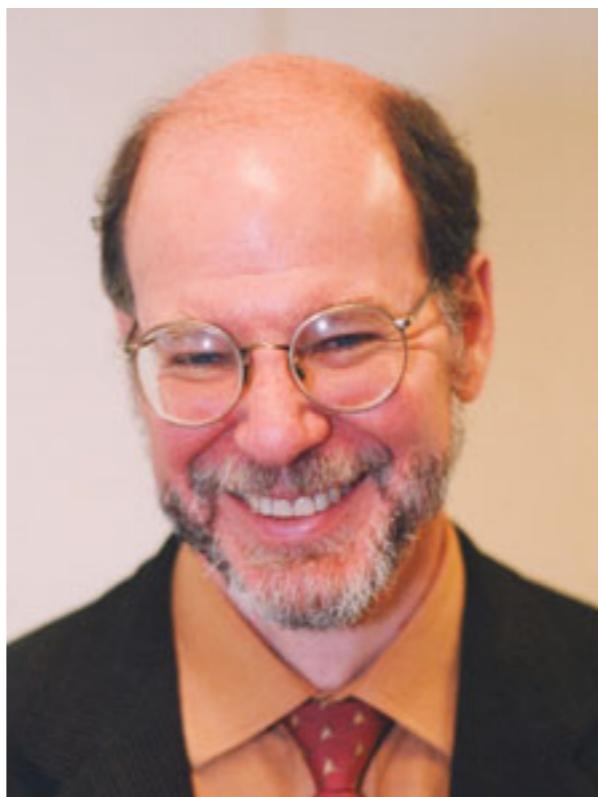


# Bio393: Genetic Analysis

Step-wise genetic analysis



**Bob Horvitz**

# **Step-wise genetic analysis**

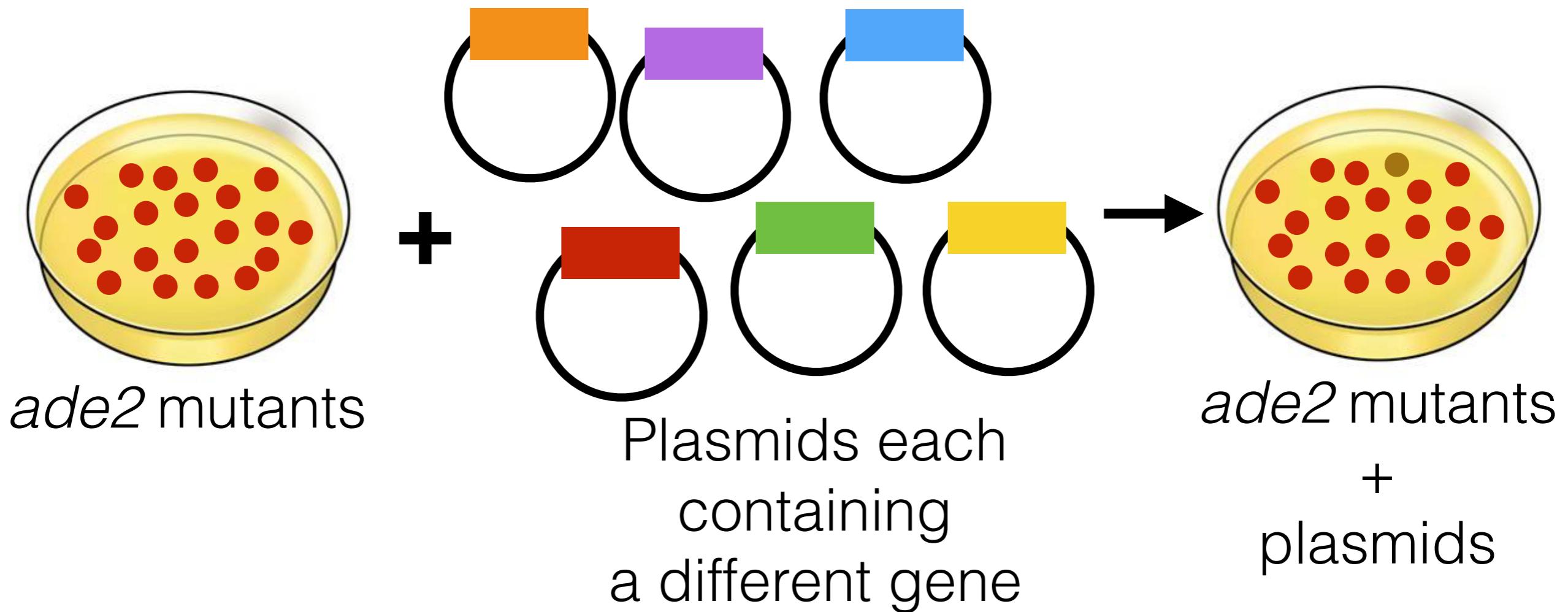
- 1. Define the problem**
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- 8. Single-gene phenotype?**
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- 11. Define the nature of the mutant allele(s): gene dosage**
- 12. Perform non-complementation screens**
- 13. Define the null phenotype**

# 14. Clone the gene

***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

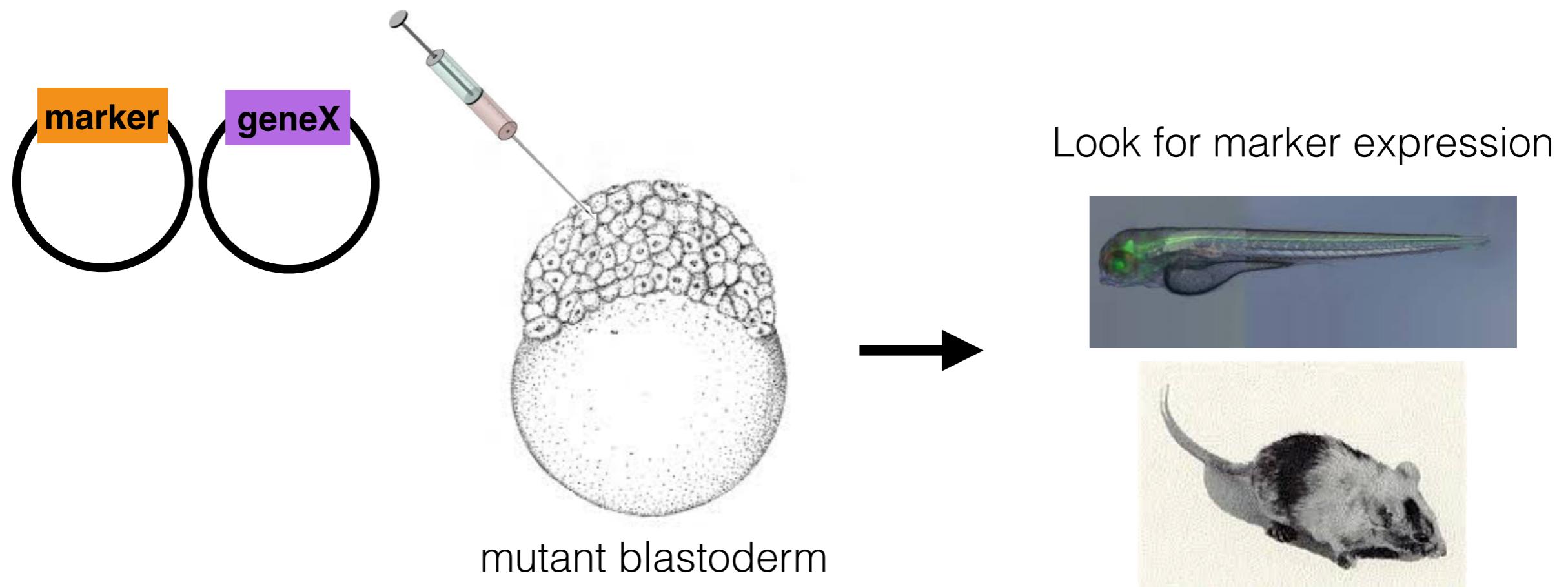


Caveat: overexpression bypass suppressors and not stable



Caveat: overexpression bypass suppressor and variable expression

# Cloning by complementation in fish and mice

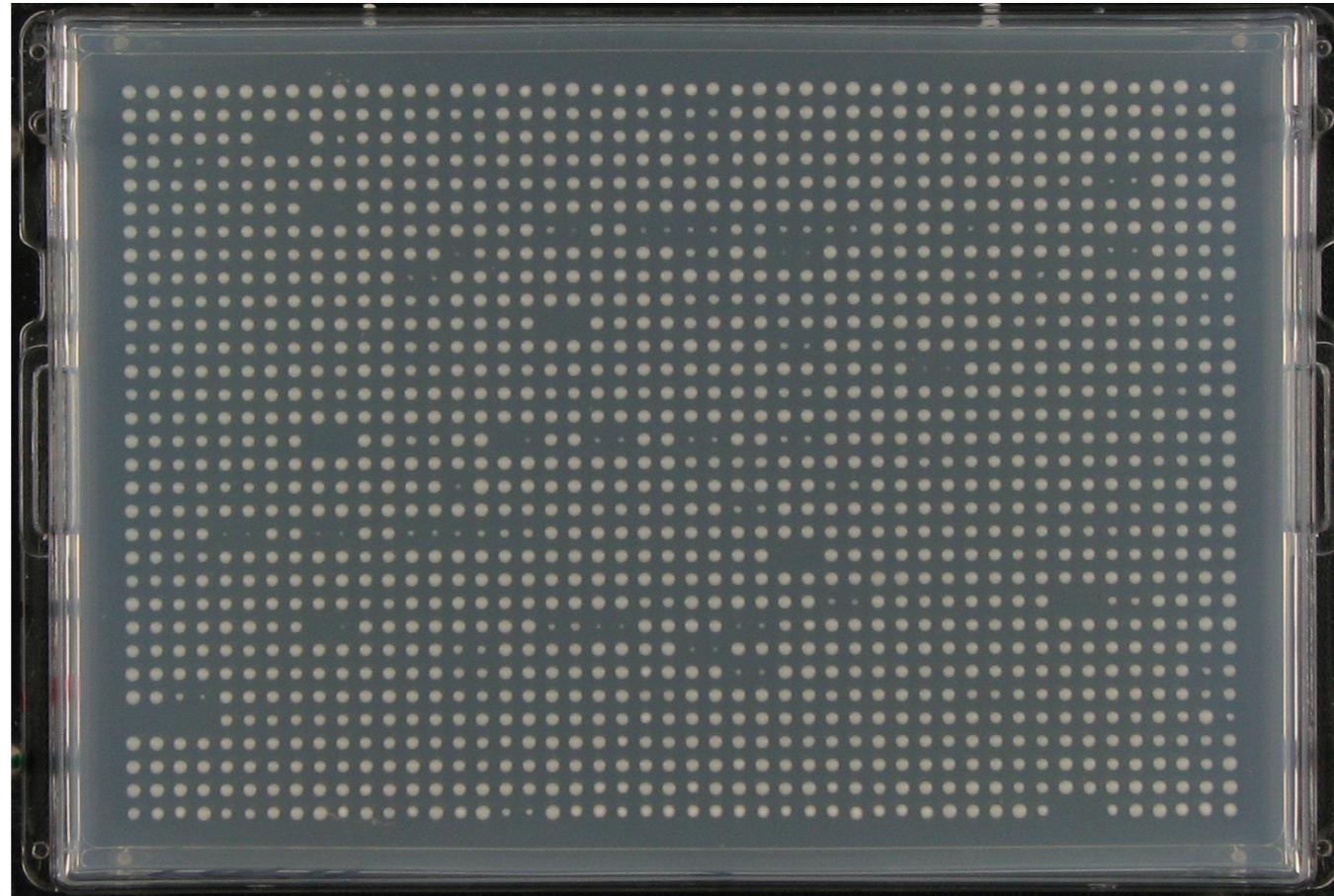


Caveat: overexpression bypass suppressors  
and variable expression

# **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**



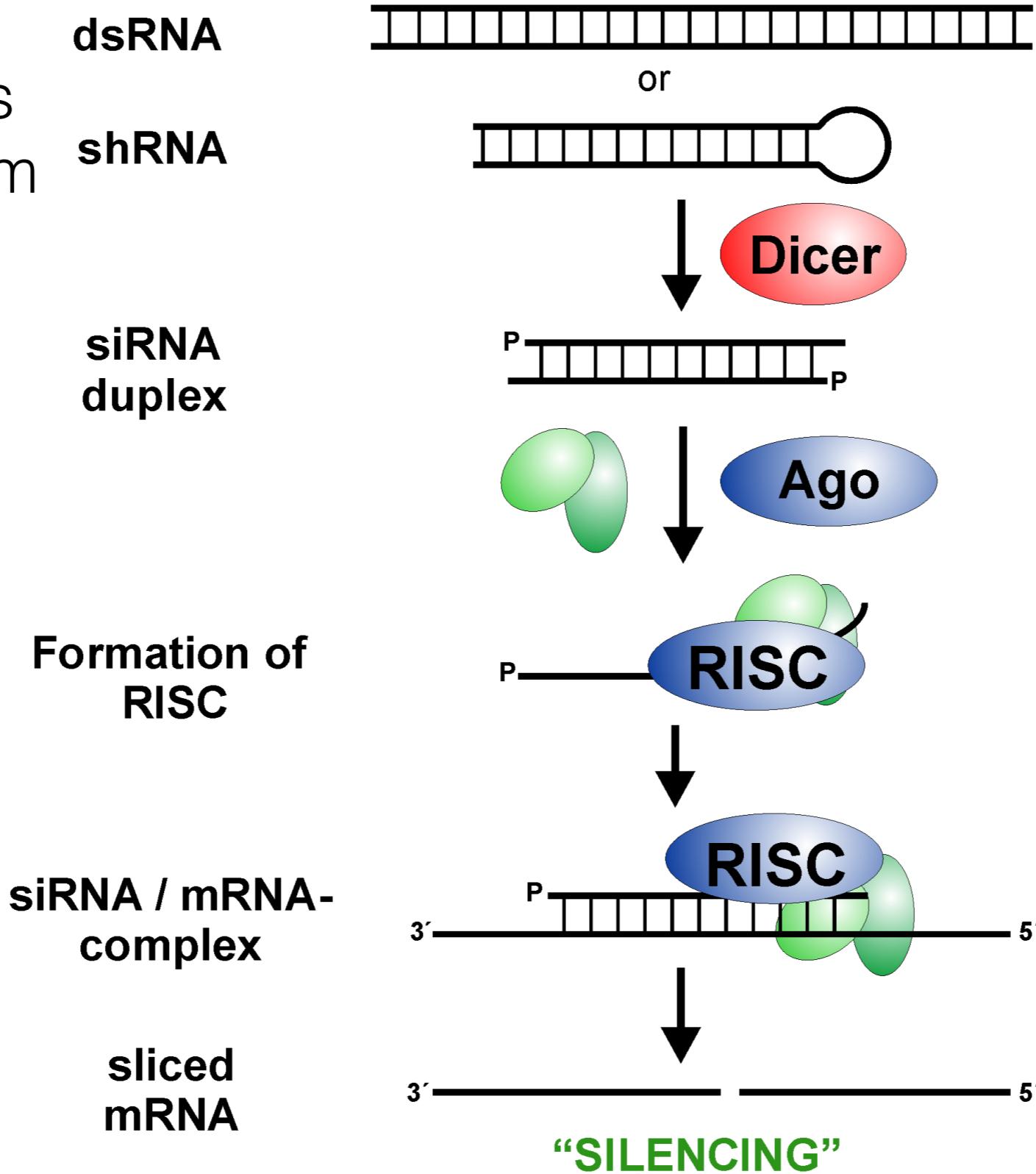
Score them all!!!

Phenotype is everything!

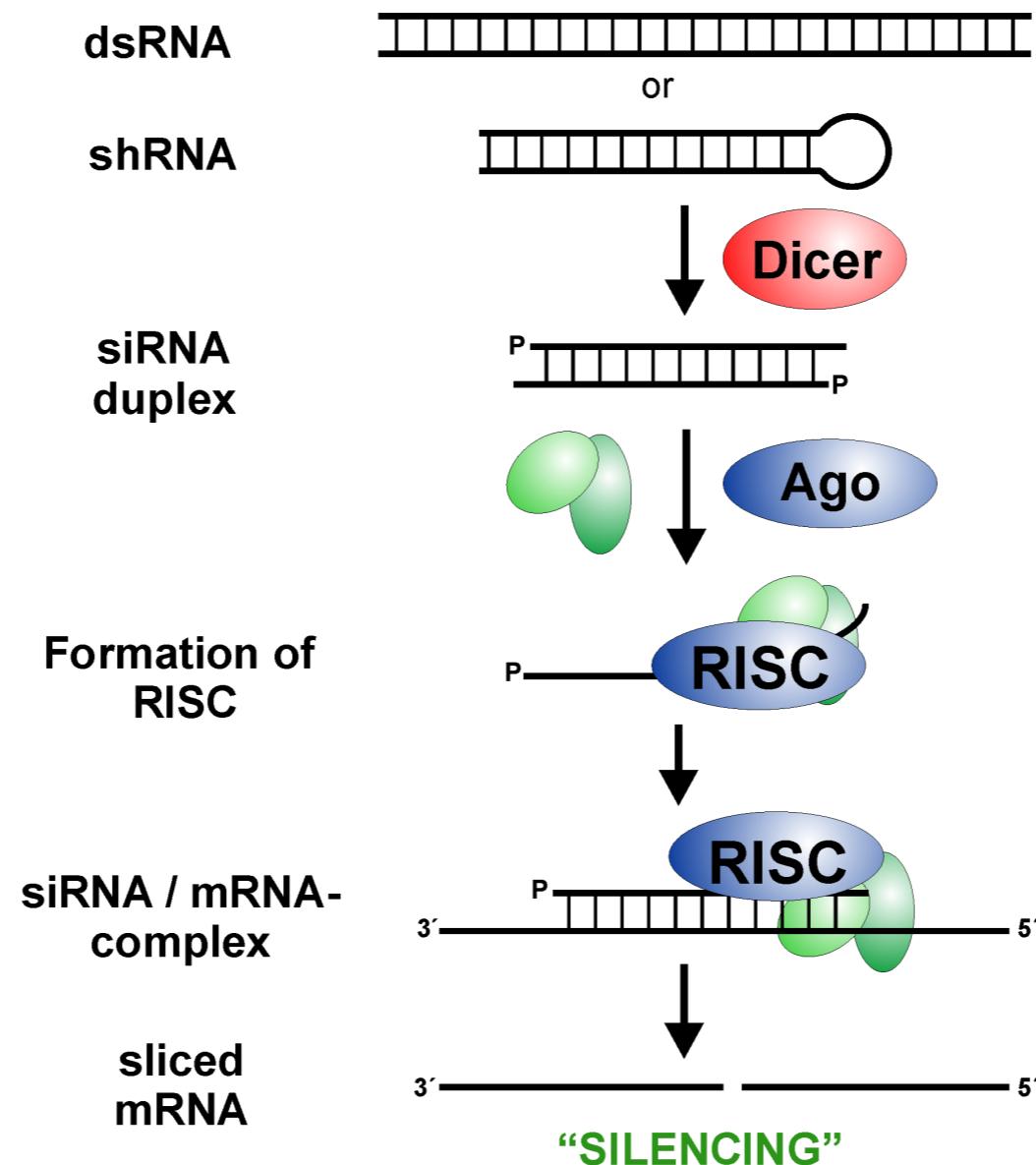
It has to be scalable, quantitative, reproducible,  
and accurate

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

You have to  
get these RNAs  
into the organism



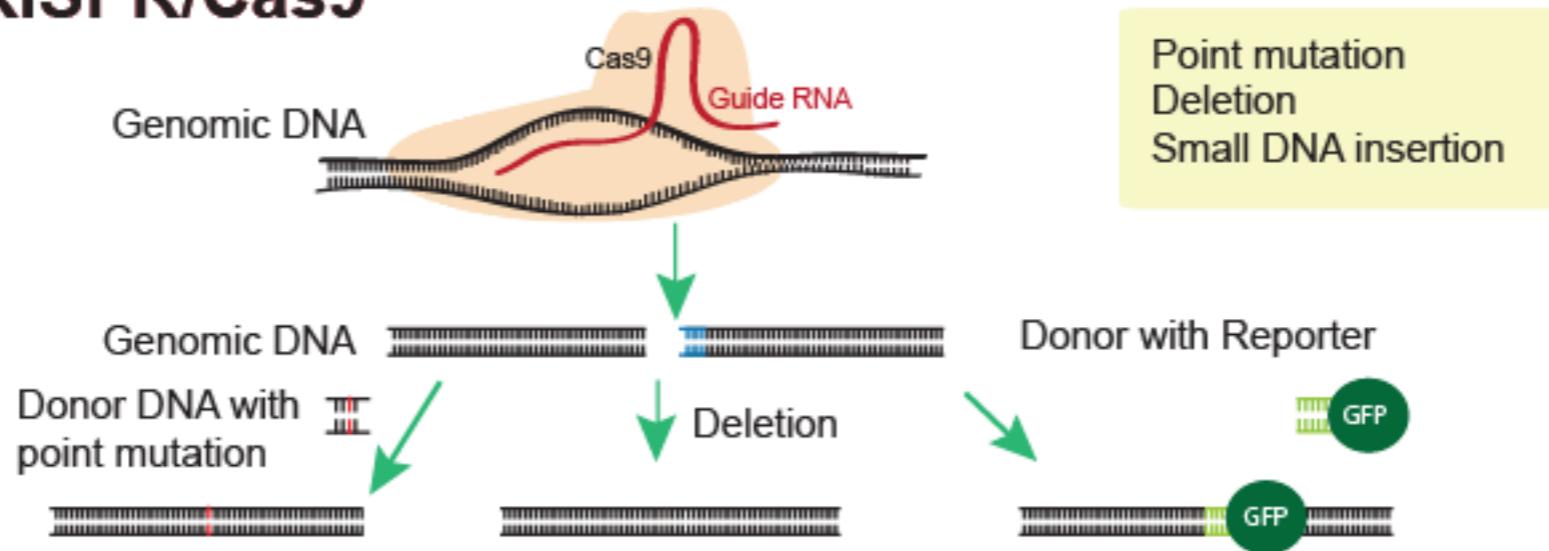
# RNA interference (RNAi) can be used to inactivate most genes in a variety of organisms



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

## CRISPR/Cas9



Caveat: Sometimes not specific (off-target effects)

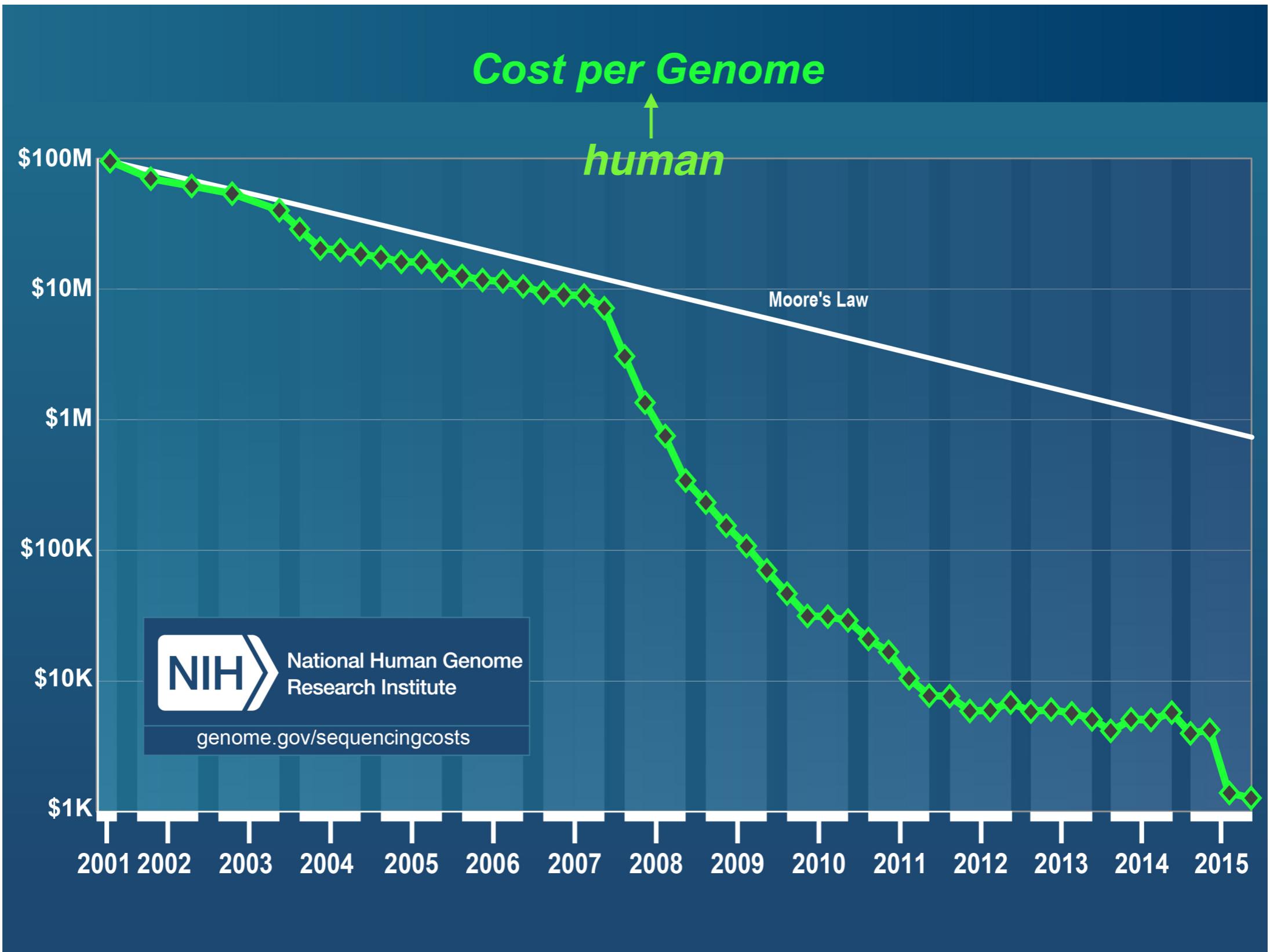
# Generate “mutations” by reverse genetic RNAi or CRISPR for “all” genes in an organism

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

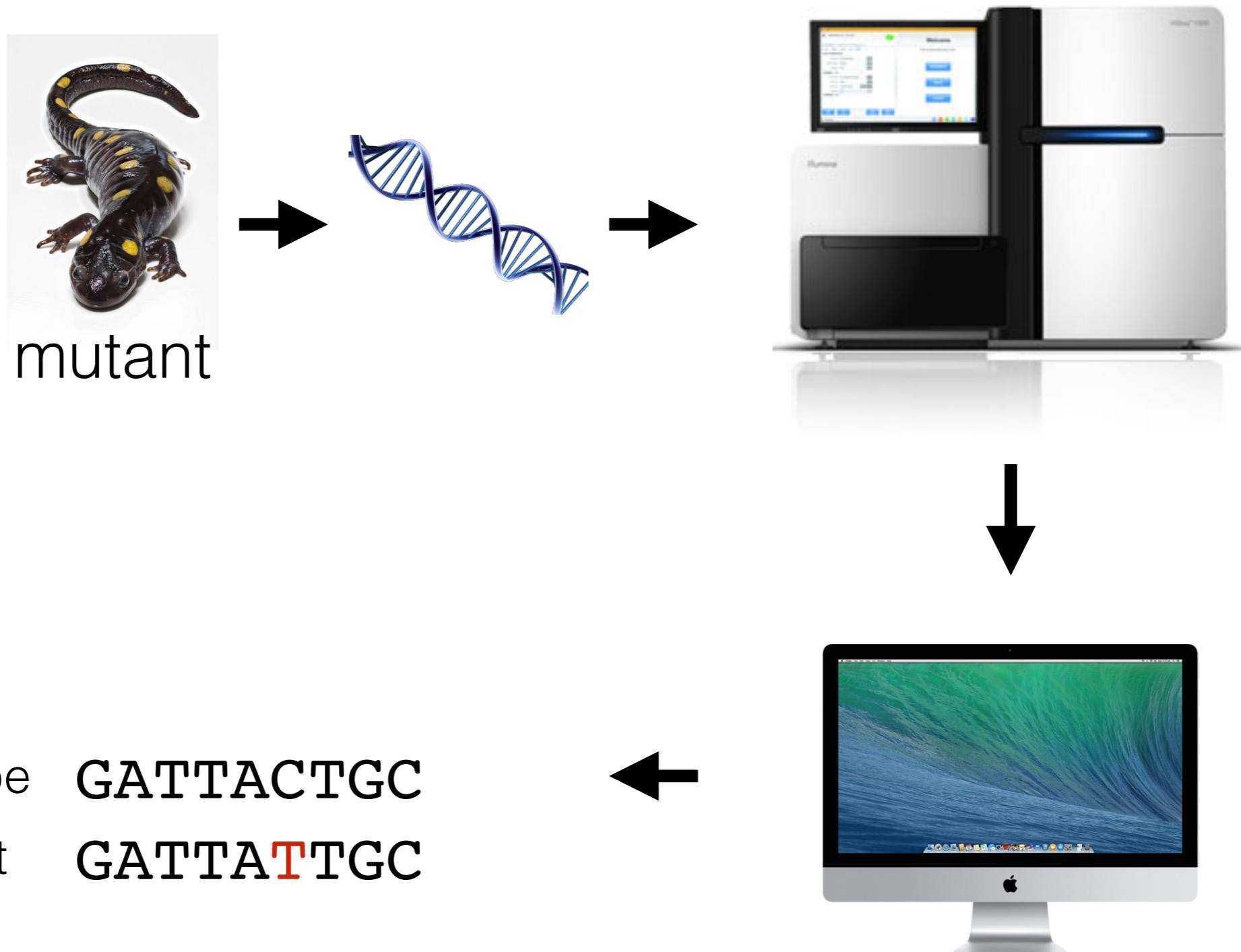
# **14. Clone the gene**

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

# Clone by sequencing

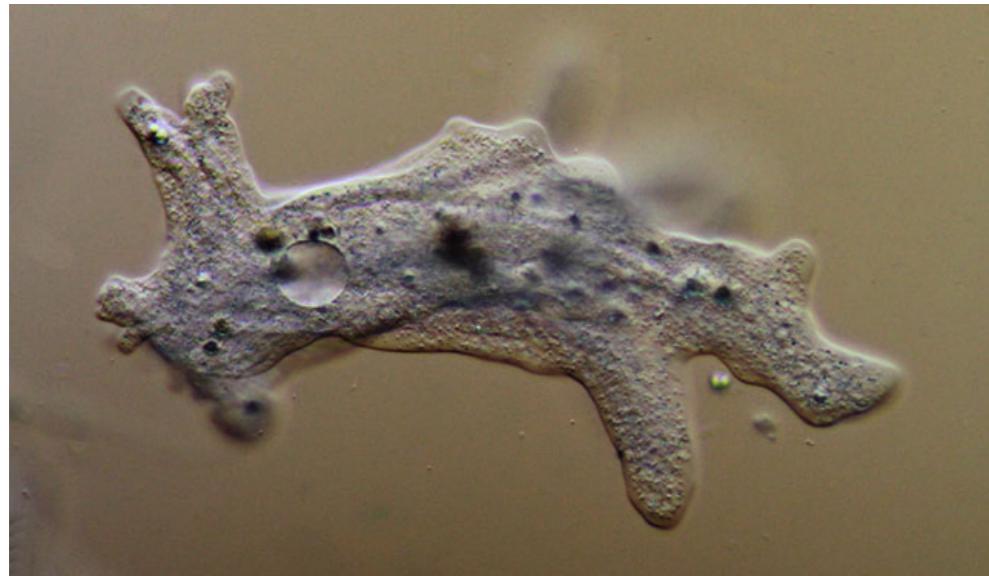


# Clone by sequencing



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?**

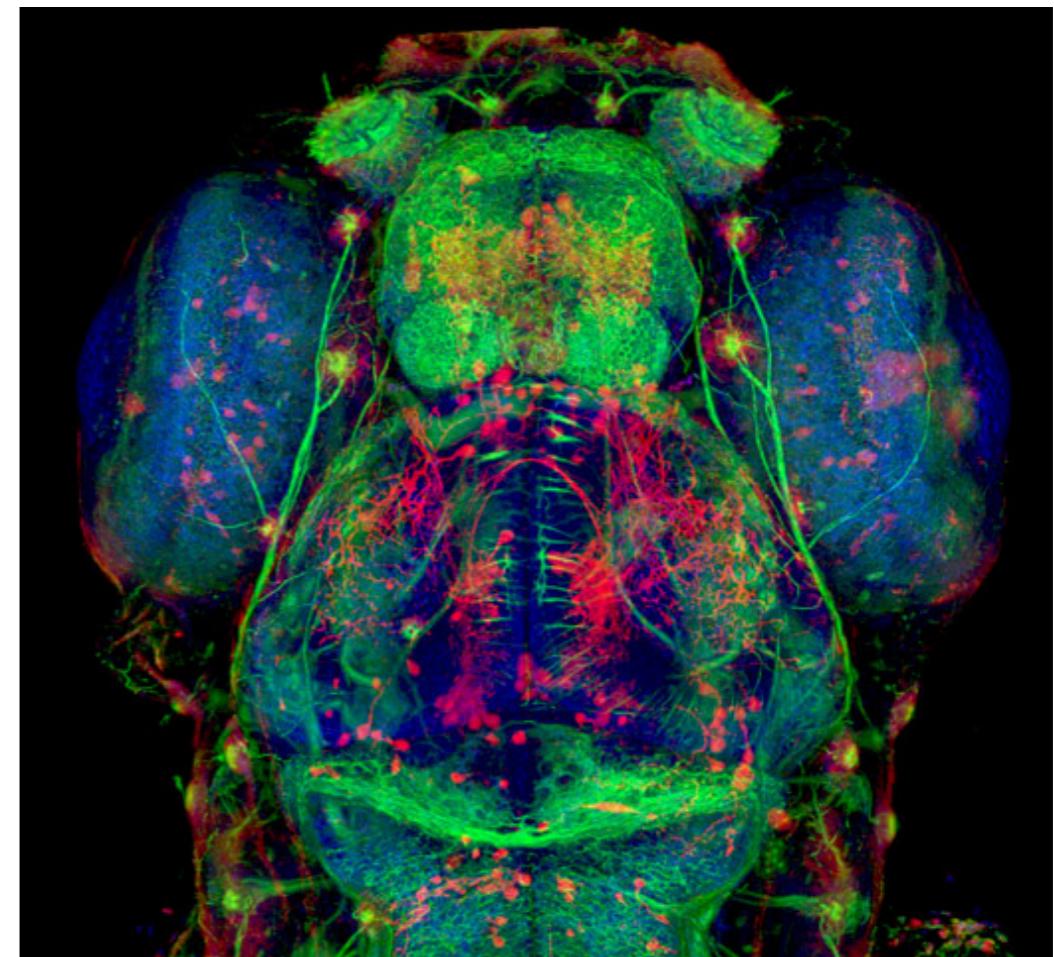
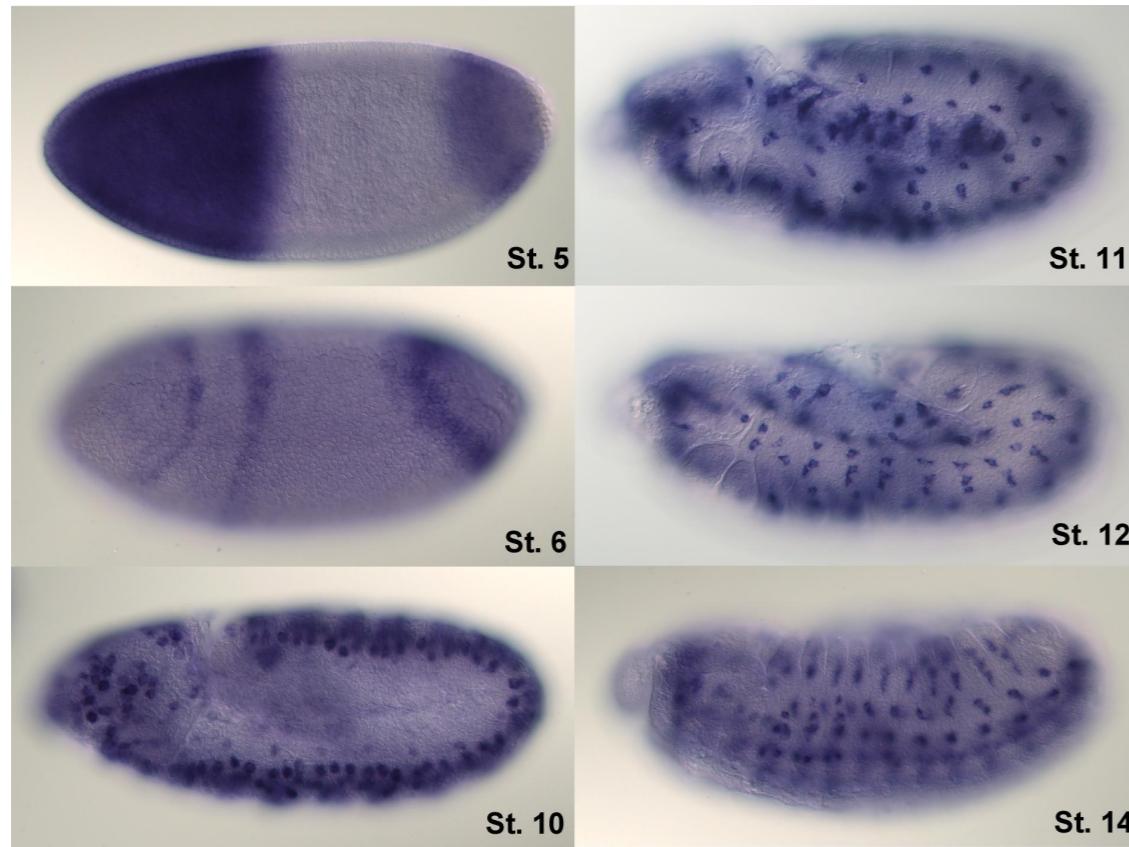
1. RNAi of gene X has same phenotype as our mutant
2. Mutation in gene X with rescue of mutant phenotype in gene X
3. Independent screens for engulfment mutants get multiple alleles of gene X

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

# 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:

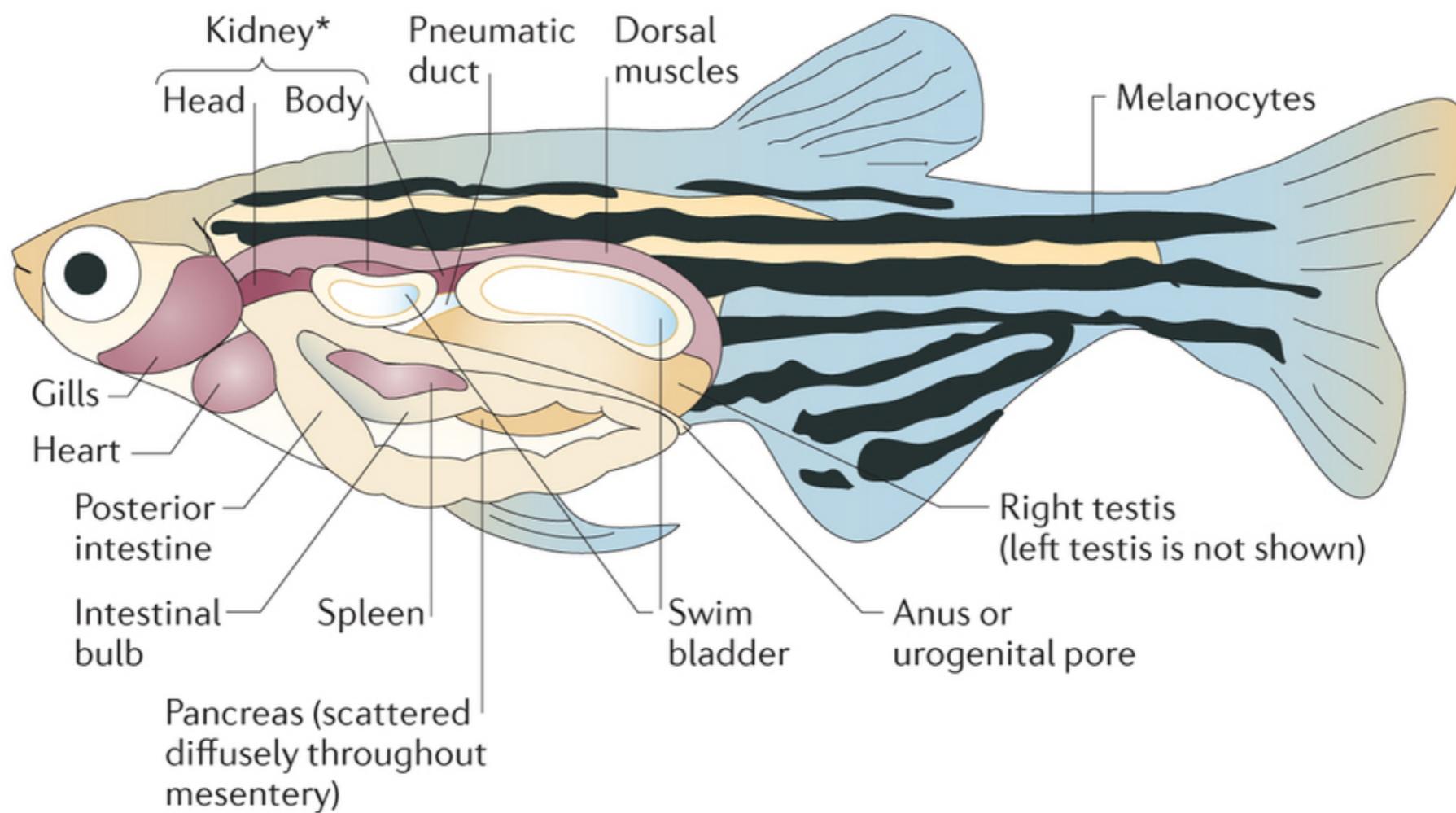


**Where a gene is expressed might not be where it acts?**

# 16. Determine site of gene action

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

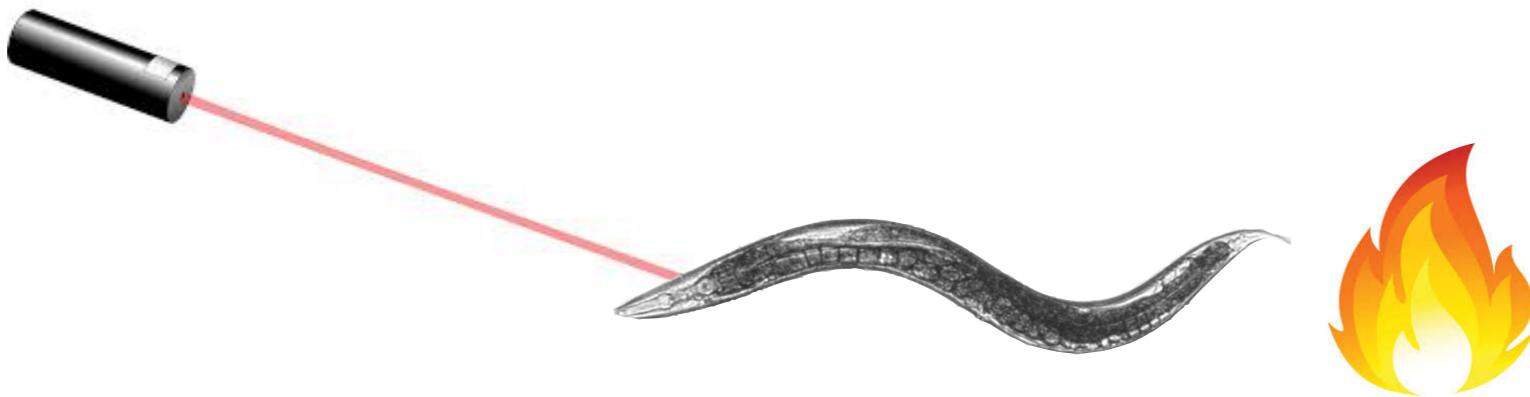
1. Rescue a mutant phenotype in a specific cell, organ, or tissue
2. Mosaic analysis (cell autonomy experiments)



# 17. Determine time of gene action

When does the gene function?

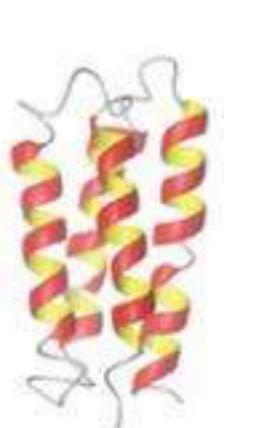
1. Induce expression to rescue a mutant phenotype at a specific time



# 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



**WT**

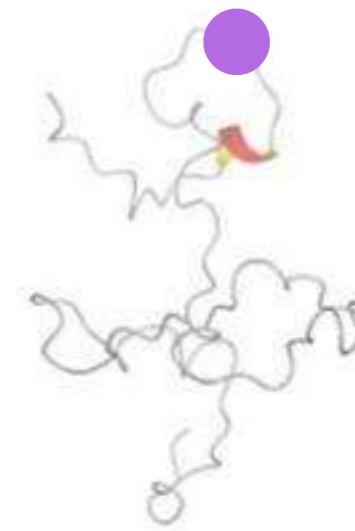


**Mutant**

Permissive temperature



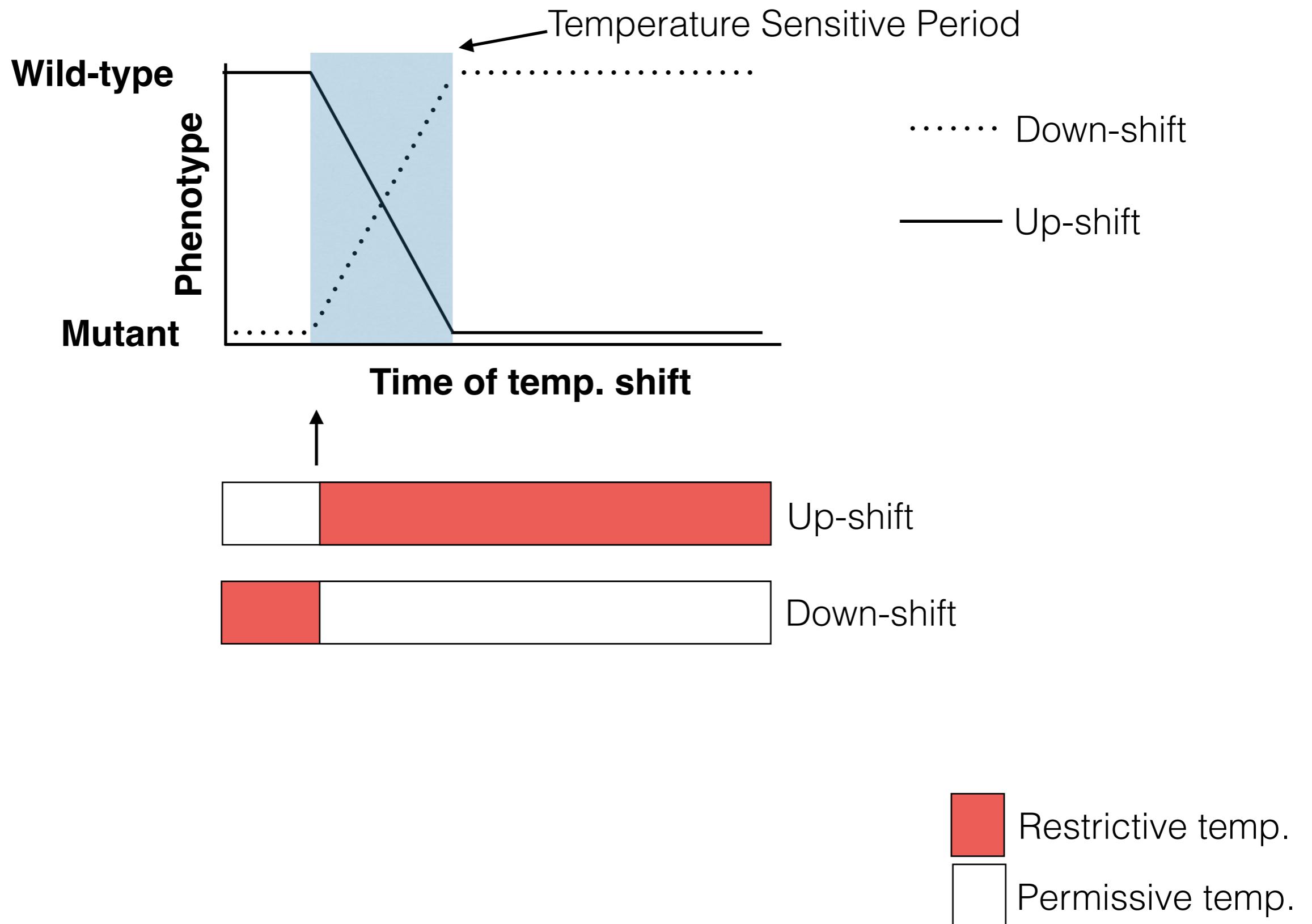
**WT**



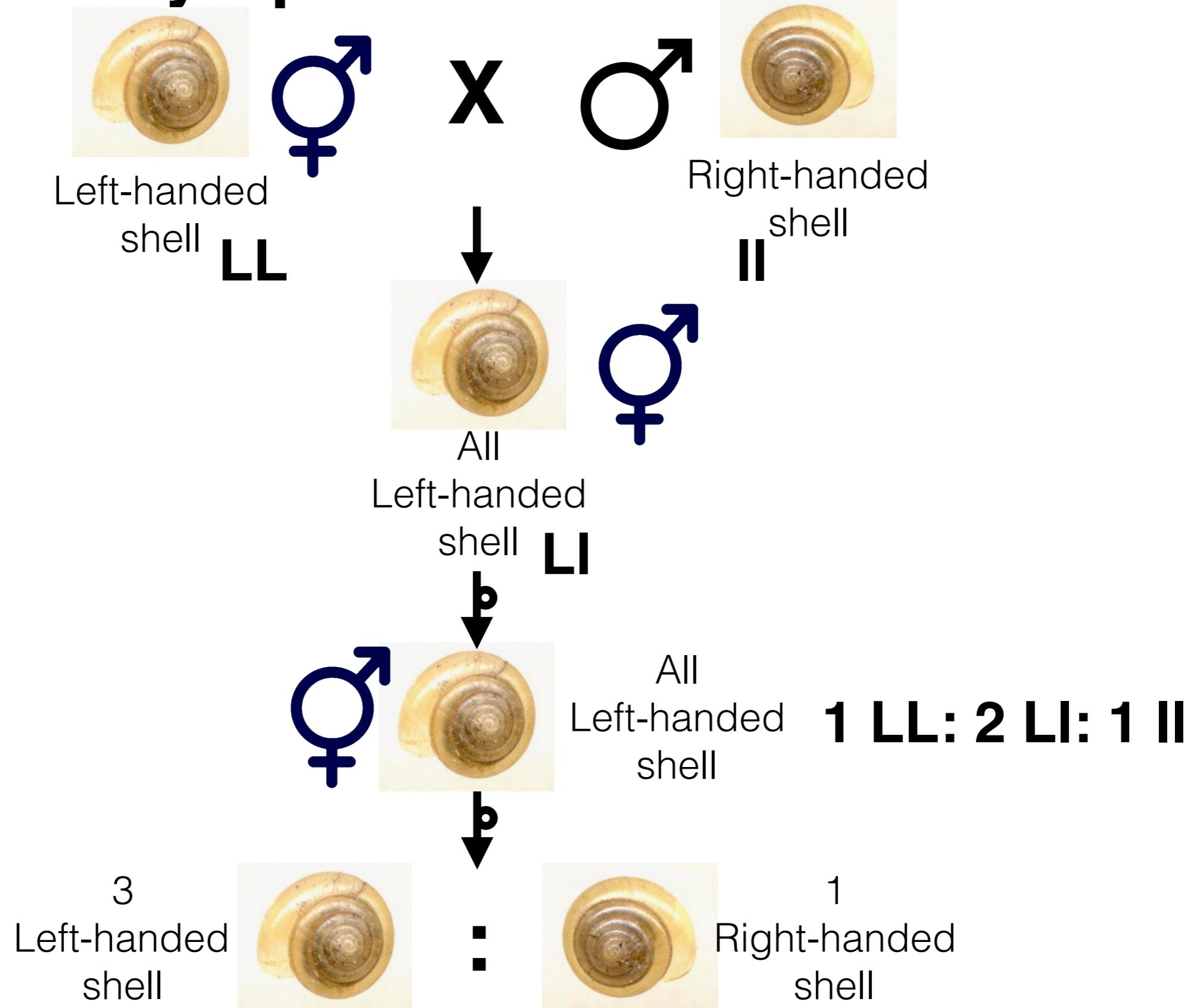
**Mutant**

Restrictive temperature

# 17. Determine time of gene action

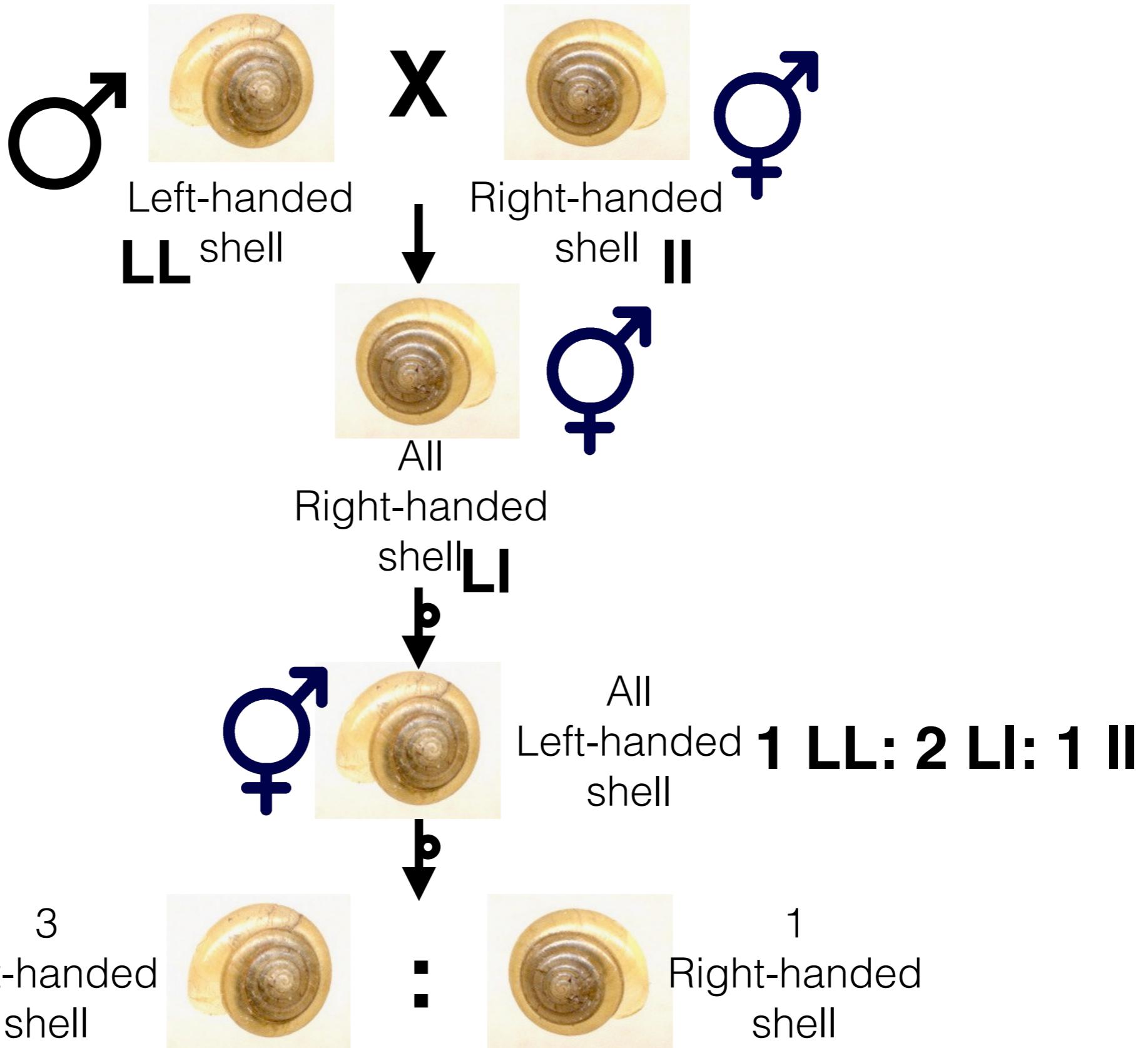


# 18. Determine if there are maternal effects or cytoplasmic inheritance



# 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



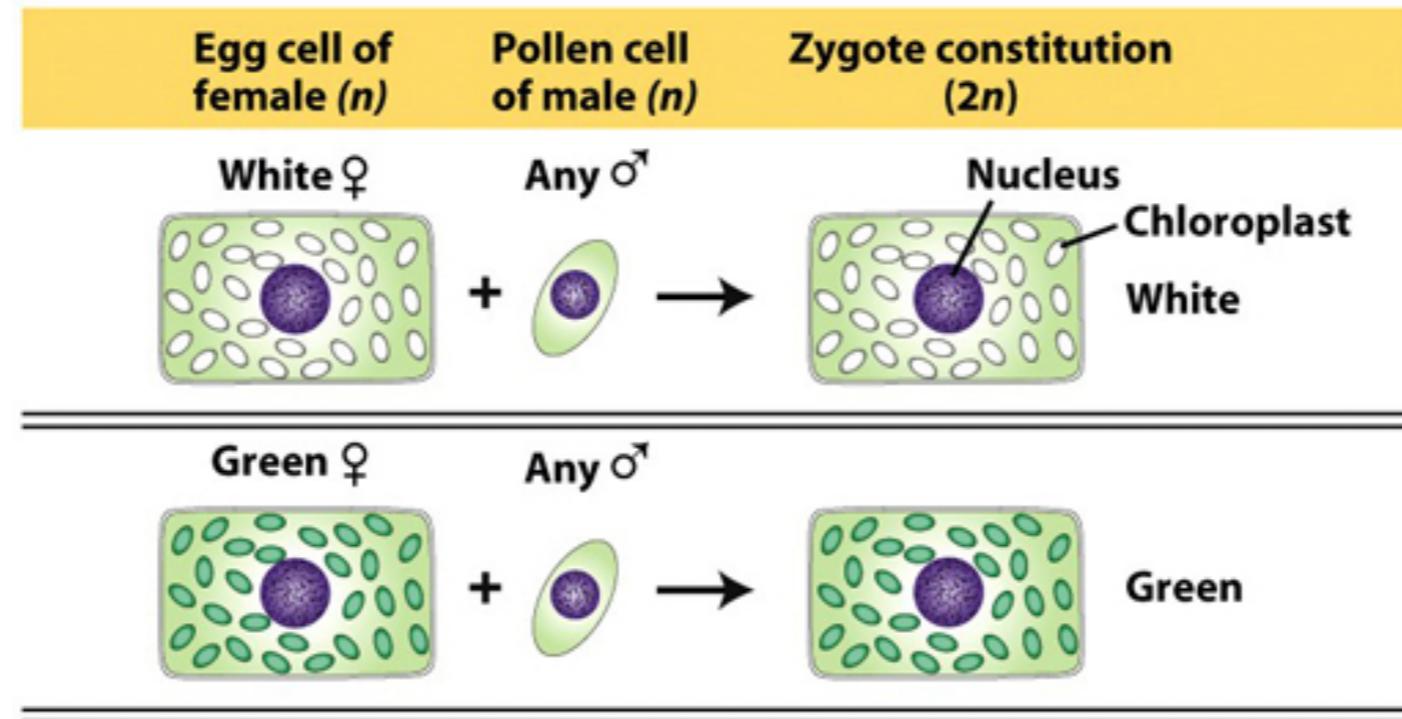
Plastid inheritance in  
*Mirabilis jalapa*

Only the color  
of the stem (mother)  
matters

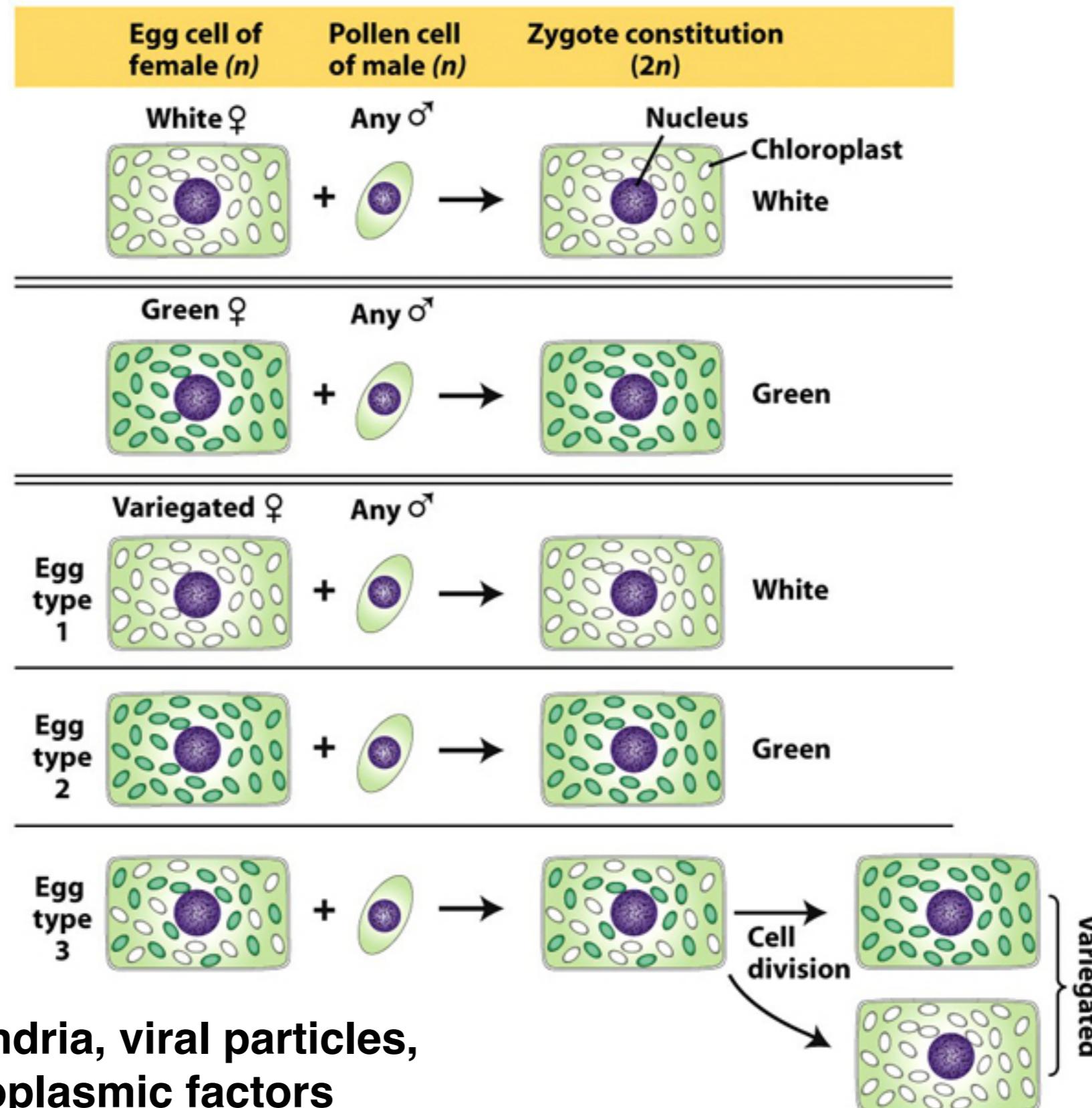
White, green, or  
variegated



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



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



# 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**

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