

CSI Wildlife:

Using forensic science
in wildlife



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CSI TV



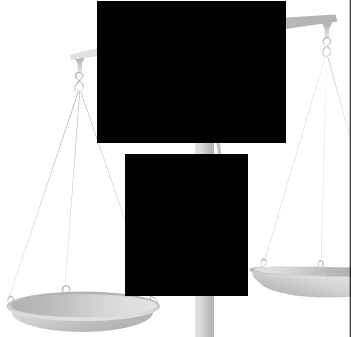
CSI Wildlife

- Techniques
 - Necropsy



CSI Wildlife

- Techniques
 - Necropsy
 - Microscopy



CSI Wildlife

■ Techniques

- Necropsy
- Microscopy
- DNA fingerprints

Cat Mutilations - Salt Lake City

Cat Mutilations - Salt Lake City

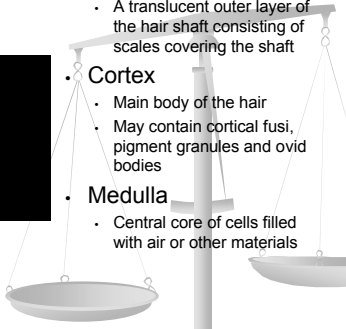
- "Careful forensics work in each case will determine the cause of death."
- "It is not known if all of these cats were killed by the same perpetrator — human or wild animal. Forensics testing is the key to eliminate the likelihood that the cats were killed by dogs or wild animals such as coyotes, cougars or great horned owls, and to identify cases that might involve a human assailant."

Dr. Randall Lockwood
Humane Society U.S.

Cat Mutilations - Salt Lake City

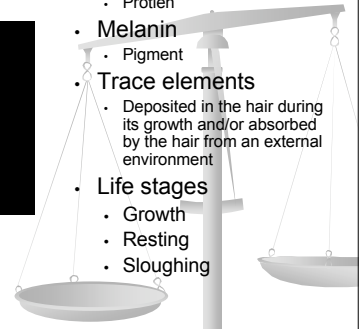
Hair Structure

- **Cuticle**
 - A translucent outer layer of the hair shaft consisting of scales covering the shaft
- **Cortex**
 - Main body of the hair
 - May contain cortical fusi, pigment granules and ovoid bodies
- **Medulla**
 - Central core of cells filled with air or other materials



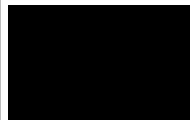
Hair Structure

- **Keratin**
 - Protein
- **Melanin**
 - Pigment
- **Trace elements**
 - Deposited in the hair during its growth and/or absorbed by the hair from an external environment
- **Life stages**
 - Growth
 - Resting
 - Sloughing



Hair Structure - Scales

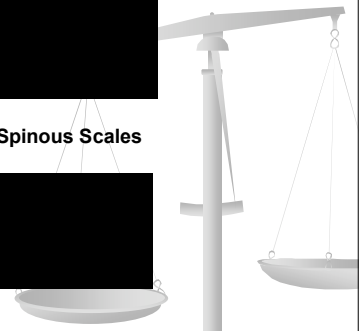
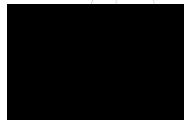
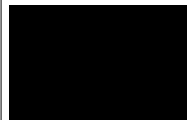
Coronal Scales

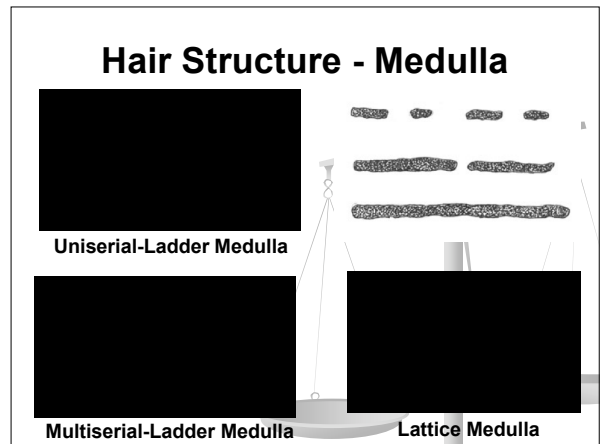
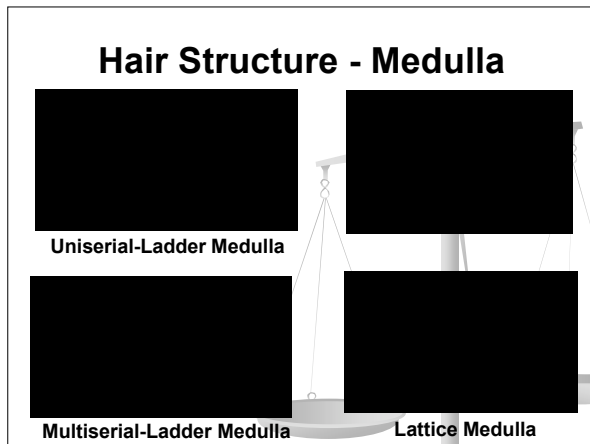
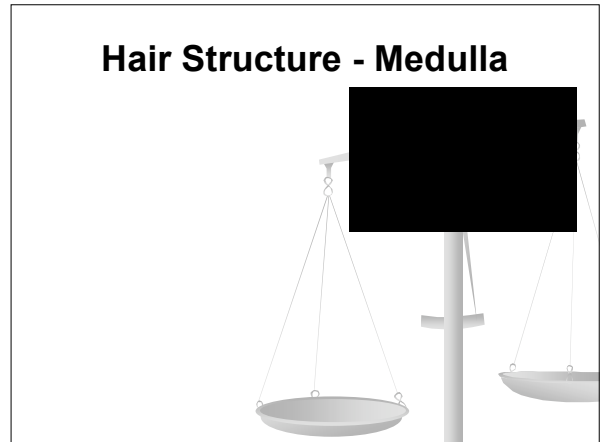
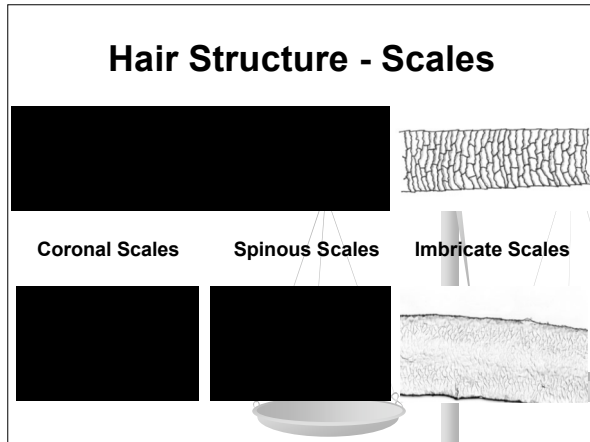


Hair Structure - Scales

Coronal Scales

Spinous Scales





Hair Structure - Cortex



- Main body of the hair
 - composed of elongated and fusiform (spindle-shaped) cells.
 - cortical fusi
 - Irregular-shaped airspaces of varying sizes
 - commonly found near the root of a mature human hair

Hair Structure - Cortex



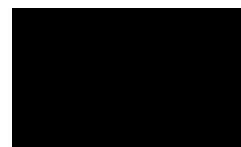
- Main body of the hair
 - Pigment granules
 - Small, dark, and solid structures that are granular in appearance
 - Considerably smaller than cortical fusi.
 - Vary in color, size, and distribution in a single hair.
 - In humans, commonly distributed toward the cuticle
 - Except in red-haired individuals.
 - Animal hairs
 - Pigment granules commonly distributed toward the medulla

Hair Structure - Cortex

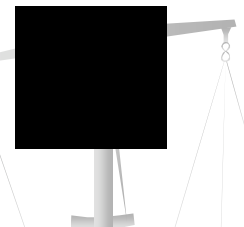


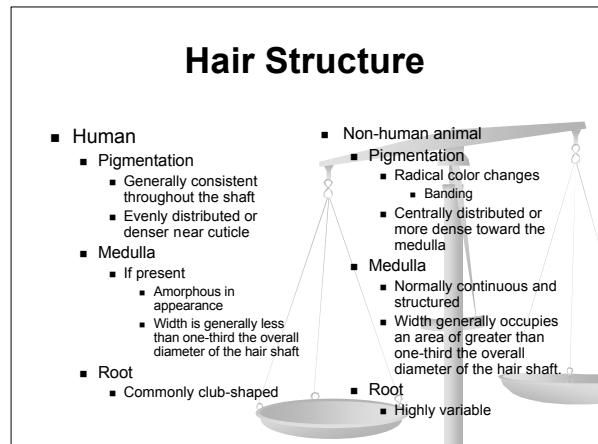
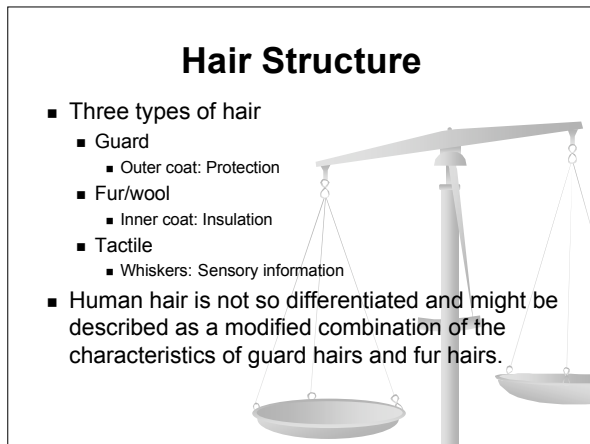
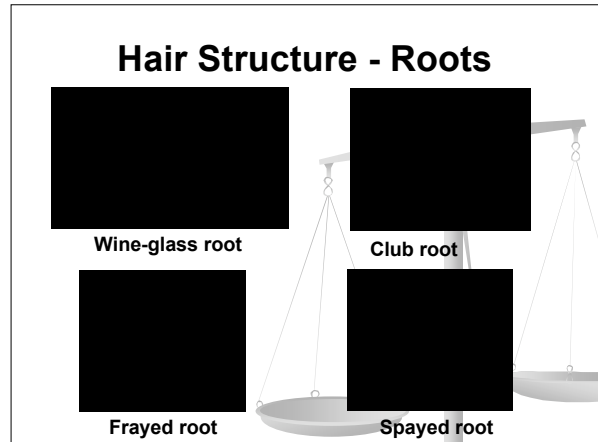
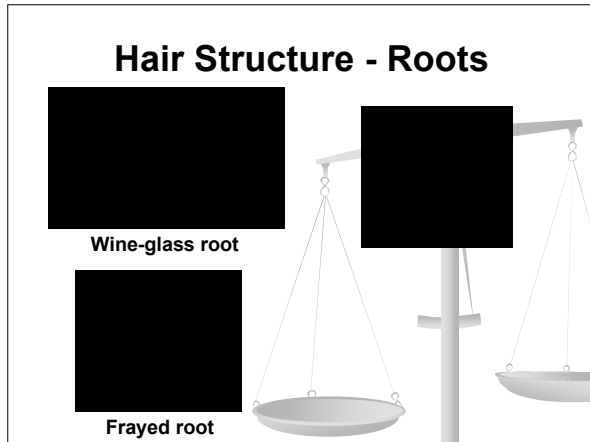
- Main body of the hair
 - Ovoid bodies
 - Larger than pigment granules
 - Solid structures
 - Spherical to oval in shape
 - Very regular margins.
 - Abundant in some cattle and dog hairs as well as in other animal hairs.
 - Found in varying degrees in human hairs

Hair Structure - Roots

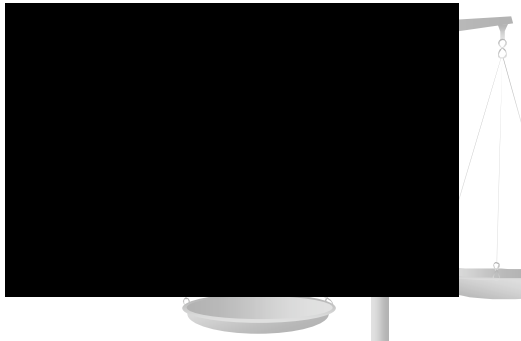


Wine-glass root

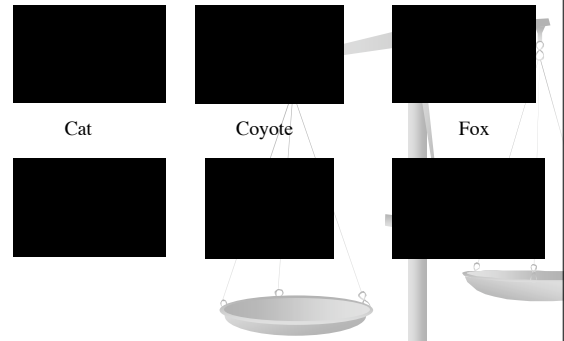




Cat Mutilations - Salt Lake City



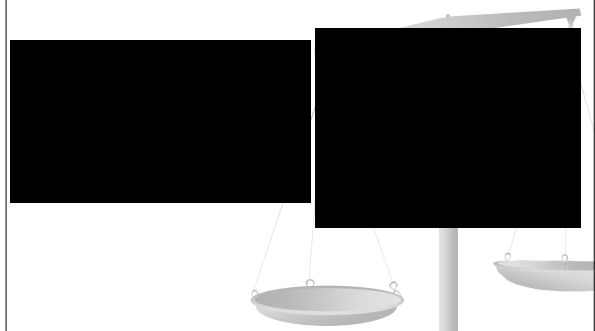
Cat Mutilations - Salt Lake City



Cat Mutilations - Bothell, WA



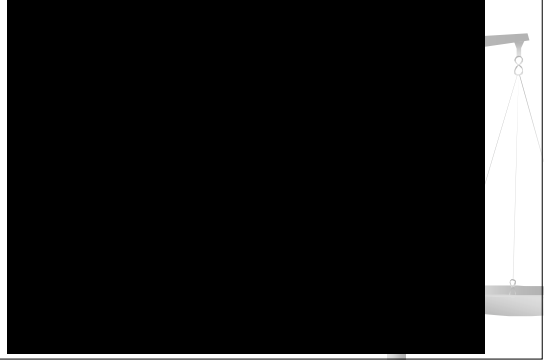
Cat Mutilations - Bothell, WA



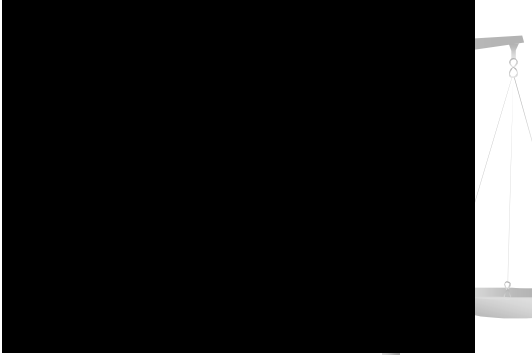
Cat Mutilations - Bothell, WA



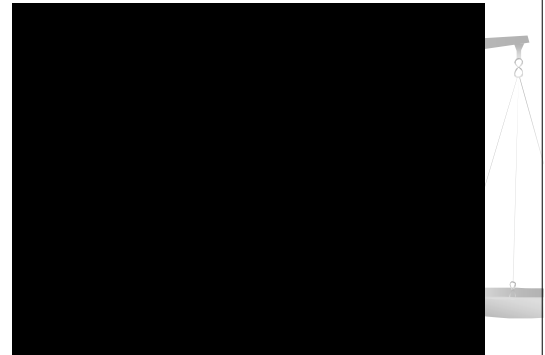
Cat Mutilations - Bothell, WA



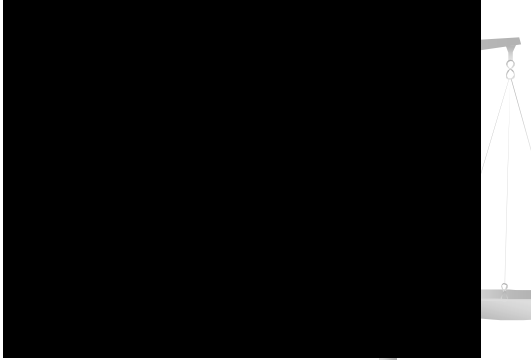
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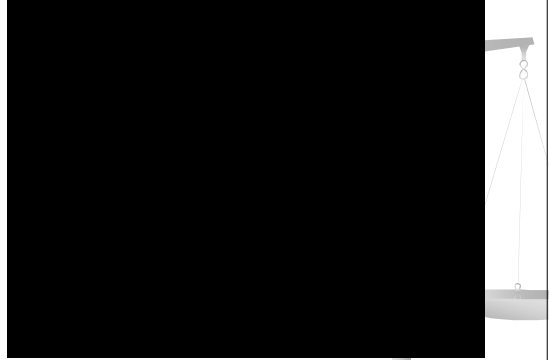
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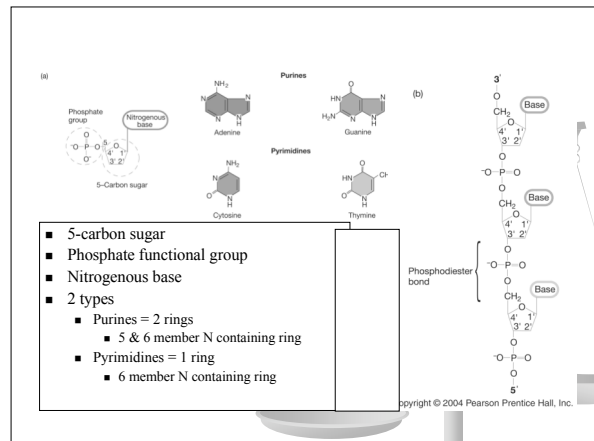
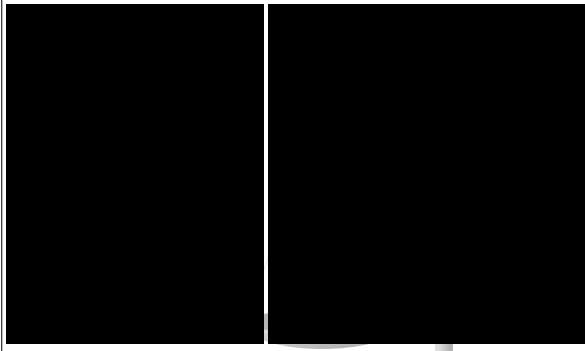
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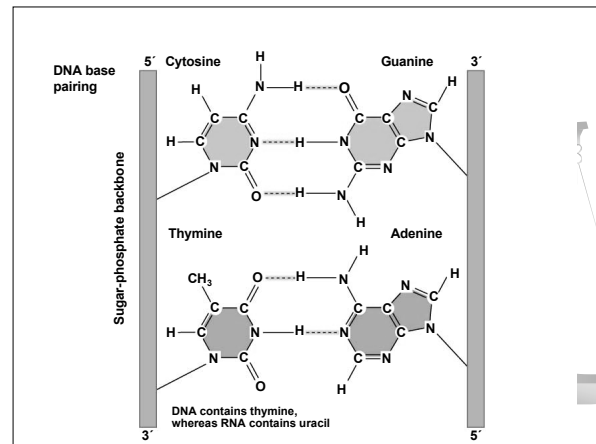
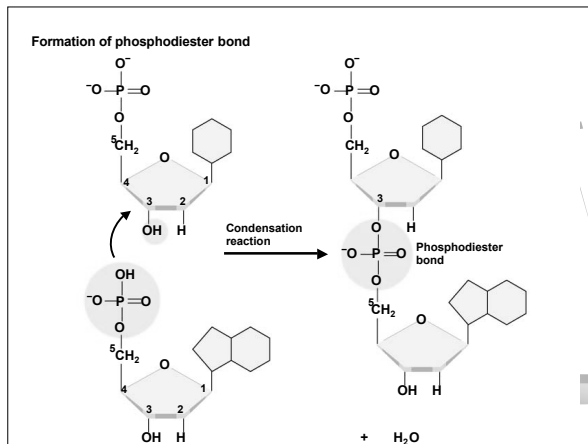
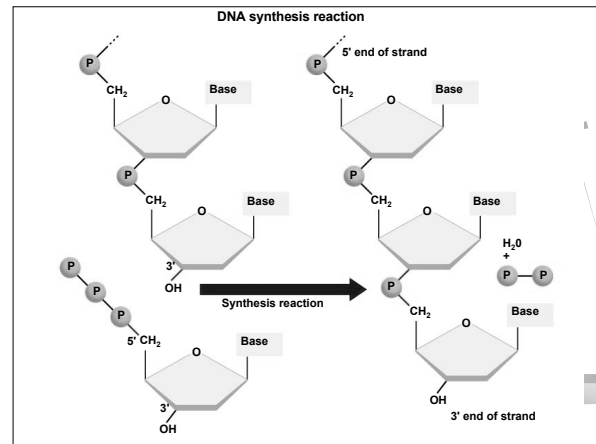
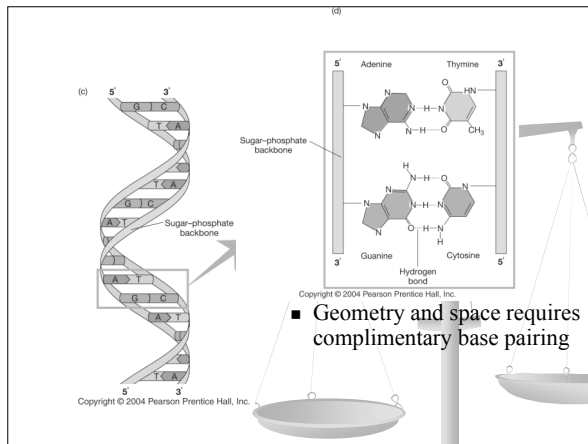


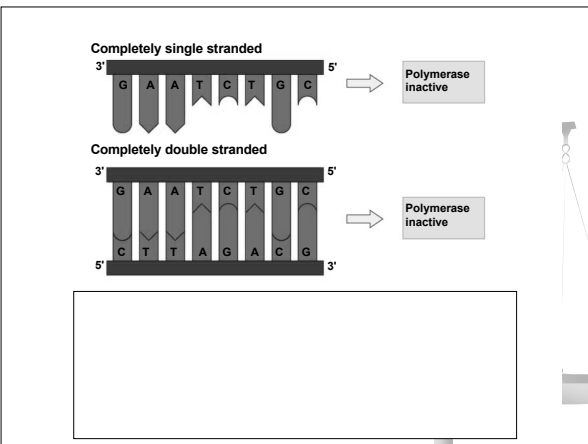
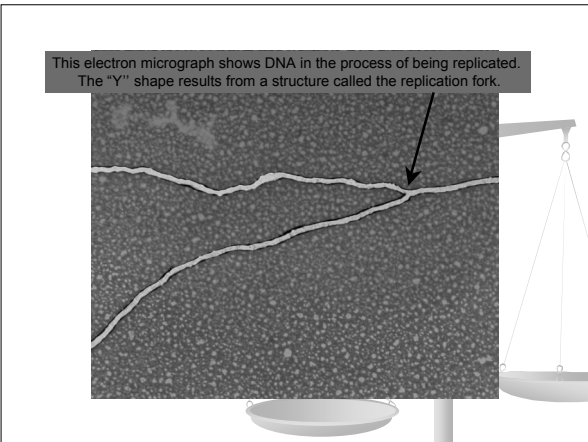
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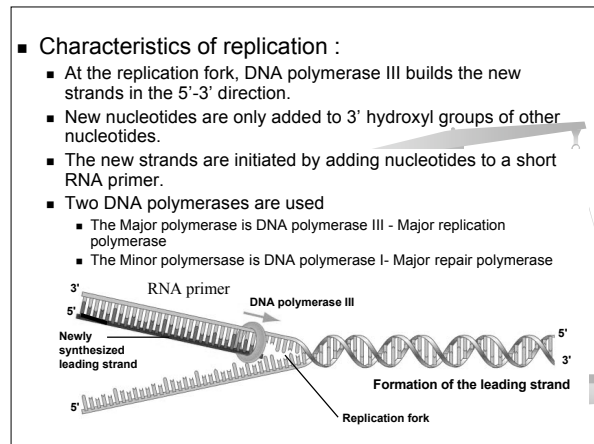
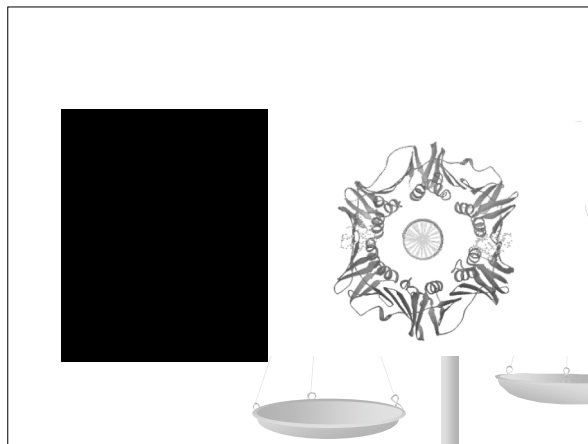
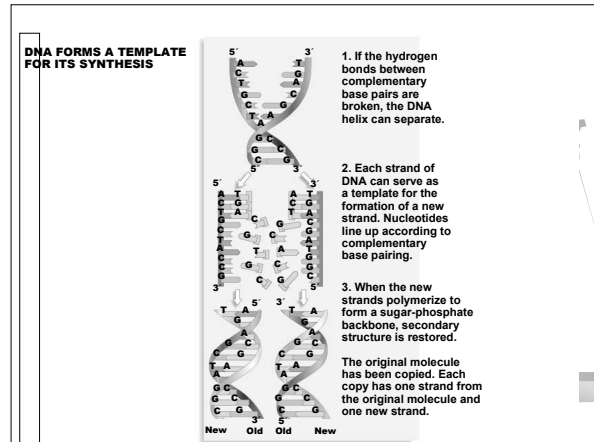
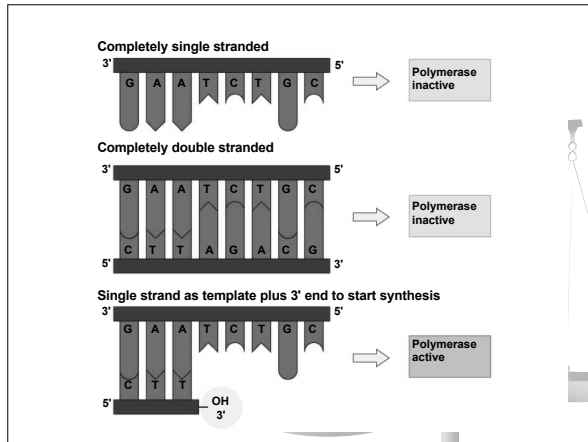


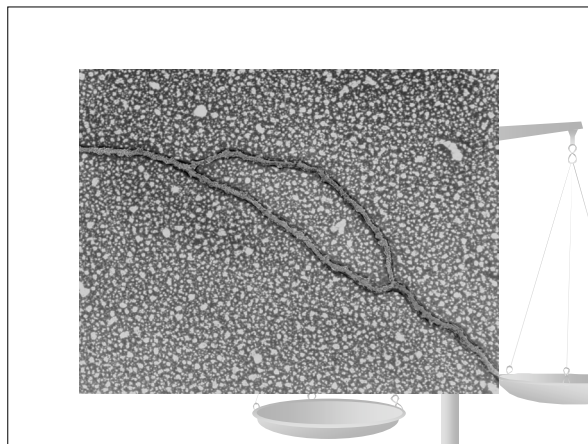
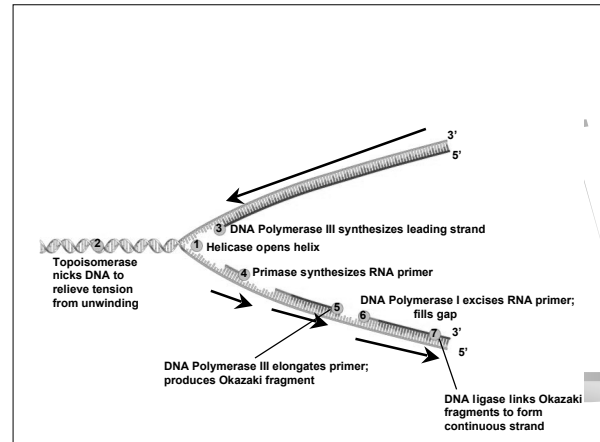
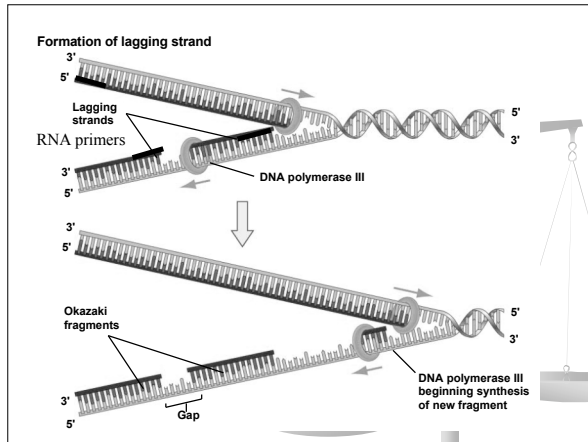
Cat Mutilations - Bothell, WA









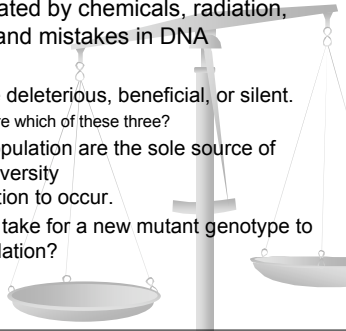


■ There's no workman, whatsoever he be, that may both work well and hastily

■ Chaucer

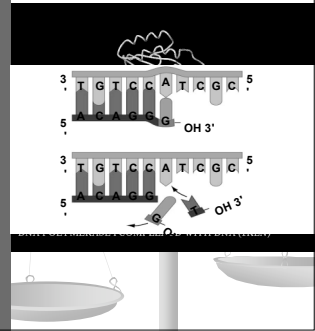
Mutation and DNA Repair Mechanisms

- Mutations are created by chemicals, radiation, errors in meiosis and mistakes in DNA replication.
 - Mutations can be deleterious, beneficial, or silent.
 - Most mutations are which of these three?
 - Mutations in a population are the sole source of unique genetic diversity that allows evolution to occur.
 - How long does it take for a new mutant genotype to get into the population?



Mutation and DNA Repair Mechanisms

- DNA Repair Mechanisms
 - DNA polymerase I and II proofread and correct point mutations during replication.
 - DNA Poly I
 - Fills gaps in lagging strand
 - Fills gaps in DNA repair
 - DNA Poly II
 - Excises mutated DNA post replication



Mutation and DNA Repair Mechanisms

- DNA Repair Mechanisms
 - Repair enzymes identify the correct template strand by its methyl groups.
 - Following complete synthesis and proof reading additional enzymes add methyl group to several of the adenosines of the new strand

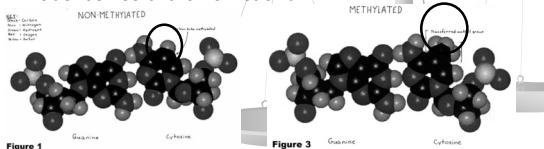
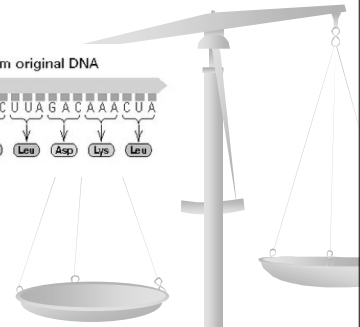
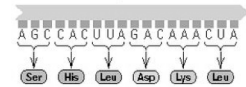


Figure 1

Figure 3

Mutations

mRNA from original DNA



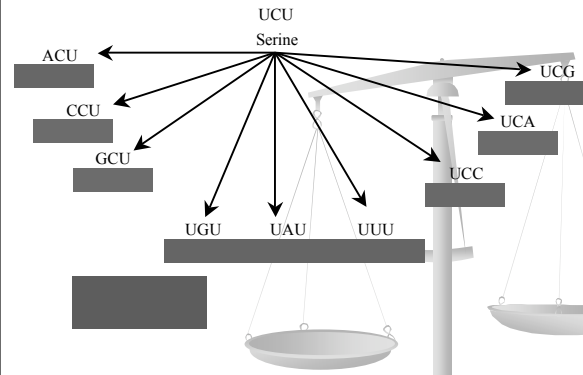
Redundancy

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

•64 different codons can be created but there are only 20 exist

•Why does most redundancy occurs in the third position

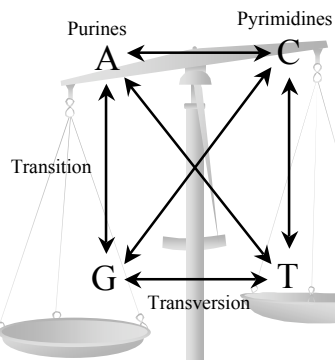
Mutations



Mutations

Point Mutations

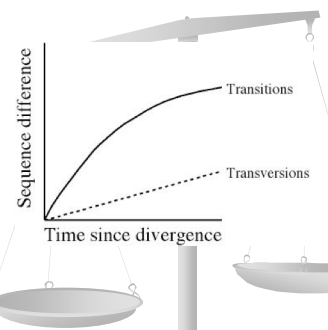
- DNA polymerase makes mistakes during synthesis or ironically, in repair.



Mutations

Point Mutations

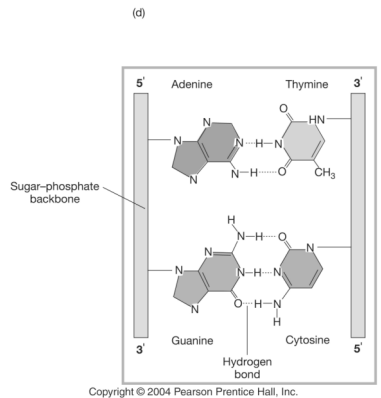
- Transitions outnumber transversions approximately 2:1
- WHY???
- Why does sequence difference asymptote?



Mutations

Point Mutations

- The different hydrogen bonding patterns produces different degrees of disruption to the helical structure of DNA.

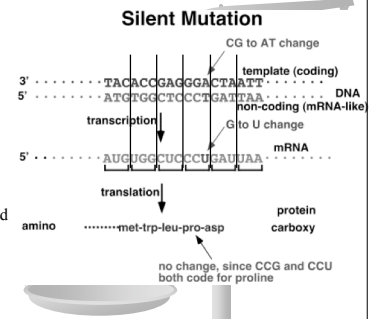


Mutations

Point Mutations

Silent Mutation

- Switching of one nucleotide for another
- Does not change the amino acid
 - Redundancy
- No effect on organism
- Very important in evolutionary studies and forensics

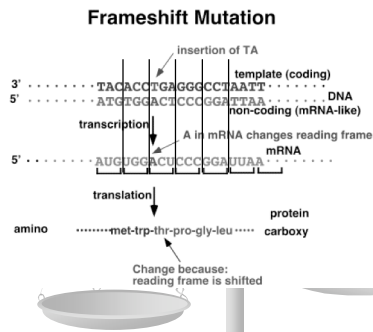


Mutations

Point Mutations

Frameshifts

- Insertion or deletion of a nucleotide
- Major consequences because it changes all of the downstream codons.
- Most often deleterious



Mutations

Base insertion-frameshift mutation

Met Gly Ile Lys Val Leu His Tyr
 ATG GGG ATT AAG GTG CTG CAC TAT

↓

ATG GGG ATT AAG AGT GCT GCA CCT
 Met Gly Ile Lys Ser Ala Ala Pro

Base deletion-frameshift mutation

Met Gly Ile Lys Val Leu His Tyr
 ATG GGG ATT AAG GTG CTG CAC TAT

↓

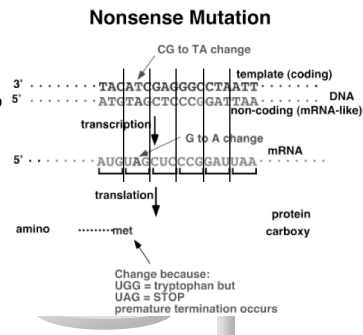
ATG GGG ATT AAG TGC TGC ACT ATA
 Met Gly Thr Lys Cys Cys Thr Ile

Mutations

Point Mutations

Nonsense

- Creation of a stop codon
- Major consequences as it stops production of the protein.
- Most often deleterious

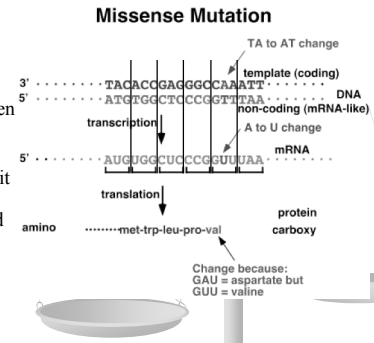


Mutations

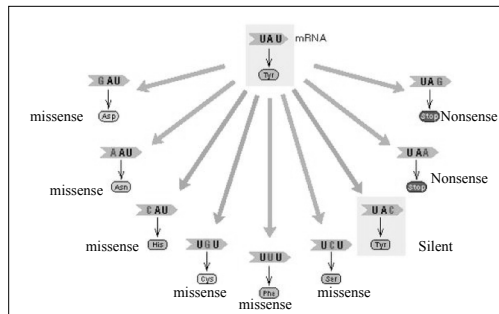
Point Mutations

Missense

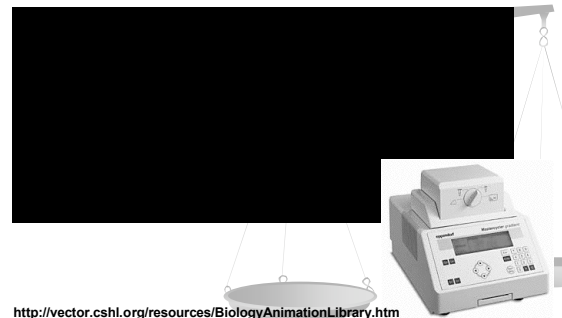
- Switching between nucleotides
- Variable consequences as it only changes a single amino acid of the protein.
- Most often deleterious



Mutations



PCR



PCR

■ Methods

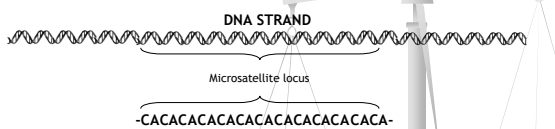
- Determine DNA sequence of regions flanking the locus
- Construct primers
 - Primer length depends upon type of marker
- Use PCR to make multiple copies
 - dNTPs, Taq polymerase, $MgCl_2$
- Run through gel matrix and visualize with radioactivity, fluorescence

MICROSATELLITES

WHAT ARE THEY?
HOW ARE THEY USED?

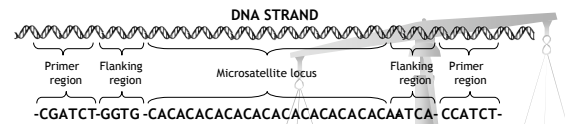
Microsatellites

- short tandem repeats (1-6 base pairs long) of “junk” DNA



- allelic state defined by fragment length (# of base pairs)
 - example fragment is 26 bp long
- fragment length can be measured using a genetic analyzer

Microsatellites



- primer regions are typically conserved & not as prone to mutation
 - primers can be used within genera
- high mutation rates
 - results in polymorphic loci (many different alleles possible)

Microsatellites

High mutation rates result in multiple alleles at each locus:

- mutations are usually insertions & deletions of the repeat unit

CACACACACACACACACACACACA = 26 base pairs long
CACACACACACACACACACACACACA = 30 base pairs long
CACACACACACACACACACACA = 20 base pairs long
CACACACACACACACACACACA = 24 base pairs long
... and so on

With multiple alleles & many loci, individual genotypes can be created...

Microsatellites

- individual genotypes can be created by typing individuals at many loci
- diploid organisms get 1 allele from mother & 1 allele from father
 - e.g., typing an individual at 3 loci

LOCUS 1

CACACACACACACA = 16

CACACACACACACA = 16

LOCUS 2

TCTCTCTCTC = 10

TCTCTCTCTCTCTC = 14

LOCUS 3

CACACACACACACACACA = 20

CACACACACACACA = 16

Microsatellites

- What are they?
 - Repetitive units of 2 - 5 bp
 - SSR - Simple Sequence Repeats
 - STR - Simple Tandem Repeats
 - VNTR - Variable Number of Tandem Repeats
- Mutation rates
 - 10^{-2} - 10^{-4}
 - Due to replication slippage
 - In lagging strand synthesis repeat unit slips out of pairing after polymerase dissociation
 - Repair then adds new repeat
 - Unequal crossing over in meiosis

Microsatellites

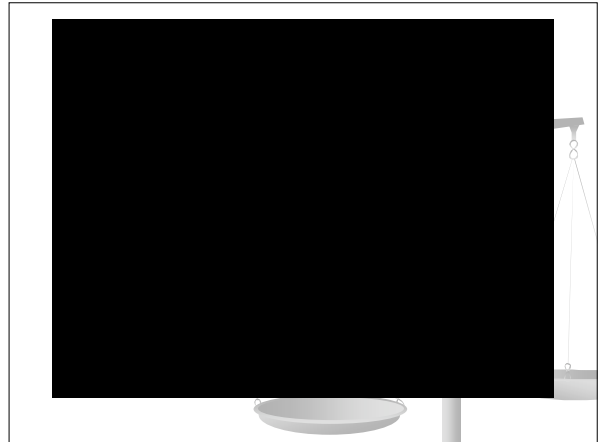
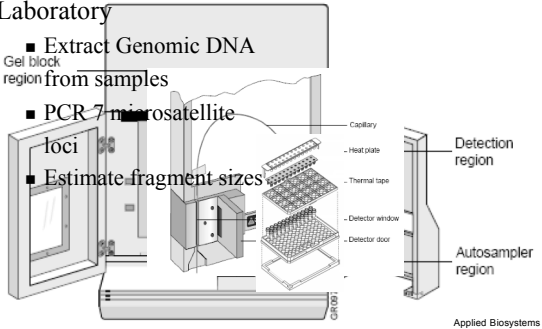
- repeat this for many individuals...

Individual	Locus 1		Locus 2		Locus 3	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
1	10	10	16	16	22	24
2	8	10	12	14	24	24
3	10	10	16	16	22	22
4	8	8	12	14	22	26
5	10	10	12	16	22	24

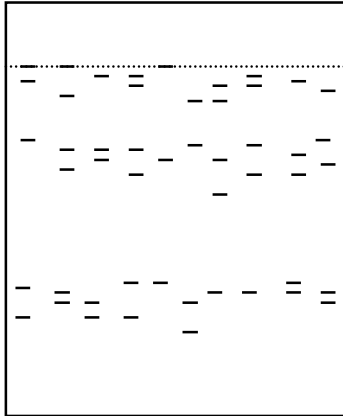
Methods

Laboratory

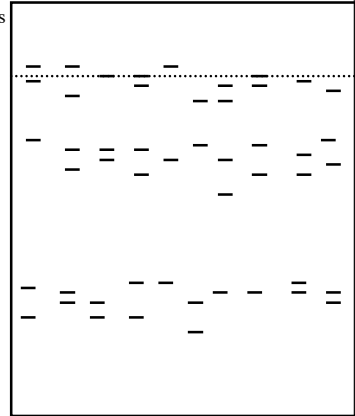
- Extract Genomic DNA
- PCR 7 microsatellite loci
- Estimate fragment sizes

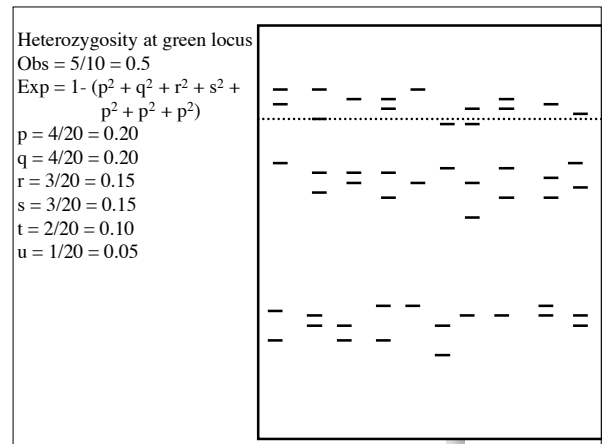
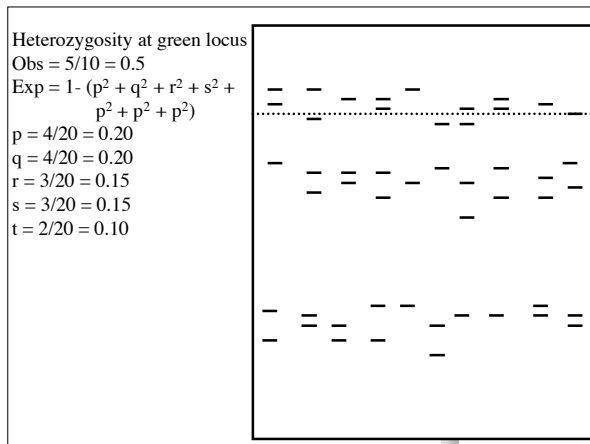
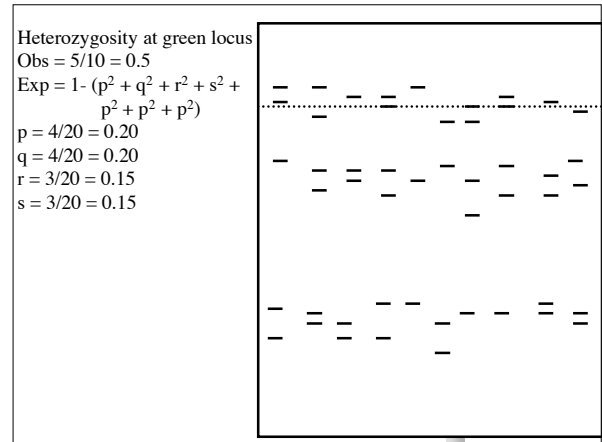
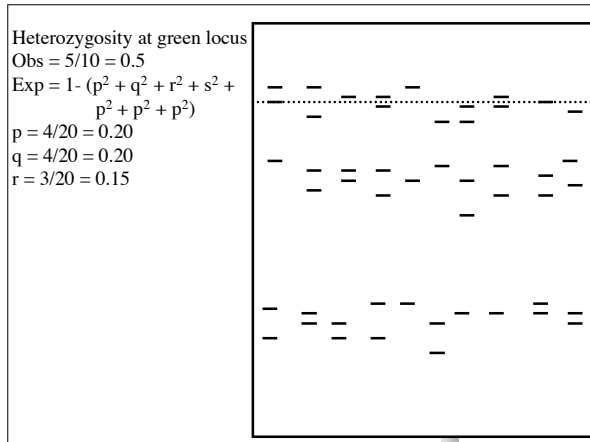


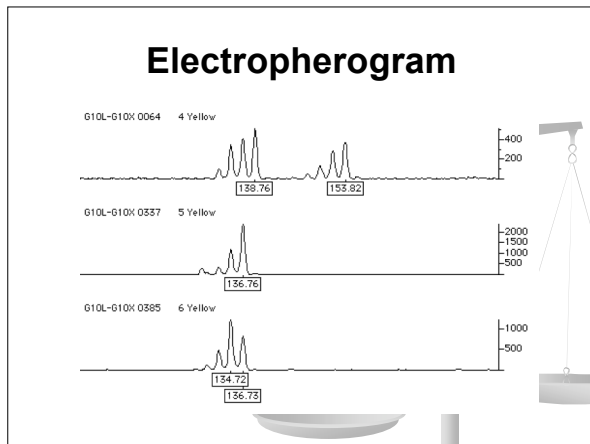
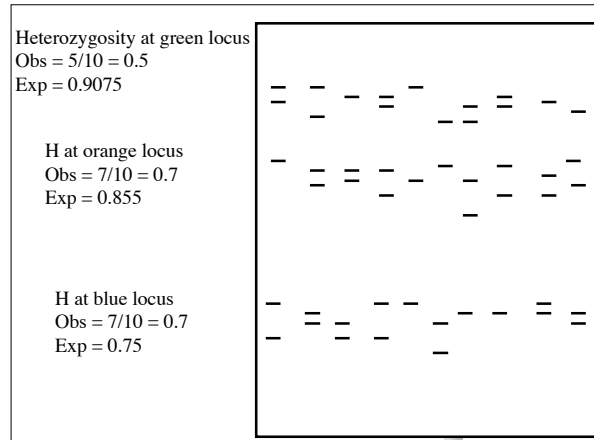
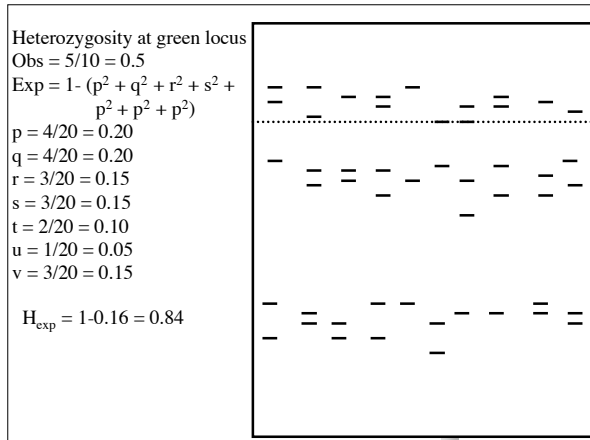
Heterozygosity at green locus
 Obs = 5/10 = 0.5
 Exp = $1 - (p^2 + q^2 + r^2 + s^2 + p^2 + p^2 + p^2)$
 $p = 4/20 = 0.20$



Heterozygosity at green locus
 Obs = 5/10 = 0.5
 Exp = $1 - (p^2 + q^2 + r^2 + s^2 + p^2 + p^2 + p^2)$
 $p = 4/20 = 0.20$
 $q = 4/20 = 0.20$







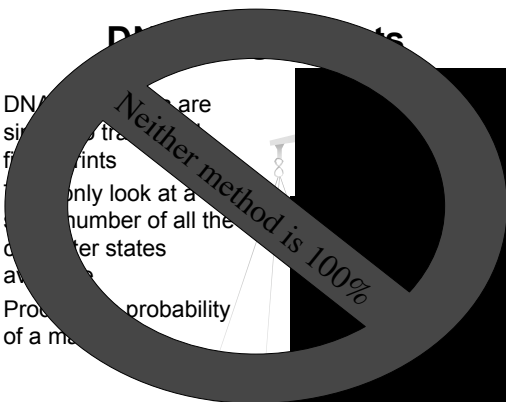
Microsatellites

- A molecular marker that can be used to identify & characterize individuals
- Uses
 - Parentage
 - Dispersal rates
 - Population boundaries
 - Forensic identification
 - Estimating population size
- Problems
 - Expensive to develop
 - Often requires development of new primers for each new species examined

DNA

- DNA are similar to fingerprints
- Only look at a small number of all the possible states
- Probability of a match

Neither method is 100%



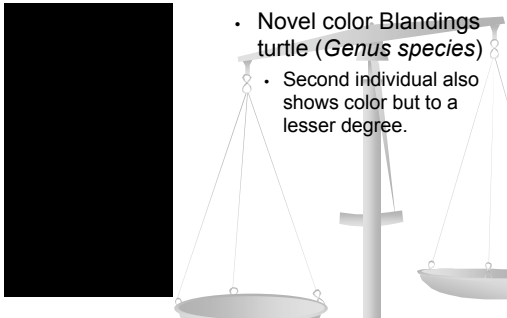
Blandings turtle poaching

- Sting operation for wildlife poaching
 - U.S. Fish and Wildlife Service
 - Michigan State Police



Blandings turtle poaching

- Novel color Blandings turtle (*Genus species*)
- Second individual also shows color but to a lesser degree.



Blandings turtle poaching

- Street value estimated at



Blandings turtle poaching

- Street value estimated at

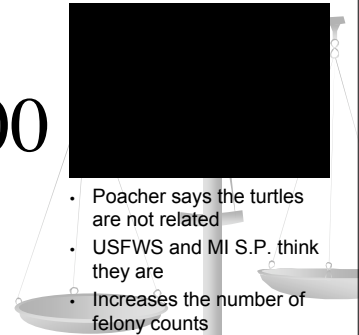
\$3,000



Blandings turtle poaching

- Street value estimated at

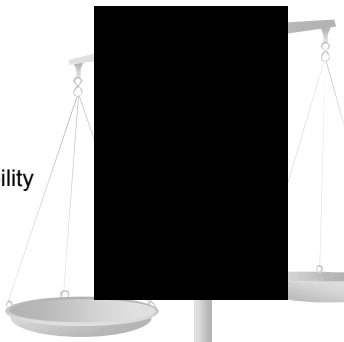
\$10,000



- Poacher says the turtles are not related
- USFWS and MI S.P. think they are
- Increases the number of felony counts

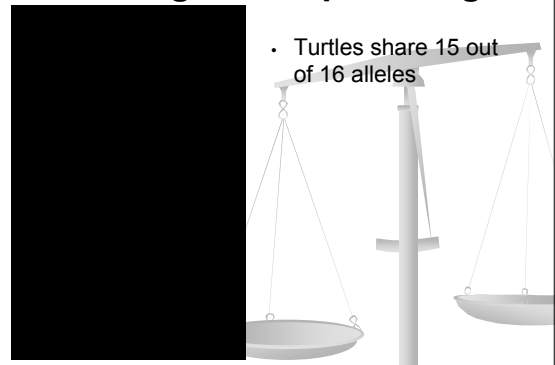
Blandings turtle poaching

- Take blood from turtles
- Extract DNA
- Produce DNA fingerprint
- Determine probability of identity

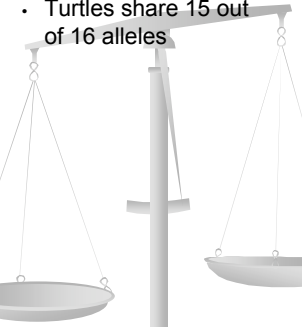


Blandings turtle poaching

- Turtles share 15 out of 16 alleles

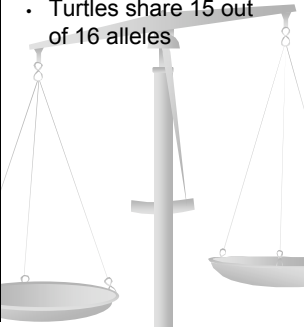


Blandings turtle poaching



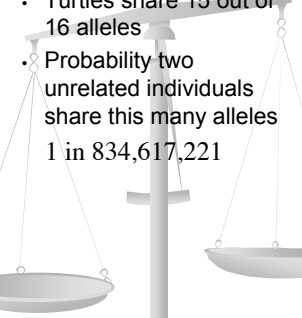
- Turtles share 15 out of 16 alleles

Blandings turtle poaching



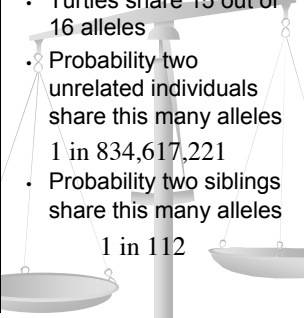
- Turtles share 15 out of 16 alleles

Blandings turtle poaching



- Turtles share 15 out of 16 alleles
- Probability two unrelated individuals share this many alleles
1 in 834,617,221

Blandings turtle poaching



- Turtles share 15 out of 16 alleles
- Probability two unrelated individuals share this many alleles
1 in 834,617,221
- Probability two siblings share this many alleles
1 in 112

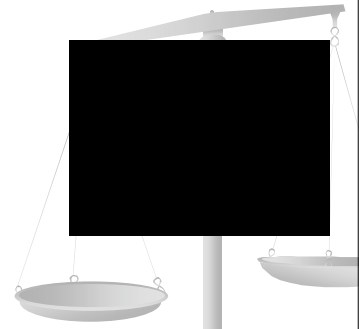
Blandings turtle poaching

- Suspect fought the case for about 14 months
- Finally plead guilty



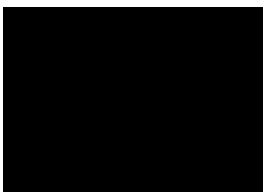
Deer Poaching

- Deer farms in Michigan
 - Hunting
 - Meat
 - Breeding
 - Velvet



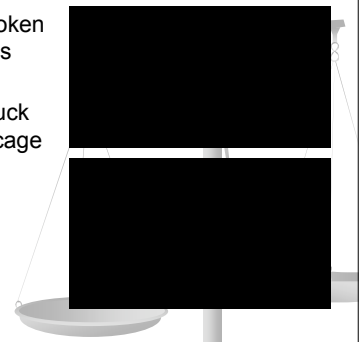
Deer Poaching

- Value of breeding males can exceed \$20,000!
- Often male is kept isolated from herd other than for breeding



Deer Poaching

- Deer farmer is woken up by shots on his property
- Finds his prize buck missing from its cage
- Does find blood



Deer Poaching



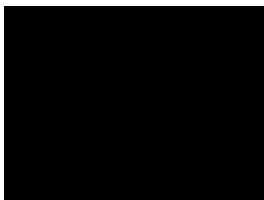
- Multiple other drag marks from other parts of property to drive way
- Owner calls in MI DNR Conservation Officer

Deer Poaching

- Finds bloody trail on paved road leading away from property
- Follows trail for 7 MILES straight to suspects house!!!
- Finds 5 deer hanging on property and hearts in the back of pickup truck



Deer Poaching

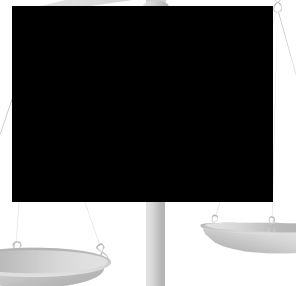


- CO brings bloody leaves to lab from the bucks pen
- Extract DNA from blood on leaves
- Compare DNA fingerprints

Deer Poaching

- 100% of alleles match
- Probability of this happening for two individuals

1
in
16,385,942,441



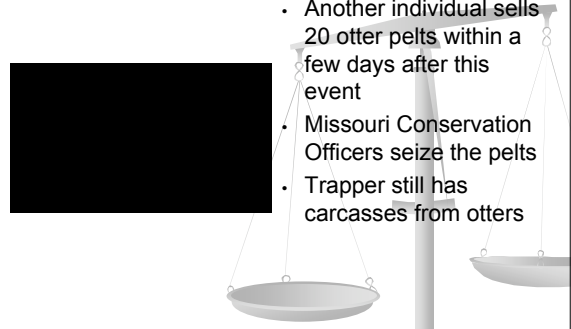
Otter Pelt Theft

- Otters are a nuisance in Missouri
- Essentially unlimited harvest
- A trapper reports 20 otter pelts stolen from his drying shed



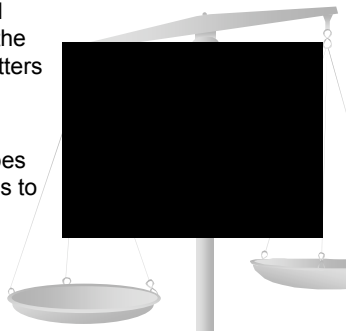
Otter Pelt Theft

- Another individual sells 20 otter pelts within a few days after this event
- Missouri Conservation Officers seize the pelts
- Trapper still has carcasses from otters



Otter Pelt Theft

- We are sent small piece of pelt and the tails of all of the otters
- Extract DNA from both samples
- Compare genotypes from pelts and tails to determine if they match

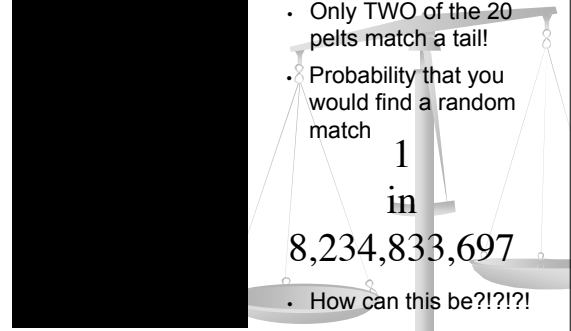


Otter Pelt Theft

- Only TWO of the 20 pelts match a tail!
- Probability that you would find a random match

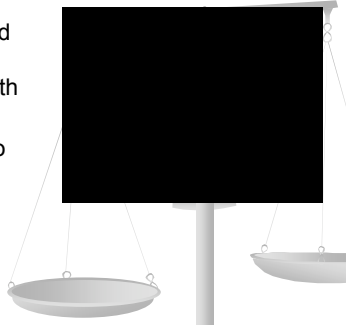
1
in
8,234,833,697

- How can this be?!?!?



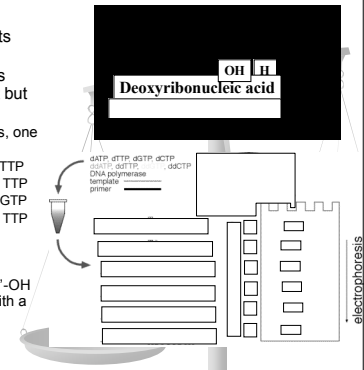
Otter Pelt Theft

- Further police investigation found that the individual traded 18 pelts with his friends!
- Kept the other two because of their value!



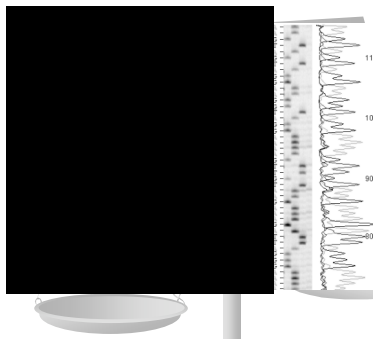
DNA Sequencing

- Requirements
 - Separate DNA fragments differing by 1 bp
 - Produce DNA fragments with same starting point but different end points
 - Four different reactions, one for each nucleotide
 - ddATP, dGTP, dCTP, dTTP
 - ddGTP, ATP, CTP, TTP
 - ddTTP, ATP, CTP, GTP
 - ddCTP, ATP, GTP, TTP
 - ddNTP stops addition of nucleotides because its 3'-OH is replaced with a 3'-H

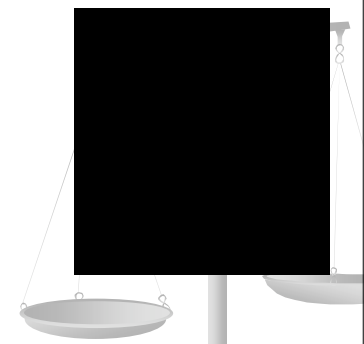


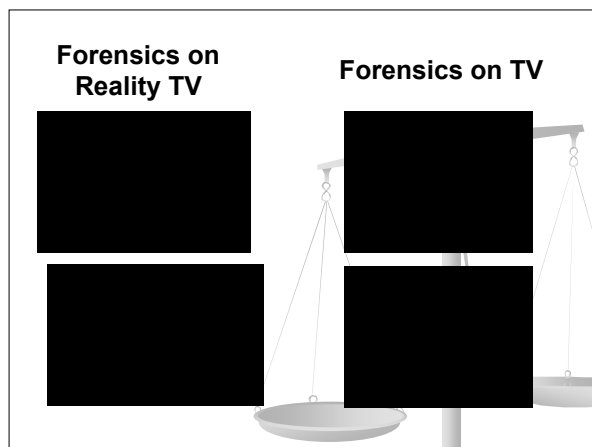
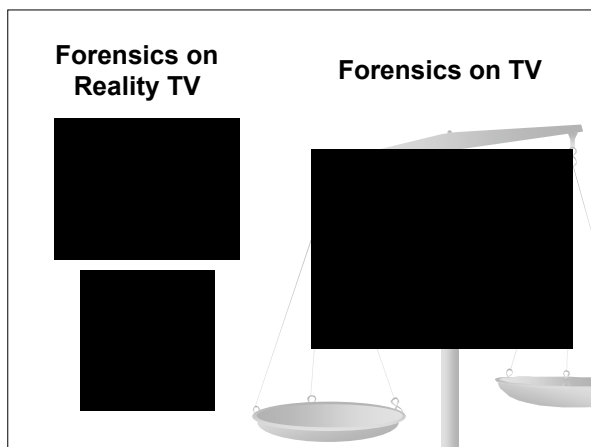
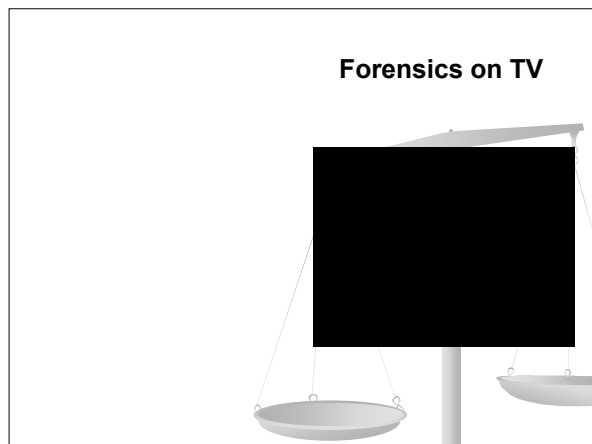
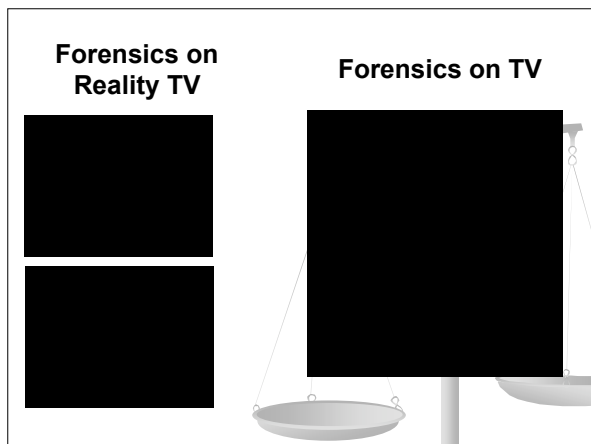
DNA Sequencing

- Visualization
 - The ddNTP is labeled either with radioactivity or fluorescent dye



Forensics on TV





What forensics isn't!



What forensics isn't! What forensics is!

