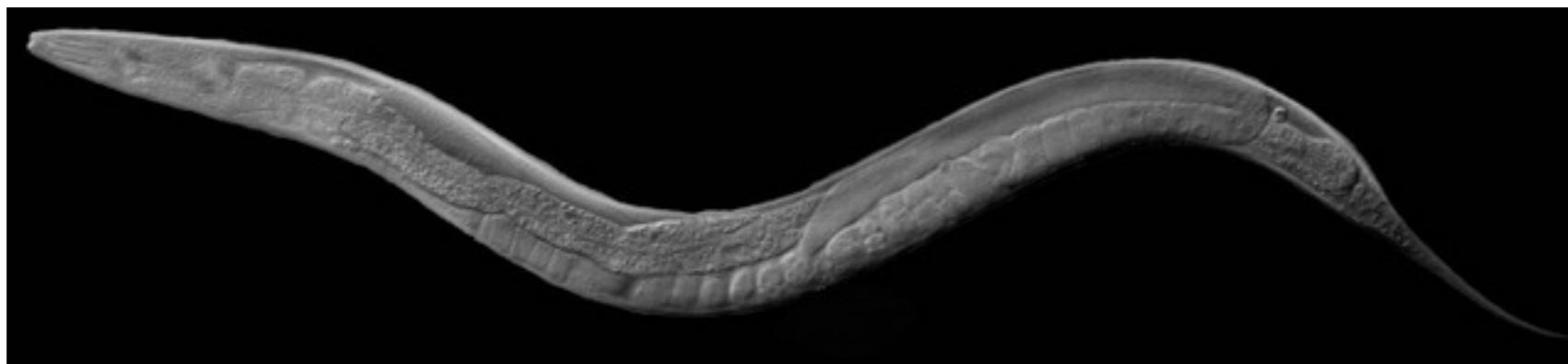
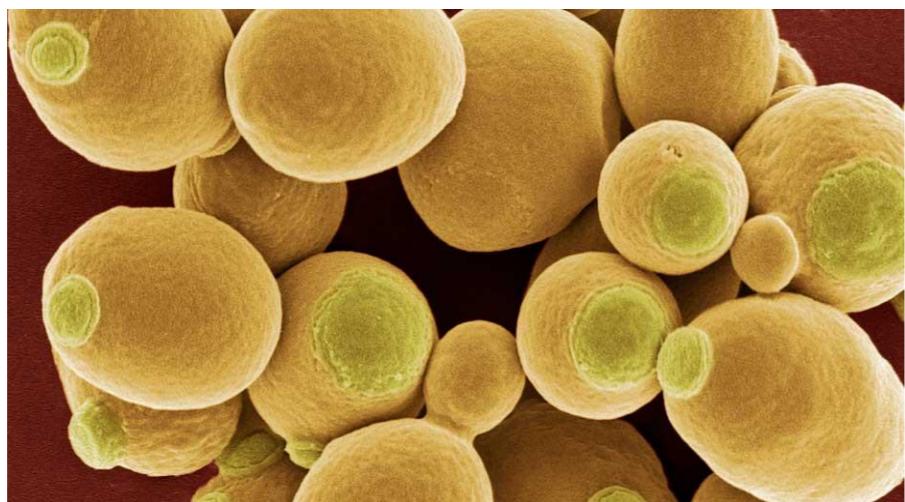


# Bio393: Genetic Analysis

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**

# **Why do we want mutants?**

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

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

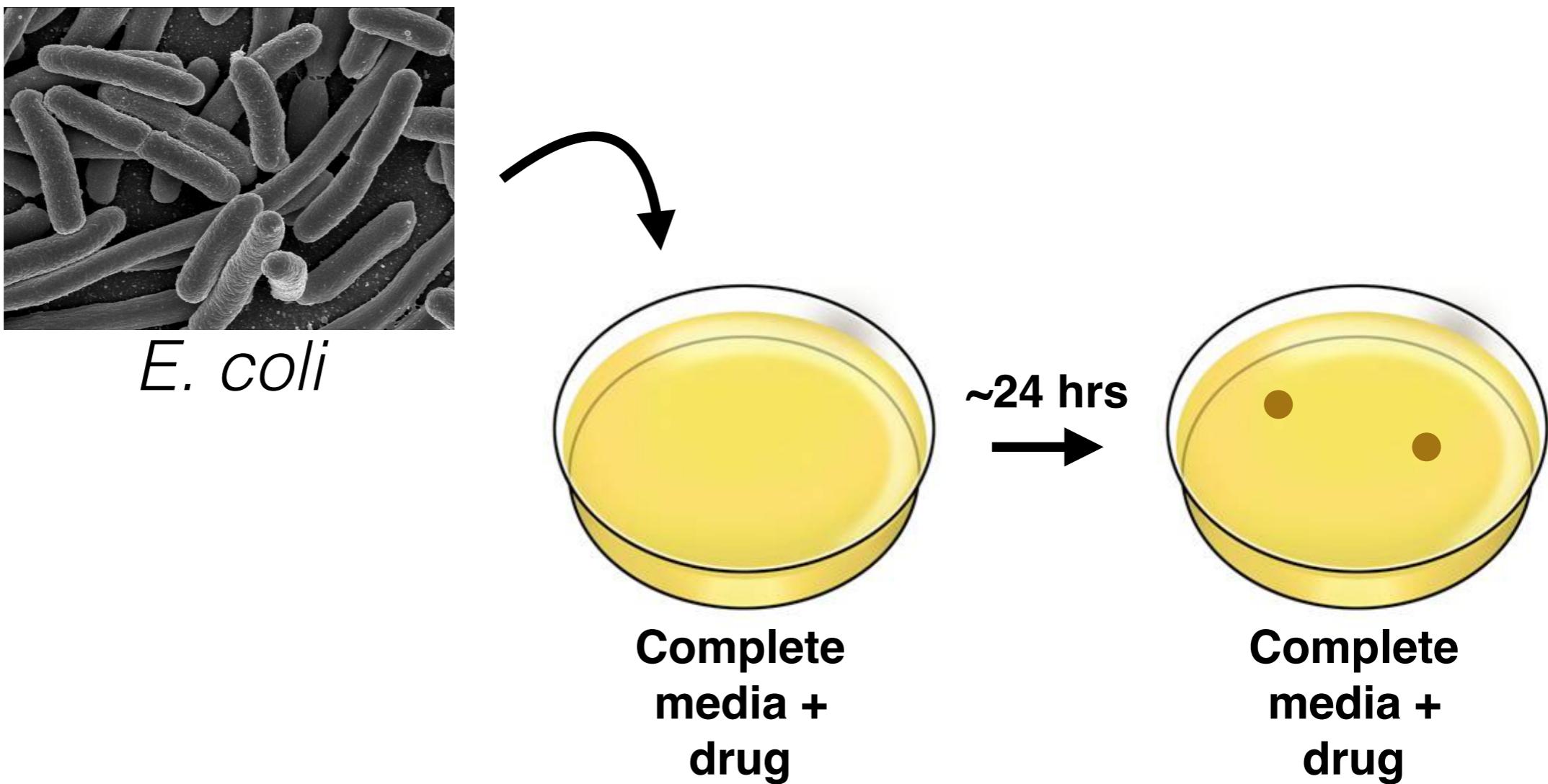
**Selection:** You only get the mutants you want

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

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

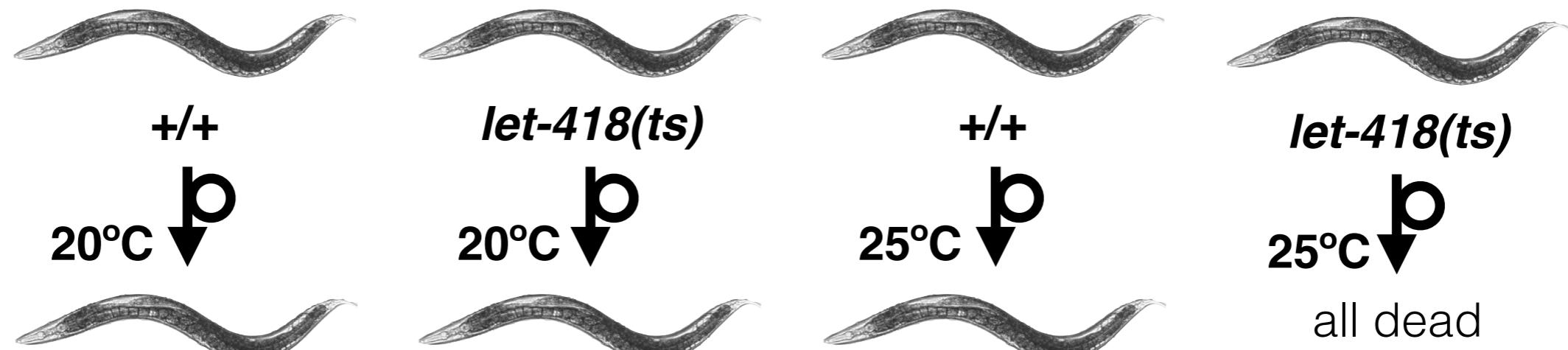
# Two ways to isolate mutants: selection or screen

## Selection:

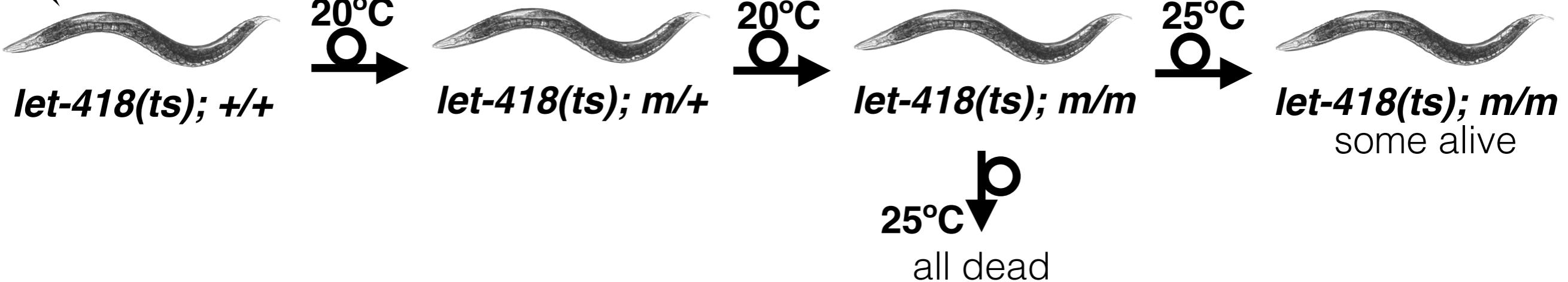


# Two ways to isolate mutants: selection or screen

## Selection:

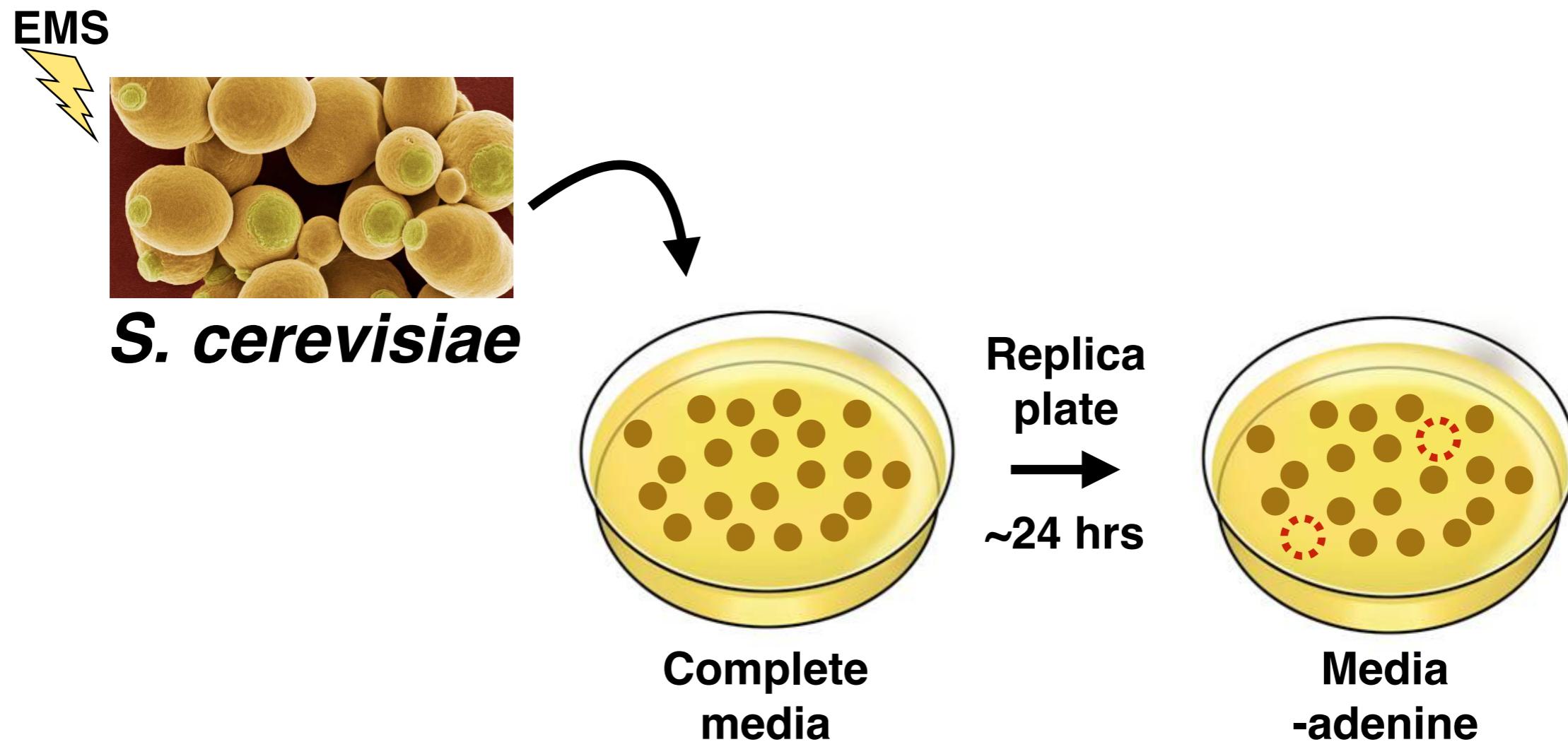


EMS  
⚡



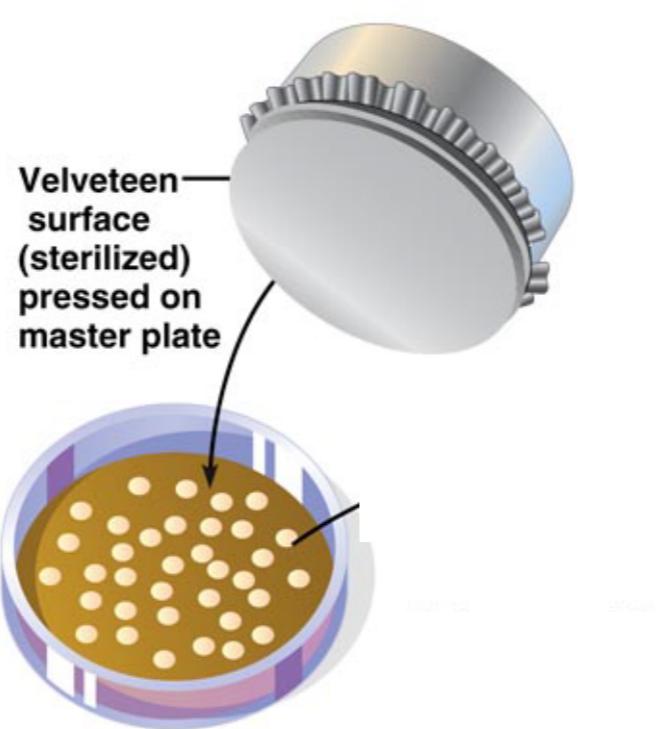
# Two ways to isolate mutants: selection or screen

## Screen:



Why not directly plate on -adenine media?

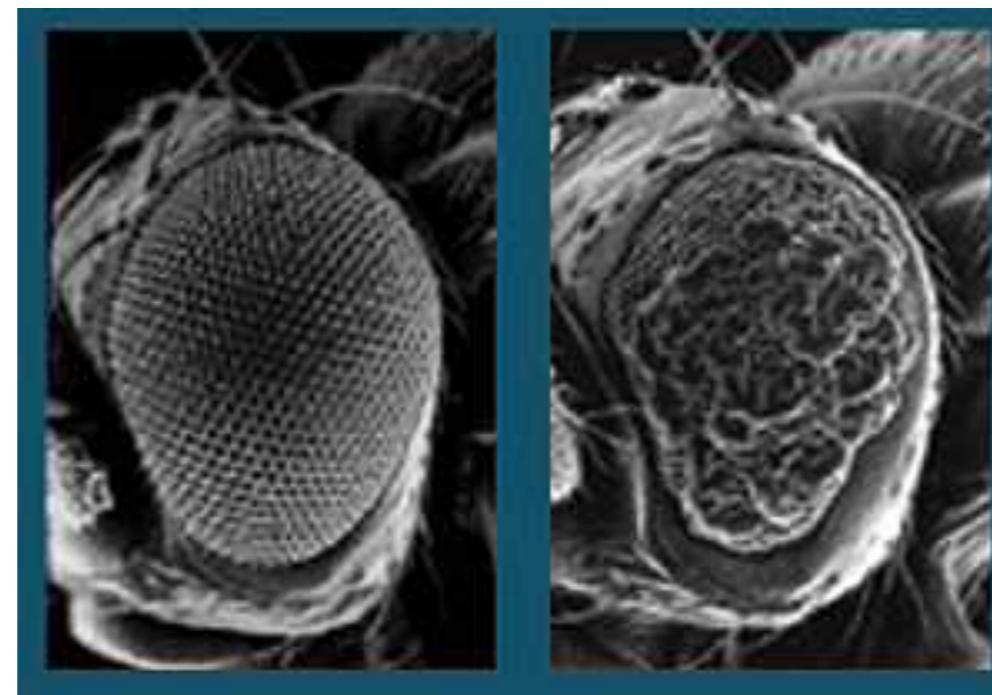
# Replica plating



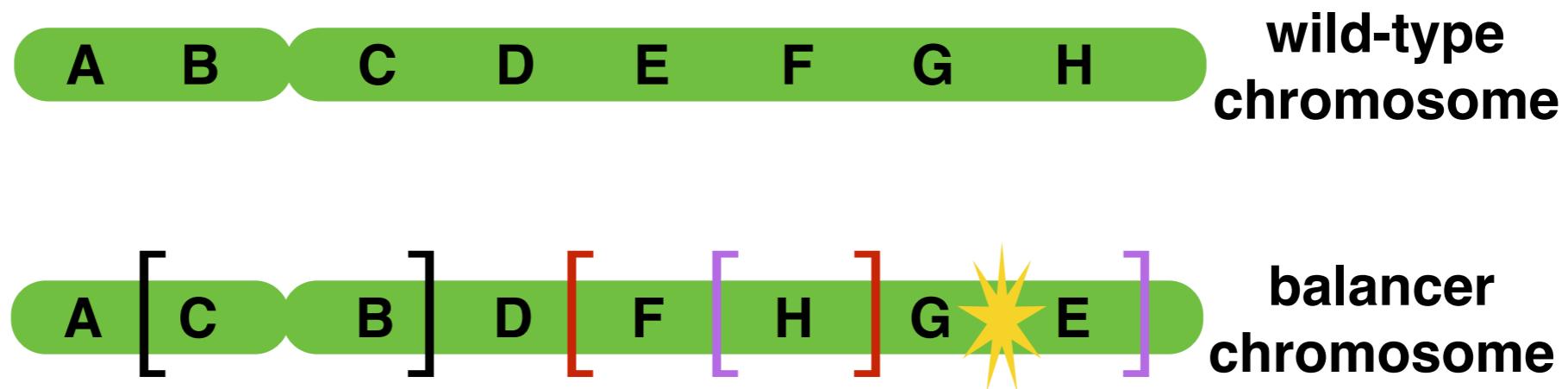
# Two ways to isolate mutants: selection or screen



*D. melanogaster*



# *Drosophila* have balancer chromosomes



Every balancer chromosome:

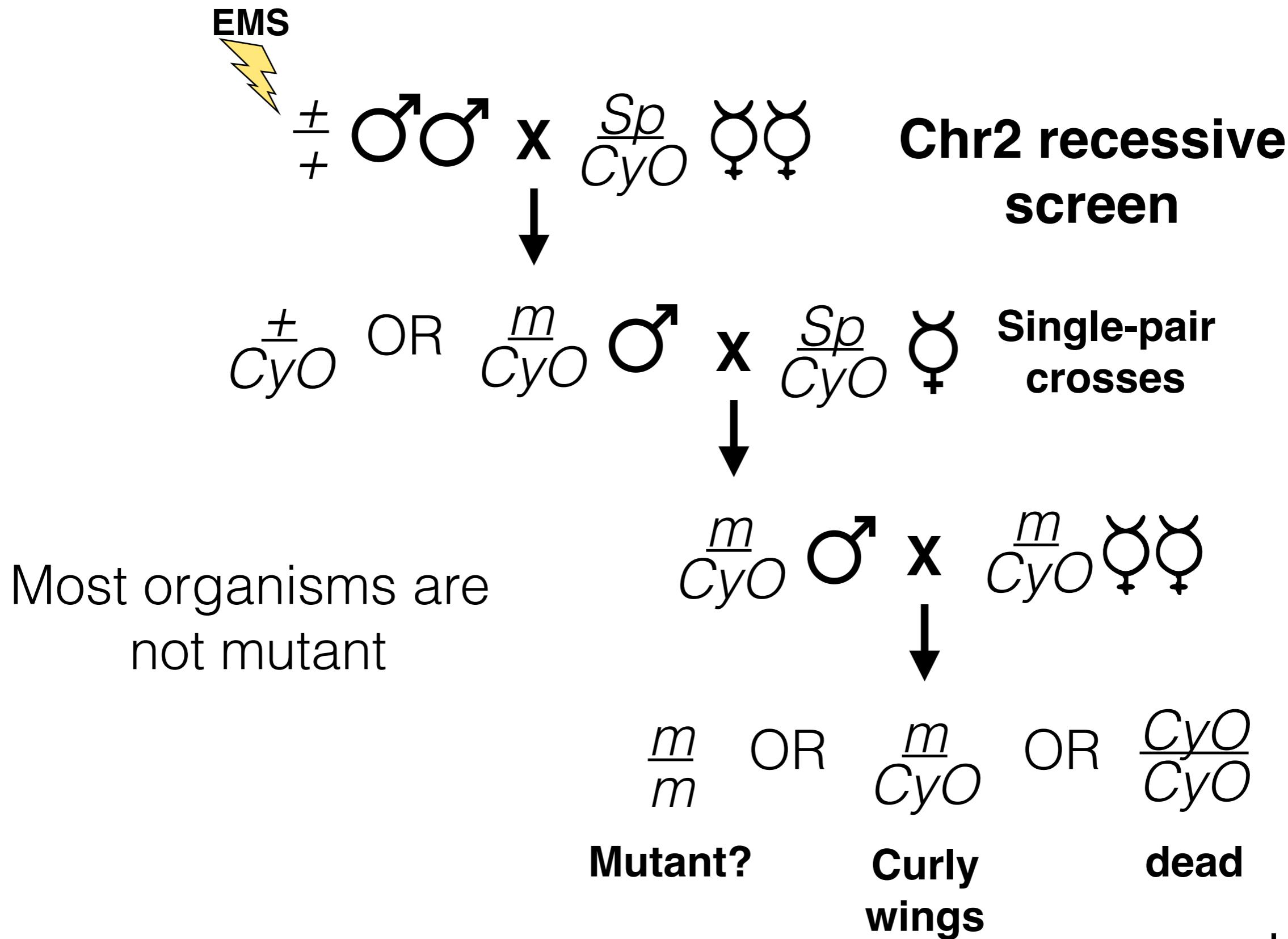
1. has many inversions to eliminate recombination
2. confers an easily scored dominant phenotype
3. is recessive lethal



**Sp**  
***CyO***

# 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  $\frac{m}{+}$



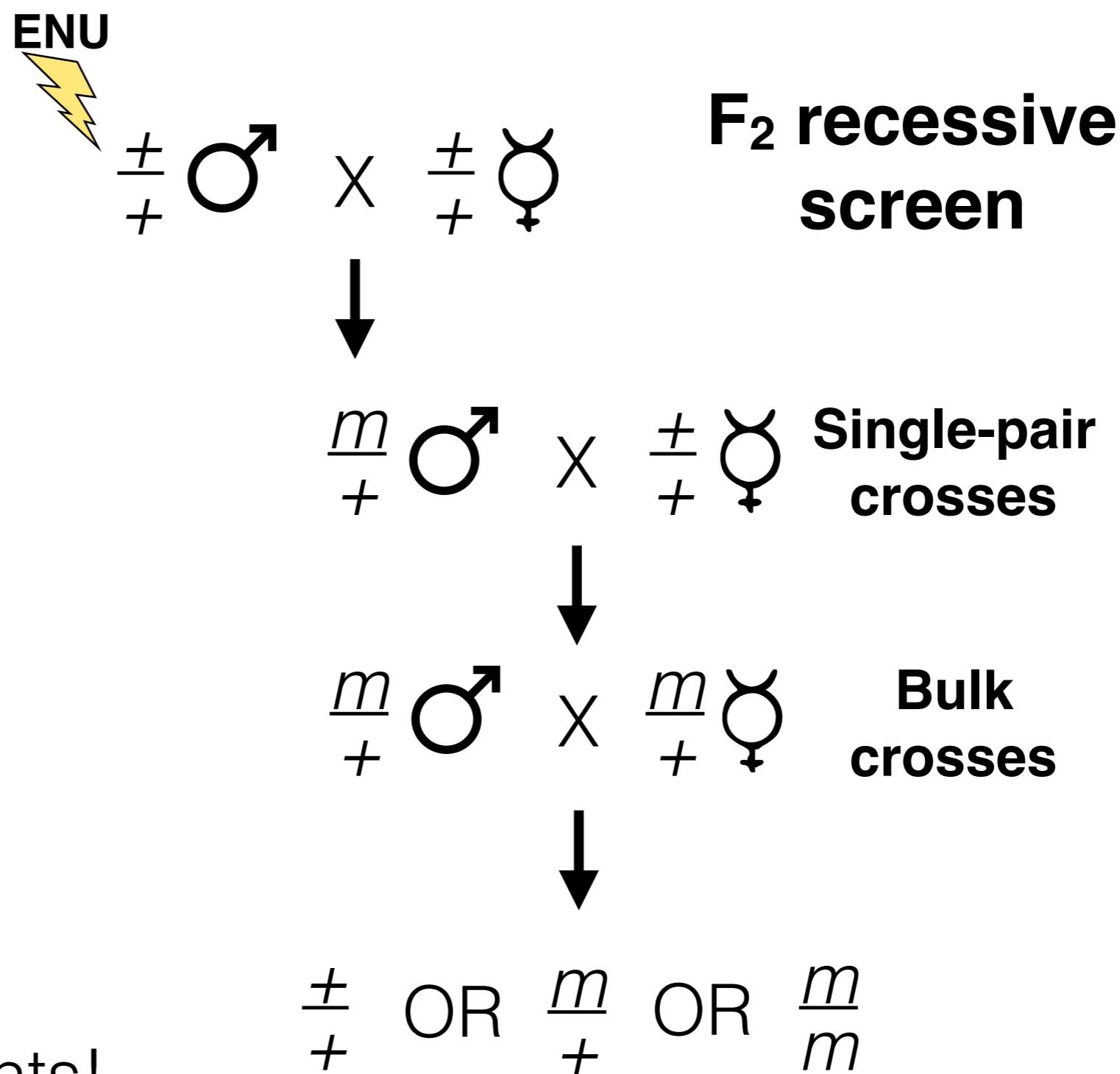
$\pm$  OR  $\frac{m}{+}$  OR  $\frac{m}{m}$

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:



Hunt for your mutants!

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

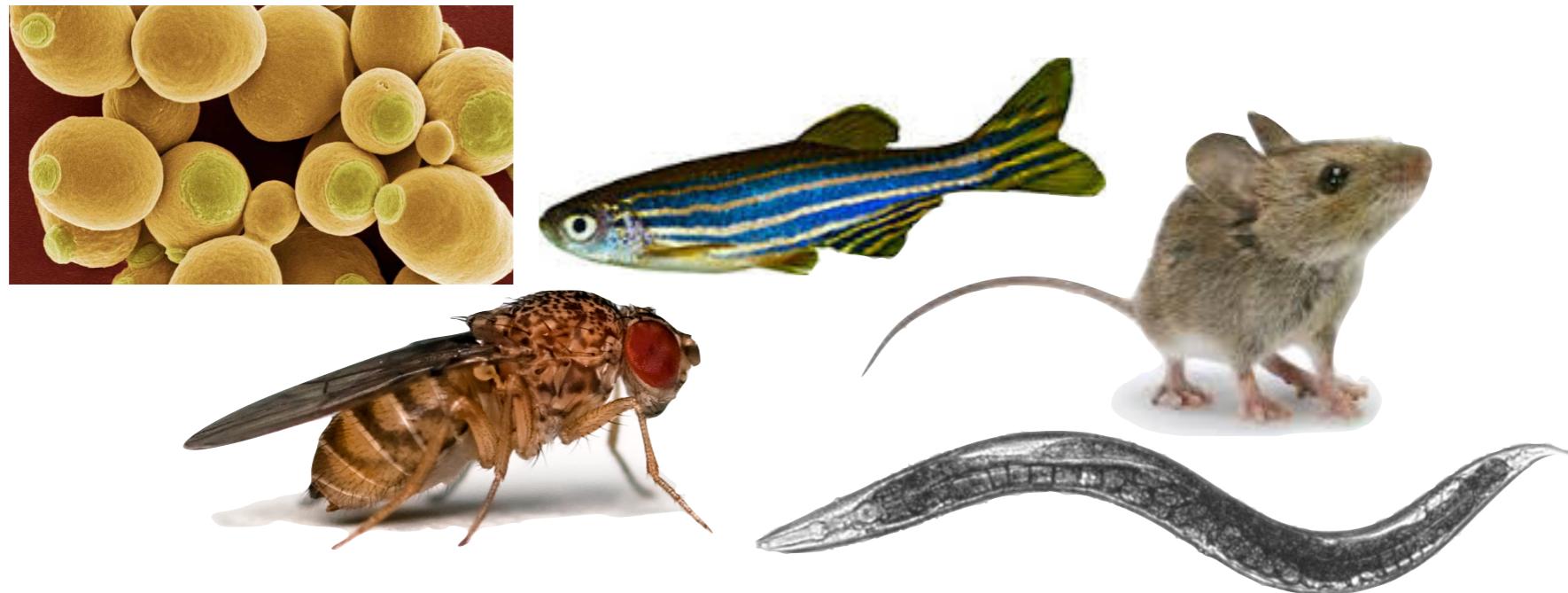
Recessive alleles teach us about:

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

Dominant alleles teach us about:

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

# What happens when we mutagenize strains?

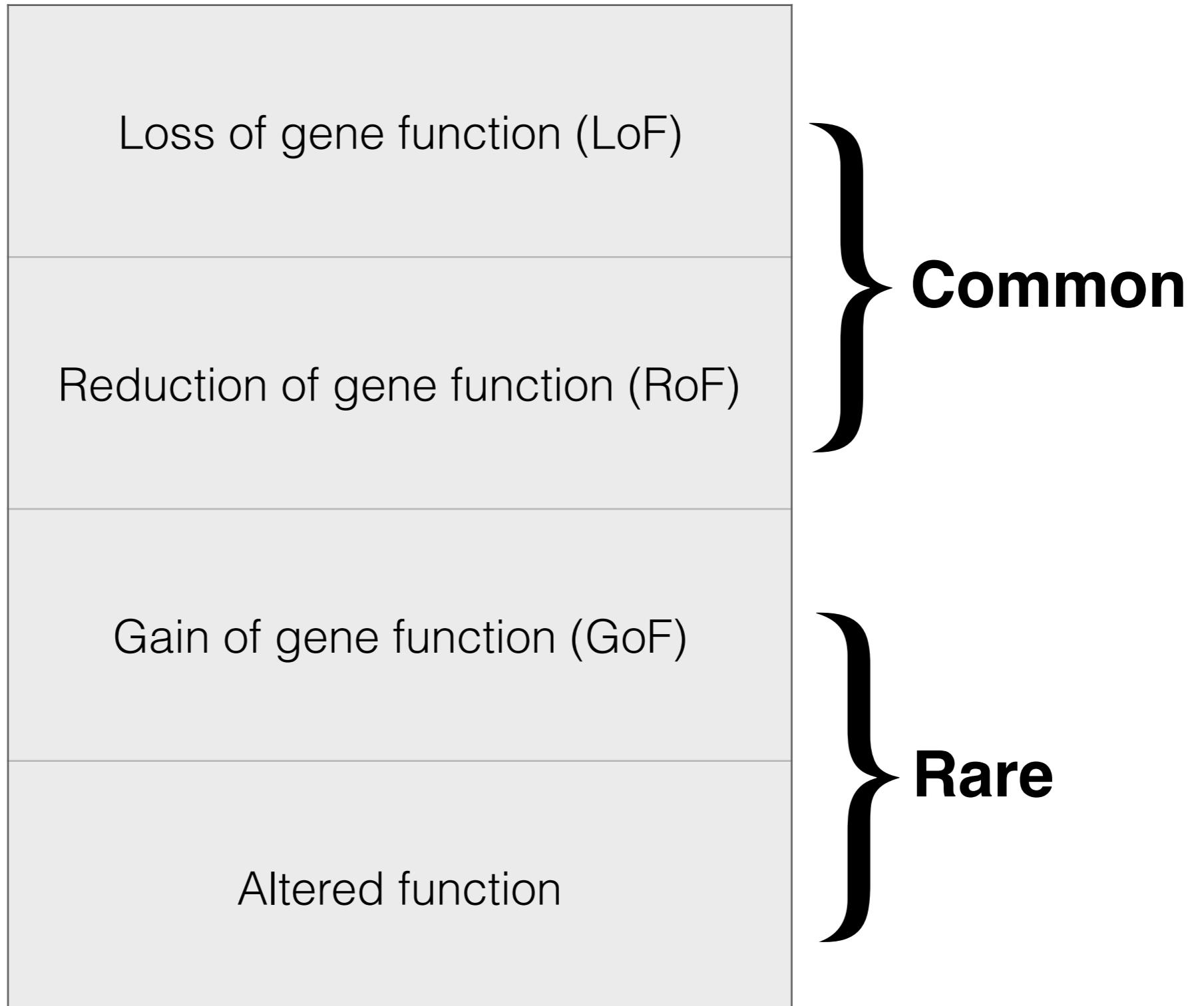


Mutations occur in the DNA of somatic and germline cells

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 recessive phenotype  
in yeast, *C. elegans*, *Drosophila*, and mice?**

**What does a mutation do to gene function?**



**Dominant or recessive  
correlates with mutation type most times**



**Hermann Muller**



# Muller's morphs - gene dosage tests

Loss of gene function (LoF)	amorph, nullomorph
Reduction of gene function (RoF)	hypomorph
Gain of gene function (GoF)	hypermorph
Altered function	neomorph, antimorph

m = mutation of gene

△ = deletion of gene

+ = normal copy of gene

= = Phenotype is equivalent

> = Phenotype is more mutant than

< = Phenotype is less mutant than

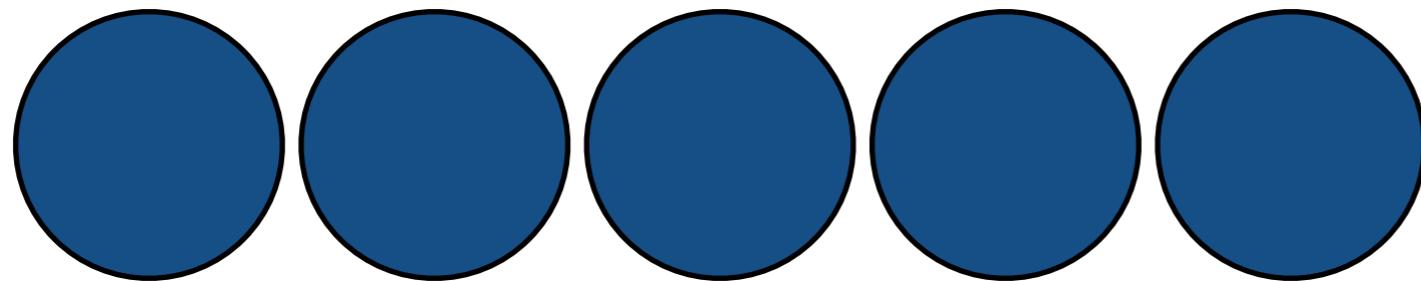
# **How do you get strains that are more or less mutant?**

## **Incomplete penetrance**

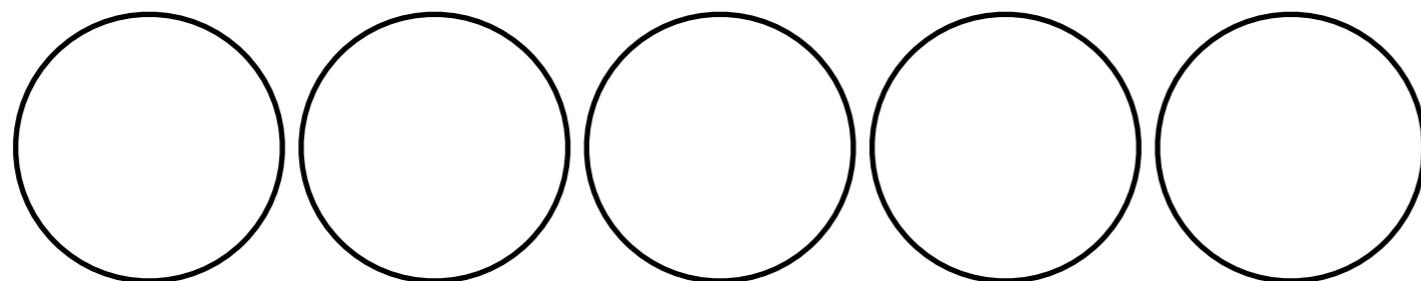
Even when a mutant has mutant alleles, it has the wild-type phenotype.

## **Variable expressivity**

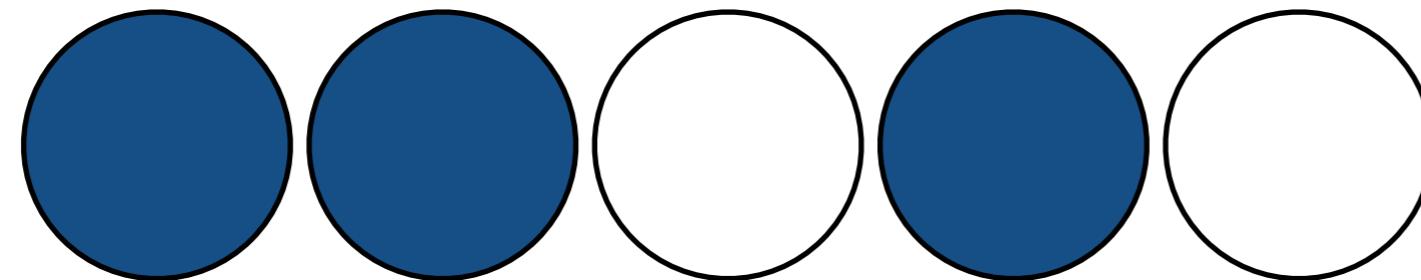
If the mutant has the mutant phenotype, the severity of the mutant phenotype varies from individual to individual.



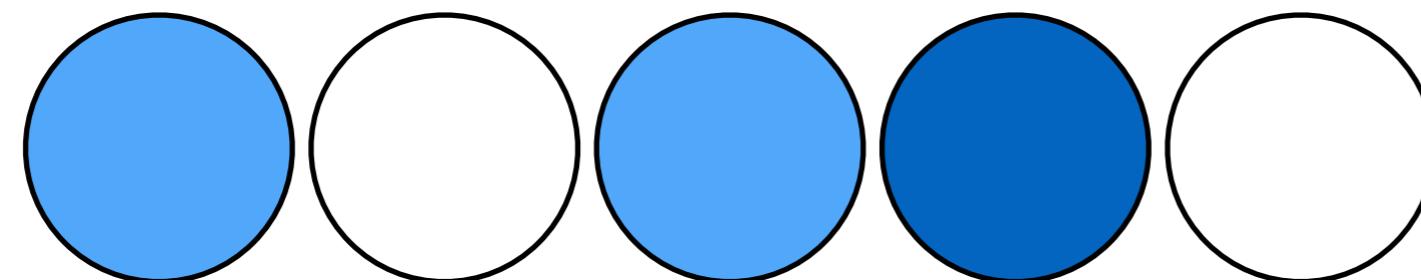
Wild-type



Mutant



Incomplete  
penetrance



Variable  
expressivity



Wild-type worms have one vulva



Multivulva mutant worms have multiple vulvae

**Incomplete penetrance is when not every mutant animal has the mutant phenotype**

117/129 animals are multivulva  
91% penetrant



Wild-type worms have one vulva



Multivulva mutant worms have multiple vulvae

**Variable expressivity is when each mutant animal is not completely mutant**

An animal only has two extra vulvae instead of three.

# Muller's morphs - gene dosage tests

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Altered function	neomorph, antimorph

m = mutation of gene

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= = Phenotype is equivalent

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# Recessive mutant phenotypes

$$\frac{m}{m} > \frac{m}{+} = \frac{+}{+}$$

*amorph*, *null*, or *nullamorph* = mutant causes a complete loss of gene function

$$\frac{m}{m} = \frac{m}{\Delta} > \frac{m}{+} = \frac{\Delta}{+} = \frac{+}{+}$$

*hypomorph* = mutant causes a partial loss of gene function

$$\frac{m}{\Delta} > \frac{m}{m} > \frac{m}{+} = \frac{\Delta}{+} = \frac{+}{+}$$

$m$  = mutation of gene

$\Delta$  = deletion of gene

$+$  = normal copy of gene

$=$  = Phenotype is equivalent

$>$  = Phenotype is more mutant than

$<$  = Phenotype is less mutant than

# Muller's morphs - gene dosage tests

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# Dominant mutant phenotypes

$$\frac{m}{m} \geq \frac{m}{+} > \frac{+}{+}$$

*haploinsufficient* = two wild-type copies are required for normal function

$$\frac{\Delta}{+} \geq \frac{m}{+} > \frac{+}{+}$$

m = mutation of gene

$\Delta$  = deletion of gene

+ = normal copy of gene

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# Muller's morphs - gene dosage tests

Loss of gene function (LoF)	amorph, nullomorph
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# Dominant mutant phenotypes

$$\frac{m}{m} \geq \frac{m}{+} > \frac{+}{+}$$

*hypermorph* = mutant causes an increase in wild-type function

$$\frac{m}{m} > \frac{m}{+} > \frac{m}{+} \geq \frac{+}{+} > \frac{+}{+}$$

m = mutation of gene

Δ = deletion of gene

+ = normal copy of gene

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# Muller's morphs - gene dosage tests

Loss of gene function (LoF)	amorph, nullomorph
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# Dominant mutant phenotypes

$$\frac{m}{m} \geq \frac{m}{+} > \frac{+}{+}$$

*neomorph* = mutant causes function unrelated to normal gene function (abnormal function)

$$\frac{m}{m} \geq \frac{m}{+} = \frac{m}{\Delta} = \frac{m}{\begin{matrix} + \\ - \end{matrix}}$$

m = mutation of gene

Δ = deletion of gene

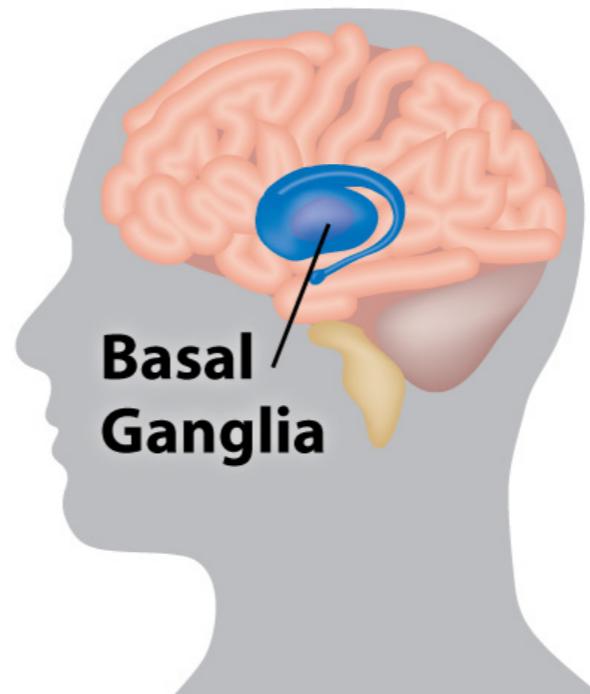
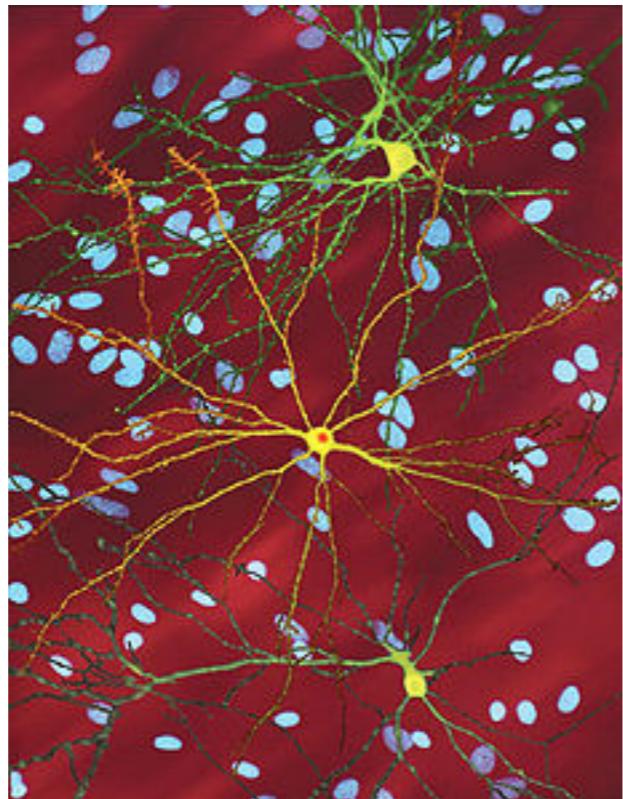
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# Huntington's disease is caused by a neomorphic gain of function



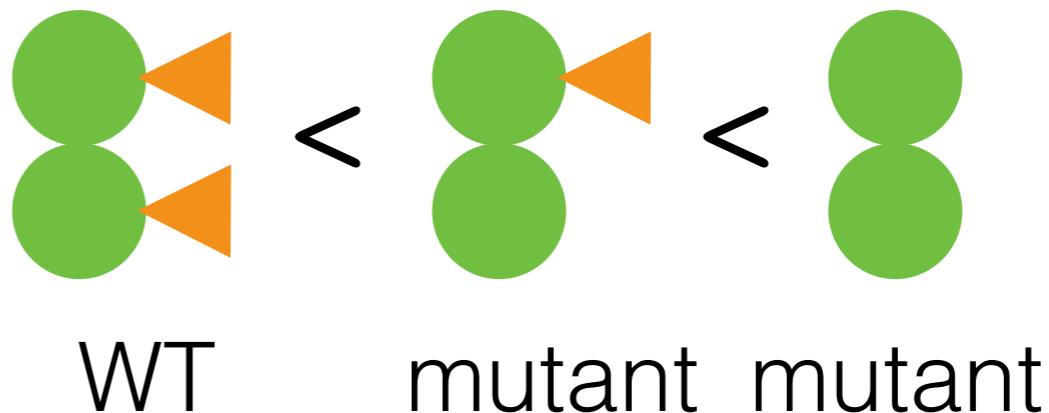
The pathogenic increase in glutamine repeats causes protein aggregation. This phenomenon has nothing to do with normal protein function.

# Dominant mutant phenotypes

$$\frac{m}{m} \geq \frac{m}{+} > \frac{+}{+}$$

*antimorph* = mutant causes dominant loss of gene function  
dominant negative

$$\frac{m}{+} < \frac{m}{+} < \frac{m}{m} \leq \frac{m}{\Delta}$$



m = mutation of gene

$\Delta$  = deletion of gene

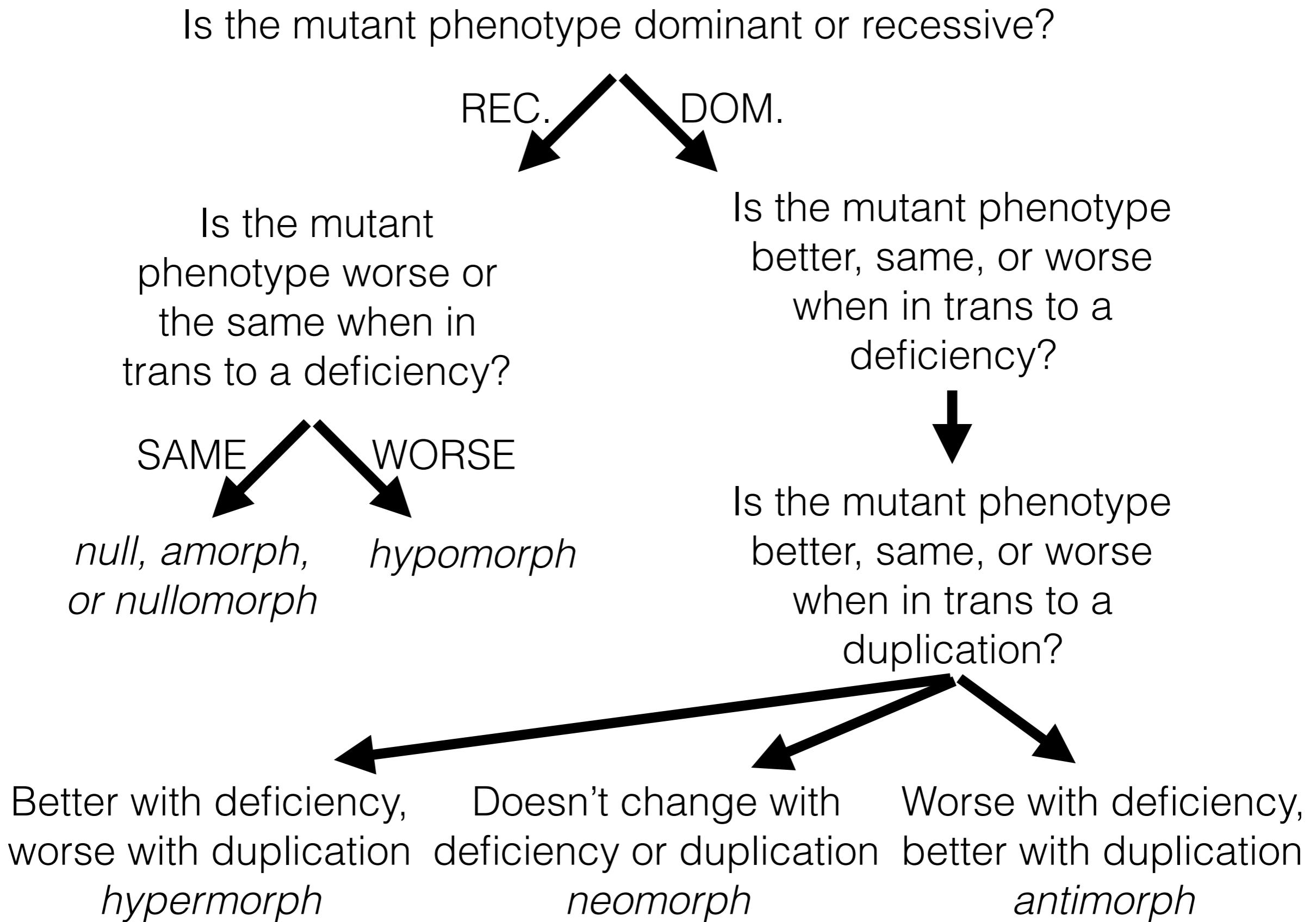
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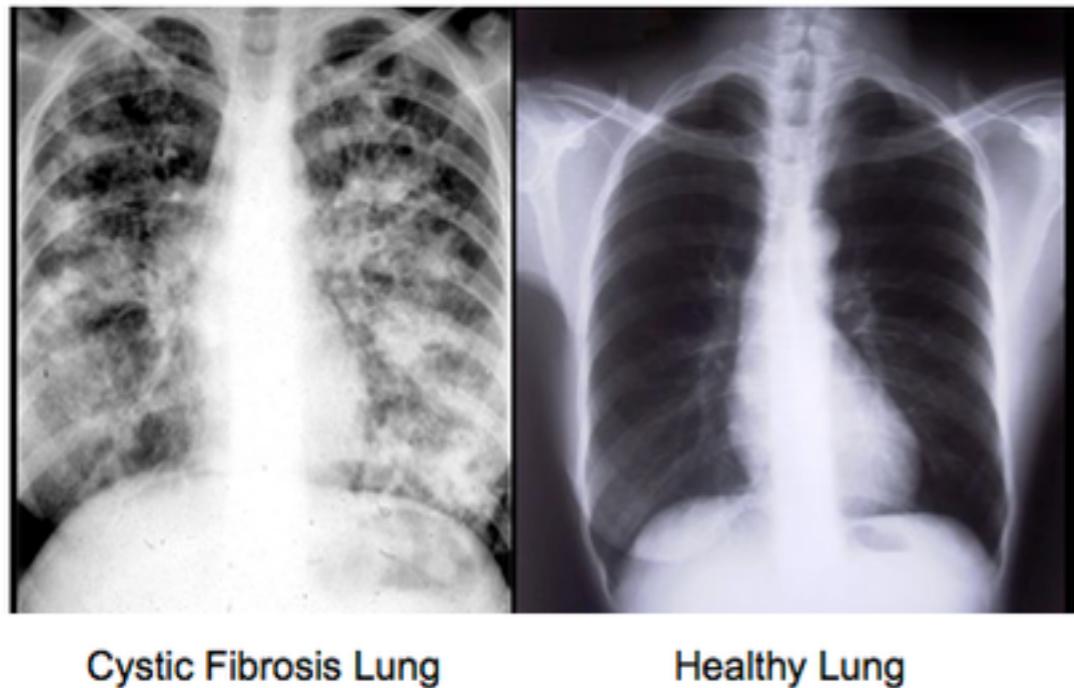
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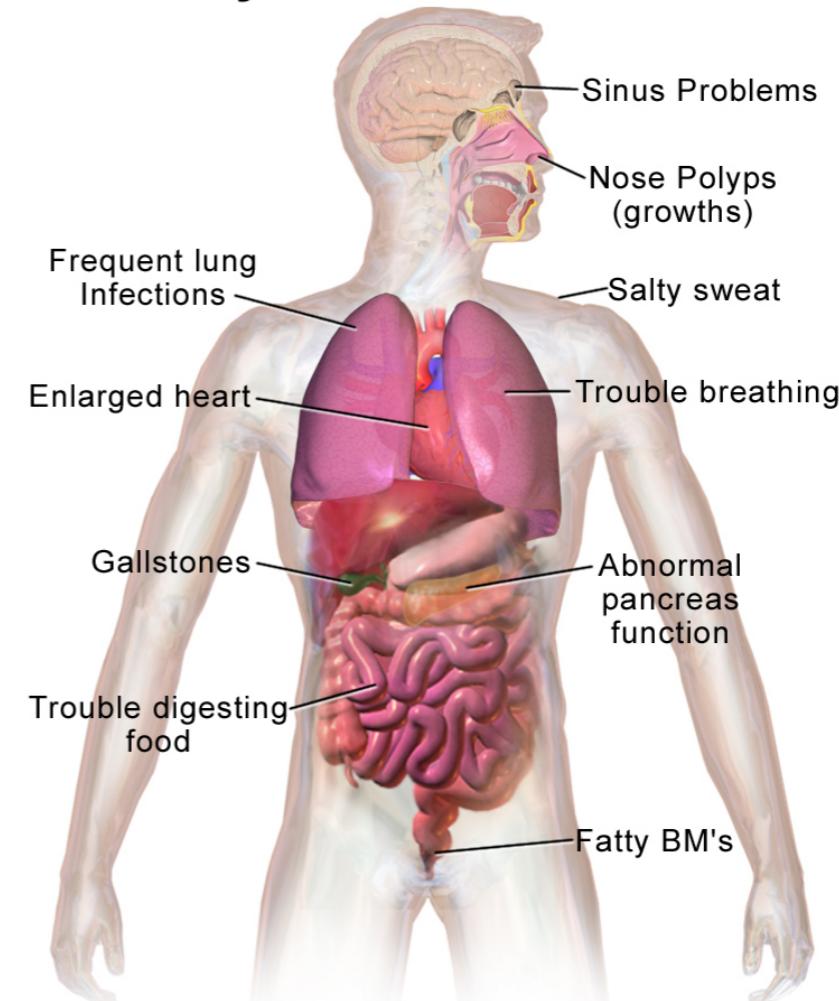
# Flow chart for gene dosage studies



# What about cystic fibrosis and today's topic?

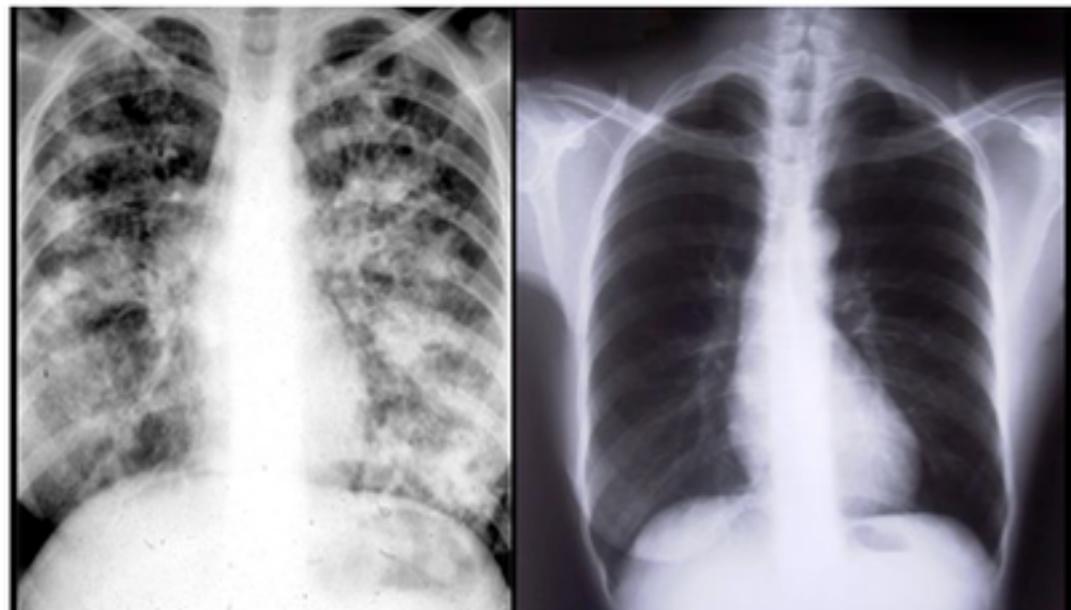


## Health Problems with Cystic Fibrosis



1. Autosomal recessive disorder
2. Not caused by chromosomal aberrations or meiotic NDJ
3. Mapped to chromosome 7

# CF is an autosomal recessive disorder



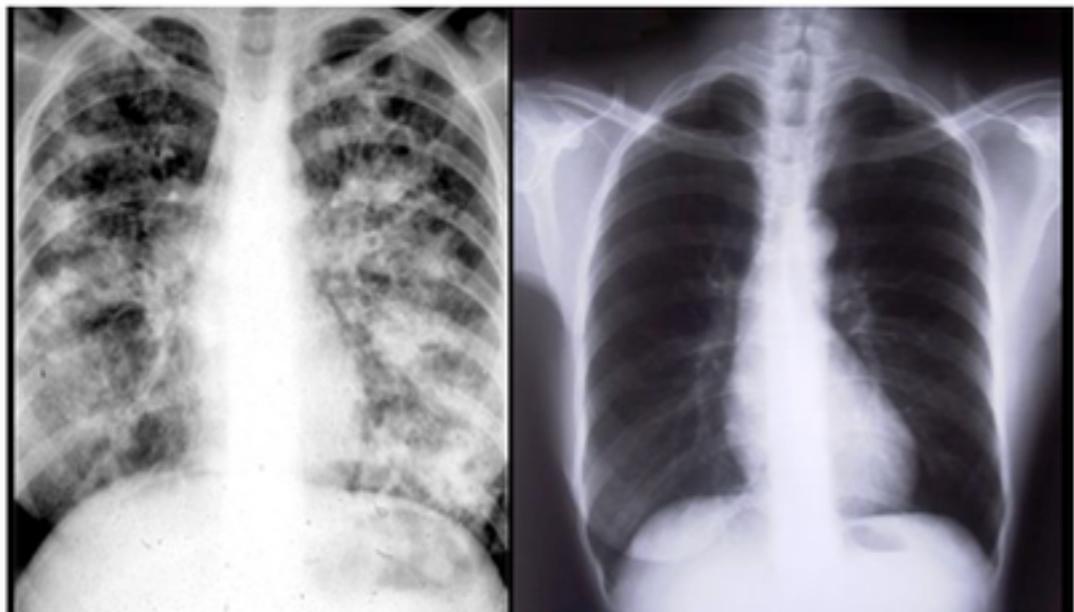
null or hypomorph?

Cystic Fibrosis Lung

Healthy Lung

CF allele	Severity	Survival (yrs)	Prevalence in pop.
F508del	High	36.3	~83%
G542X	High	36.3	~5%
I507del	High	36.3	~0.8%
R347P	Medium	50.0	~0.6%

# CF is an autosomal recessive disorder



Cystic Fibrosis Lung

Healthy Lung

null or hypomorph

How do we do gene dosage tests in humans?