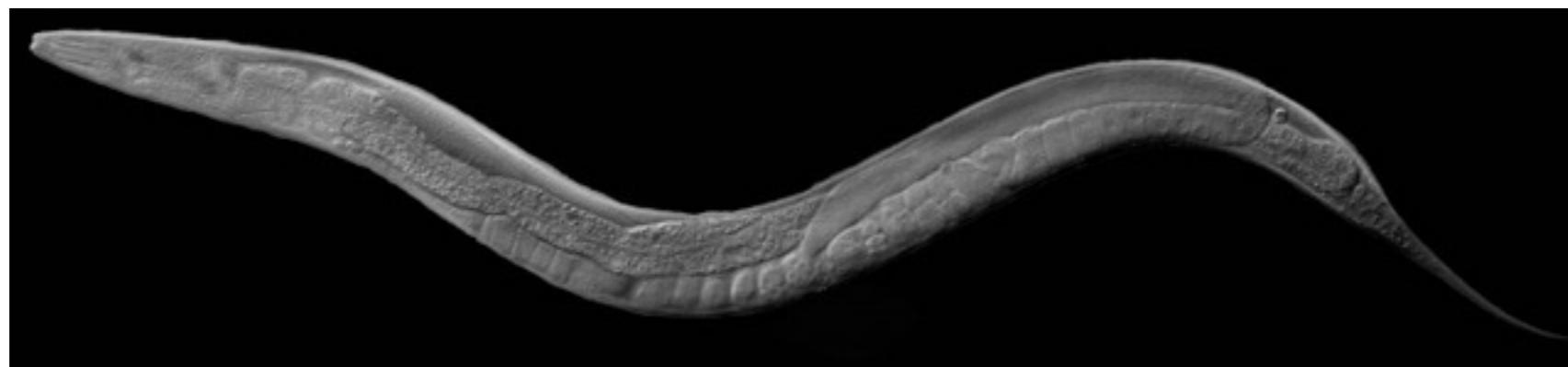
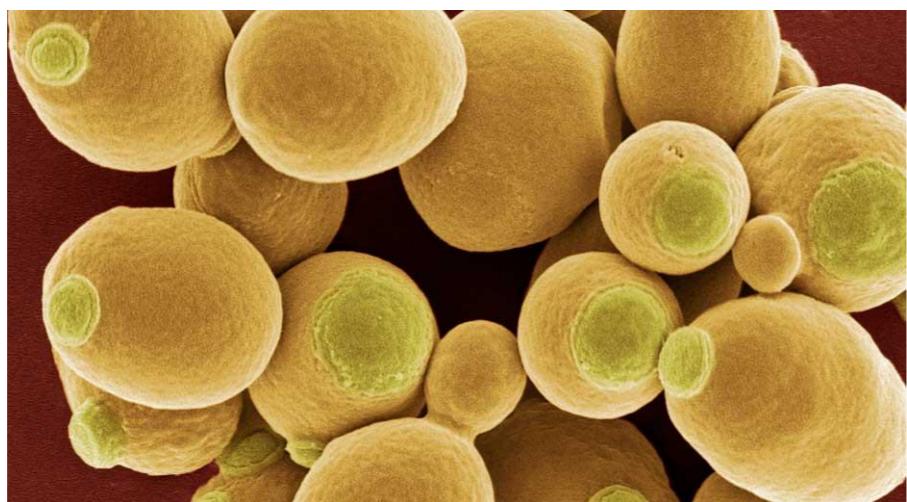


Screens, selections, mutants, dosage



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

- *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

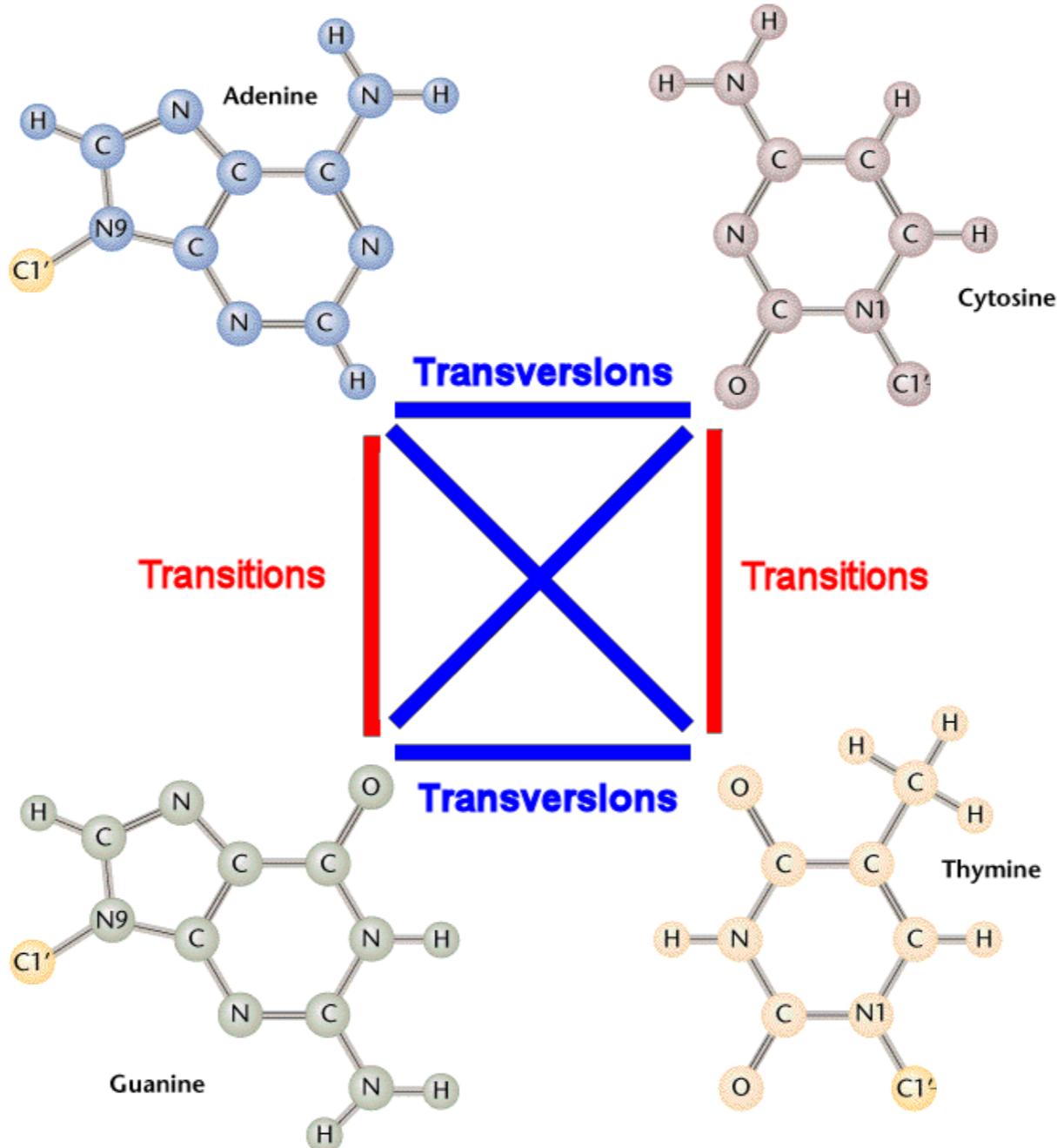
Negatives: Strong effects, not full mutation spectrum open, less efficient than mutagen

CRISPR/Cas9, targeted mutations

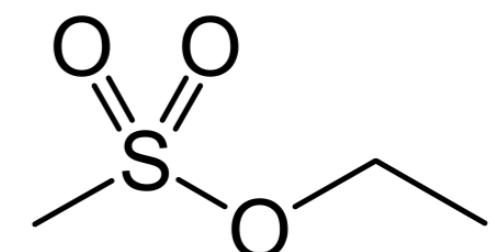
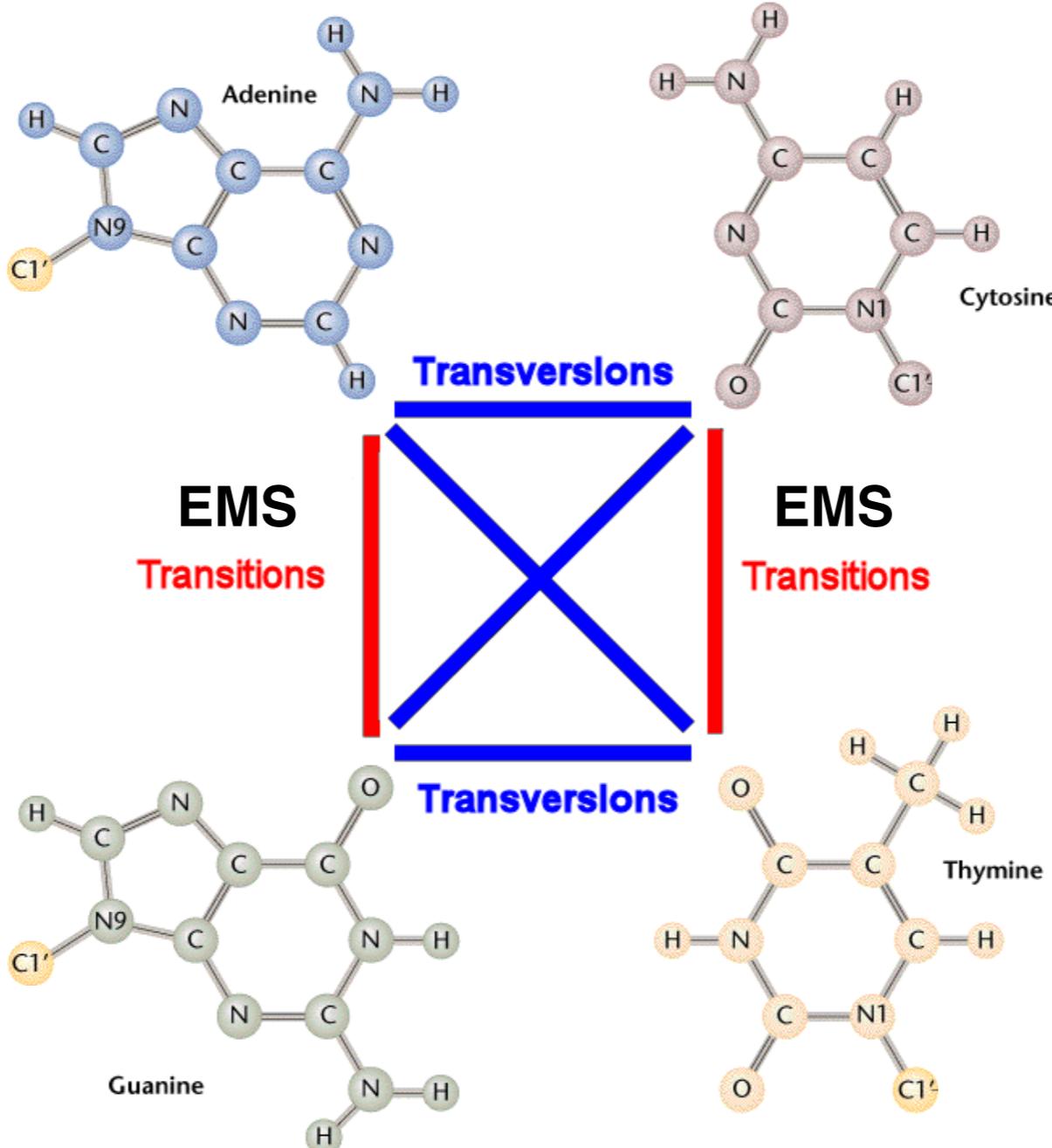
Positives: Targeted, Designable, Defined genetic background, Scalable

Negatives: Off target effects? Delivery?

Single-base substitutions

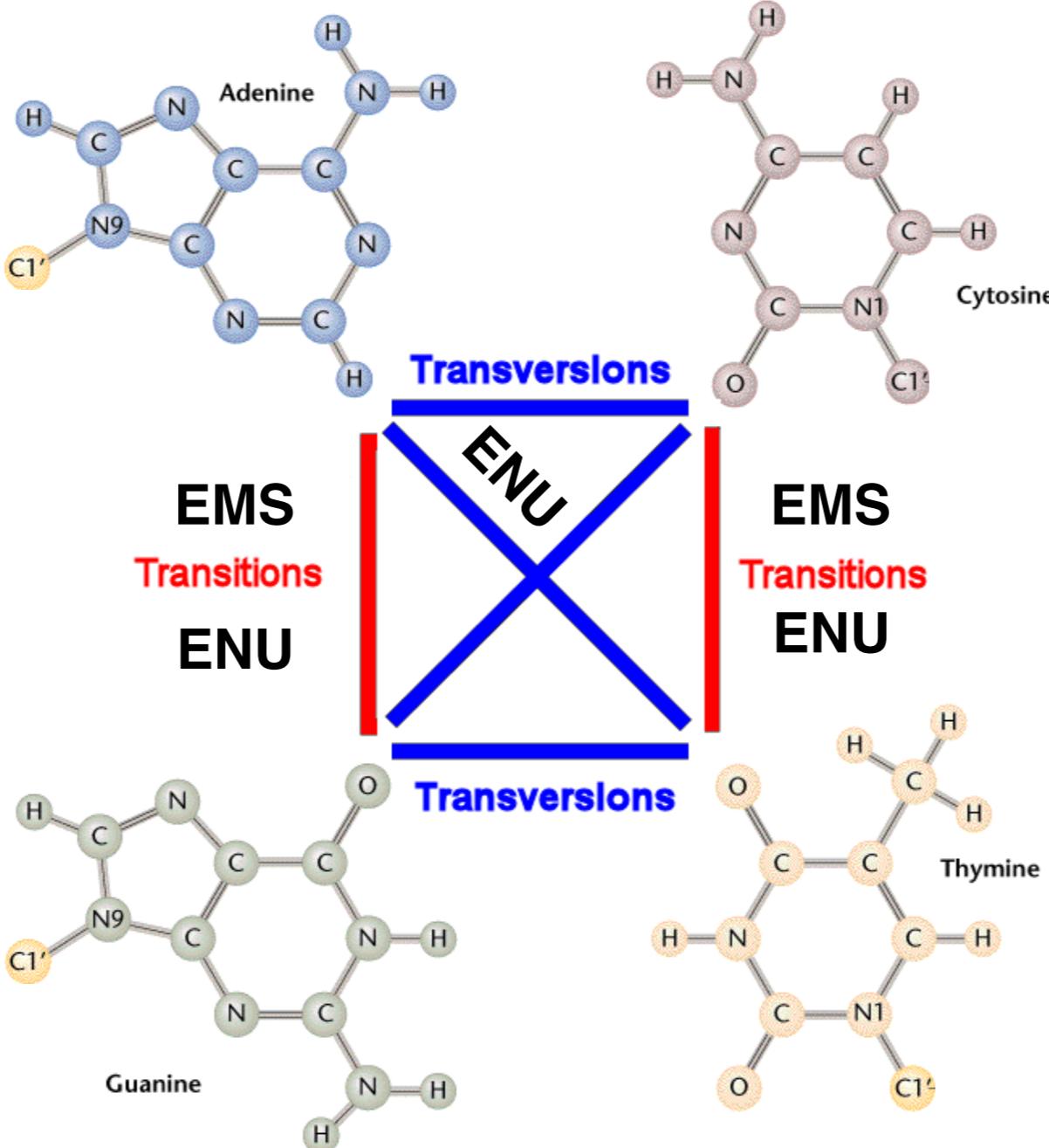


Single-base substitutions

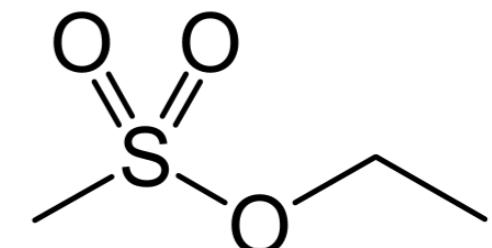


Ethyl methanesulfonate
(EMS)

Single-base substitutions



**Ethyl-nitrosurea
(ENU)**



**Ethyl methanesulfonate
(EMS)**

Two ways to isolate mutants: selection or screen

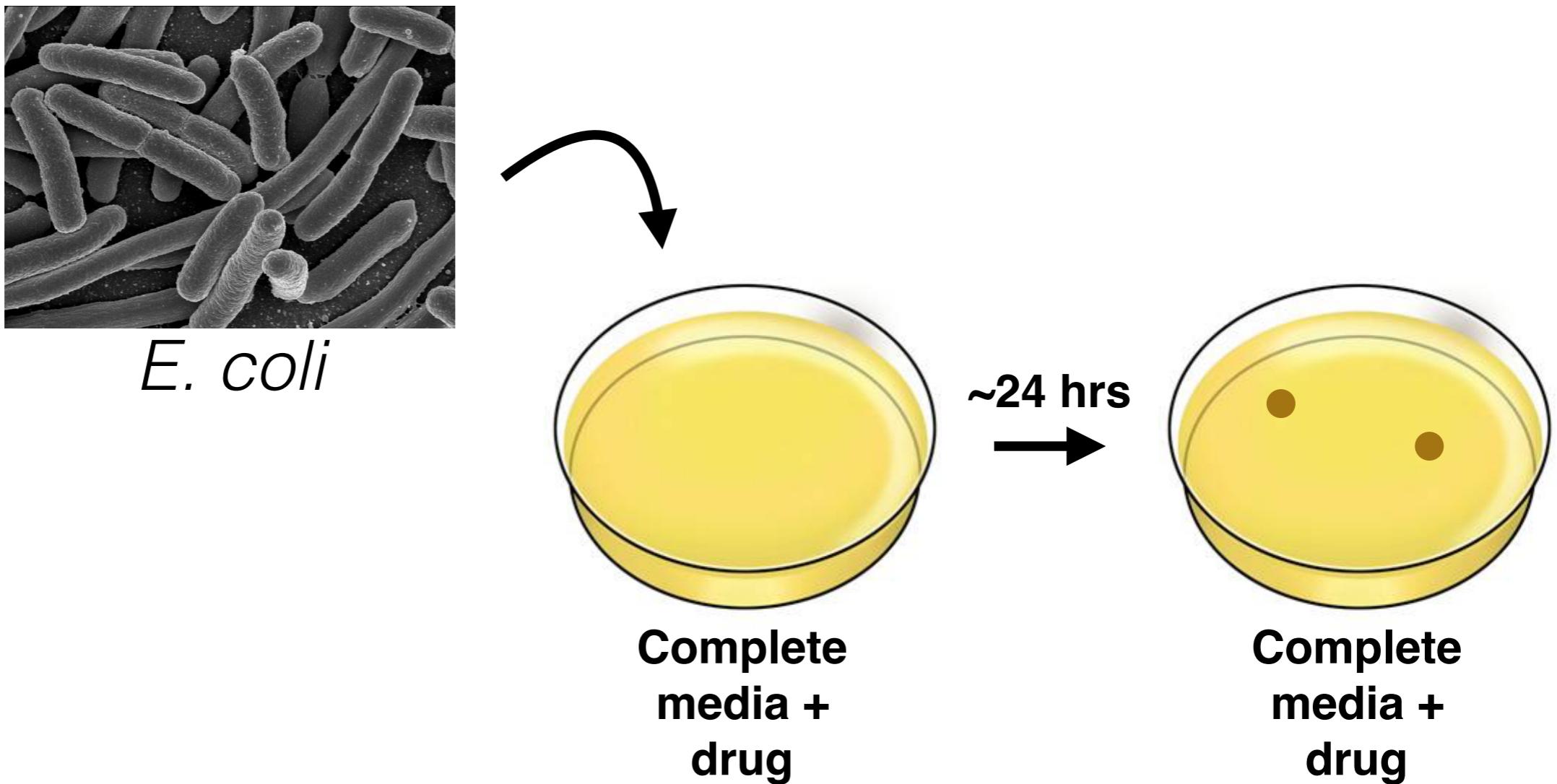
Selection: You only see mutants

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

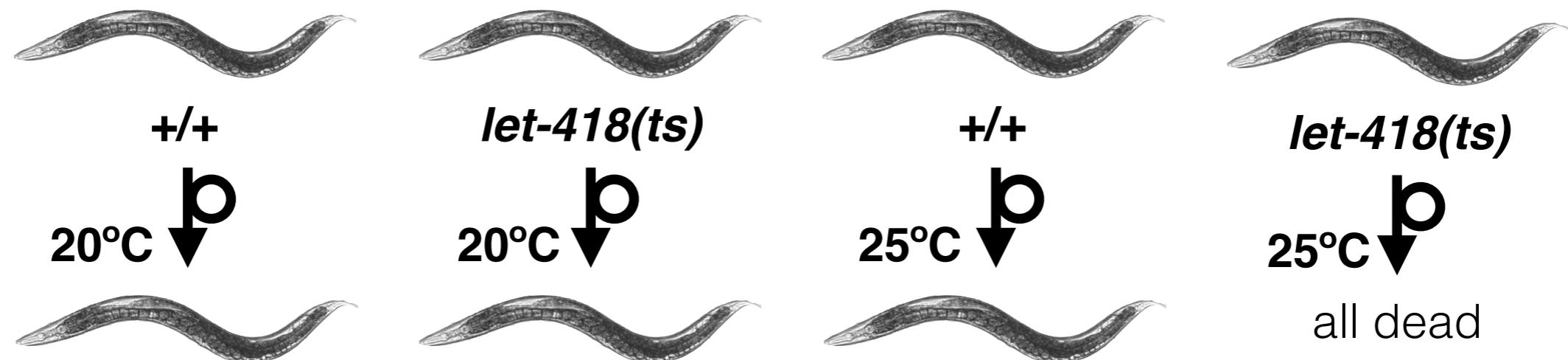
Selection:



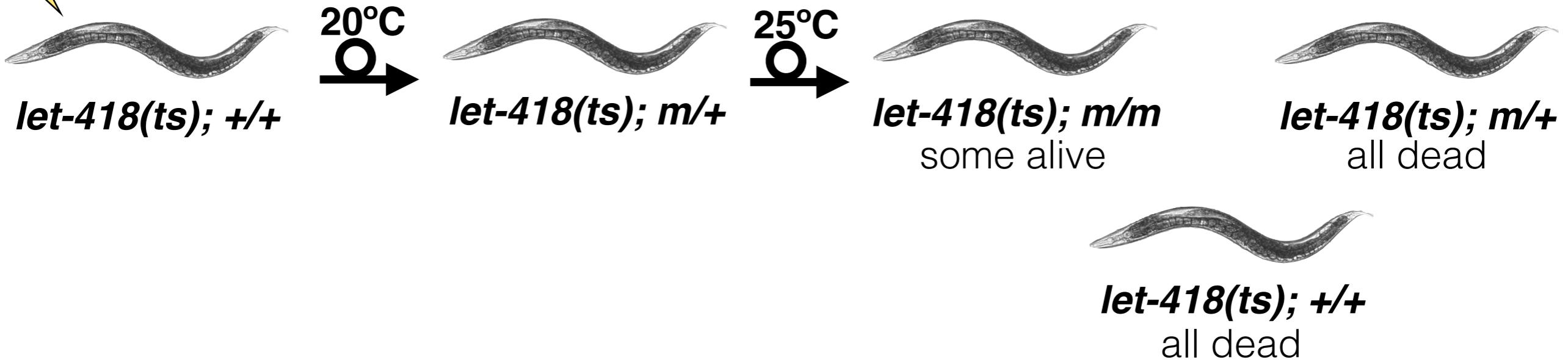
Goal: Find mutants that can grow on a specific drug

Two ways to isolate mutants: selection or screen

Selection:



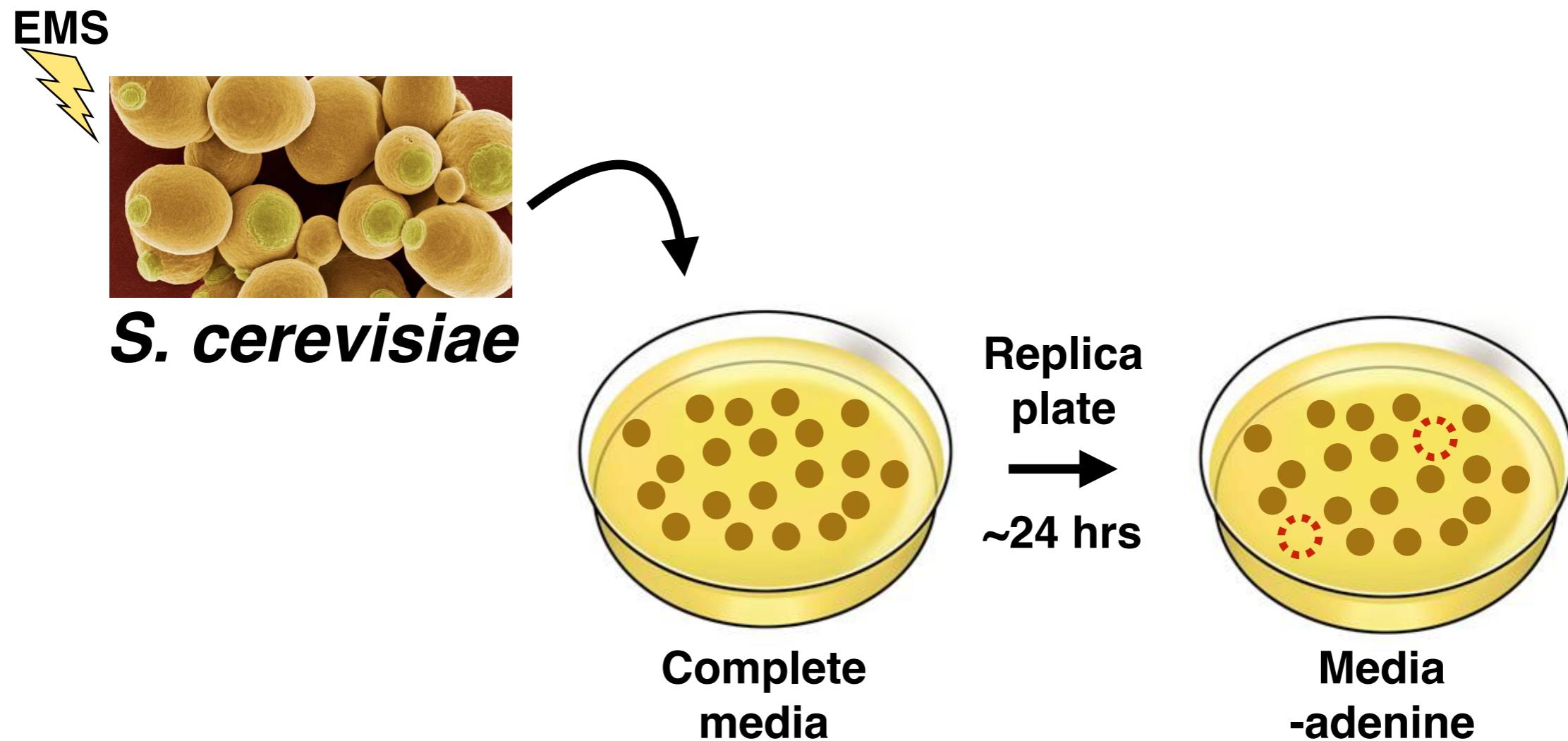
EMS
⚡



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

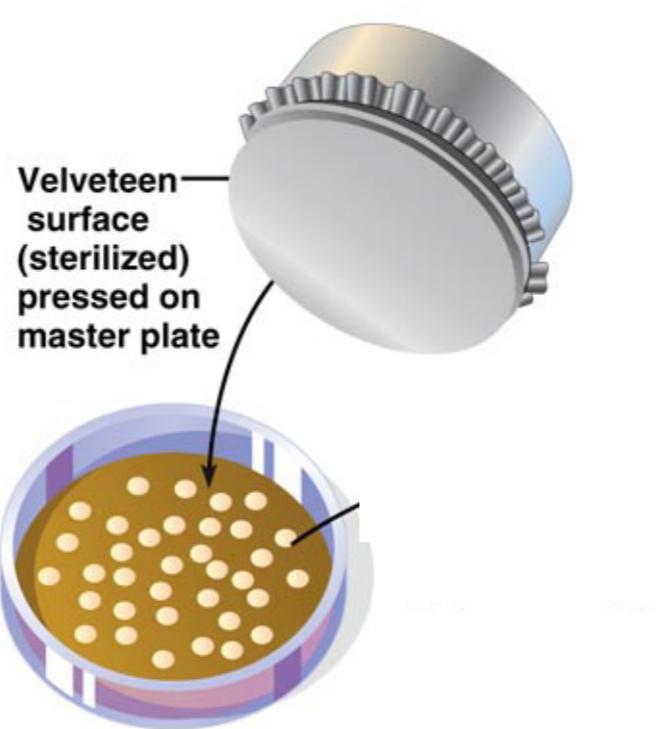
Two ways to isolate mutants: selection or screen

Screen:



Why not directly plate on media lacking adenine?

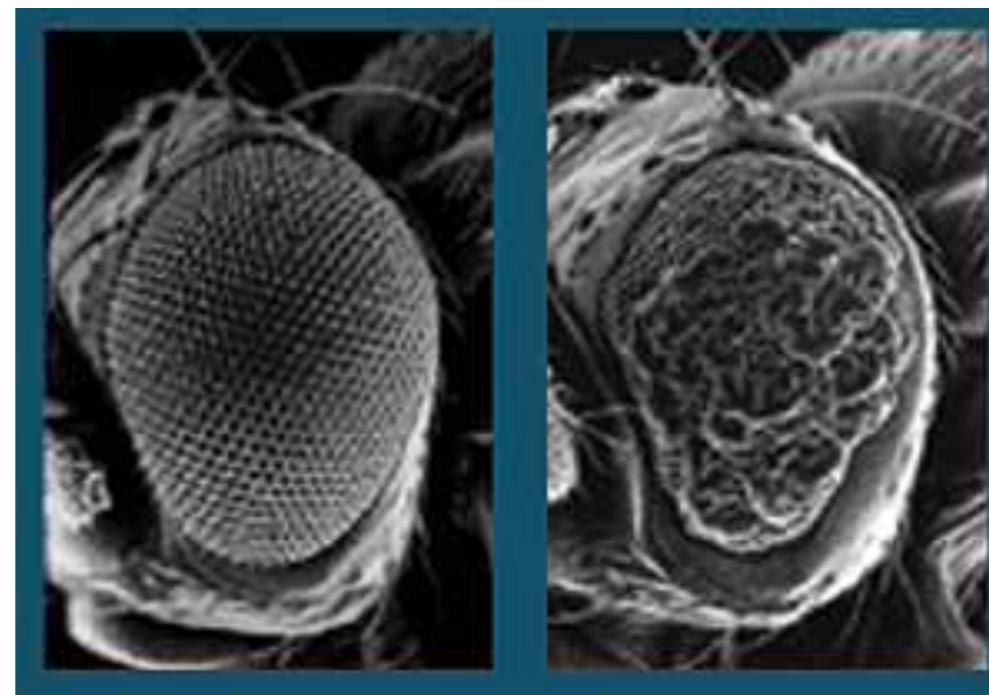
Replica plating



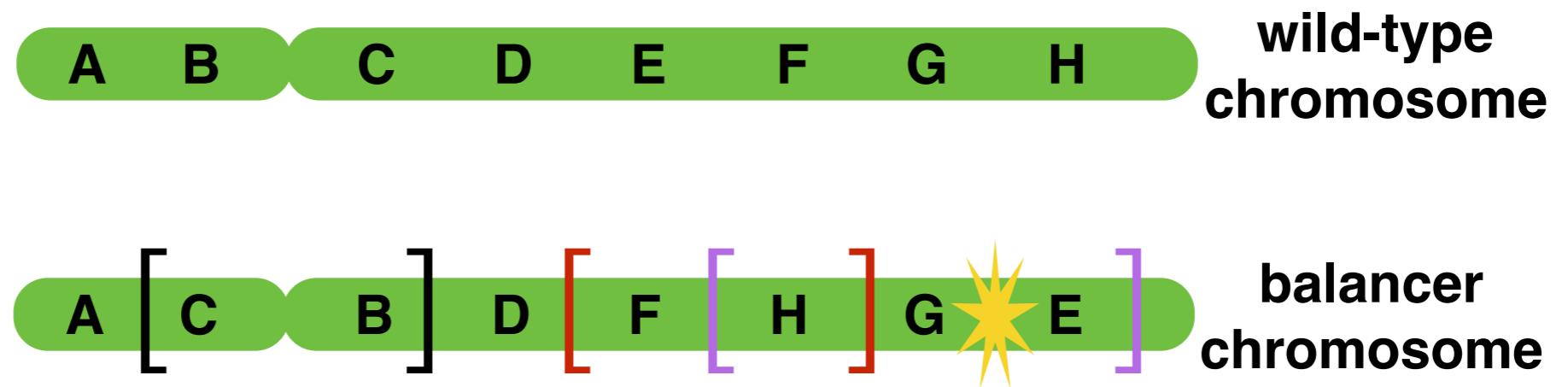
Two ways to isolate mutants: selection or screen



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**



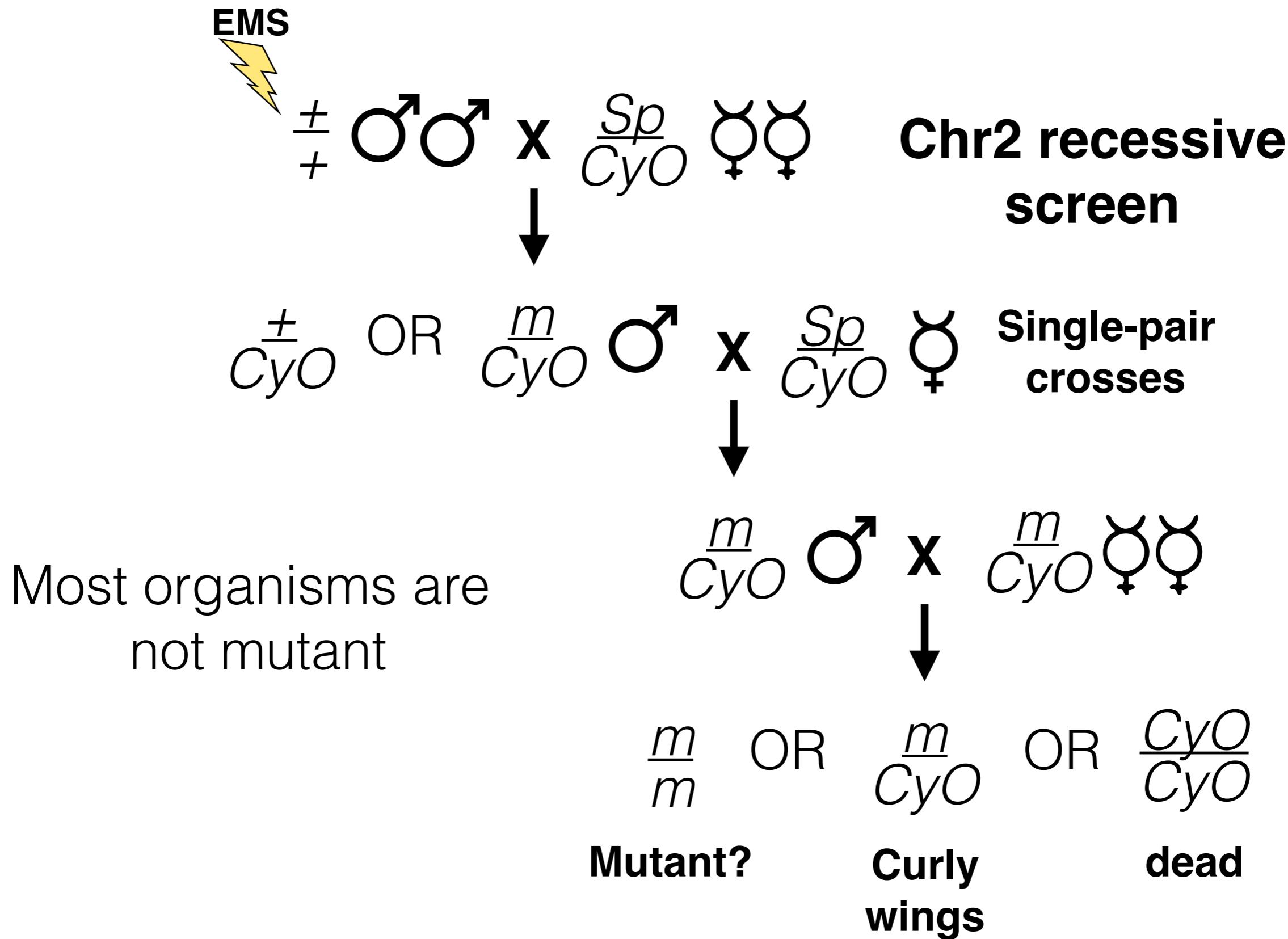
**Sp
*CyO***

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 $\frac{m}{+}$



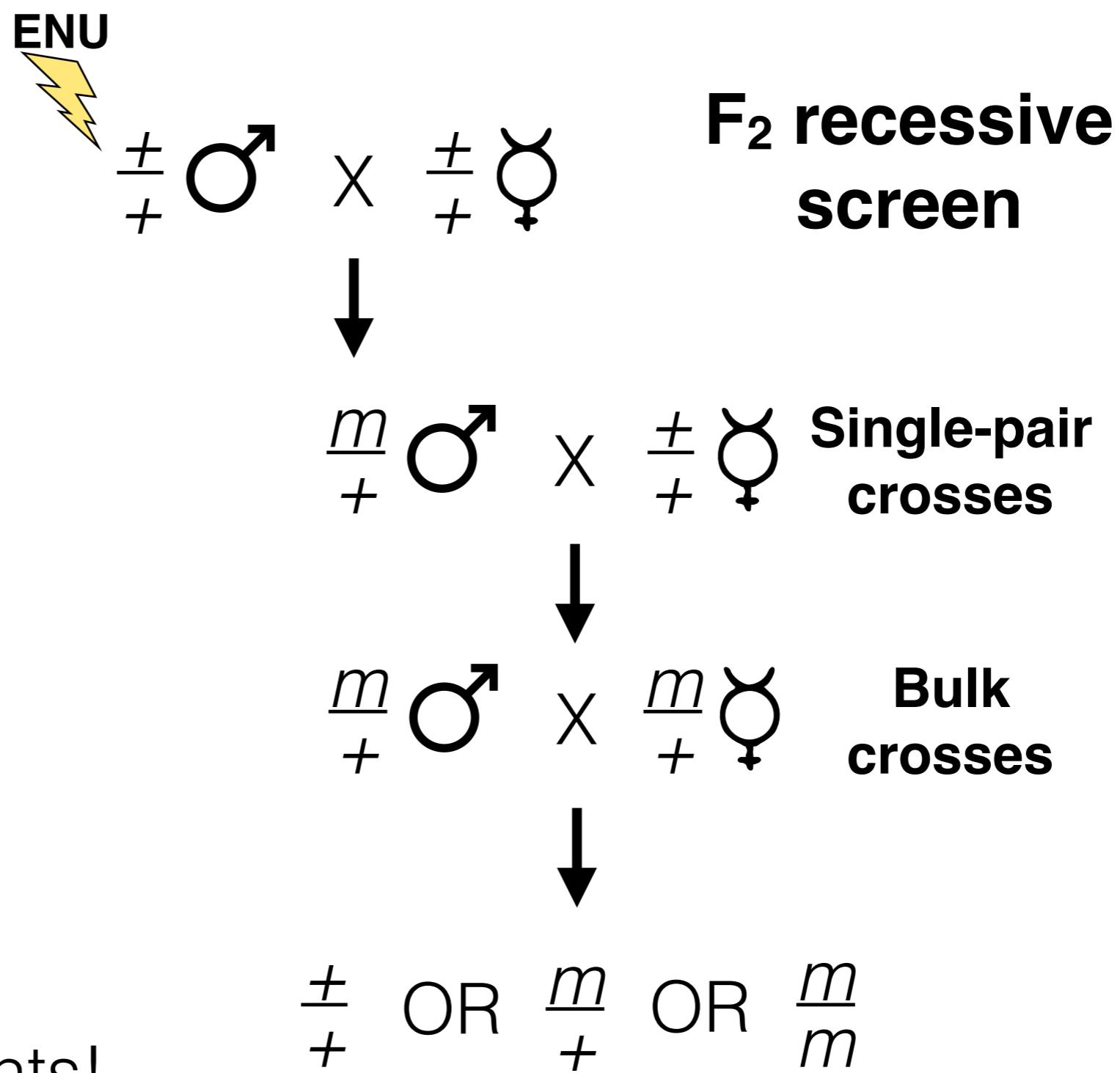
\pm OR $\frac{m}{+}$ OR $\frac{m}{m}$

F₂ non-clonal
screen

Hunt for your mutants!

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

Screen:



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

Alleles that confer dominant traits teach us about:

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

What happens when we mutagenize strains?

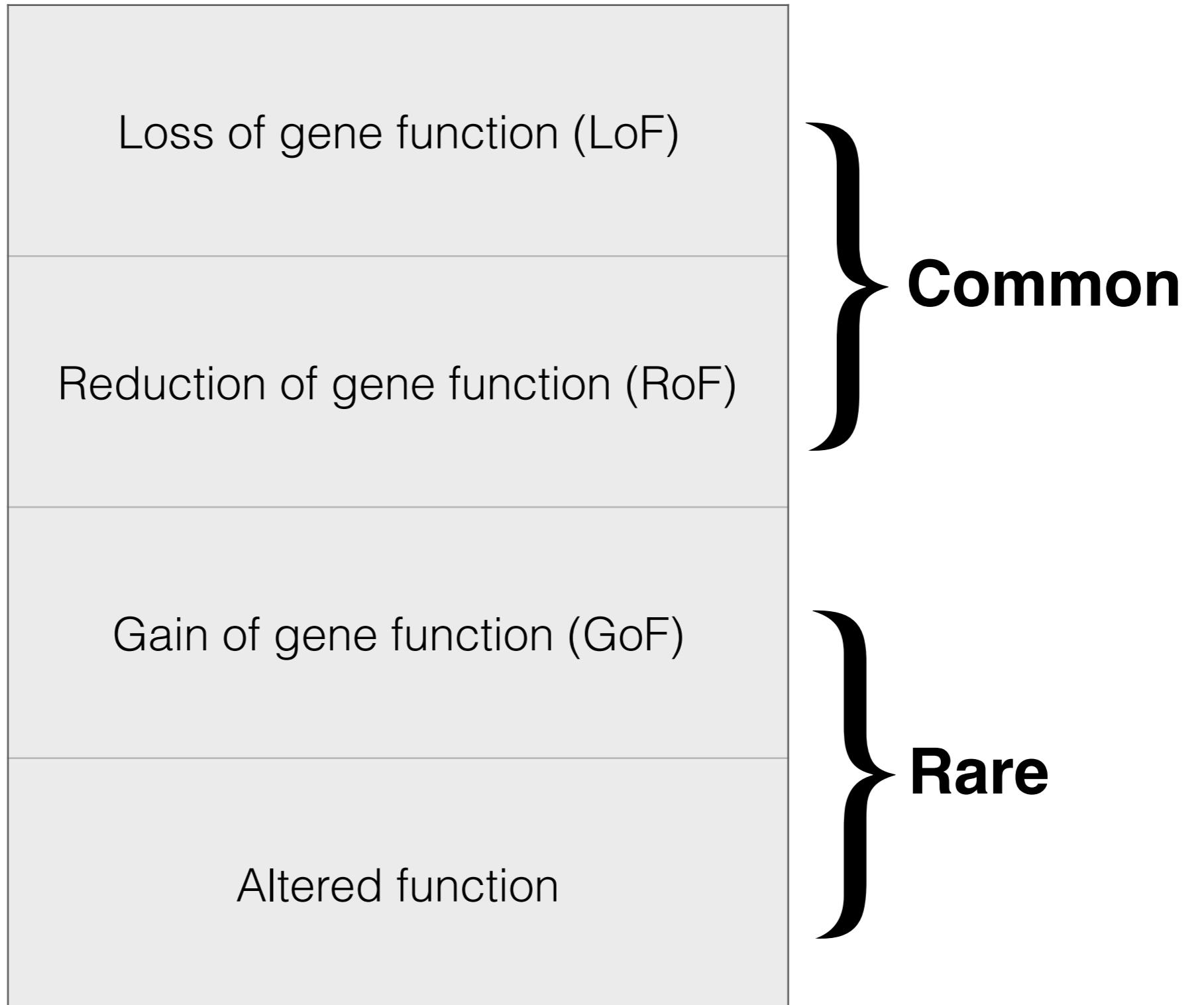


Mutations occur in the DNA of somatic and germline cells

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