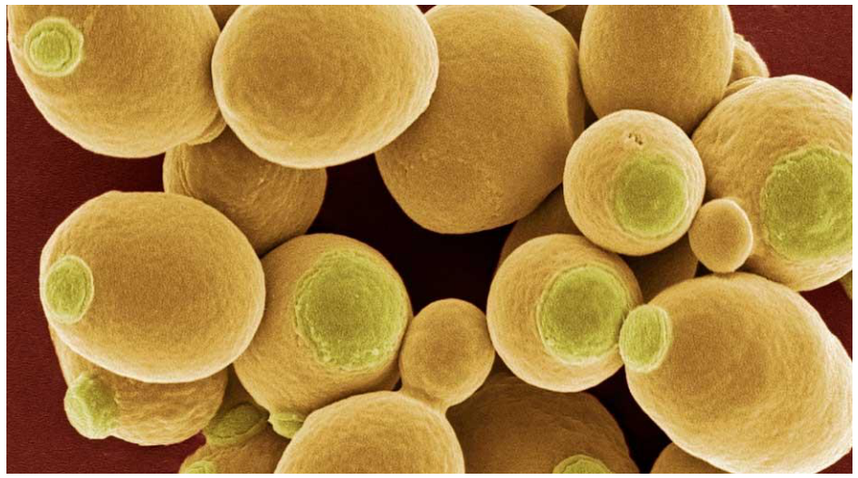
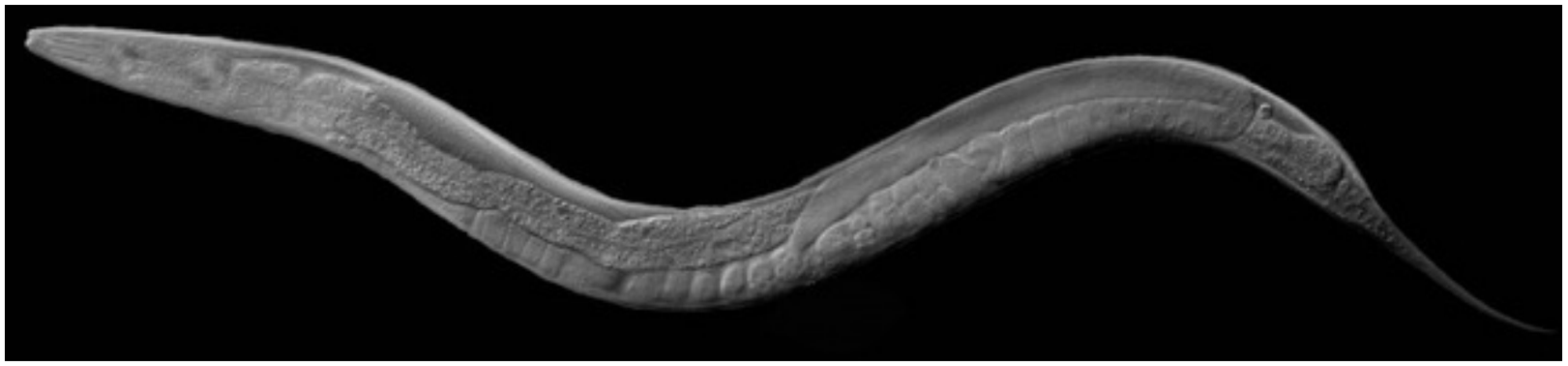
Bio393: Genetic Analysis

Screens, selections, mutants, dosage





Lecture 3

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

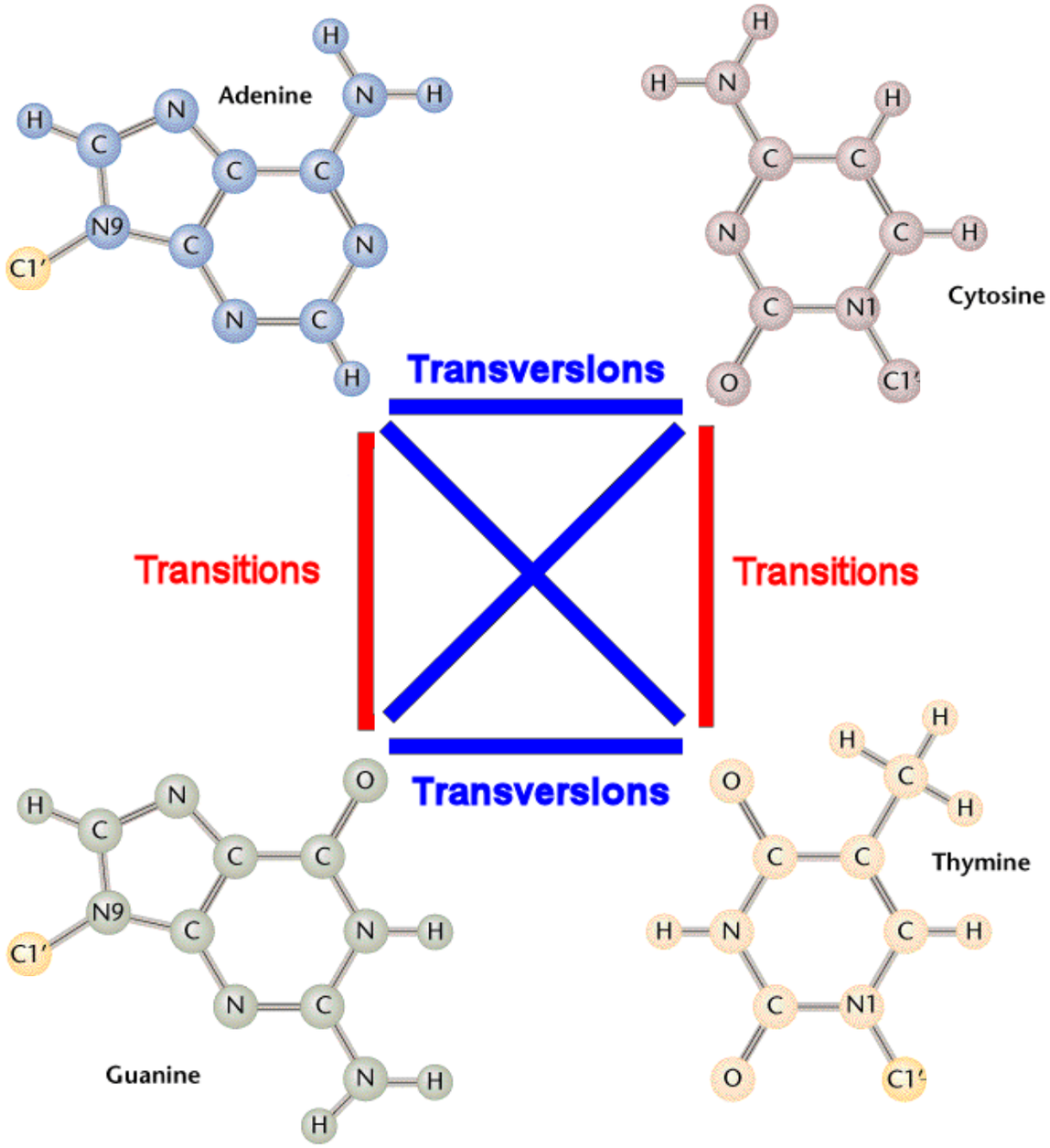
Transposons

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

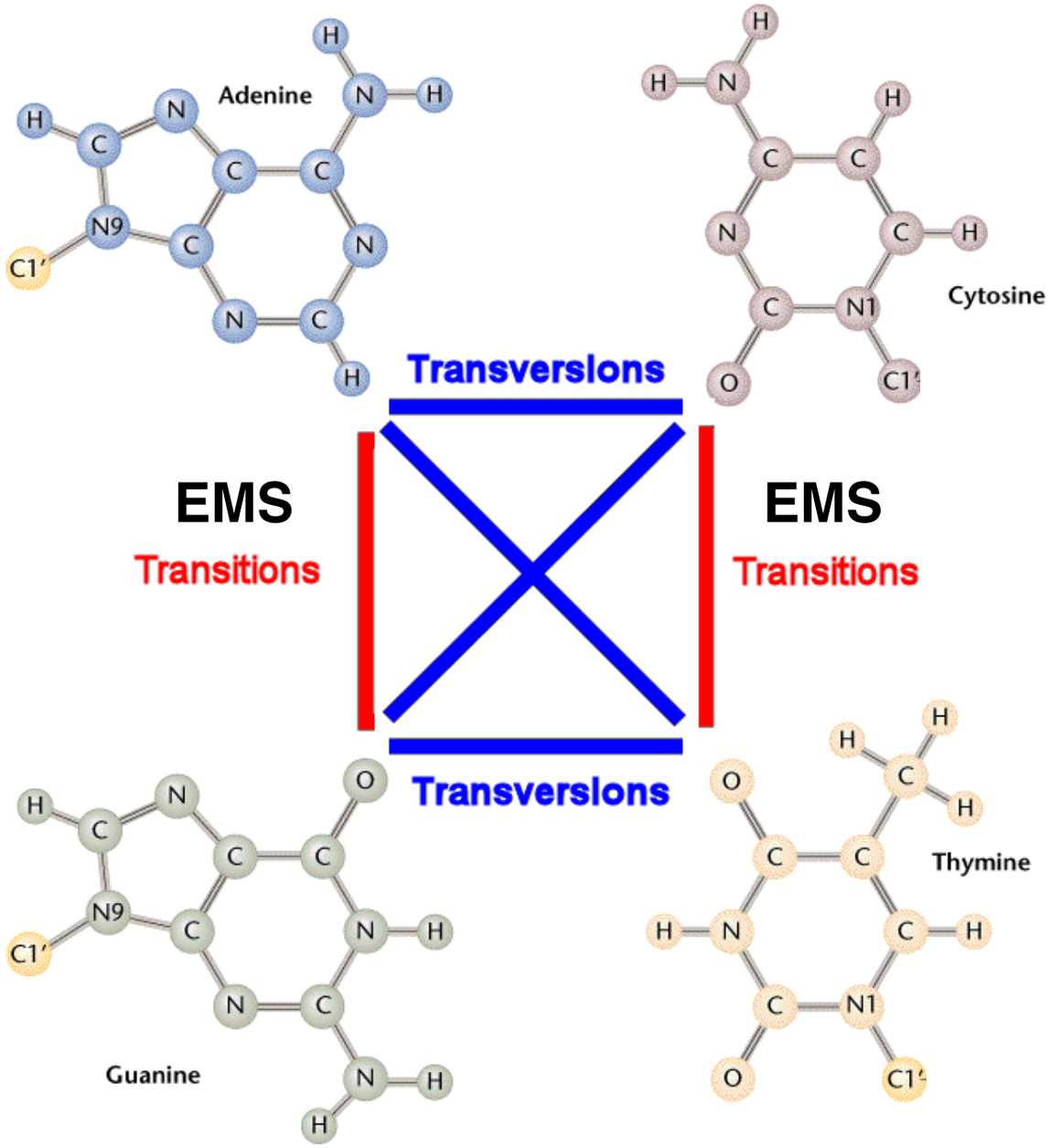
CRISPR/Cas9, targeted mutations

Positives: Targeted, Designable, Defined genetic background, Scalable
Negatives: Off target effects? Delivery?

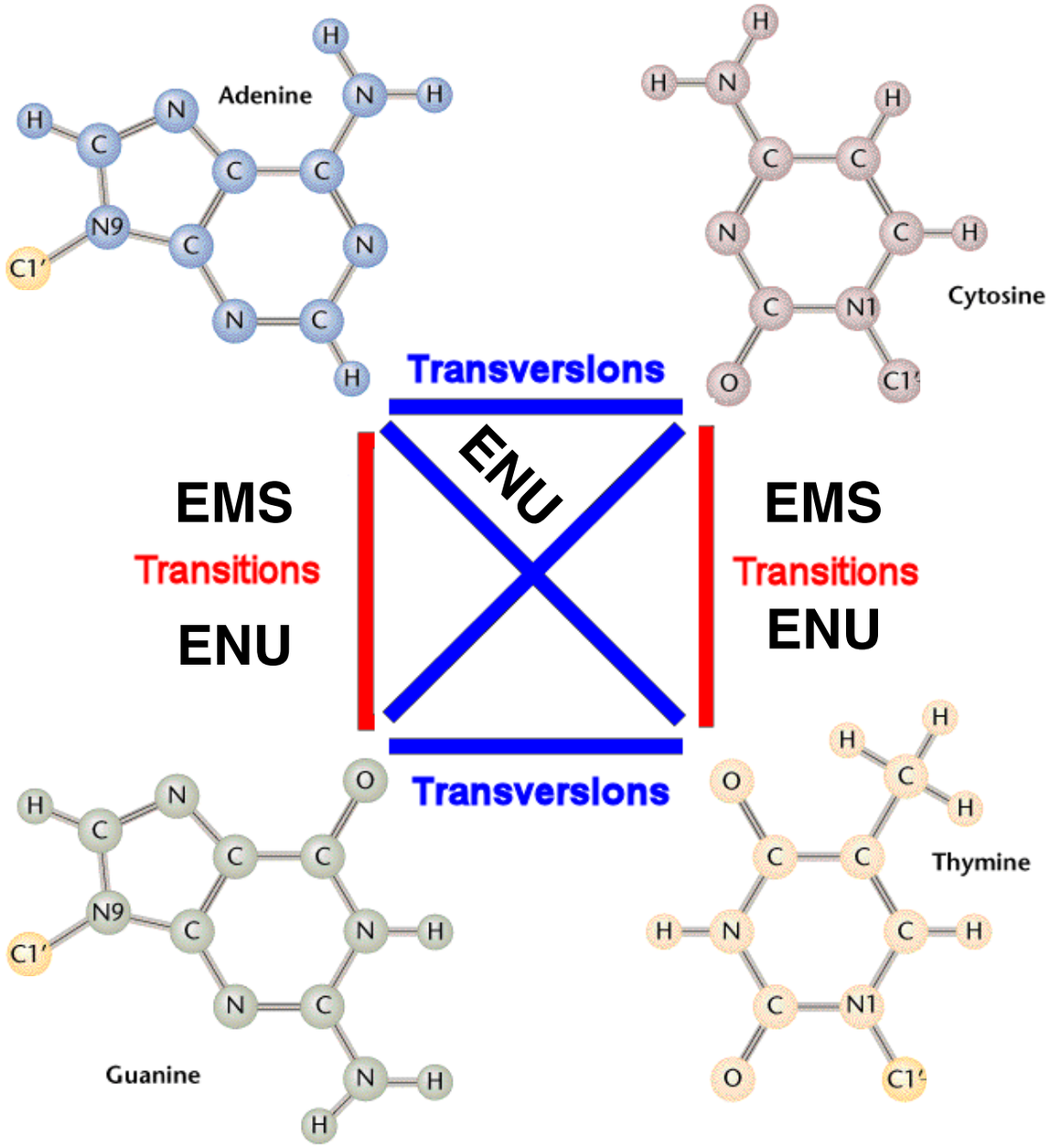
Single-base substitutions



Single-base substitutions



Single-base substitutions



Two ways to isolate mutants: selection or screen

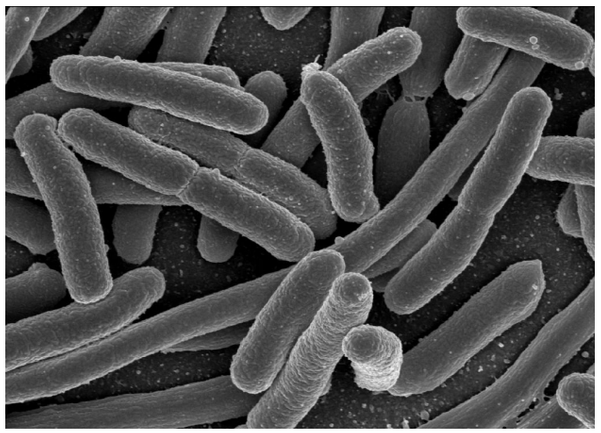
Selection: You only get the mutants you want

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

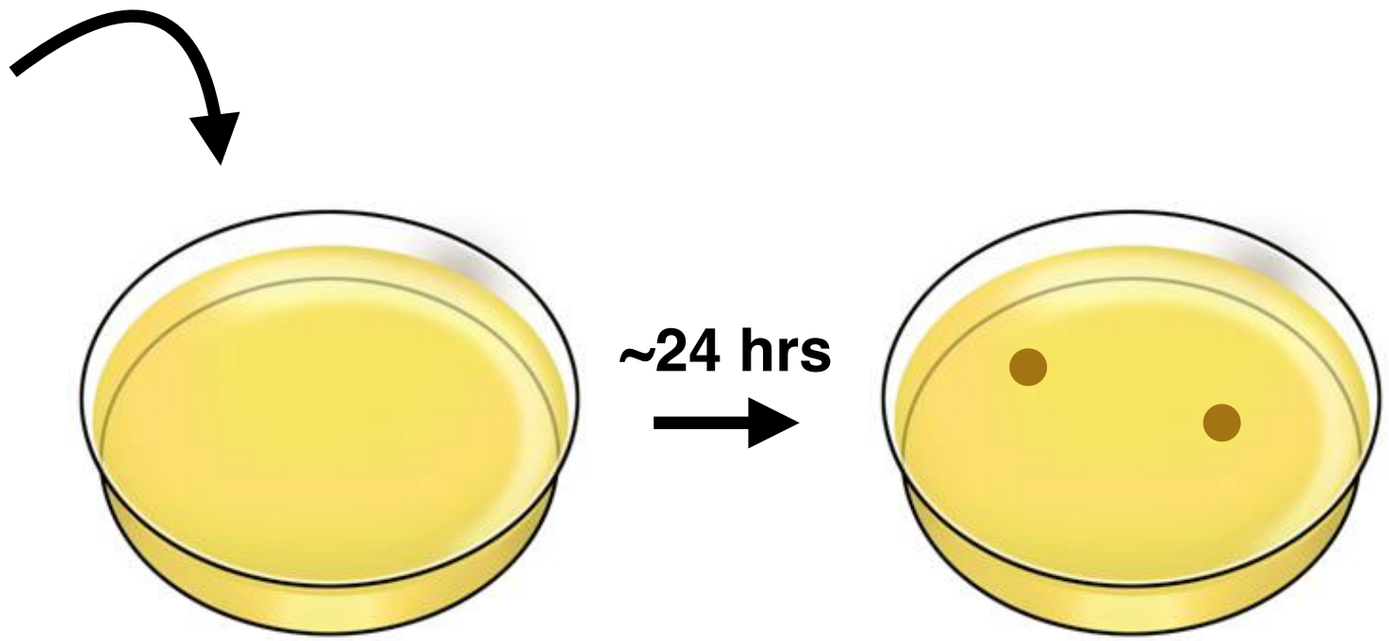
You don’t always get what you want!

Two ways to isolate mutants: selection or screen

Selection:

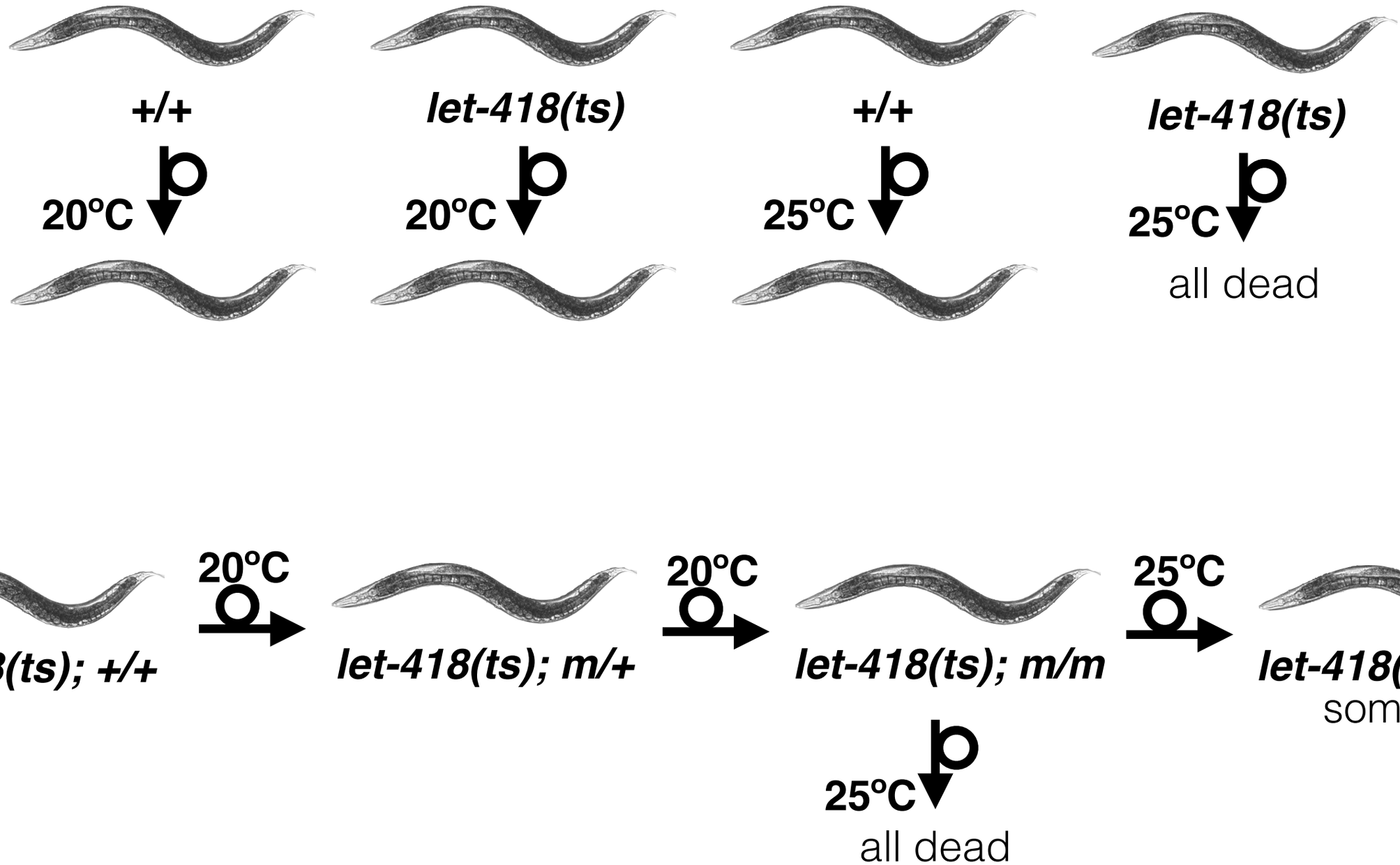


E. coli



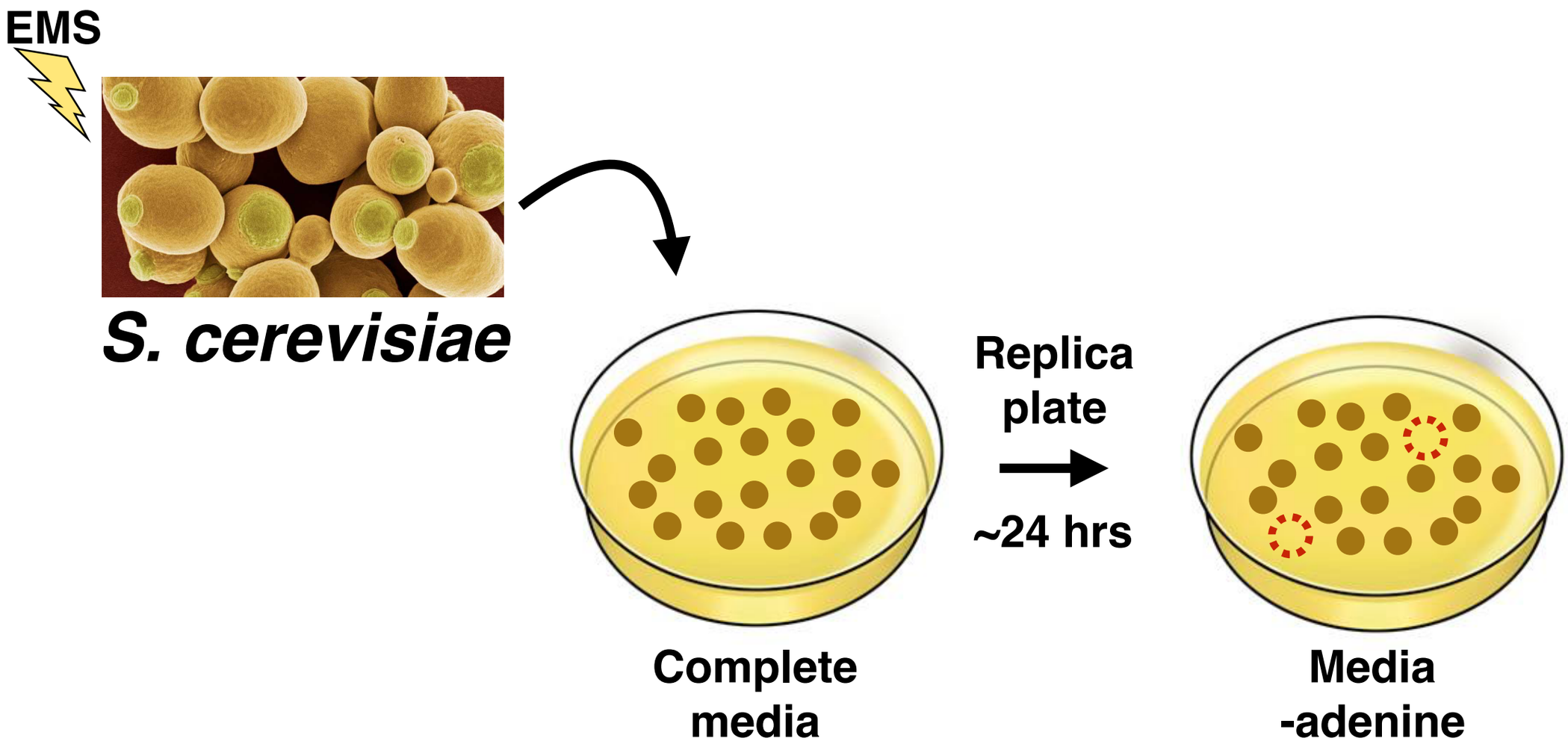
Two ways to isolate mutants: selection or screen

Selection:



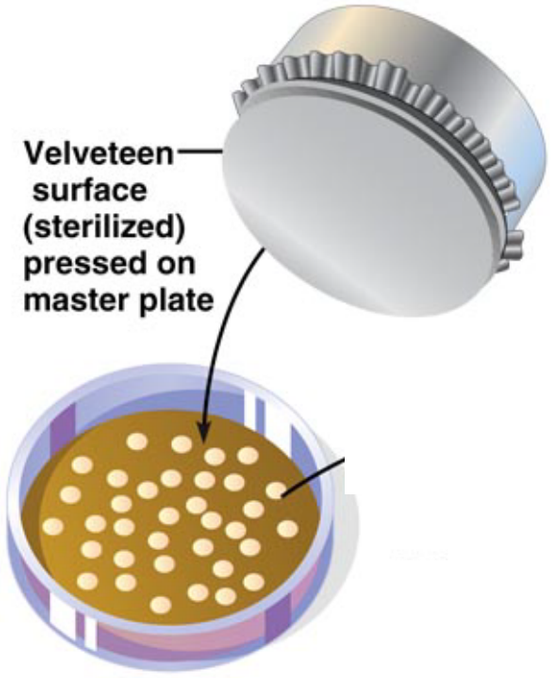
Two ways to isolate mutants: selection or screen

Screen:



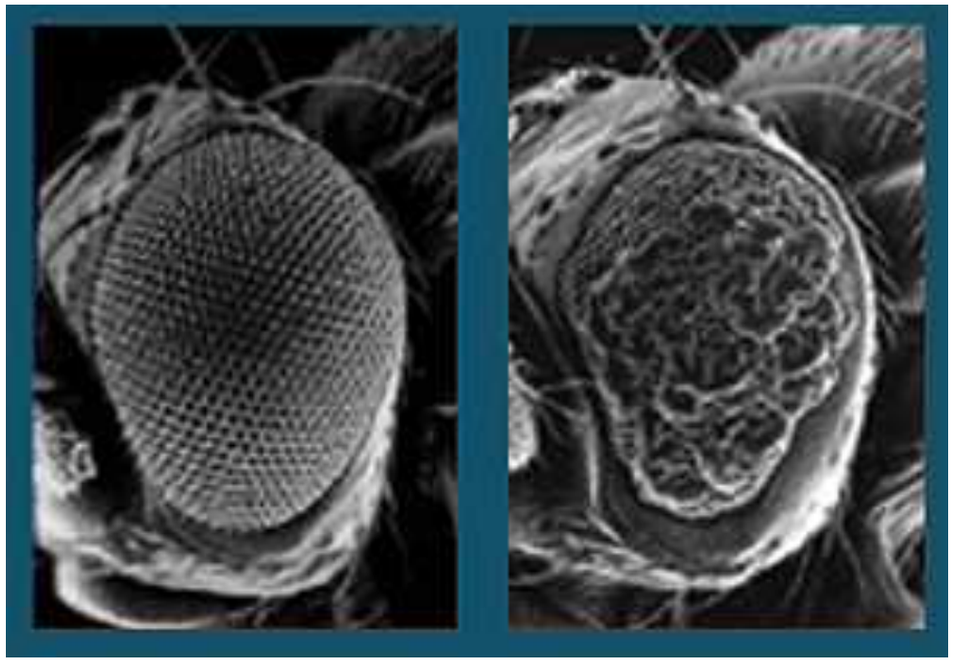
Why not directly plate on -adenine media?

Replica plating

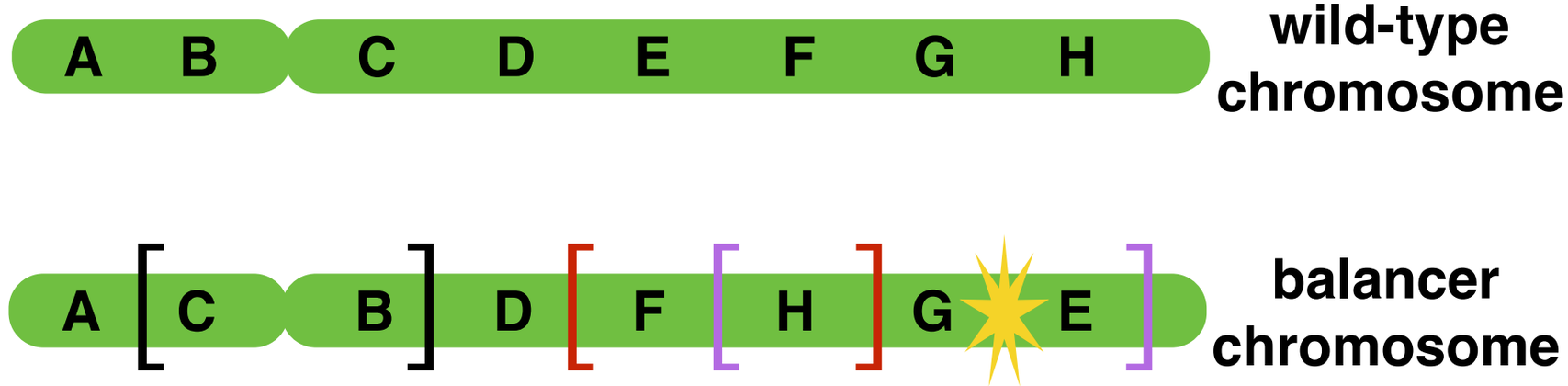


Two ways to isolate mutants: selection or screen

D. melanogaster



Drosophila have balancer chromosomes



Every balancer chromosome:

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

Sp

CyO

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

EMS

$\frac{+}{+}$ ♂♂ x $\frac{Sp}{CyO}$ ♀♀ **Chr2 recessive screen**

$\frac{+}{CyO}$ OR $\frac{m}{CyO}$ ♂ x $\frac{Sp}{CyO}$ ♀ **Single-pair crosses**

Most organisms are not mutant

$\frac{m}{CyO}$ ♂ x $\frac{m}{CyO}$ ♀♀

$\frac{m}{m}$ OR $\frac{m}{CyO}$ OR $\frac{CyO}{CyO}$

Mutant? **Curly wings** **dead**

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

EMS

$\frac{\pm}{+}$ ♂

♀

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

♀

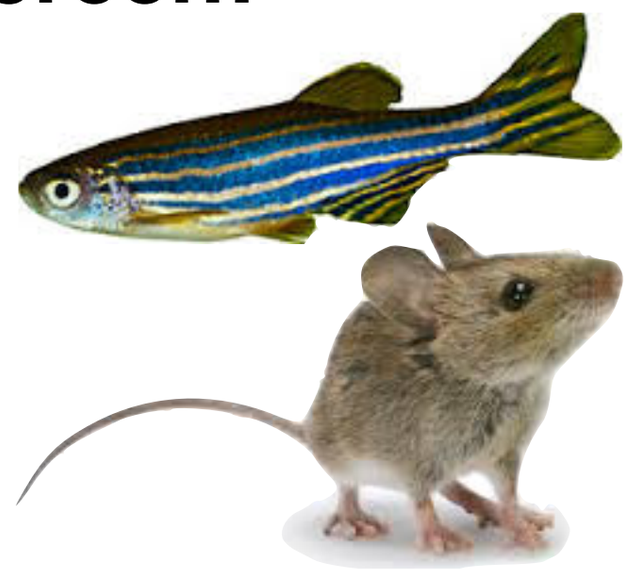
$\frac{\pm}{+}$ OR $\frac{m}{+}$ OR $\frac{m}{m}$

F_2 non-clonal screen

Hunt for your mutants!

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

Screen:



ENU

$\frac{\pm}{+}$♂ x $\frac{\pm}{+}$♀ **F_2 recessive screen**

↓

$\frac{m}{+}$♂ x $\frac{\pm}{+}$♀ **Single-pair crosses**

↓

$\frac{m}{+}$♂ x $\frac{m}{+}$♀ **Bulk crosses**

↓

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

Hunt for your mutants!

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

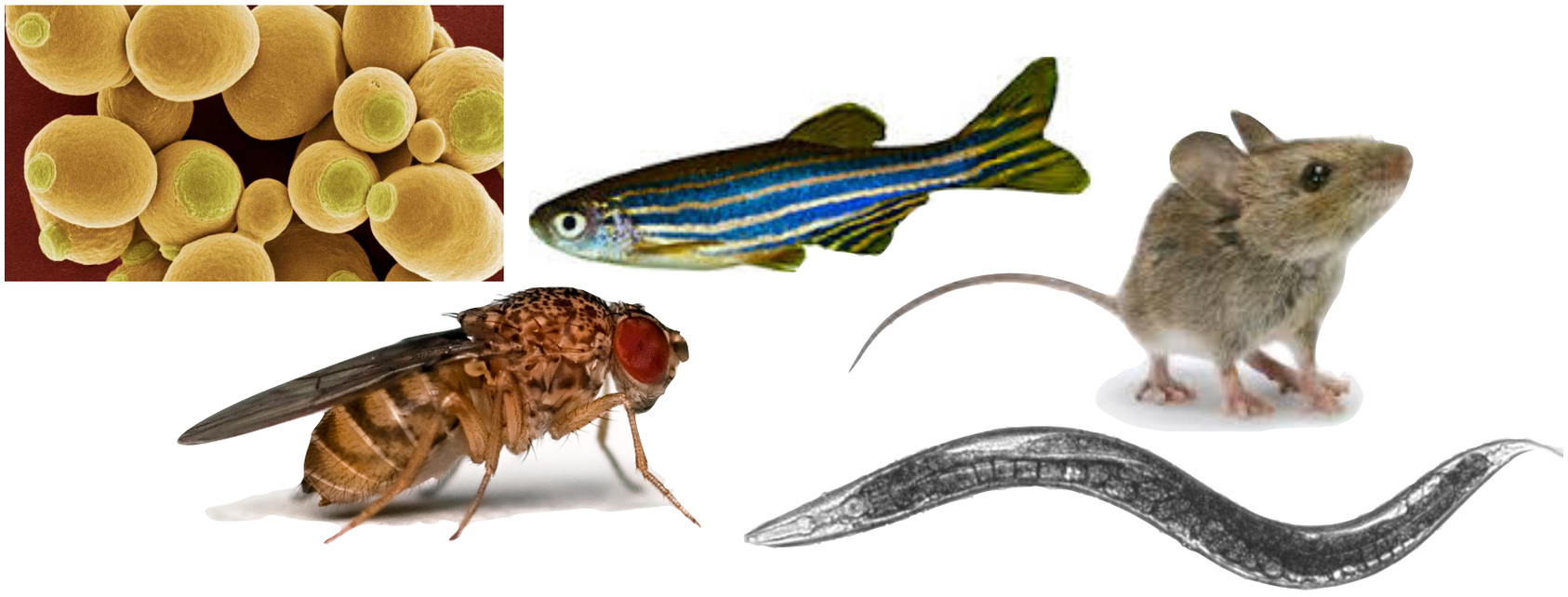
Alleles that confer recessive traits teach us about:

- 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?