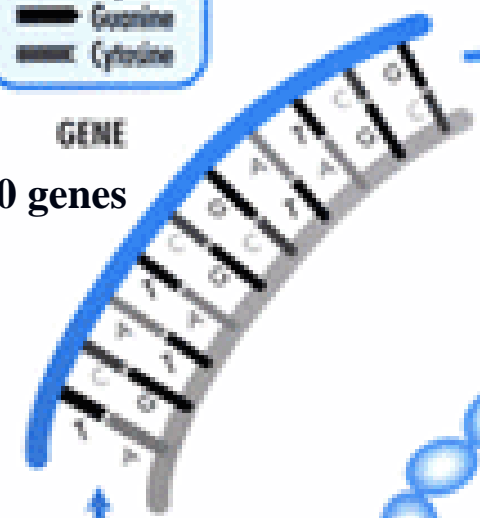
CELL



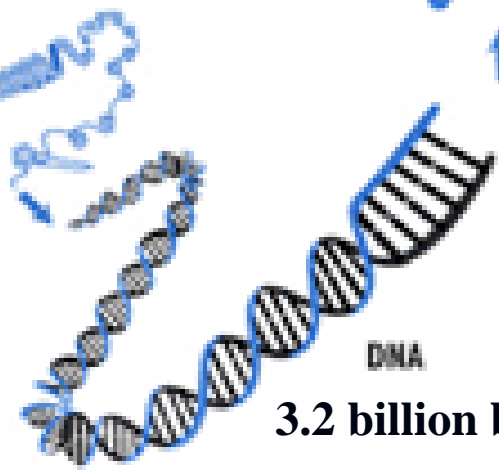
CHROMOSOME
23 pairs

- Adenine
- Thymine
- Guanine
- Cytosine

GENE
~25,000 genes

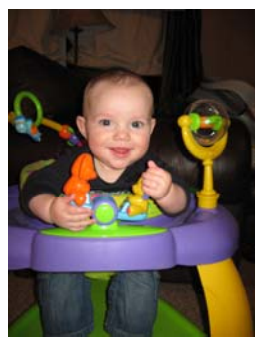


PROTEIN



DNA

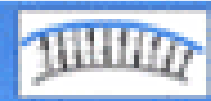
3.2 billion bp



CHROMOSOME



DNA



GENE



PROTEIN

Legend: In the nucleus of every cell is a set of chromosomes, which are made of densely packed DNA.

Illustration courtesy of Greenwood Genetic Center

The basics of inheritance

- Inherit one chromosome from each parent
 - two complete sets of chromosomes
 - chromosome = DNA + proteins
- DNA - Deoxyribonucleic acid
 - Unique genetic blueprints for each individual
 - Double helix of complementary nucleotides
 - Nucleotide = base + sugar + phosphate
 - DNA nucleotides - adenine (A), cytosine (C),
guanine (G), & thymine (T)
- RNA – Ribonucleic Acid
 - RNA nucleotides – adenine (A), cytosine (C),
guanine (G) & uracil (U)

Base Pairing Rules

- Nucleotide = base + sugar + phosphate
- DNA
 - Adenine pairs with Thymine (A – T)
 - Cytosine pairs with Guanine (C – G)
- RNA
 - Adenine pairs with Uracil (A – U)
 - Cytosine pairs with Guanine (C – G)

Gene Structure



- Exon – contains part of the open reading frame of the complete protein
- Intron – not translated into protein
- Regulatory Regions
 - Promoter – facilitates transcription of a gene
 - Untranslated Region (UTR) – important for efficient translation and for controlling the rate of translation

Genes

- Average size: 3,000 bp
- Largest known human gene: Dystrophin
 - 2.4 million bp
- Estimated 25,000 genes
- Function is not yet known for >50% of discovered genes
- ~2% of genome is coding sequence

β -Globin Gene



- Mutations lead to clinically significant hemoglobinopathies
 - sickle cell anemia
 - sickle beta-thalassemia
 - homozygous β^0 -thalassemia
- 1,600 base pairs, 3 exons, 146 amino acids
- > 700 known mutations

Phenylalanine Hydroxylase



- 99% of mutations causing PKU occur in Phenylalanine Hydroxylase (PAH)
- ~90,000 bp, 13 exons, 452 amino acids
- > 500 reported mutations

Cystic Fibrosis Transmembrane Conductance Regulator



- Mutations in CFTR may cause Cystic Fibrosis
- ~250,000 base pairs
- 1480 amino acids
- 27 exons
- >1600 known mutations

From a gene to a protein: The Central Dogma

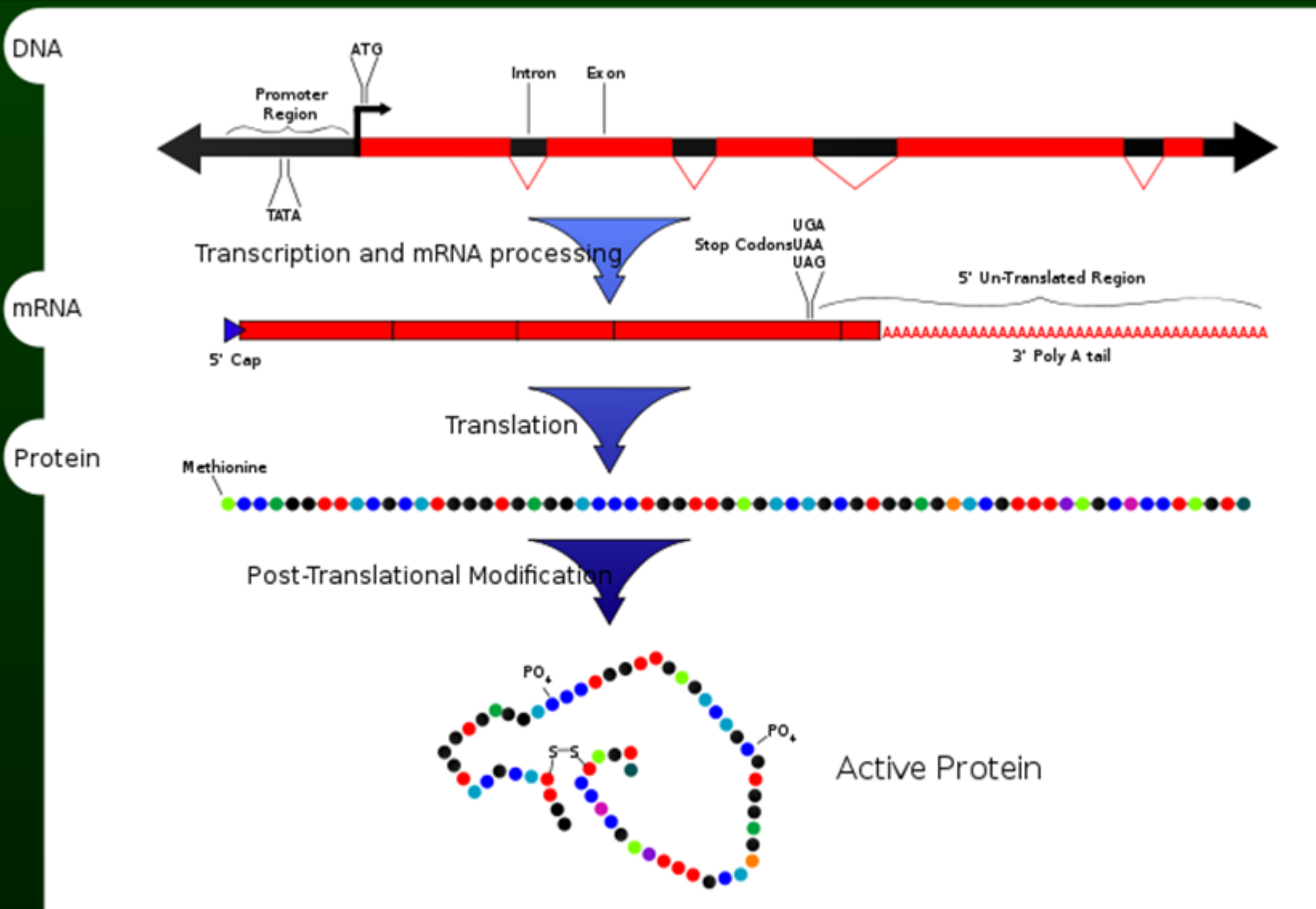


Figure: http://en.wikipedia.org/wiki/Central_dogma_of_molecular_biology

The Genetic Code

2 →

	U	C	A	G	
U	UUU Phenylalanine UUC Alanine UUG Leucine UUA Leucine	UCU Serine UCC Serine UCA Serine UCG Serine	UAU Tyrosine UAC Tyrosine UAA Stop UAG Stop	UGU Cysteine UGC Cysteine UGA Stop UGG Tryptophan	U C A G
C	CUU Leucine CUC Leucine CUA Leucine CUG Leucine	CCU Proline CCC Proline CCA Proline CCG Proline	CAU Histidine CAC Histidine CAA Glutamine CAG Glutamine	CGU Arginine CGC Arginine CGA Arginine CGG Arginine	U C A G
A	AUU Isoleucine AUC Isoleucine AUA Isoleucine AUG Methionine	ACU Threonine ACC Threonine ACA Threonine ACG Threonine	AAU Asparagine AAC Asparagine AAA Lysine AAG Lysine	AGU Serine AGC Serine AGA Arginine AGG Arginine	U C A G
G	GUU Valine GUC Valine GUA Valine GUG Valine	GCU Alanine GCC Alanine GCA Alanine GCG Alanine	GAU Aspartic acid GAC Aspartic acid GAA Glutamic acid GAG Glutamic acid	GGU Glycine GGC Glycine GGA Glycine GGG Glycine	U C A G

3 ↓



- * Methionine
- Threonine
- Glutamic Acid
- Leucine
- Arginine
- Serine
- * Stop

Mutations

- Human genome is 99.9% identical across people
- Mutation = Any change in the DNA sequence
- Mutations are the source of differences between individuals

Mutations can be....

- **Harmful** - Disease causing
 - Sickle cell anemia
 - Phenylketonuria (PKU)
 - Cystic fibrosis
- **Helpful** – Adaptability
 - Color patterns for camouflage
 - disease resistance
- **Neutral** – ‘silent’ or polymorphic
 - useful as markers
 - Identification, Forensics, Paternity
 - Gene mapping
 - Population studies

Single Nucleotide Polymorphisms (SNP's)

- Silent – do not alter amino acid **GCC** → **GCG**
- Missense – substitution of an amino acid **AAA** → **AAC**
- Nonsense – creates a stop codon and causes premature termination of translation **TAT** → **TAG**
- RNA processing – affects processing of RNA transcript
 - Includes splice-site mutations
- Regulatory – occurs in promoter or other regulatory region

Deletions & Insertions

- Deletions – loss of nucleotides
- Insertions – gain of nucleotides
- Duplication – copy

TAGT → **TT**

AAA → **AGAA**

TAT → **TATTAT**

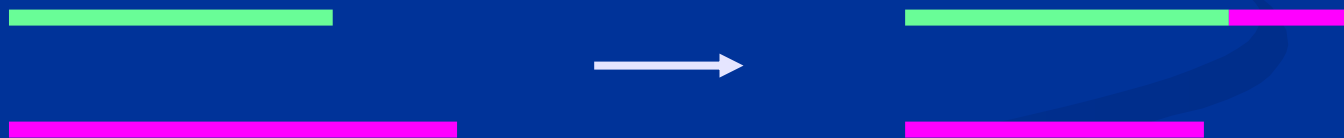
- Small – one to a few nucleotides
 - Generally caused by mispairing in DNA replication
- Large – 20 to thousands of nucleotides
 - Likely caused by unequal crossing over in replication
- Can cause frame-shift mutations
 - Will change reading frame

Chromosomal Rearrangements

Inversions – large segment of a chromosome



Translocations – between non-homologous chromosome



Basic Molecular Techniques

- DNA Extraction
- Electrophoresis
- Hybridization
- Restriction Digestion
- Polymerase Chain Reaction
- DNA Sequencing

Nucleic Acid Extraction

- Lyse cells
- Purify nucleic acid

Electrophoresis

- Separate nucleic acid/protein in an electric field
- Migration controlled by size and charge of molecule

Hybridization

- Utilizes base pairing of complementary, single-stranded nucleic acids into a double-stranded molecule



- Nucleic acid (DNA or RNA) is denatured
- Single-stranded probe is added
- Probe hybridizes to complementary DNA

Polymerase Chain Reaction

- Polymerase Chain Reaction - PCR
 - Make millions of copies of target DNA from a very small amount of DNA

PCR Steps:

- Denaturation – Two DNA strands are separated
- Annealing - ‘Primers’ target a region on a chromosome
- Extension – Target DNA is copied

View web demo:

<http://www.sumanasinc.com/webcontent/animations/content/pcr.html>

Limitations of PCR

Unilateral amplification looks homozygous

- Actually hemizygous
- Caused by mutations in the primer site or large deletions that contain the primer site
- Sensitive to contamination
 - Be careful to use proper precautions & care
 - QA/QC for molecular testing to be discussed by Dr. Cordovado

Restriction Digestion

Restriction enzyme recognizes DNA sequence and cleaves the DNA strand

Bsu36 I recognition site:



Normal

ATG GTG CAC CTG ACT CCT GAG GAG AAG

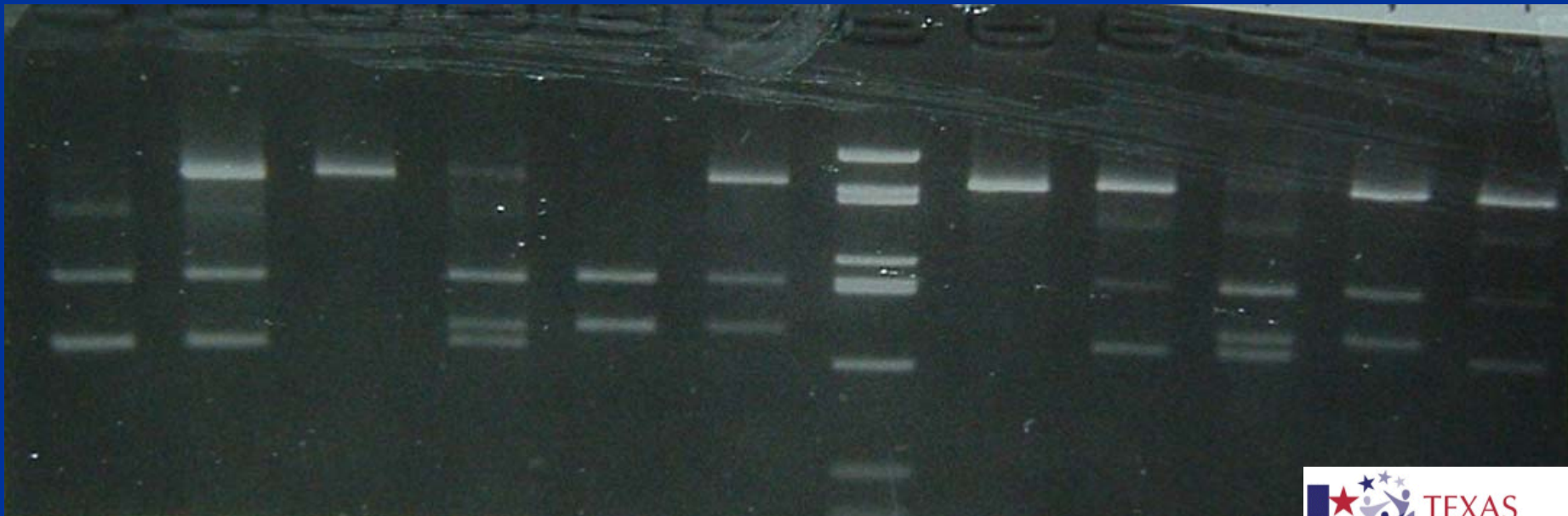
Hb S allele

ATG GTG CAC CTG ACT CCT GTG GAG AAG

Restriction Fragment Length Polymorphism Analysis

Combines PCR, restriction digestion and electrophoresis

Controls						Specimens				
+/+	+/S	SS	+/C	CC	SC	SS	+/S	+/C	SC	+/S



DNA Sequencing



↓ Heat to denature



↓ Sequencing Primer



↓ Taq, dNTPs, ddNTPs



→ Electrophoresis

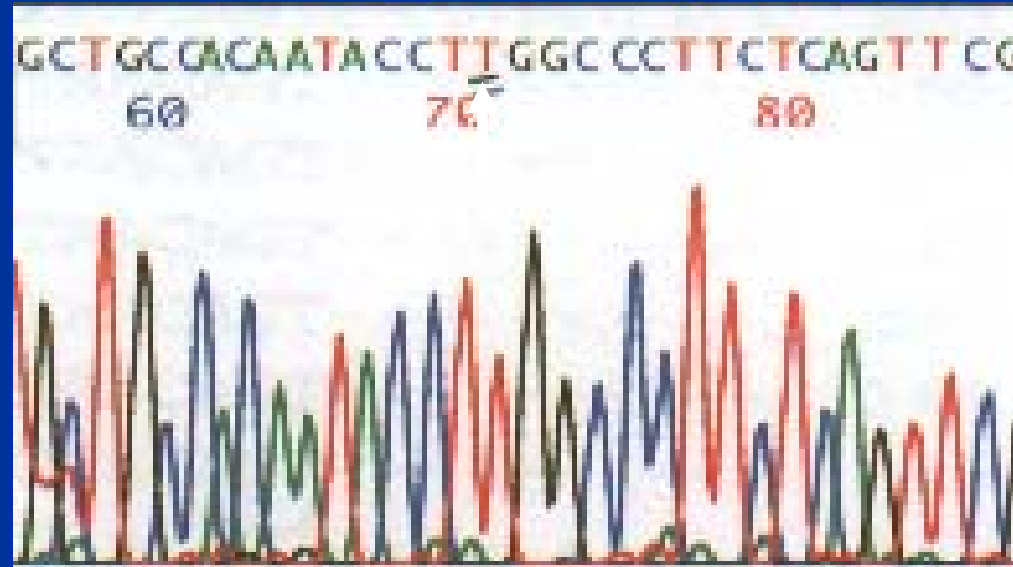
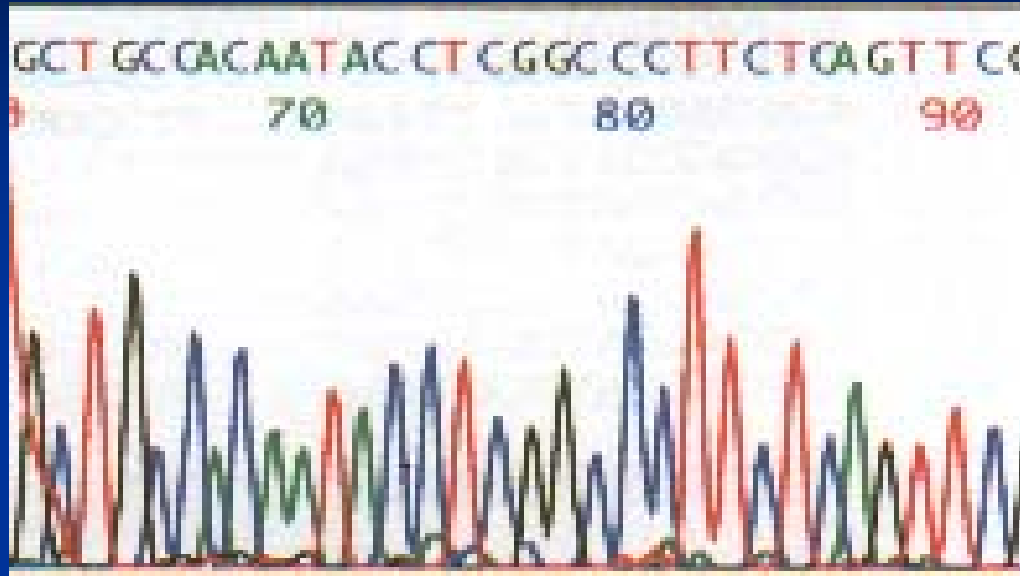
DNA Cycle Sequencing

View web demo:

<http://www.dnalc.org/ddnalc/resources/shockwave/cycseq.html>

http://trc.ucdavis.edu/biosci10v/bis10v/media/ch11/sequencer_v2.html

DNA Sequencing to identify SNP



Summary

- Basics of inheritance
 - DNA to proteins
- Various types of mutations
- Basic molecular techniques that serve as the building blocks for more complex procedures