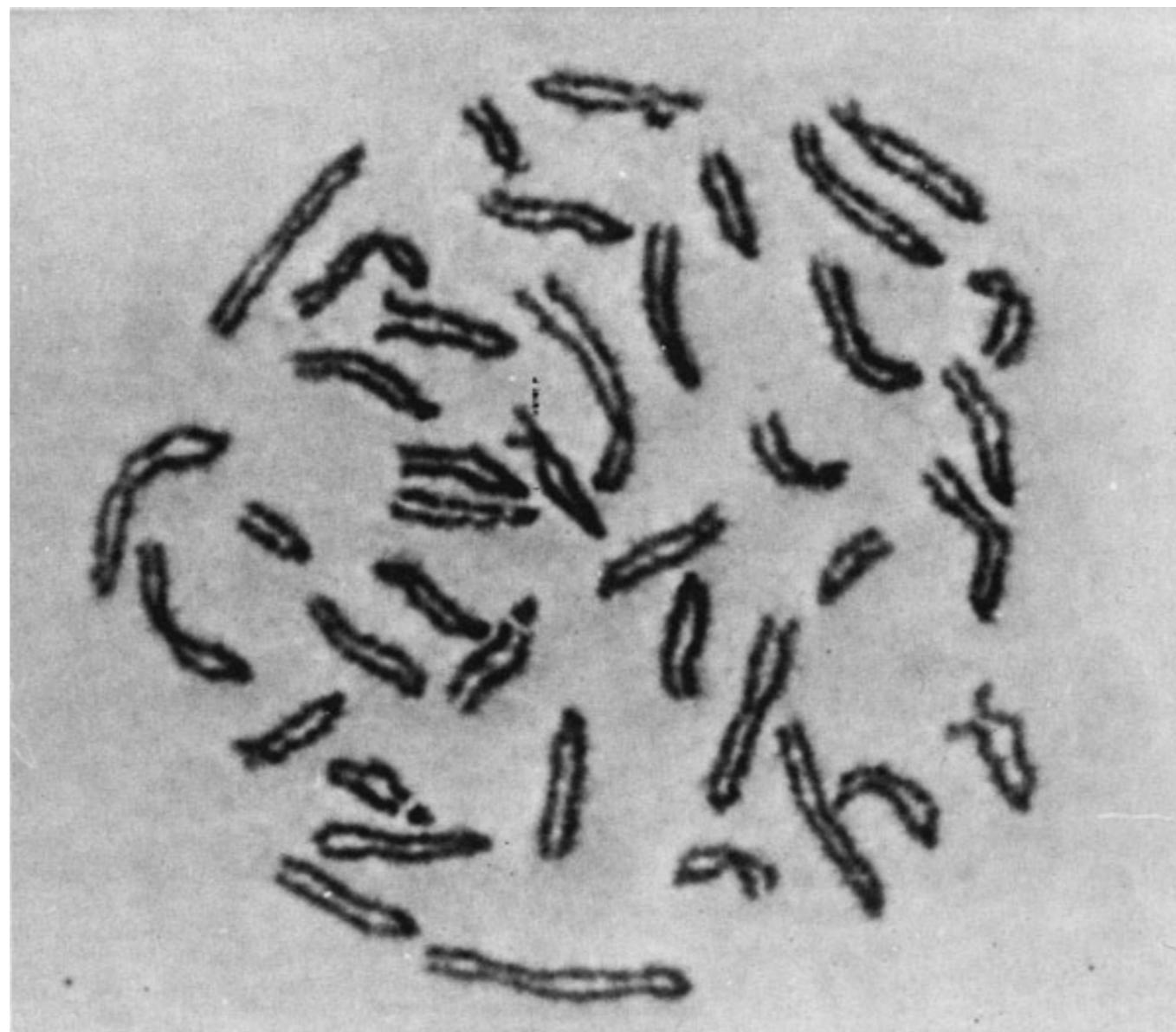
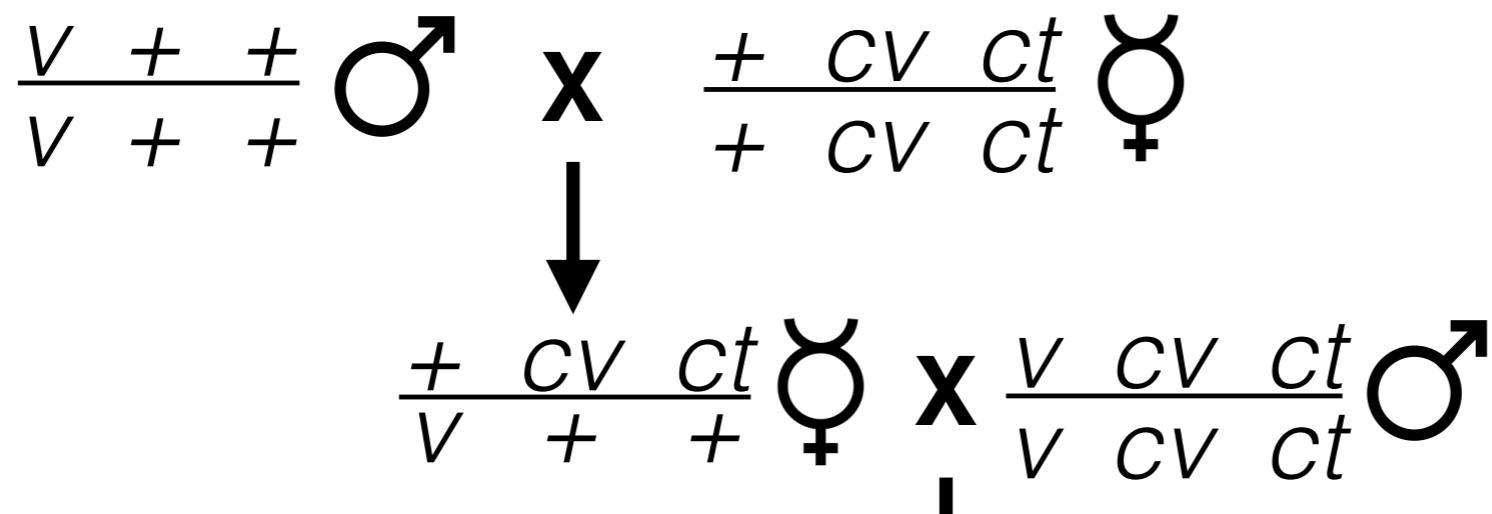


# Bio393: Genetic Analysis

Chromosome theory, recombination, and mapping



# A three-factor cross



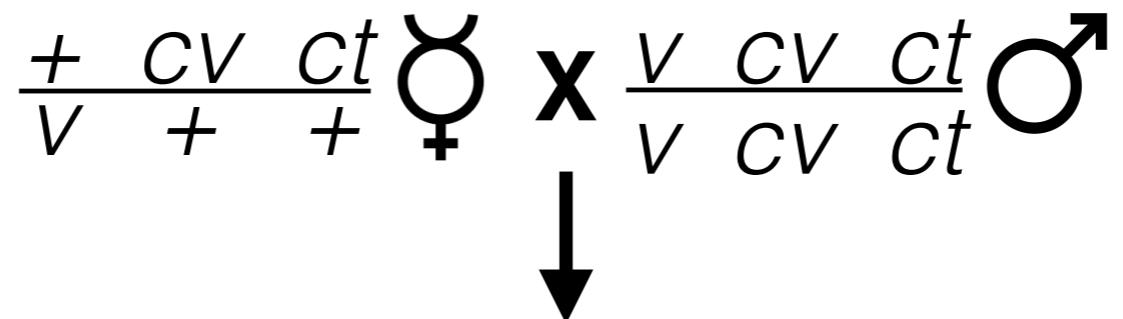
Eye Phenotype	Crossvein Phenotype	Cut Phenotype	Number of offspring
Red	No crossvein	Cut wing	580
Vermillion	Crossvein	Normal wing	592
Red	Crossvein	Cut wing	40
Vermillion	No crossvein	Normal wing	45
Red	Crossvein	Normal wing	94
Vermillion	No crossvein	Cut wing	89
Red	No crossvein	Normal wing	5
Vermillion	Crossvein	Cut wing	3

v = vermillion eyes

ct = cut wings

cv= crossveinless wings

+ = red eyes and normal wings



Eye Phenotype	Crossvein Phenotype	Cut Phenotype	Number of offspring
Red	No crossvein	Cut wing	580
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Vermillion	Crossvein	Cut wing	3

$+ \quad CV \quad ct$       **P**  
 $v \quad + \quad +$       **P**

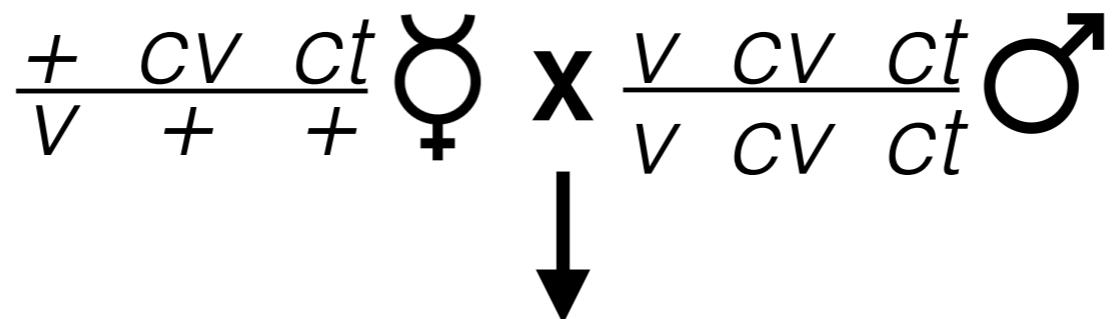
1. Determine parental class, label

$v$  = vermillion eyes

$ct$  = cut wings

$cv$  = crossveinless wings

$+$  = red eyes and normal wings



Eye Phenotype	Crossvein Phenotype	Cut Phenotype	Number of offspring
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Red	No crossvein	Normal wing	5
Vermillion	Crossvein	Cut wing	3

+   CV   ct   P  
 V   +   +   P  
 R   R   R   R  
 R   R   R   R  
 R   R   R   R

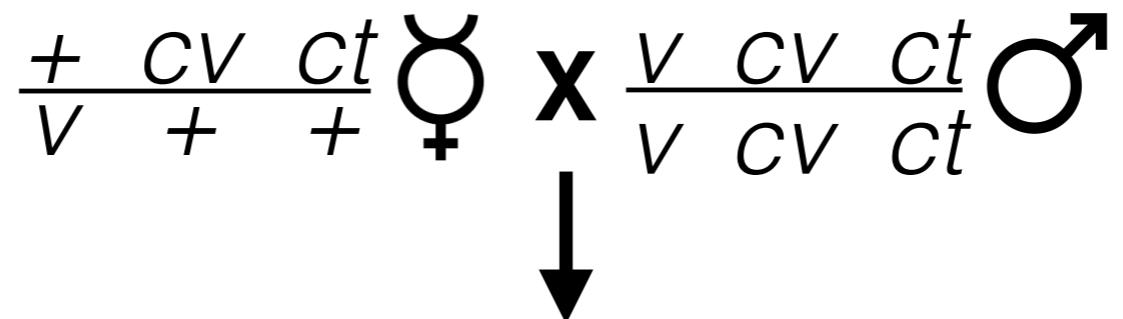
1. Determine parental class, label
2. Are all classes present?

v = vermillion eyes

ct = cut wings

cv= crossveinless wings

+ = red eyes and normal wings



Eye Phenotype	Crossvein Phenotype	Cut Phenotype	Number of offspring
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Vermillion	Crossvein	Cut wing	3

+ CV ct      P  
 V + +      P  
 R      R  
 R      R  
 R      R  
 + CV +      R  
 V + ct      R

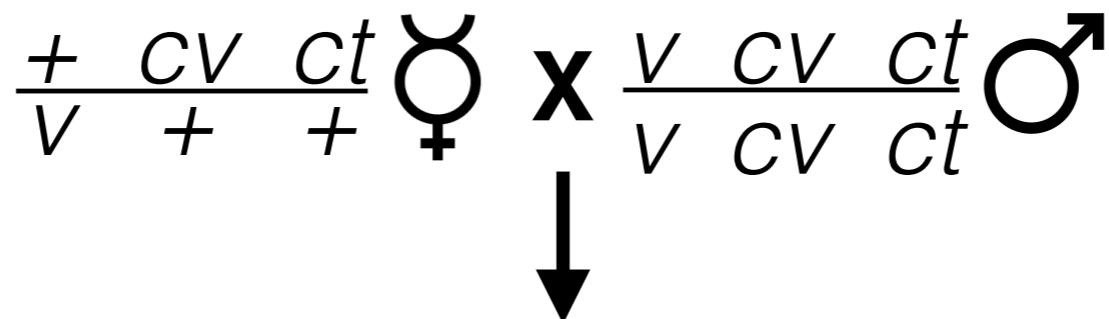
1. Determine parental class, label
2. Are all classes present?
3. Least abundant class is double recombinant, tells gene in middle

v = vermillion eyes

ct = cut wings

cv= crossveinless wings

+ = red eyes and normal wings



Eye Phenotype	Crossvein Phenotype	Cut Phenotype	Number of offspring
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Red	Crossvein	Normal wing	94
Vermillion	No crossvein	Cut wing	89
Red	No crossvein	Normal wing	5
Vermillion	Crossvein	Cut wing	3

+ CV ct	P
V + +	P
+ + ct	R
V CV +	R
+ + +	R
V CV ct	R
+ CV +	R
V + ct	R

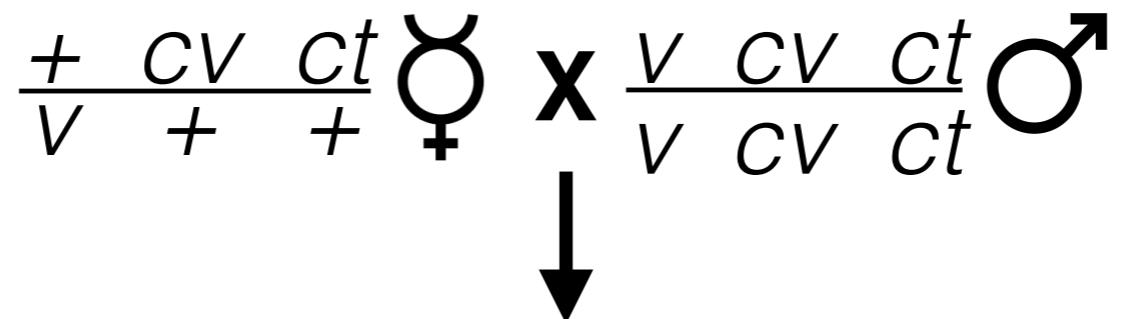
1. Determine parental class, label
2. Are all classes present?
3. Least abundant class is double recombinant, tells gene in middle
4. Write out the genotypes of the offspring

v = vermillion eyes

ct = cut wings

cv= crossveinless wings

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Eye Phenotype	Crossvein Phenotype	Cut Phenotype	Number of offspring
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Vermillion	Crossvein	Cut wing	3

$+ \quad CV \quad ct$  P  
 $v \quad + \quad +$  P  
 $+ \quad + \quad ct$  R  
 $v \quad CV \quad +$  R  
 $+ \quad + \quad +$  R  
 $v \quad CV \quad ct$  R  
 $+ \quad CV \quad +$  R  
 $v \quad + \quad ct$  R



1448 total progeny

1. Determine parental class, label
2. Are all classes present?
3. Least abundant class is double recombinant, tells gene in middle
4. Write out the genotypes of the offspring
5. Calculate distance from one gene to middle gene **v to ct**

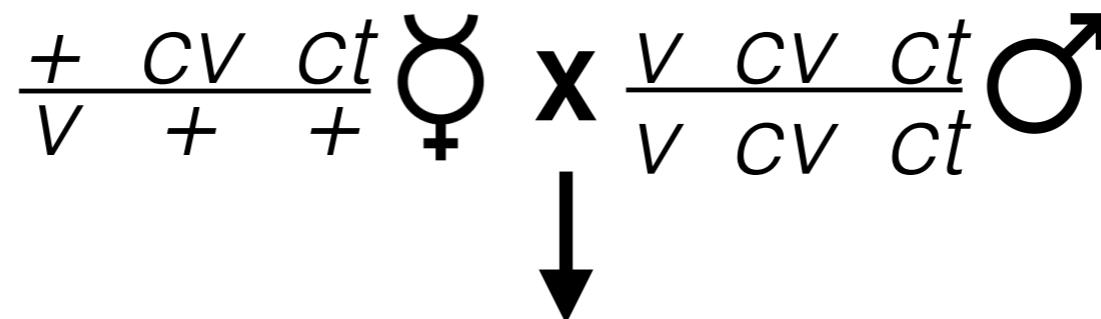
$v$  = vermillion eyes

$ct$  = cut wings

$cv$  = crossveinless wings

$+$  = red eyes and normal wings

$$\frac{94+89+5+3}{1448} \times 100 = 13.2\%$$



Eye Phenotype	Crossvein Phenotype	Cut Phenotype	Number of offspring
Red	No crossvein	Cut wing	580
Vermillion	Crossvein	Normal wing	592
Red	Crossvein	Cut wing	40
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$+ \quad CV \quad ct$  P  
 $v \quad + \quad +$  P  
 $+ \quad + \quad ct$  R ←  
 $v \quad CV \quad +$  R ←  
 $+ \quad + \quad +$  R  
 $v \quad CV \quad ct$  R ←  
 $+ \quad CV \quad +$  R ←  
 $v \quad + \quad ct$  R ←

1448 total progeny

1. Determine parental class, label
2. Are all classes present?
3. Least abundant class is double recombinant, tells gene in middle
4. Write out the genotypes of the offspring
5. Calculate distance from one gene to middle gene
6. Calculate distance from the other gene to middle gene **cv to ct**

v = vermillion eyes

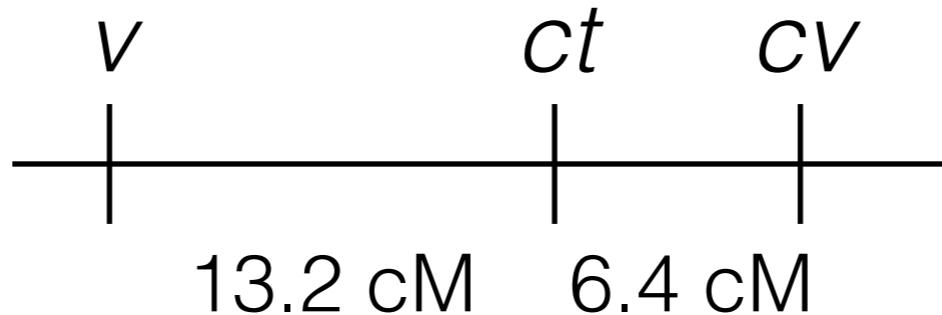
ct = cut wings

cv = crossveinless wings

+ = red eyes and normal wings

$$\frac{40+45+5+3}{1448} \times 100 = 6.4\%$$

## Our first genetic map

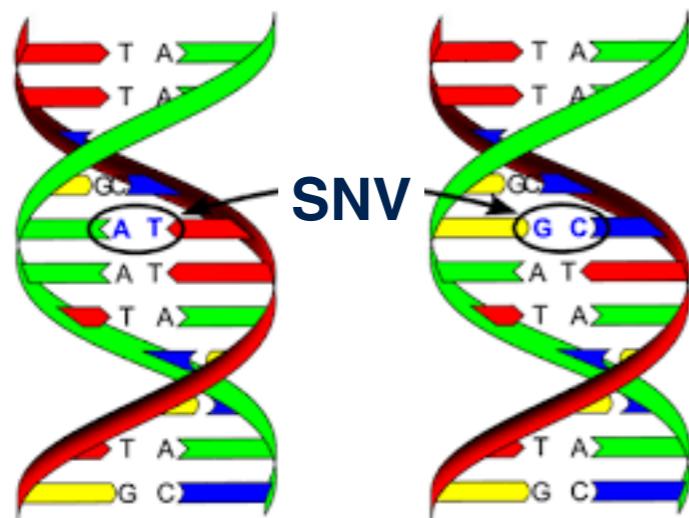


1. Order by least abundant class
2. Arbitrary which genes on ends
3. Class *v* to *cv* undercounts because double recombinants look like parentals

**We have a better way!**

# Molecular markers are often used for genetic mapping

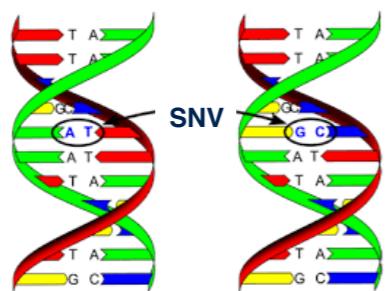
- Single nucleotide variants



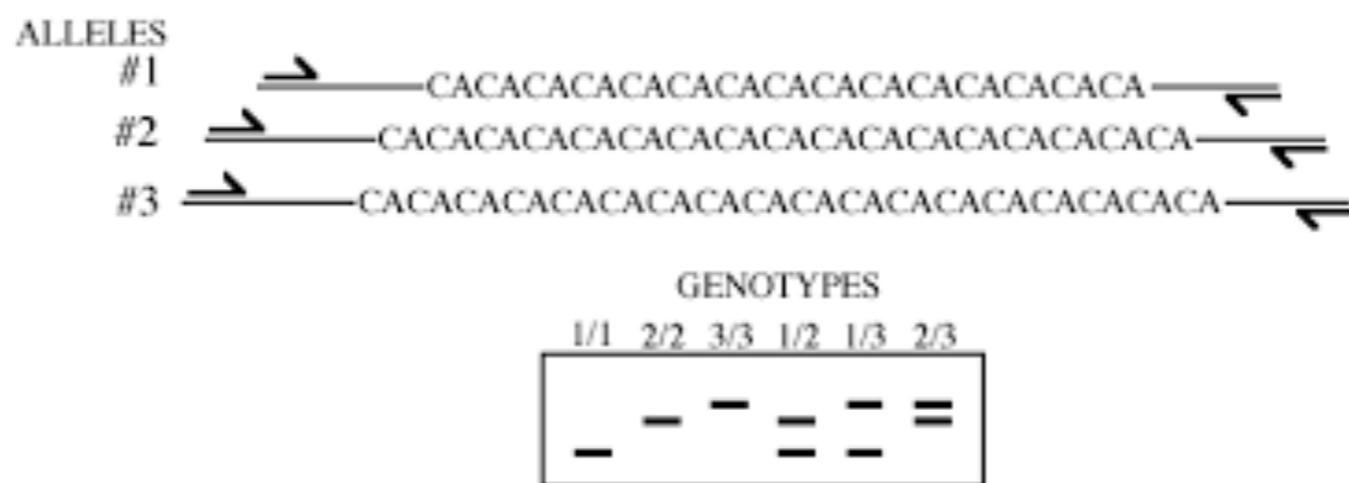
Detected by sequencing,  
hybridization (array), or PCR.

# Molecular markers are often used for genetic mapping

- Single nucleotide variants

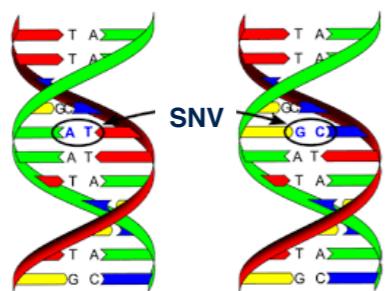


- Microsatellite repeats



# Molecular markers are often used for genetic mapping

- Single nucleotide variants

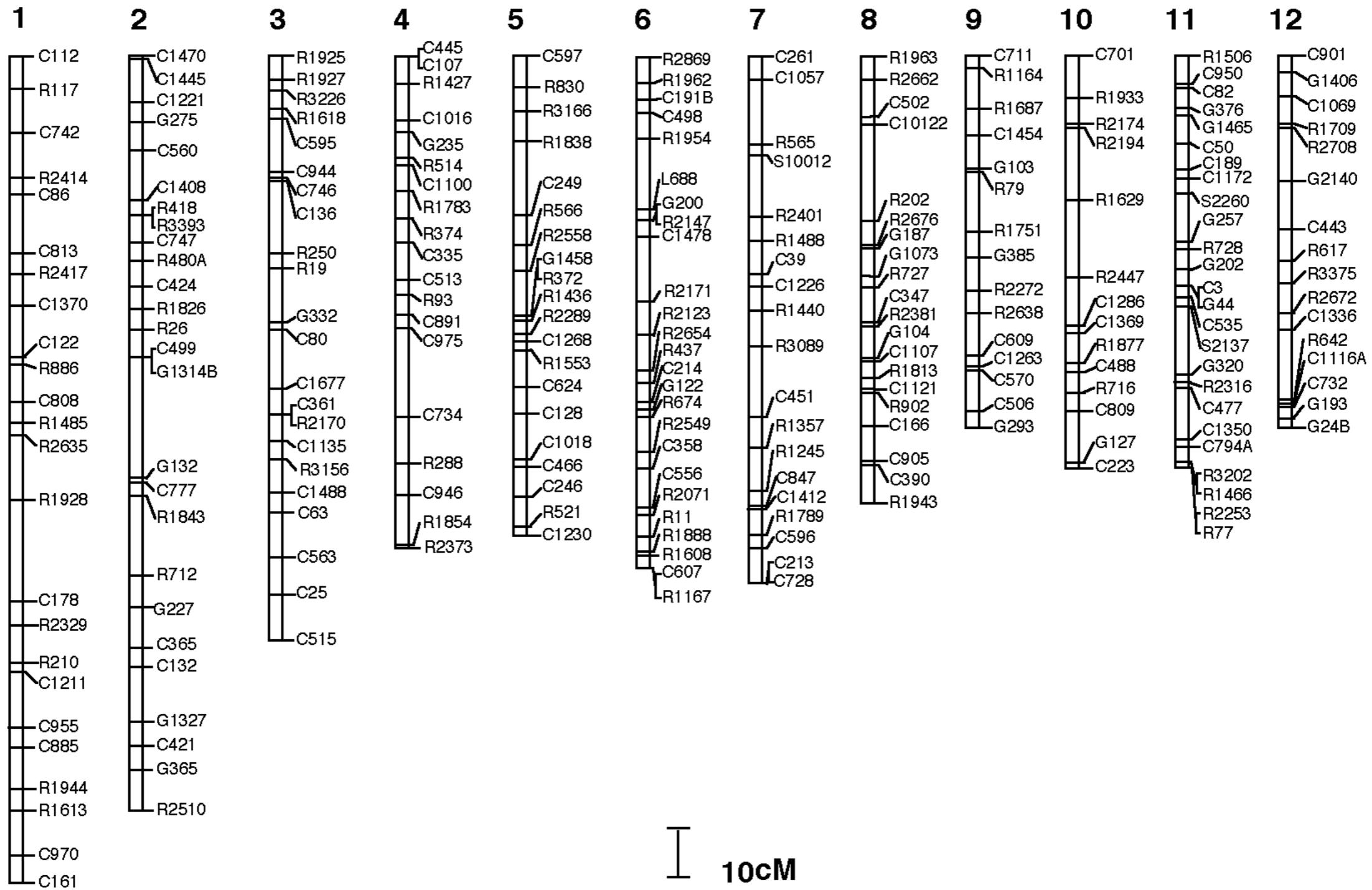


- Microsatellite repeats

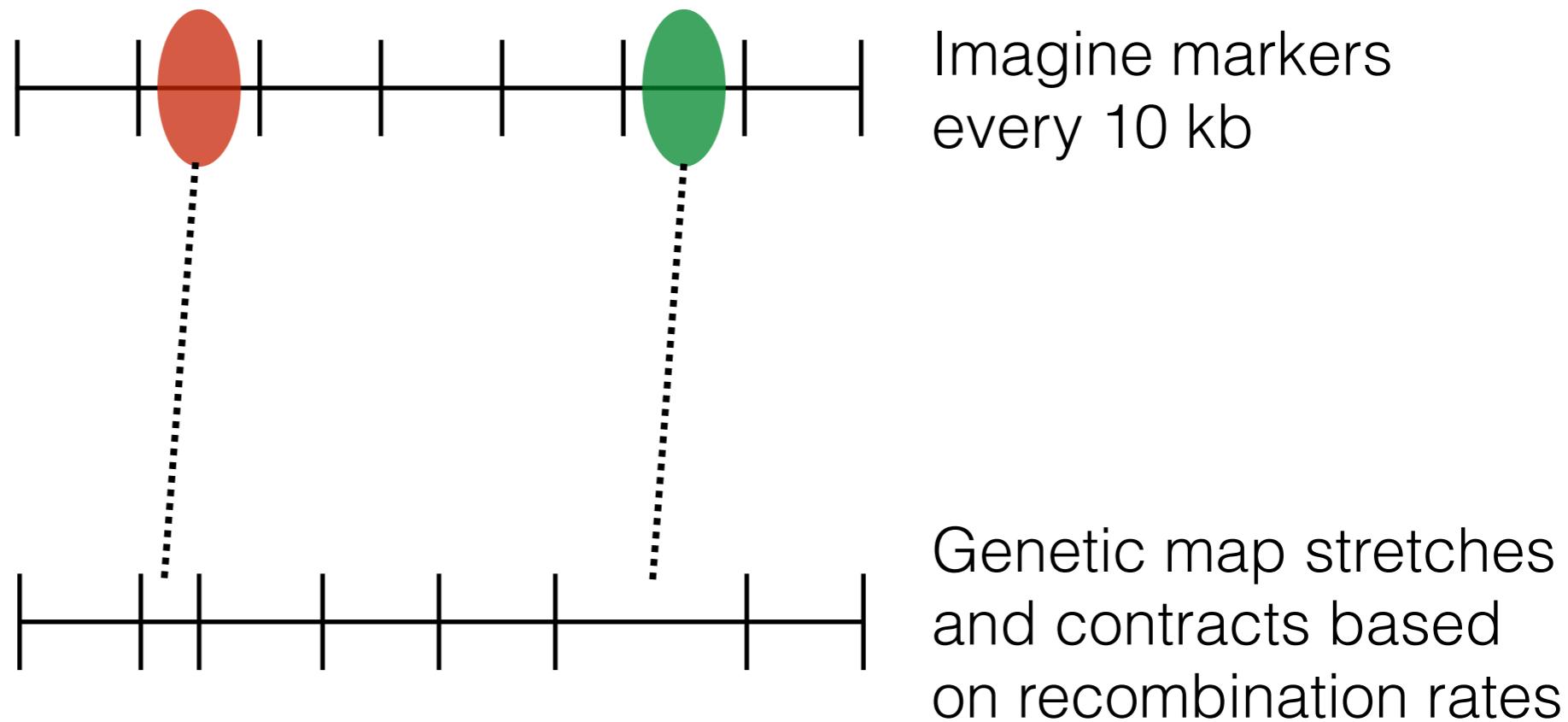


- Insertion/deletion variants

# Molecular markers are used most of the time



# What do regions of more or less recombination do to the linkage map?

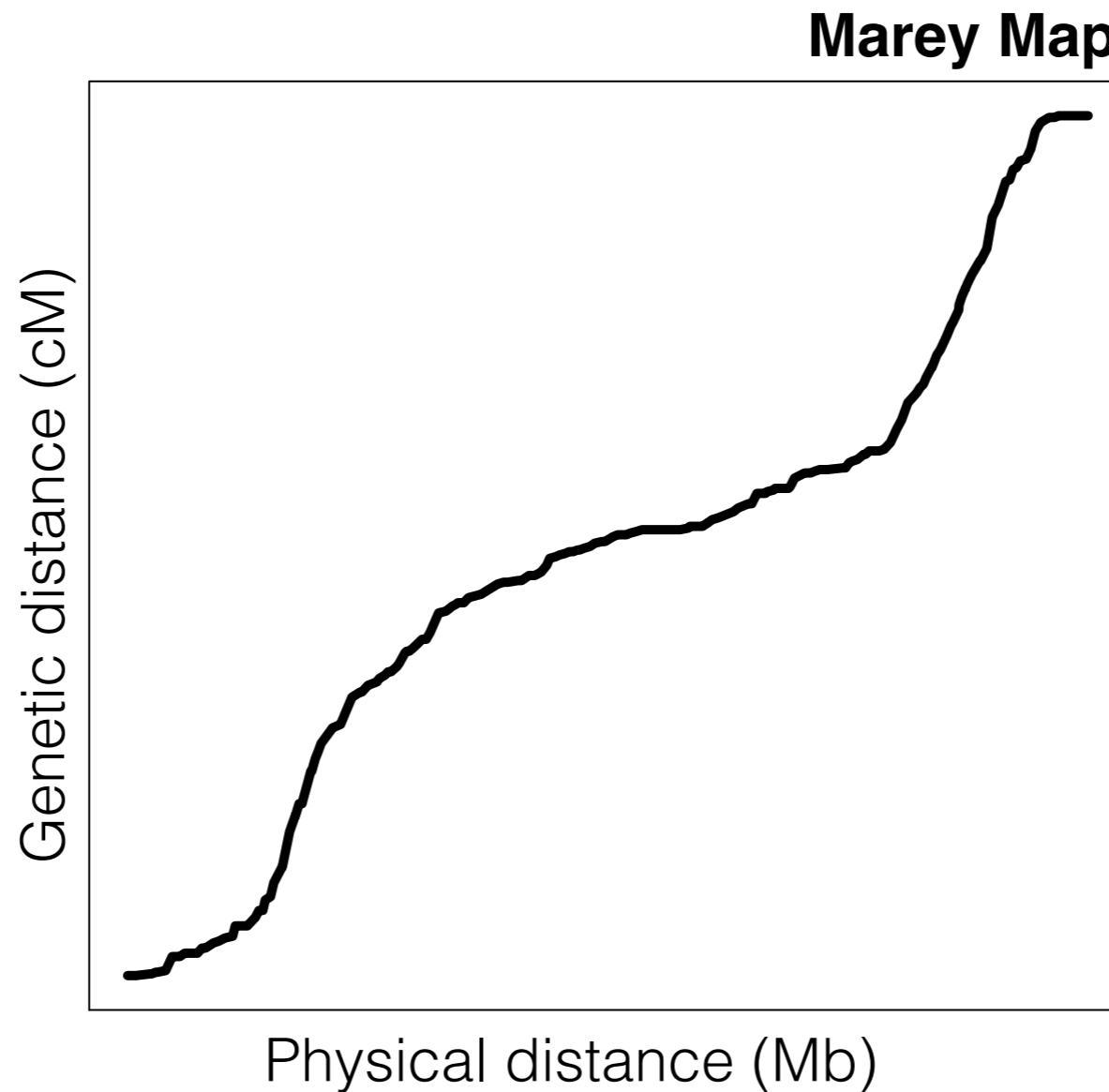


Less recombination (cold spot)



More recombination (hot spot)

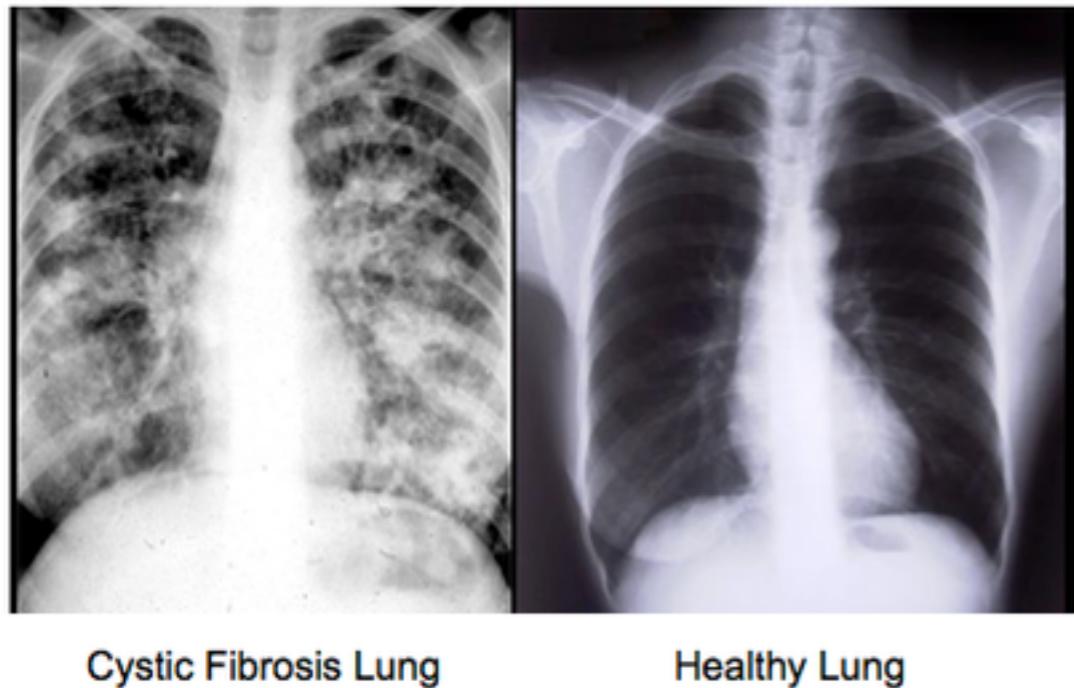
# What do regions of more or less recombination do to the linkage map?



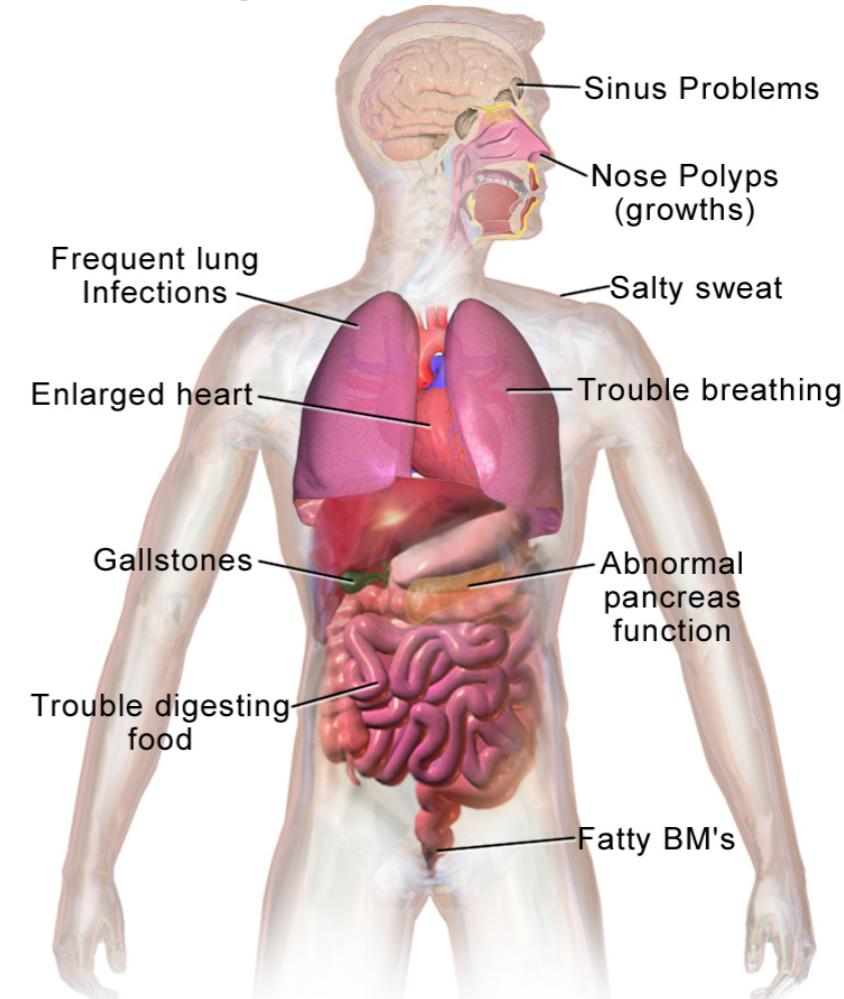
**Why do we care?**

**Is this technique “old-fashioned”?**

# What about cystic fibrosis and today's topic?



## Health Problems with Cystic Fibrosis



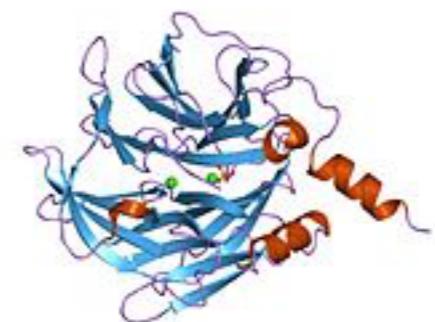
Where is the gene that when mutated causes cystic fibrosis?

How would you map it?

# Linkage to allozymes

Allozymes are enzymes with activities that vary from person to person

*PON1* is a hydrolase used for detoxifying cells.  
Activity varies from person to person.



68 families with at least two children with and without CF were phenotyped for *PON1* activity and CF.

Found linkage to *PON1* and chromosome 7

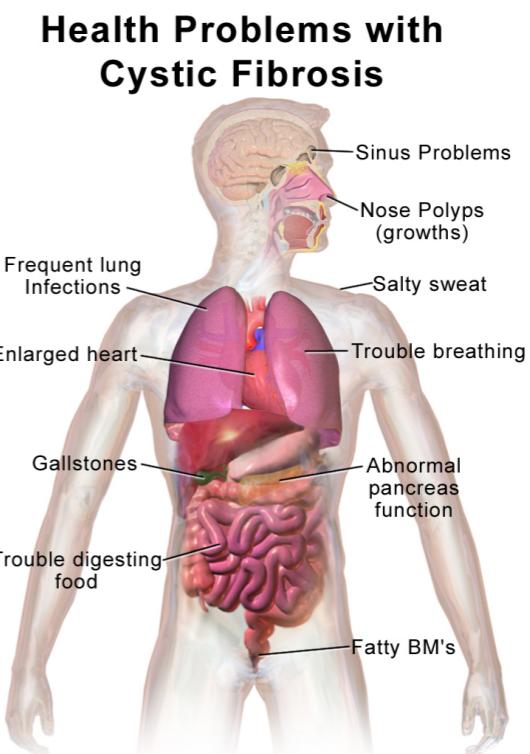
# What have we learned so far?

CF is rare – 1/10,000 births

Autosomal recessive disorder

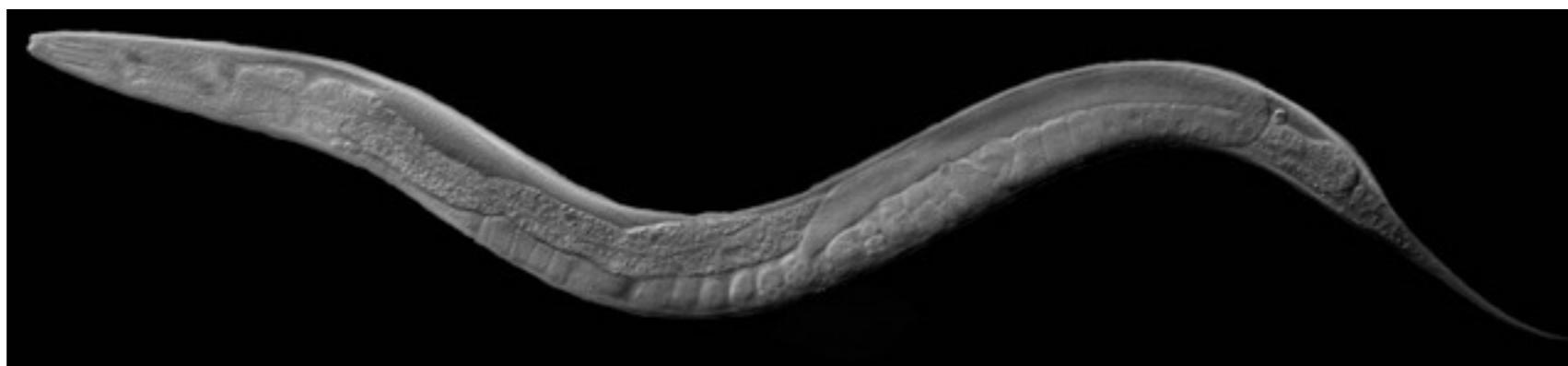
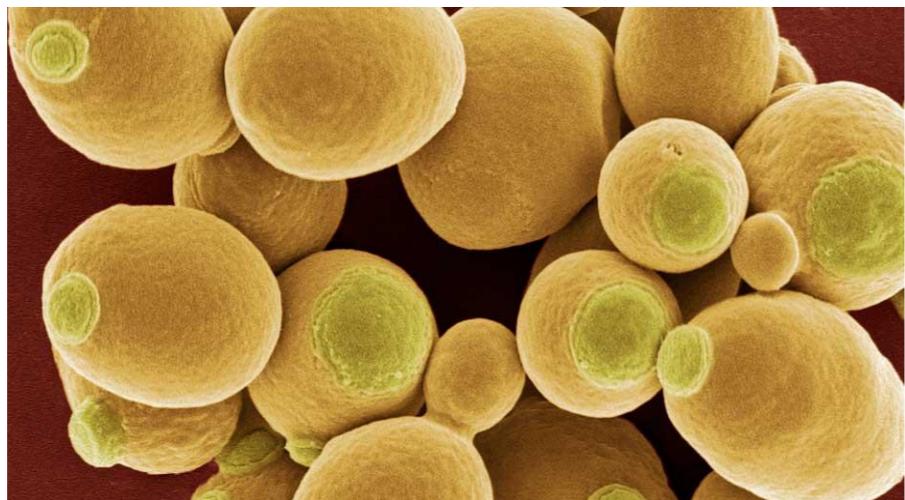
Not caused by chromosomal aberrations or NDJ

Linked to *PON1* on chromosome 7



# Bio393: Genetic Analysis

Screens, selections, mutants, dosage



# Where do all those mutant strains come from?

## Natural

- Made by random errors of DNA repair, replication, transcription, recombination, etc.
- Made by natural mutagens (UV, etc.)
- Variants present in a population
- Rare or common

## Induced

- Made by mutagens  
(e.g. ethyl methanesulfonate (EMS), N-ethyl-N-nitrosourea (ENU), X-ray irradiation)

**Genomes are full of mutations**

# **Spectrum of mutations**

Single-base substitutions

Multiple bases affected

Large chromosome abnormalities

# Spectrum of mutations

Single-base substitutions

Multiple bases affected

Large chromosome abnormalities

- *Translocations, inversions, duplications, deletions*

# Spectrum of mutations

Single-base substitutions

Multiple bases affected

- *Indels, affect coding and non-coding parts of gene, frameshift mutations in coding*

Large chromosome abnormalities

- *Translocations, inversions, duplications, deletions*

# Spectrum of mutations

## Single-base substitutions

- *Silent (synonymous)*
- *Missense (nonsynonymous)*
- *Nonsense*

## Multiple bases affected

- *Indels, affect coding and non-coding parts of gene, frameshift mutations in coding*

## Large chromosome abnormalities

- *Translocations, inversions, duplications, deletions*

# **Why do we want mutants?**

- Teaches us about gene function
- Teaches us about evolution
- Map other mutations

# Which mutagen to choose?

## No mutagen

Positives: very rare changes, fewer background mutations

Negatives: Rare = slow, many generations to find mutant

## UV, ionizing radiation

Positives: Strong mutagen, large effects

Negatives: Lots of mutations, need to clean up background, causes sickness and sterility

## EMS, ENU, base altering mutagens

Positives: Not too strong mutagens, dose sensitive, focal perturbations to genes

Negatives: Lots of mutations, need to clean up background, causes sickness and sterility

## Transposons

Positives: Strong effects on gene function (big piece of DNA), easily found in genome

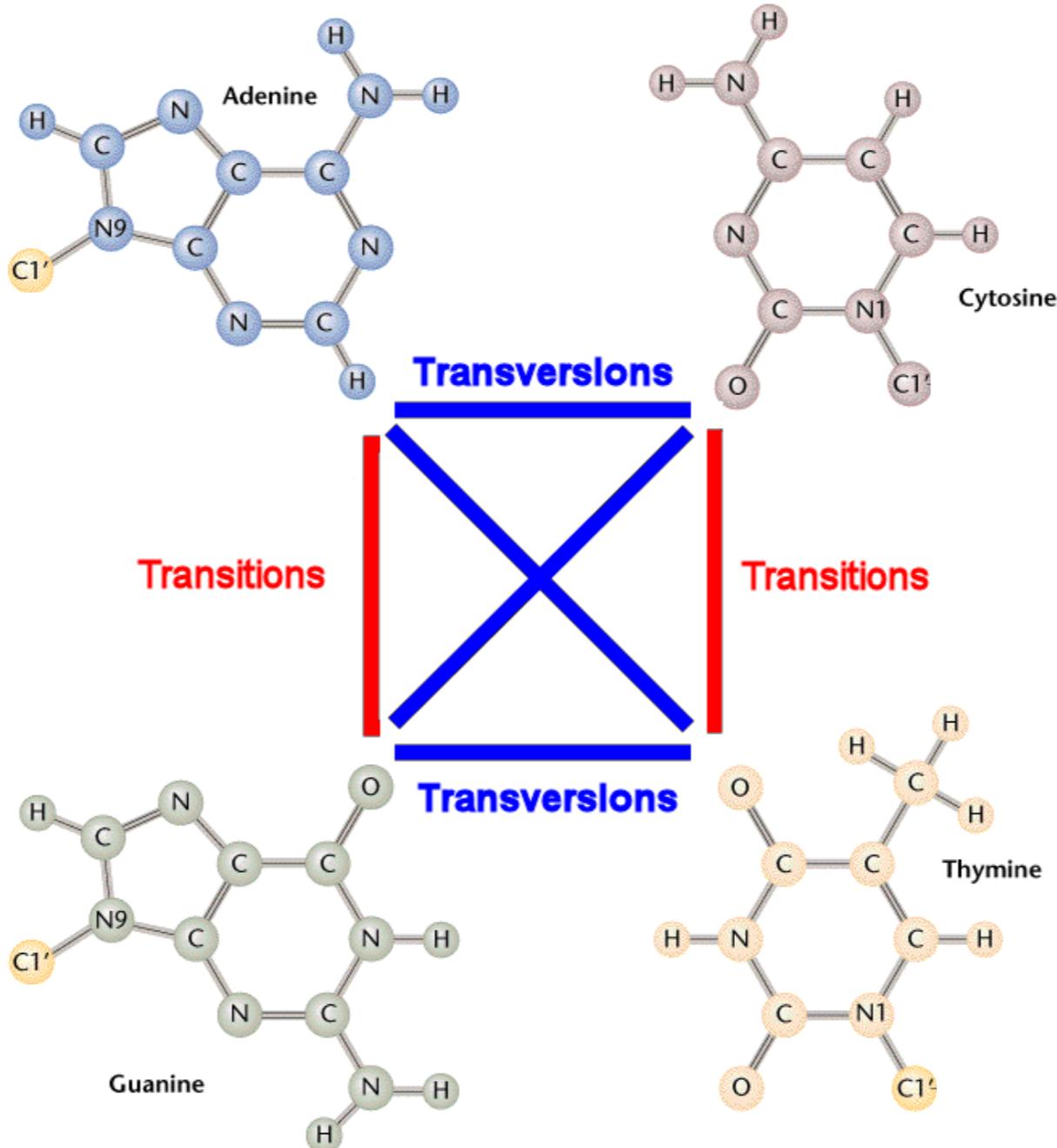
Negatives: Strong effects, not full mutation spectrum open, less efficient than mutagen

## CRISPR/Cas9, targeted mutations

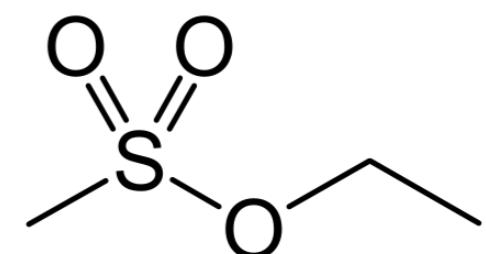
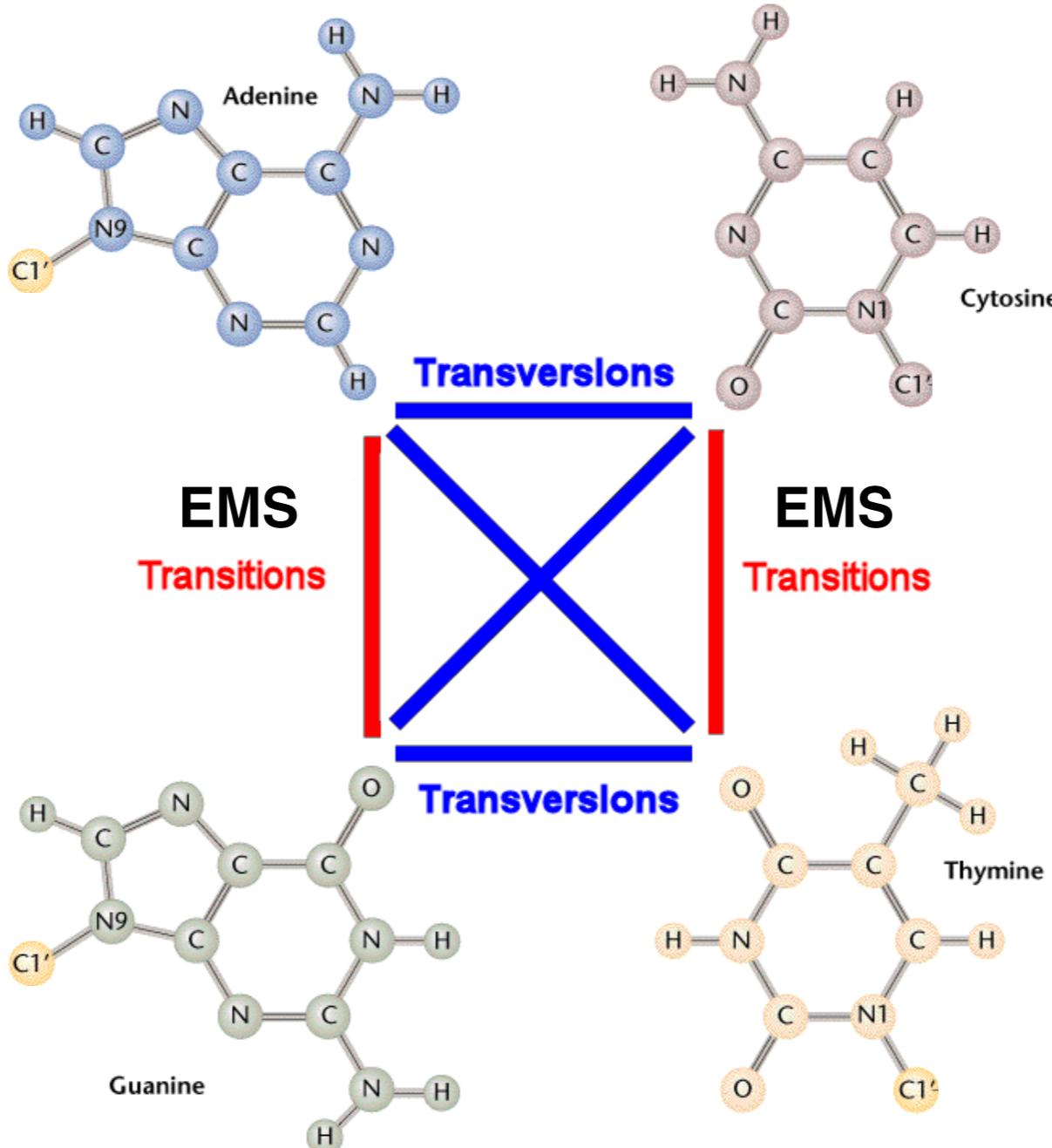
Positives: Targeted, Designable, Defined genetic background, Scalable

Negatives: Off target effects? Delivery?

# Single-base substitutions

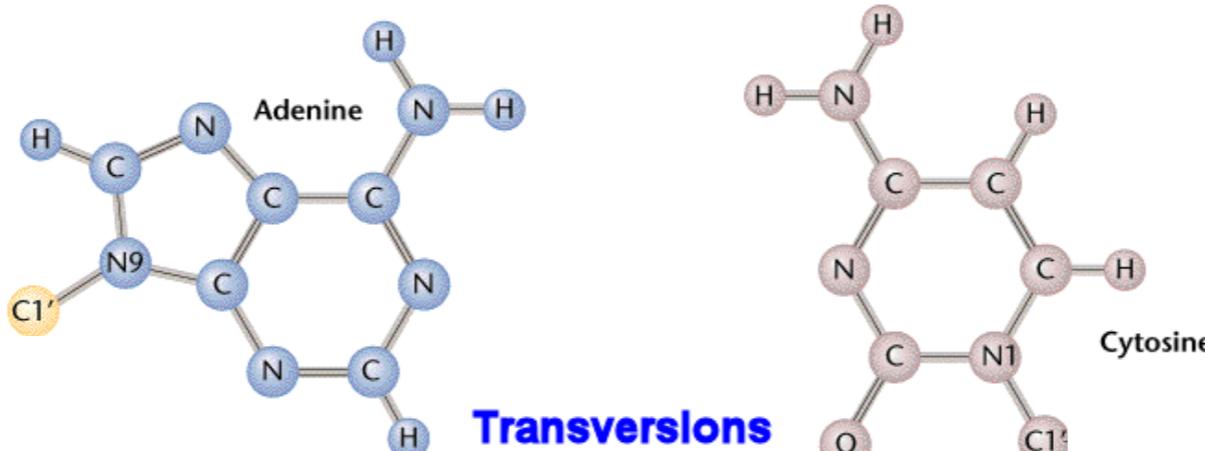


# Single-base substitutions

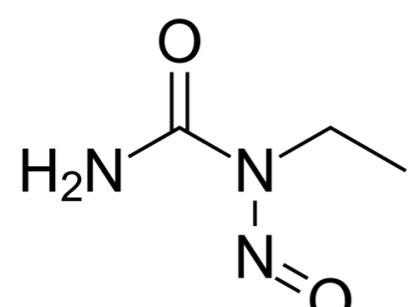
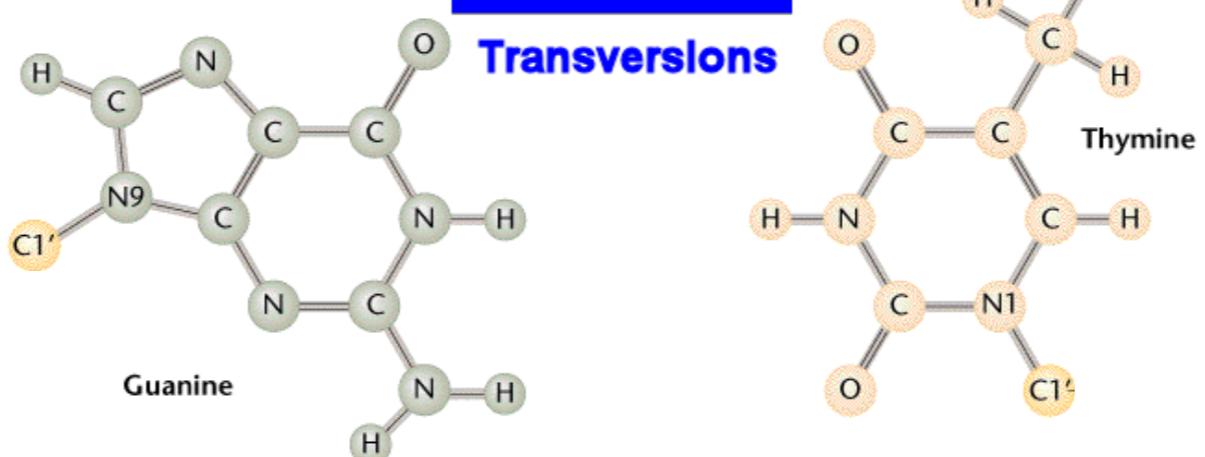


Ethyl methanesulfonate  
(EMS)

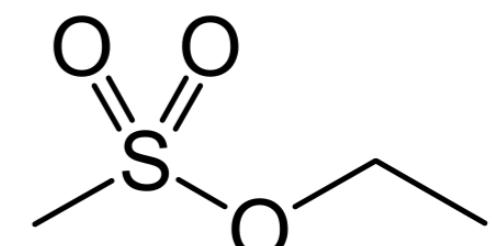
# Single-base substitutions



**EMS**  
Transitions  
**ENU**



**Ethyl-nitrosourea  
(ENU)**



**Ethyl methanesulfonate  
(EMS)**

# **Two ways to isolate mutants: selection or screen**

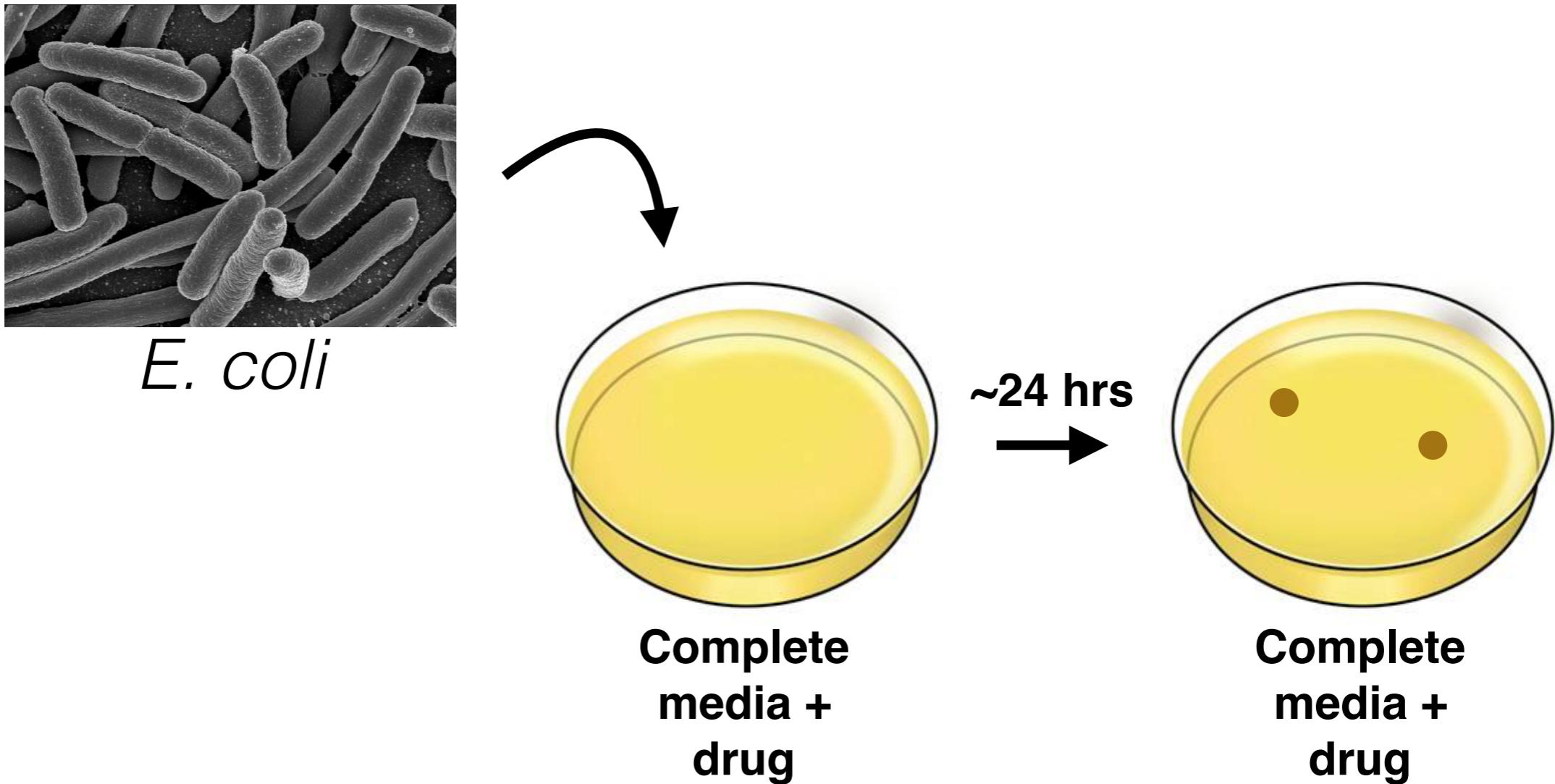
**Selection:** You only see mutants

**Screen:** You need to look through lots of wild-type animals to find the rare mutants.

**You don't always get what you want!**

# Two ways to isolate mutants: selection or screen

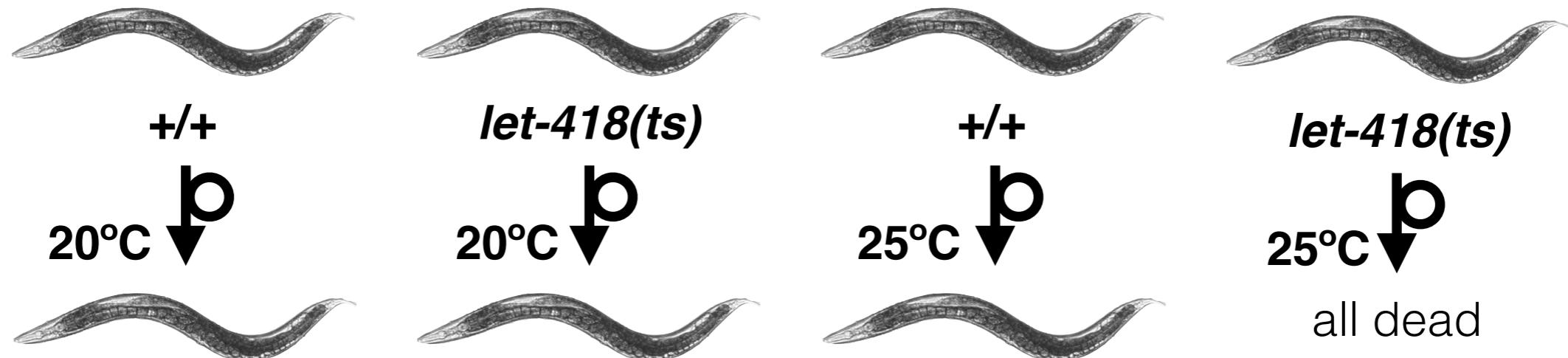
## Selection:



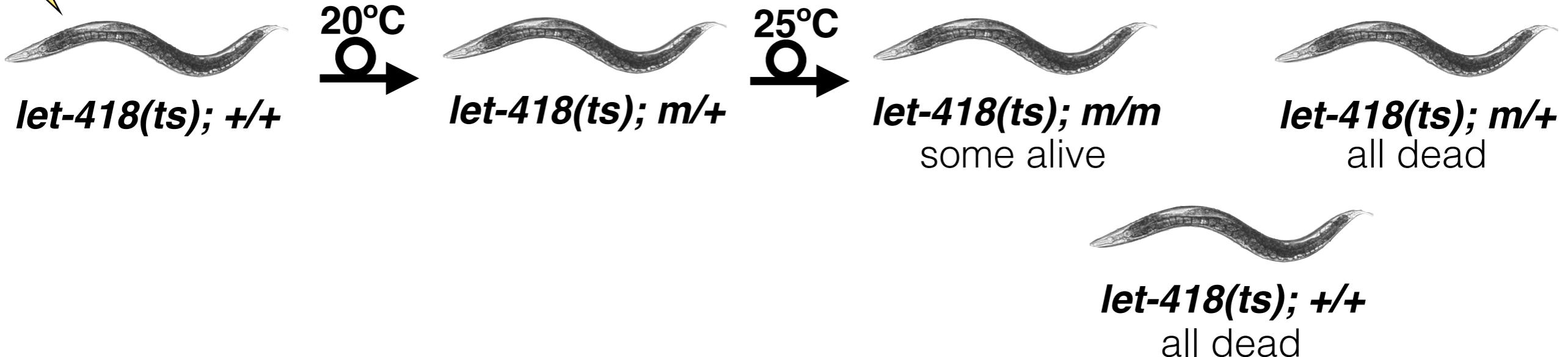
Goal: Find mutants that can grow on a specific drug

# Two ways to isolate mutants: selection or screen

## Selection:



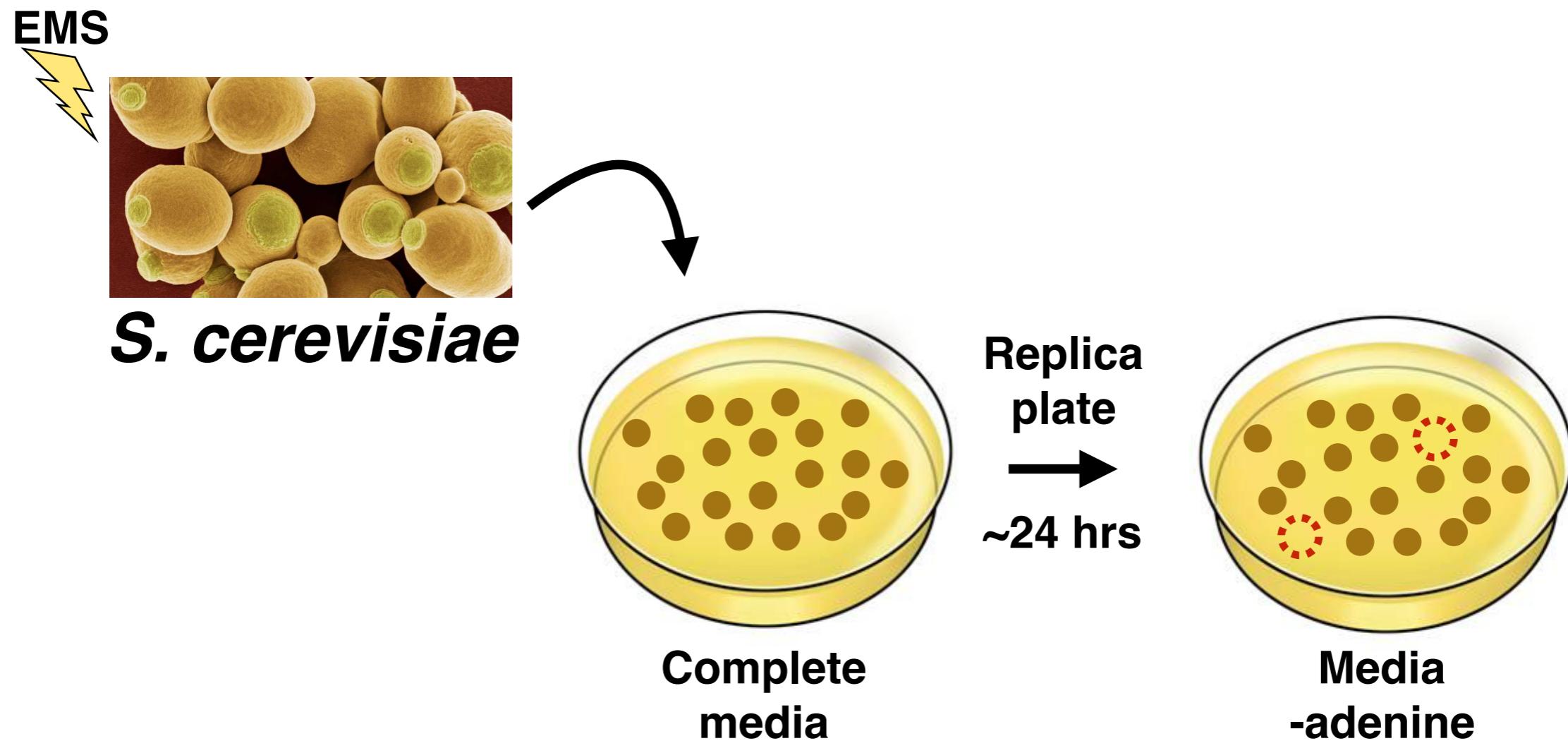
EMS  
⚡



Goal: Find mutants that can grow at high temp. with *let-418*

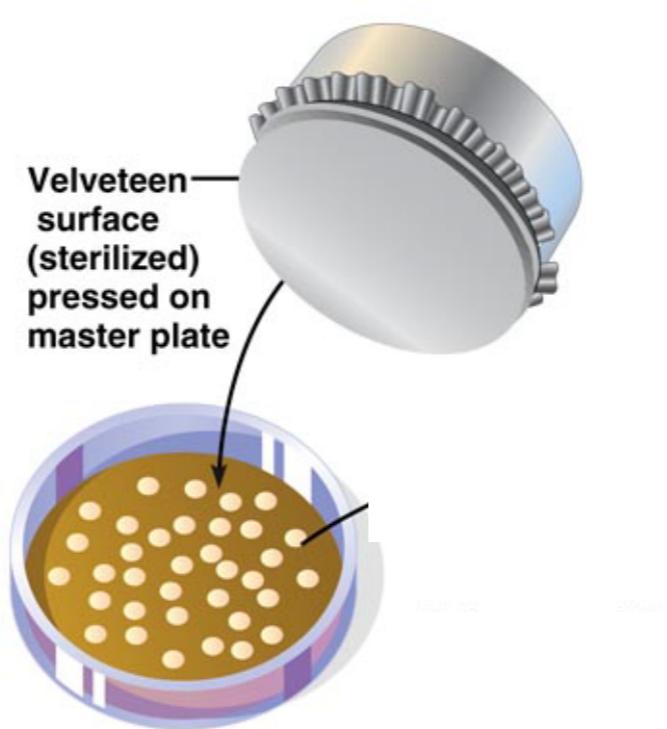
# Two ways to isolate mutants: selection or screen

Screen:



Why not directly plate on media lacking adenine?

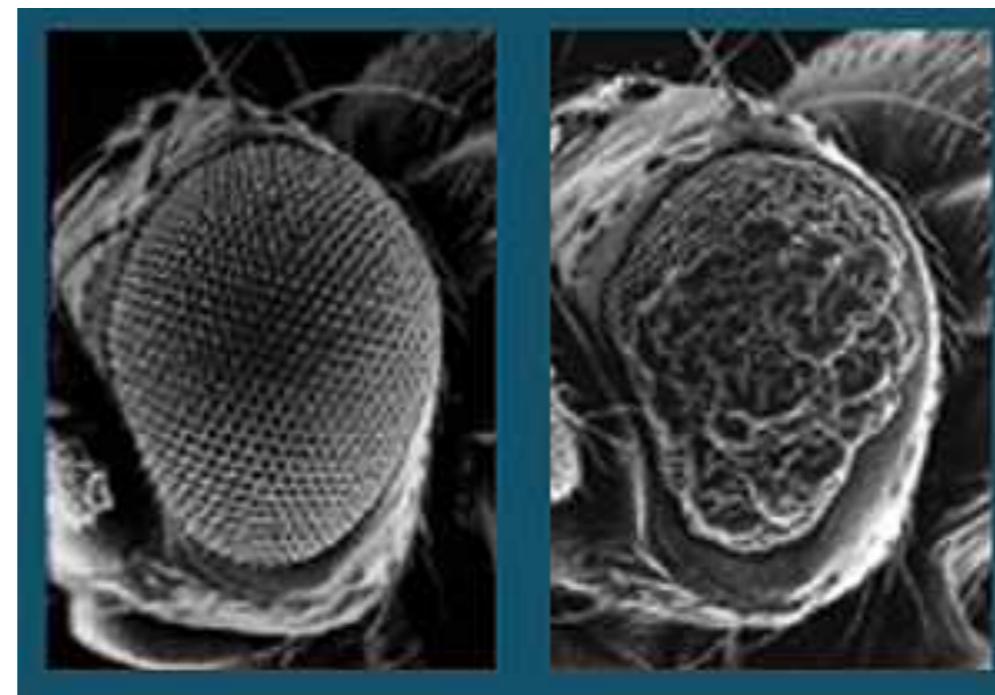
# Replica plating



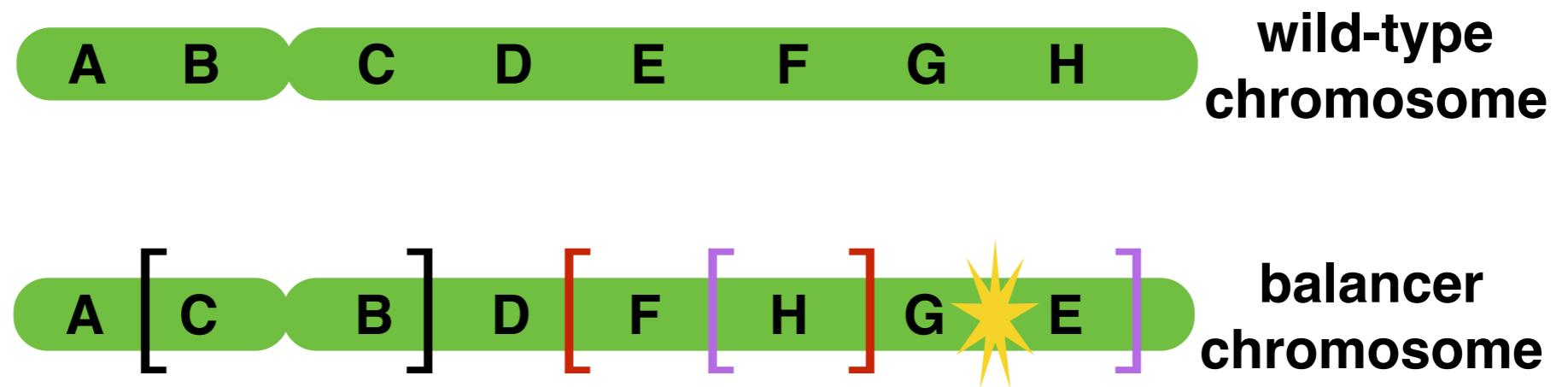
# Two ways to isolate mutants: selection or screen



*D. melanogaster*



# *Drosophila* have balancer chromosomes



Every balancer chromosome:

1. has many inversions to eliminate recombinant progeny
2. confers an easily scored dominant phenotype
3. is recessive lethal

# ***Cyo* is a second chromosome balancer**



*Sp*  
*CyO*

# ***Cyo* is a second chromosome balancer**



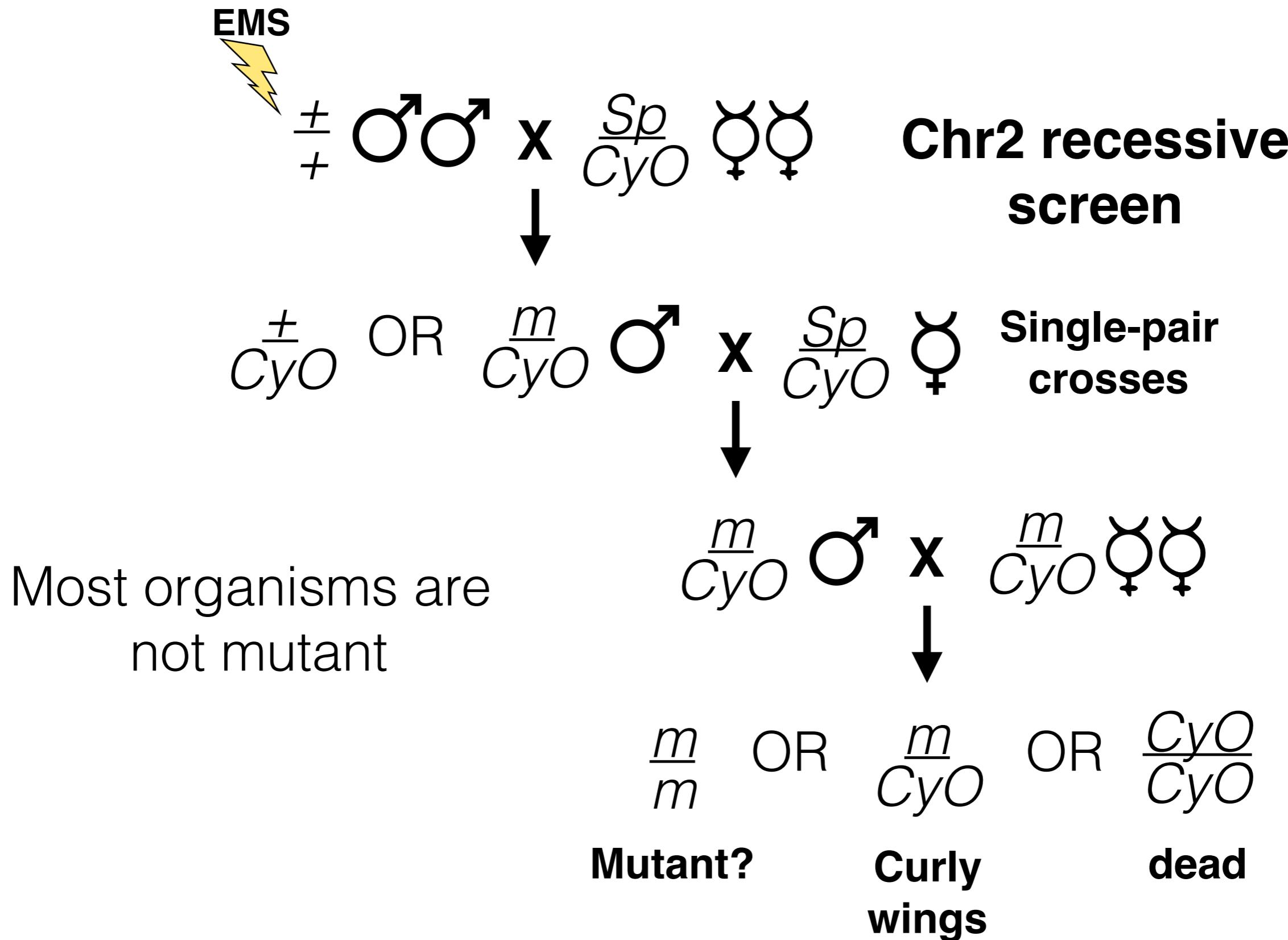
**Sp  
*CyO***

Every balancer chromosome:

1. has many inversions to eliminate recombinant progeny
2. confers an easily scored dominant phenotype
3. is recessive lethal

# Two ways to isolate mutants: selection or screen

## Balancer chromosomes



# Two ways to isolate mutants: selection or screen no balancer chromosomes but selfing



EMS



$\pm$  ♂  
 $+$  ♀



$\pm$  OR  $\frac{m}{+}$



$\pm$  OR  $\frac{m}{+}$  OR  $\frac{m}{m}$

F<sub>2</sub> non-clonal  
screen

Hunt for your mutants!

# Two ways to isolate mutants: selection or screen no balancer chromosomes and no selfing

Screen:



ENU



$$\frac{\pm}{+} \text{♂} \times \frac{\pm}{+} \text{♀}$$

**F<sub>2</sub> recessive screen**



$$\frac{m}{+} \text{♂} \times \frac{\pm}{+} \text{♀}$$

**Single-pair crosses**



$$\frac{m}{+} \text{♂} \times \frac{m}{+} \text{♀}$$

**Bulk crosses**



$$\frac{\pm}{+} \text{ OR } \frac{m}{+} \text{ OR } \frac{m}{m}$$

Hunt for your mutants!

# **Why do we look for alleles that confer dominant or recessive traits?**

Alleles that confer recessive traits teach us about:

- Gene function (Break it to understand it)
- Loss of function
- Pathway genetics (Lecture 6)

Alleles that confer dominant traits teach us about:

- Pathway genetics
- Gain of function (next)
- Function

# What happens when we mutagenize strains?



Mutations occur in the DNA of somatic and germline cells

Mutations are “random”  
and are only inherited when they occur in germline cells

**How would you screen or select for mutants  
that cause a dominant or a recessive phenotype  
in yeast, *C. elegans*, *Drosophila*, and mice?**