Molecular Markers

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CRITFC Genetics Training
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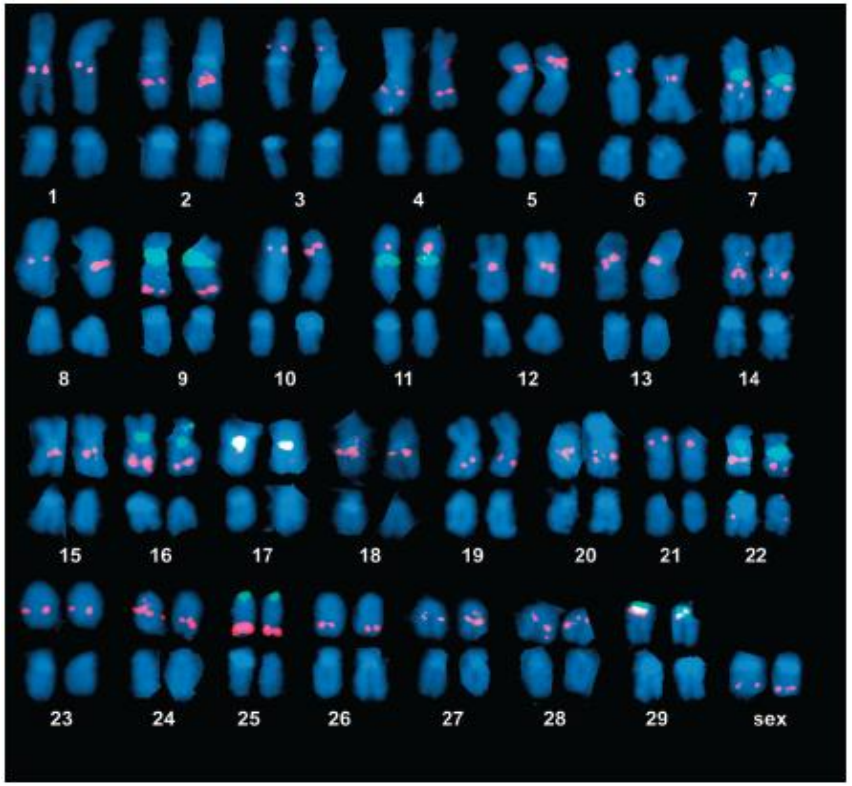
Molecular Markers

- Review of Inheritance
- Allozyme Marker
- Mitochondrial DNA
- Microsatellite Marker
- SNP Marker

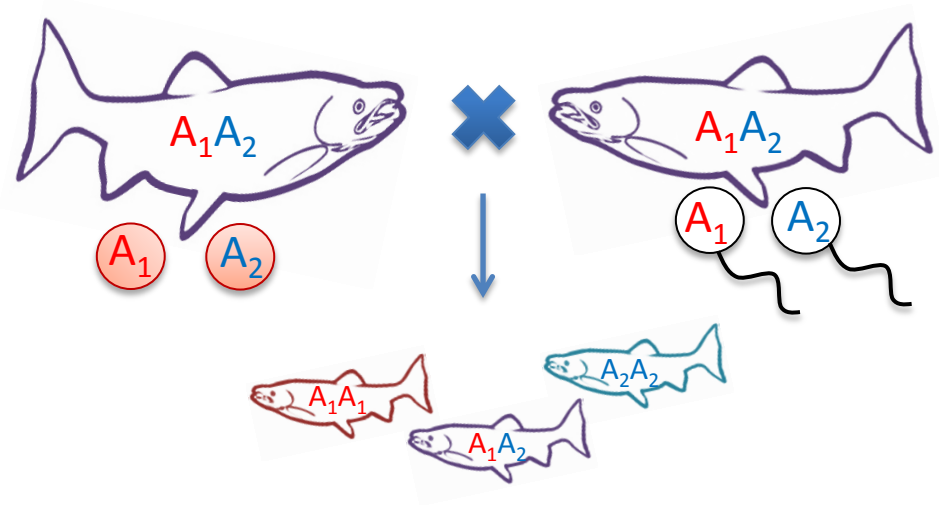
Inheritance

- Diploid organisms have two copies of each chromosome, one inherited from mom and one from dad
- Genes are positioned on chromosomes, thus two copies of each gene are present, one from mom and one from dad
- Each copy/variant is called an allele

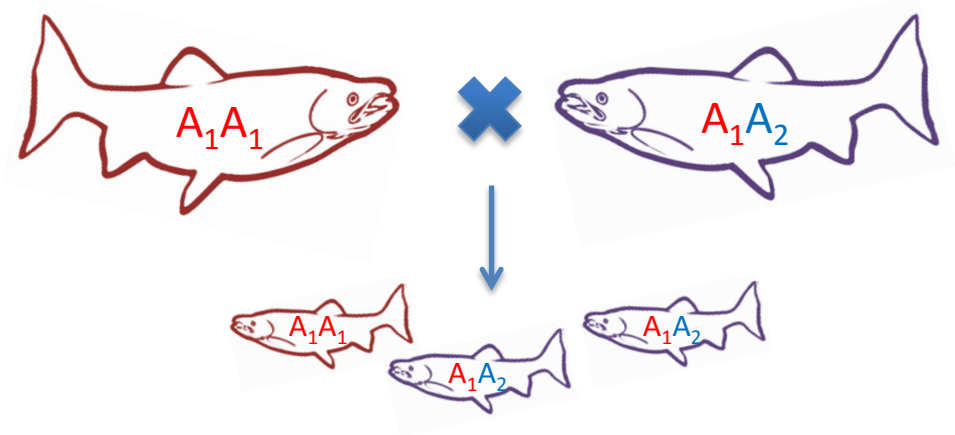


Rainbow trout karyotype
Phillips et al. 2006

Inheritance



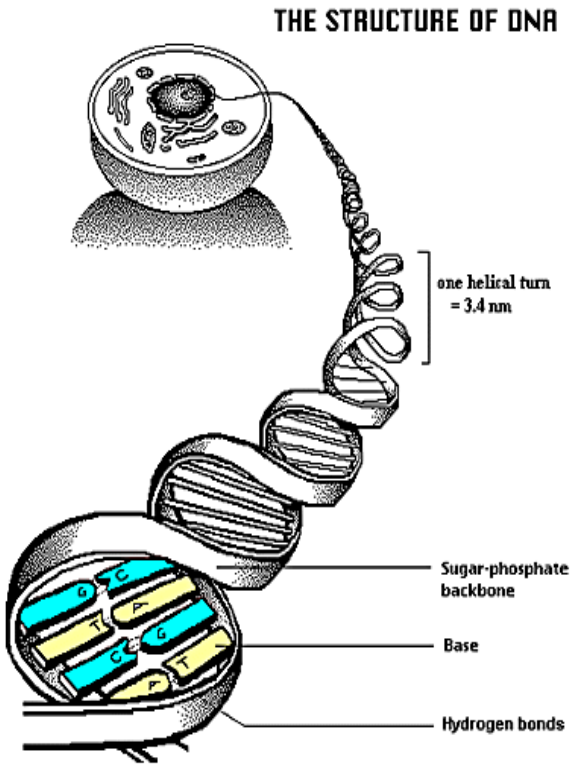
Male Gametes (frequency)	Female Gametes (frequency)	
	A_1(p)	A_2(q)
A_1(p)	A_1A_1	A_1A_2
A_2(q)	A_1A_2	A_2A_2

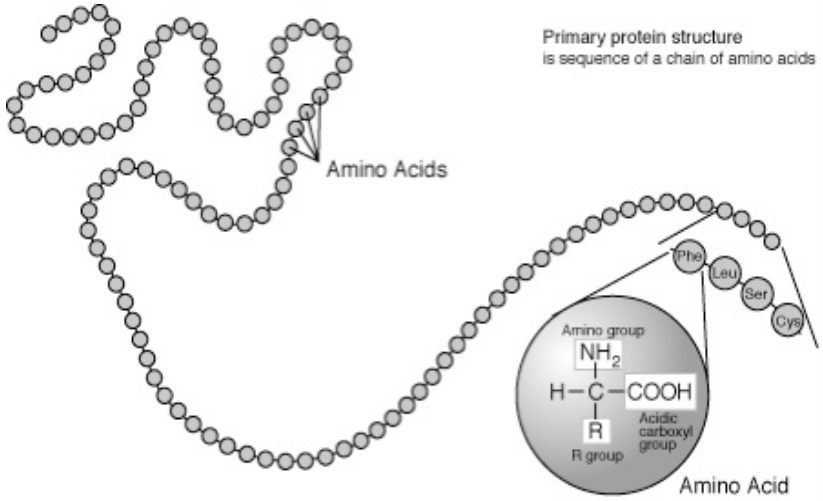


Male Gametes (frequency)	Female Gametes (frequency)	
	A_1(p)	A_1(p)
A_1(p)	A_1A_1	A_1A_1
A_2(q)	A_1A_2	A_1A_2

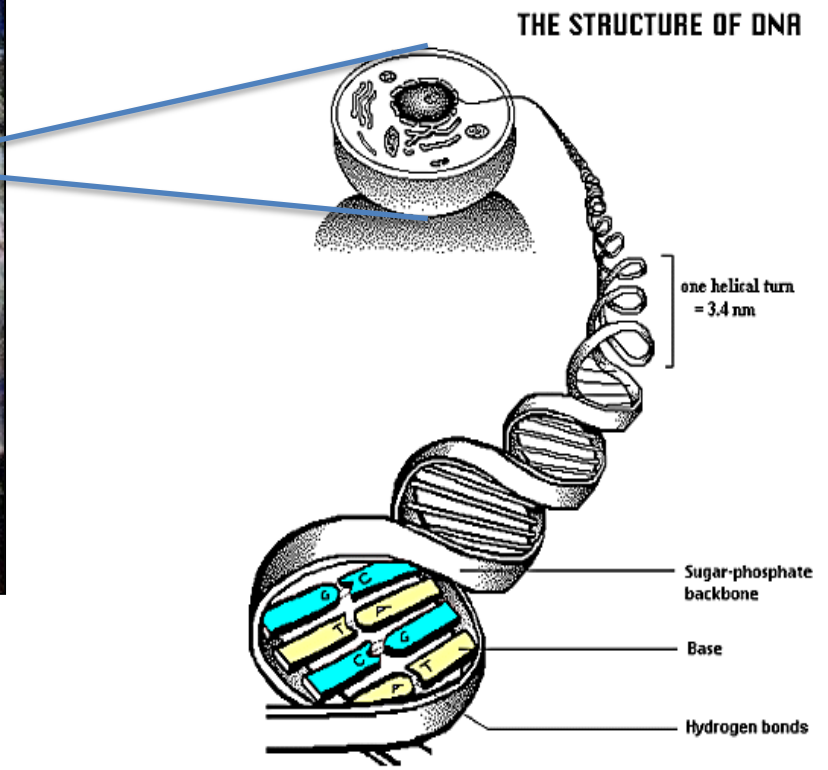
Central Dogma of Molecular Biology

DNA ➔RNA ➔ Protein





biotech.matcmadison.edu



Fun Fact

Salmonid genome = ~3,000,000,000 bp

If each basepair is 1mm long, the entire salmon genome could be lined up to span the distance of the entire Columbia river, and still overflow into the Pacific Ocean an additional 1/3 its length.

Allozyme Markers

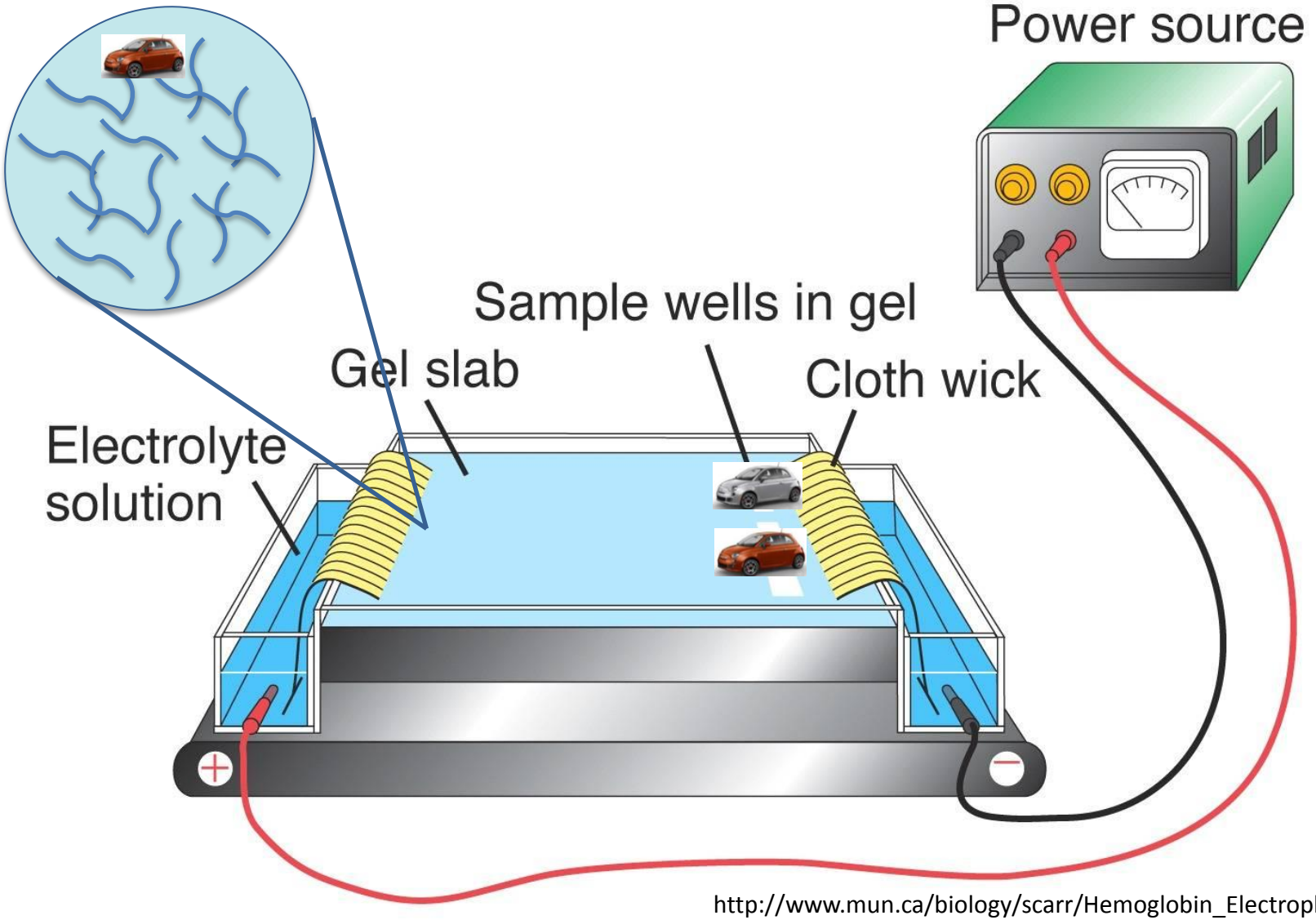
- Proteins are involved in carrying out various biological processes
- Changes in the genetic code (DNA) can have an affect on the physical properties of the protein

Allozyme Markers

- Allozymes are variants of the same protein

- Each variant (allele) has unique physical properties that allow us to differentiate them using electrophoresis

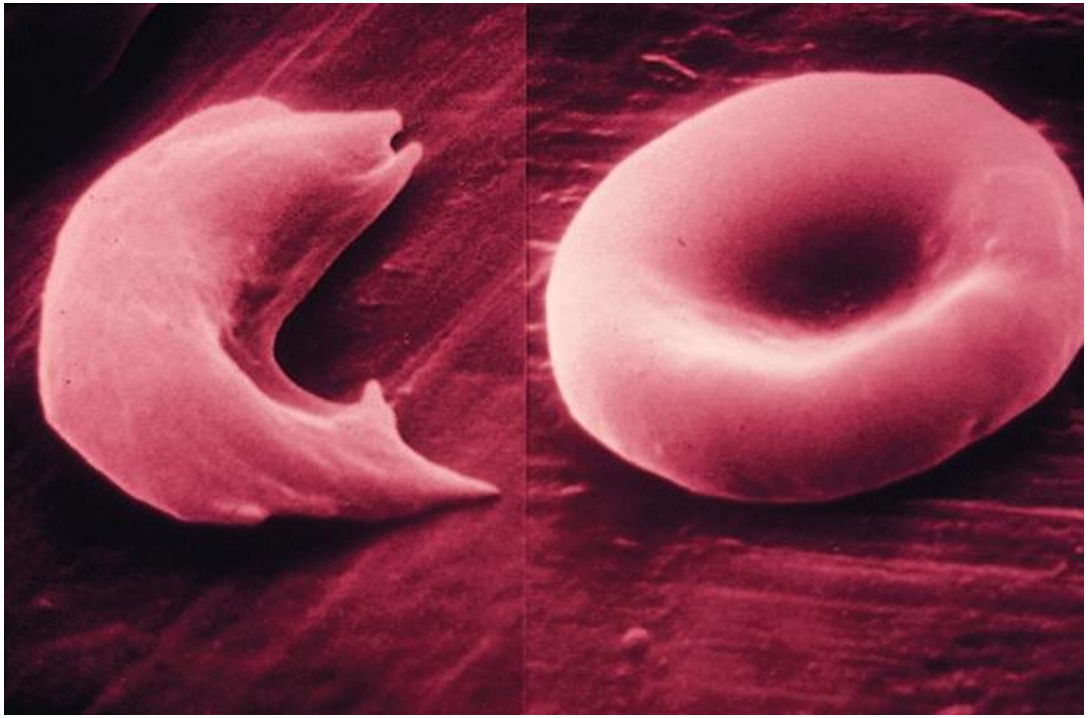
Electrophoresis



http://www.mun.ca/biology/scarr/Hemoglobin_Electrophoresis.html

Allozyme Markers

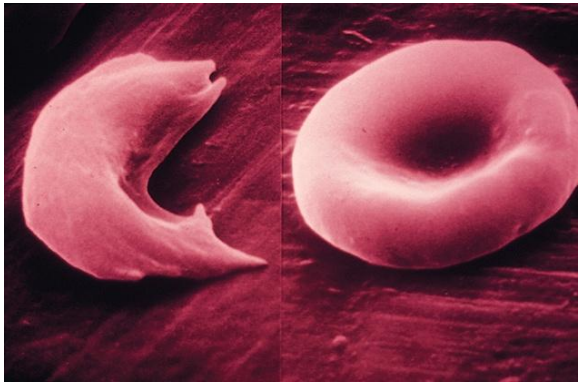
- Example: Sickle Cell hemoglobin

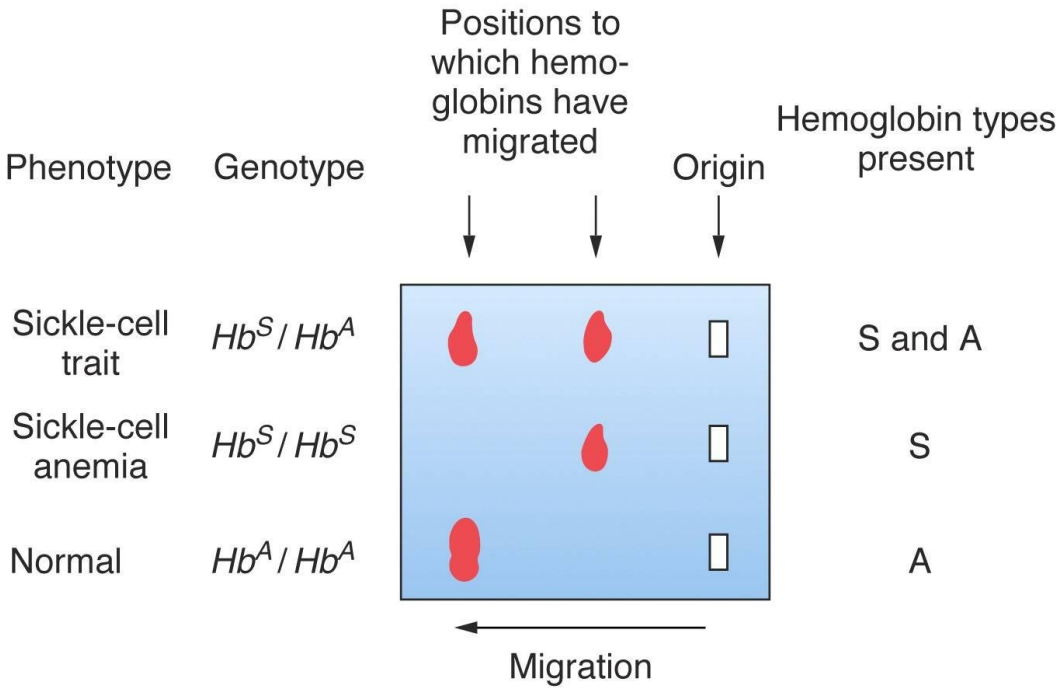


- Glu>Val in Hb^A beta-globin > Hb^S

http://www.mun.ca/biology/scarr/Hemoglobin_Electrophoresis.html

Allozyme Markers





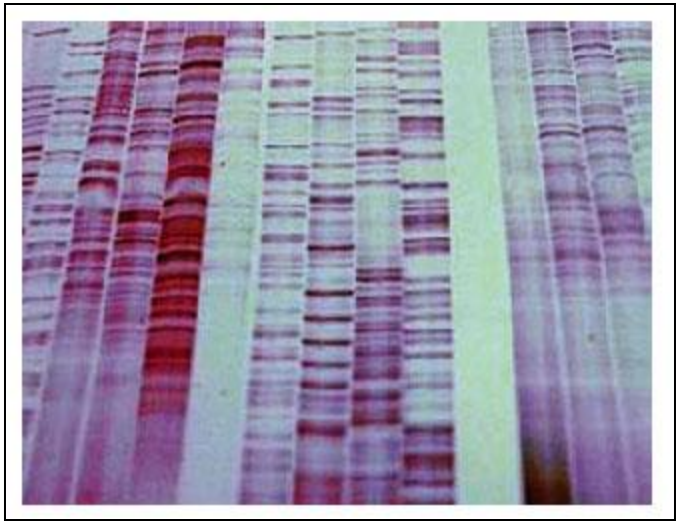
http://www.mun.ca/biology/scarr/Hemoglobin_Electrophoresis.html

Allozyme Markers

- The pioneering marker of molecular genetics
- Pros
  - Multi-allelic/co-dominant marker
  - Easy to replicate across labs
  - Requires no DNA sequence information
- Cons
  - Protein isolation can be time consuming and expensive
  - Large amount of tissue required (lethal sampling)
  - Susceptible to environmental variation (tissue specific protein expression levels)
  - Not considered genetic marker (phenotypic marker)

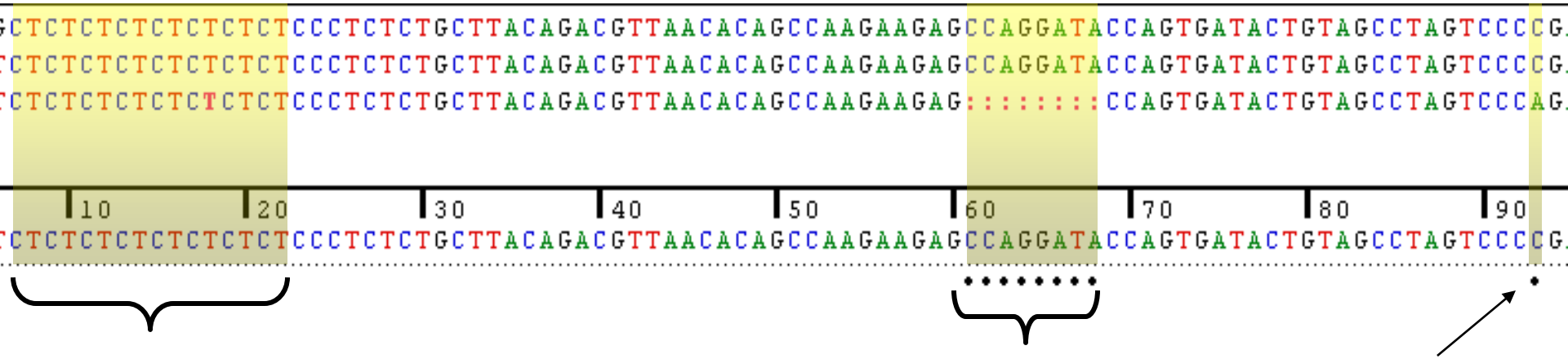
DNA Sequence and Genetic Markers

- Investigating variation at the DNA sequence level
- Required DNA sequencing technology to immerge
- Once sequences could be obtained, informative genetic markers were discovered



http://www.nature.com/nmeth/journal/v5/n1/full/nmeth1154.html

DNA Sequence Variation



microsatellite

- di-nucleotide “CT” repeat
- di- and tetra-nucleotide markers common

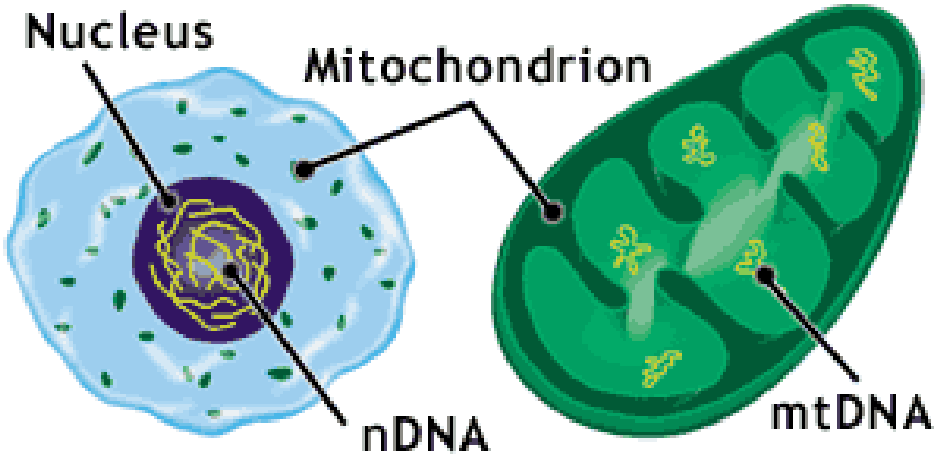
insertion/deletion (indel)

- Ex. 8 bp deletion
- 1 or more nucleotides

SNP

- Any variation of the 4 nucleotides

Mitochondrial vs Nuclear DNA



http://www.mda.org/publications/images/q92resup_mitochondrion.gif

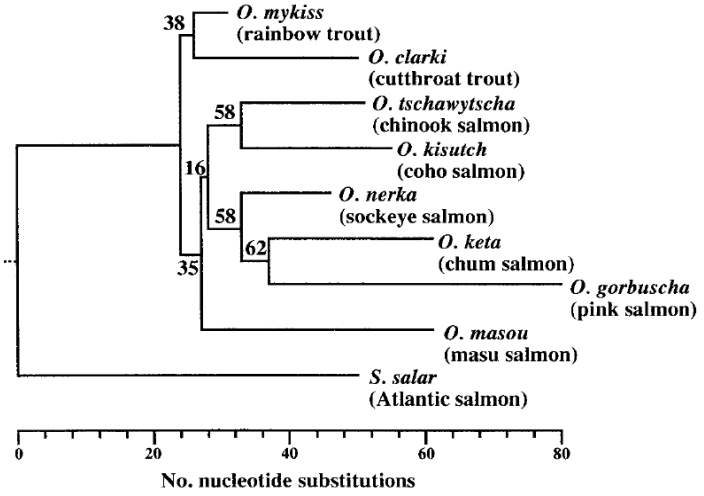
Mitochondrial DNA

- Maternally inherited
  - mtDNA in sperm destroyed at fertilization
  - Maternal lineages can be traced back in time
- Susceptible to oxidative damage and increased mutation rates
  - Allows for assessment of genetic relationships among individuals, species, and across taxa

Mitochondrial DNA

- Remains of Jesse James identified using mtDNA sequence that was identical to two maternal relatives

shs.umsystem.edu



Kitano et al. 1997

- Salmo gairdneri -> Oncorhynchus mykiss

Mitochondrial DNA

- Pros
  - More copies per cell than nuclear DNA
    - Paleontology
  - No recombination/Maternally inherited/High mutation rates
    - Good resolution of taxonomic relationships
  - Sequence effort comparable to nuclear DNA
- Cons
  - Requires sequencing of long stretches of DNA
  - Only maternally inherited, so can’t be used to identify paternity

Microsatellite Markers

Microsatellite markers are repeating sequences of 2-6 base pairs of DNA and can be hyper-variable compared to other markers

1 TGCCGTGCATATATCGAGCTATT (3X)=23bp

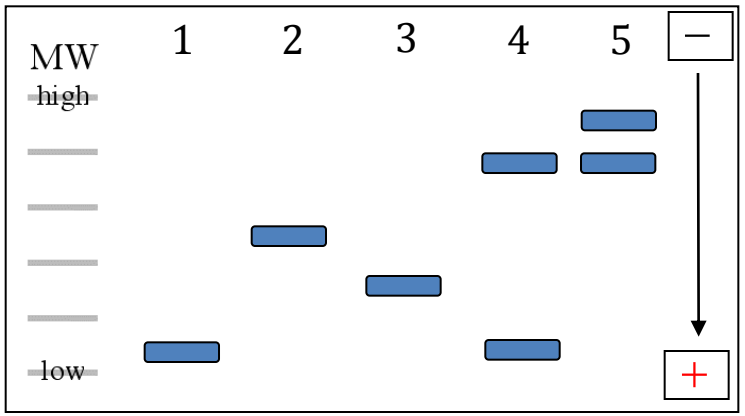
2 TGCCGTGCATATATATATATCGAGCTATT (6X)=29bp

3 TGCCGTGCATATATATATCGAGCTATT (5X)=27bp

4 TGCCGTGCATATATCGAGCTATT (3X)=23bp
TGCCGTGCATATATATATATATATATCGAGCTATT (8X)=33bp

5 TGCCGTGCATATATATATATATATATCGAGCTATT (8X)=33bp
TGCCGTGCATATATATATATATATATATCGAGCTATT (9X)=35bp

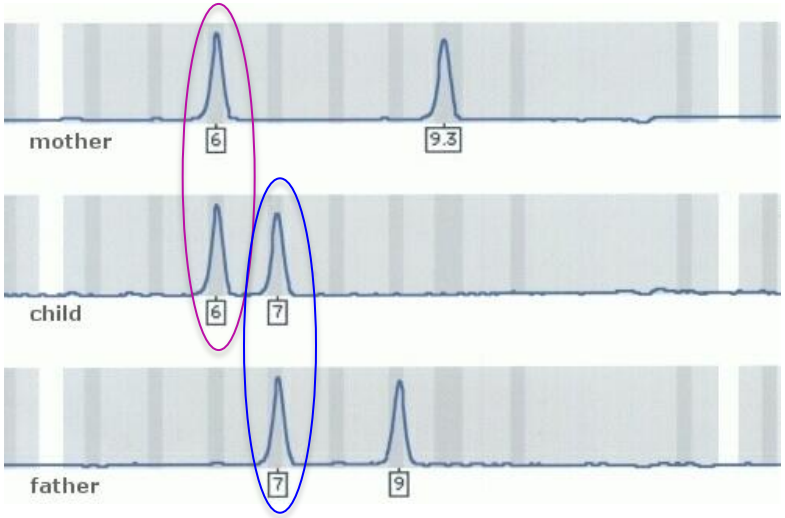
Can be scored using electrophoresis just like allozyme markers.



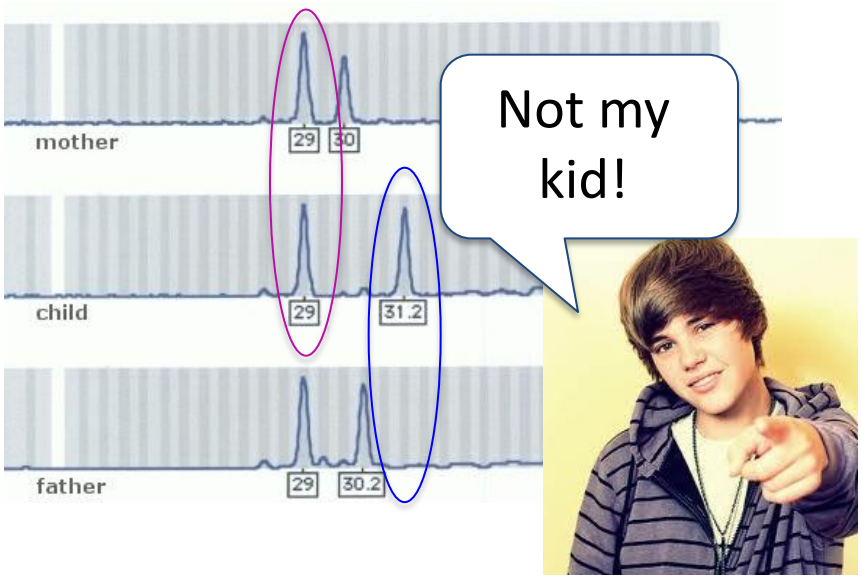
Microsatellite Markers

- Example: Paternity testing

Case 1



Case 2



http://www.paternity.be/information_EN.html

Microsatellite Markers

- Pros
  - Extremely variable
    - > 20 alleles for one locus
    - Codominant
  - Moderately abundant in genome
  - Potentially neutral and adaptive loci
  - Moderately easy to genotype
    - Less time and effort to genotype than allozyme markers
  - Little tissue required (non-lethal sampling)
- Cons
  - Difficult to discover
    - Requires DNA sequence
  - Difficult to standardize and exchange data across labs
    - PCR variation
    - Scoring variation

SNP Markers

- A single-nucleotide polymorphism (SNP) is a variation in DNA, when a single nucleotide (A,T,C, or G) within a given sequence differs between homologous chromosomes or between individuals at homologous loci

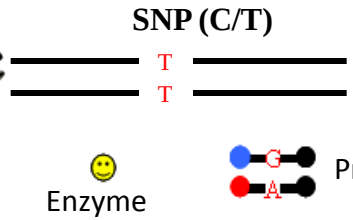
ATG GCT TCG ATC GAT CTA
ATG GCC TCG ATC GAT CTA

ATG GCT ACG ATC GAC CTA
ATG GCT ACG ATC GAC CTA

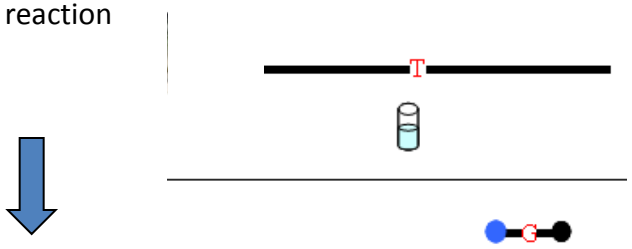
SNP Markers

- Can be detected in many ways including electrophoresis and sequencing
- Can be rapidly/massively genotyped using high-throughput methods

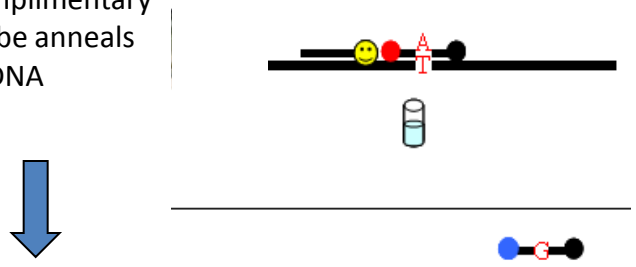
SNP Genotyping



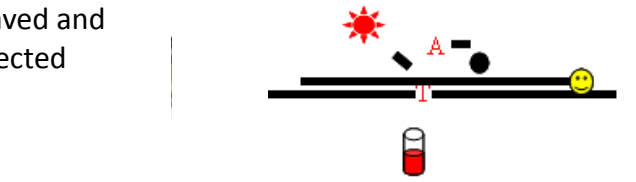
Components of PCR reaction

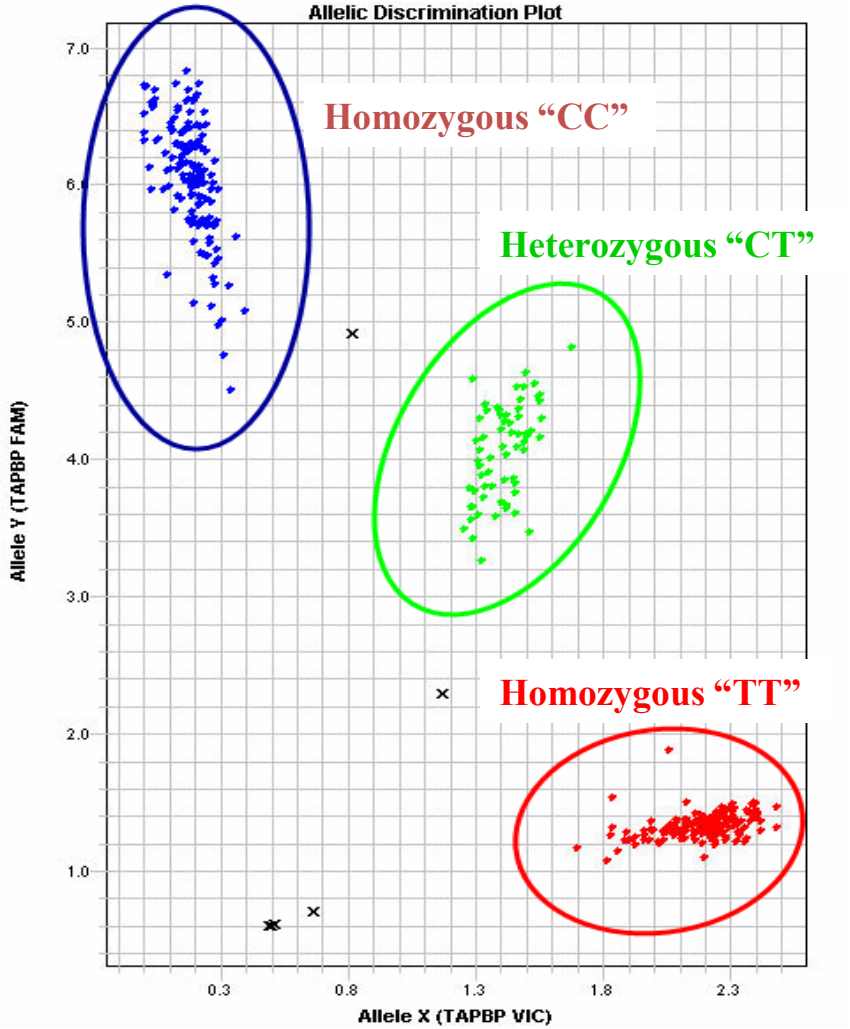


Complimentary probe anneals to DNA



Fluorescent tag cleaved and detected

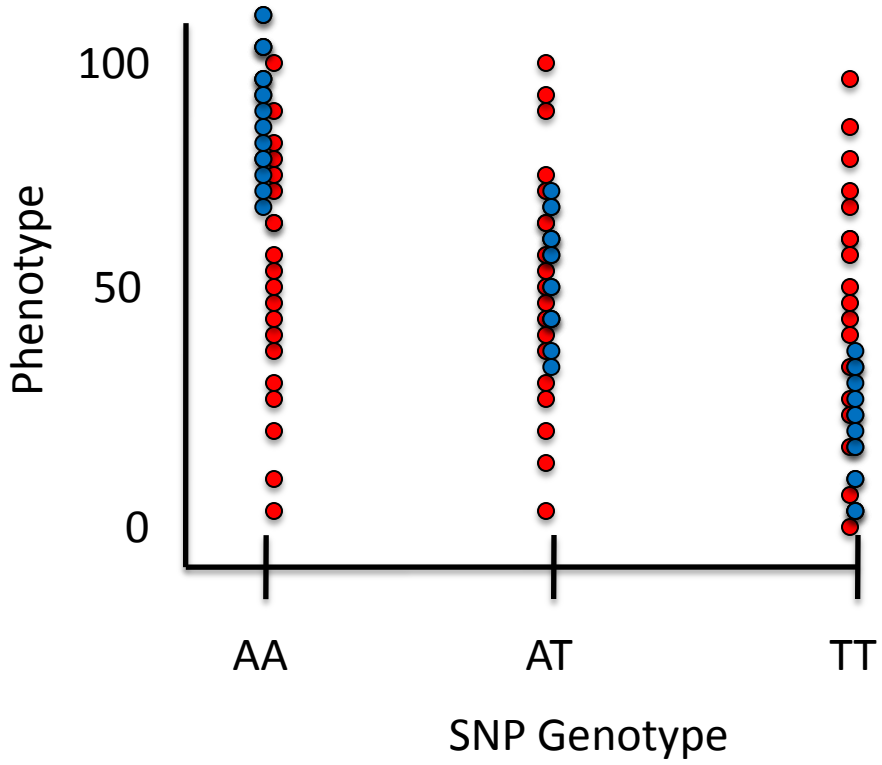


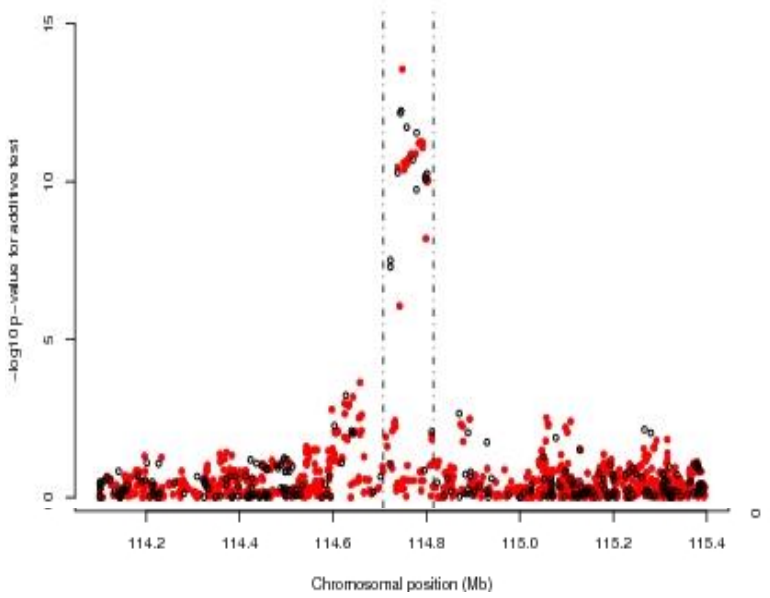


Adapted from C. Smith

SNP Markers

- Testing for statistical associations between genotype and phenotype along the genome





SNP Markers

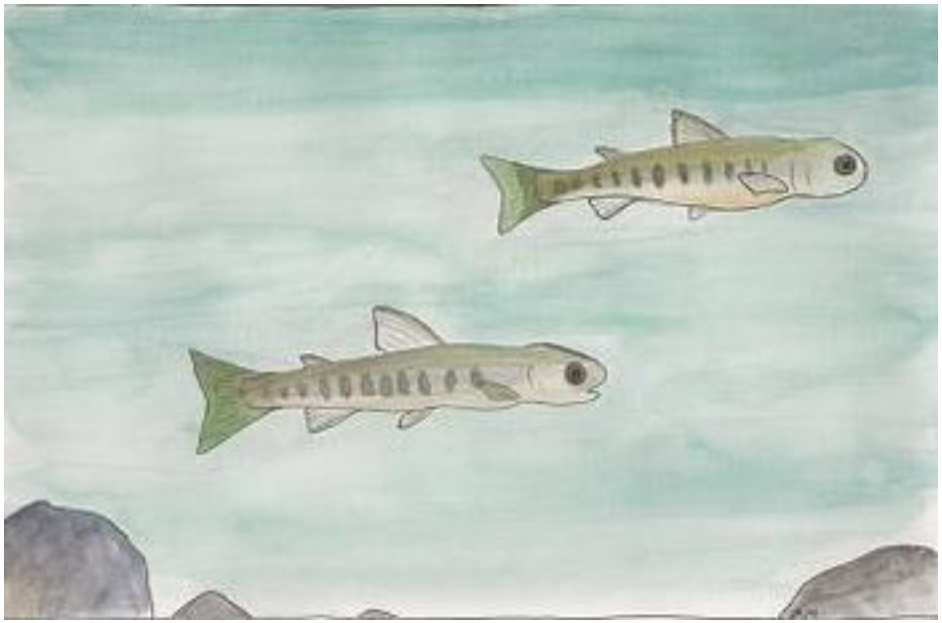
- Can be used for estimating:
  - Parentage
  - Genetic diversity
  - Genetic distance
  - Genetic mapping

SNP Markers

- Pros
  - Codominant markers
  - Most abundant markers in the genome/easy to discover
  - Easy to interrogate with current high-throughput technology
  - requires little tissue
  - Highly reproducible between labs, easy to standardize, easy exchange of data
  - Can be adaptive and neutral loci

- Cons
  - Not as variable as microsatellites
    - Only two alleles per locus
  - Requires DNA sequence
  - High up front discovery/operating cost (new technology is making this cheaper)

Questions?



http://www.streamexplorers.org/atf/cf/SMOLT-OR-PARR.jpg