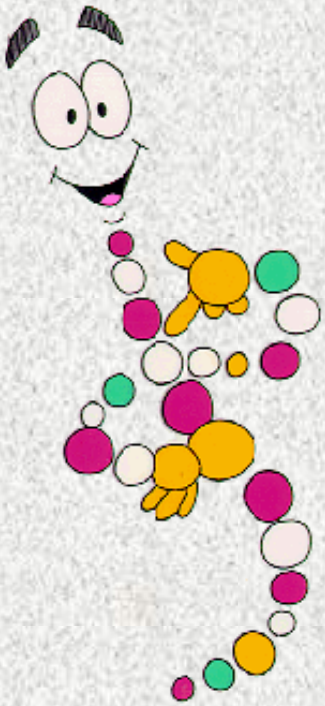
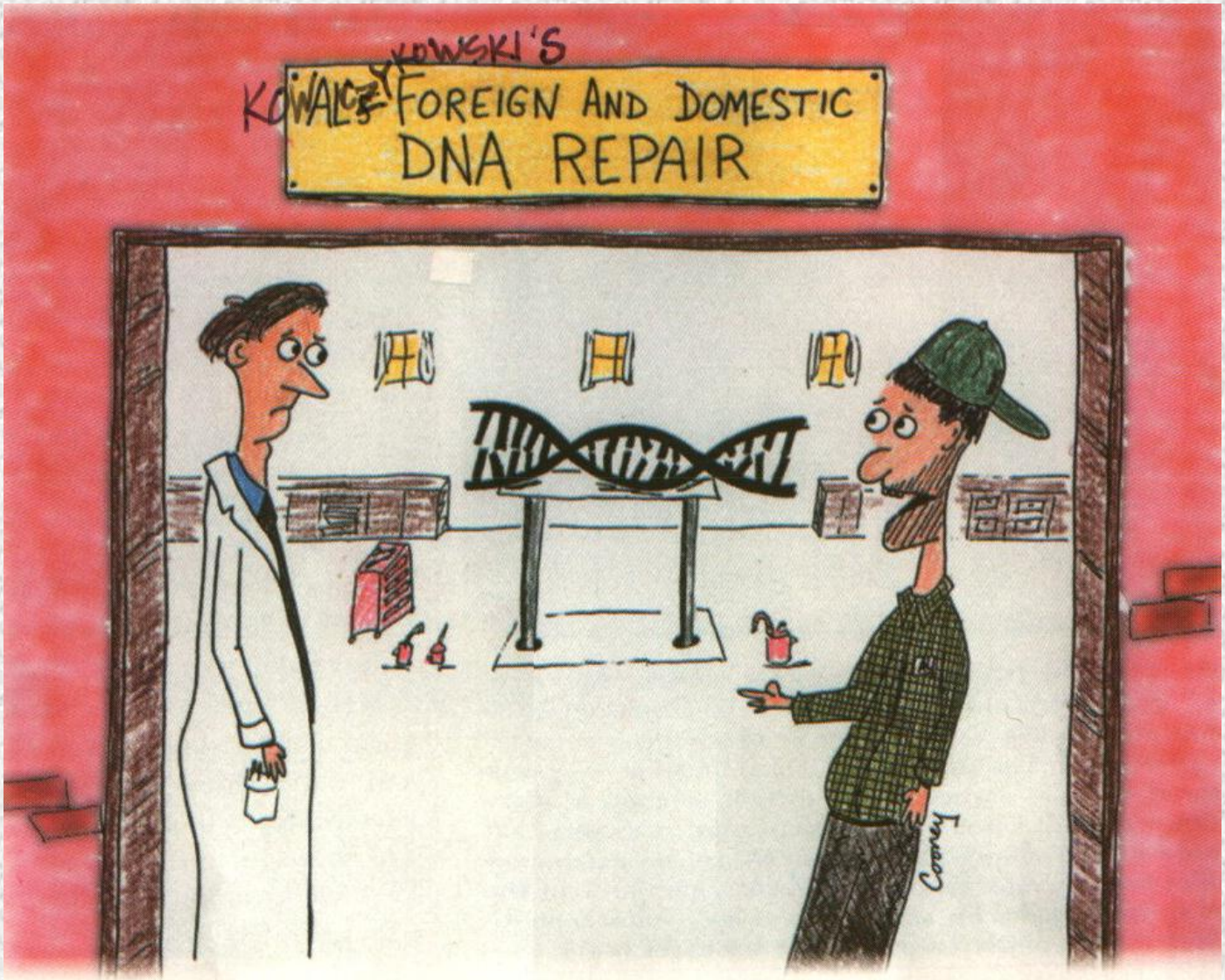
Genetics 101:





"You're lucky nobody was injured. Your base pairs are out of alignment and that has your reading frames all messed up."

Overview

Introduction

Important terminology

DNA extraction, gel electrophoresis, PCR

Allozymes (Protein electrophoresis)

RFLP

AFLP

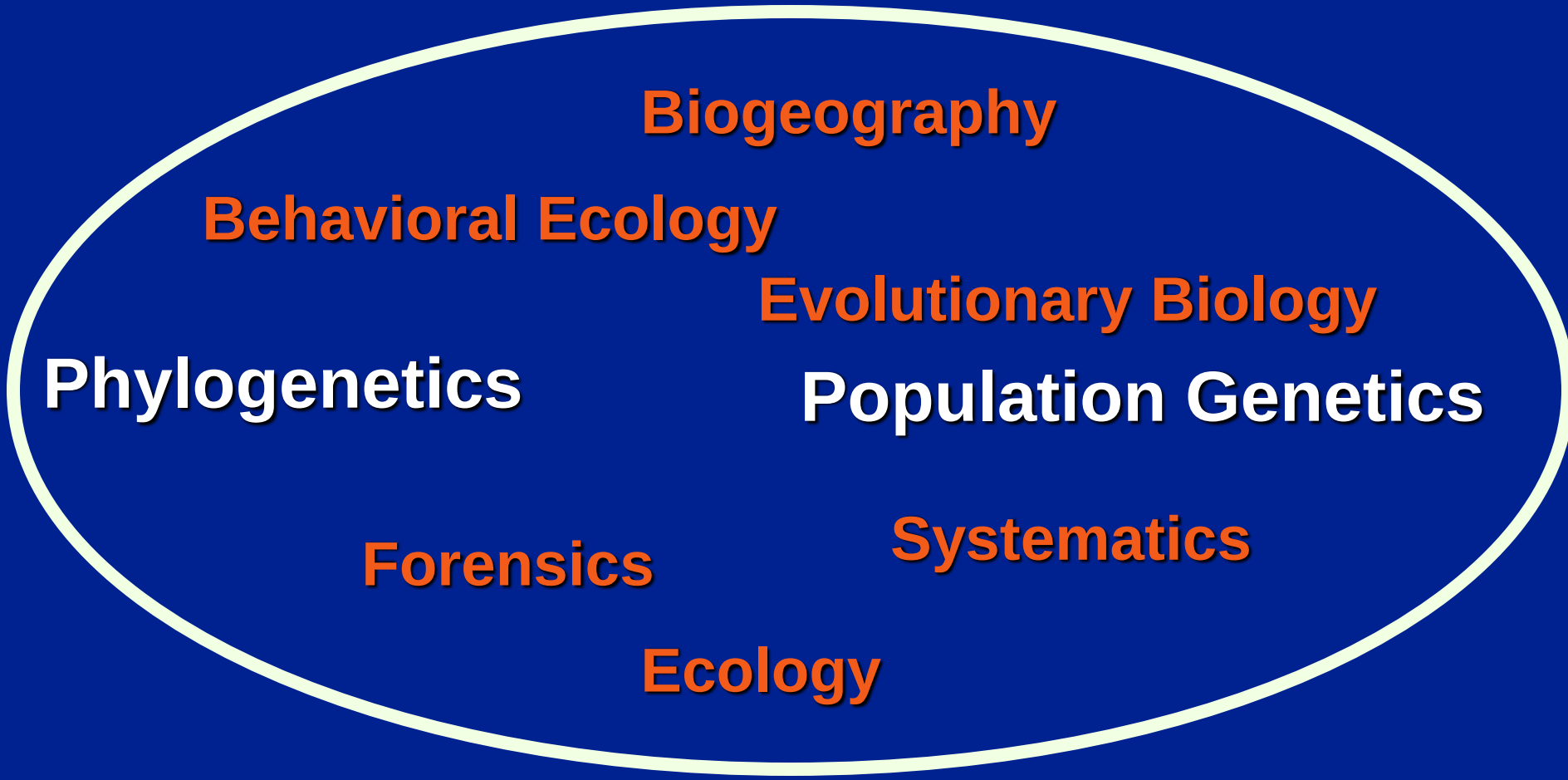
Sequencing

Microsatellites

SNPs

Costs, Sample Collection

Conservation Genetics



Goal: Provide information for conservation and management

Useful Books

- **Frankham et al 2002 (2010), Introduction to conservation genetics. Cambridge Univ. Press**

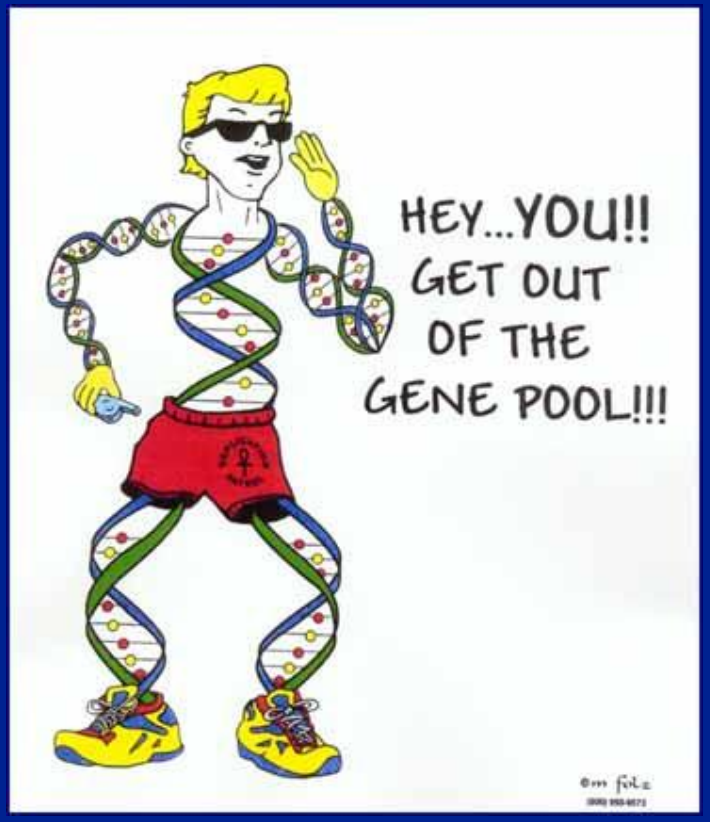
- **Allendorf and Luikart 2007 Conservation and the Genetics of Populations. Blackwell Publishing**

Perspective

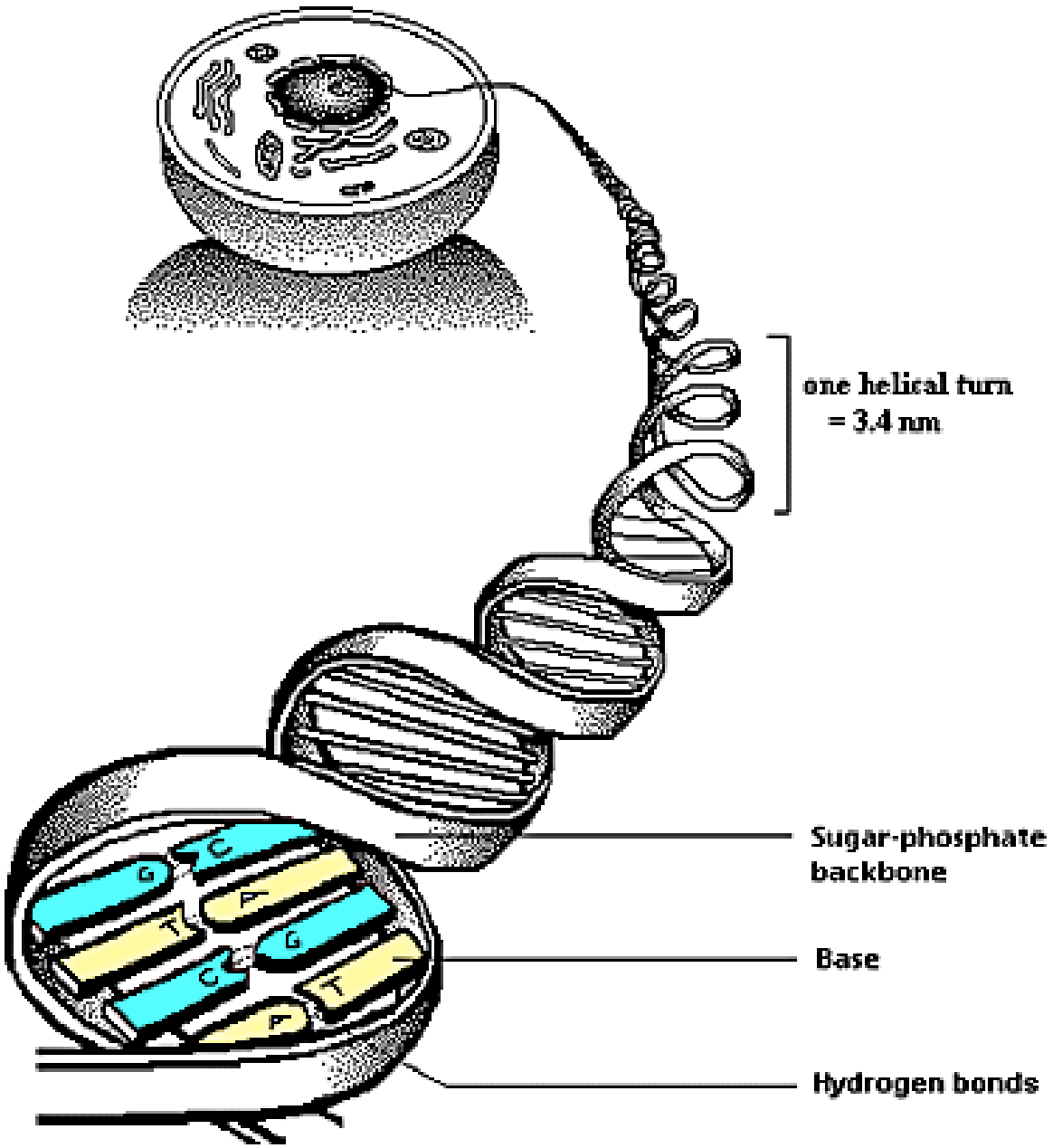
“Over the short term, most species are threatened by extrinsic and environmental factors. Ultimately, the viability of species that survive the short term demographic and environmental threats may depend upon the genetic variability they possess, and genetic variation can interact with environmental factors in such a way that the two cannot be viewed as independent effects.”

Mace et al. 1996

Important terminology



THE STRUCTURE OF DNA



Two Types of DNA

Mitochondrion

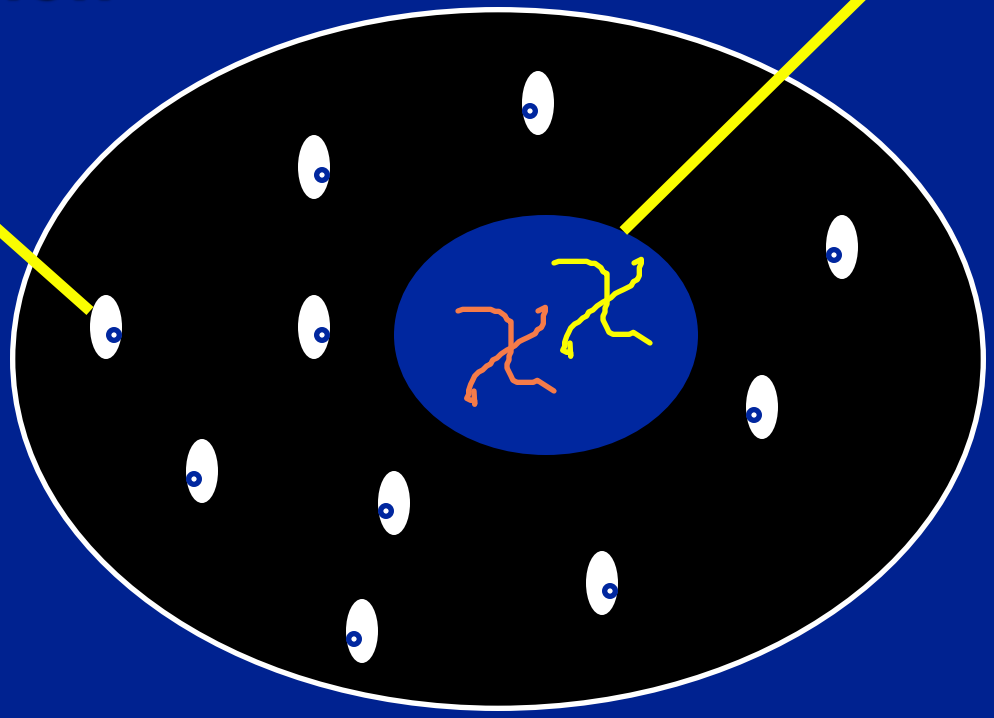
MtDNA

Maternal Inheritance

Nucleus

nDNA

Biparental Inheritance



Animal Cell

Plants:
mtDNA
nDNA
Chloroplast DNA

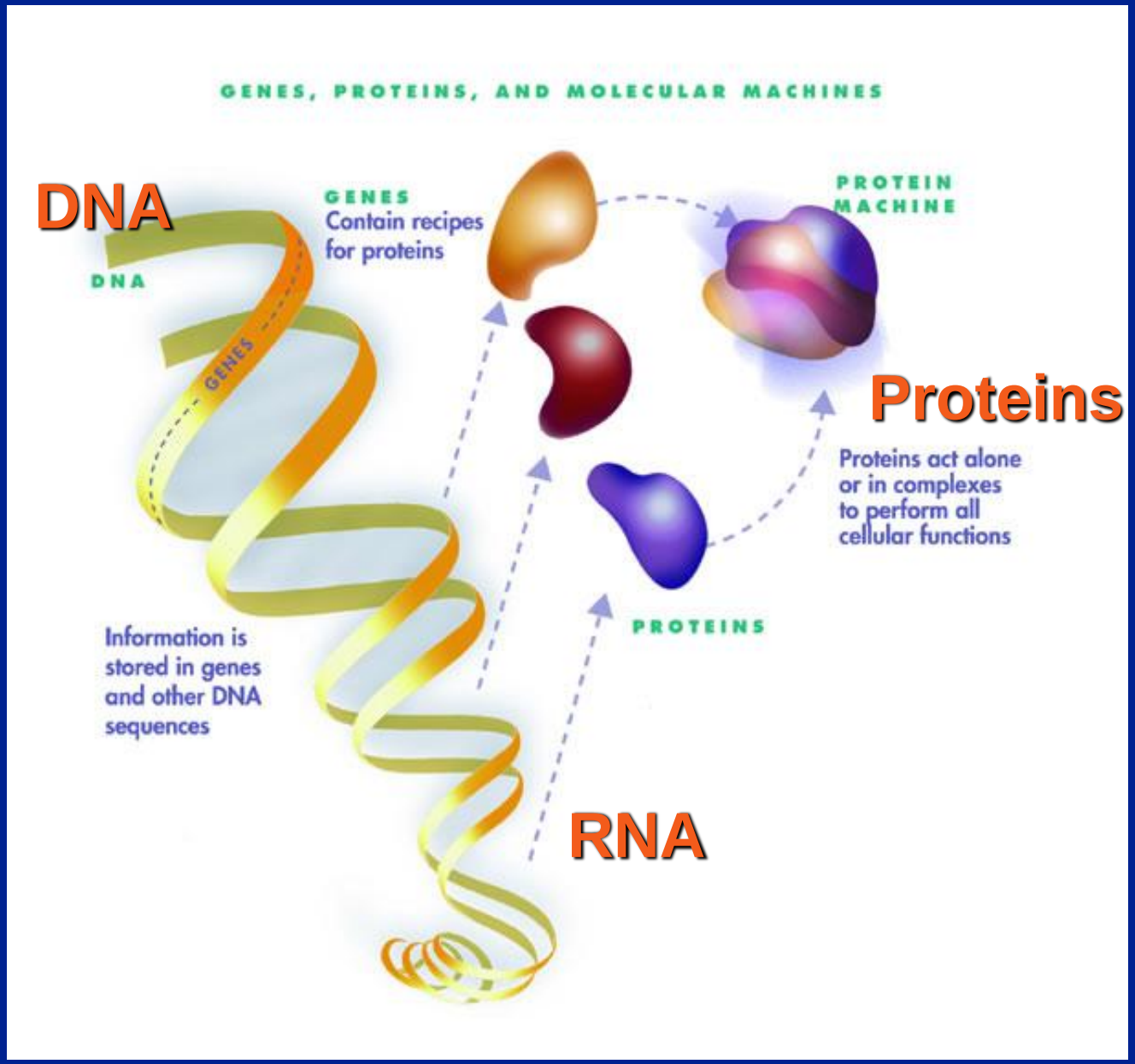
What is genetic diversity?

DNA sequence differences at the same physical location

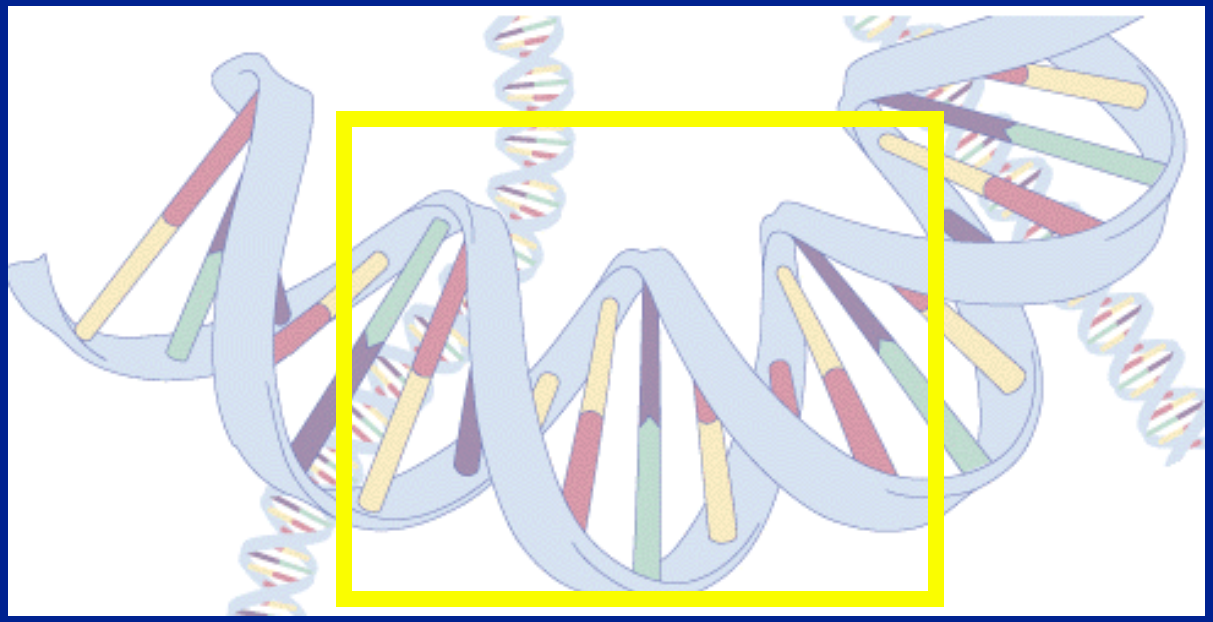
Example:

Sample 1	GATCC ATCGA TCTGGC A
Sample 2	GATCA ATCGG TCTGAT A

Proteins


GENES, PROTEINS, AND MOLECULAR MACHINES
DNA
DNA
GENES
GENES
Contain recipes
for proteins
PROTEIN
MACHINE
Proteins
Proteins act alone
or in complexes
to perform all
cellular functions
PROTEINS
Information is
stored in genes
and other DNA
sequences
RNA

Locus



A physical location in the genome

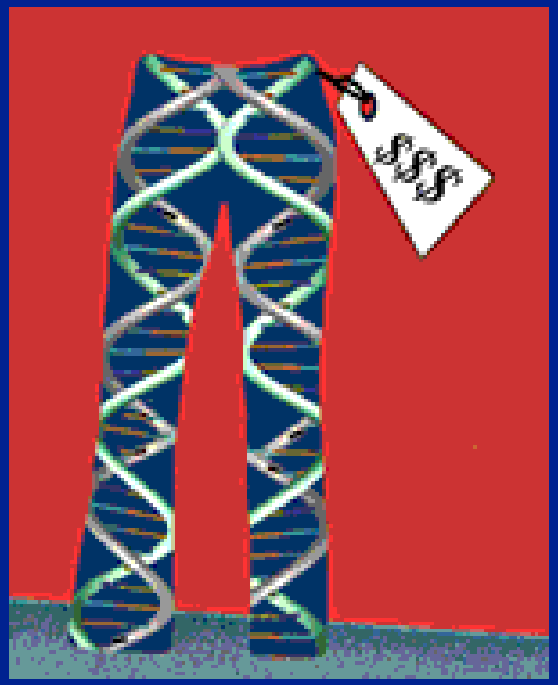
Neutral Loci vs Loci under selection

- **Genes are loci influenced by natural selection.**

- **Other sections of DNA with no known function are neutral.**

Genes:

Pieces of DNA that code for a protein



Genotype - Allelic combination at one locus or multiple loci

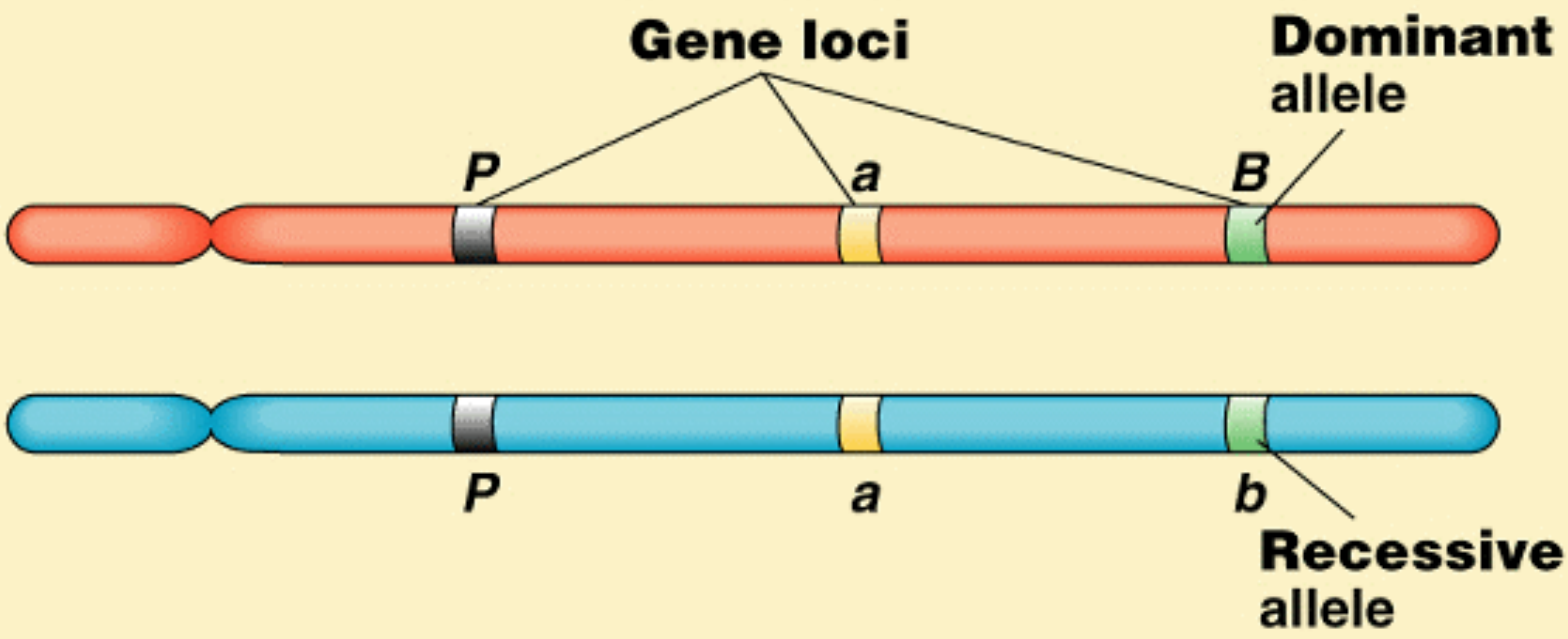
Father's Genotype - Aa

Mother's Genotype Aa

	Allele 1 A	Allele 2 a
A	AA 	Aa 
a	Aa 	aa 

Heterozygote

Homozygote

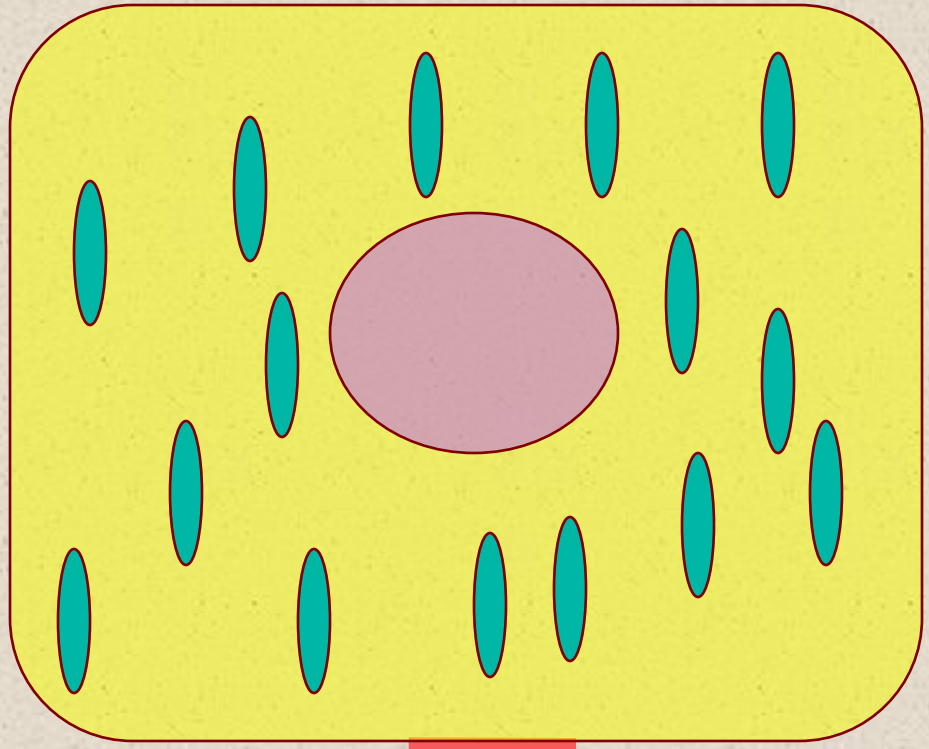


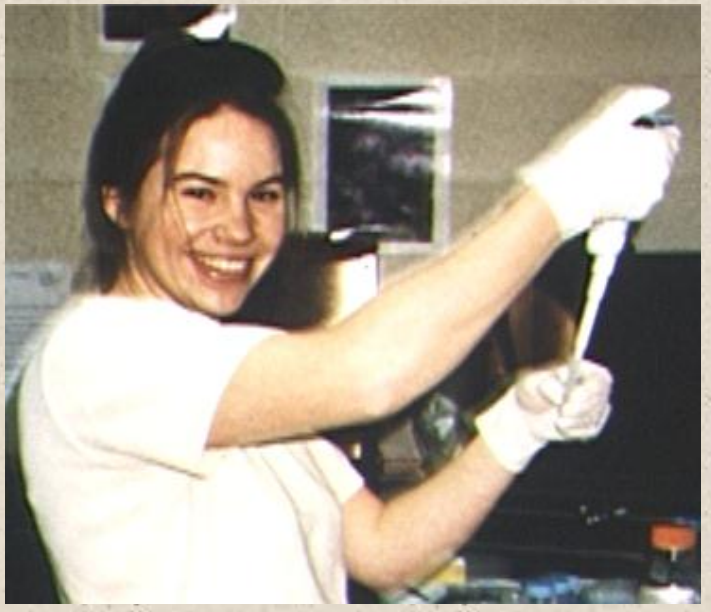
Genotype: *PP* *aa* *Bb*

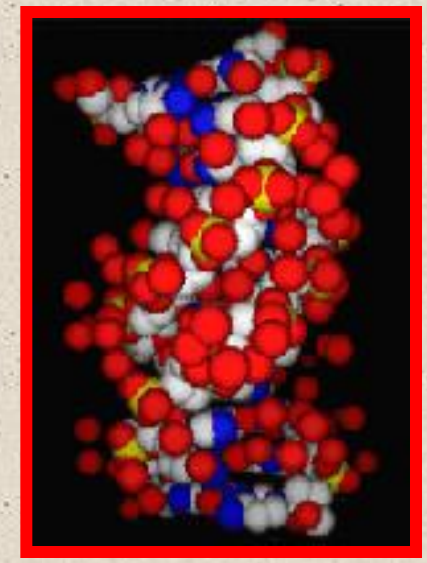
Homozygous for the dominant allele

Homozygous for the recessive allele

Heterozygous





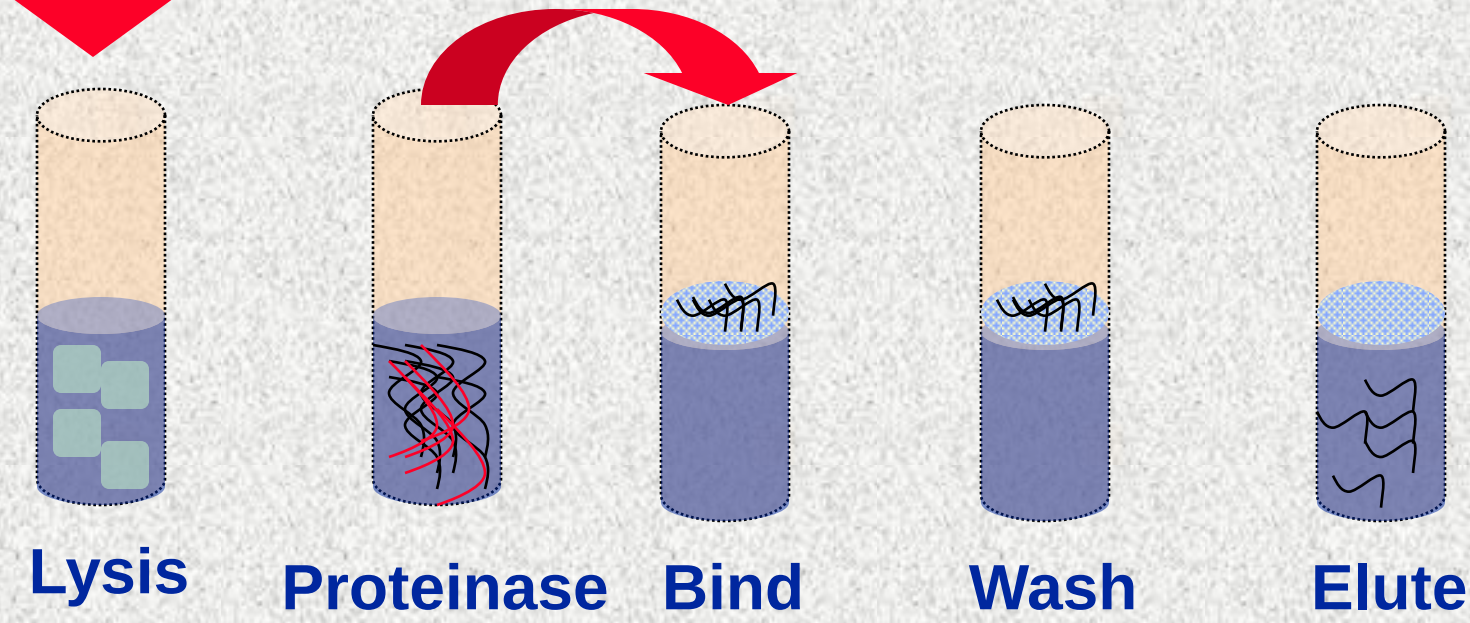


DNA Extraction

DNA Extraction:

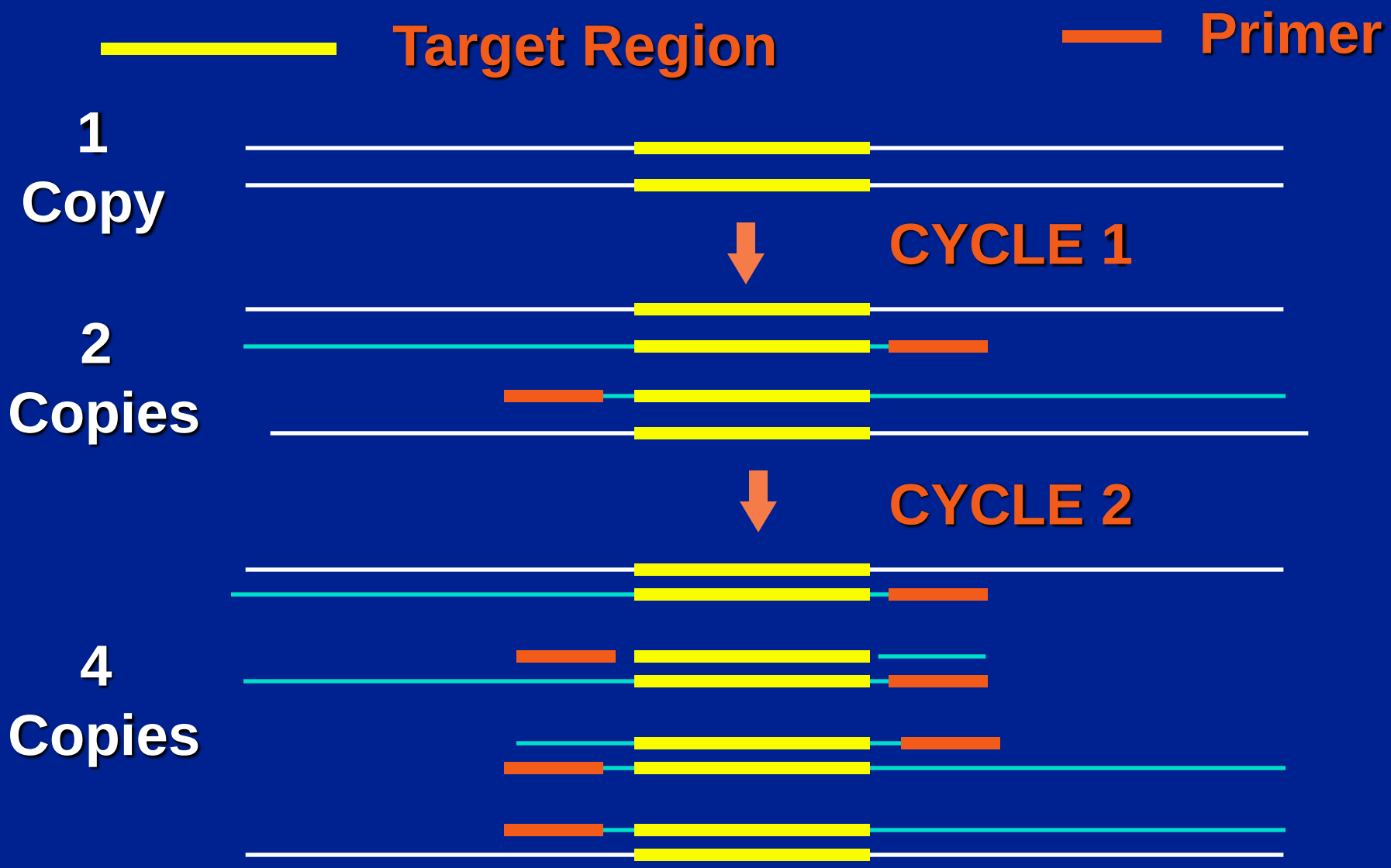
Blood	Tissue	Bone
Hair	Feces	Urine
Feathers	Scales	Skin

Silica Based method



Other Options: Chelex, Phenol/Chloroform

Polymerase Chain Reaction (PCR)

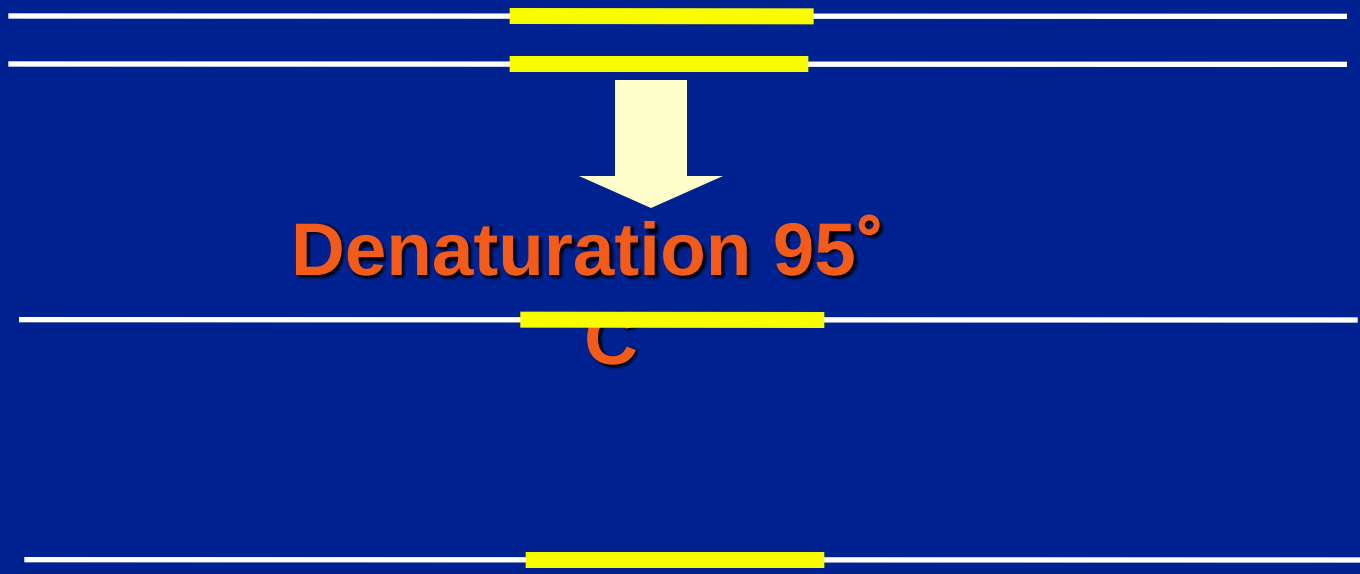


(Mullis 1982)

Double stranded DNA

Target Region

Double stranded DNA



Denaturation 95°C

Target Region

Double stranded DNA

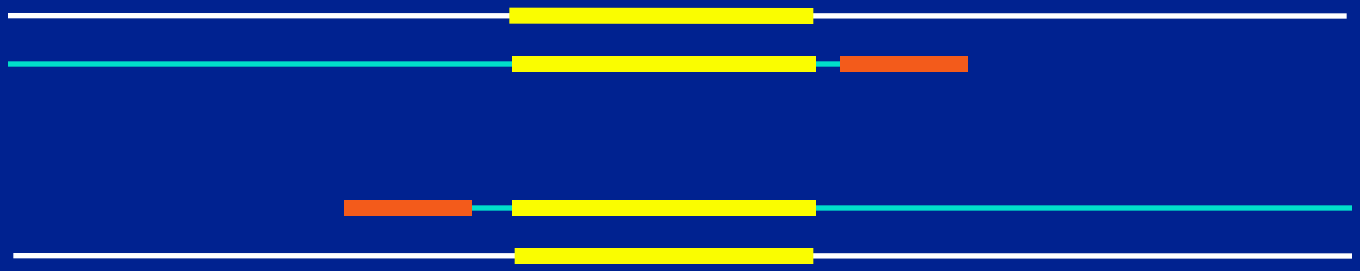
Annealing (~44 ° – 62 ° C)

Target Region

Primer

Double stranded DNA

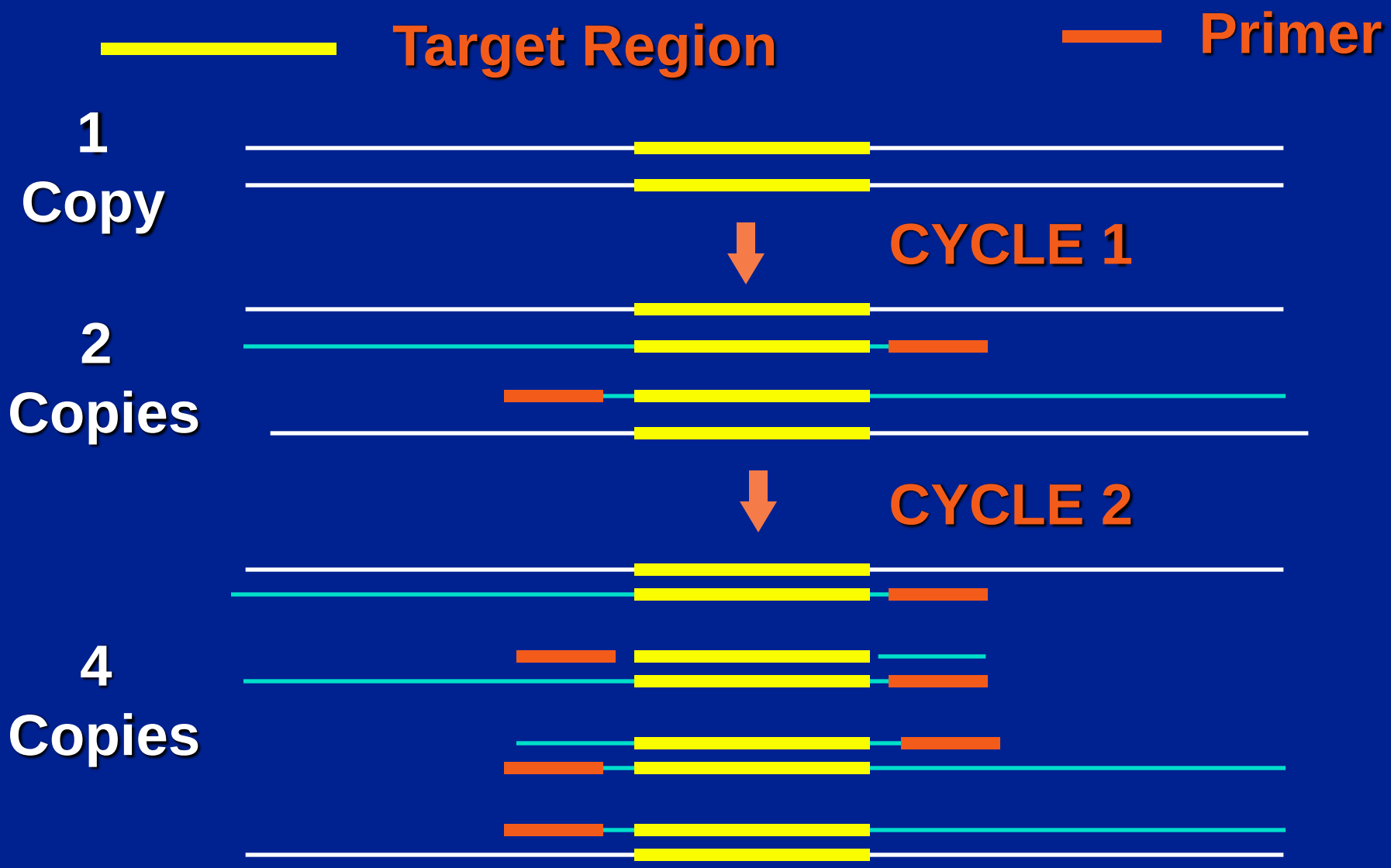
DNA Synthesis 72° C



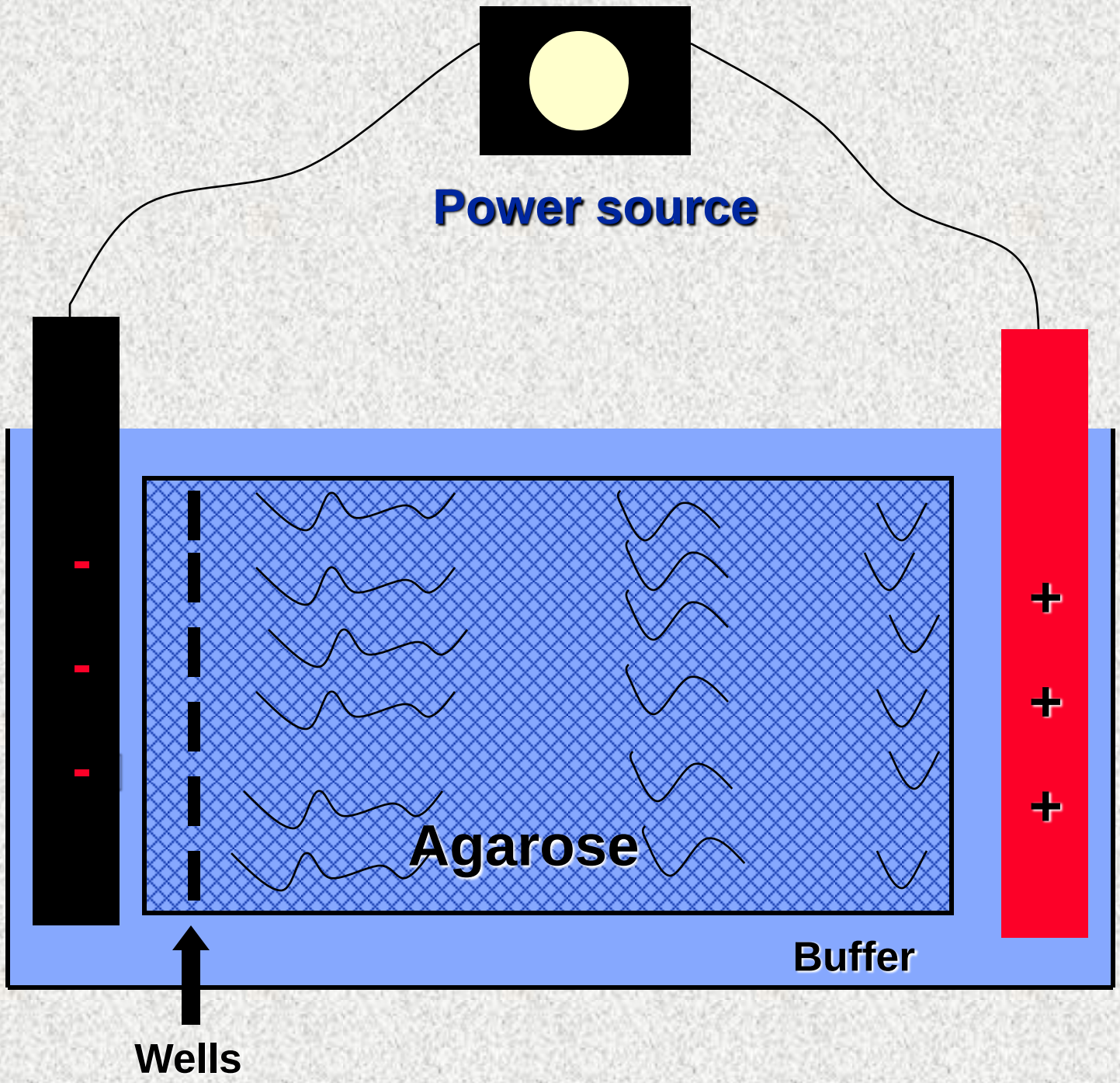
Target Region

Primer

Polymerase Chain Reaction (PCR)



(Mullis 1982)

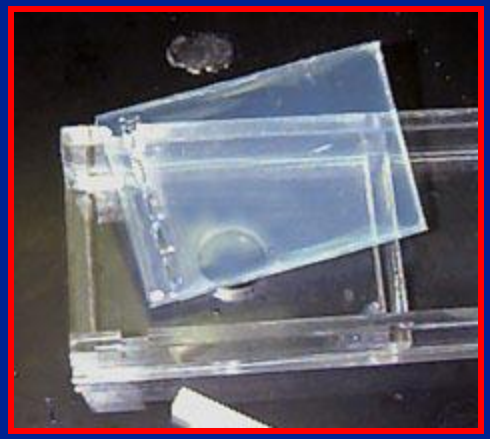

Power source
-
-
-
+
+
+
Agarose
Buffer
Wells

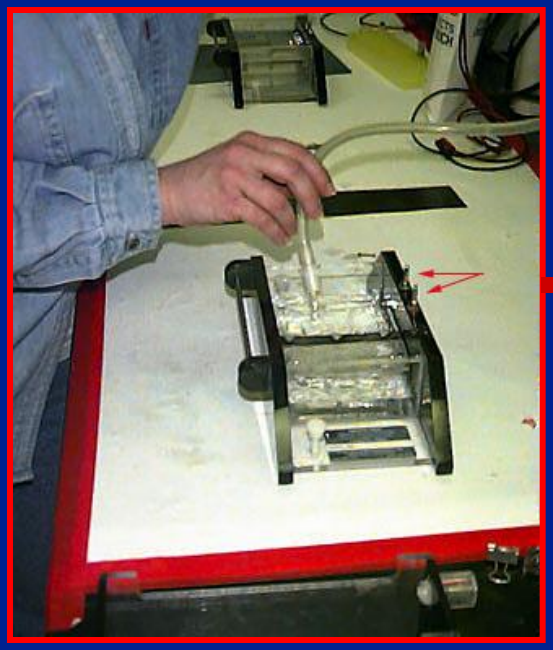

SeaKem
LE
Midwest Scientific

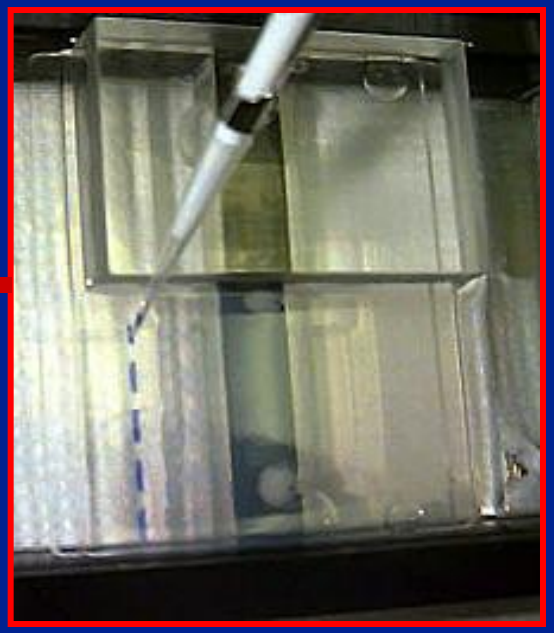

E

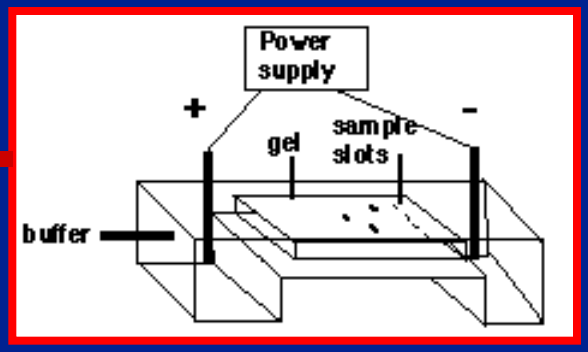

UV Stratalinker
1800


Comb

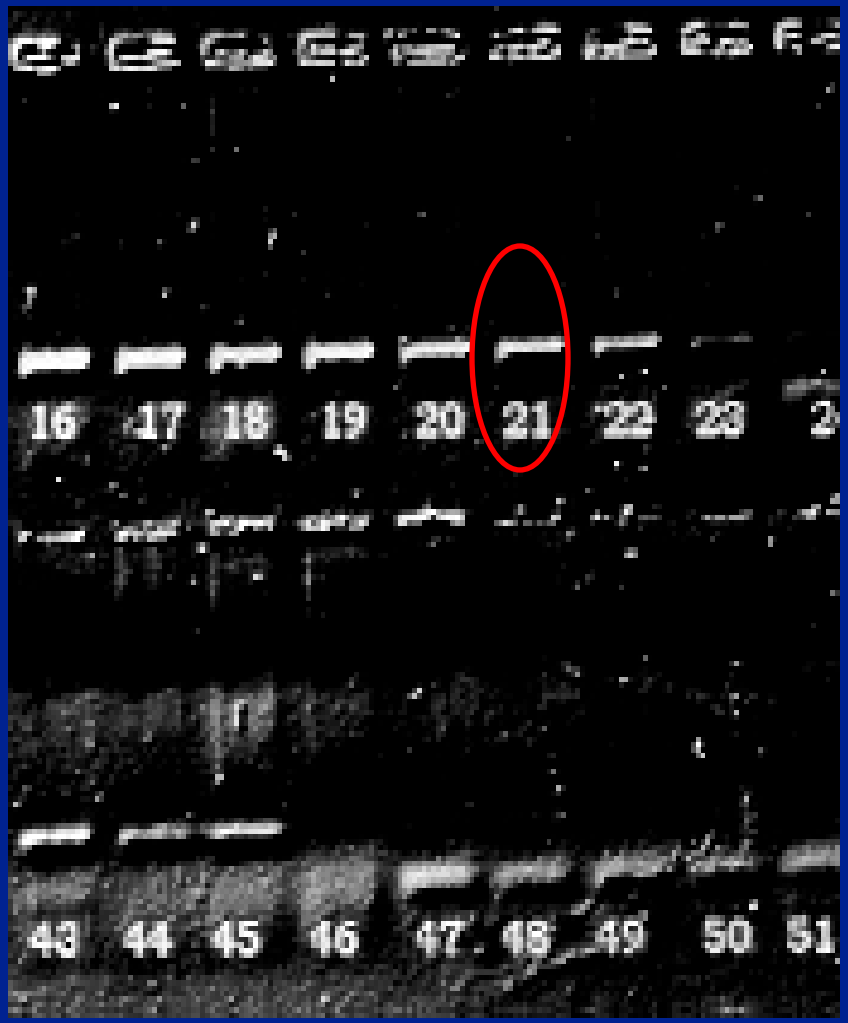




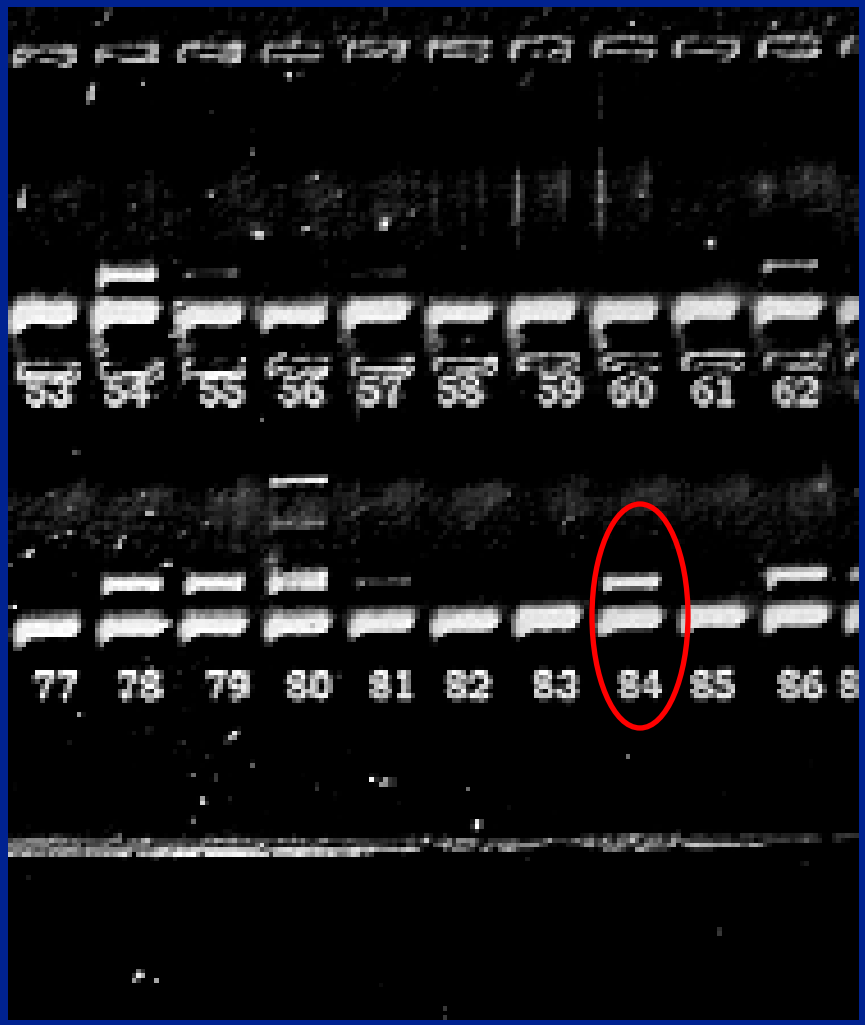



Power supply
+
-
gel
sample slots
buffer

Species ID (mtDNA)

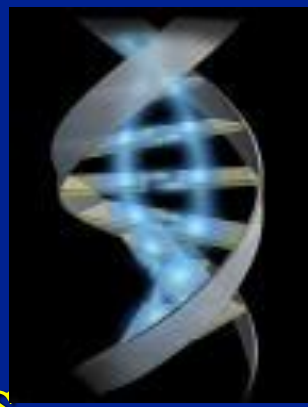


Locus A (nDNA)



Techniques for Detecting Genetic Variation

- **Allozyme analysis - proteins**
- **Restriction Fragment Length Polymorphism Analysis (RFLP)**
- **Random Amplified Polymorphic DNA (RAPDs)**
- **Amplified Fragment Length Polymorphism Analysis (AFLP)**
- **DNA sequencing**
- **Microsatellite analysis**
- **Single Nucleotide Polymorphism Analysis (SNPs) or SCN - single copy nuclear analysis**



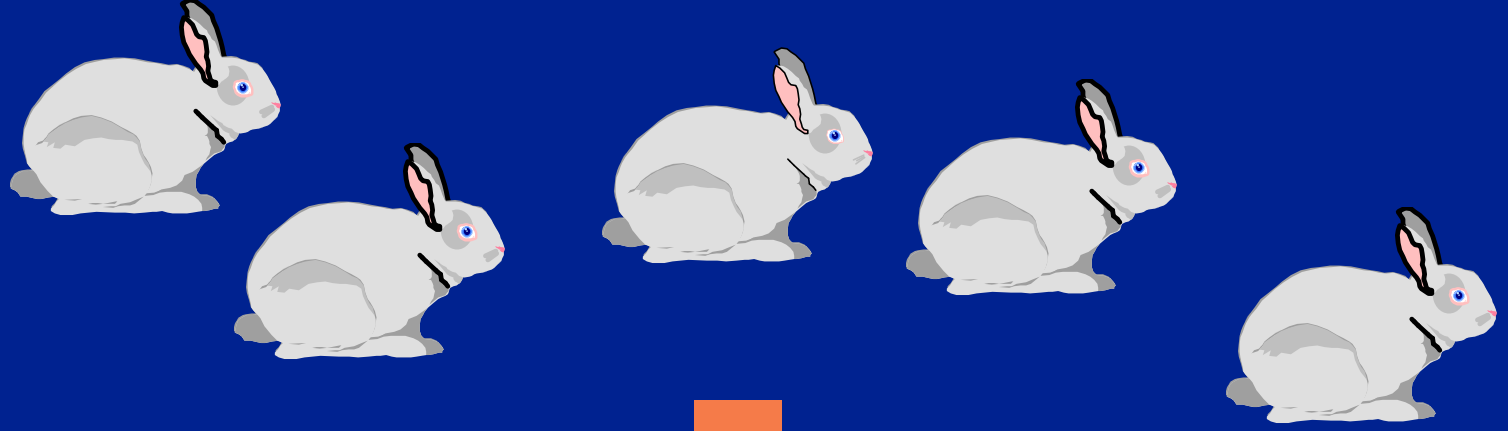
Restriction Fragment Length Polymorphism Analysis (RFLP)

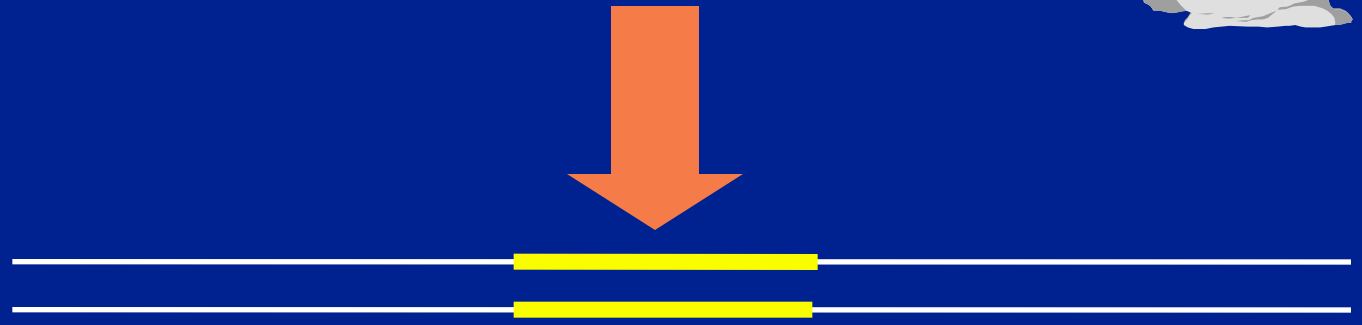
Restriction Enzymes cut DNA at specific locations

Example: EcoR1 enzyme cuts at 5' G*AATTC '3
3' CTTAA*G '5

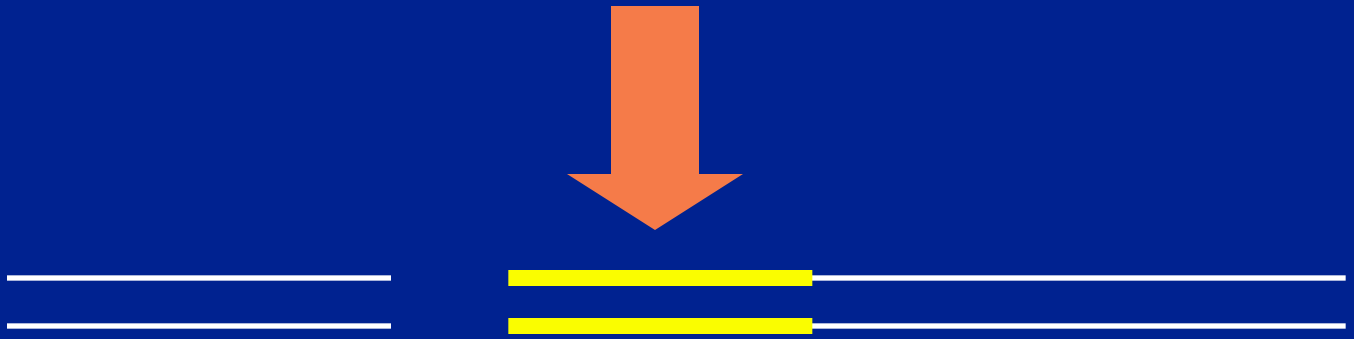
A -5' GATCGGATTCCCTGAACTAG '3
3' CTAGCCTAAGGGACTTGATC '5

B- 5' GATCGAATTCCCTGAACTAG '3
3' CTAGCTTAAGGGACTTGATC '5



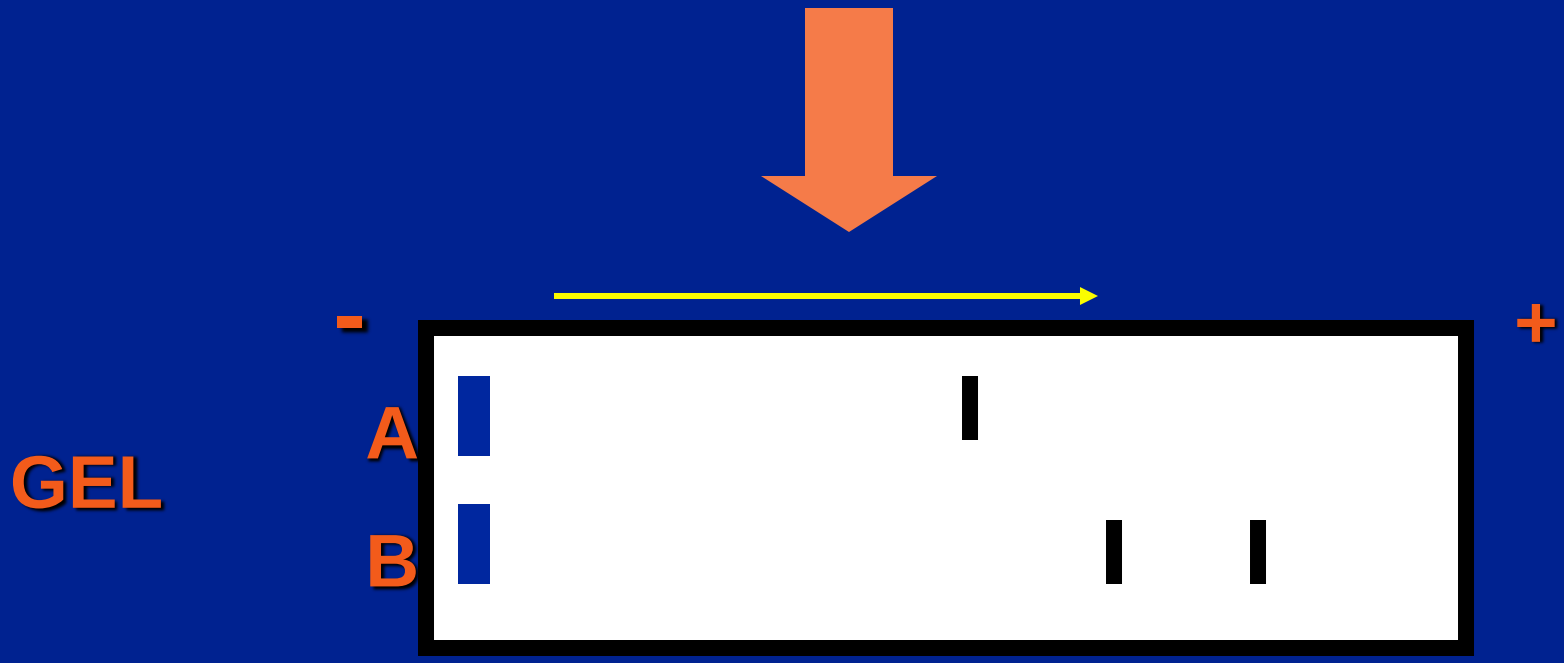


PCR of known DNA locus or use whole mtDNA



Cut DNA overnight with Restriction enzymes

Run out on agarose or acyrlamide gel



Uses: assay of diversity, phylogenetic character, Population structure, species ID

Advantages and Disadvantages

- Small amounts of DNA required when PCR based
- Fast and Accurate
- Less expensive and time consuming than sequencing

- Moderately expensive
- Limited detail
- No direct sequence data

For example exercises see (Blackett family 1 and 2):

www.biology.arizona.edu/human_bio/activities/blackett/introduction.html

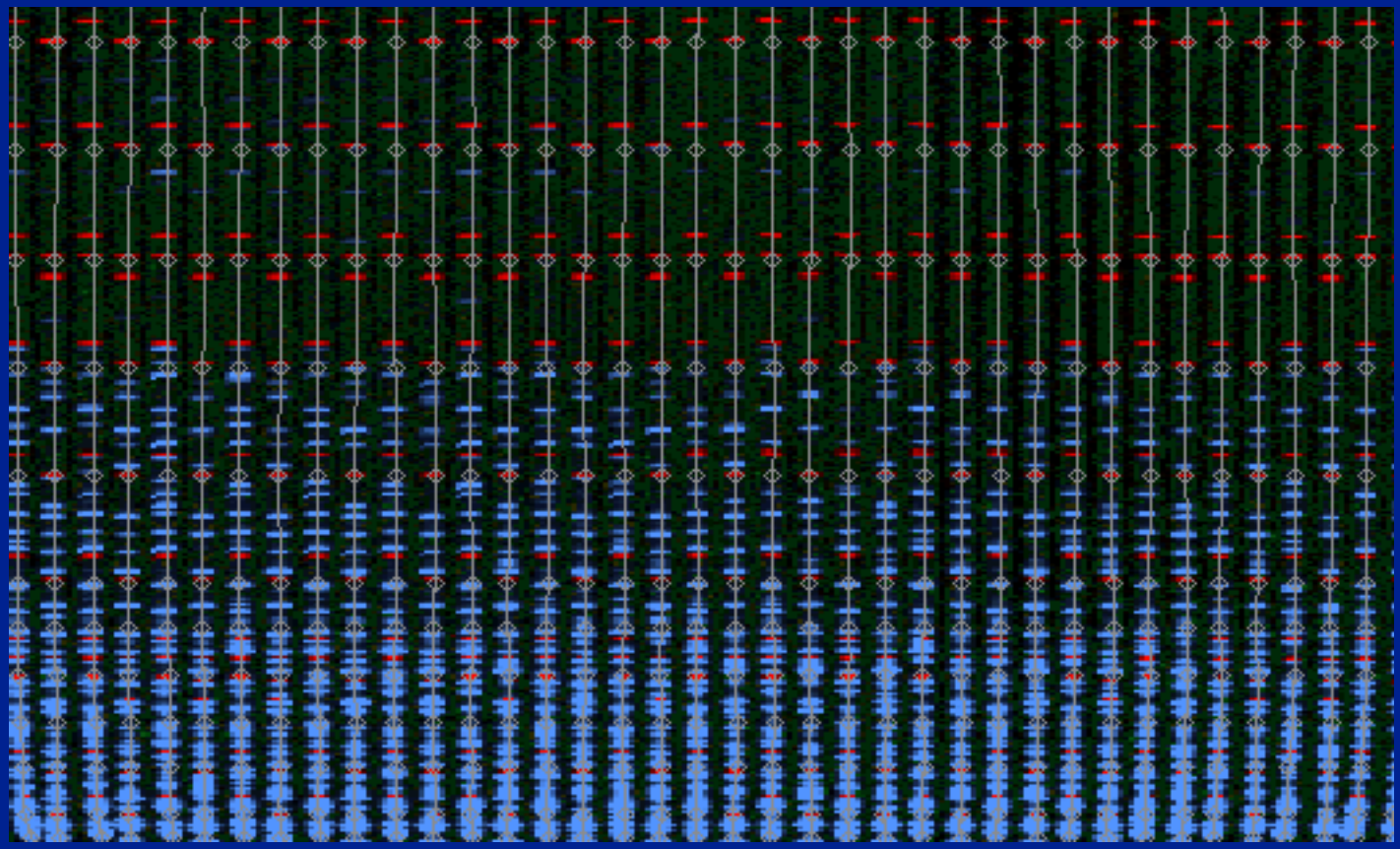
Random Amplified Polymorphic DNA (RAPDs)

- Method detects DNA sequence changes in PCR primer binding sites.
- Use random primers to amplify genomic DNA
- Run PCR products out on a gel and look for differences in banding patterns

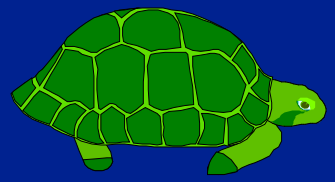
- Strengths: quick, inexpensive, surveys multiple loci, no need for prior knowledge about the DNA sequence, high variation
- Weaknesses: Doesn't transfer between labs, can be difficult to reproduce, can't identify specific loci

- Replaced by AFLP

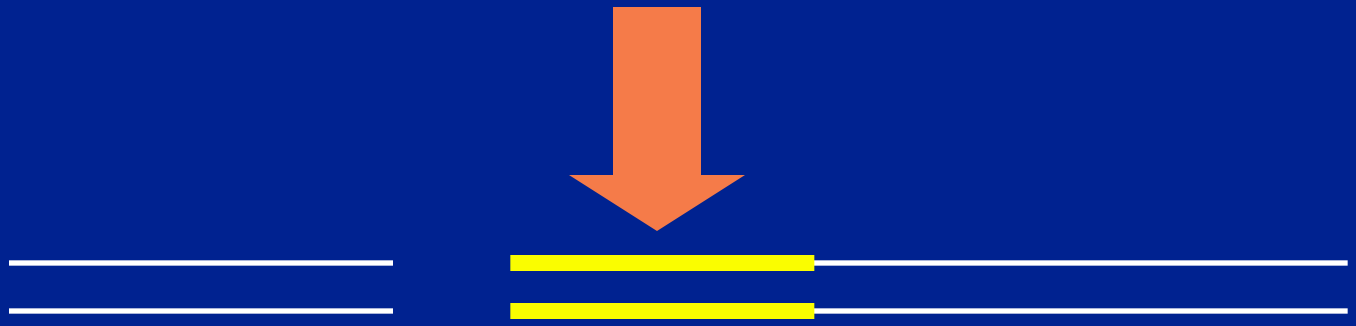
Amplified Fragment Length Polymorphism Analysis (AFLP)



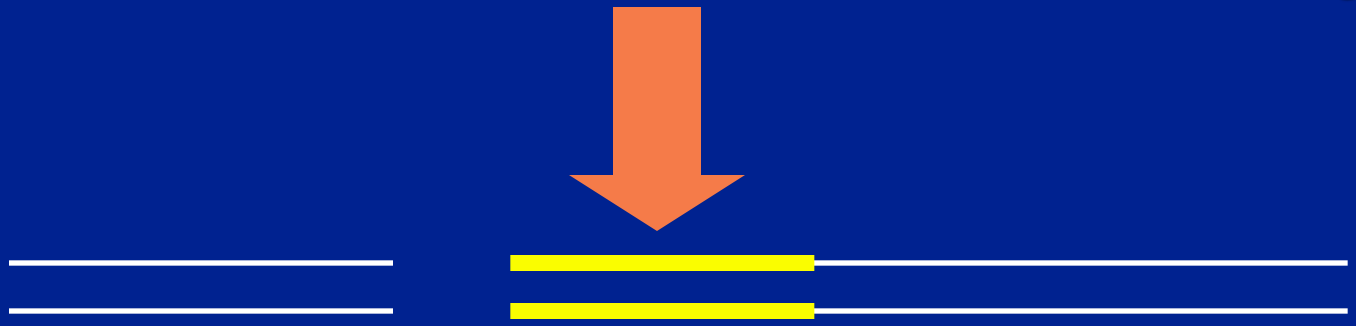
(Kim 2001)



(N = varies with application)

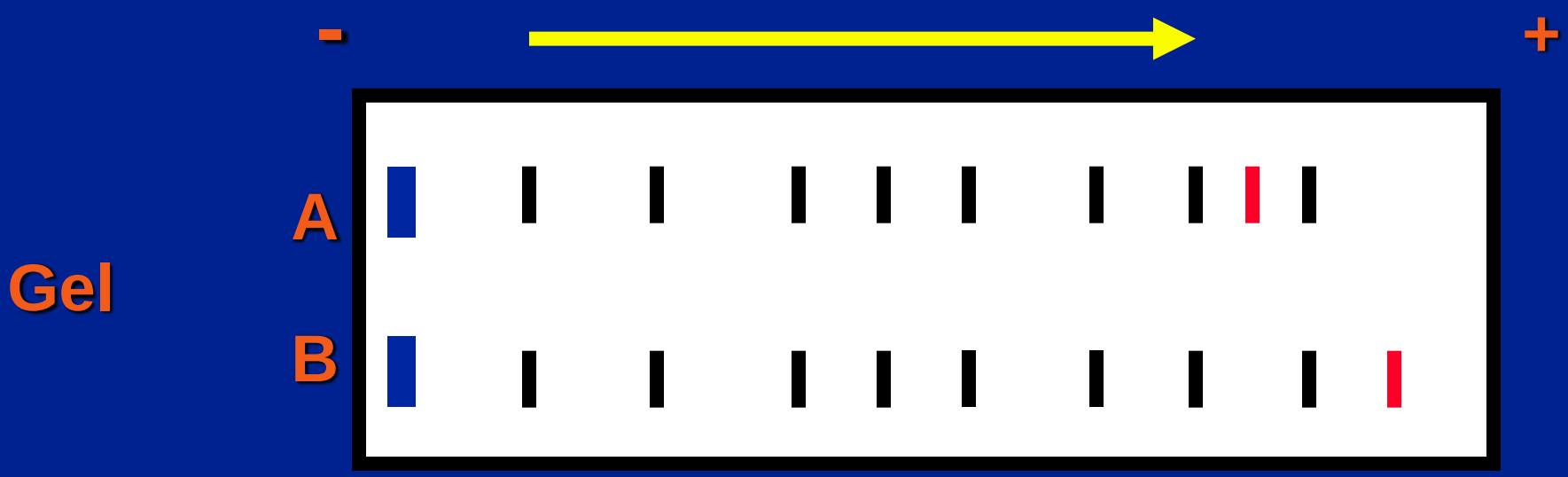


Cut mtDNA or nDNA with restriction enzymes



Amplify with PCR using labeling

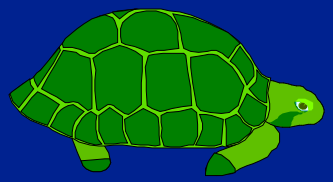
Run on high resolution acrylamide gel and compare fingerprints

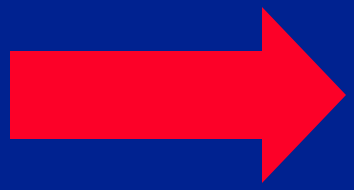


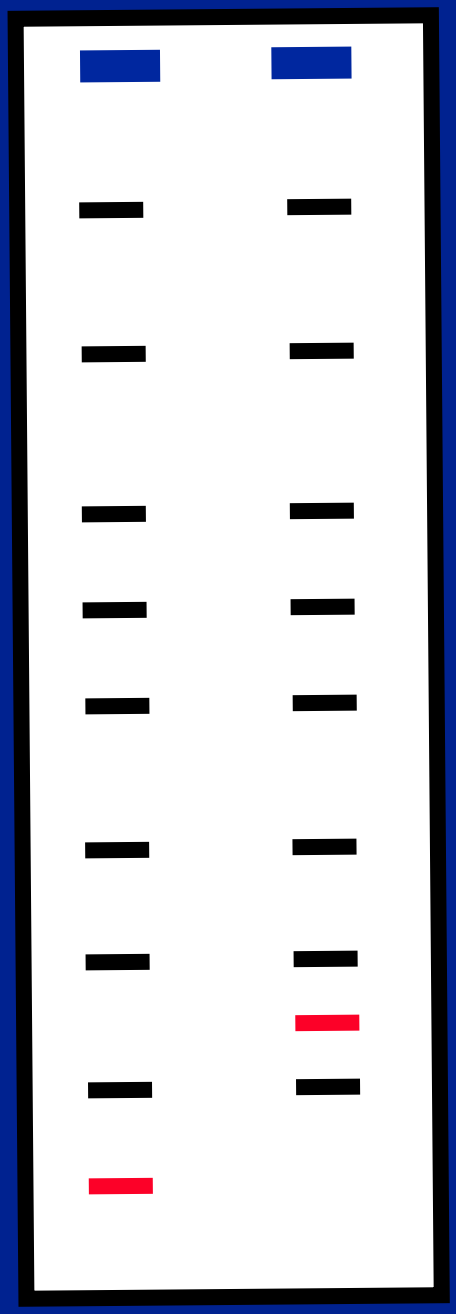
Looking for presence/absence of bands

DNA fingerprinting

(Individual ID)

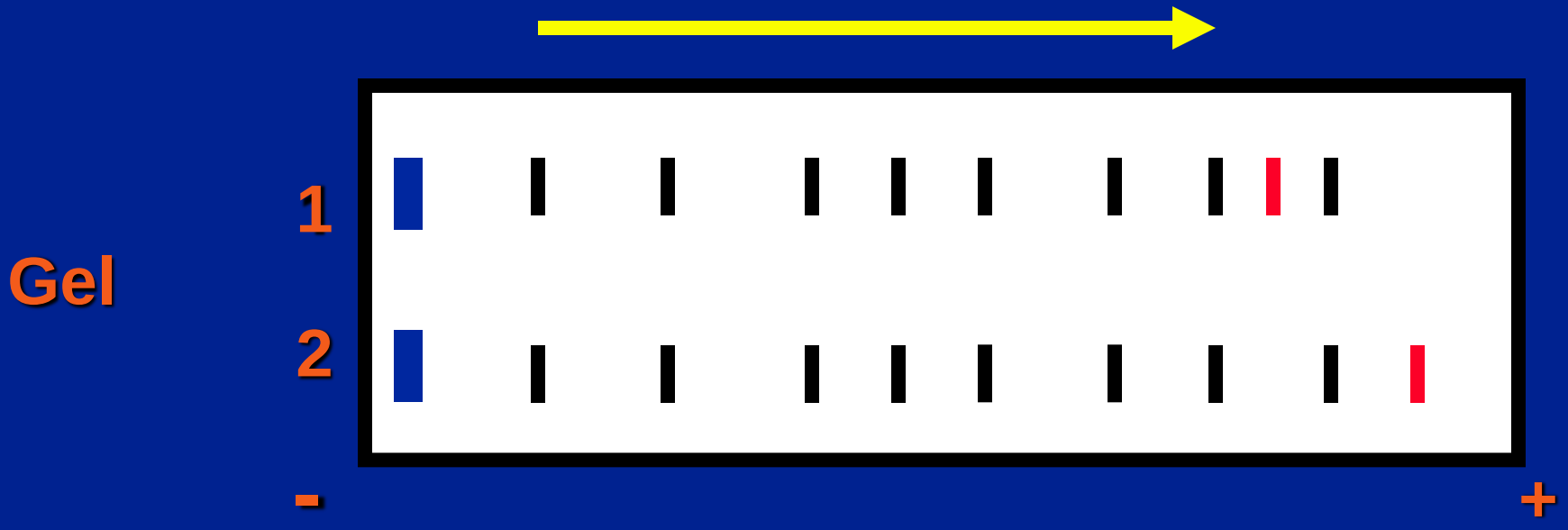






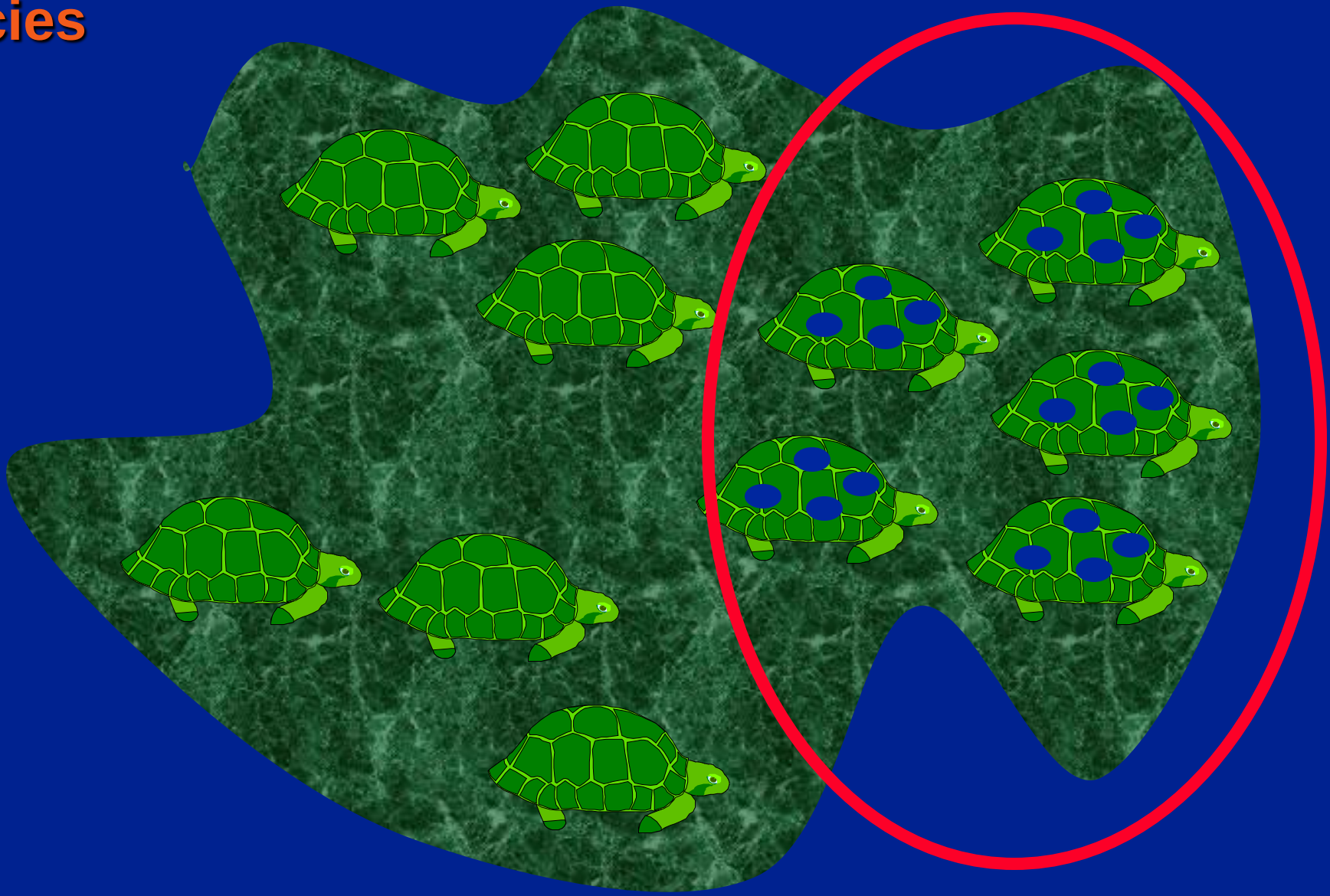
Band sharing - measure of genetic distance:

matching bands/average total # of bands



Population differentiation
Relatedness

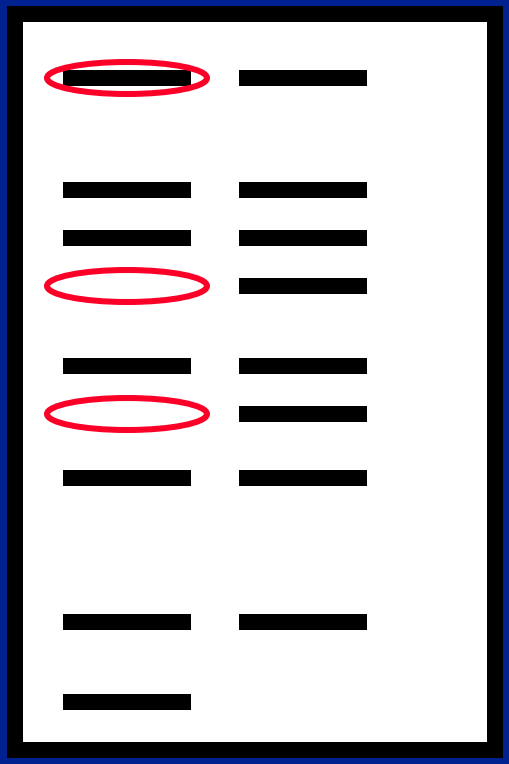
Identify species



Advantages and Disadvantages

- Inexpensive
- Assays entire genome
- High resolution
- Both mtDNA and nDNA
- 10 - 100 times the markers
- Can use non-specific markers
- Faster than other fingerprinting methods

- Requires multiple steps can be technically challenging
- Dominant markers



Molecular Ecology (2007) **16**, 3737–3758 doi: 10.1111/j.1365-294X.2007.03435.x

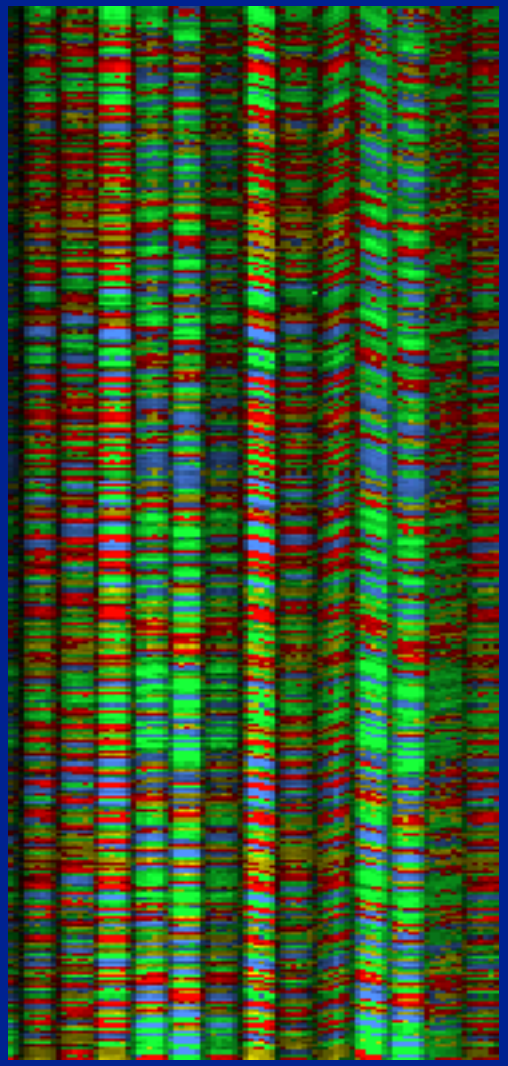
INVITED REVIEW

Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists

A. BONIN,*† D. EHRICH‡ and S. MANEL†

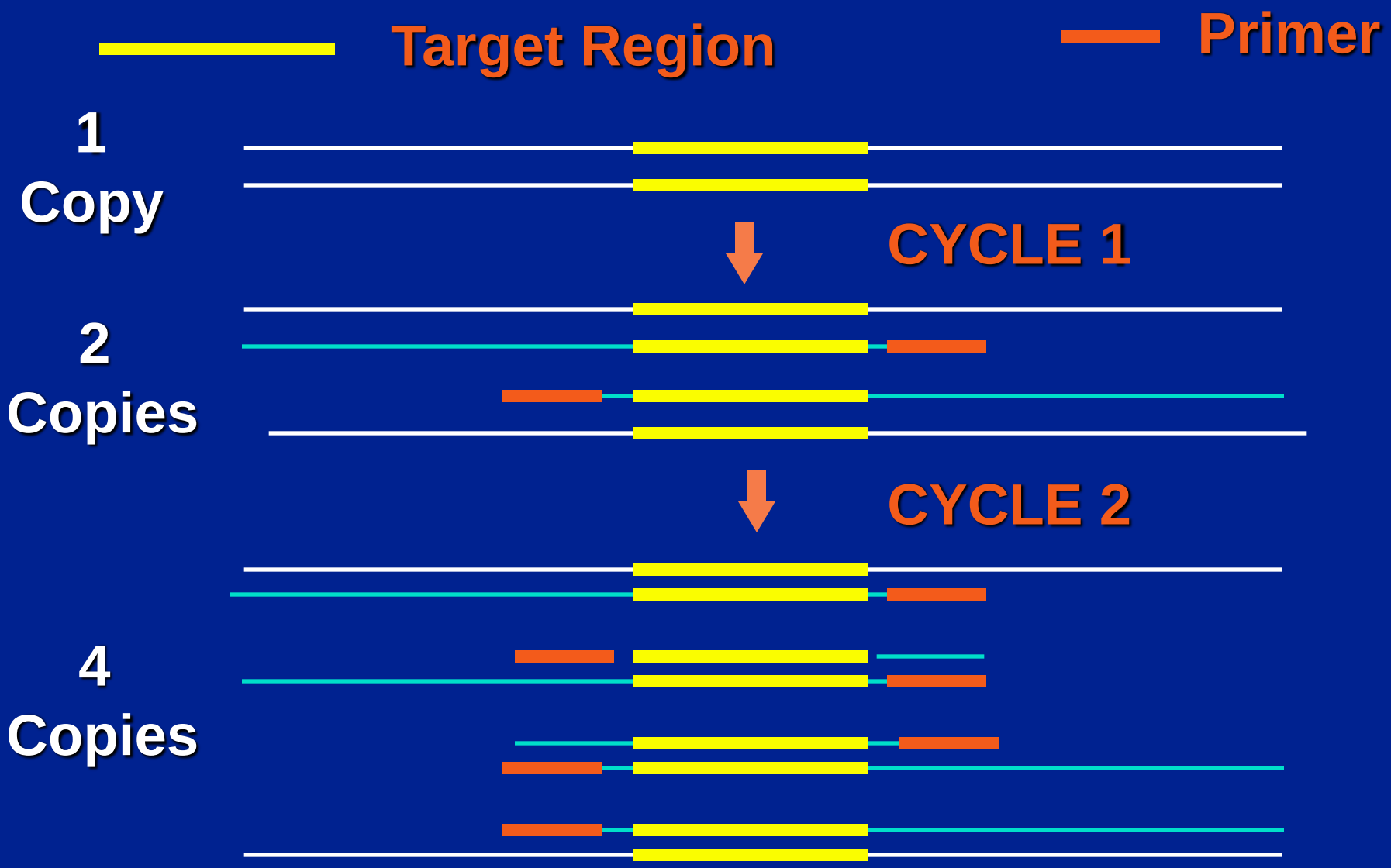
**Diversity Arrays Technology P/L, PO Box 7141, Yarralumla, ACT 2600, Australia, †Laboratoire d'Ecologie Alpine, CNRS-UMR 5553, Université Joseph Fourier, BP 53, 38041 Grenoble cedex 09, France, ‡National Centre for Biosystematics, Natural History Museum, University of Oslo, PO Box 1172, Blindern, NO-0318 Oslo, Norway*

ABI Fluorescent DNA Sequencing

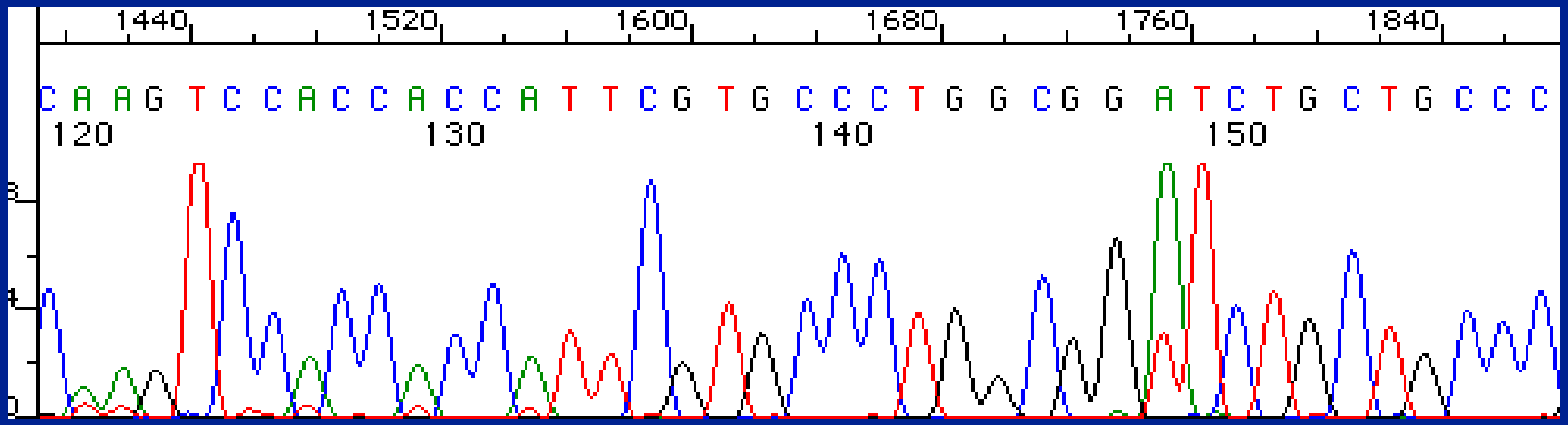


Polymerase Chain Reaction (PCR)

- Makes thousands of copies of a particular DNA target region



(Mullis 1982)

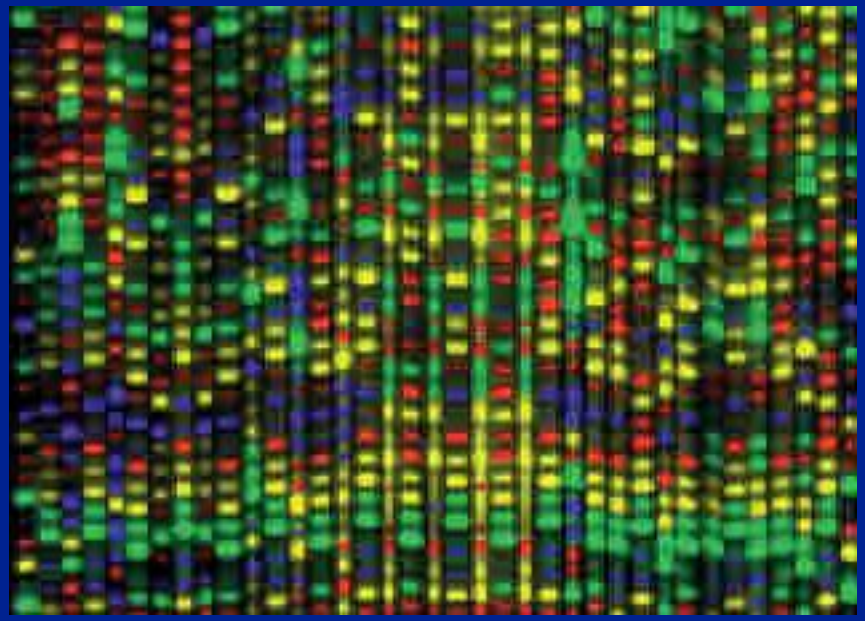

1440
1520
1600
1680
1760
1840
C A A G T C C A C C A C C A T T C G T G C C C T G G C G G A T C T G C T G C C C
120
130
140
150

C

A

G

T



Main Uses

- **Phylogenetic/Systematic analyses**
- **Hybridization analyses**
- **Phylogeography**
- **MtDNA - female gene flow**

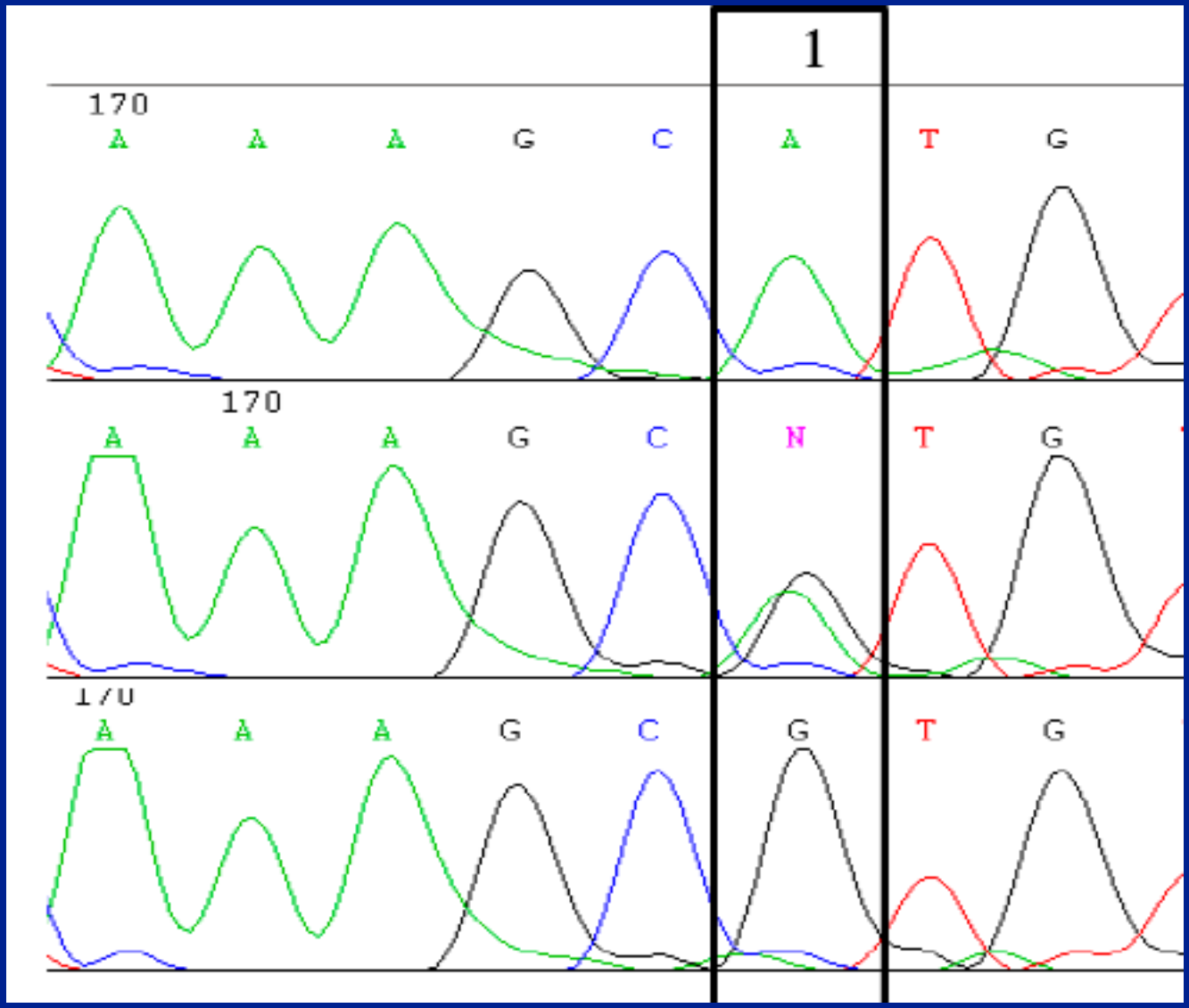
Advantages and Disadvantages

- Resolution down to base pair, Shows all genetic variation in the area sequenced
- mtDNA or nDNA
- Now sequencing largely automated and fast

- Variation is generally low so many base pairs are required
- Sequencing nDNA genes is more difficult and expensive
- Primers can be time consuming to produce when working on a little known species
- Analyzing data is time consuming

Single Nucleotide Polymorphism Analysis (SNPs)

- **Identify sites scattered throughout the nuclear genome that are polymorphic for taxa of interest.**
- **Generate data from many (> 50) polymorphic loci**
- **Uses: Phylogenetic/systematic questions, individual ID, population structure**
- **Benefits: Multiple independent markers, nDNA biparental inheritance, genotypes transferable, mutational model better understood, automation possible**
- **Problems: Difficult to find these polymorphic sites, does not easily transfer to new taxa**



Vignal et al 2002 Genet. Sel. Evol 34:275

Useful References

Review

- **Morin et al 2004 TREE 19:208**
- **Brumfeld et al 2003 TREE 18:249**

Applications

- **Humans Samella et al 2008 Plos one 10:e3519**
- **Wolves Seddon et al 2005 ME 14:503**
- **Orsini et al 2008 ME 17:2629**
- **Salmon Narum et al 2008 ME 17:3464**

Microsatellites

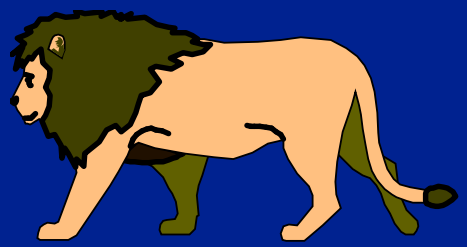
simple sequence repeats of 6 base pairs or less

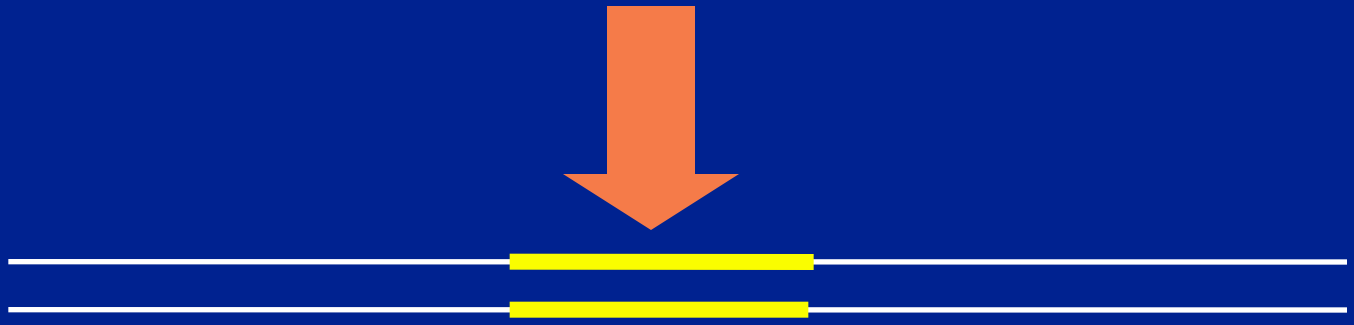
Example:

GCTAATC CACACACACACACACACACA TACTT

GCTAATC CACACACACACA TACTT

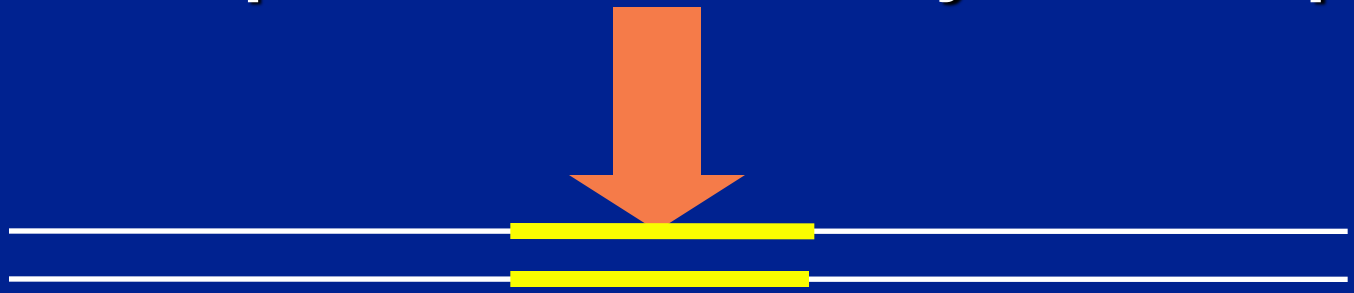
Microsatellites are thought to be non-coding



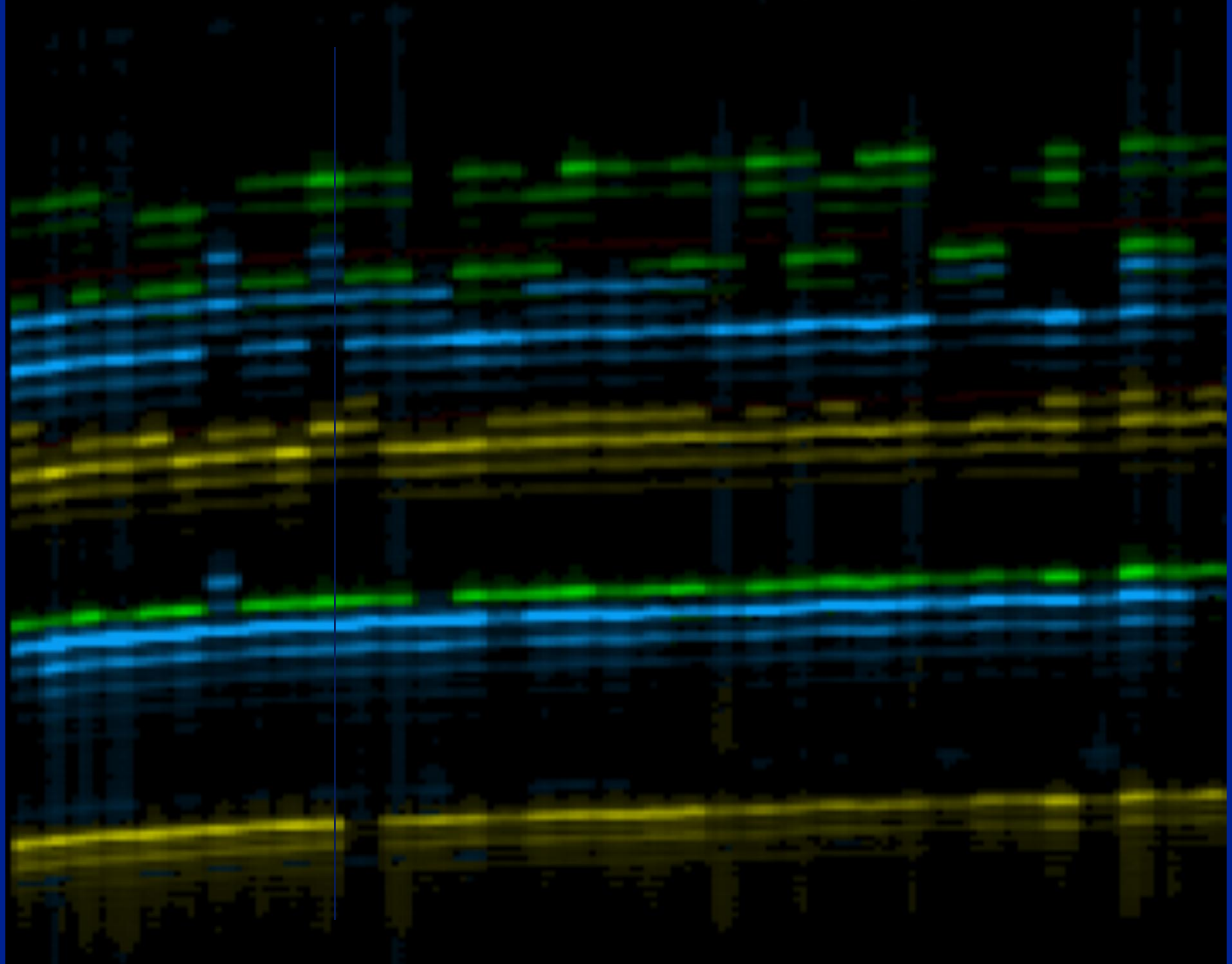


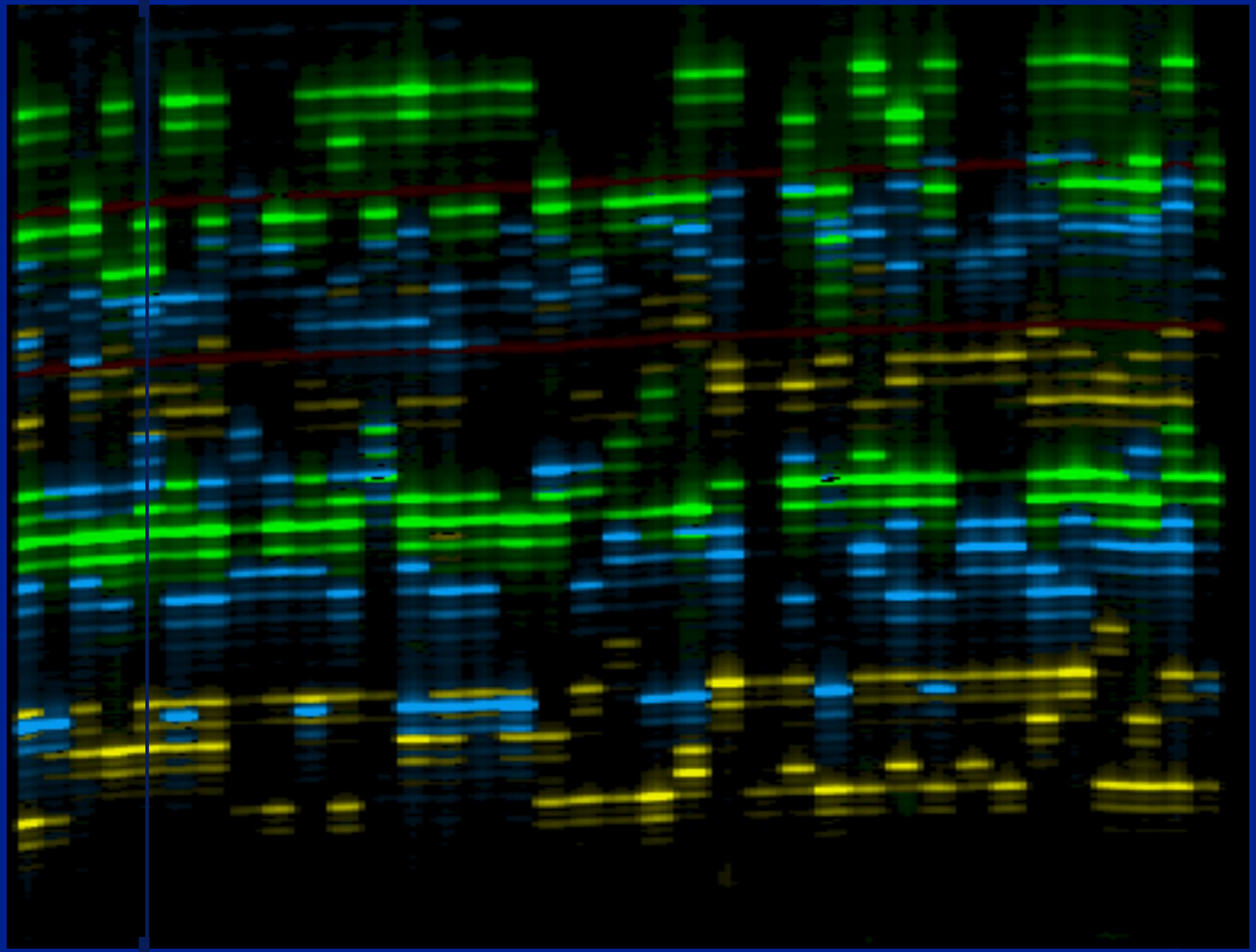
Identification of microsatellite loci thru cloning

Or transfer primers from closely related species

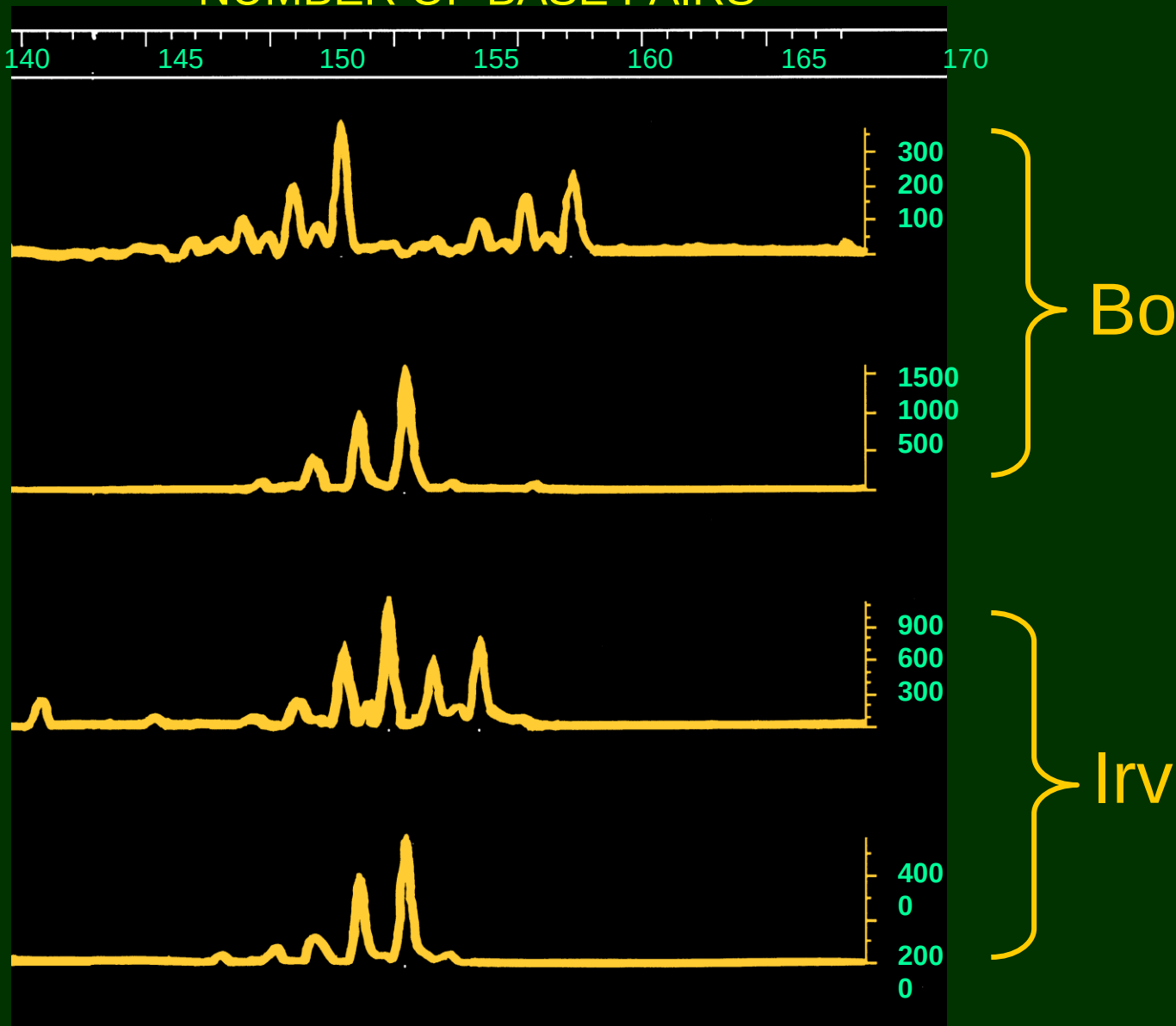


PCR of microsatellite





INDIVIDUAL ID
MICROSATELLITE GEL PLOT

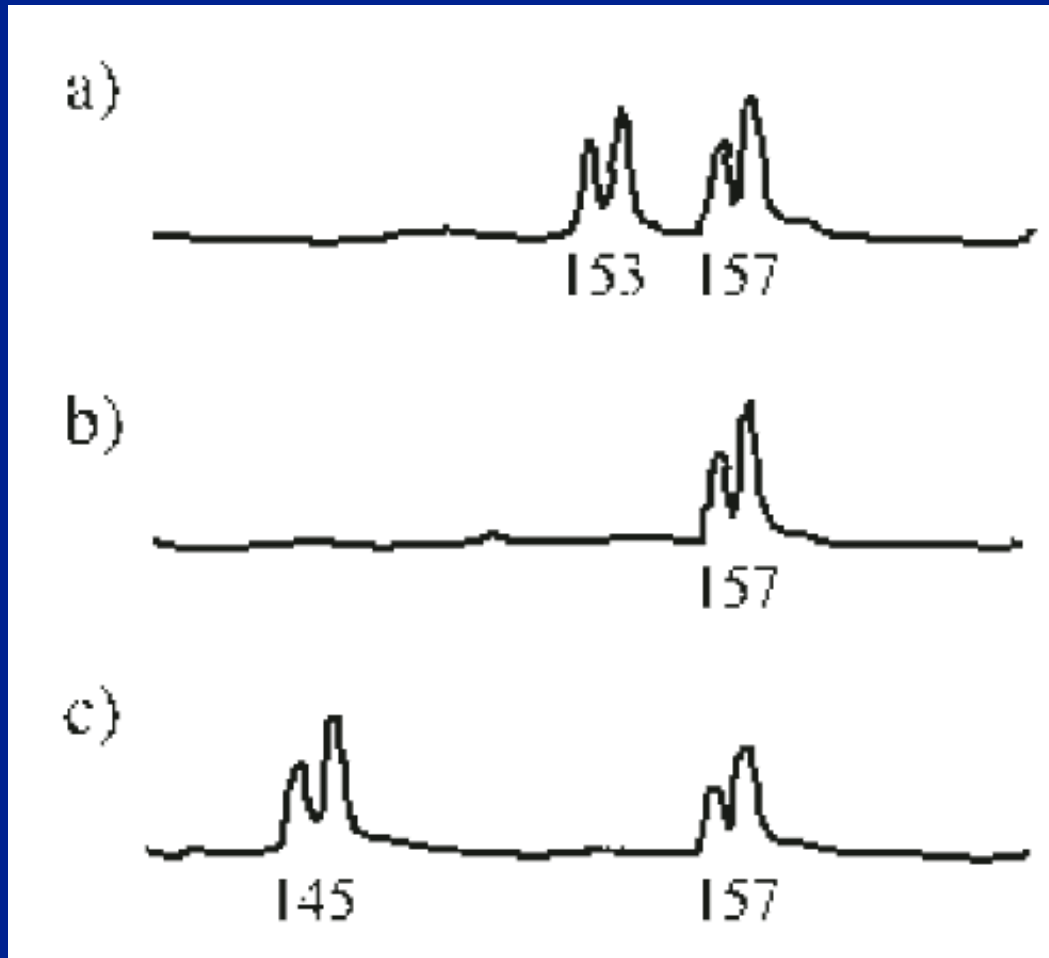


Genotyping errors

a) Correct genotype

b) Allelic dropout

c) False allele



Uses

- Genetic Diversity
- Population structure
- Gene Flow
- Species ID
- Individual ID
- Paternity/maternity/pedigree analysis
- Relatedness

Strengths and Weaknesses

- **Highly polymorphic**
- **Multiplexing possible**
- **Cheap to genotype**
- **Many applications**

- **Mutation mechanism complex**
- **PCR genotyping errors**
- **Null Alleles**
- **Not good for phylogenetic questions**
- **Accurate sizing requires carefull training**
- **Difficult to calibrate allele sizes between labs**

Individual ID

Principle: Every individual has a unique DNA fingerprint (collection of alleles).

Requires 3 - 15 microsatellite loci

When genetic diversity is high - 3 – 5 microsatellite loci are needed

When diversity is very low 10 - 15 loci are needed

Sex ID

Principle: To determine gender, it is necessary to verify the presence of the sex chromosomes.

In mammals, XX - female, XY - male

Birds - XZ - female, XO - male

Method: use PCR and run products on gel to look for Sex chromosomes

Sample Collection and Storage

- **Blood - 1) lysis buffer storage (5 volumes buffer, 1 volume blood) can store at room temp indefinitely (Longmire et al 1999) or 2) purple top EDTA tubes - fridge 3 months, frozen years, 2 - 5 mls is plenty, can work with much less (100 ul), avoid freeze thawing**

- **Hair - place in coin envelope, add to ziplock bag with silica beads, room temperature or freezer storage, avoid freeze thawing, for nDNA extract within 6 months if possible (Roon et al 2003)**

- **Tissue - 1) freezing, 2) 90 - 100% ethanol, 3) lysis buffer, 4) silica dessicant. For methods 2 - 4 important to cut tissue into small pieces (1 cm^3). Hole punch size is sufficient.**

- **Scat - 1) store in 90 - 100% ethanol (4 volumes ethanol:1 volume scat Murphy et al 2002), 2) DETs buffer (Fratzen et al 1998), 3) silica (Wasser et al 1997), and freezing (Murphy et al 2000)**