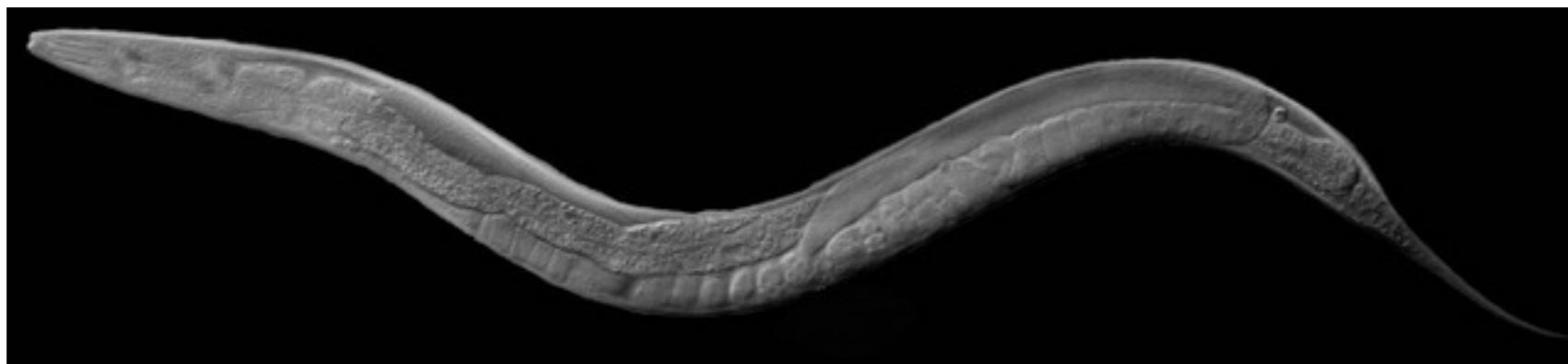
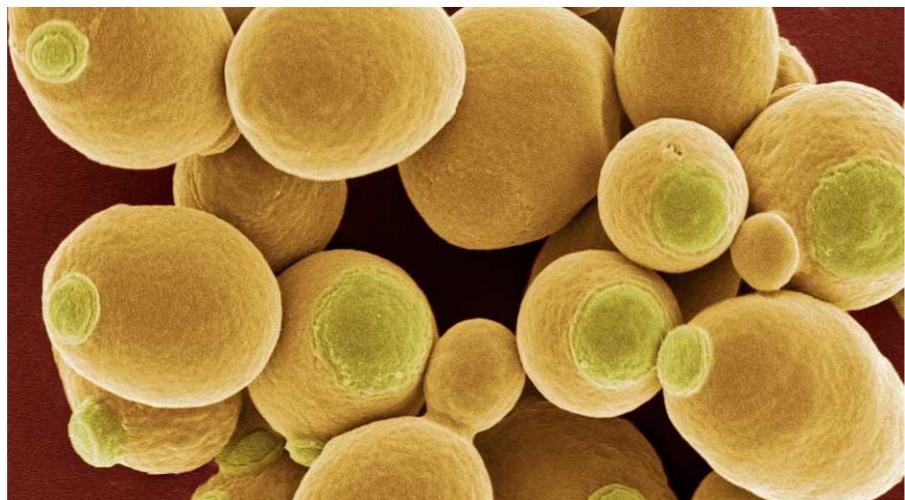


Bio393: Genetic Analysis

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

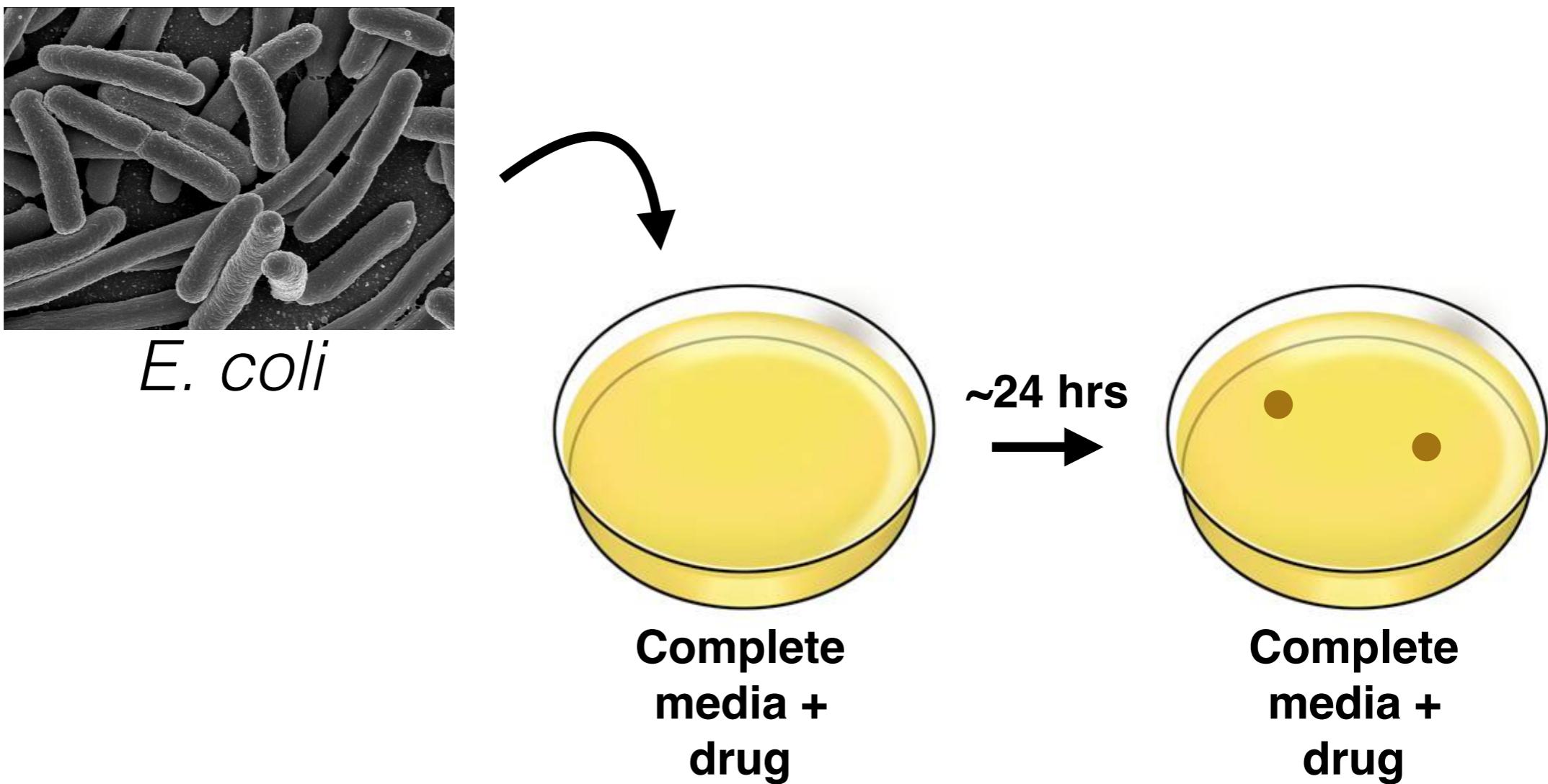
Selection: You only get the mutants you want

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

You don't always get what you want!

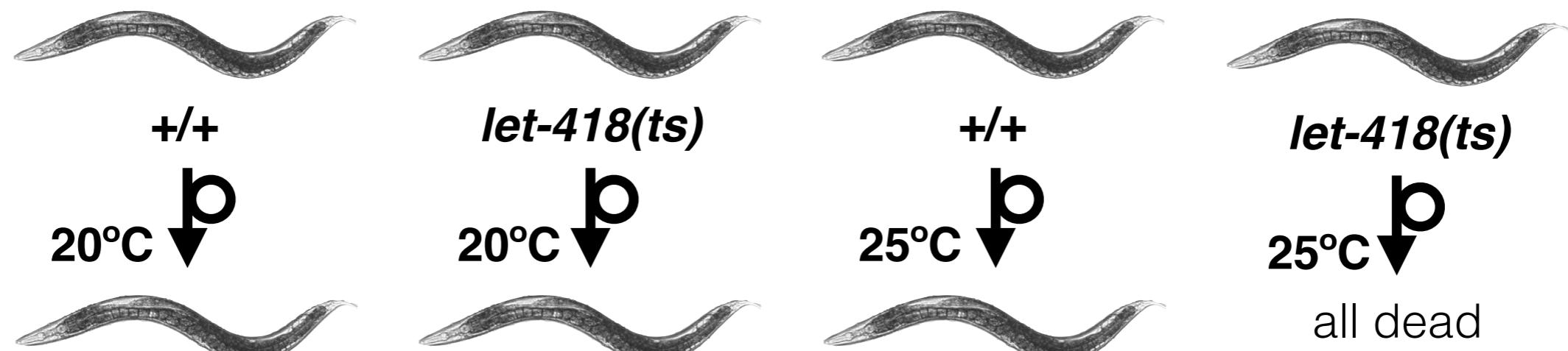
Two ways to isolate mutants: selection or screen

Selection:

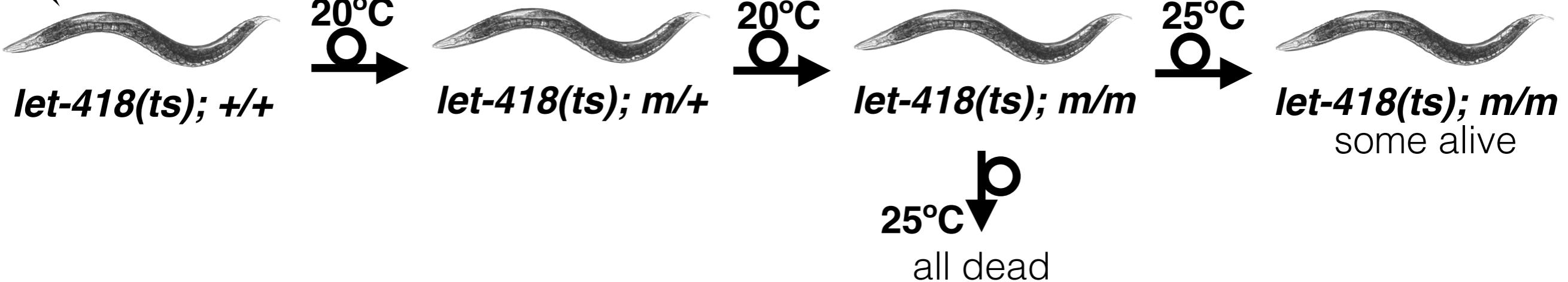


Two ways to isolate mutants: selection or screen

Selection:

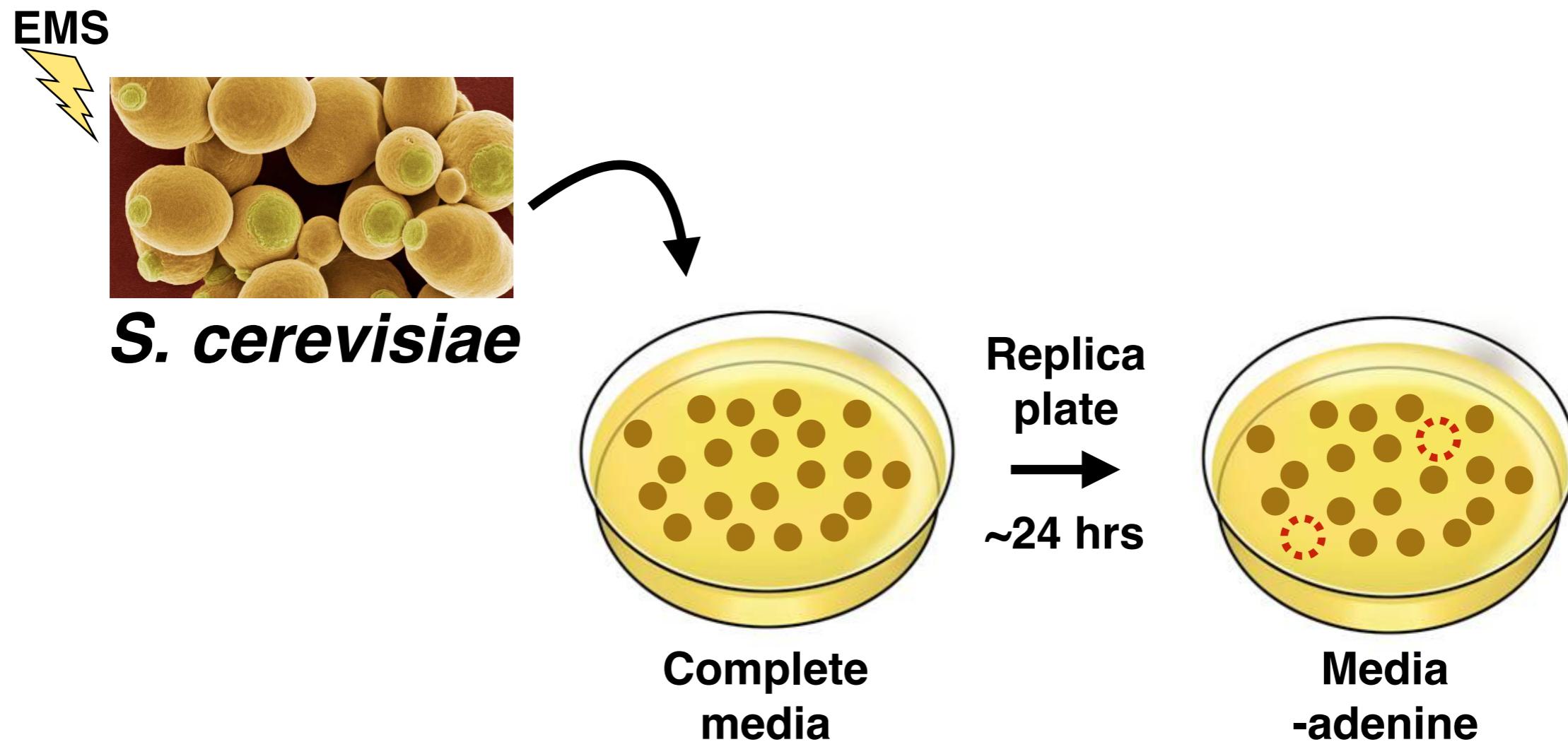


EMS
⚡



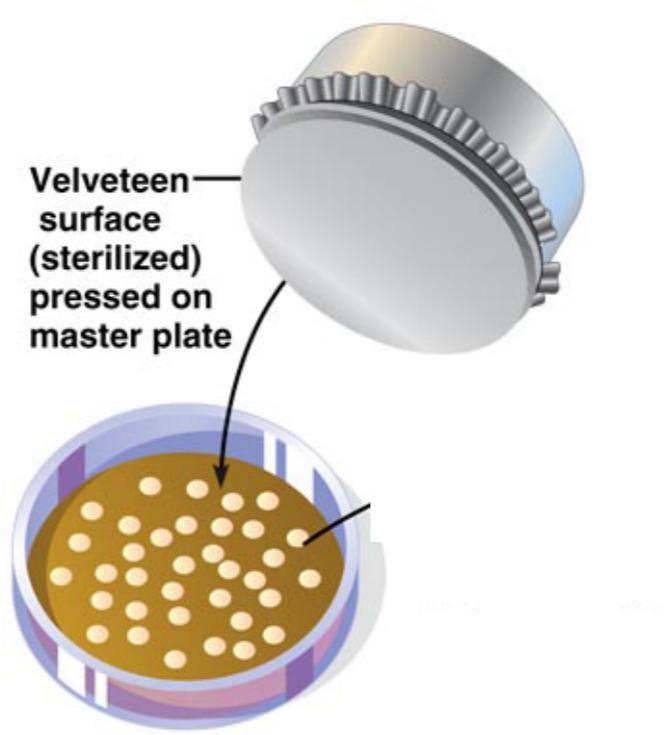
Two ways to isolate mutants: selection or screen

Screen:



Why not directly plate on -adenine media?

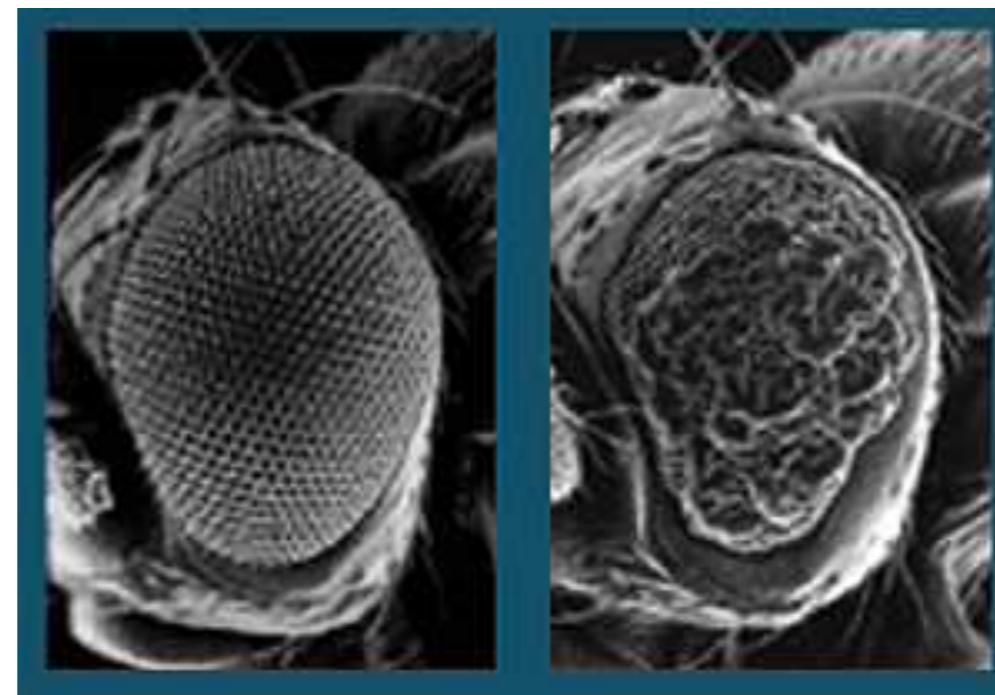
Replica plating



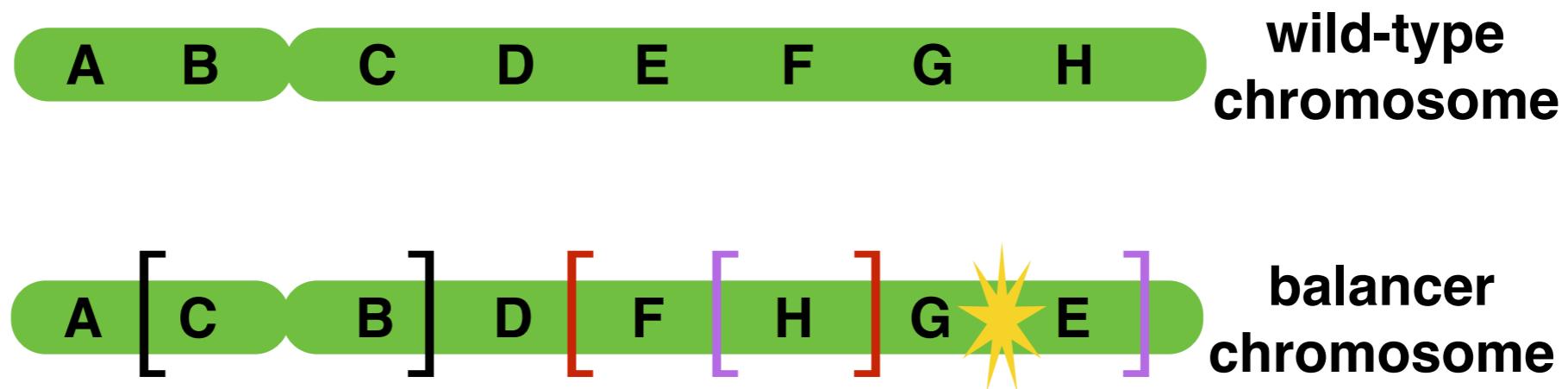
Two ways to isolate mutants: selection or screen



D. melanogaster



Drosophila have balancer chromosomes



Every balancer chromosome:

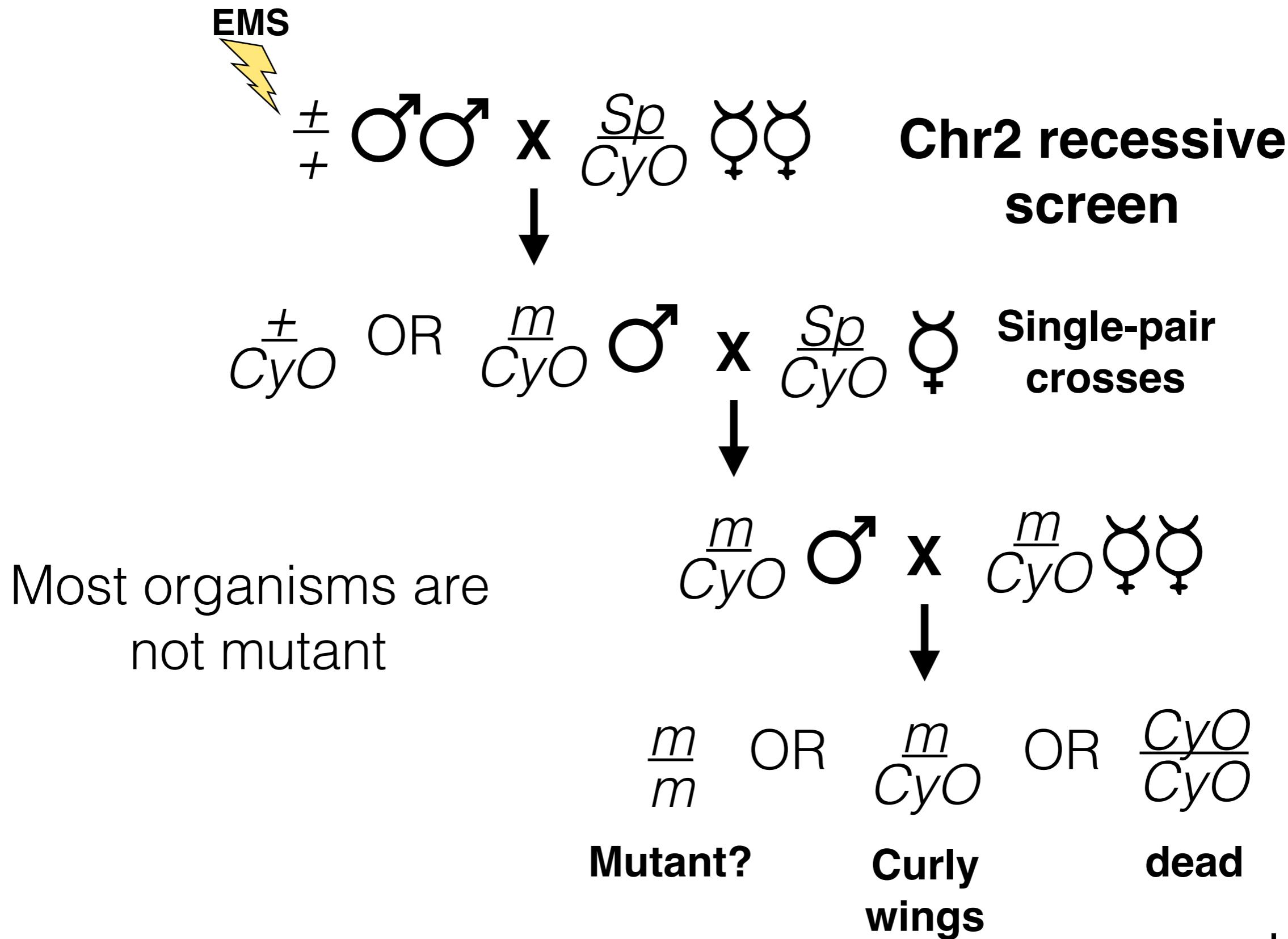
1. has many inversions to eliminate recombination
2. confers an easily scored dominant phenotype
3. is recessive lethal



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 $\frac{m}{+}$



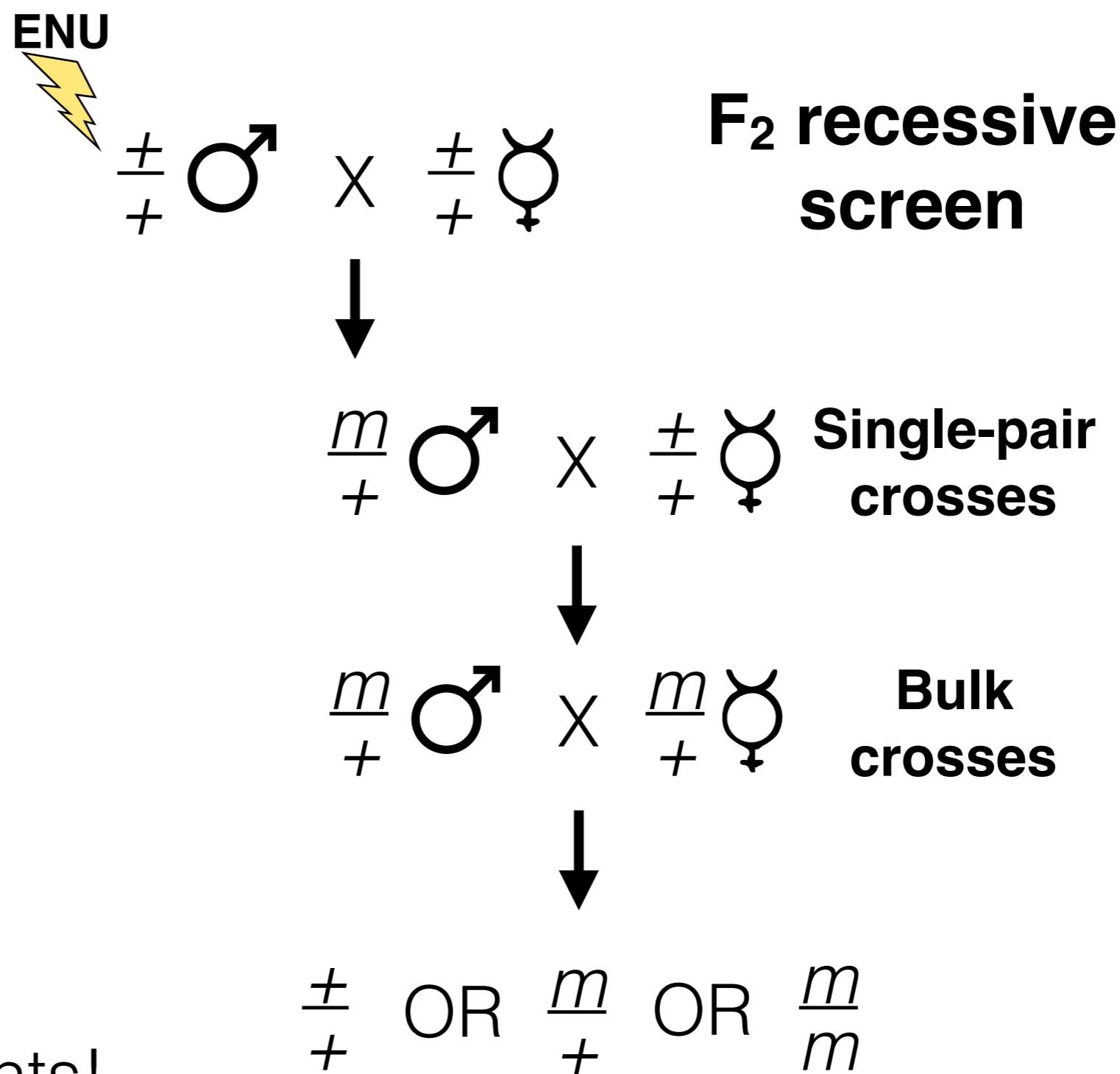
\pm OR $\frac{m}{+}$ OR $\frac{m}{m}$

F₂ 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?

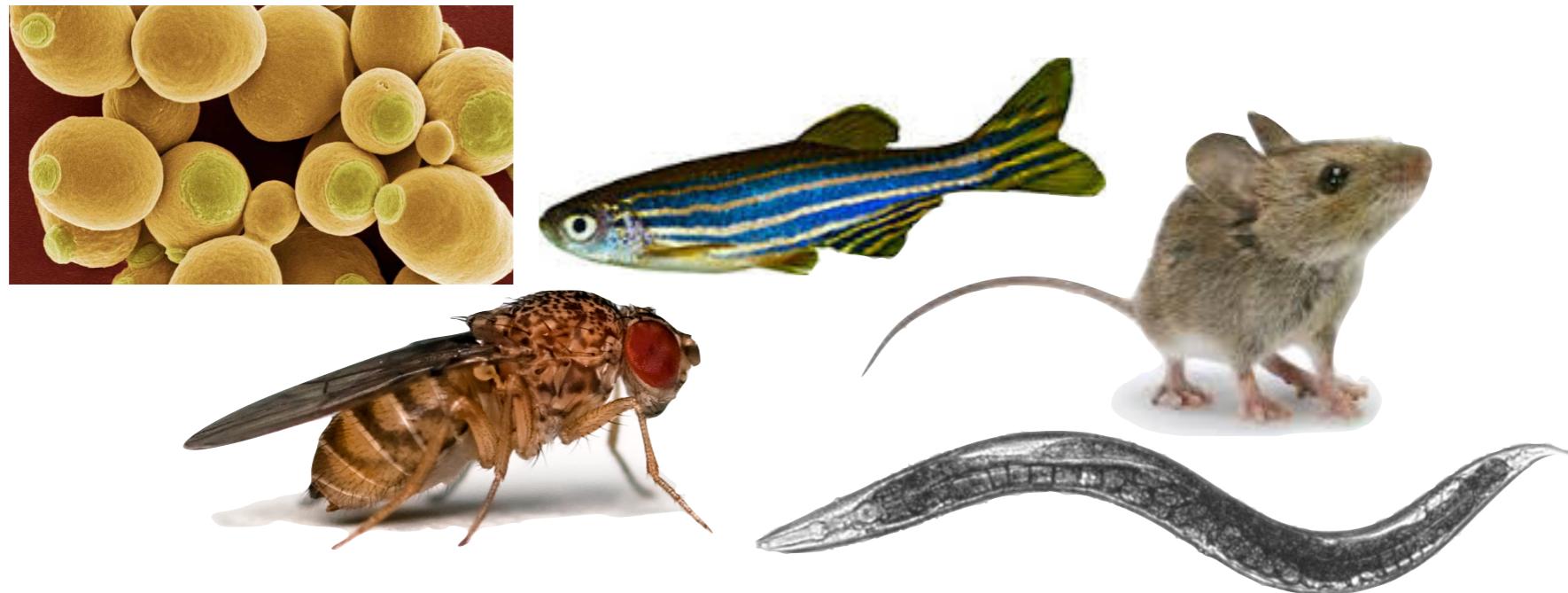
Recessive alleles teach us about:

- 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?

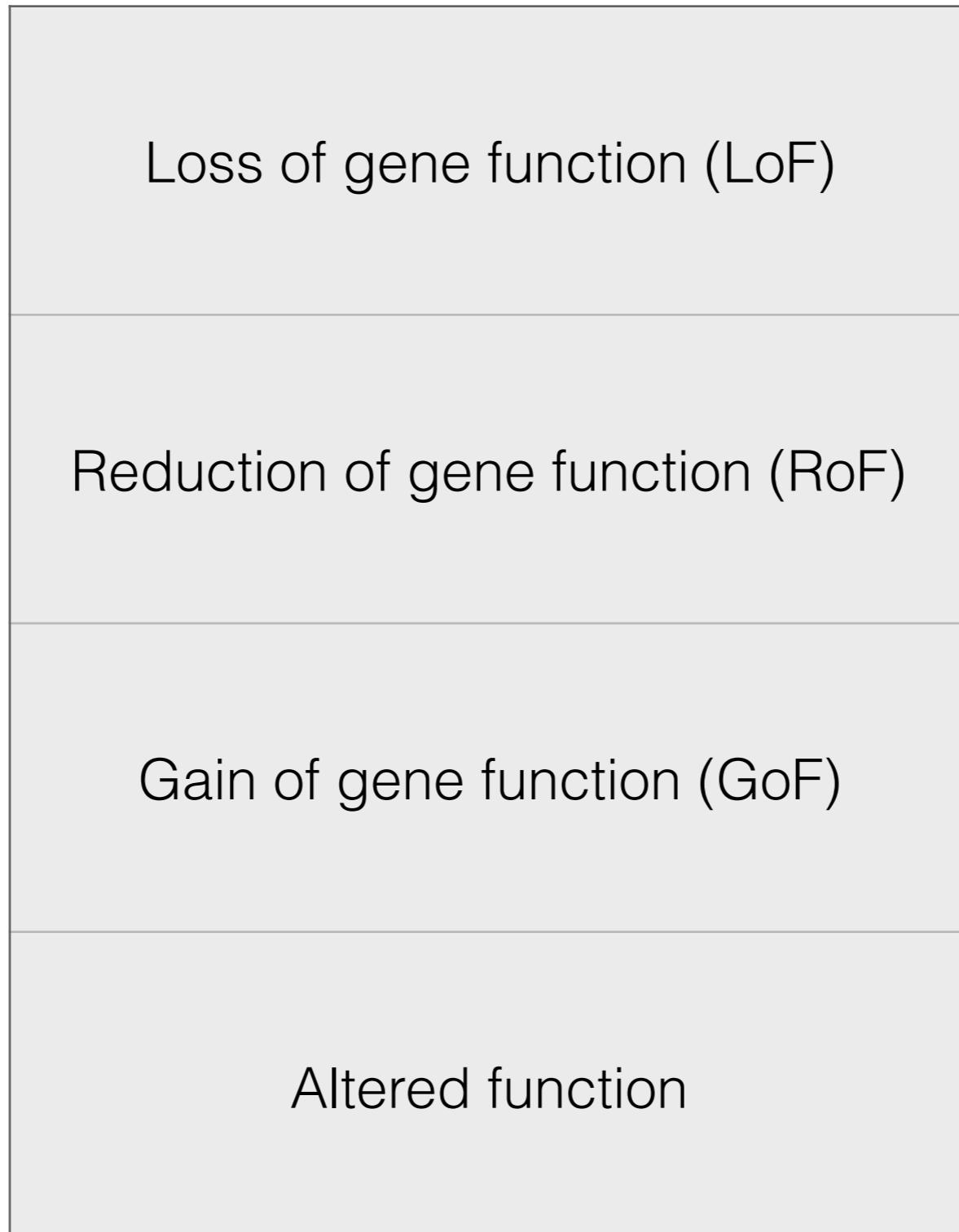


Mutations occur in the DNA of somatic and germline cells

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?



Common

Rare

**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

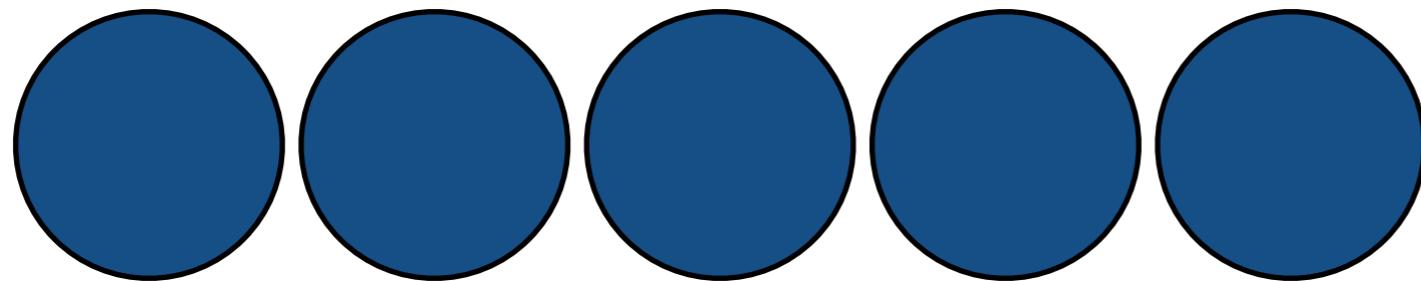
How do you get strains that are more or less mutant?

Incomplete penetrance

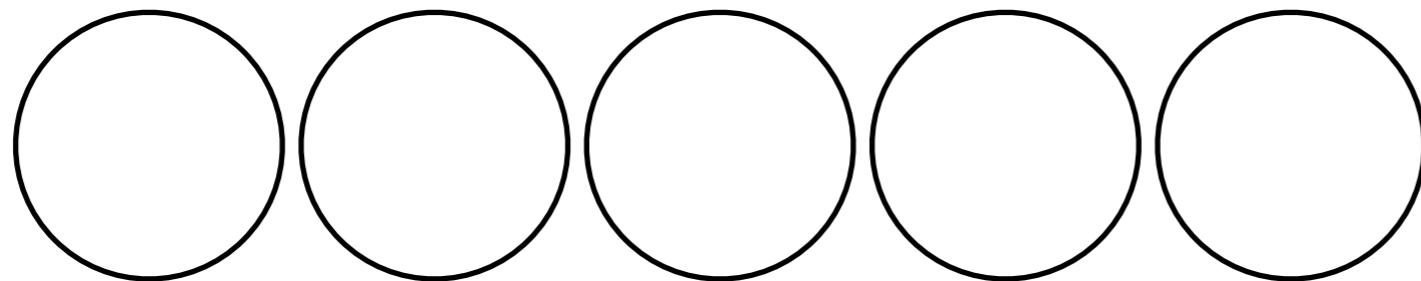
Even when a mutant has mutant alleles, it has the wild-type phenotype.

Variable expressivity

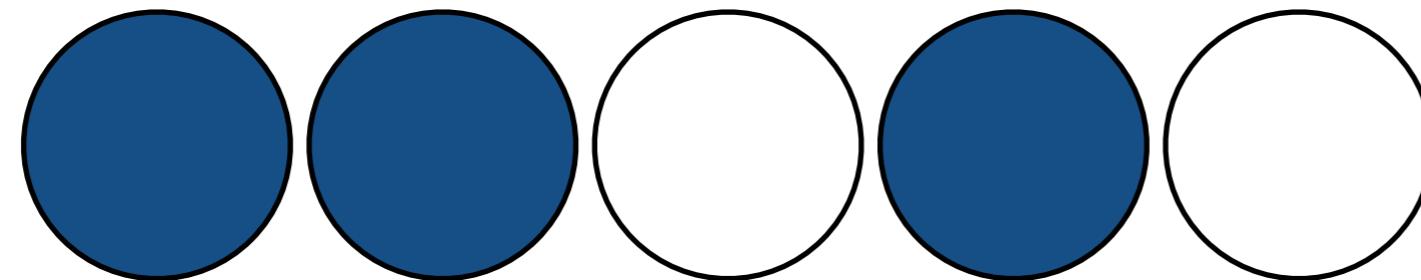
If the mutant has the mutant phenotype, the severity of the mutant phenotype varies from individual to individual.



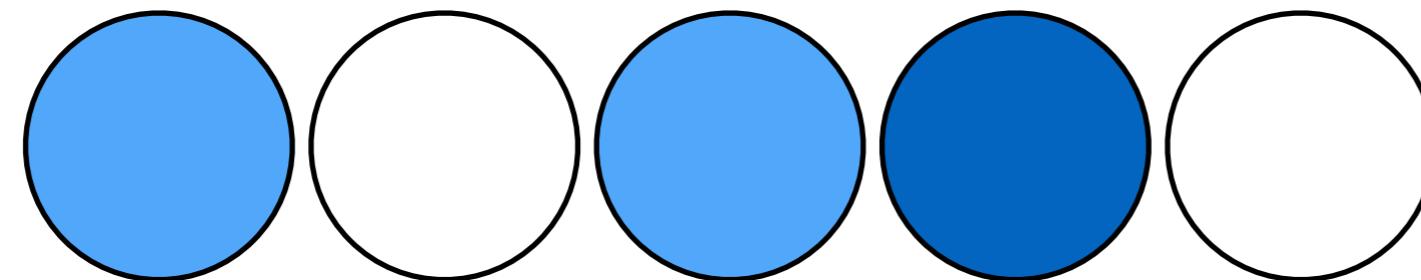
Wild-type



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

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

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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

Δ = deletion of gene

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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

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

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Δ = deletion of gene

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Muller's morphs - gene dosage tests

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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

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Muller's morphs - gene dosage tests

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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

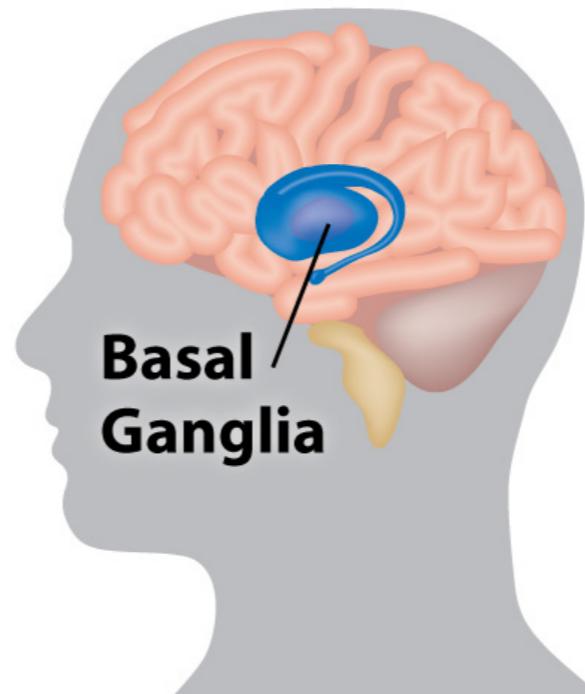
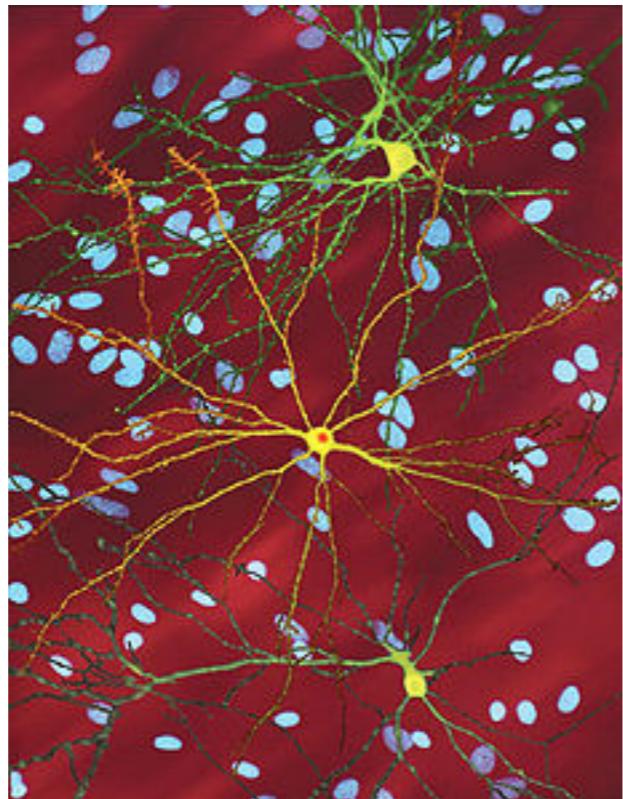
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Huntington's disease is caused by a neomorphic gain of function



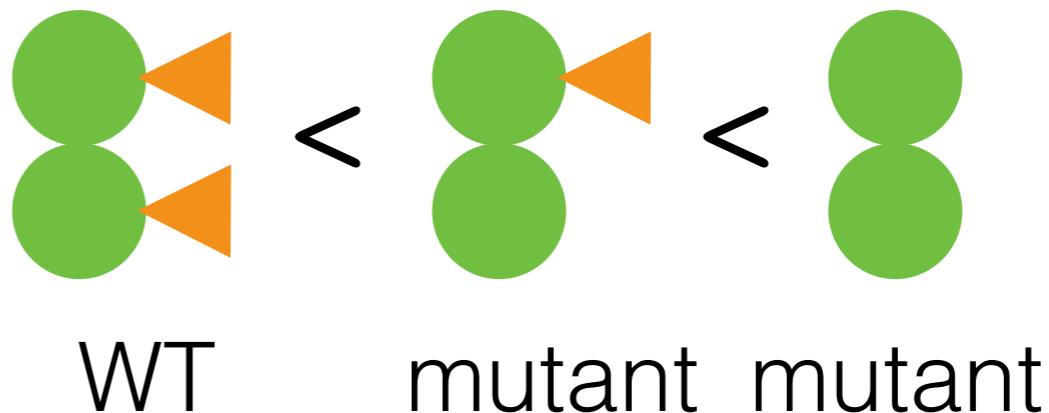
The pathogenic increase in glutamine repeats causes protein aggregation. This phenomenon has nothing to do with normal protein function.

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

Δ = deletion of gene

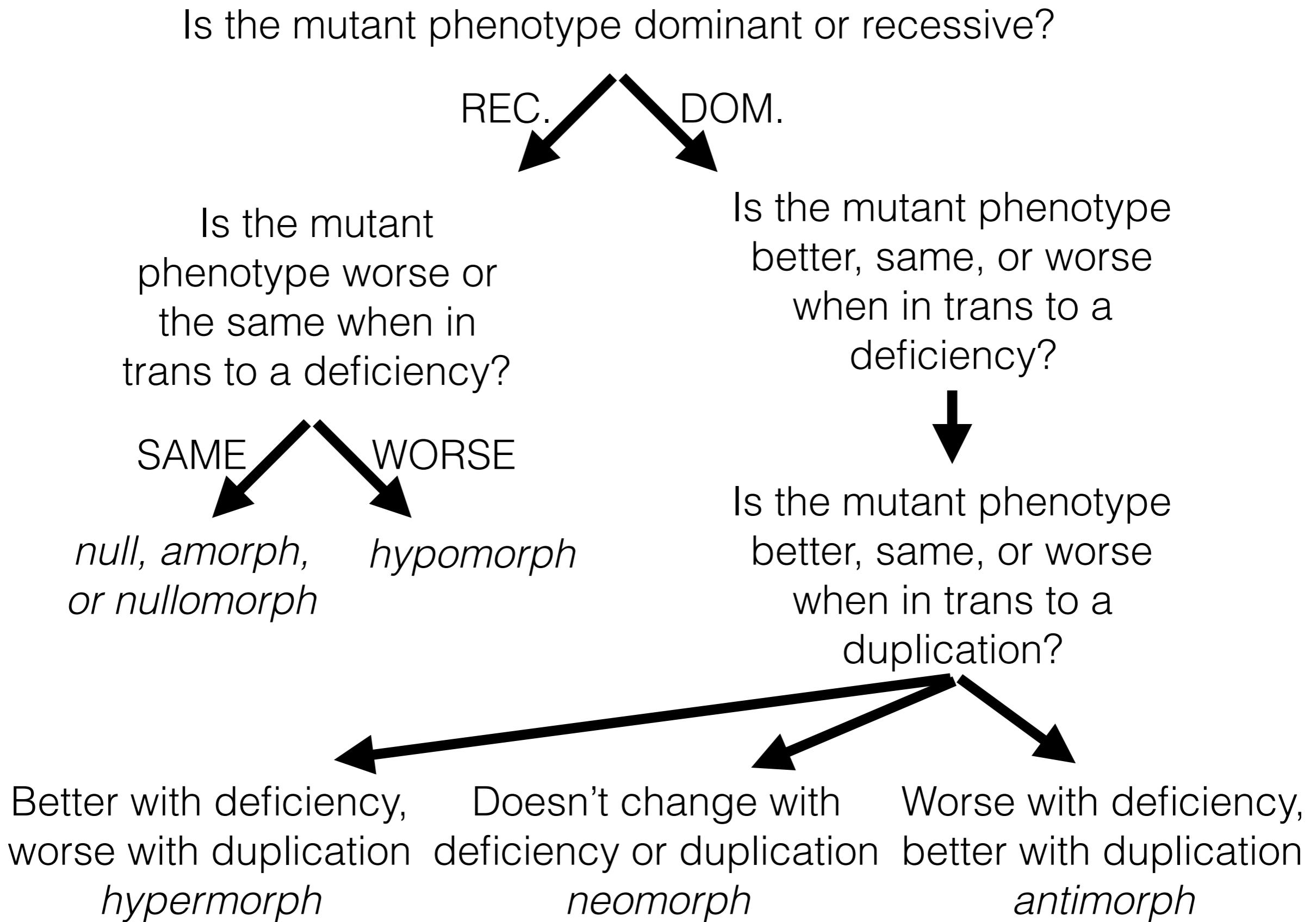
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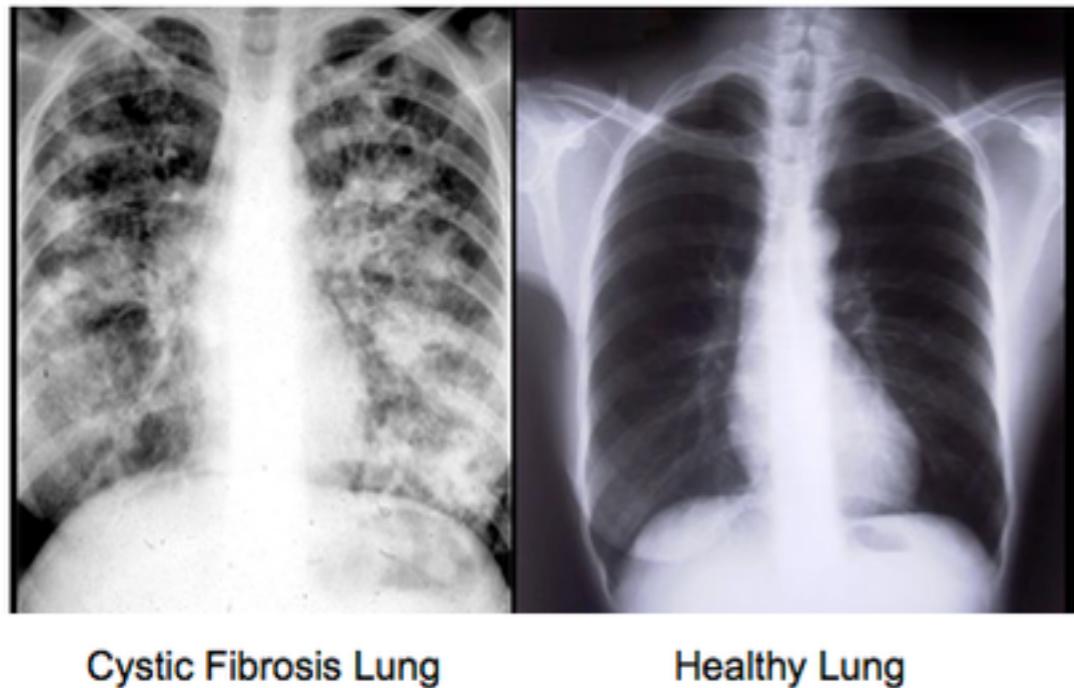
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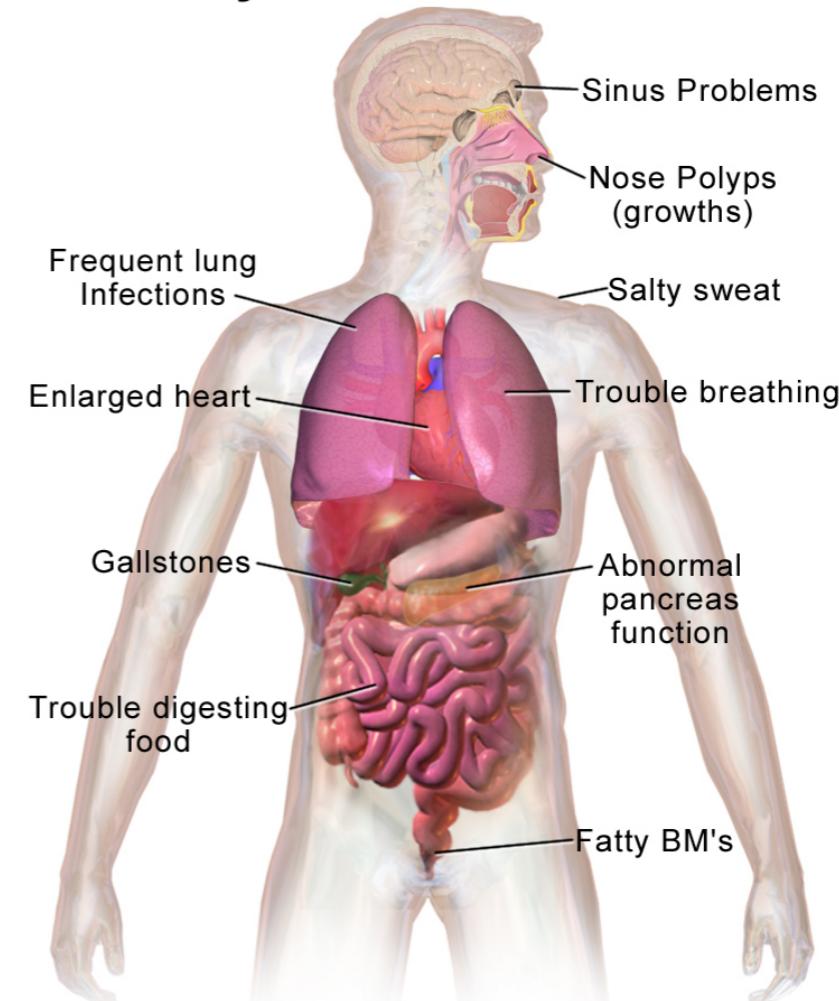
Flow chart for gene dosage studies



What about cystic fibrosis and today's topic?

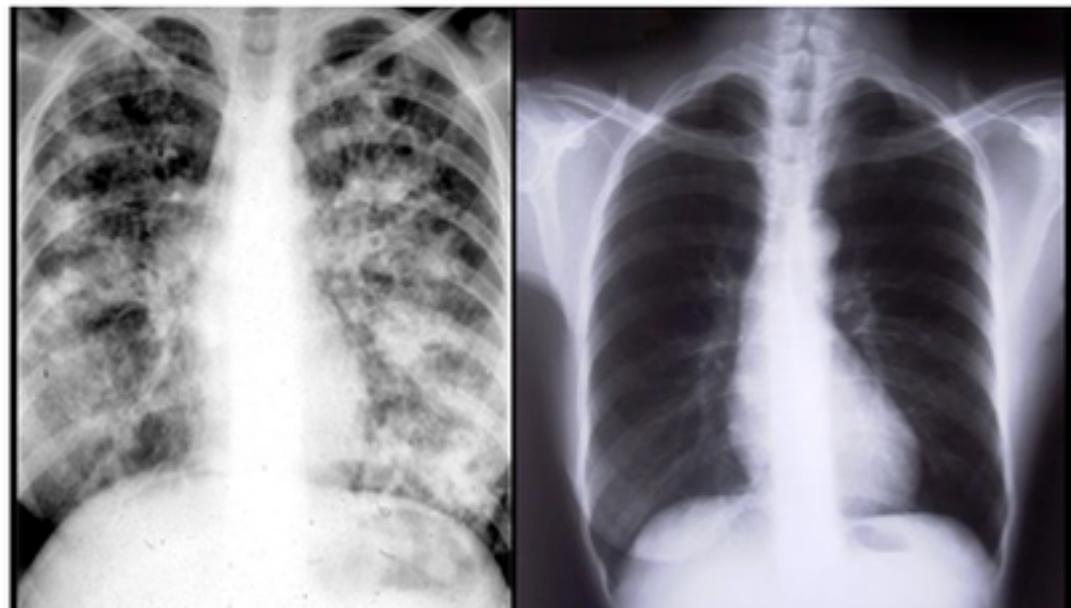


Health Problems with Cystic Fibrosis



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

CF is an autosomal recessive disorder



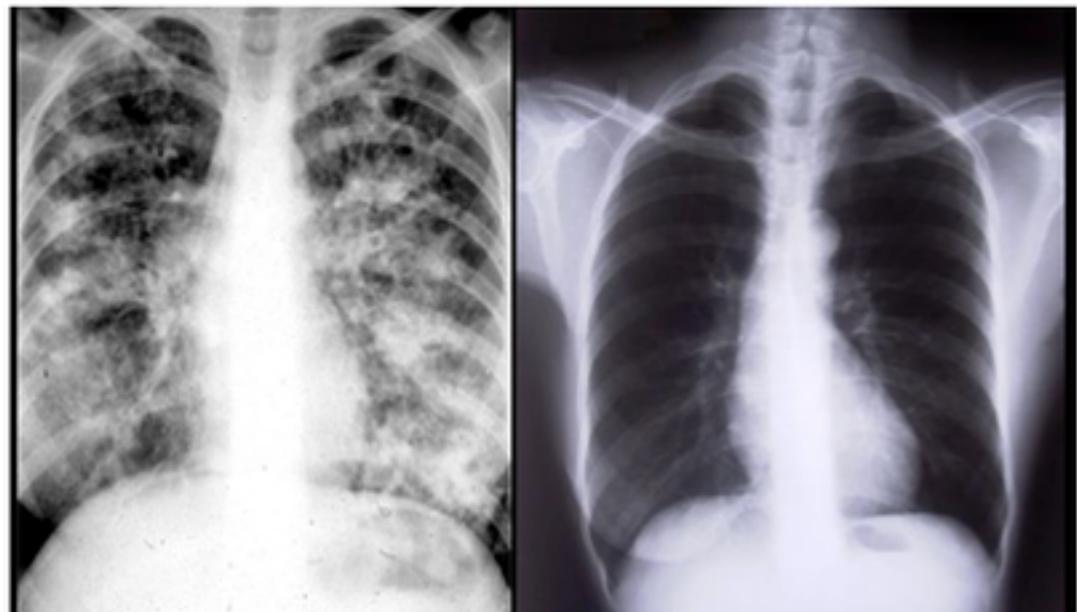
null or hypomorph?

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?