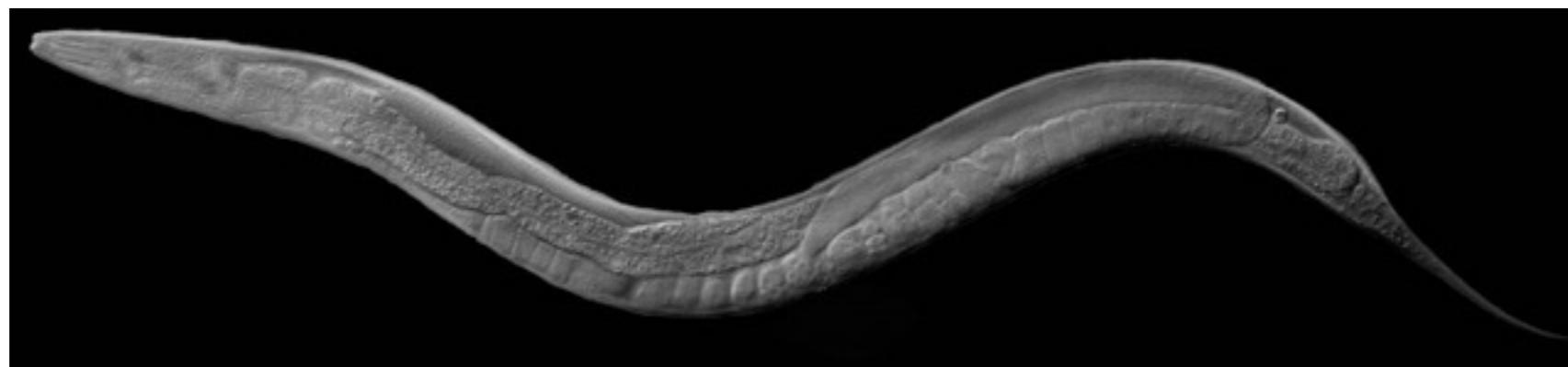
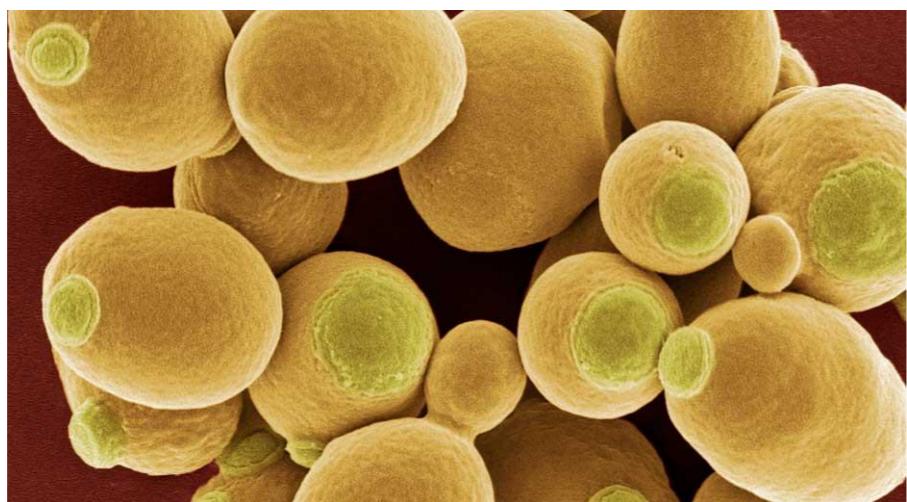


Screens, selections, mutants, dosage



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

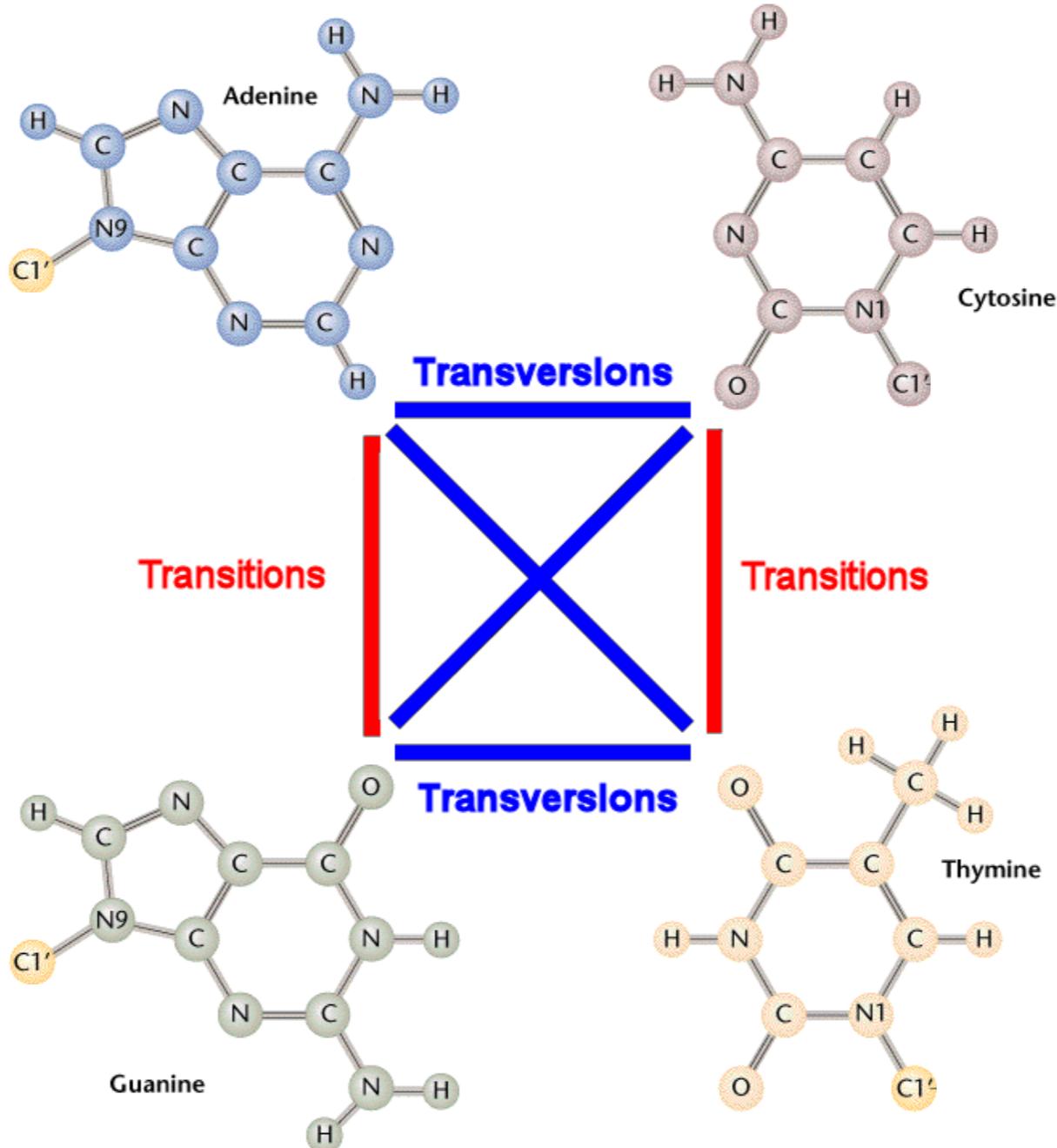
Negatives: Strong effects, not full mutation spectrum open, less efficient than mutagen

CRISPR/Cas9, targeted mutations

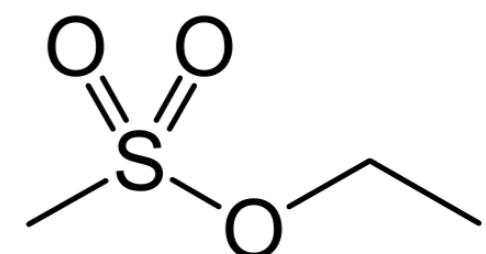
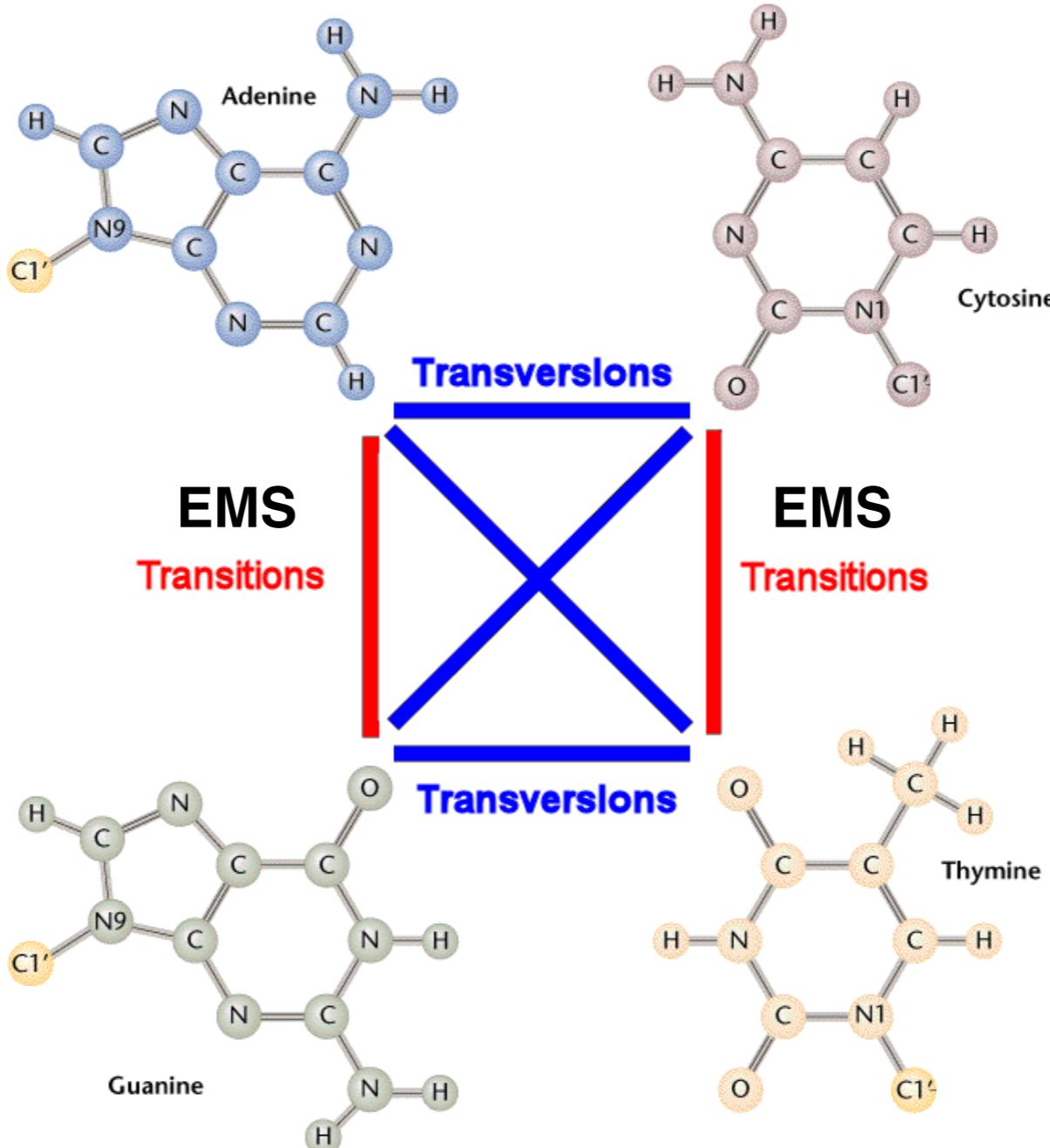
Positives: Targeted, Designable, Defined genetic background, Scalable

Negatives: Off target effects? Delivery?

Single-base substitutions

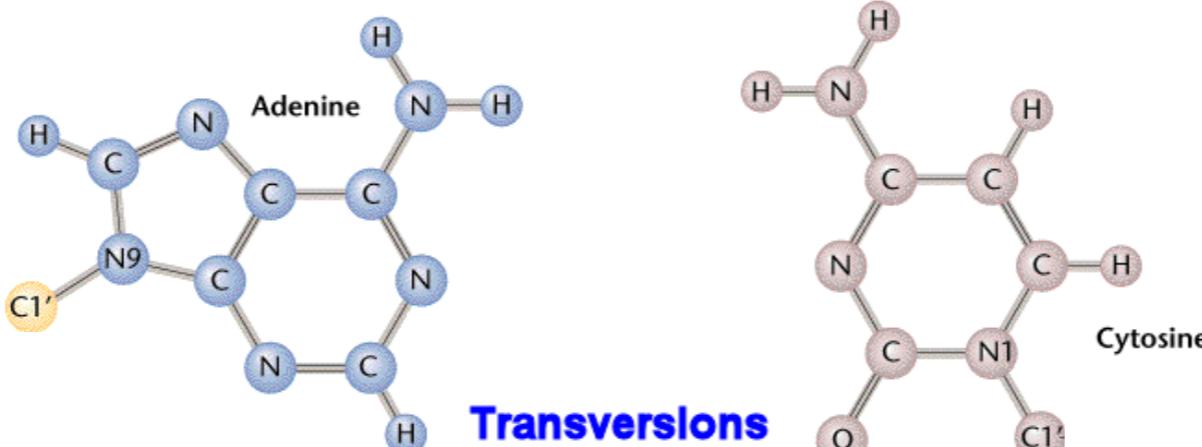


Single-base substitutions



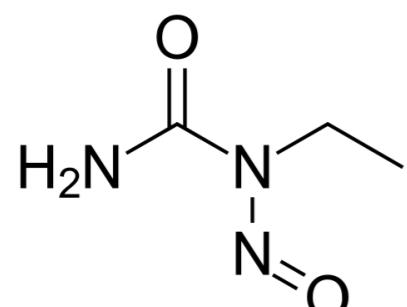
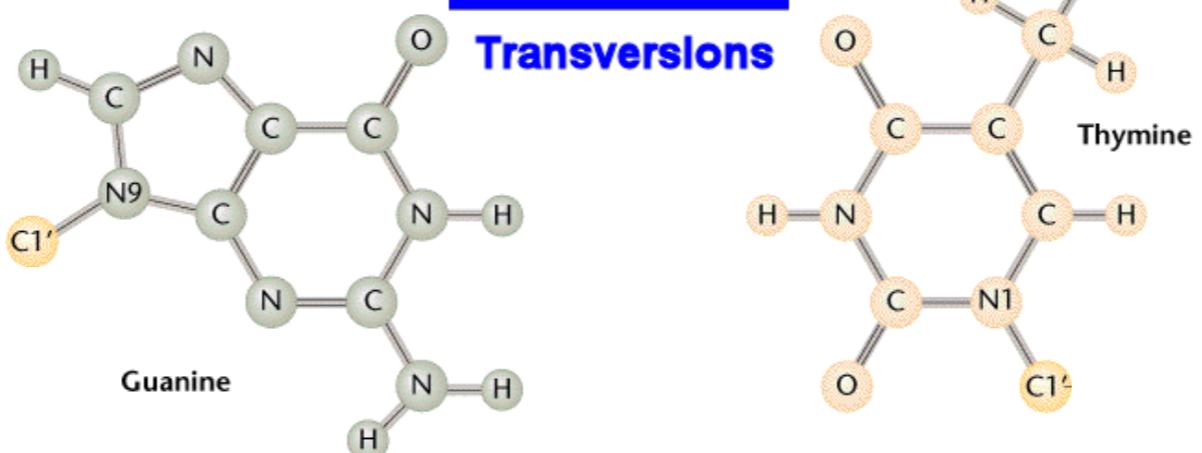
Ethyl methanesulfonate
(EMS)

Single-base substitutions

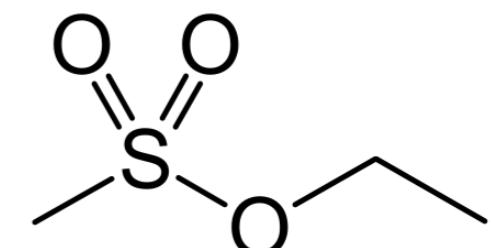


EMS
Transitions

ENU



Ethyl-nitrosourea
(ENU)



Ethyl methanesulfonate
(EMS)

Two ways to isolate mutants: selection or screen

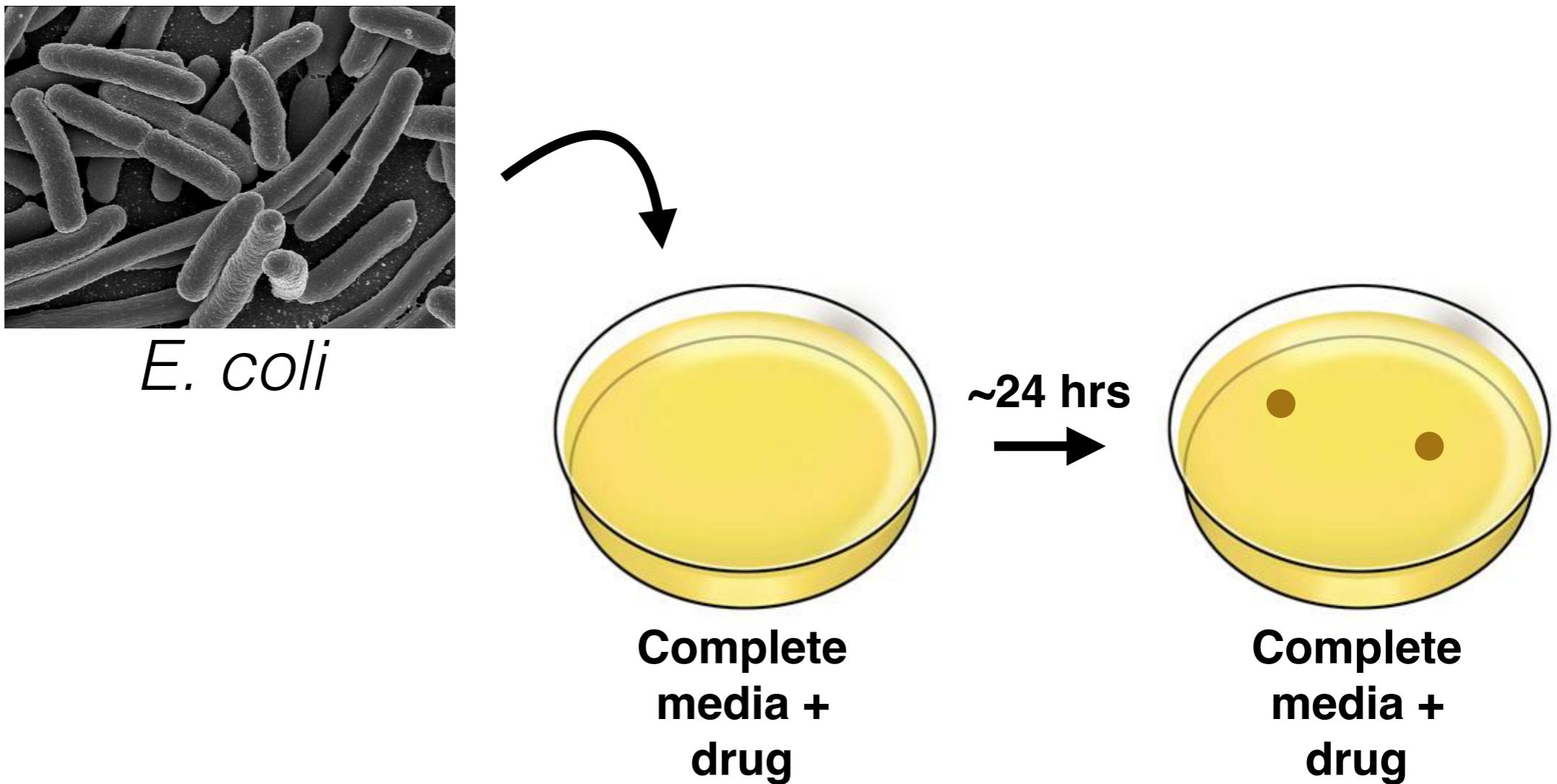
Selection: You only see mutants

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

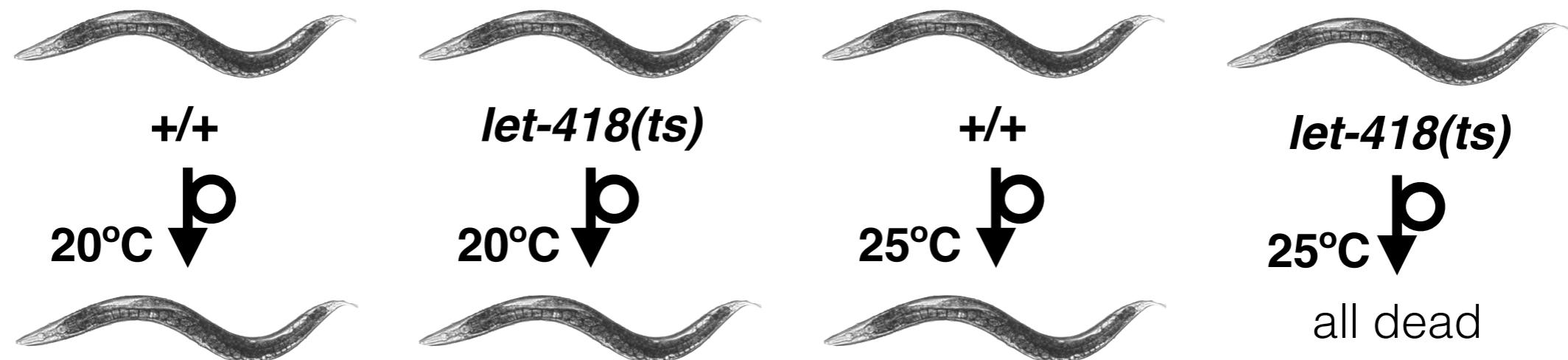
Selection:



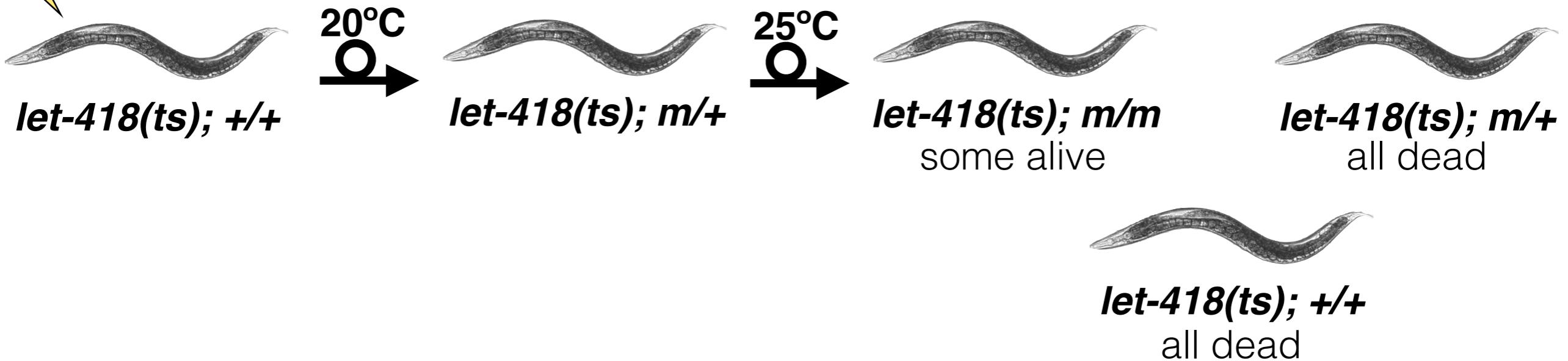
Goal: Find mutants that can grow on a specific drug

Two ways to isolate mutants: selection or screen

Selection:



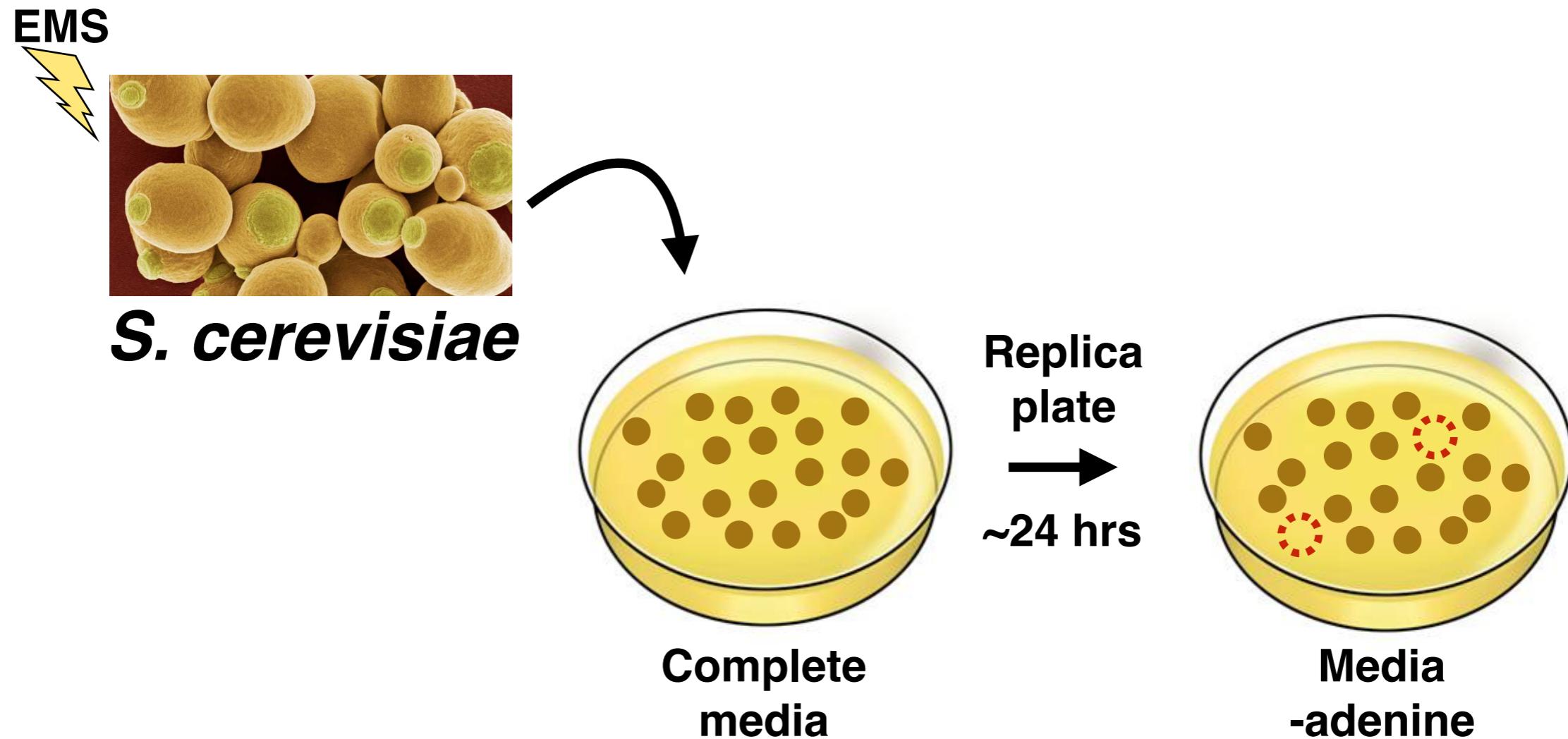
EMS
⚡



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

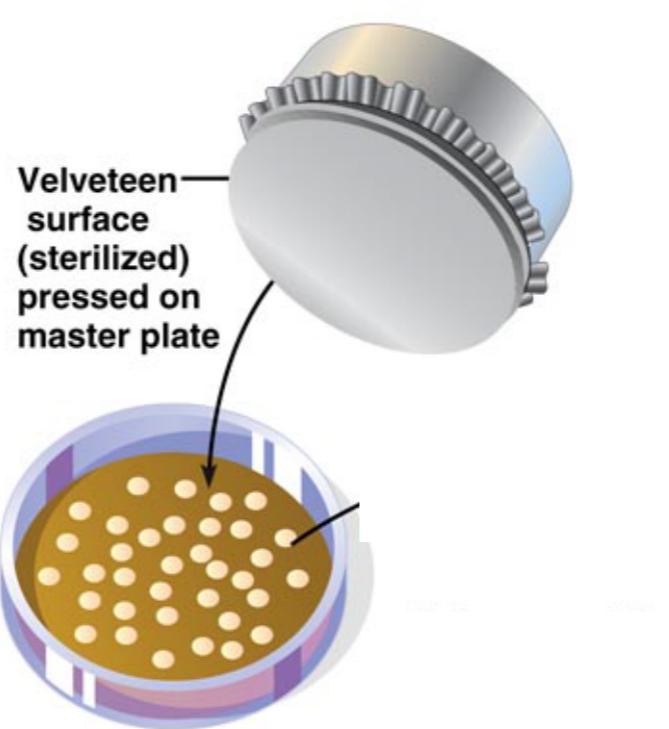
Two ways to isolate mutants: selection or screen

Screen:



Why not directly plate on media lacking adenine?

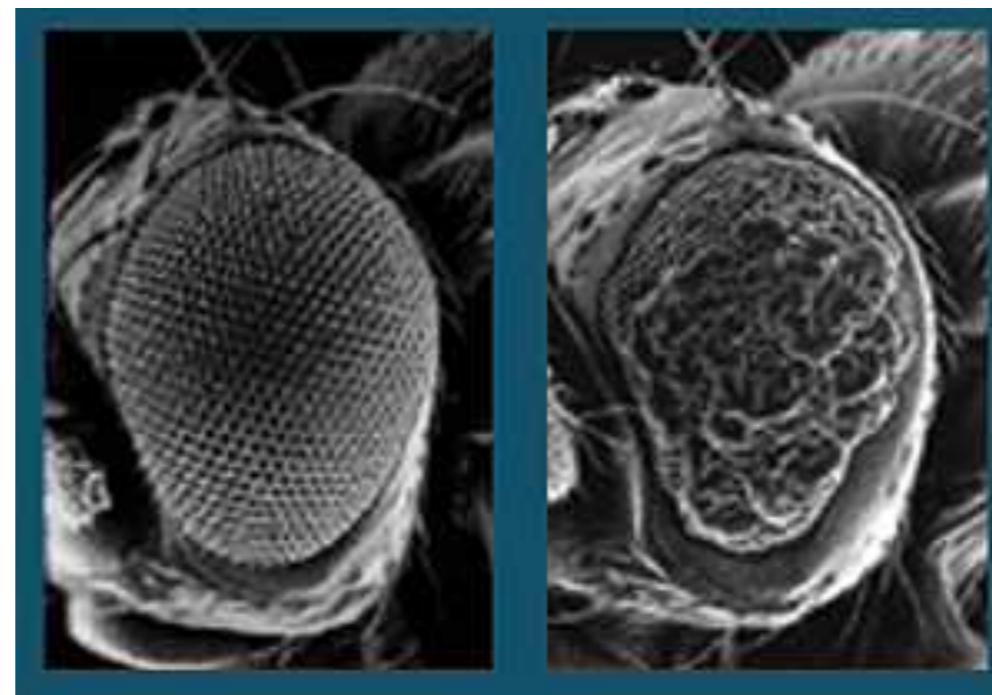
Replica plating



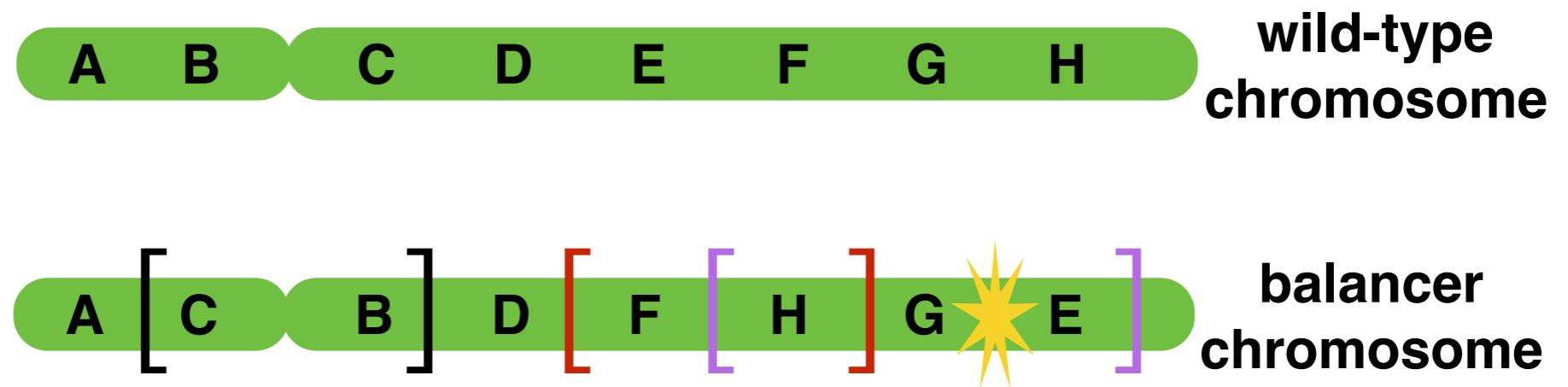
Two ways to isolate mutants: selection or screen



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**



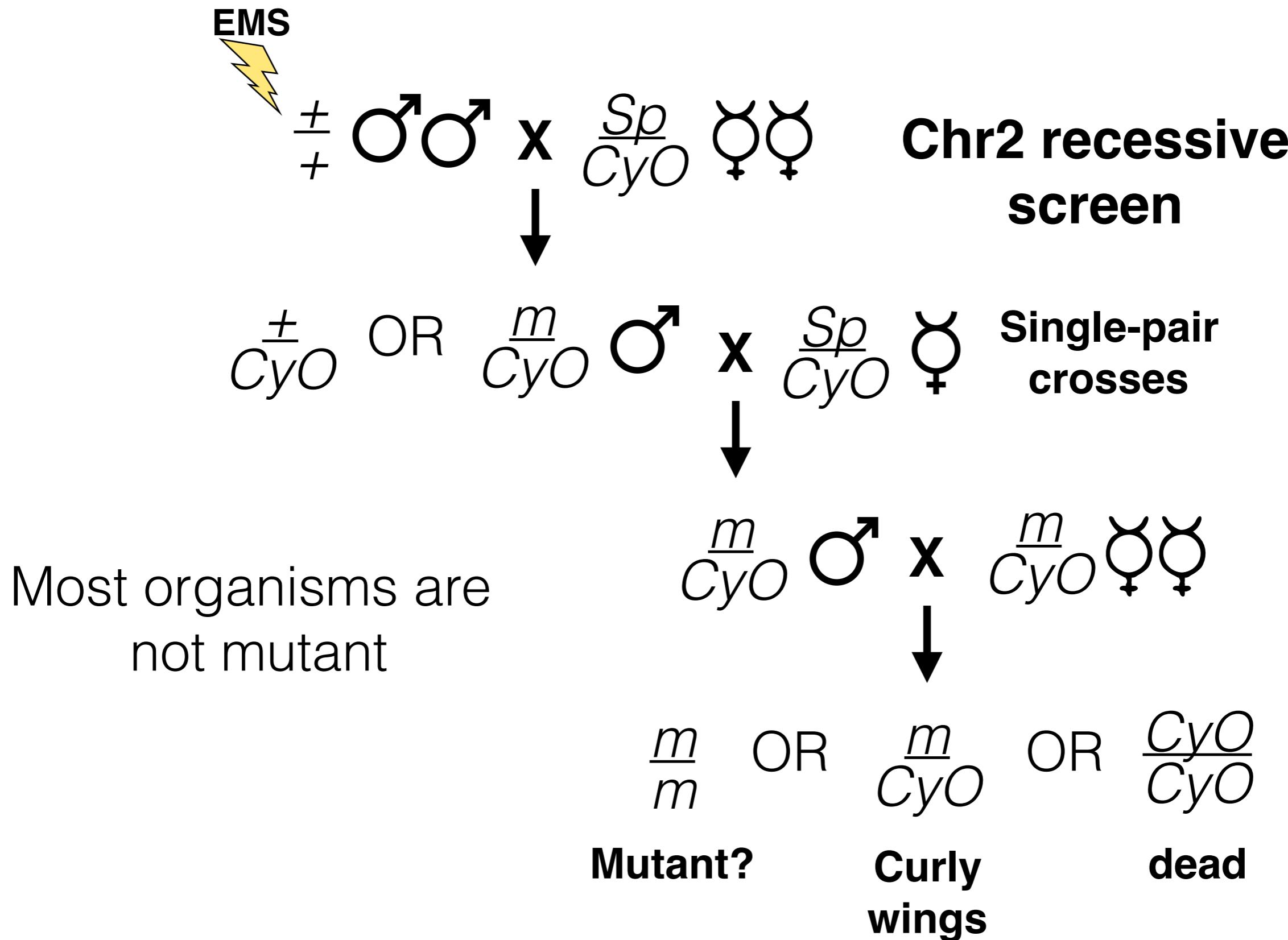
**Sp
*CyO***

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 $\frac{m}{m}$

F₂ non-clonal
screen

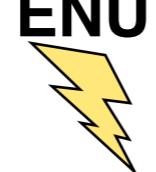
Hunt for your mutants!

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

Screen:



ENU



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

F₂ 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?

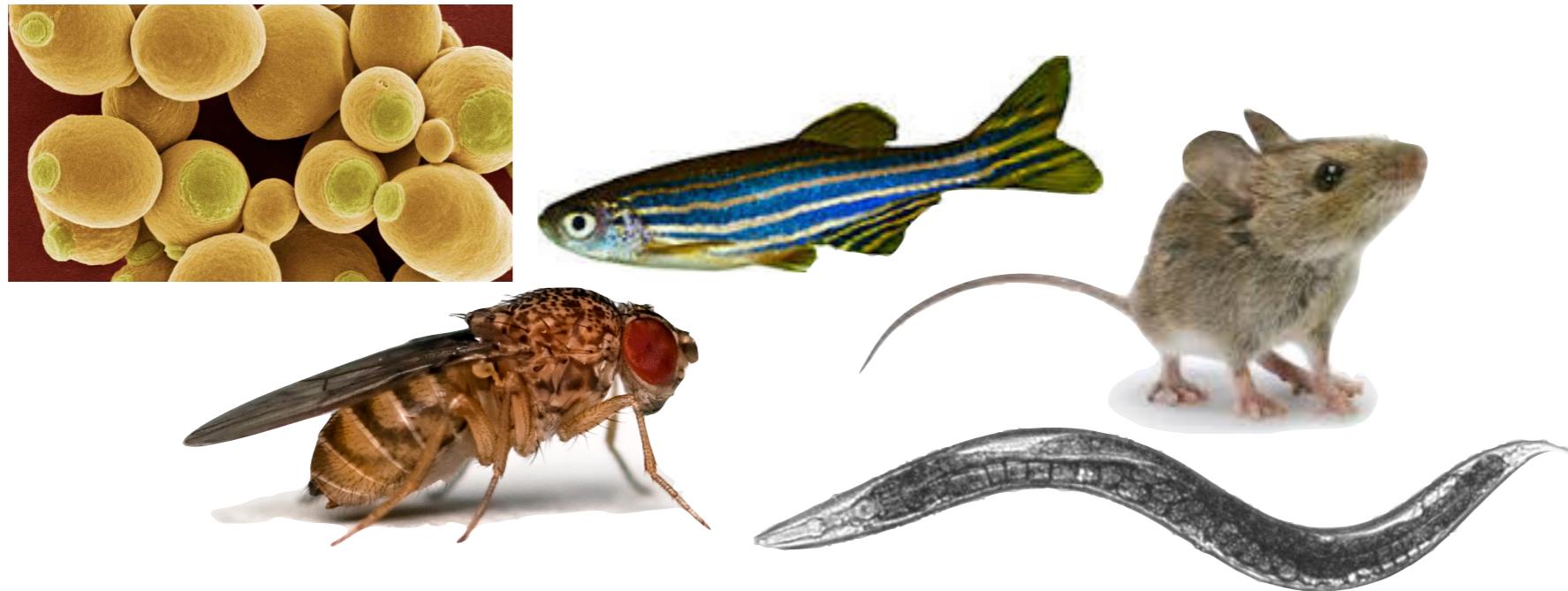
Alleles that confer recessive traits teach us about:

- 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?

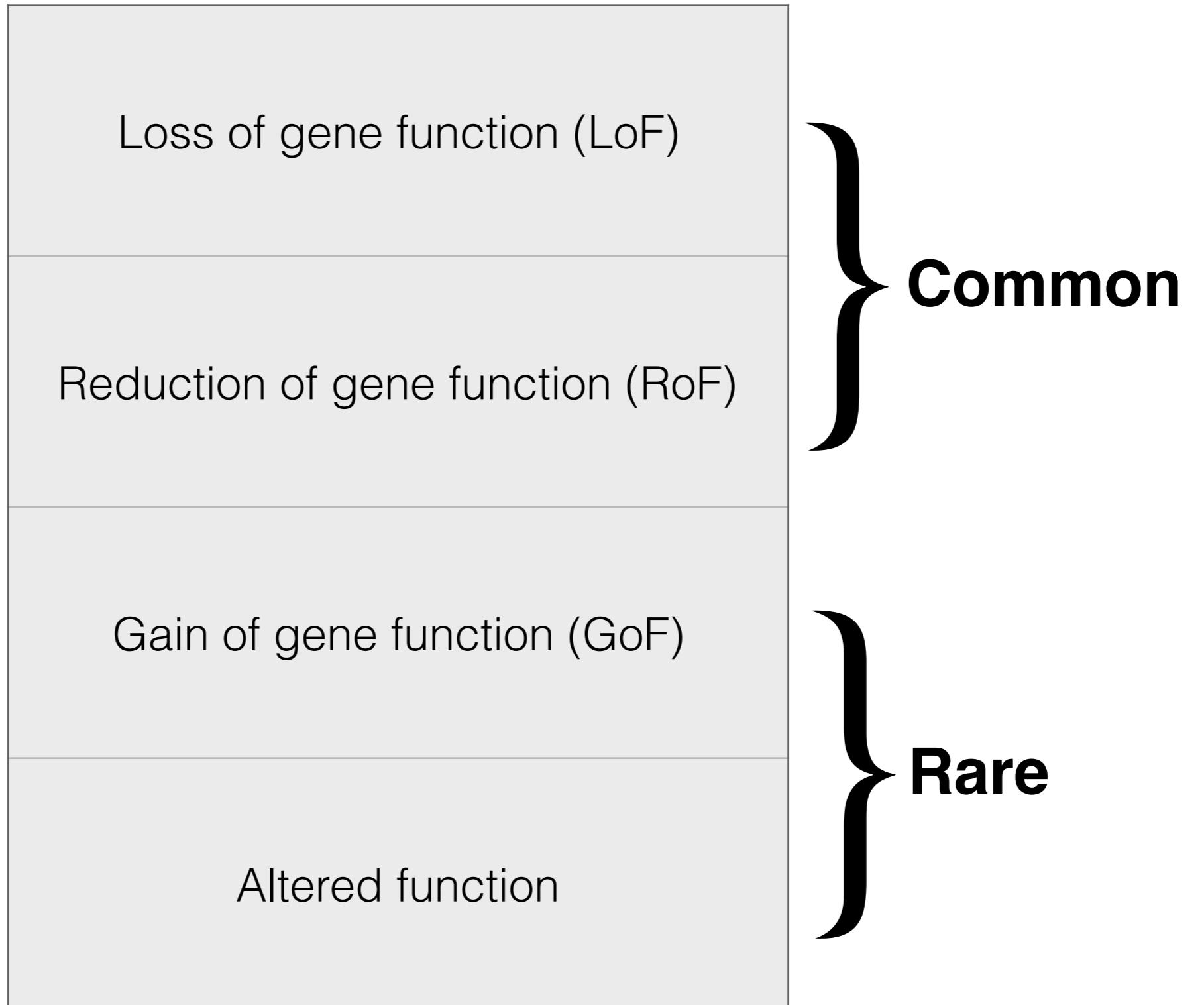


Mutations occur in the DNA of somatic and germline cells

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?**

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 allele of gene

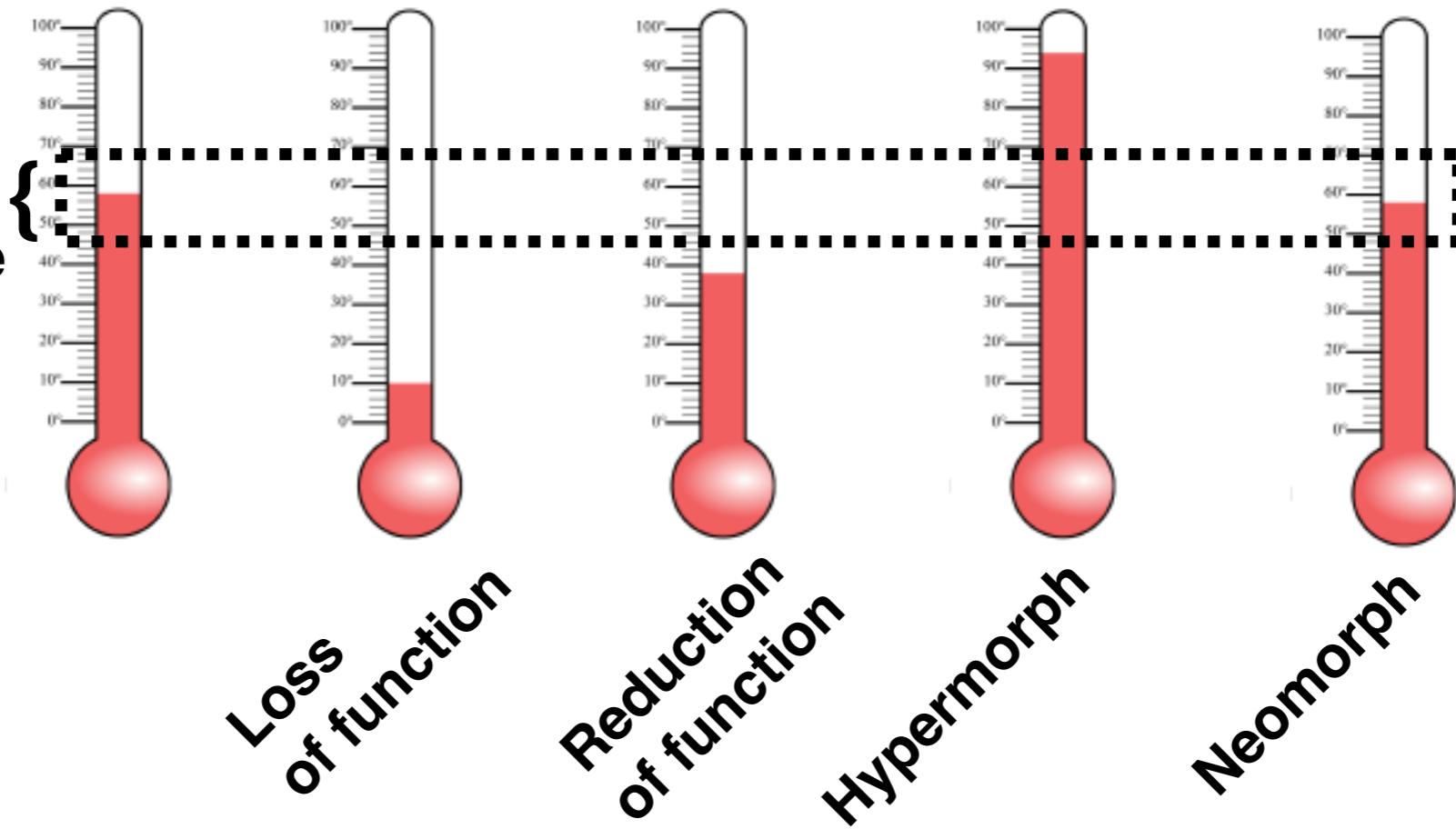
= = Phenotype is equivalent

> = Phenotype is more mutant than

< = Phenotype is less mutant than

A wild-type phenotype is a reading of the amount of gene function

Range of gene function that confers a wild-type phenotype



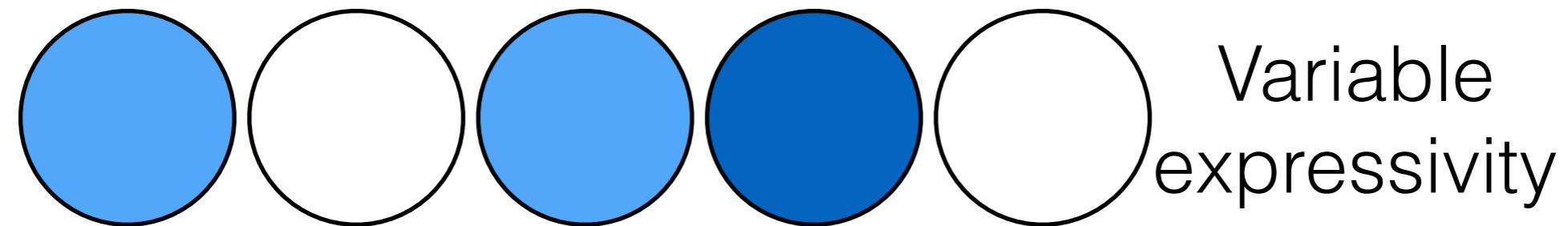
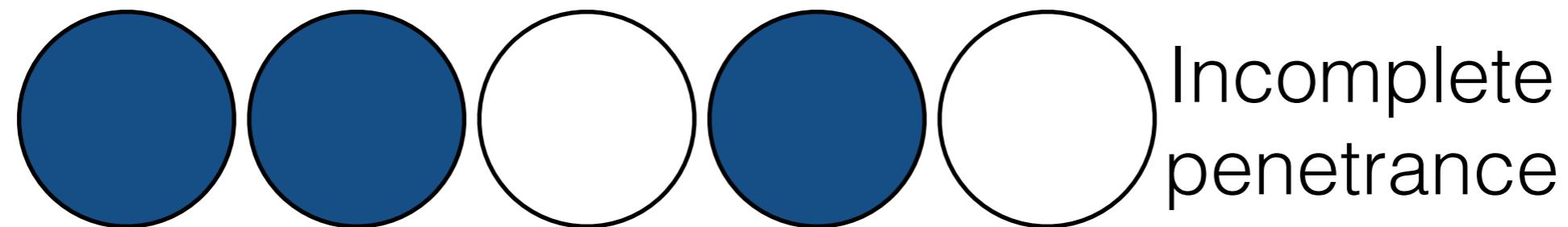
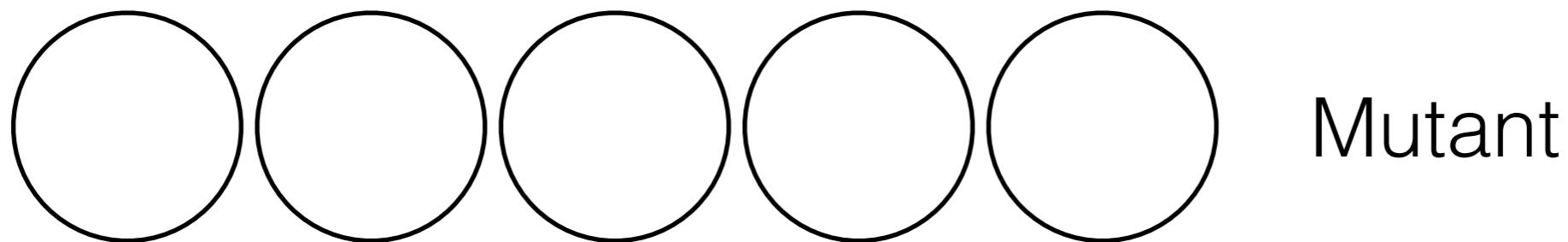
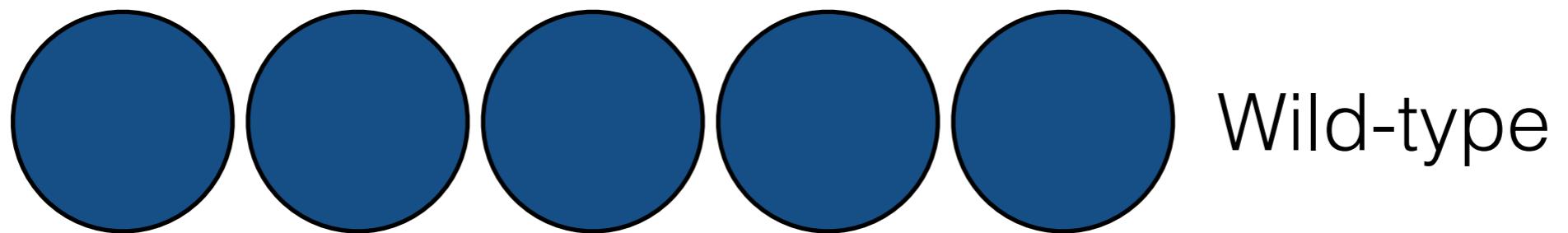
How do you get strains that are more or less mutant?

Incomplete penetrance

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

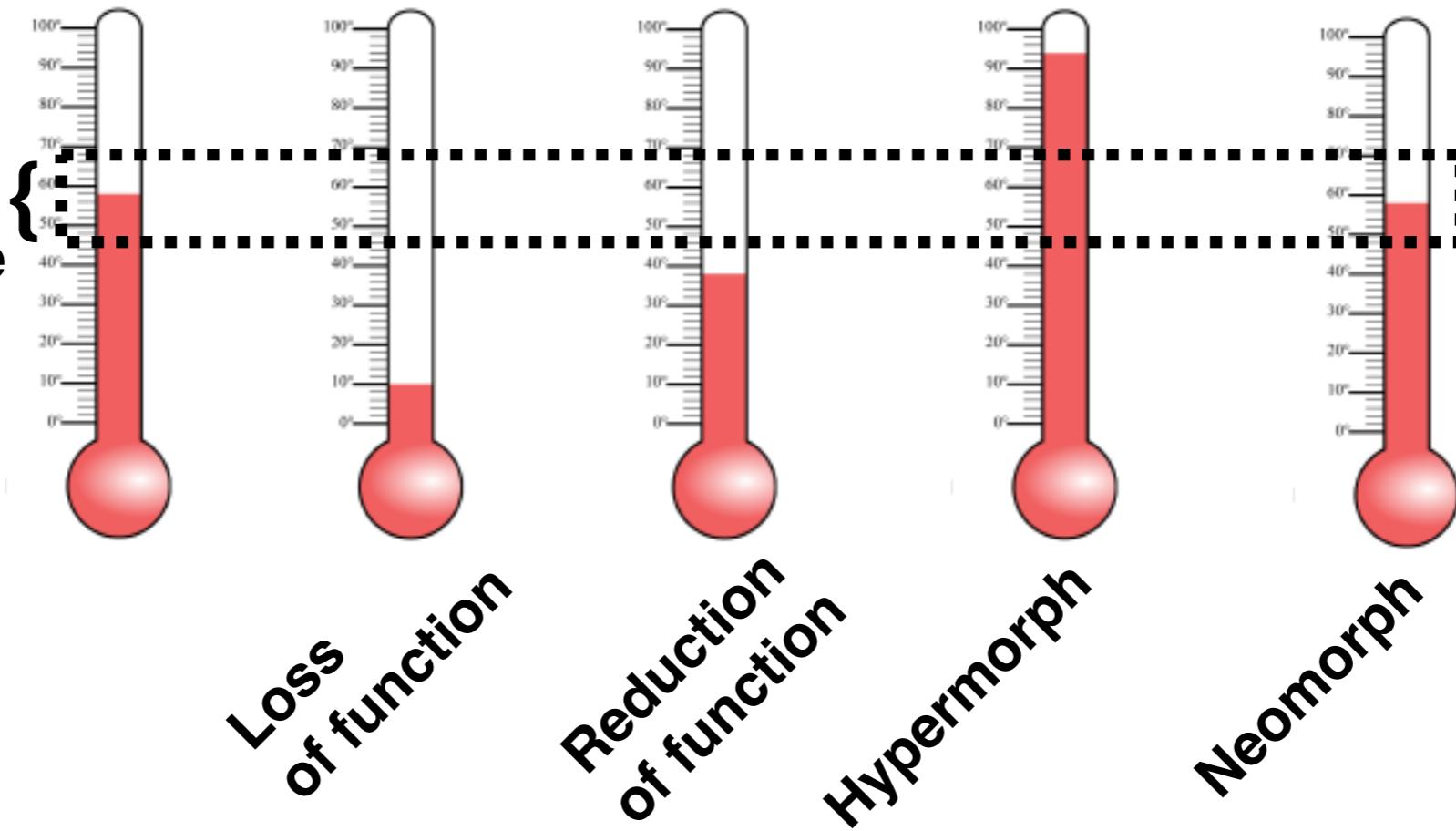
Variable expressivity

If the mutant expresses the mutant phenotype, the severity of the mutant phenotype differs from individual to individual.



A wild-type phenotype is a reading of the amount of normal gene function

Range of gene function that confers a wild-type phenotype





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

A mutant 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

m = mutation of gene

△ = deletion of gene

+ = normal allele of gene

= = Phenotype is equivalent

> = Phenotype is more mutant than

< = Phenotype is less mutant than

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

$+$ = normal allele of gene

= = Phenotype is equivalent

> = Phenotype is more mutant than

< = Phenotype is less mutant than

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 allele of gene

= = Phenotype is equivalent

> = Phenotype is more mutant than

< = Phenotype is less mutant than

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

Δ = deletion of gene

+ = normal allele of gene

= = Phenotype is equivalent

> = Phenotype is more mutant than

< = Phenotype is less mutant than

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 allele of gene

= = Phenotype is equivalent

> = Phenotype is more mutant than

< = Phenotype is less mutant than

Dominant mutant phenotypes

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

hypermorph = mutant causes an increase in wild-type function

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

m = mutation of gene

Δ = deletion of gene

+ = normal allele of gene

= = Phenotype is equivalent

> = Phenotype is more mutant than

< = Phenotype is less mutant than

Hypermorphic mutations cause an increase of wild-type function



Wild-type



Too much signaling

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 allele of gene

= = Phenotype is equivalent

> = Phenotype is more mutant than

< = Phenotype is less mutant than

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

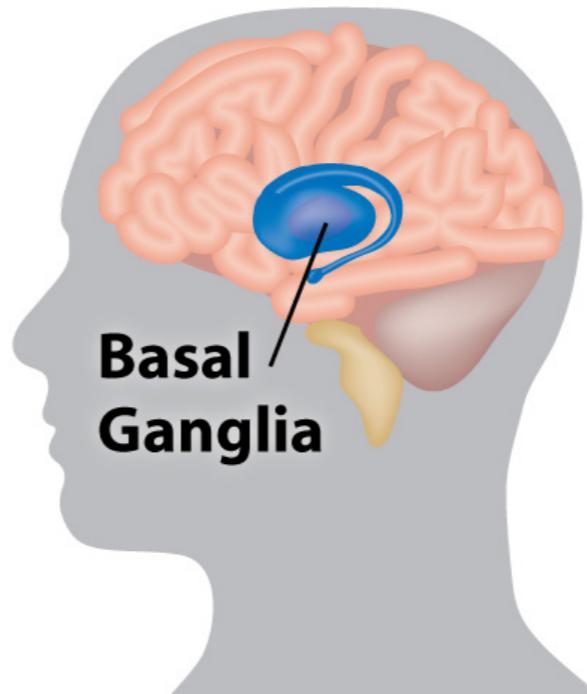
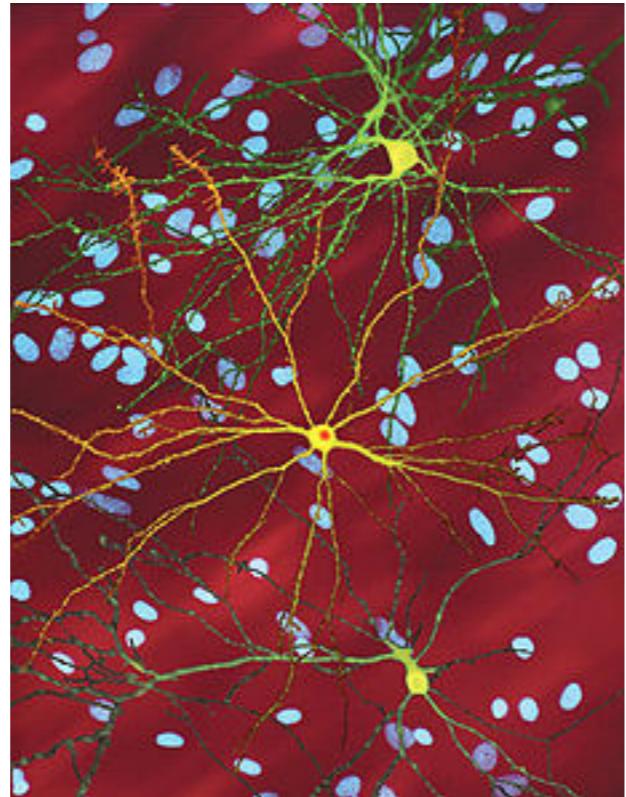
+ = normal allele of gene

= = Phenotype is equivalent

> = Phenotype is more mutant than

< = Phenotype is less mutant than

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

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

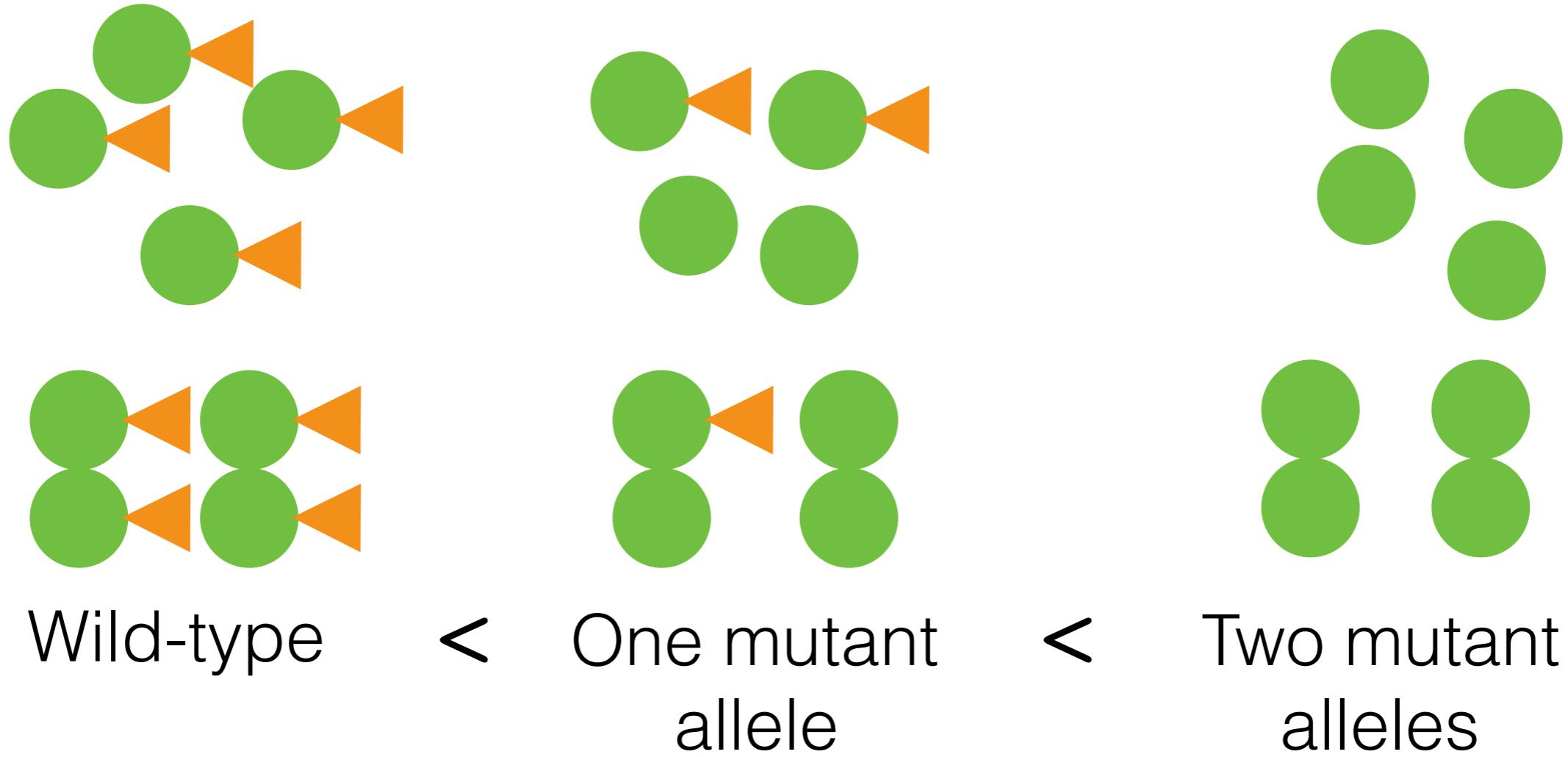
antimorph = mutant causes dominant loss of gene function
dominant negative

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

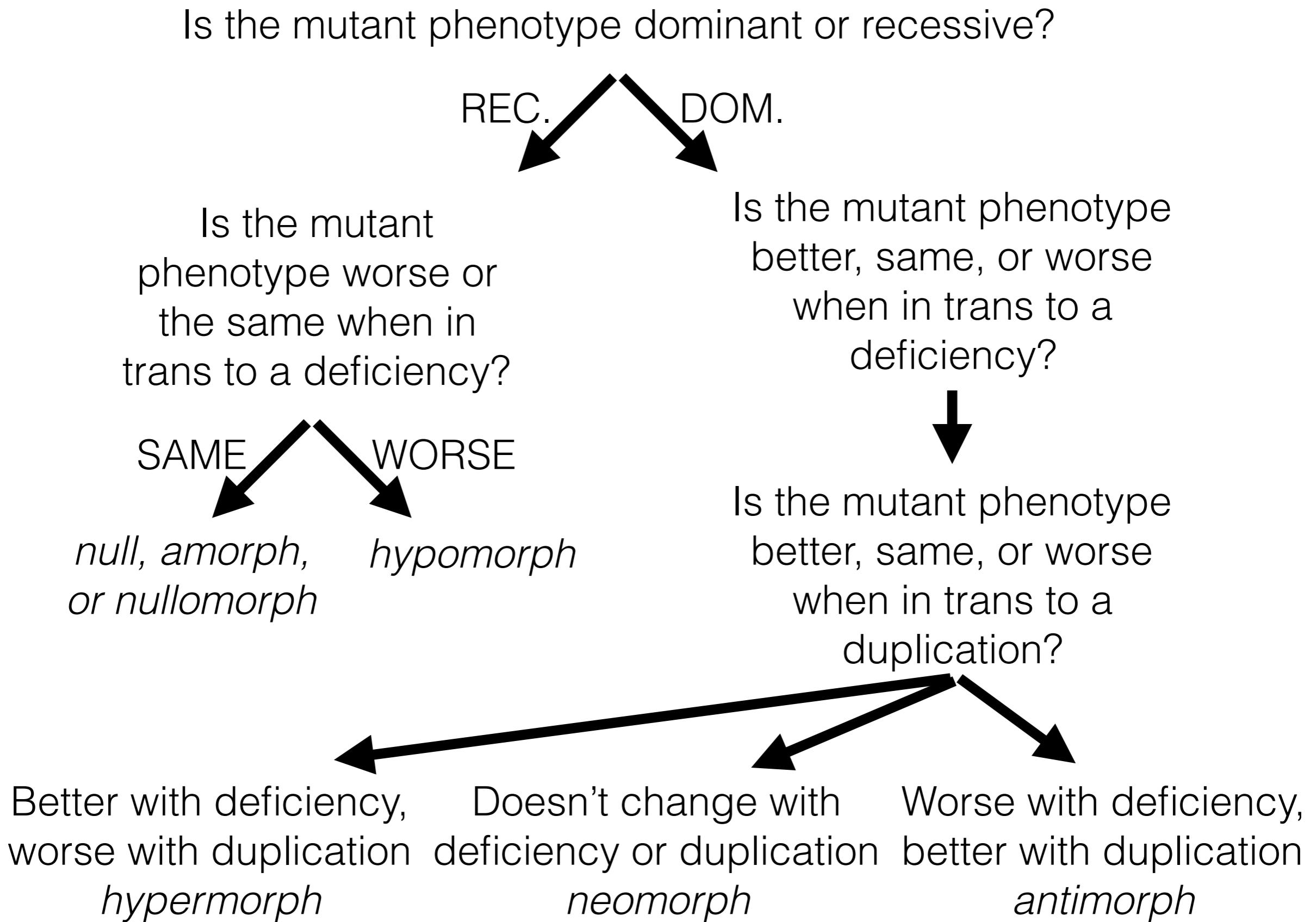
m = mutation of gene
 Δ = deletion of gene
 $+$ = normal allele of gene

= = Phenotype is equivalent
 $>$ = Phenotype is more mutant than
 $<$ = Phenotype is less mutant than

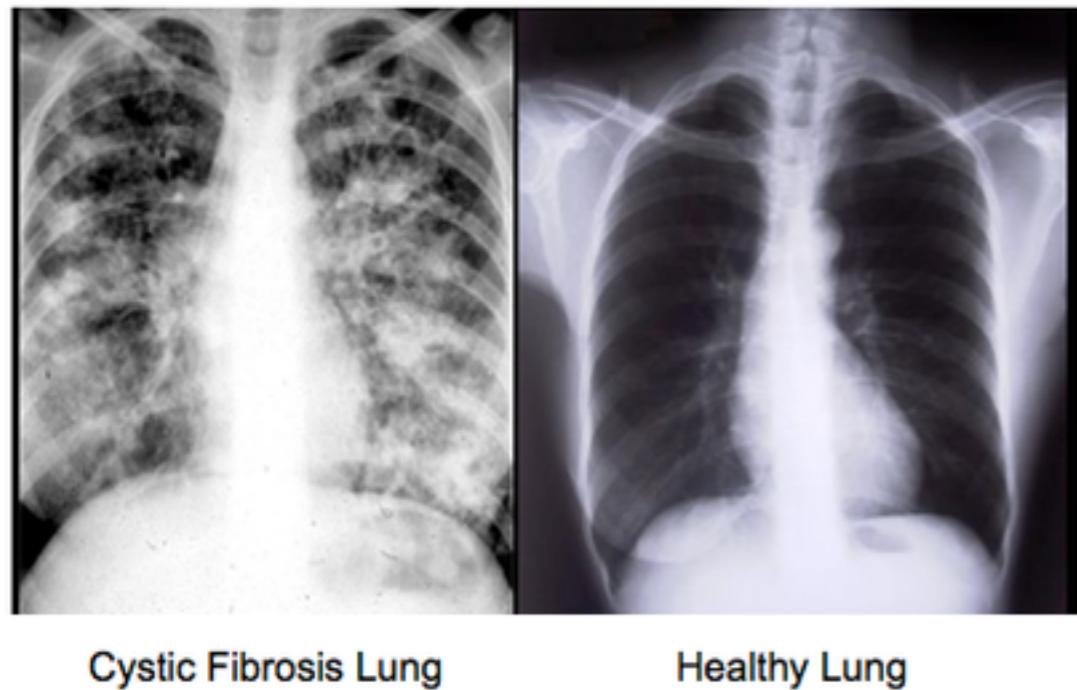
Antimorphs or dominant negatives compete with wild-type function



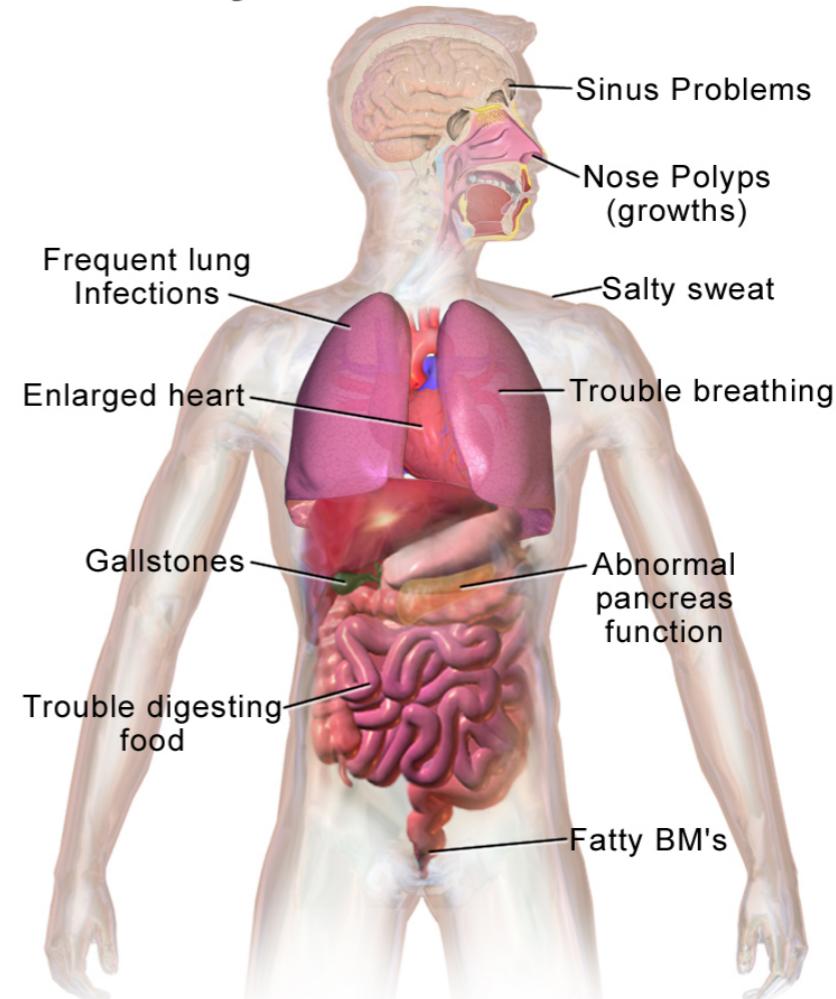
Flow chart for gene dosage studies



What about cystic fibrosis and today's topic?

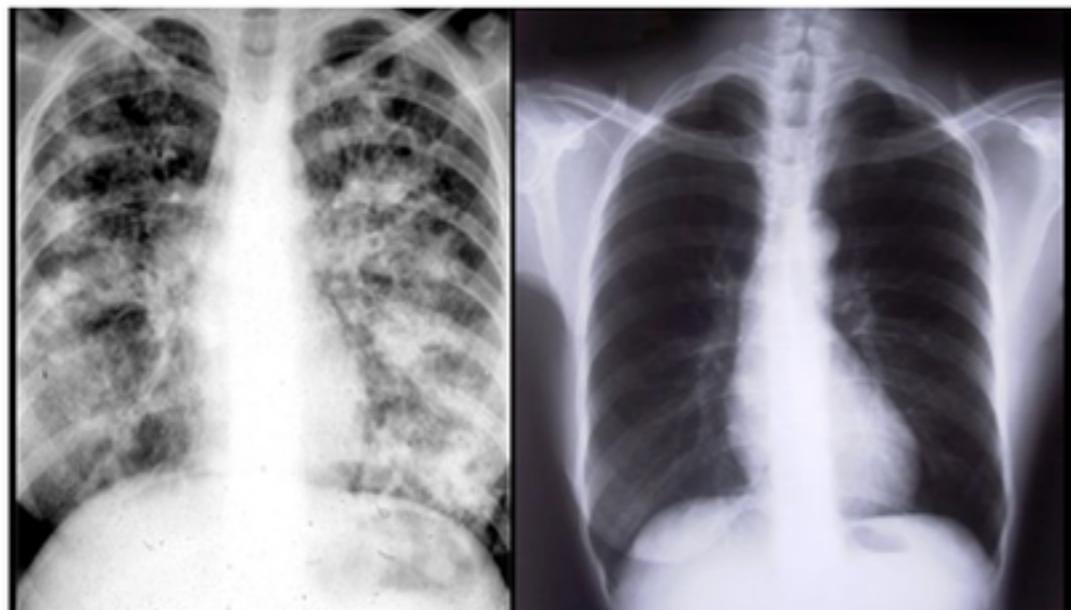


Health Problems with Cystic Fibrosis



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

CF is an autosomal recessive disorder



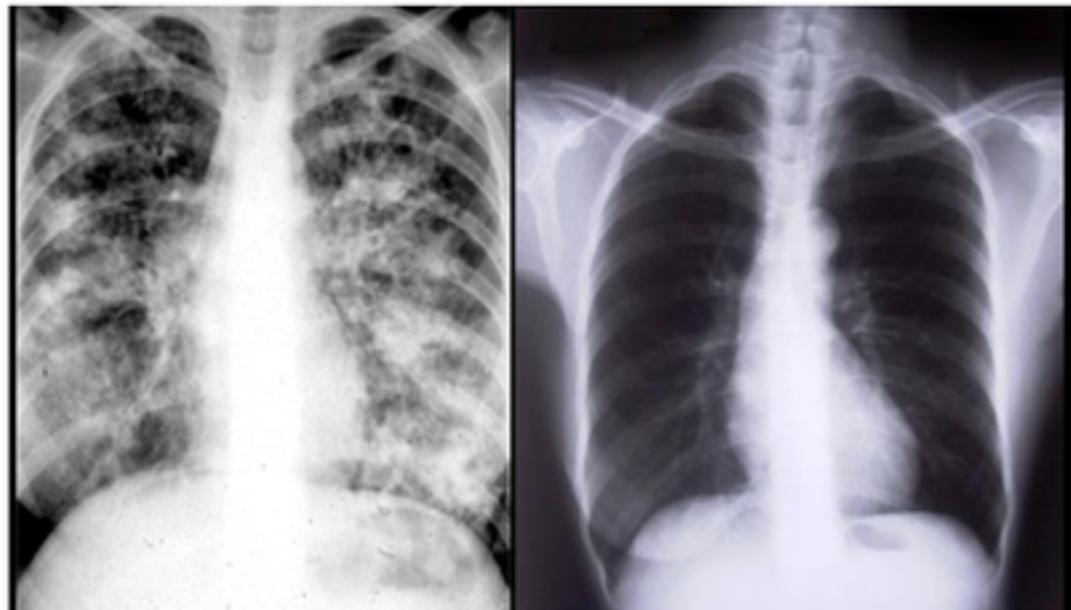
null or hypomorph?

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?