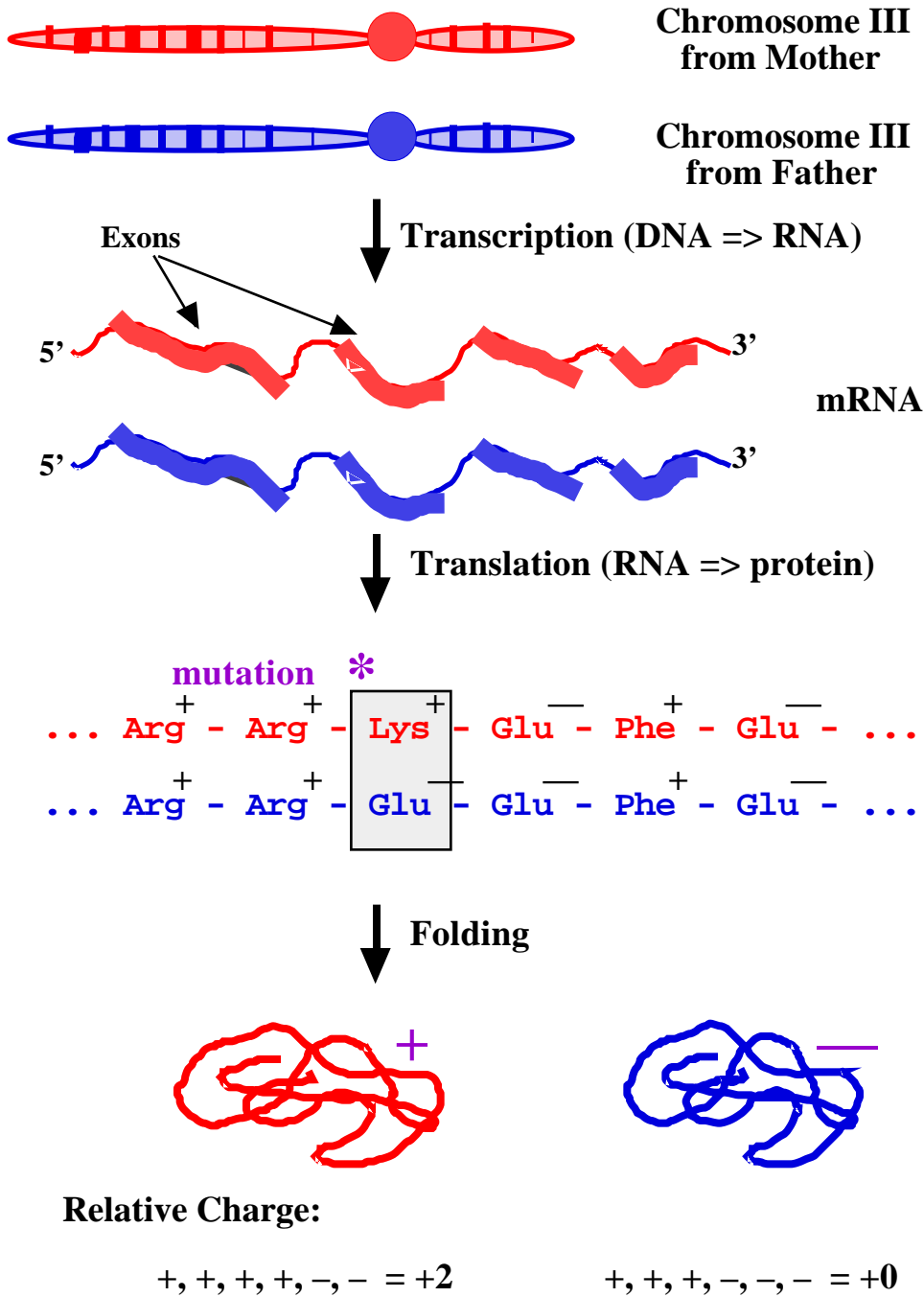
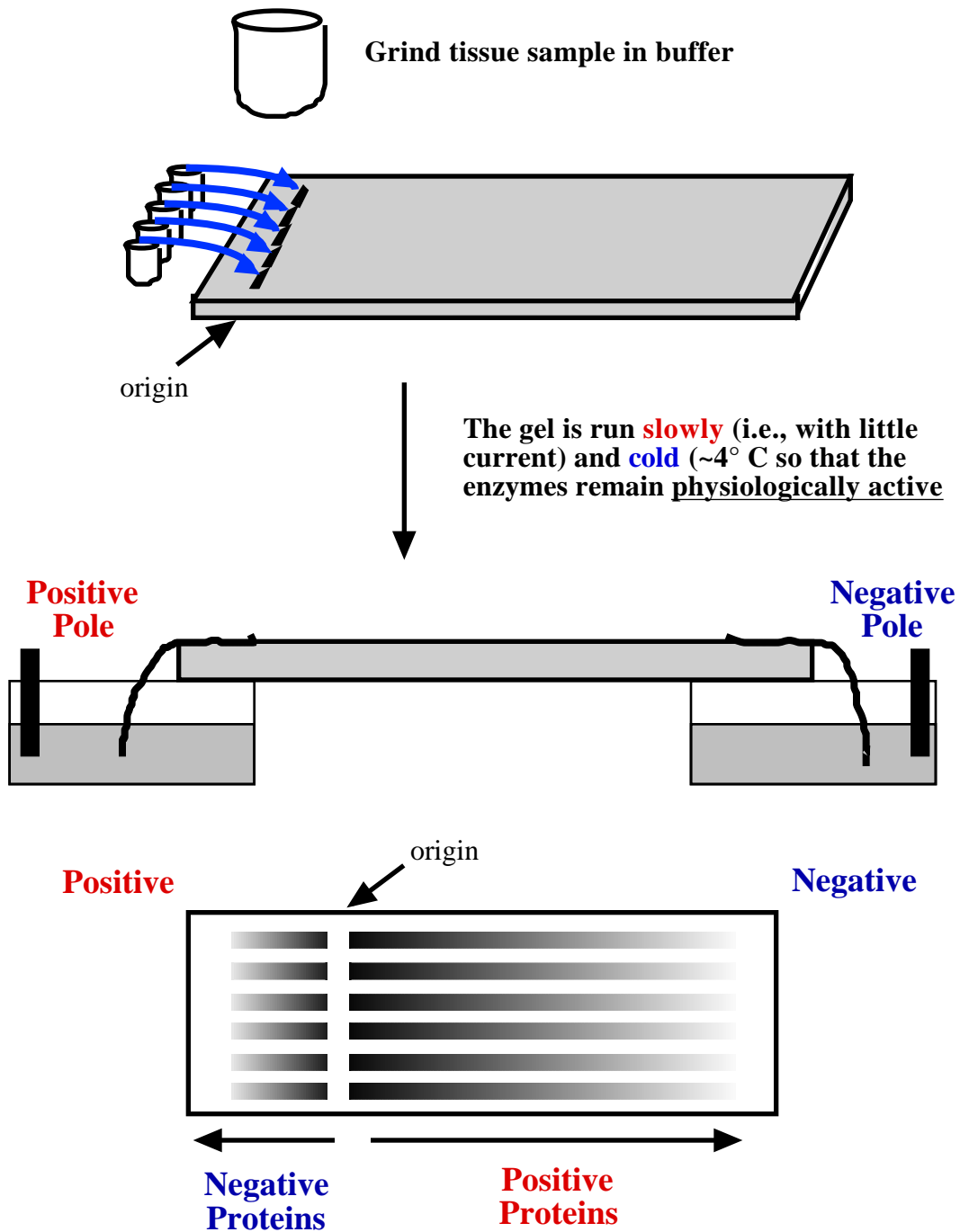


STARCH GEL ELECTROPHORESIS

Making a Protein



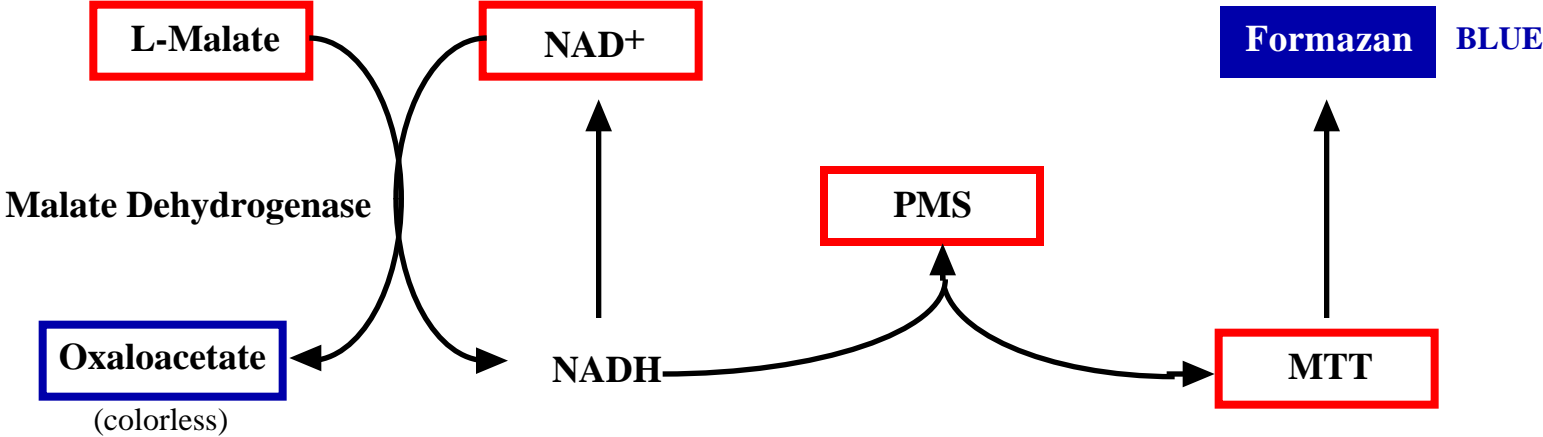
Starch Gel Electrophoresis of Proteins



After electrophoresis, all water-soluble proteins have been separated according to their **CHARGE**, **SIZE**, and **SHAPE**

Selecting a Single Protein

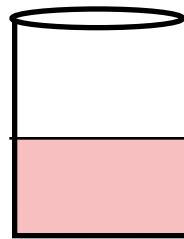
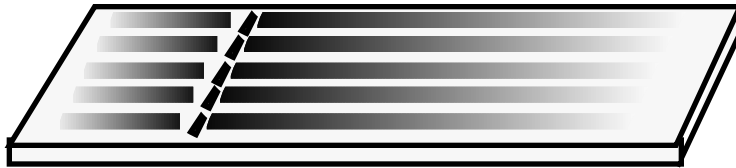
Looking for:
Malate dehydrogenase



 You Add (all colorless)

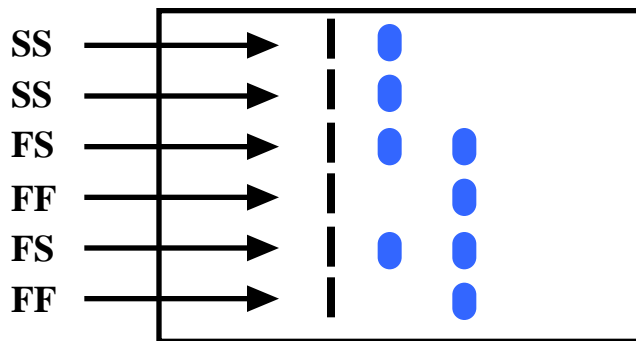
 You Make

Genotyping Isozymes



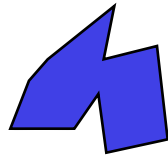
L-Malate
NAD
PMS
MTT

Mdh Genotype



Multimeric Homodimer Isozymes

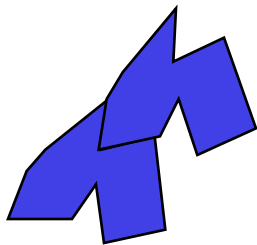
(aka, A little more complicated)



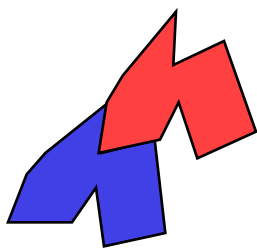
50 %

50 %

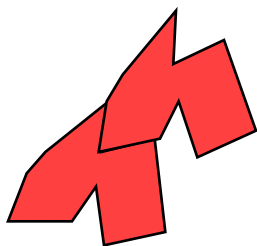
3 ways to combine



$$(0.5)(0.5) = 0.25$$



$$2(0.5)(0.5) = 0.25$$



$$(0.5)(0.5) = 0.25$$

Gel Profile of a HETEROZYGOTE

1



:

2

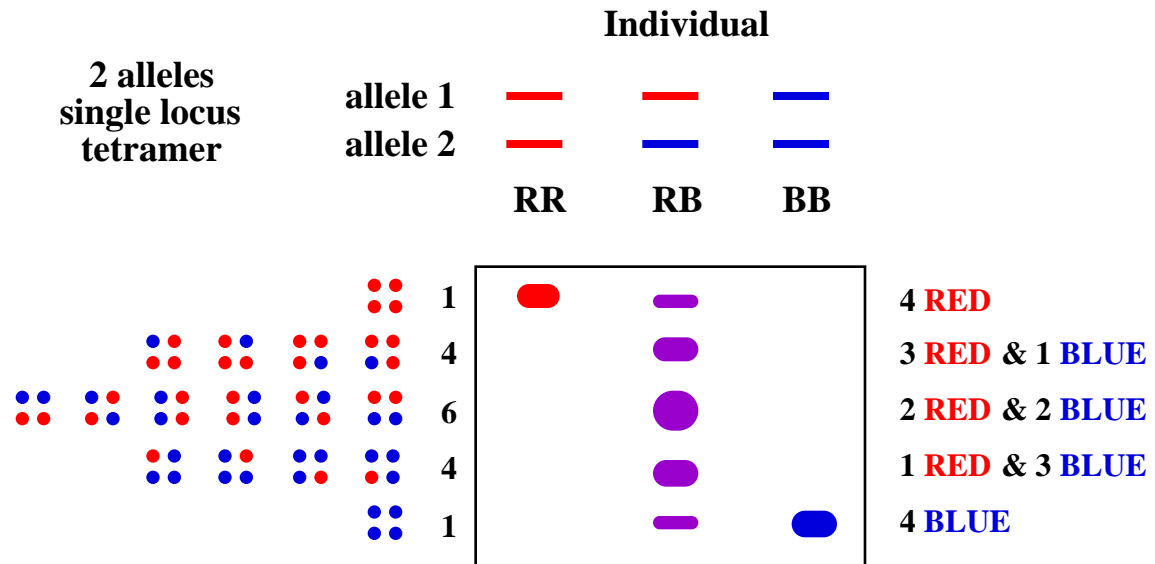


:

1



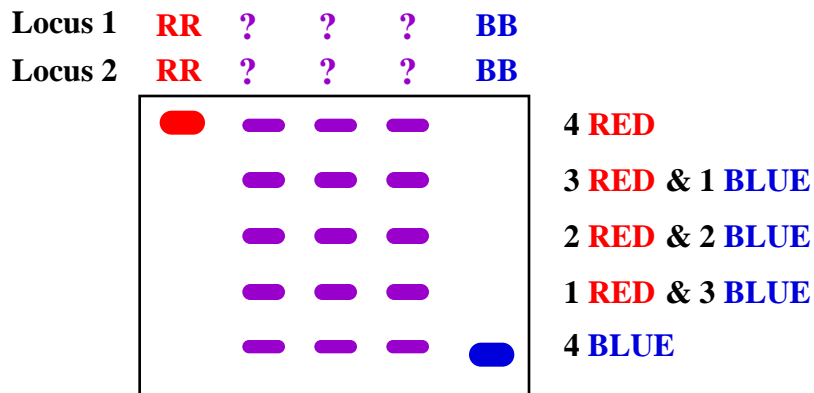
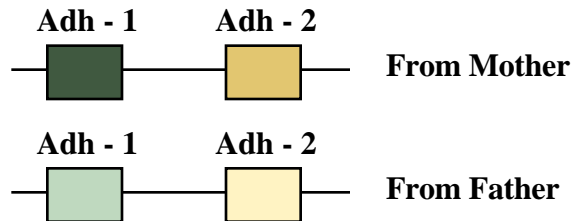
Multimeric Heteropolymers (aka, a bit more complicated)



Multimeric Heteropolymers

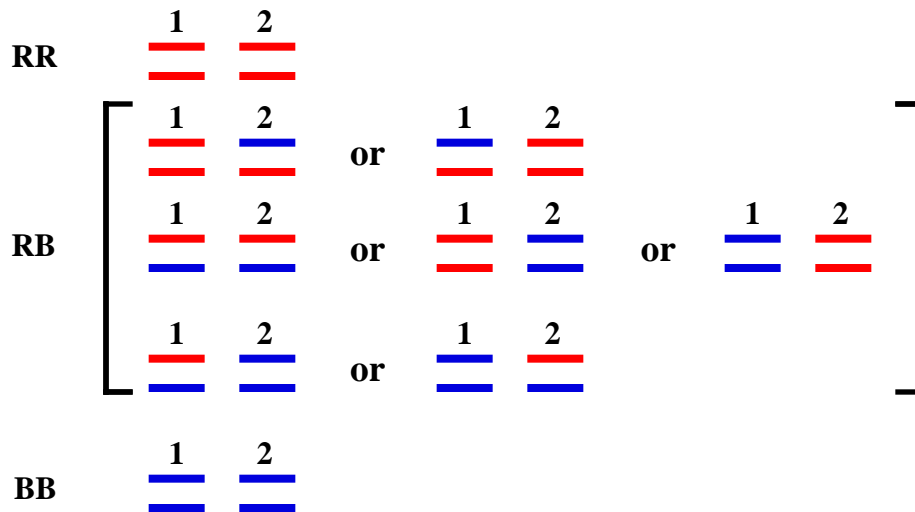
(aka, a lot more complicated)

2 alleles
2 loci
tetramer



Why don't we know?:

Apparent Genotype



**How do we get more than
one copy of a locus
producing the same protein?**

I) Gene duplication

A. Common, maybe all genes at some time

B. Maybe source of non-coding DNA (junk or garbage DNA)

C. e.g., Alcohol dehydrogenase in *Drosophila*, humans, etc.

II) Polyploidy

**A. Hybridization of two species may produce a third species
with all DNA from both parents (e.g., $2N + 2N = 4N$)**

B. Common in plants and some fish (salmon)

III) Anuploidy

A. Extra chromosome or part of chromosome

**B. e.g., trisomy 21 (Down's syndrome), XXY (Klinefelter's
syndrome)**

Positive Attributes of Starch Gel Electrophoresis

- I) SGE can be done on almost any taxa since all have enzymes involved in key pathways (i.e., Kreg's cycle and glycolysis)**

- II) Many loci can be scored (~50-100, but usually 20-40)**

- III) Loci are generally unlined (i.e., are on different chromosomes or greater than 50 centimorgans apart)**

- IV) Alleles are co-dominant (i.e., all three genotypes can be differentiated)**

- V) Loci are usually considered to be scattered about the nuclear genome (therefore estimates are for the entire genome)**

- VI) Shift in allele mobility is due to amino acid changes in protein and amino acid changes are due to mutations in DNA**

Negative Attributes of Starch Gel Electrophoresis

- I) Enzymes are part of very important biochemical pathways and may be (i.e., are) under selection**

- II) Many loci are scored but some loci are always polymorphic and some are always monomorphic**

- III) Loci are genetically unlinked but many are part of an enzyme cascade and may be functionally linked**
 - a. And often not even genetically unlinked**

- IV) Modifier loci can affect mobility and effectively make new alleles (i.e., phosphorylation)**

- V) Many regulatory linked loci are also chromosomally adjacent**

- VI) Many (i.e., most) DNA changes (including amino acid replacements) do not change mobility of protein**

When to use Starch Gel Electrophoresis?

- I) Little known about species**

- II) Quick look at genetic structure (short term)**

- III) Potential for high level of polymorphisms**

- IV) Taxa are moderately divergent (little resolving power when very close or very far)**

- V) Interested in selection on the allozyme loci themselves**

- VI) Marker of population subdivision**

- vii) Etc....**

Uses of Starch Gel Electrophoresis

- 1) Gene flow and population subdivision
- 2) Parentage:

		Lap	Pgi	Pgm-1	Pgm-2	6Pgd	Idh
Known mother		AB	AA	AA	AB	AC	AA
Offspring		BB	AB	AC	AB	AA	AB
Mother contributed		B	A	A	?	A	A
Father contributed		B	B	C	?	A	B
	1	BB	AB	BC	BB	CC	AA
Potential fathers	2	BB	BC	AC	AA	AC	AA
	3	AB	AB	AC	AA	AB	AB

Father cannot be:

#1 – 6Pgd cannot give an “A” allele

#2 – Idh cannot give an “B” allele

Father must be either # 3 or come from outside of the scored population

3) Systematics – defining species and taxa. Need to transform allele data into distance data

e.g., Nei's 78 (0 to ∞)

$$\text{Distance} = -\ln \frac{x_i y_i}{\sqrt{x_i^2 y_i^2}}$$

Where: x_i = frequency of the i^{th} allele at a locus in species x
 y_i = frequency of the i^{th} allele at a locus in species y
 = summed over all alleles at locus

Take the mean value of D over all loci

	A	B	C	D
A	—			
B	0.5	—		
C	2.6	1.8	—	
D	7.6	6.3	10.4	—

