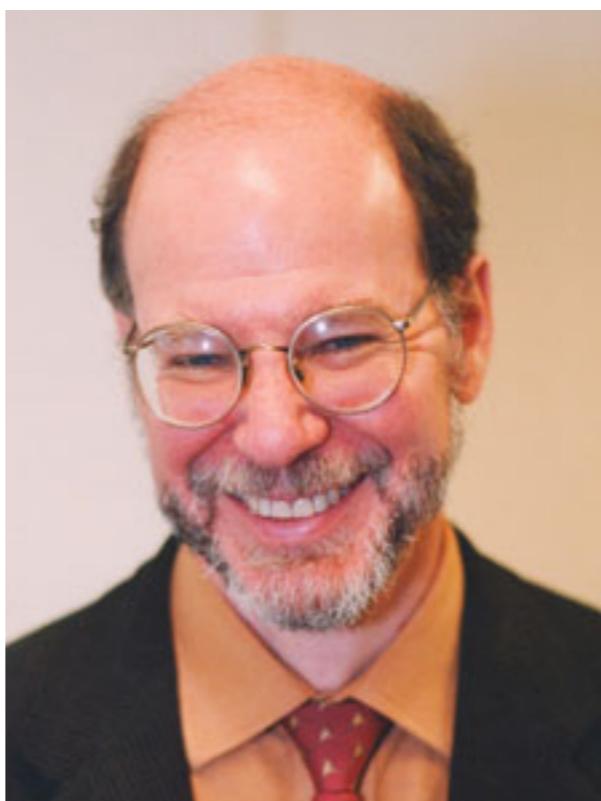


# **Step-wise genetic analysis**



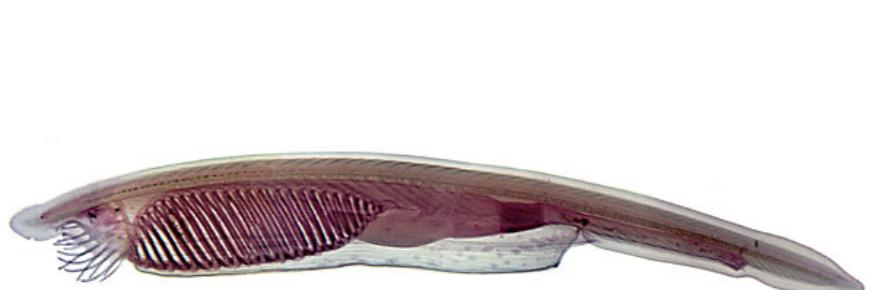
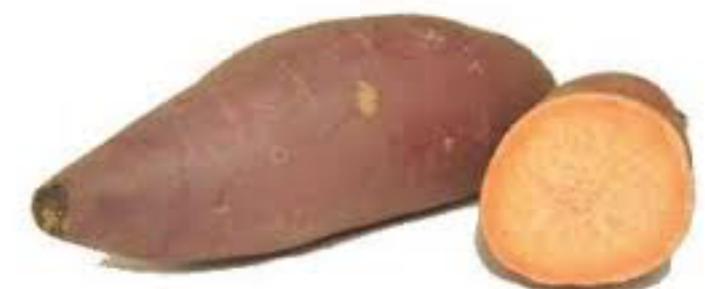
**Bob Horvitz**

# 1. Define the problem



Let the question influence the choice of organism  
(not the other way around)

# “Model organisms” are everywhere now



## 2. Choose an organism

Organism	Time to $10^6$	Space
Bacteriophage	1 hour	10 nL

$10^6$  individuals to study  $10^{-6}$  mutation rate

$$2^{20} \approx 10^6 \text{ individuals}$$

## 2. Choose an organism

Organism	Time to $10^6$	Space
Bacteriophage	1 hour	10 nL
Bacteria	15 hours	1 $\mu$ L

$10^6$  individuals to study  $10^{-6}$  mutation rate

$$2^{20} \approx 10^6 \text{ individuals}$$

## 2. Choose an organism

Organism	Time to $10^6$	Space
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Bacteria	15 hours	1 $\mu$ L
Yeast	1 day	0.1 mL

$10^6$  individuals to study  $10^{-6}$  mutation rate

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## 2. Choose an organism

Organism	Time to $10^6$	Space
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Yeast	1 day	0.1 mL
Worm	10 days	6 cm cube

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Fly	6 weeks	0.5 m cube

$10^6$  individuals to study  $10^{-6}$  mutation rate

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Mouse	3 years	Half Pancoe

$10^6$  individuals to study  $10^{-6}$  mutation rate

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## 2. Choose an organism

Organism	Time to $10^6$	Space
Bacteriophage	1 hour	10 nL
Bacteria	15 hours	1 $\mu$ L
Yeast	1 day	0.1 mL
Worm	10 days	6 cm cube
Fly	6 weeks	0.5 m cube
Mouse	3 years	Half Pancoe
Human	750 years	Chicago suburbs

$10^6$  individuals to study  $10^{-6}$  mutation rate

$$2^{20} \approx 10^6 \text{ individuals}$$

### 3. Perform a mutant hunt

To mutagenize?

Yes	No	
$10^{-3}$	$10^{-6}$	LoF mutation
$10^{-5}$ - $10^{-6}$	$10^{-8}$ - $10^{-9}$	Specific mutation

*C. elegans*



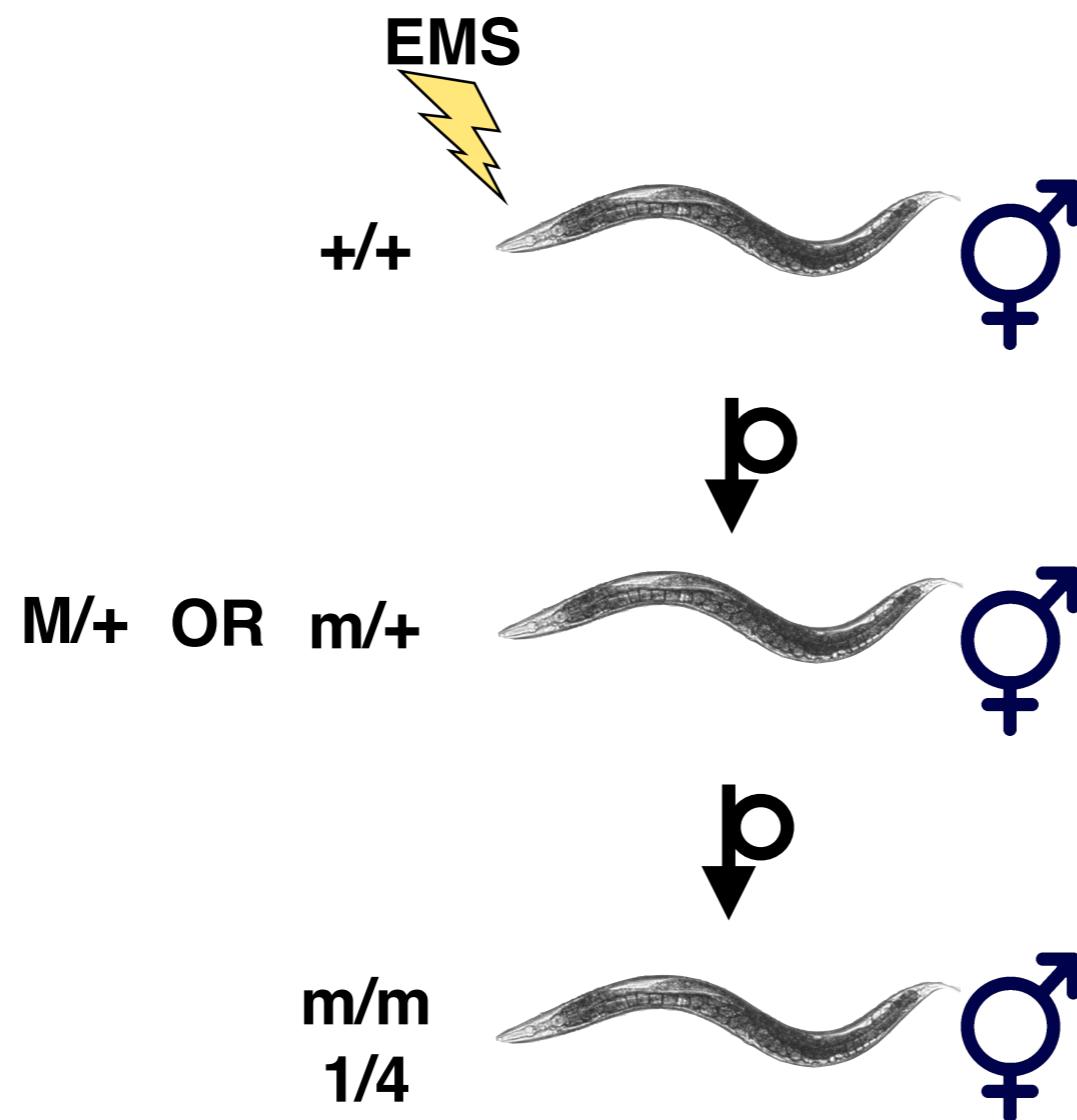
~20,000 genes  
20 LoF mutations

*D. melanogaster*



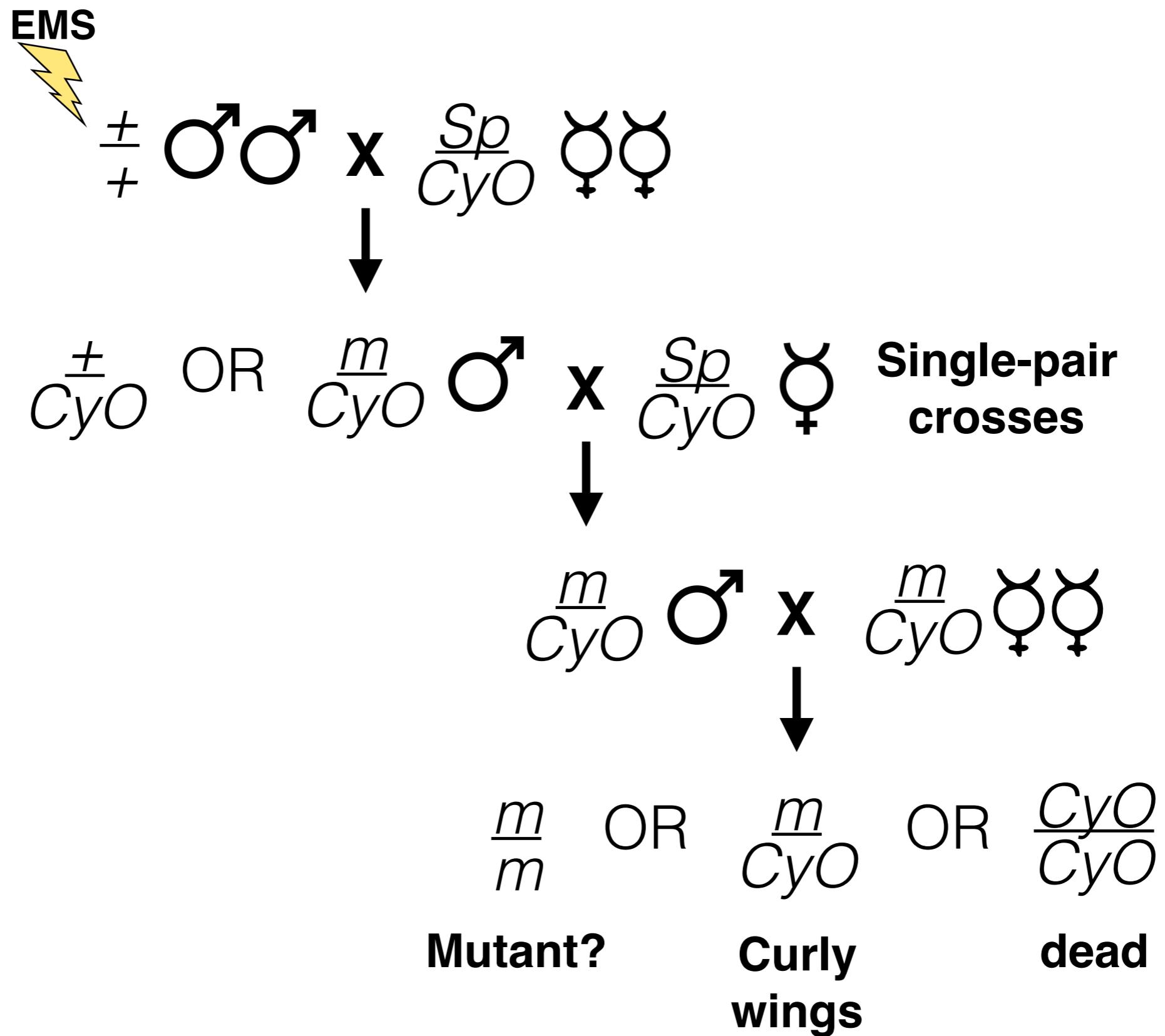
~12,000 genes  
12 LoF mutations

# Screen or selection?

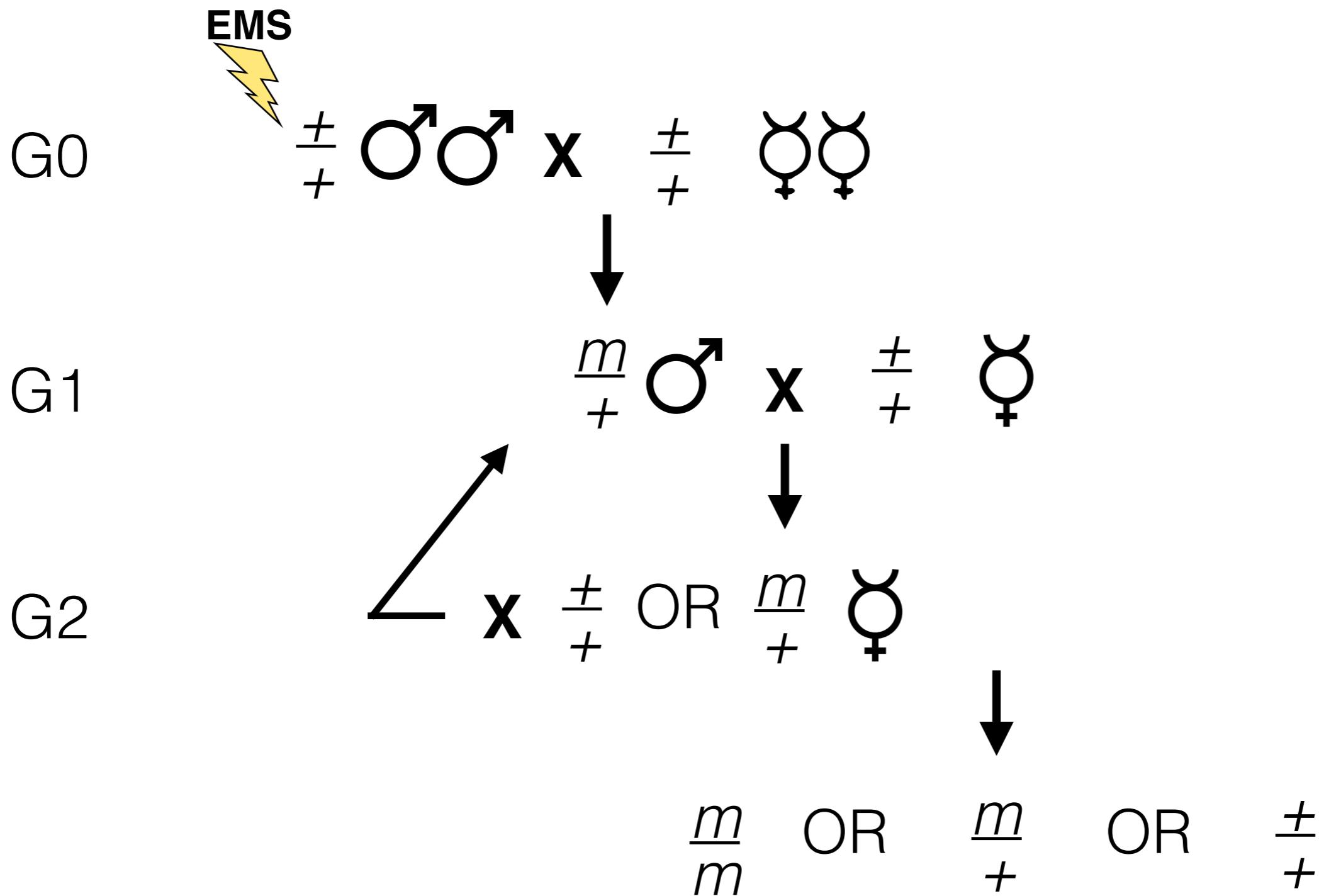


*C. elegans* screens for dominant or recessive phenotypes

# Screen or selection?



# Screen or selection?



Mouse screens for dominant or recessive phenotypes

Remember hemizygous screens too

## **4. Screen until saturation?**

Use Poisson sampling and common sense

Change mutagens

Saturation of the investigator's patience

### **Why might we miss genes?**

Numbers are too small

Pleiotropy (sterility or lethality)

Redundancy

**The most common phenotypes are sterile or dead!**

## 5. Establish a strain

True-breeding stocks

Balancers, balanced stocks

Freeze organisms

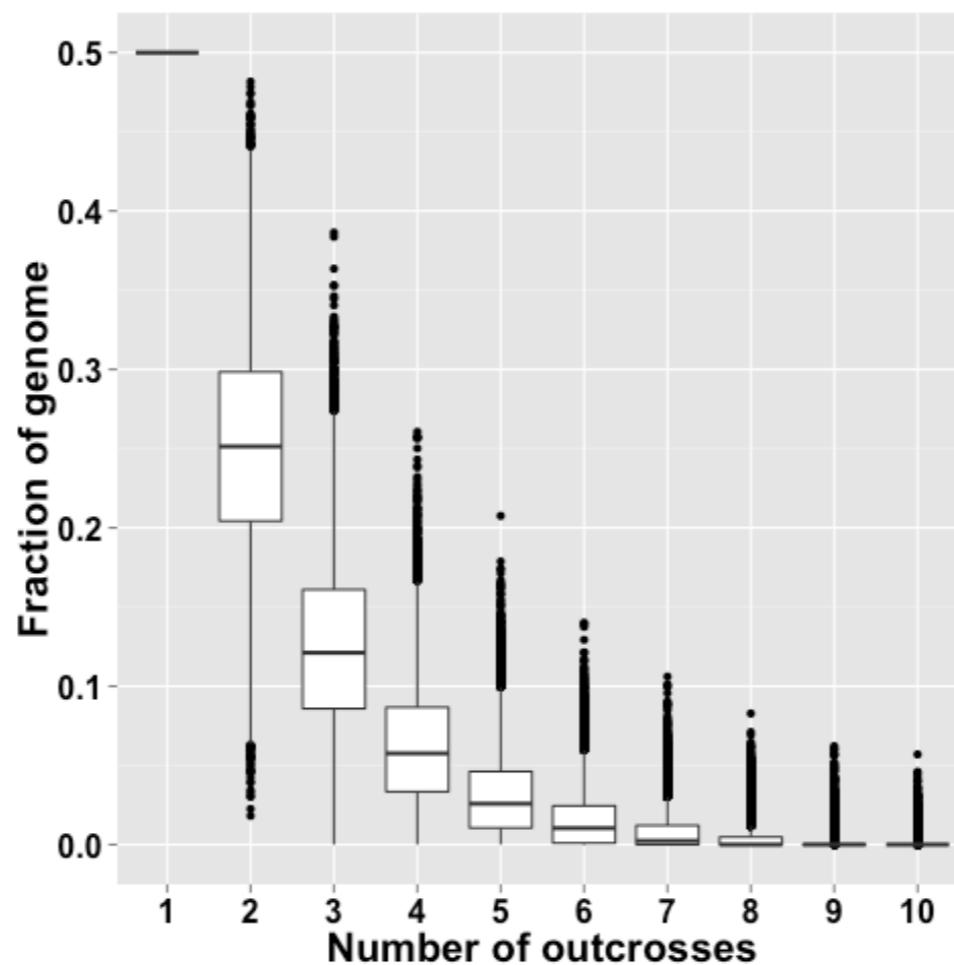
**The most common phenotypes are sterile or dead!**

# 6. Backcross and/or outcross

Mutagenesis adds hundreds of mutations randomly throughout the genome.

Backcross = cross to parent used in the screen/selection

Outcross = cross to a wild-type strain



**7. Test for dominance**

**8. Single-gene phenotype?**

**9. Mapping and complementation**

What have we discovered so far?

# 10. Characterize the phenotype

Look at the wild-type and mutant organisms *in detail*



Let's say you  
screened for mutants  
that failed to lay eggs

What could be  
mutated?

No embryos

No vulva

No vulval muscles

No neurons

Or malfunction of any vulva, muscle, or neuron

# Pleiotropy

A single mutation causes many different mutant traits

Mutation in gene X



Mutant with...

- long hairs
- disrupted sleep patterns
- slow growth
- enhanced metabolism of high fat diet

- gene X → short hair
- gene X → normal sleep
- gene X → normal growth
- gene X → fat metabolism

# **11. Define the nature of the mutant allele(s): gene dosage**

1. Dominant or recessive?
2. Frequency of mutant?
3. Where is the mutant allele in allelic series?
4. Look at deficiency heterozygotes for haploinsufficiency
5. Antagonism by wild-type copies of gene

What if you only have one mutant?

# 12. Perform non-complementation screens

EMS



$$\text{♂ } \frac{\pm}{+} \times \frac{m_1}{m_1} \text{ ♀}$$



$$\frac{m_1}{+}$$

OR

$$\frac{m_1}{m_2}$$

Common  
(wild-type)

Rare  
(mutant)

OR  $\frac{m_1}{+} \frac{+}{M}$

Rare  
(dominant)

# 13. Define the null phenotype

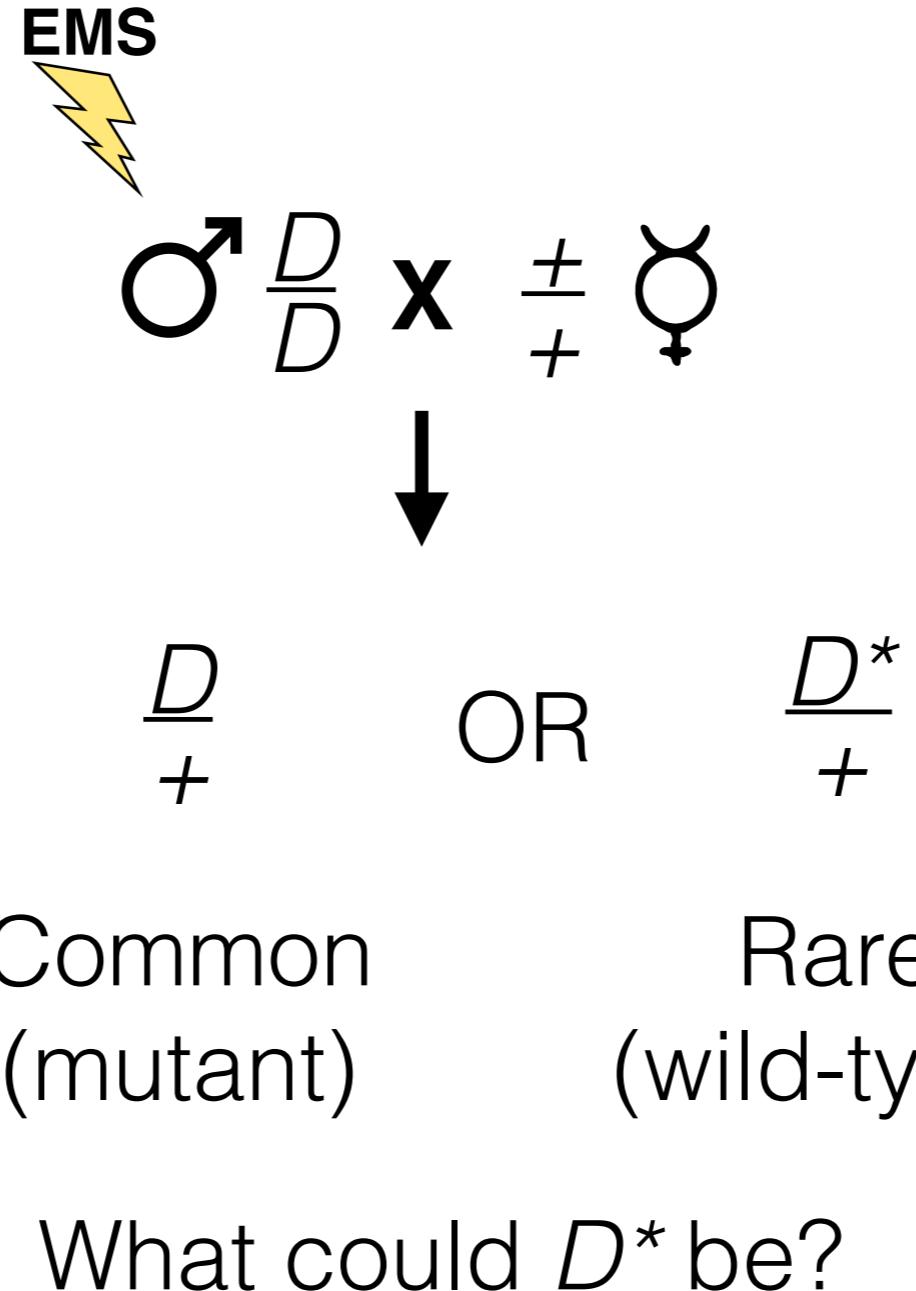
What happens with a complete loss of gene function?

Dosage studies, non-complementation screens, and characterization of the mutant phenotype tell you about the null phenotype

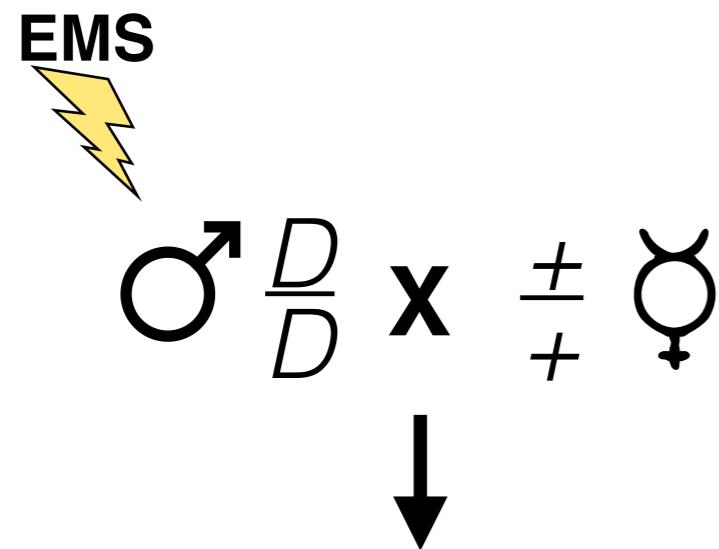
You need a null to characterize gene function. Why?

What if you have a mutant with a dominant gain-of-function phenotype?

# Cis-dominant suppressor screen

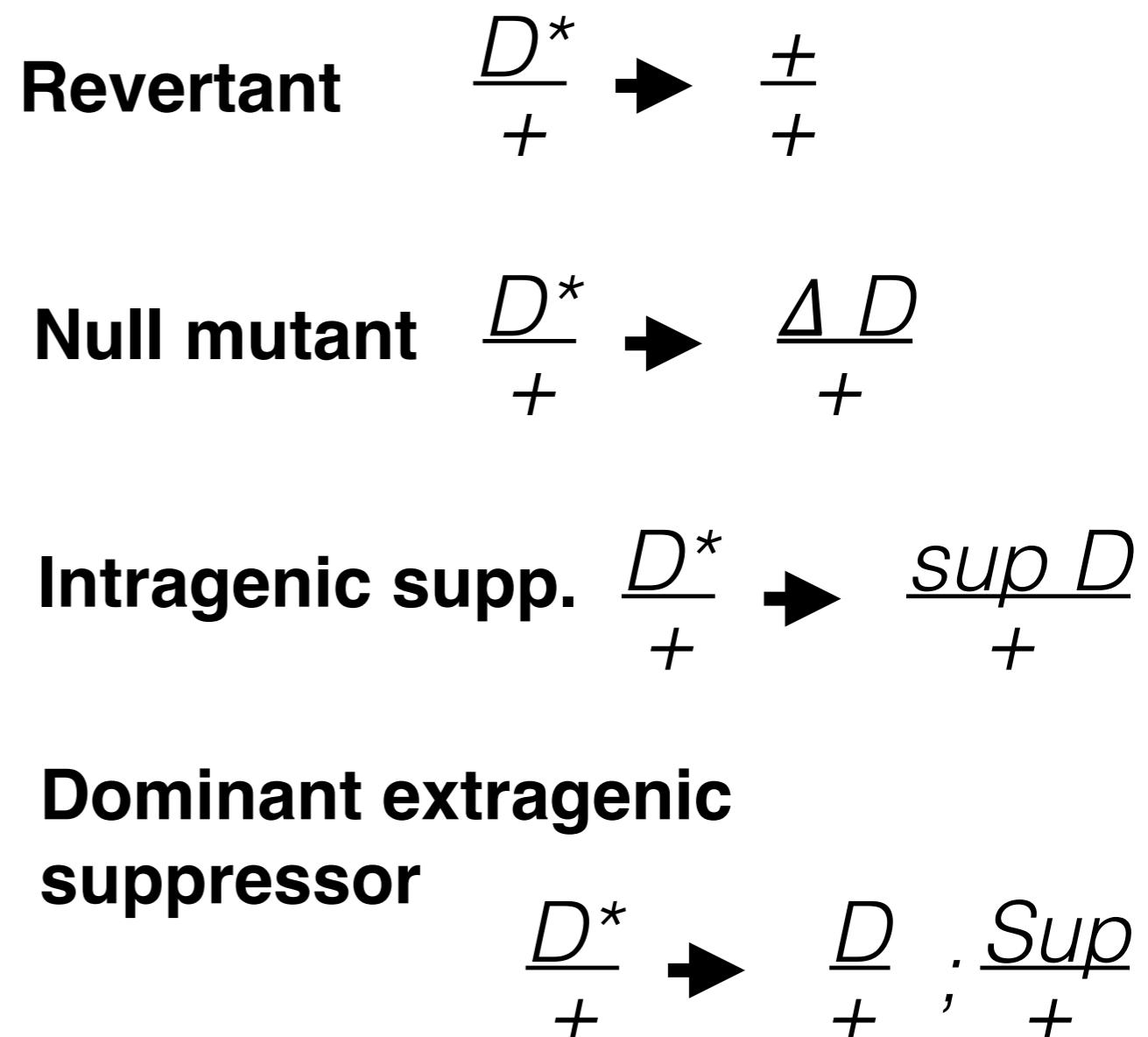


# Cis-dominant suppressor screen



$\frac{D}{+}$  OR  $\frac{D^*}{+}$

Common (mutant)      Rare (wild-type)



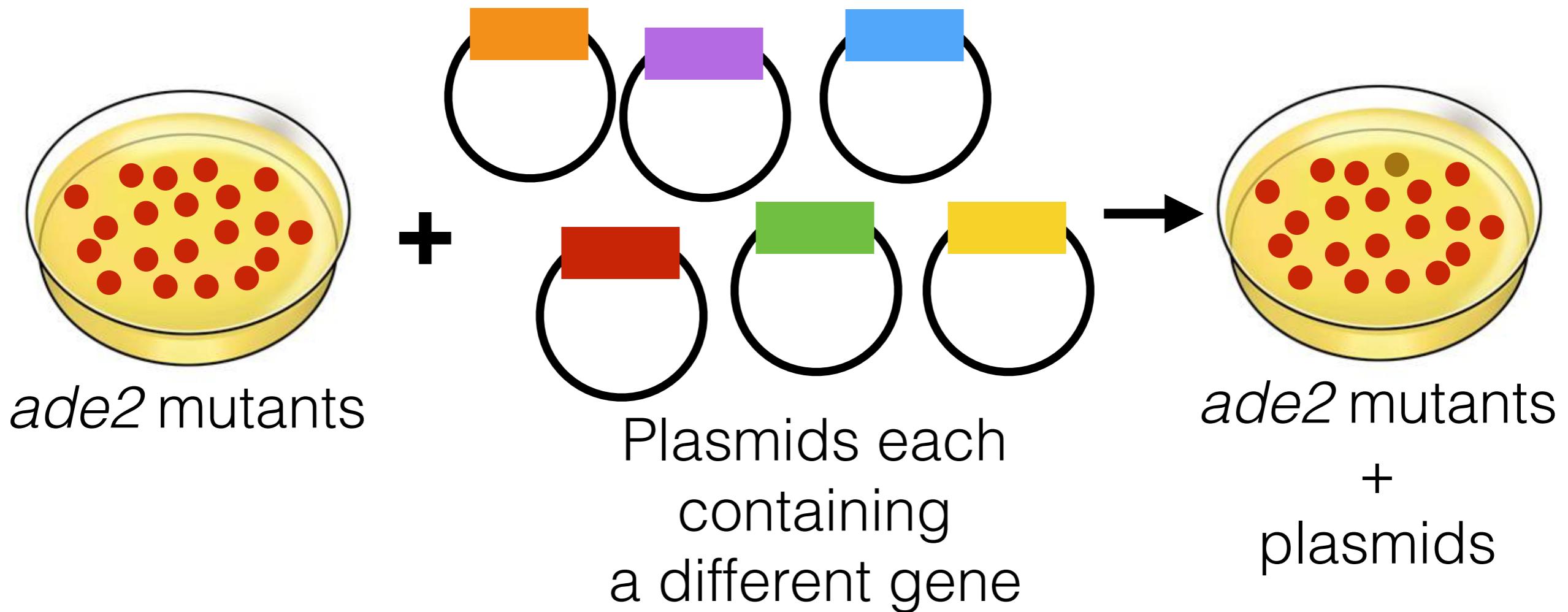
How can we tell what we got?

# 14. Clone the gene

***Identify a DNA sequence that contains  
your gene of interest***

1. Clone by complementation
2. Clone by phenocopy
3. Clone by sequencing

# Cloning by complementation in bacteria and yeast



Caveat: overexpression bypass suppressors

# Cloning by complementation in worms and flies

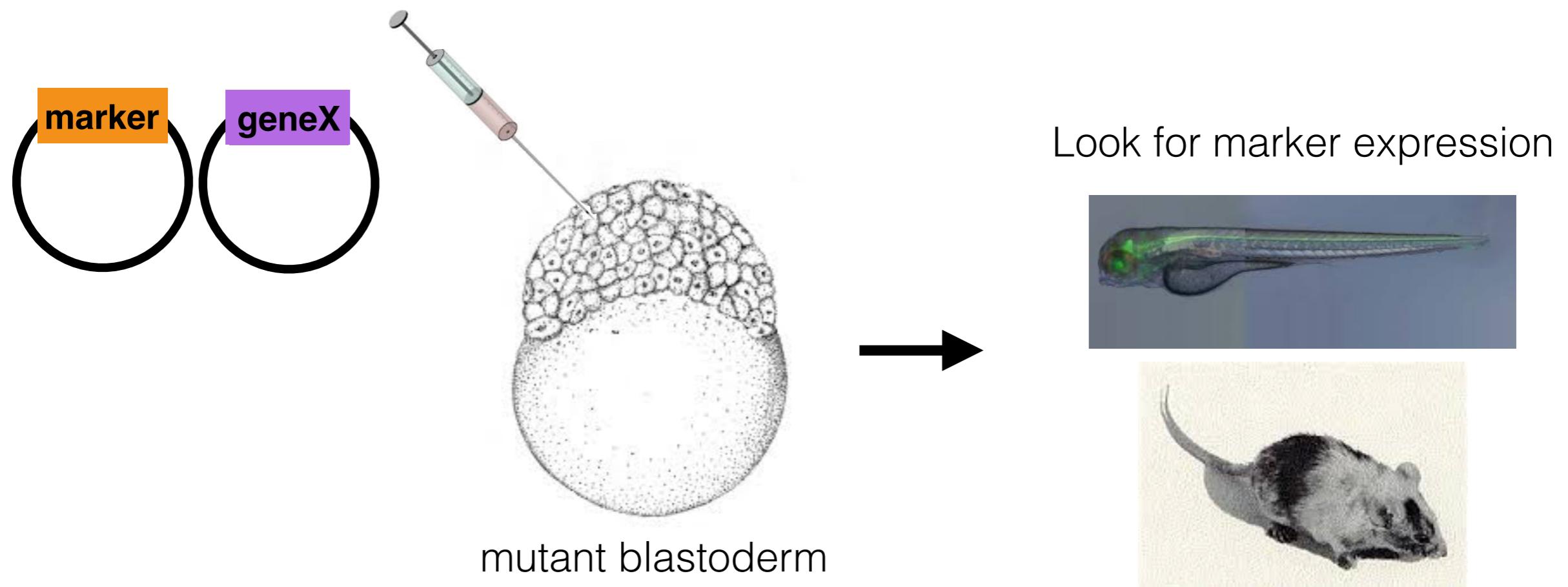


Caveat: overexpression bypass suppressors and not stable



Caveat: overexpression bypass suppressor and variable expression

# Cloning by complementation in fish and mice

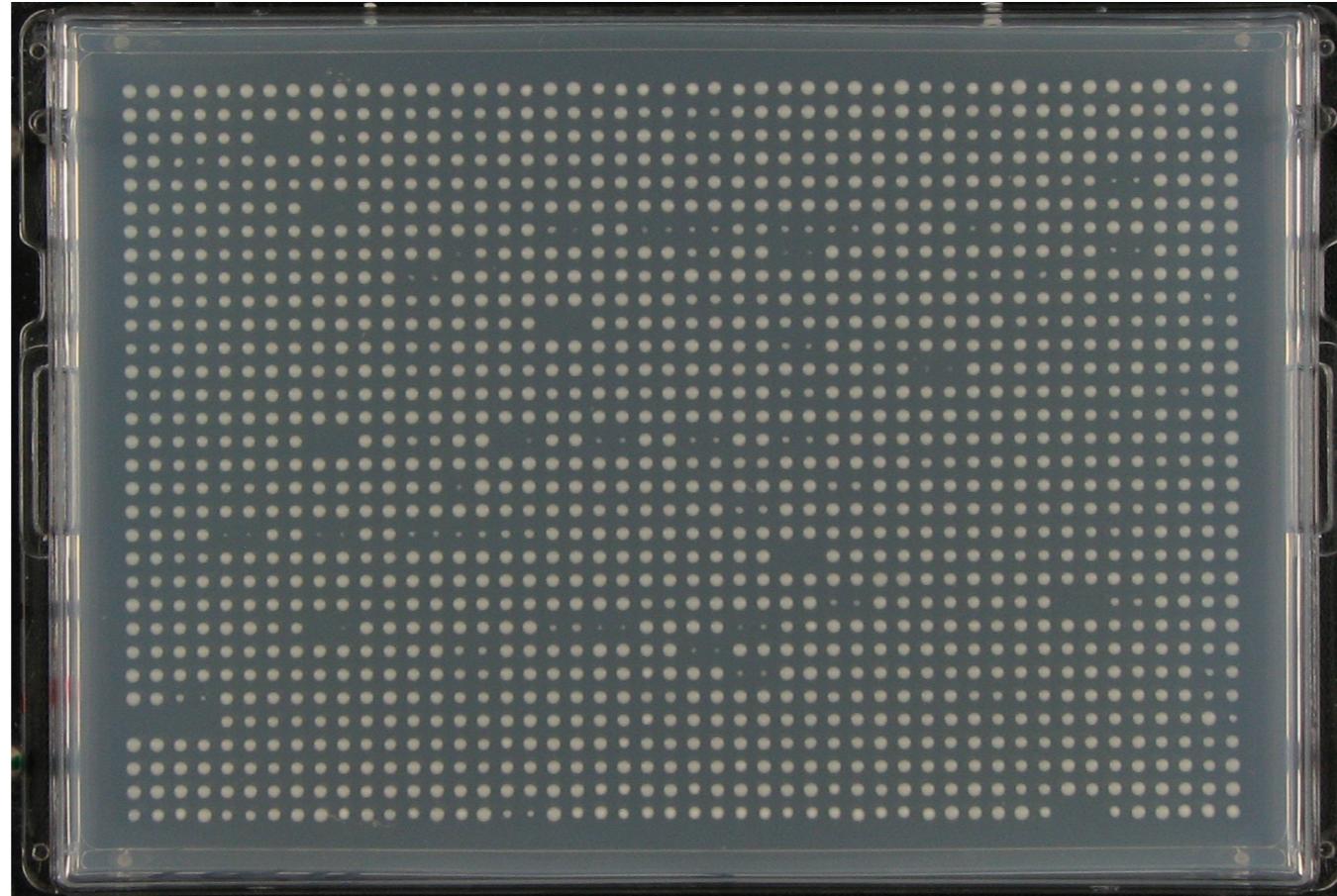


Caveat: overexpression bypass suppressors  
and variable expression

# **14. Clone the gene**

1. Clone by complementation
2. Clone by phenocopy
3. Clone by sequencing

**Most model organisms have libraries of strains  
where each strain has a unique loss-of-function mutation**



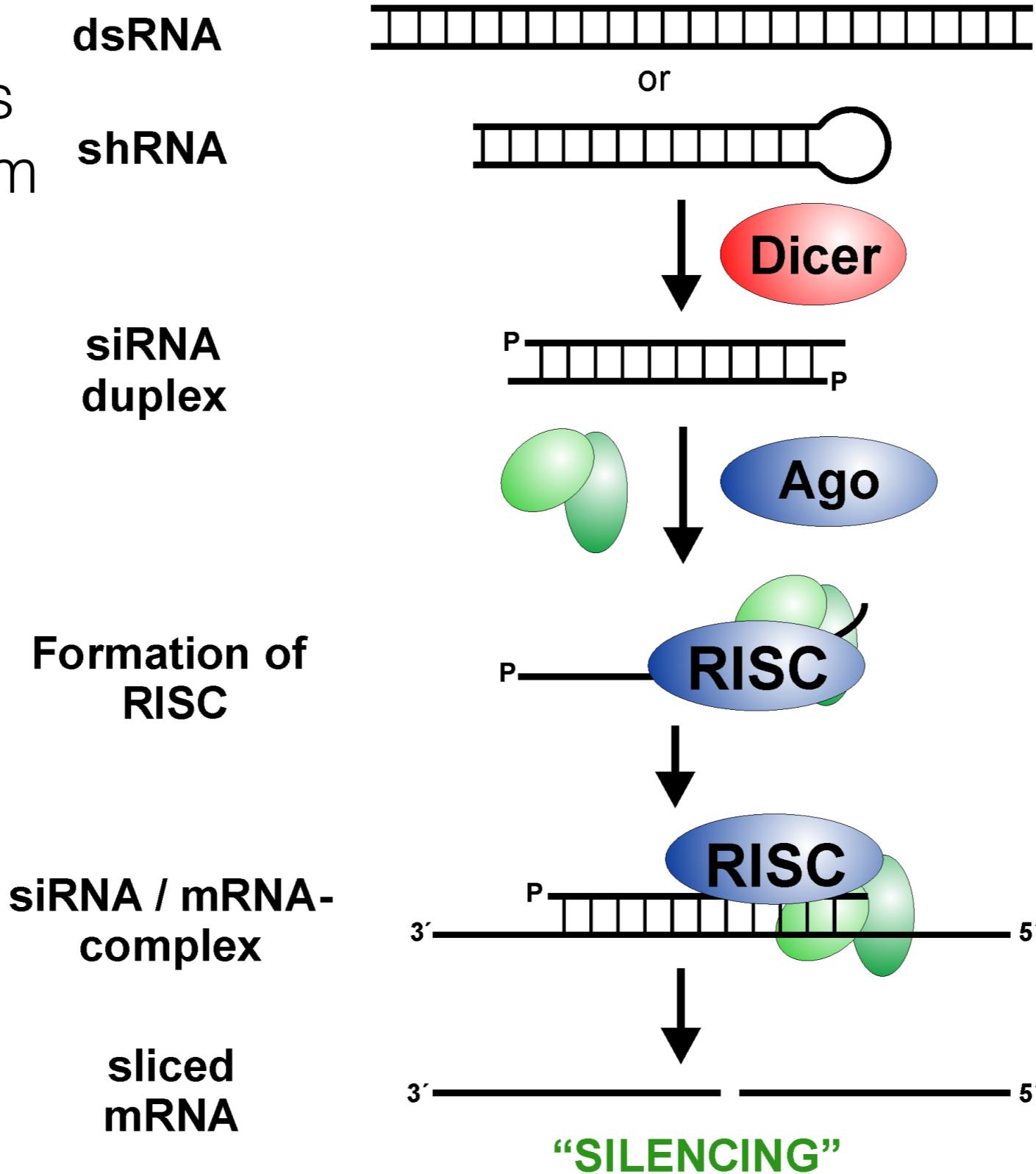
Score them all!!!

Phenotype is everything!

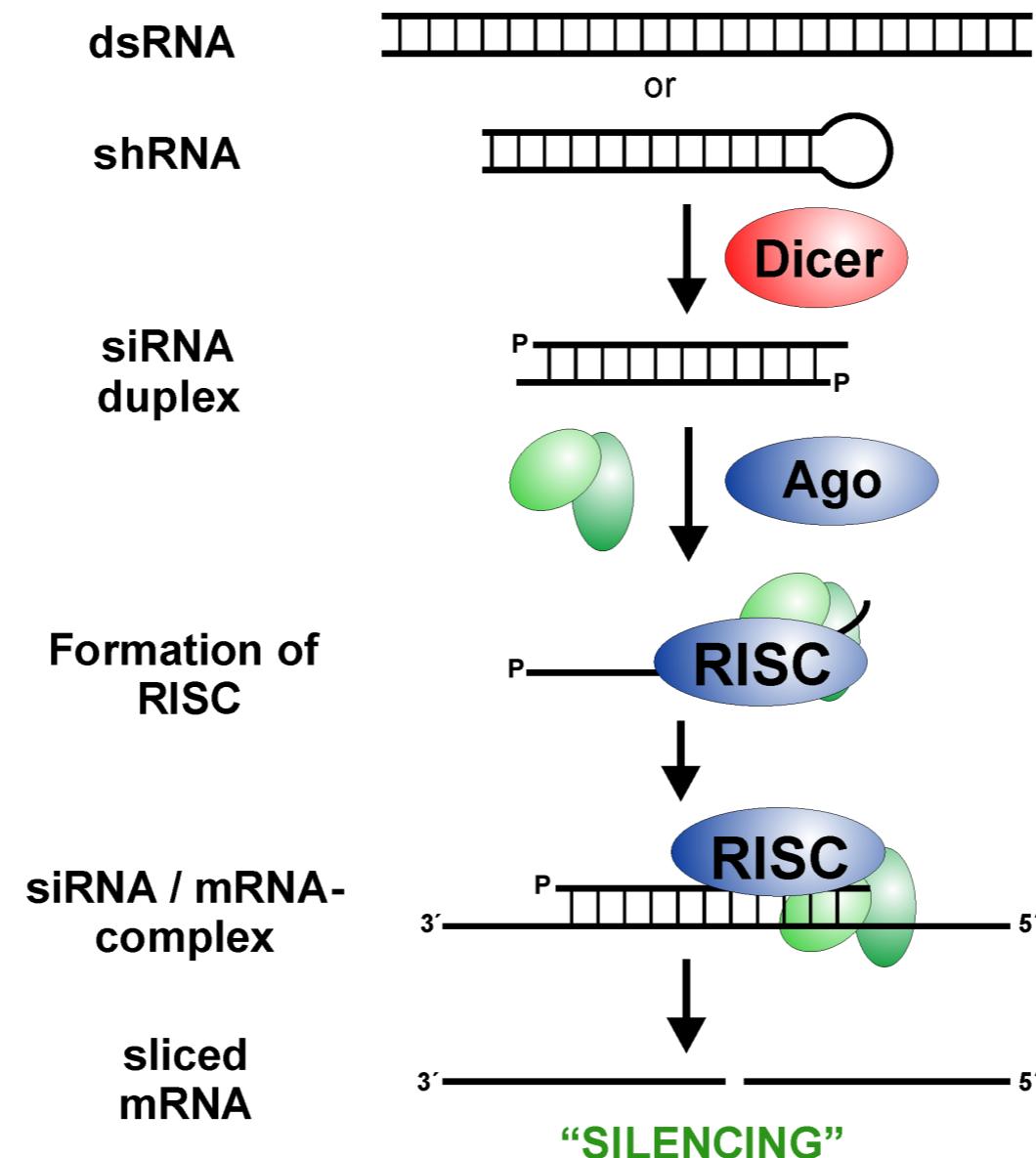
It has to be scalable, quantitative, reproducible,  
and accurate

# RNA interference (RNAi) can be used to inactivate most genes in a variety of organisms

You have to  
get these RNAs  
into the organism

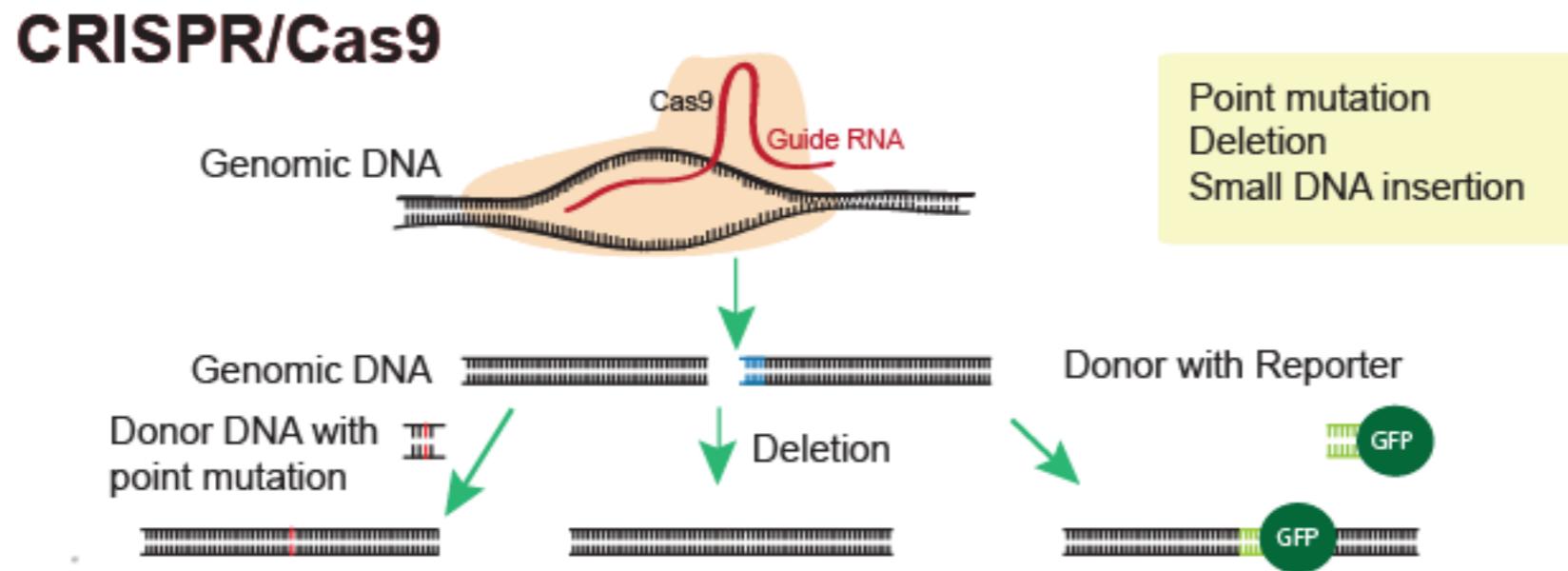


# RNA interference (RNAi) can be used to inactivate most genes in a variety of organisms



Caveat: Delivery is problematic, highly variable effects, not heritable, not loss-of-function, not specific

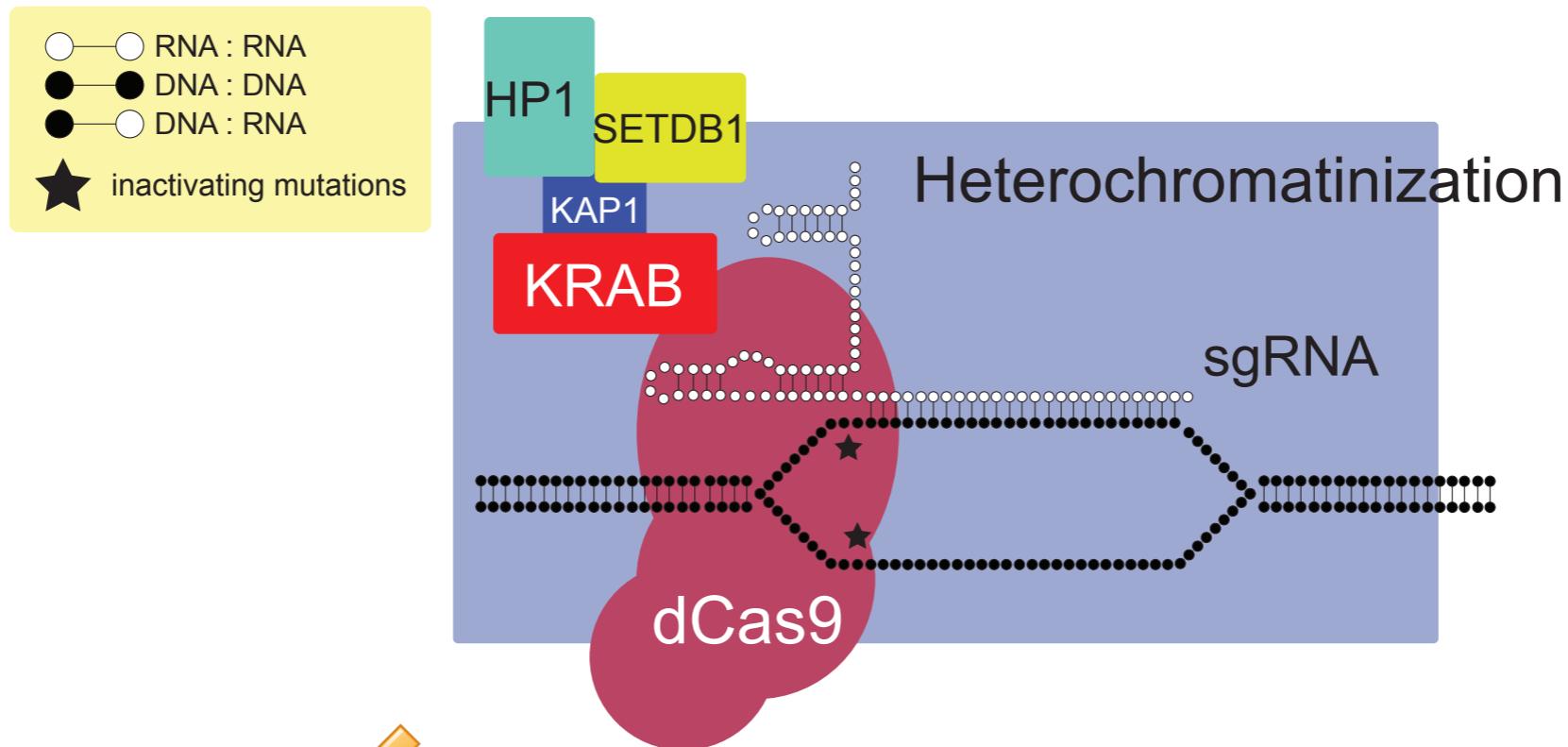
# CRISPR/cas9 can be used to create heritable loss-of-function mutations in a variety of organisms



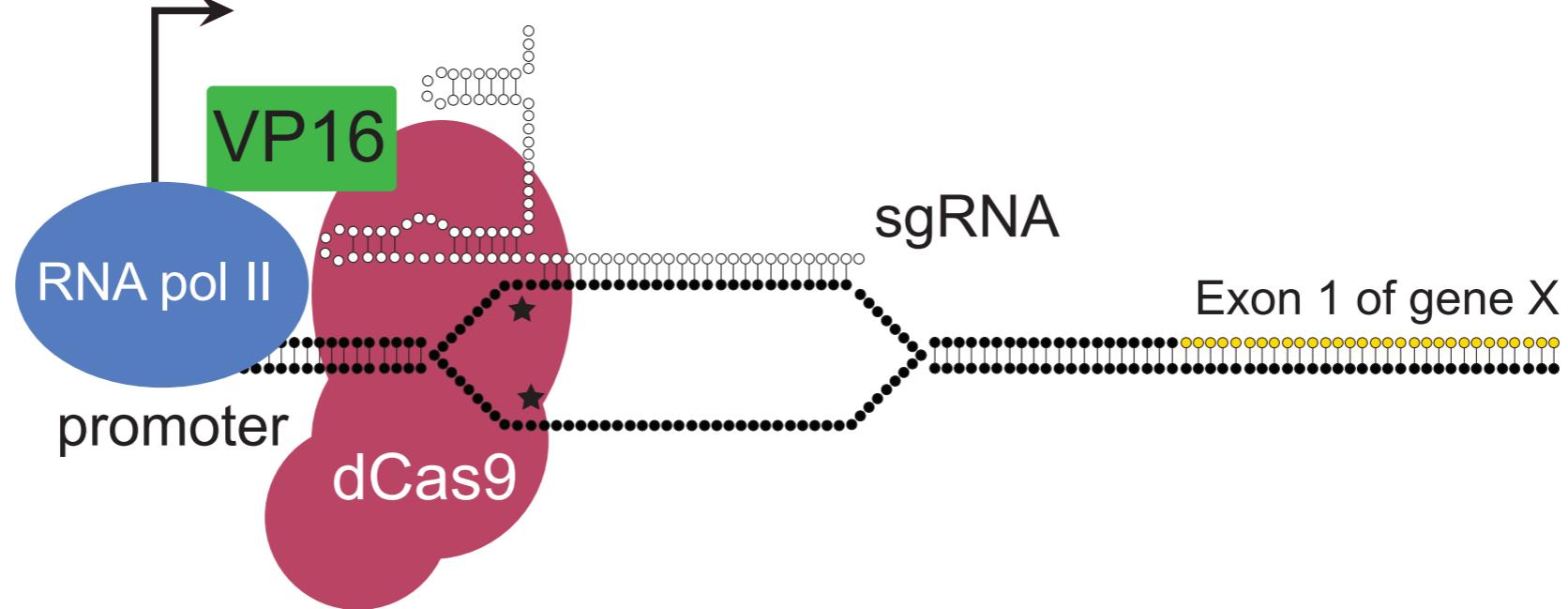
Caveat: Sometimes not specific (off-target effects)

# CRISRPi and CRISPRa can test gene function

## TRANSCRIPTION REPRESSION



## TRANSCRIPTION ACTIVATION



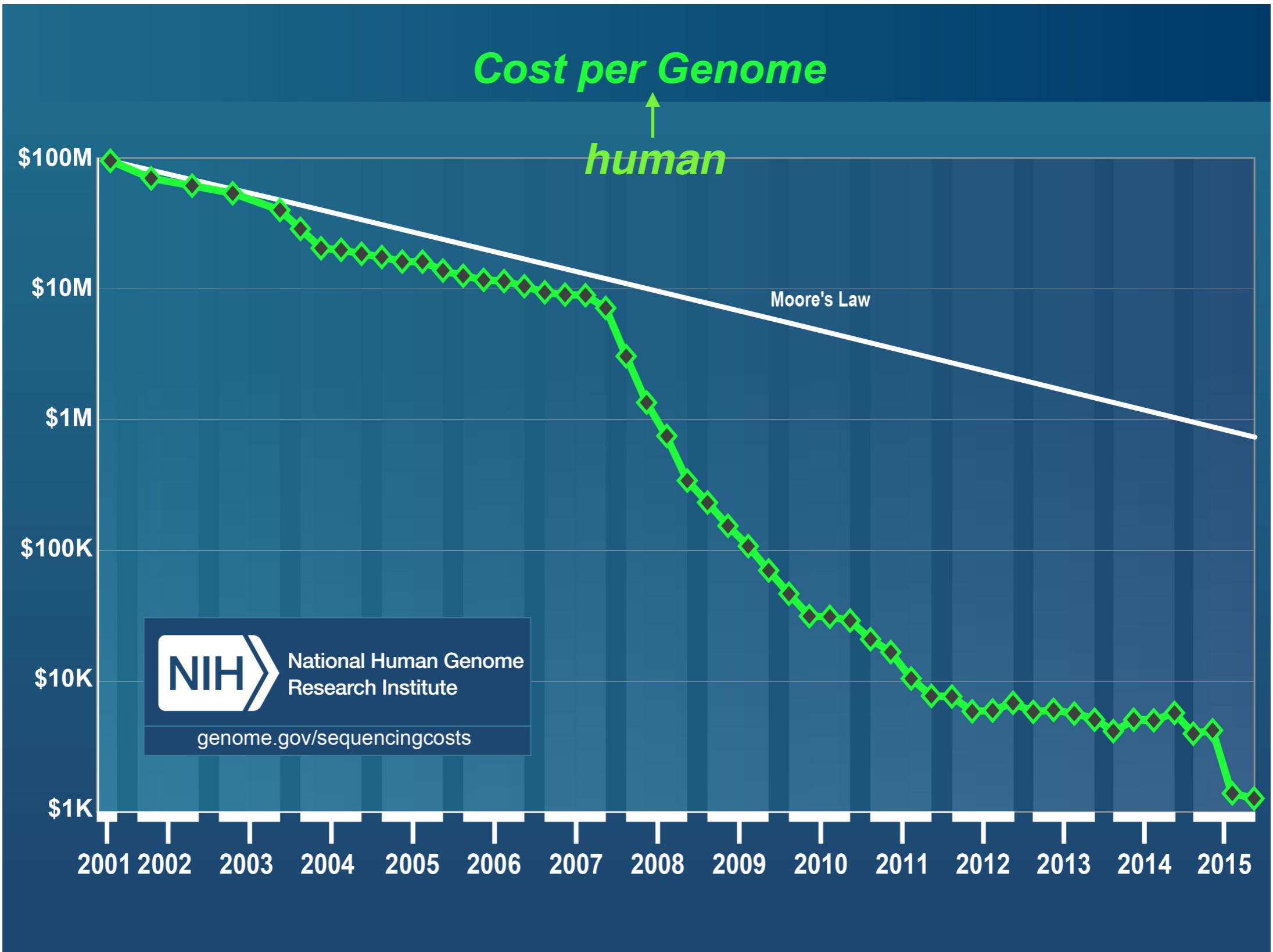
# Generate “mutations” by reverse genetic RNAi or CRISPR for “all” genes in an organism

Organism	Approx. # of genes
Yeast ( <i>S. cerevisiae</i> )	6,000
Fly ( <i>D. melanogaster</i> )	15,000
Worm ( <i>C. elegans</i> )	21,000
Zebrafish ( <i>D. rerio</i> )	26,000
Chicken ( <i>G. gallus</i> )	17,000
Mouse ( <i>M. musculus</i> )	23,000
Mustard plant ( <i>A. thaliana</i> )	28,000
Human ( <i>H. sapiens</i> )	25,000

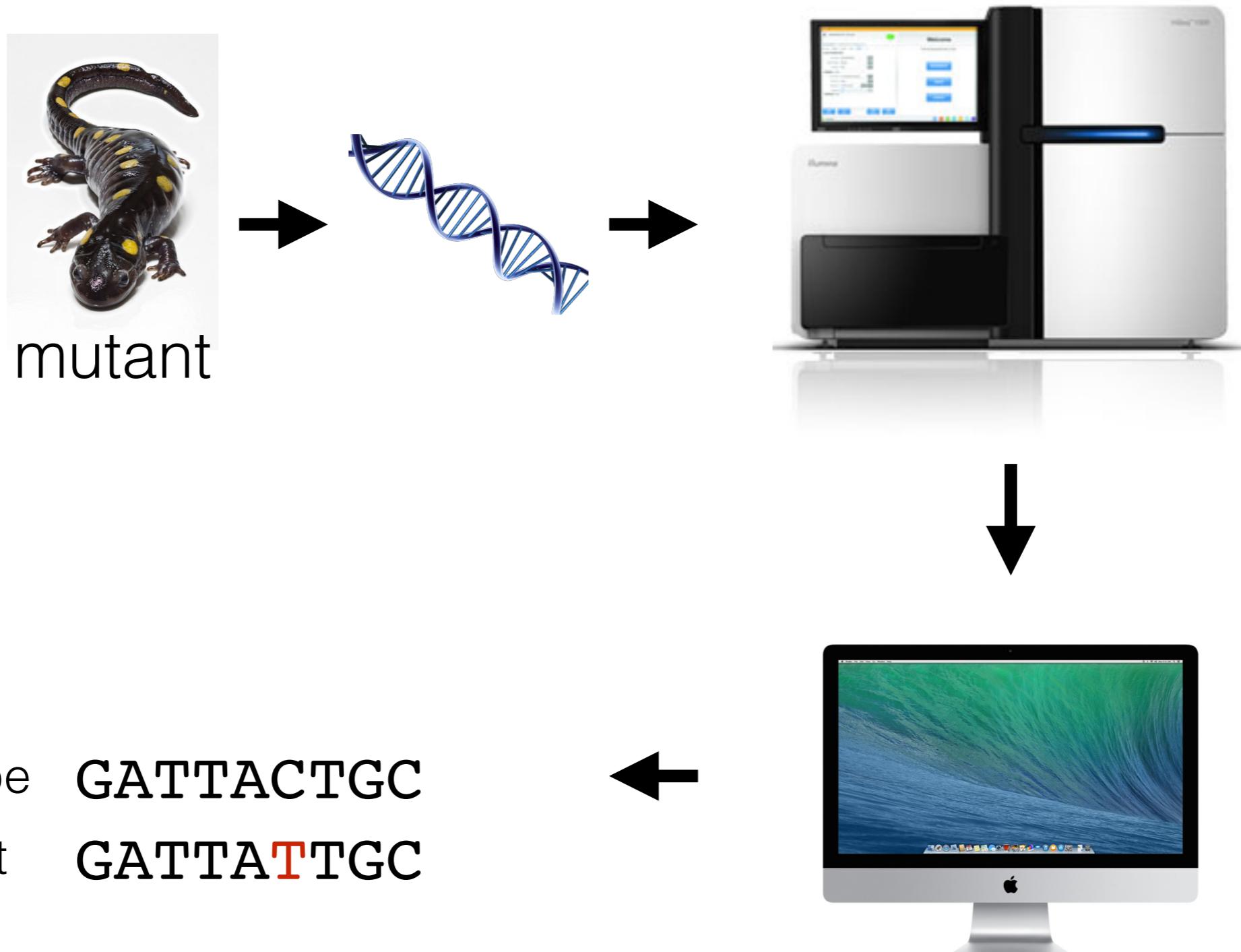
# **14. Clone the gene**

1. Clone by complementation
2. Clone by phenocopy
3. Clone by sequencing

# Clone by sequencing

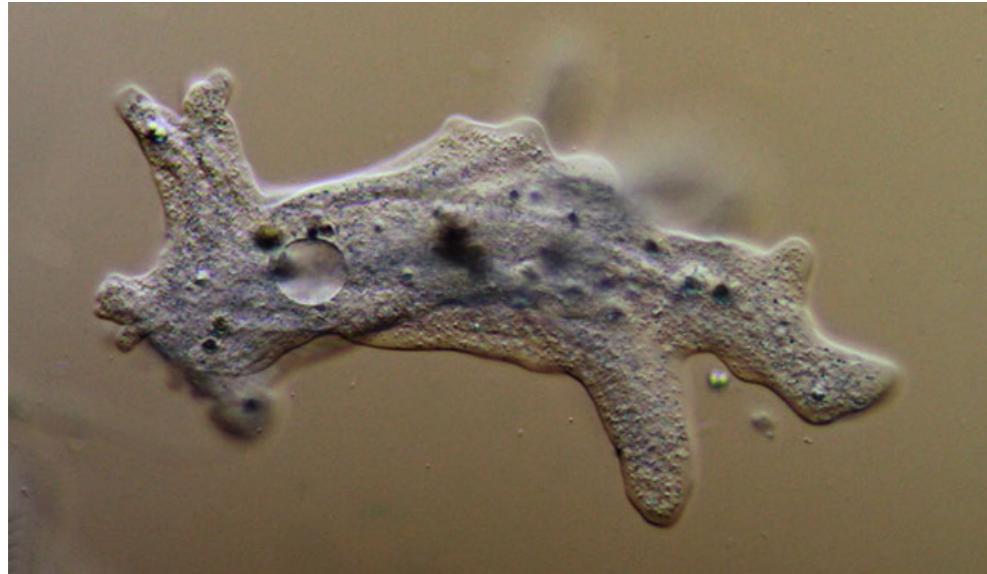


# Clone by sequencing



Need multiple non-complementing alleles and mapping

# Necessary and sufficient in the logic of cloning genes



*Amoeba proteus*

We find a mutant that doesn't engulf yeast.

It has a mutation in a signaling component, gene X.

**Is gene X mutation responsible for the yeast engulfment defect?**

1. RNAi of gene X has same phenotype as our mutant
2. Mutation in gene X with rescue of mutant phenotype in gene X
3. Independent screens for engulfment mutants get multiple alleles of gene X

**Proof requires:**  
**independent alleles or RNAi,**  
**failure to complement original mutant,**  
**phenotypic rescue**

# With a physical copy of the gene...

Add in wild-type copy to complement mutant copy

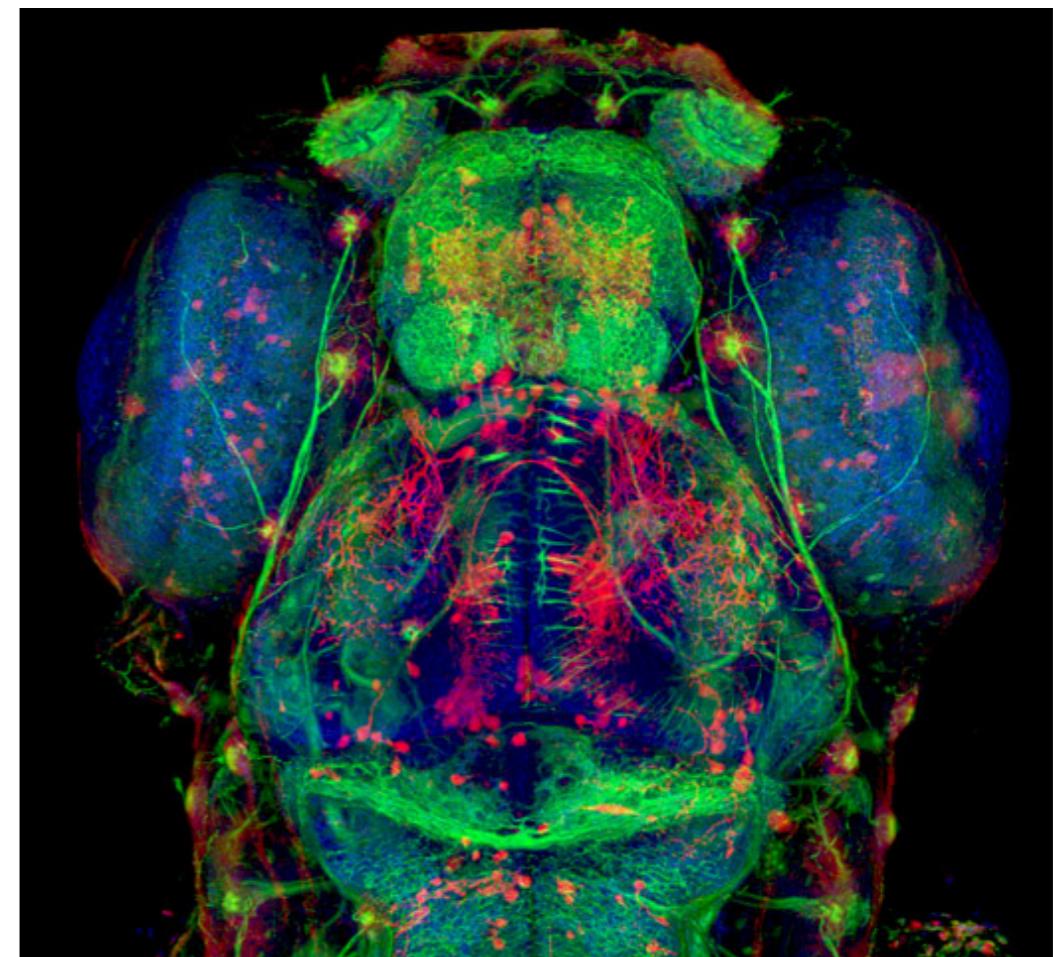
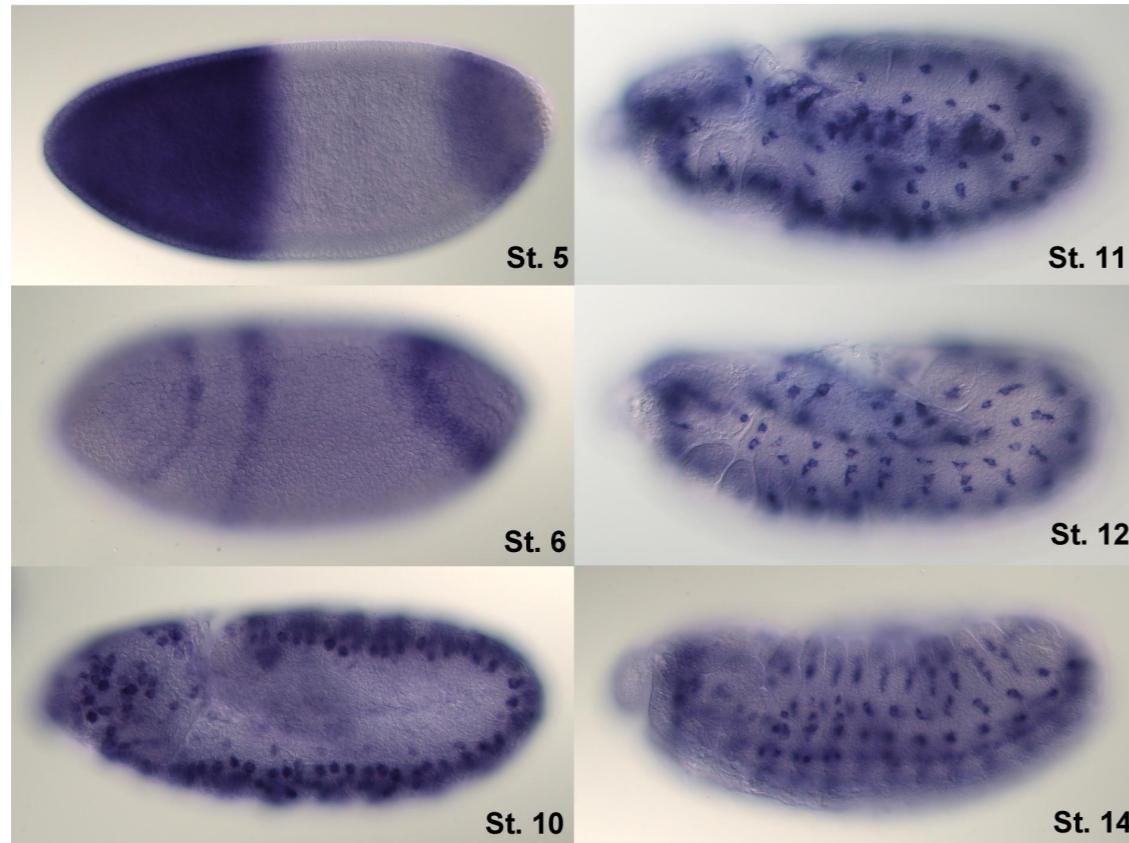
m + normal gene = Rescue of mutant  
m mutated in m phenotype  
(complementation)

m + mutant gene = **No rescue** of mutant  
m mutated in m phenotype

# 15. Determine where gene is expressed

With no transgenesis:

1. *in situ* hybridization (RNA localization)
2. Antibody immunofluorescence (protein localization)



How do we know we have the right expression pattern?

# 15. Determine where gene is expressed

With transgenesis:

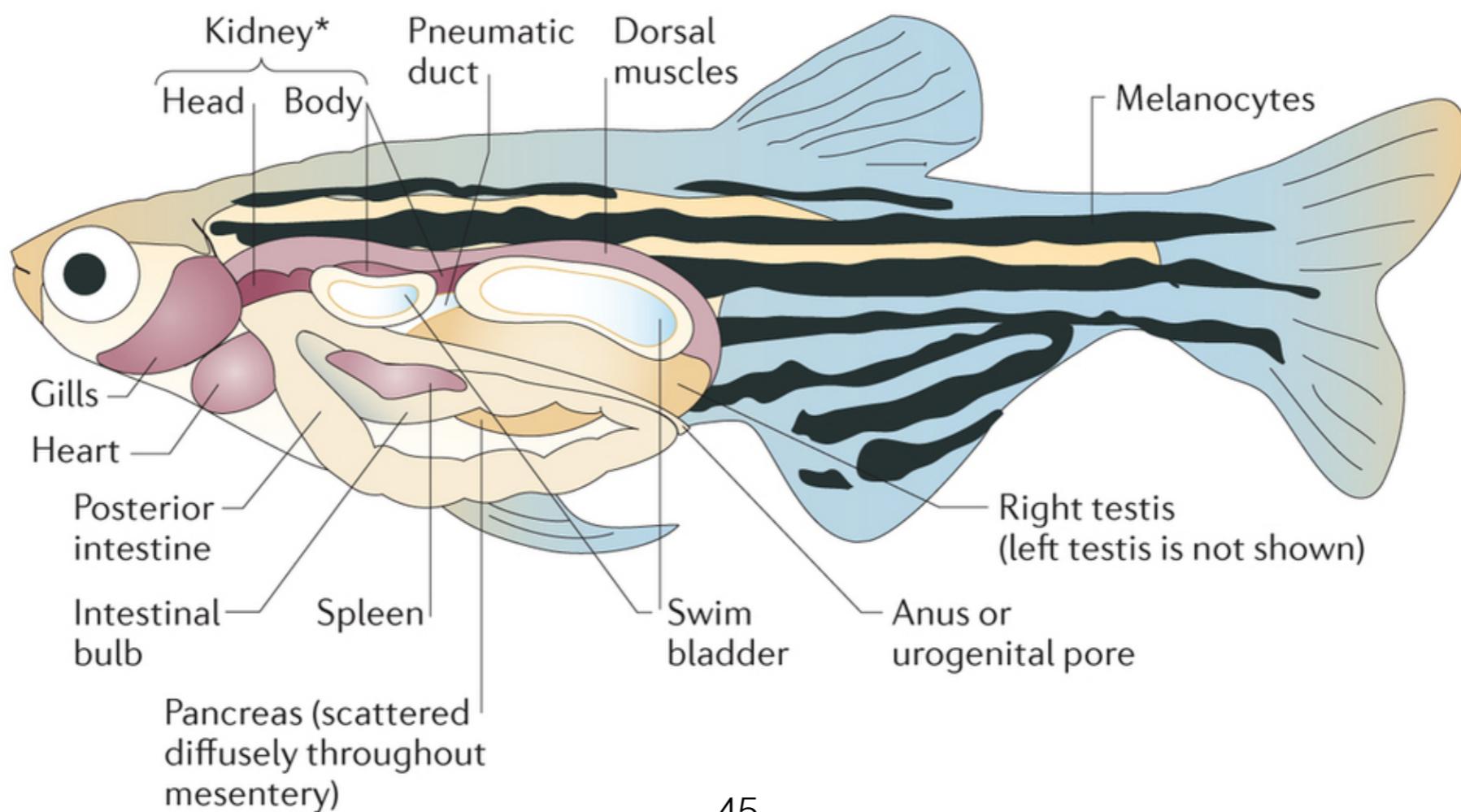


**Where a gene is expressed might not be where it acts?**

# 16. Determine site of gene action

What is the cell, organ, and/or tissue where the gene functions?

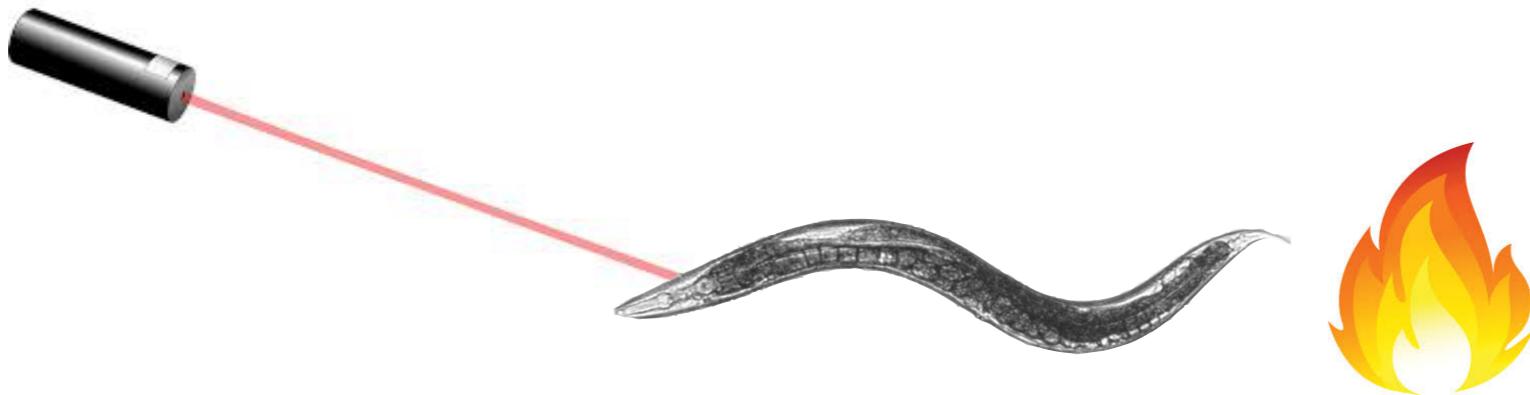
1. Rescue a mutant phenotype in a specific cell, organ, or tissue
2. Mosaic analysis (cell autonomy experiments)



# 17. Determine time of gene action

When does the gene function?

1. Induce expression to rescue a mutant phenotype at a specific time



Heat-inducible gene expression in certain tissues  
at specific times

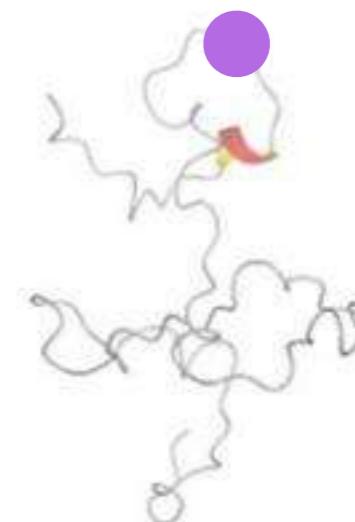
# 17. Determine time of gene action

When does the gene function?

1. Induce expression to rescue a mutant phenotype at a specific time
2. Use temperature-labile mutants to define the temperature-sensitive period

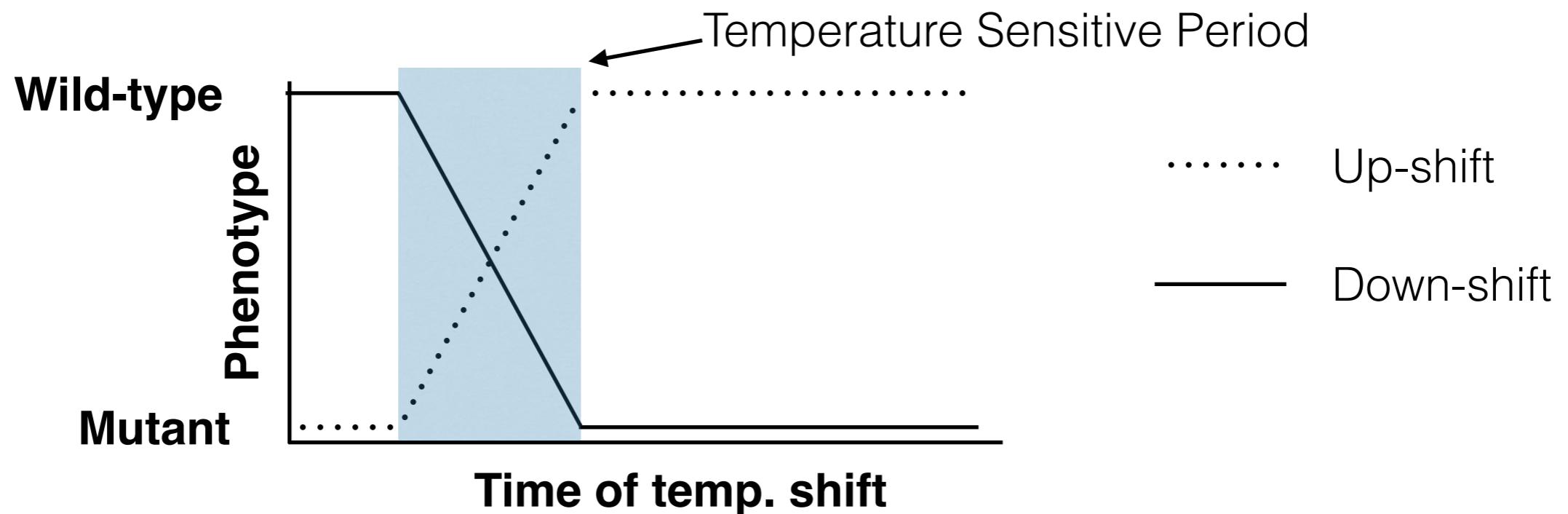


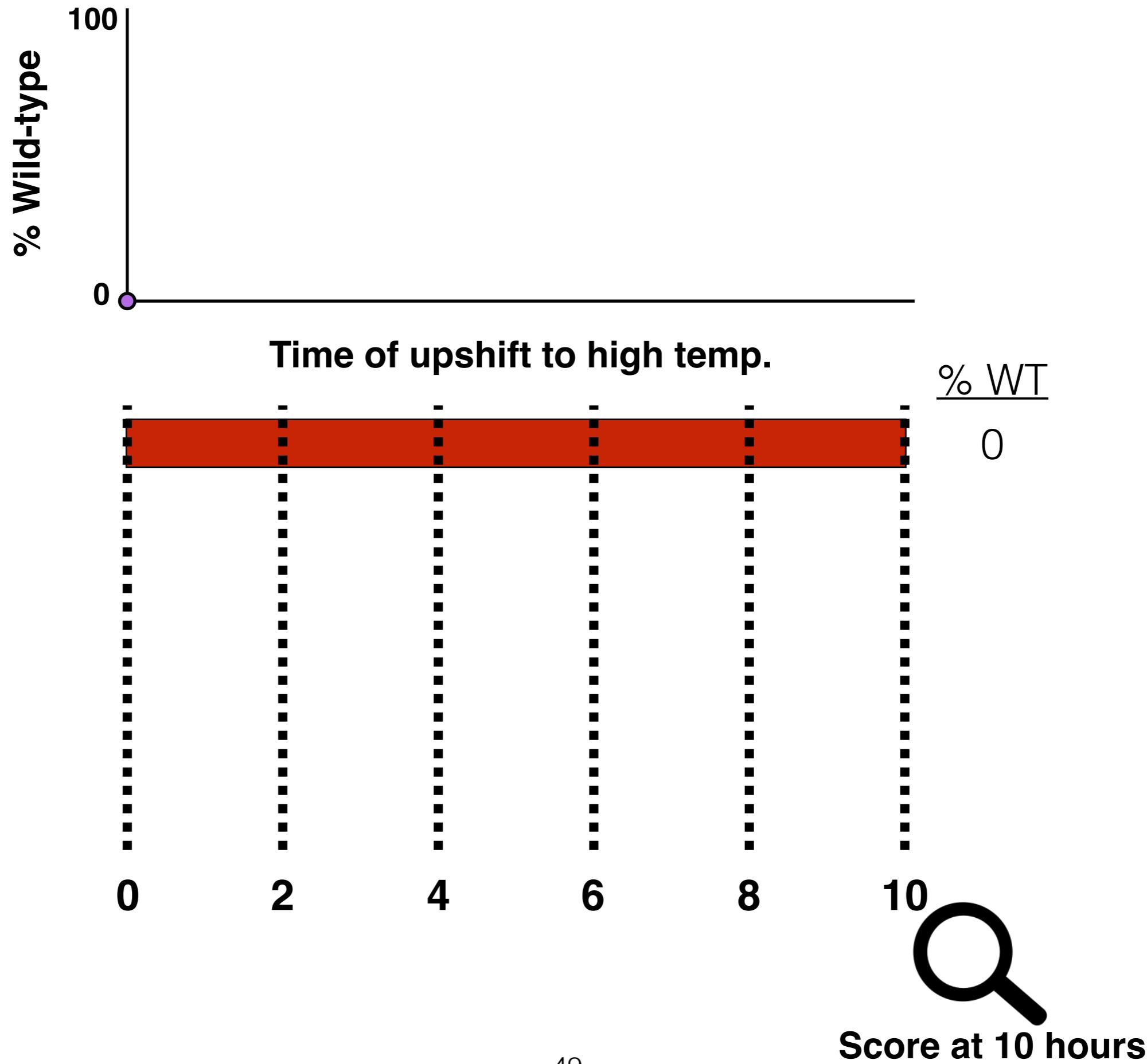
Permissive temperature

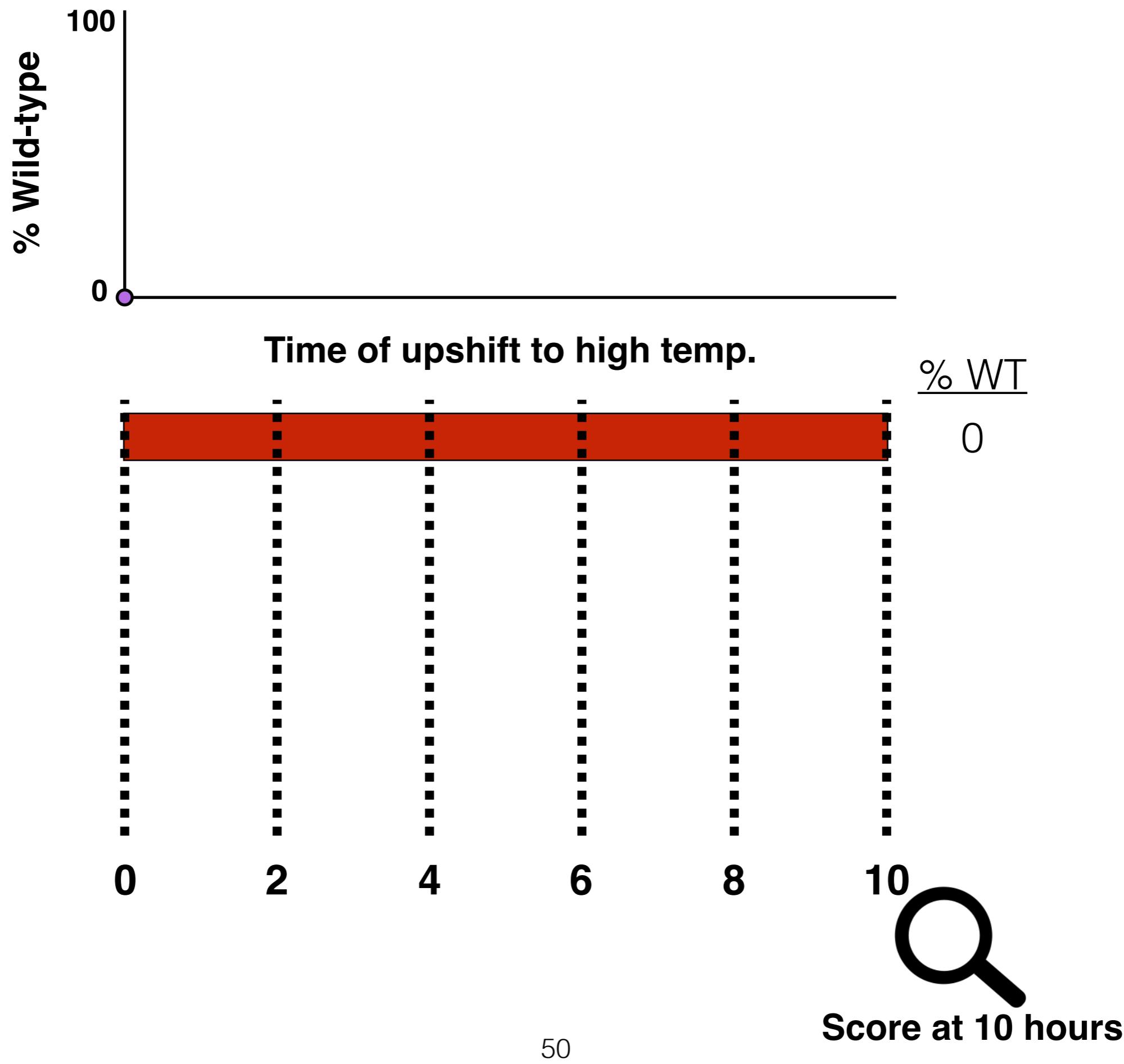


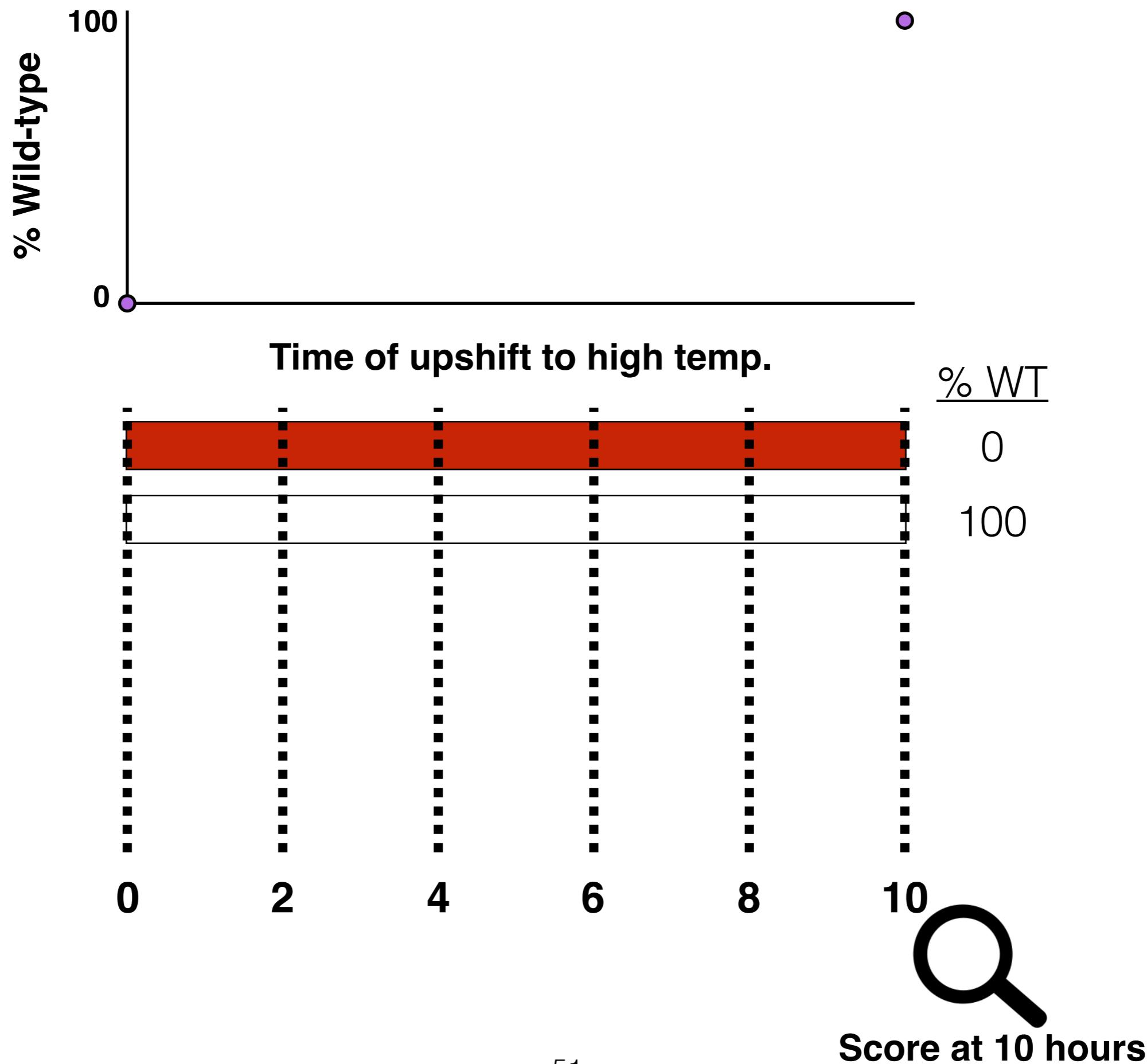
Restrictive temperature

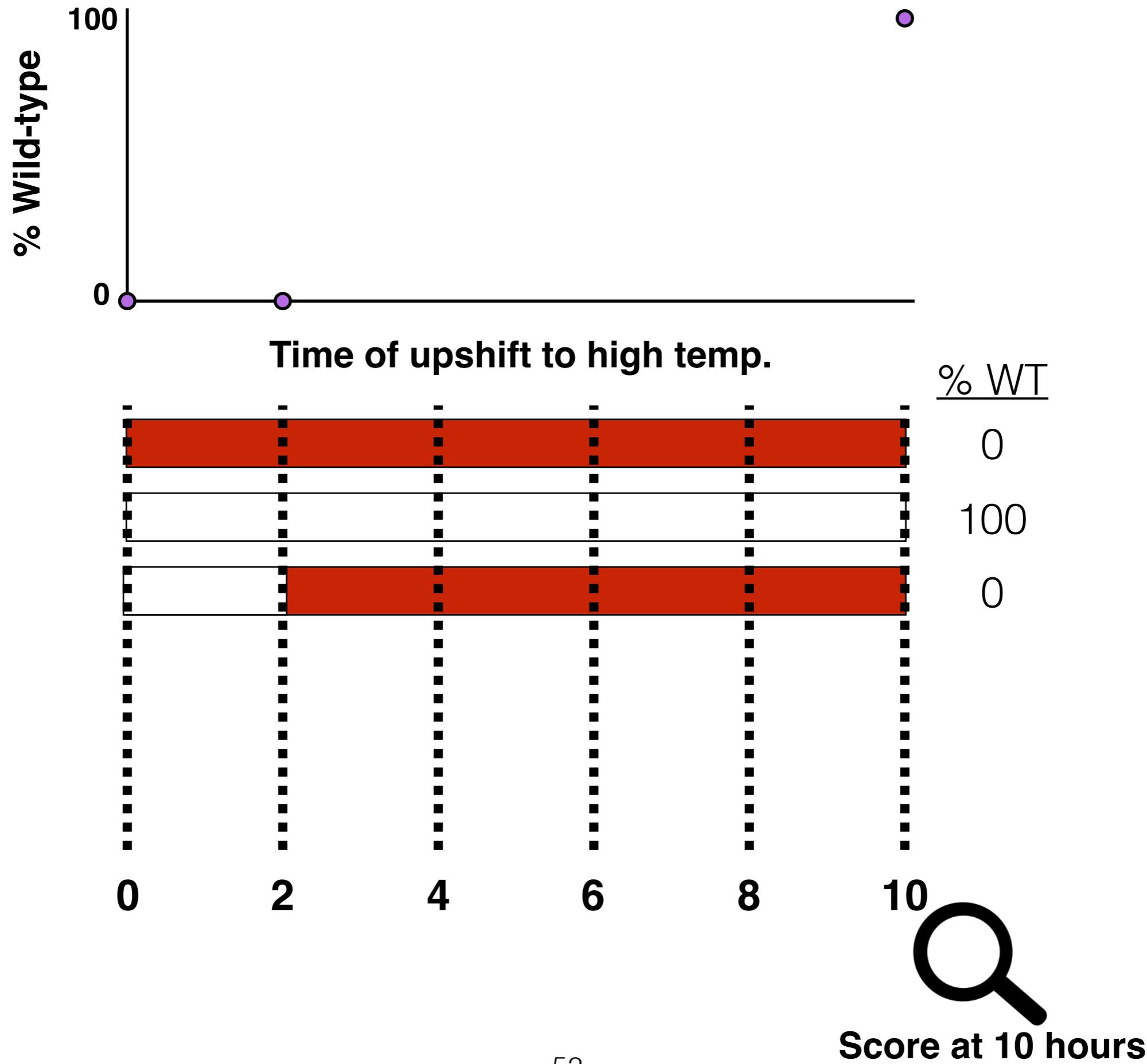
# 17. Determine time of gene action

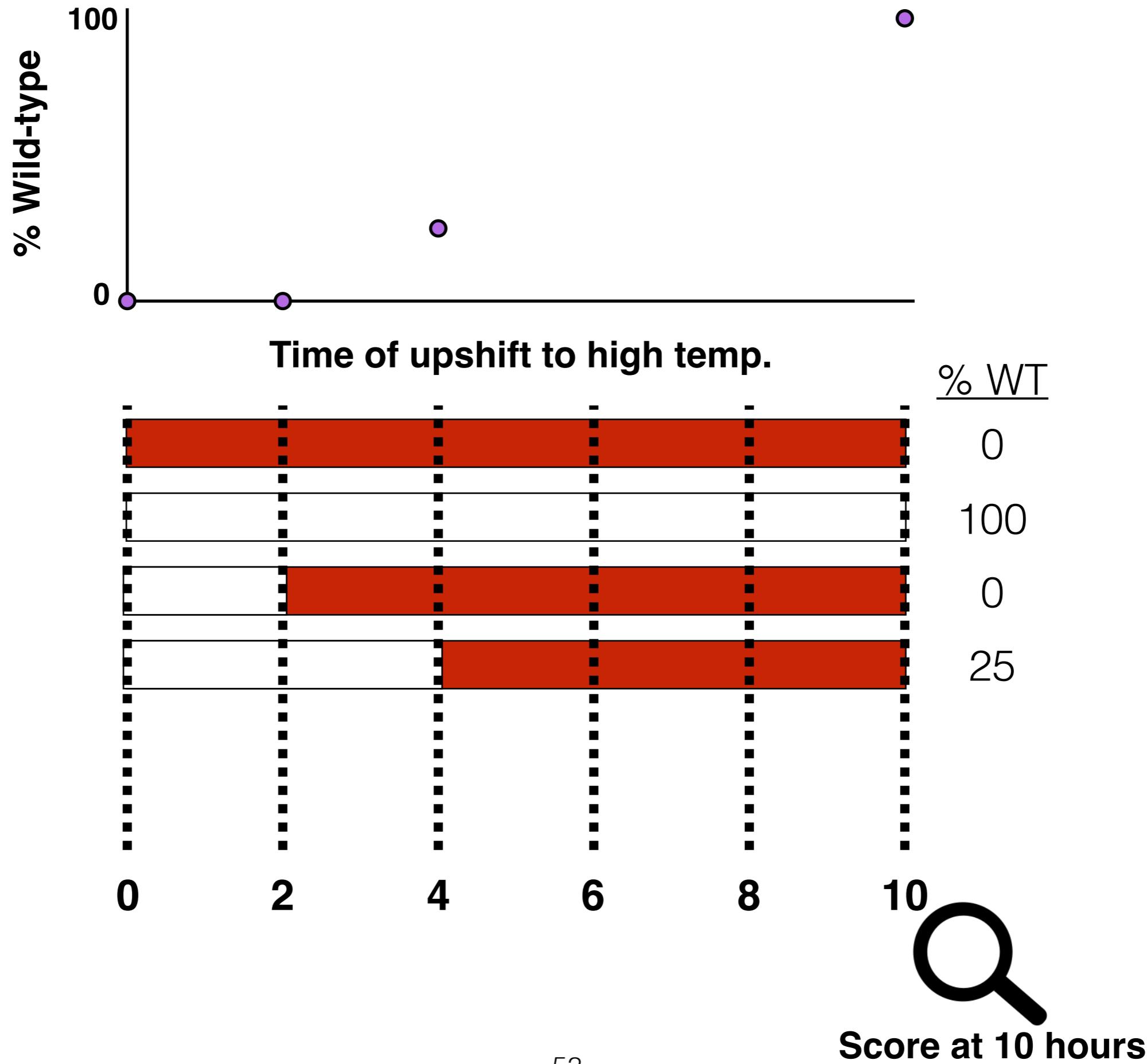


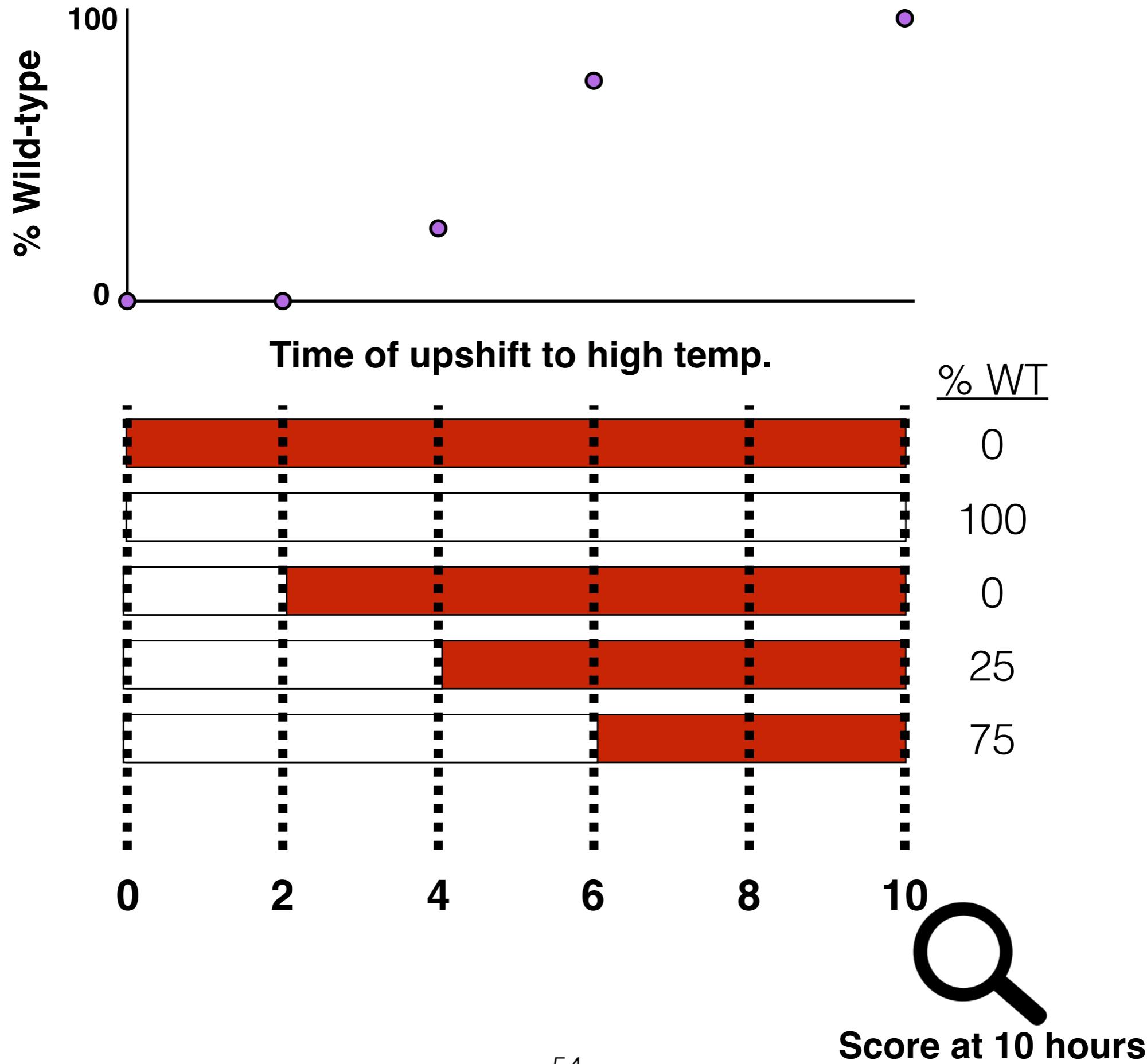


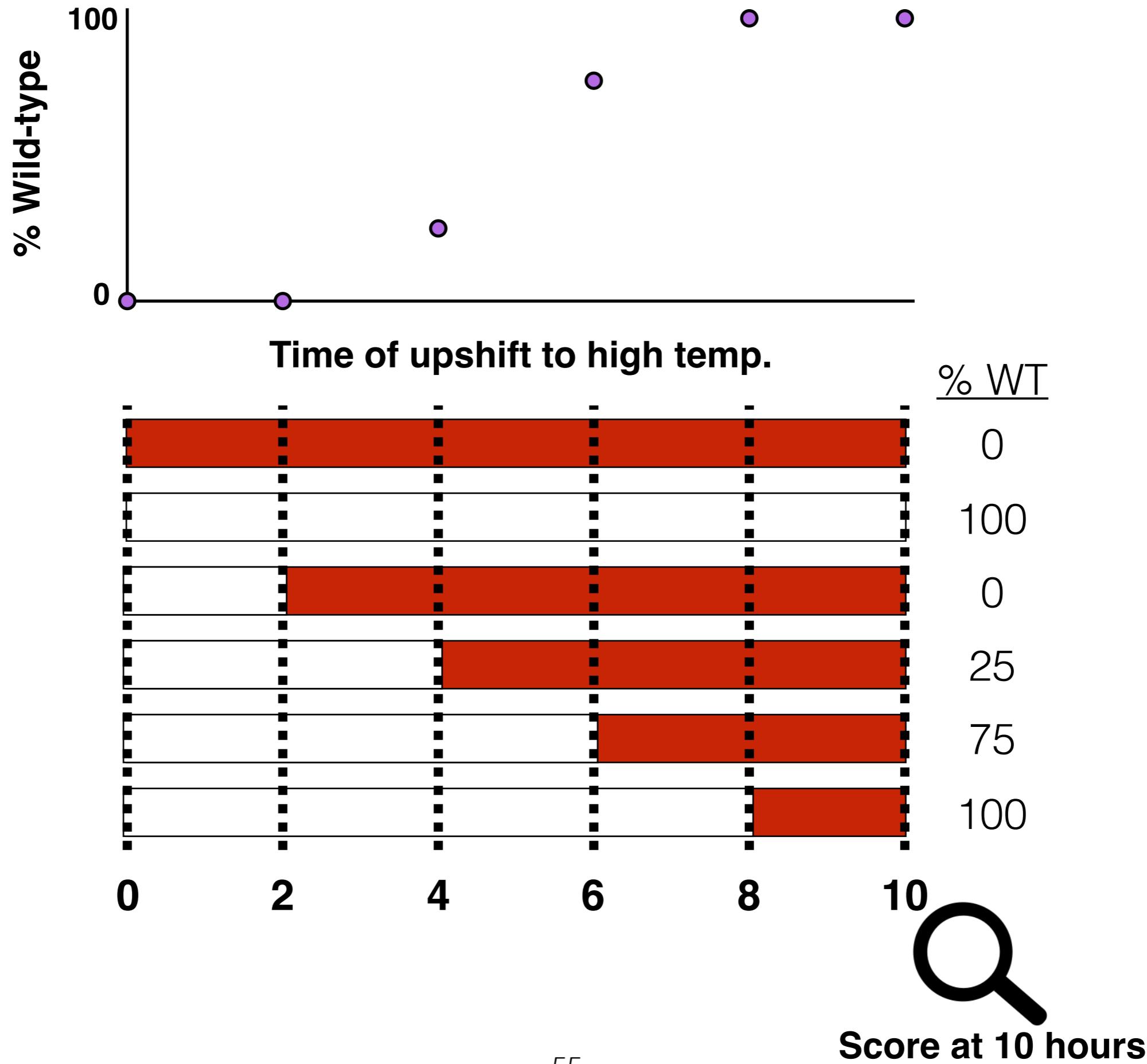


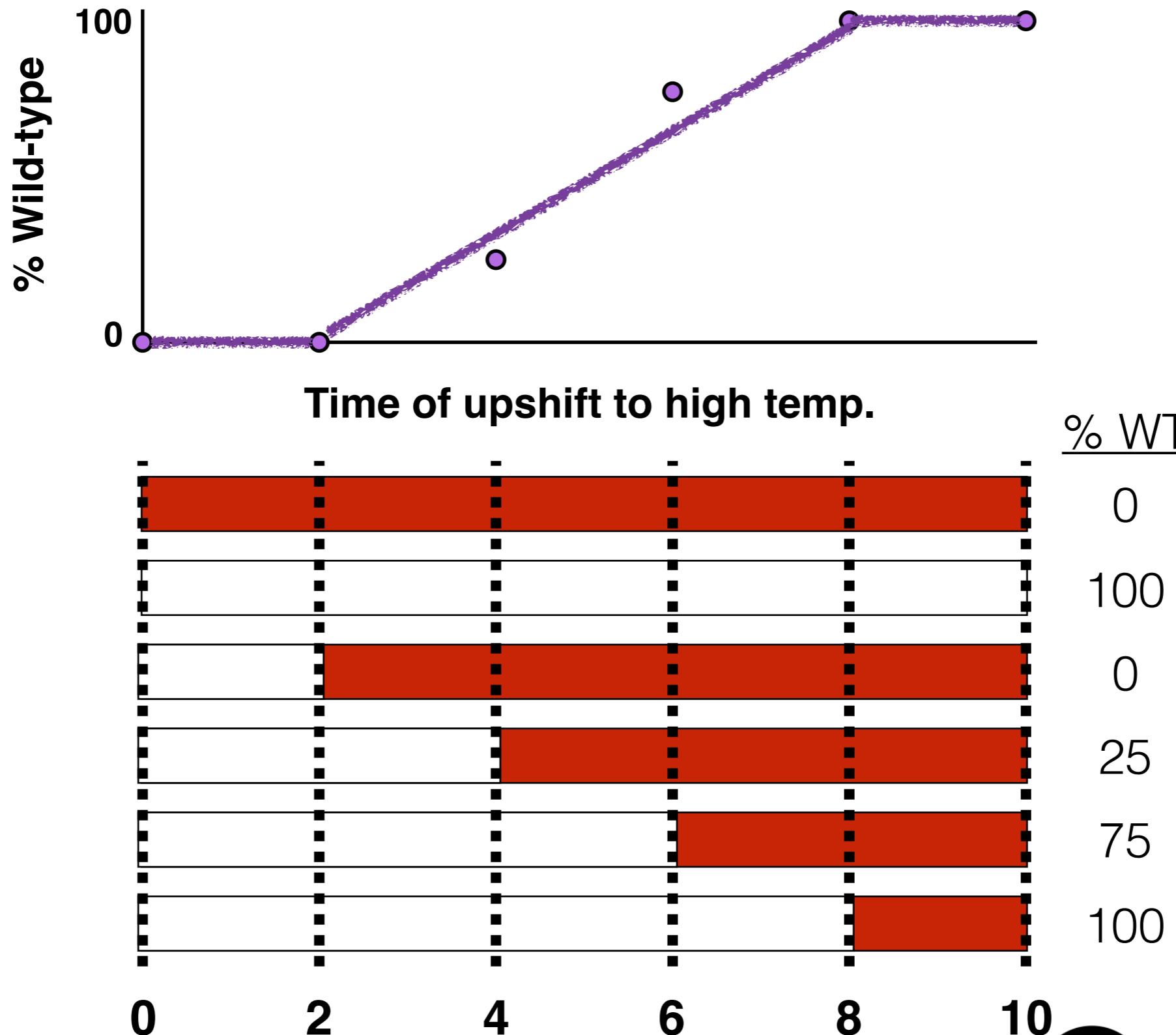




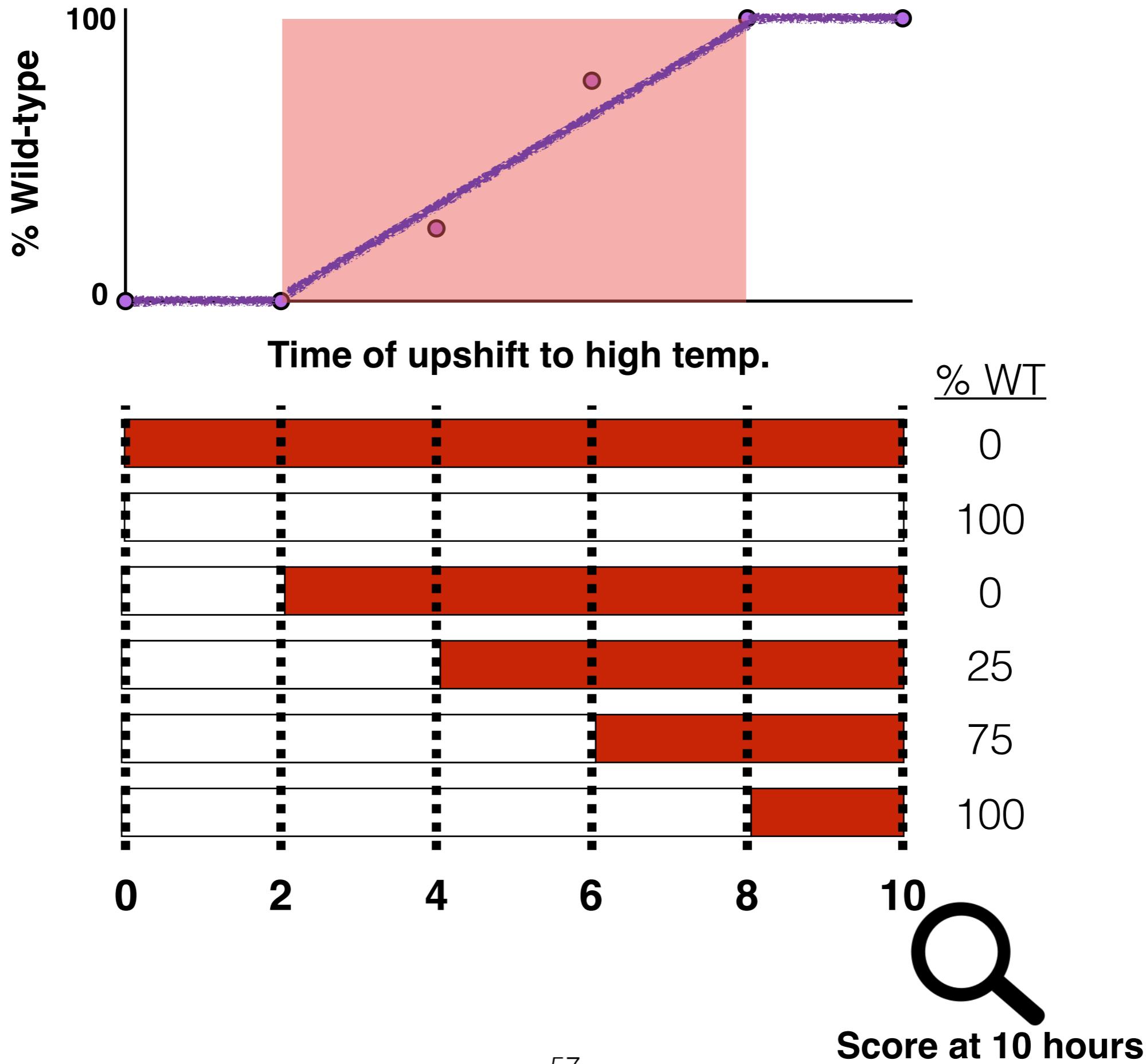


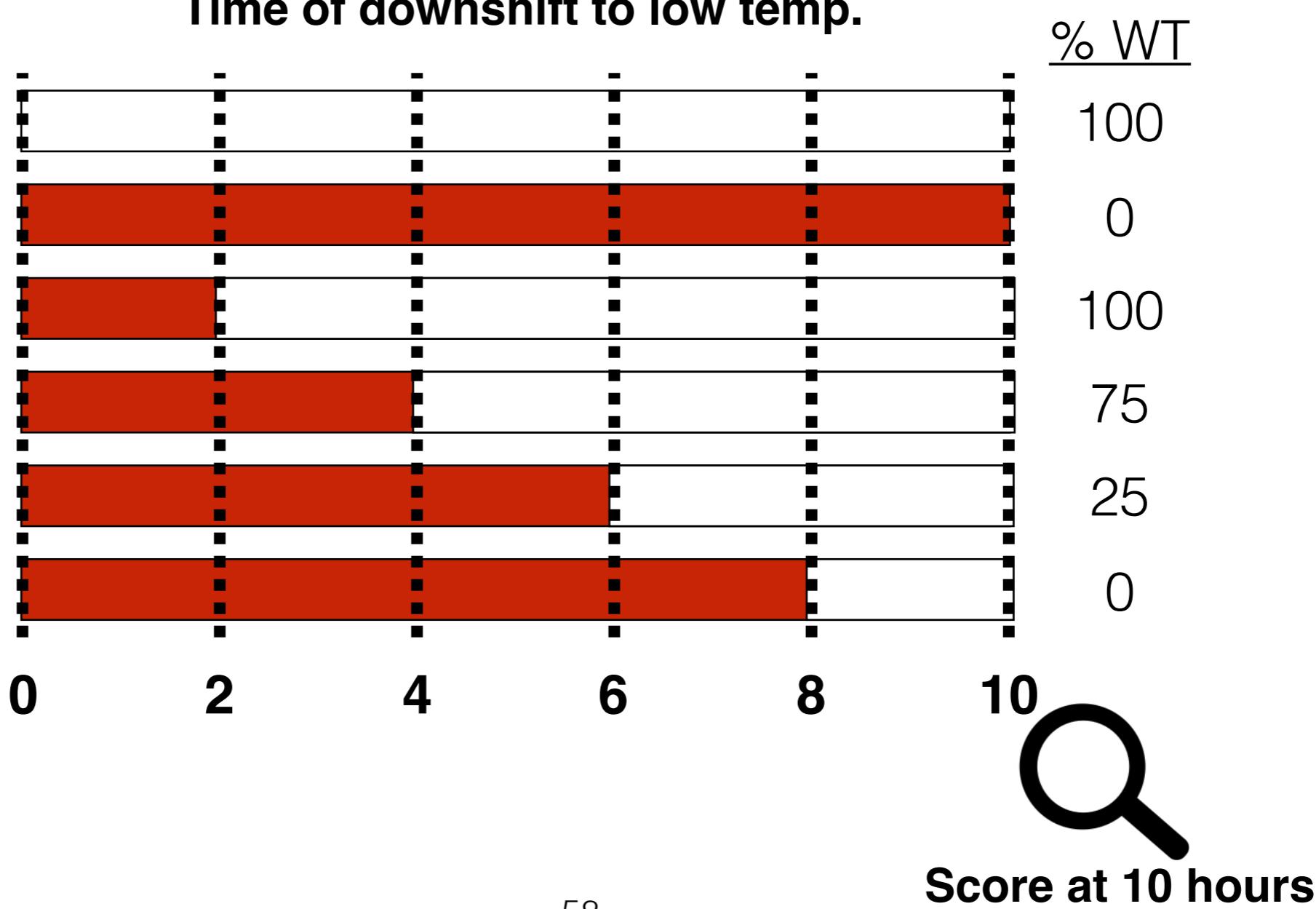
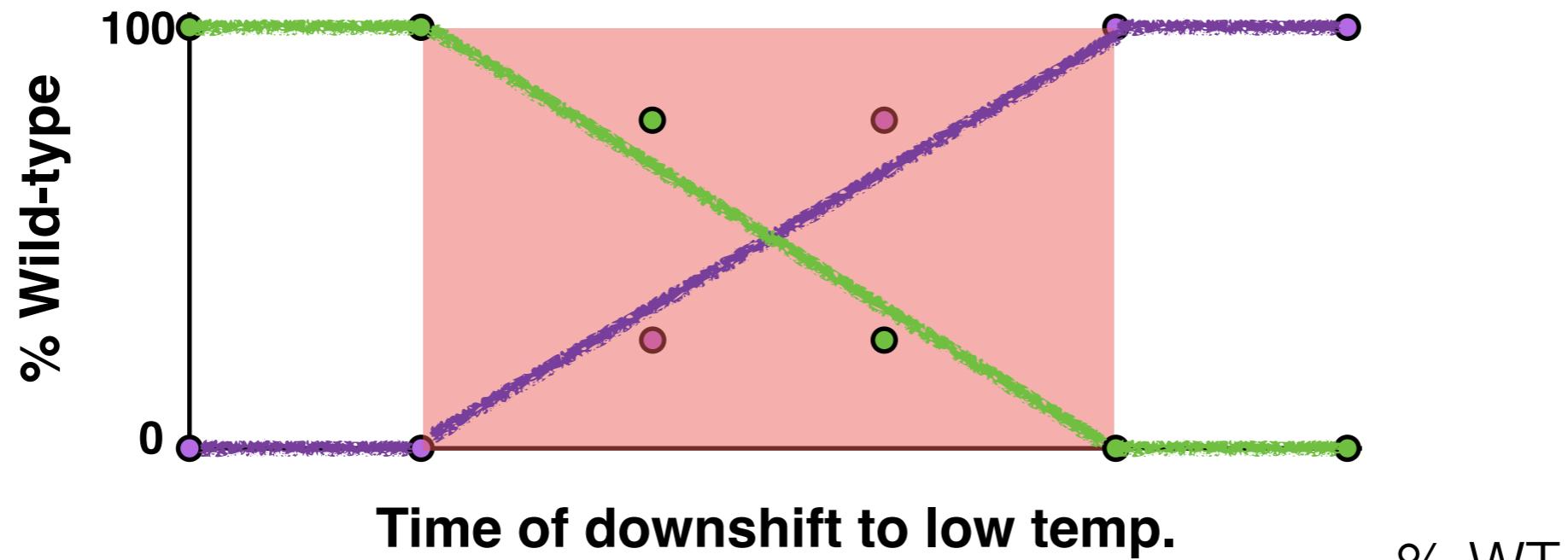




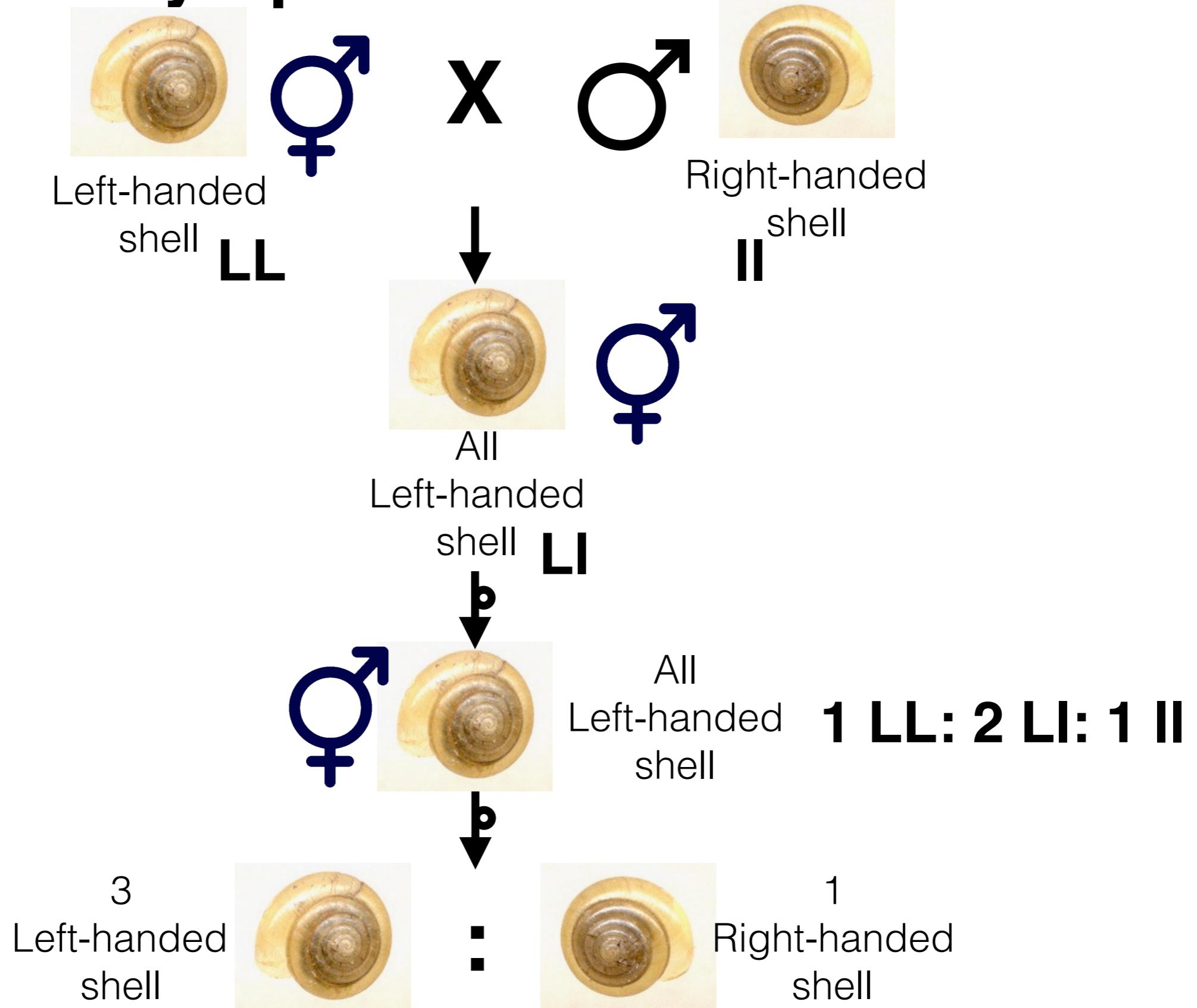


Score at 10 hours



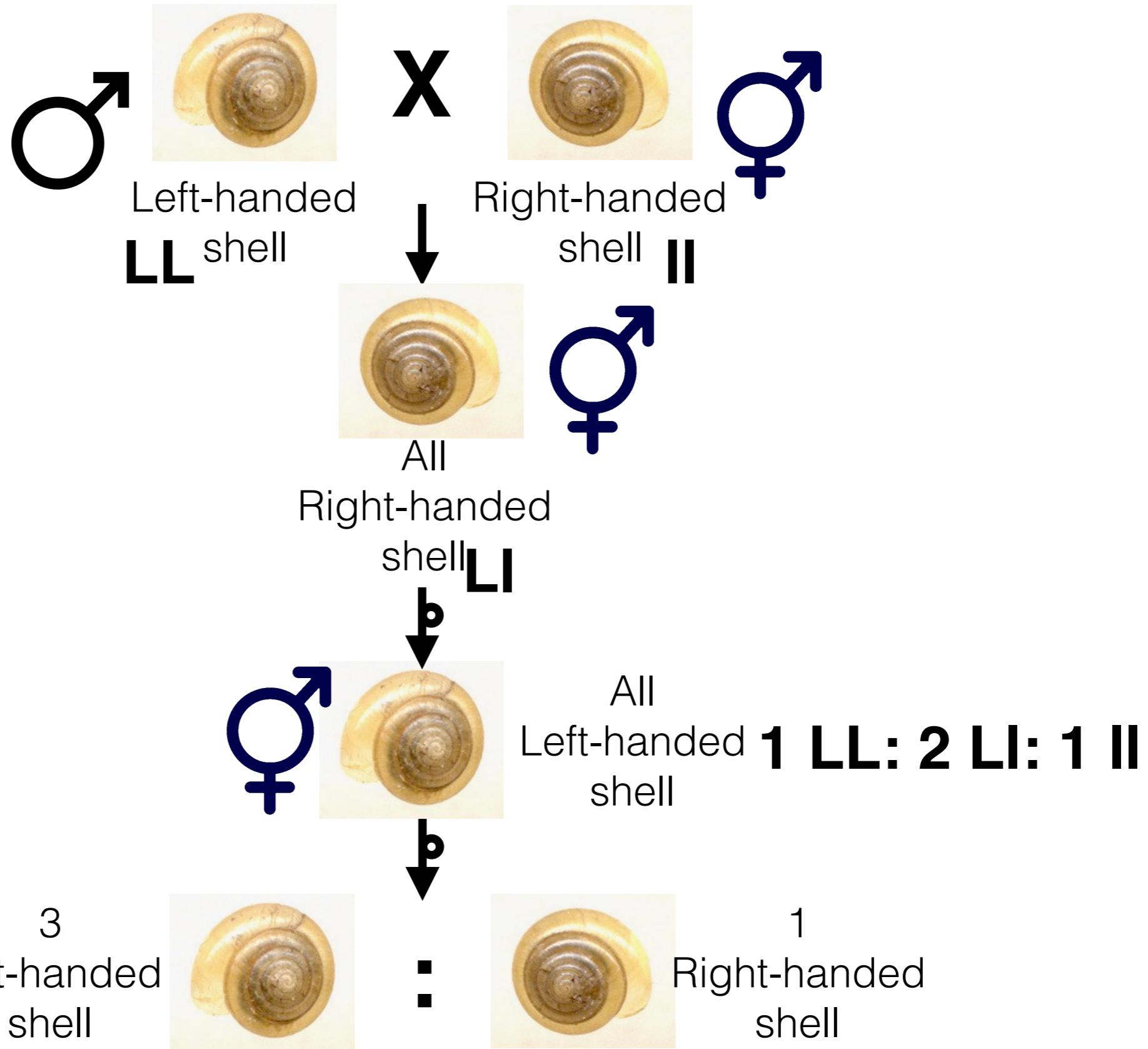


# 18. Determine if there are maternal effects or cytoplasmic inheritance



# 18. Determine if there are maternal effects or cytoplasmic inheritance

Reciprocal cross



# 18. Determine if there are maternal effects or cytoplasmic inheritance



The egg and sperm have different compositions.

# 18. Determine if there are maternal effects or cytoplasmic inheritance



Plastid inheritance in  
*Mirabilis jalapa*

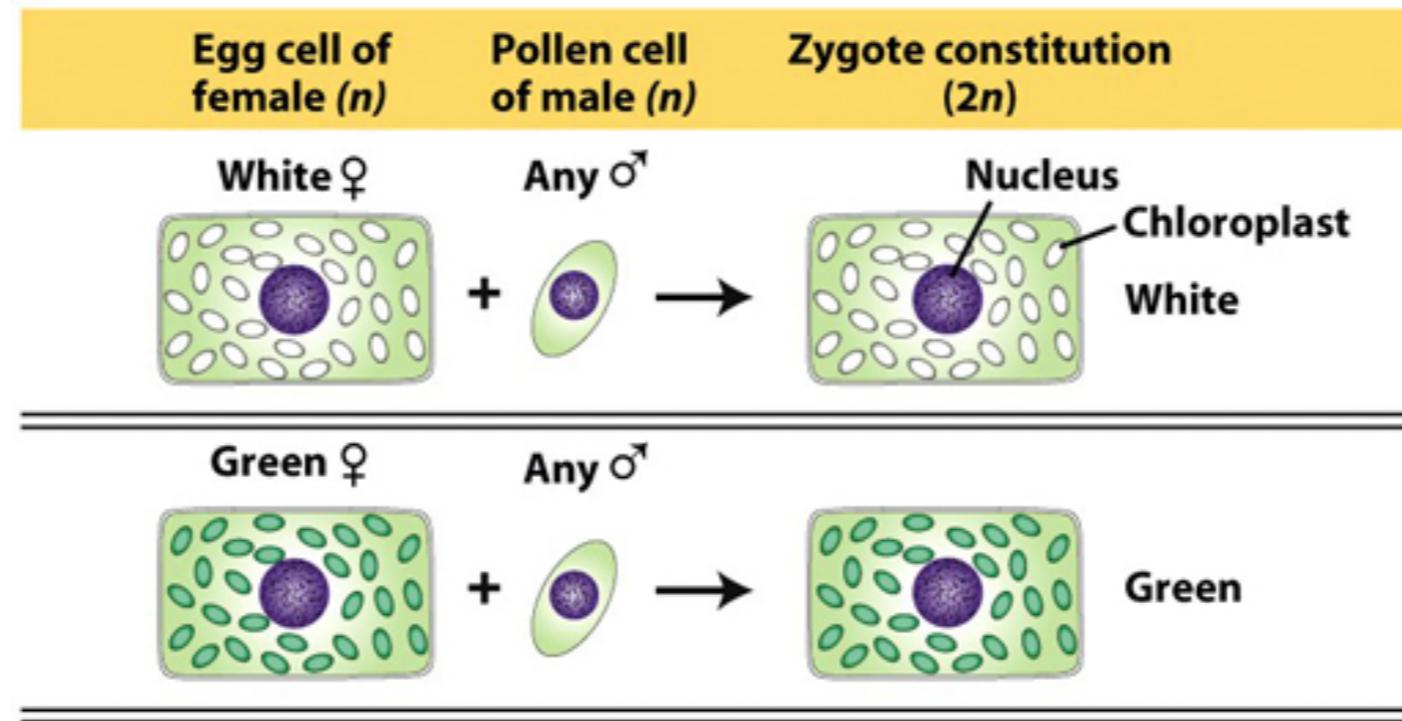
Only the color  
of the stem  
matters

White, green, or  
variegated

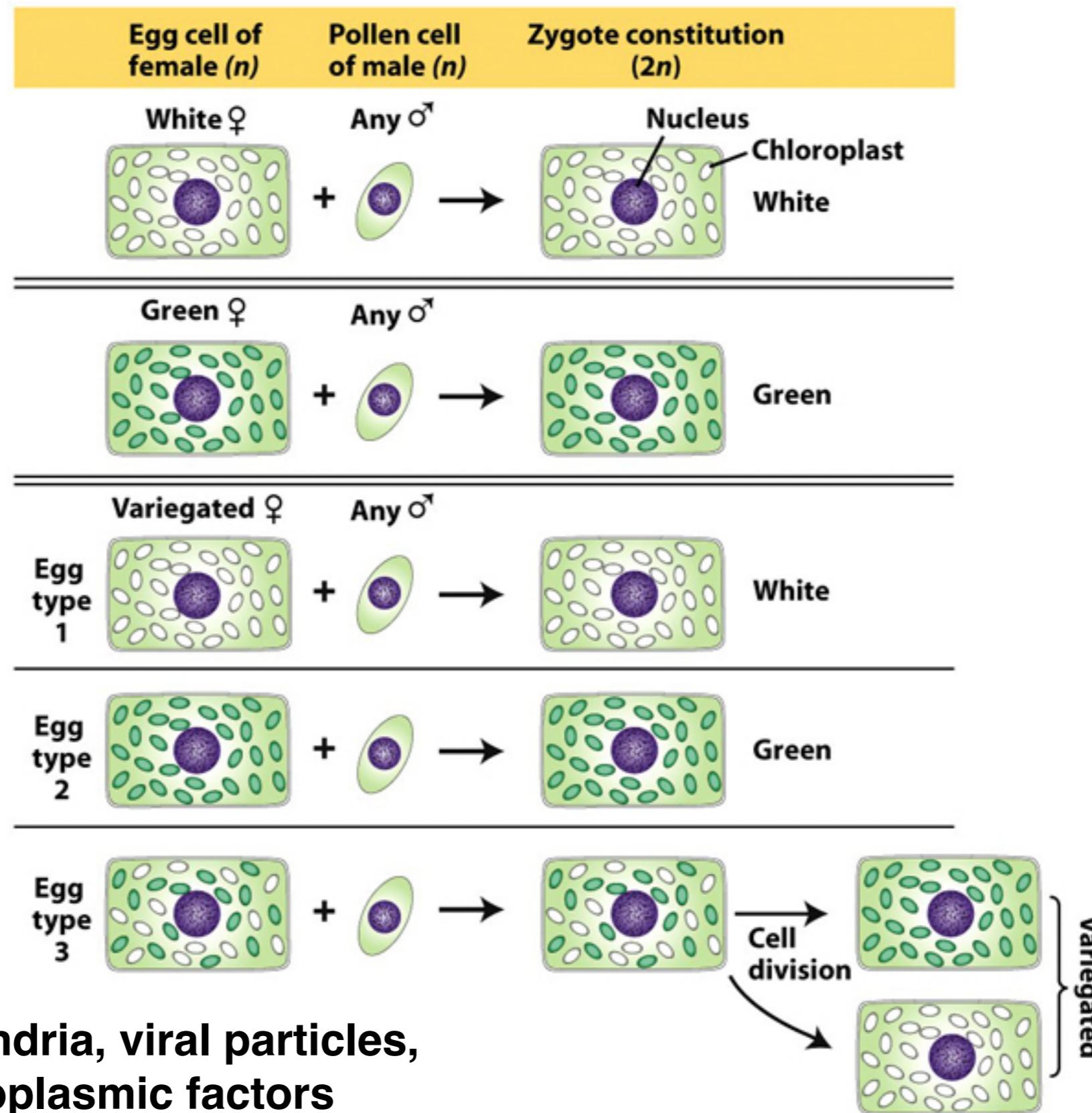


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# 18. Determine if there are maternal effects or cytoplasmic inheritance



# 18. Determine if there are maternal effects or cytoplasmic inheritance



Plastids, mitochondria, viral particles, and other cytoplasmic factors

# 19. Determine the overexpression phenotype

What happens when the wild-type individual has too much of gene X?



Overexpression *might* be useful for investigating genetic interactions

## **20. Perform an overexpression screen for additional modifiers**

1. Screen for dominant phenotypes similar to your mutant phenotype
2. Inducible overexpression of specific genes
3. Transposon-mediated overexpression screens

Find more genes by making hypermorphs

**21. Isolate enhancers and suppressors of your mutant phenotype**

**22. Investigate pathways (measure genetic interactions or epistasis)**

# Step-wise genetic analysis

1. Define the problem
2. Choose an organism
3. Perform a mutant hunt
4. Screen until saturation?
5. Establish a strain
6. Backcross and/or outcross
7. Test for dominance
8. Single-gene phenotype?
9. Mapping and complementation
10. Characterize the phenotype
11. Define the nature of the mutant allele(s): gene dosage
12. Perform non-complementation screens
13. Define the null phenotype
14. Clone the gene
15. Determine where gene is expressed
16. Determine site of gene action
17. Determine time of gene action
18. Determine if there are maternal effects or cytoplasmic inheritance
19. Determine the overexpression phenotype
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