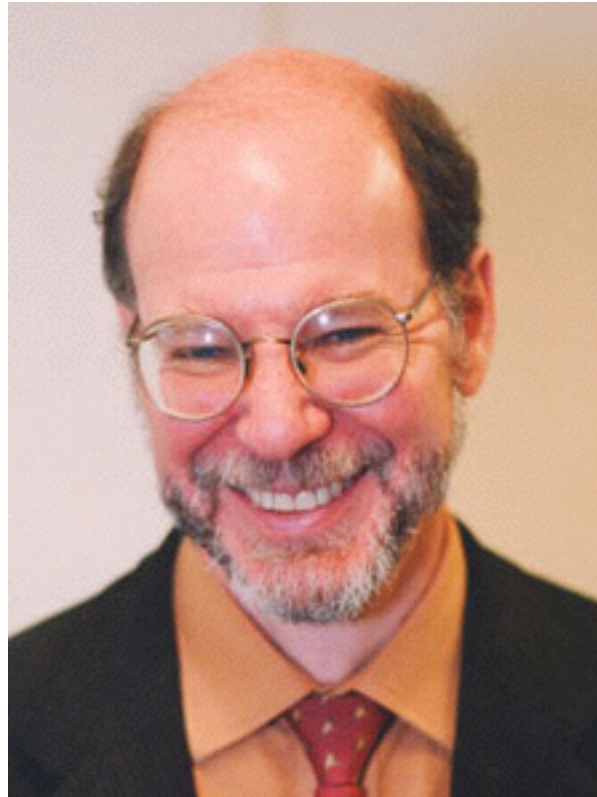# Bio393: Genetic Analysis

Step-wise genetic analysis
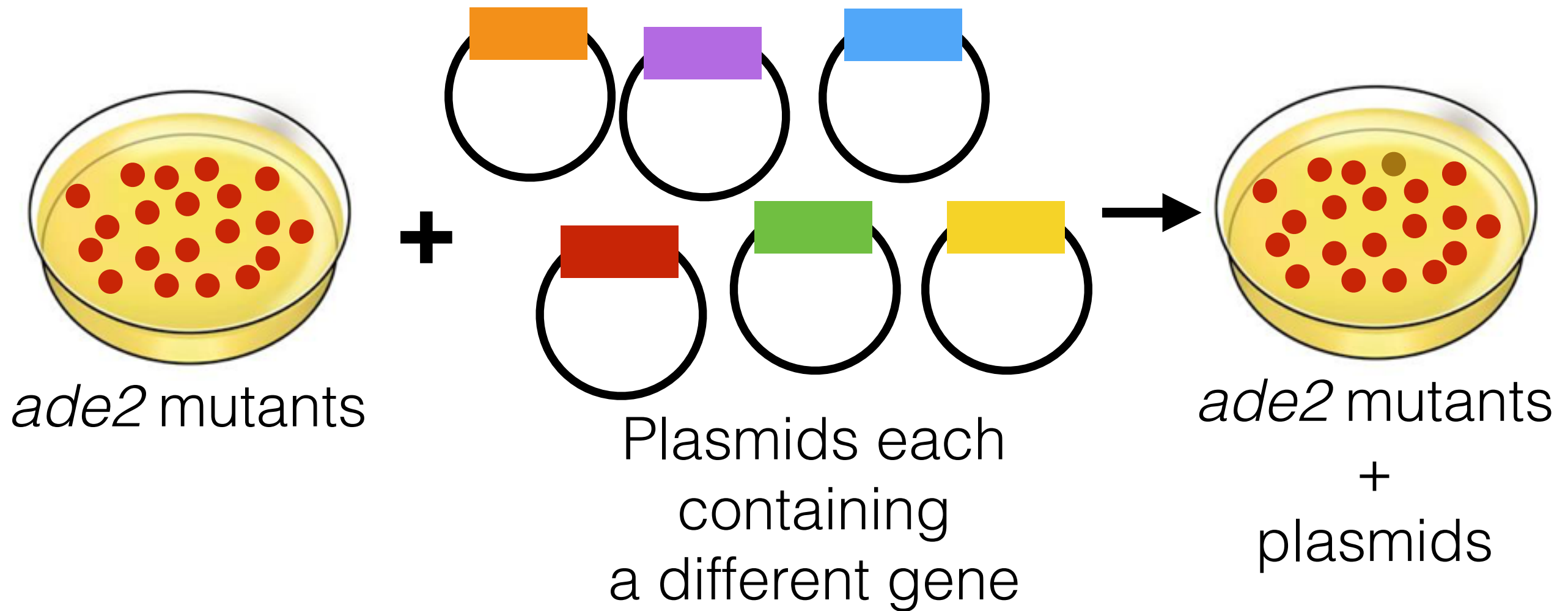

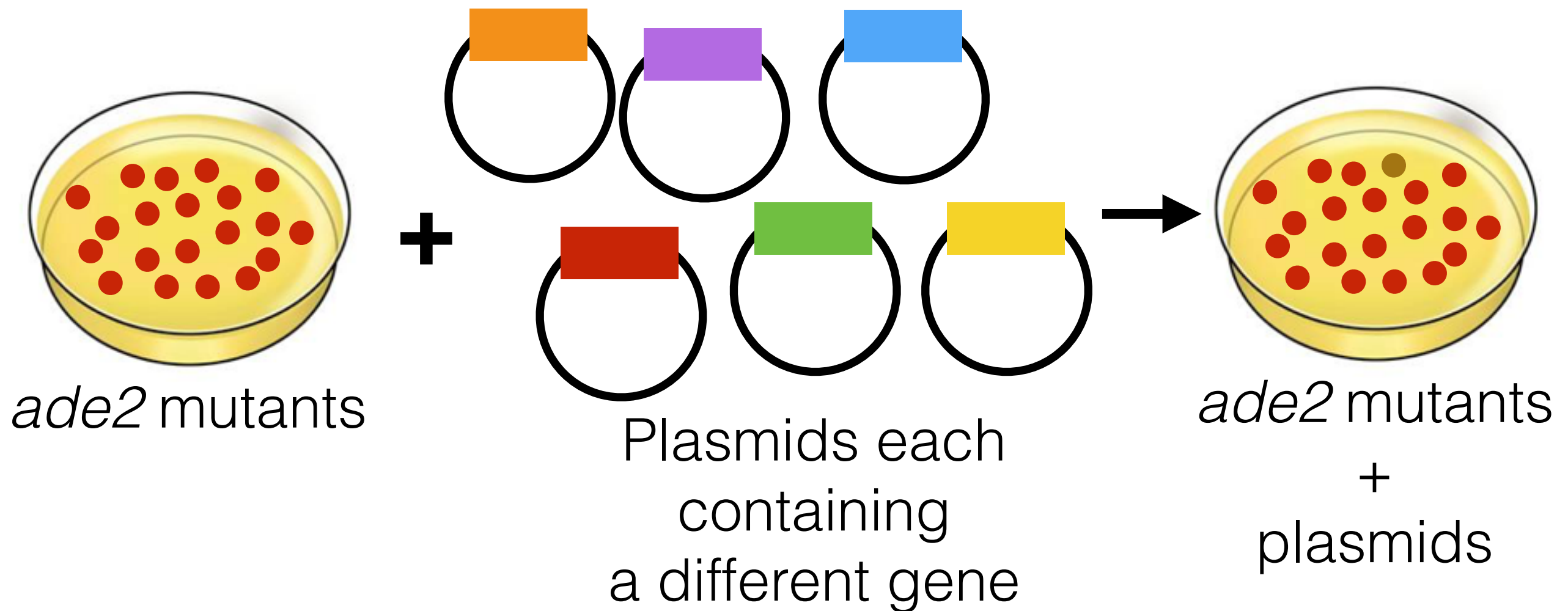**Bob Horvitz**

15. Clone the gene

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

Cloning by complementation in bacteria and yeast

ade2 mutants

+

Plasmids each containing a different gene

ade2 mutants + plasmids

Cloning by complementation in bacteria and yeast

ade2 mutants

+

Plasmids each containing a different gene

ade2 mutants
+
plasmids

Caveat: overexpression bypass suppressors

Cloning by complementation in worms and flies

geneX   GFP

Many plasmids become
an extra chromosome

geneX   mini-w

transposase

white mutant embryo

Transgenesis and rescue

Cloning by complementation in worms and flies

Many plasmids become an extra chromosome

Caveat: overexpression bypass suppressors and not stable

white mutant embryo

Transgenesis and rescue

Cloning by complementation in worms and flies

Many plasmids become an extra chromosome

Caveat: overexpression bypass suppressors and not stable

white mutant embryo

Caveat: overexpression bypass suppressor and variable expression

Transgenesis and rescue
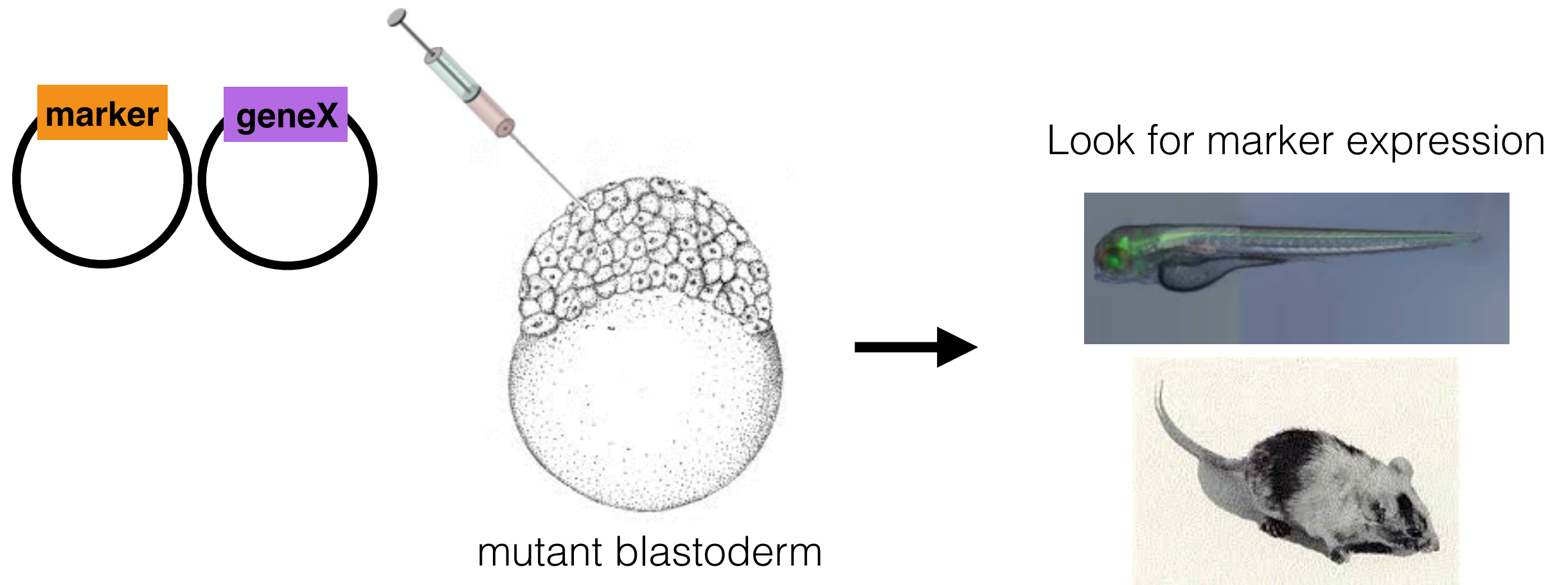
Cloning by complementation in fish and mice

marker geneX

mutant blastoderm

Look for marker expression

Cloning by complementation in fish and mice

marker   geneX

mutant blastoderm

Look for marker expression
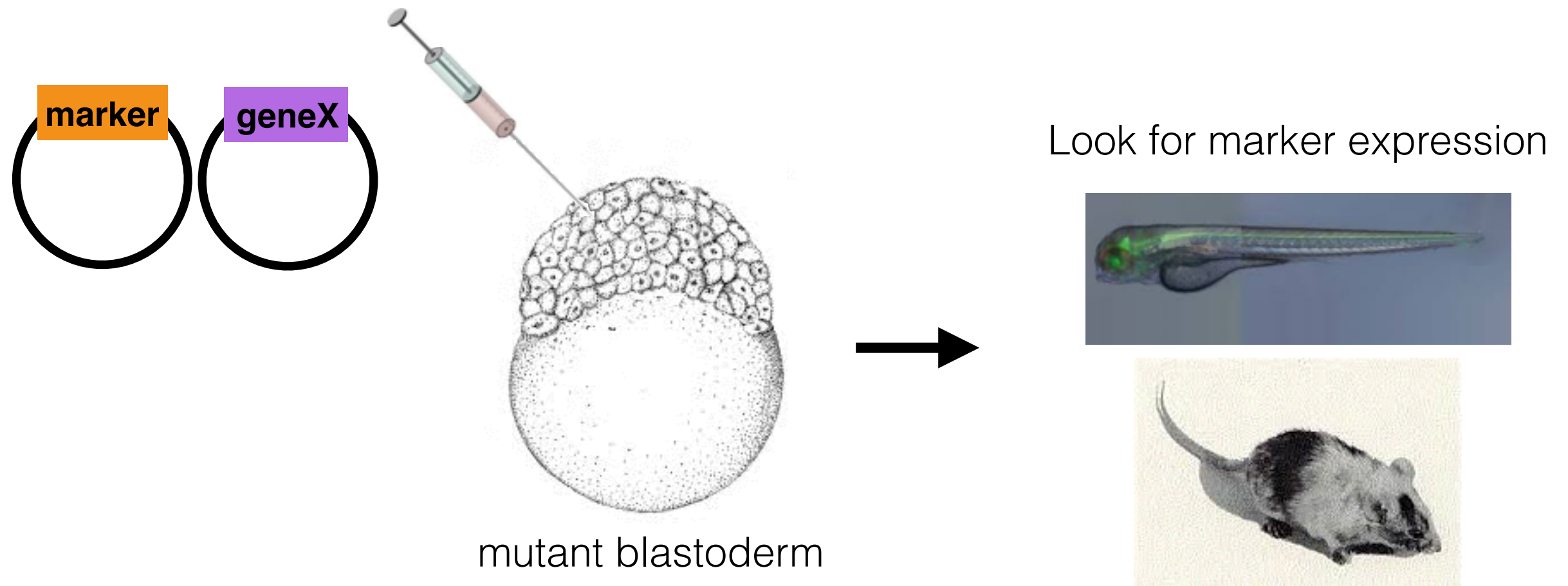
Caveat: overexpression bypass suppressors
and variable expression

15. Clone the gene

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

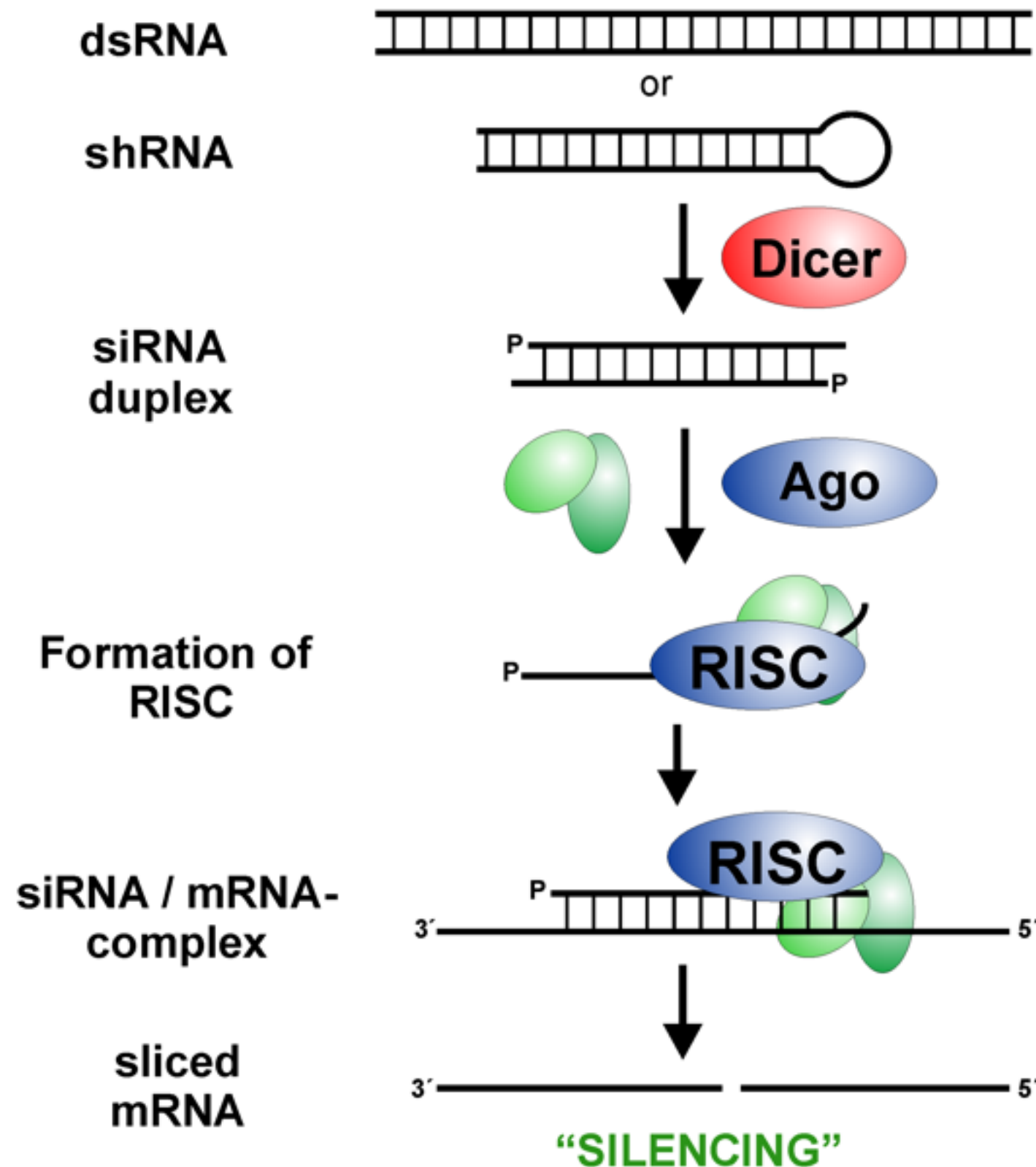# Most model organisms have libraries of strains where each strain has a unique loss-of-function mutation
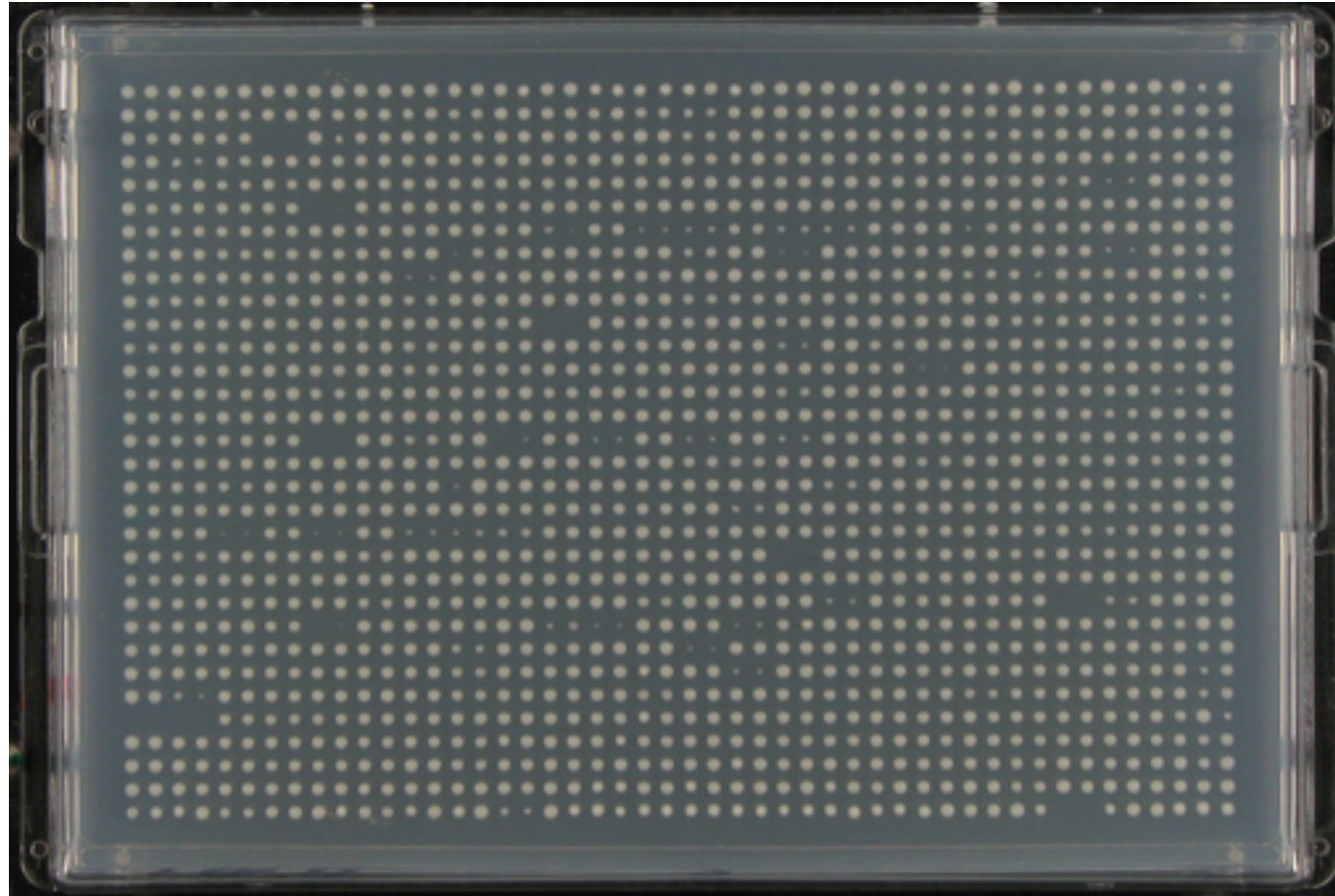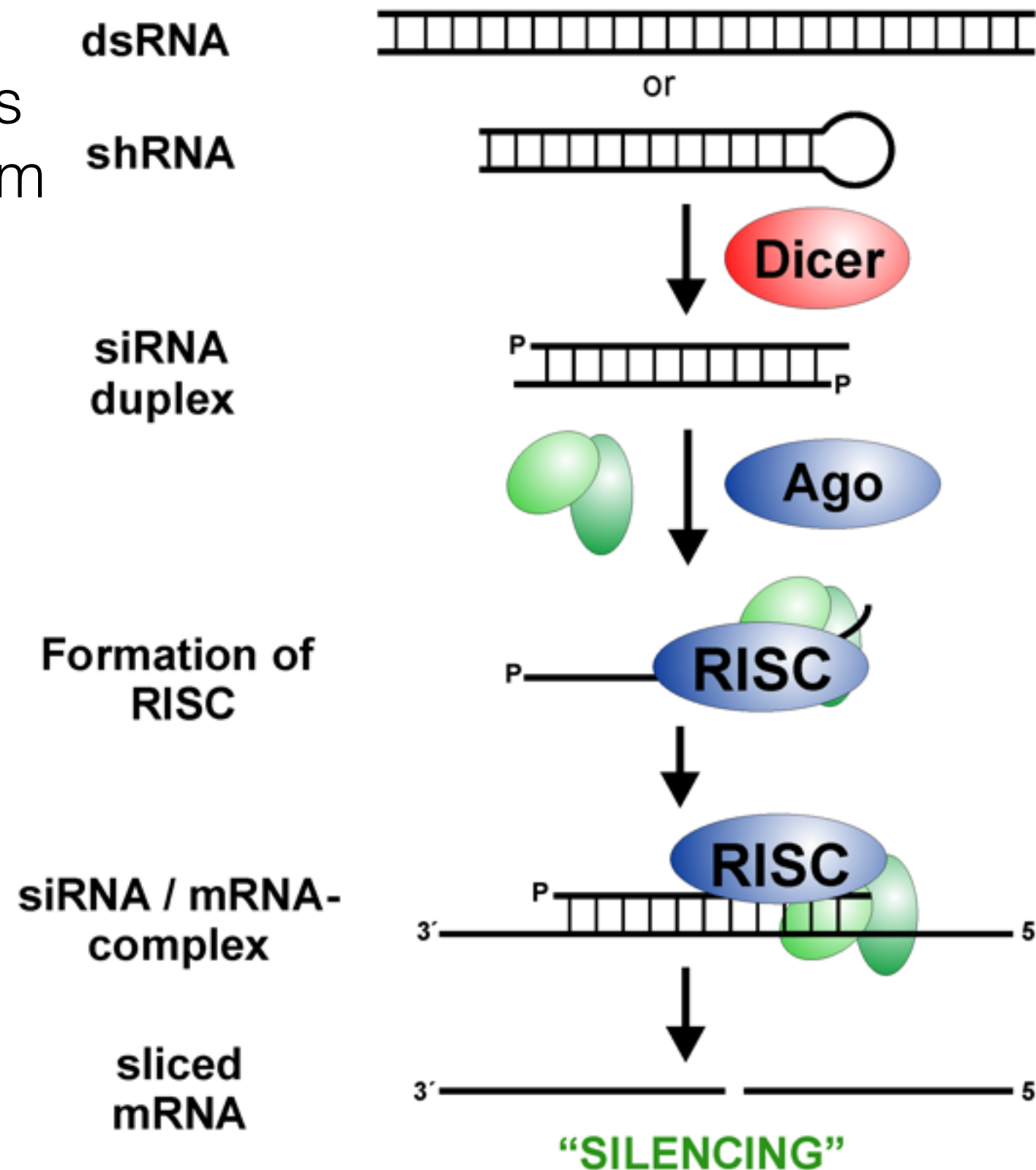

Score them all!!!

Phenotype is everything!

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

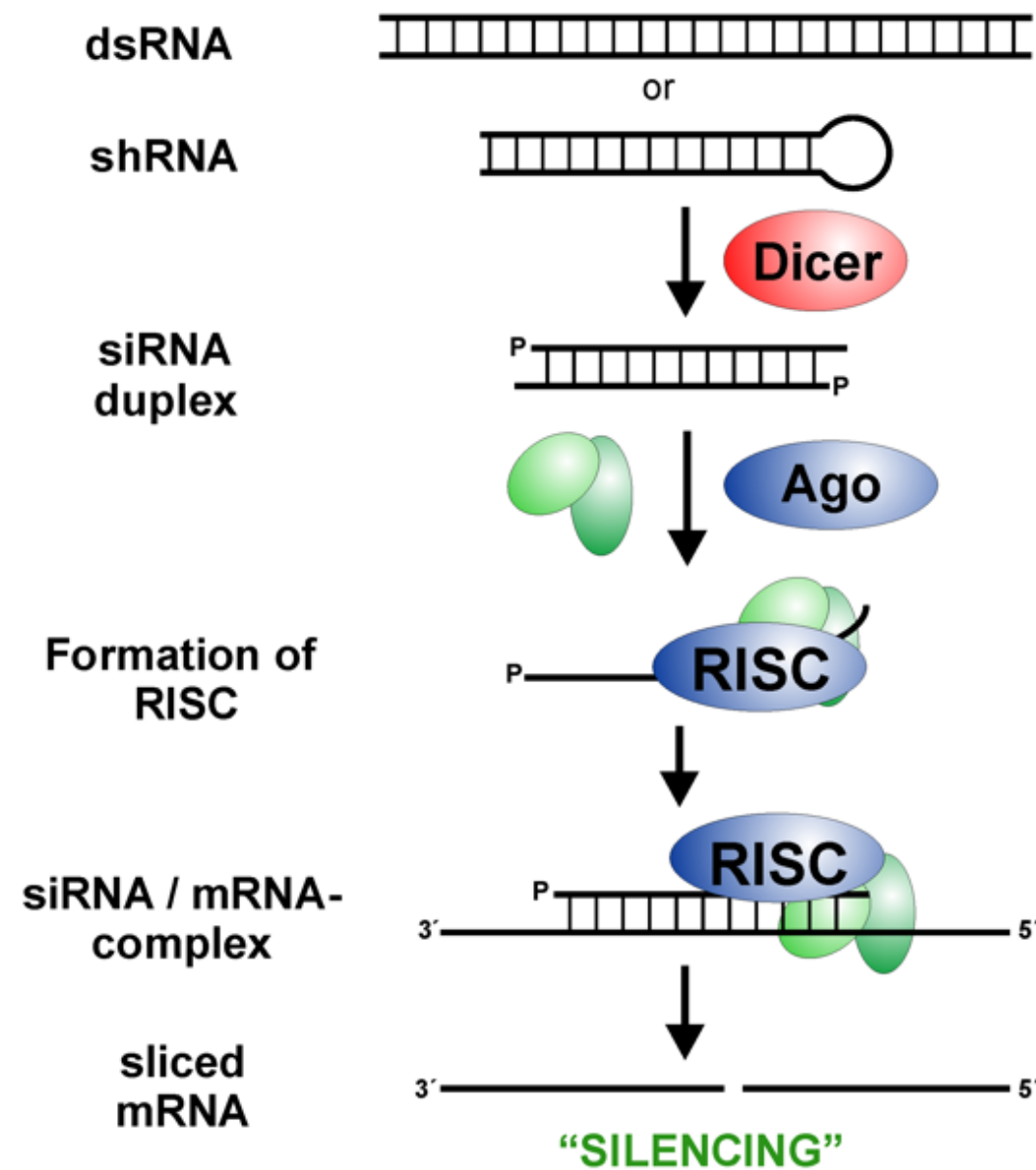# RNA interference (RNAi) can be used to inactivate most genes in a variety of organisms

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

You have to get these RNAs into the organism

dsRNA

or

shRNA

Dicer

siRNA duplex

Ago

Formation of RISC

RISC

siRNA / mRNA-complex

RISC

sliced mRNA

"SILENCING"

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

RNA interference (RNAi) can be used to inactivate most genes in a variety of organisms
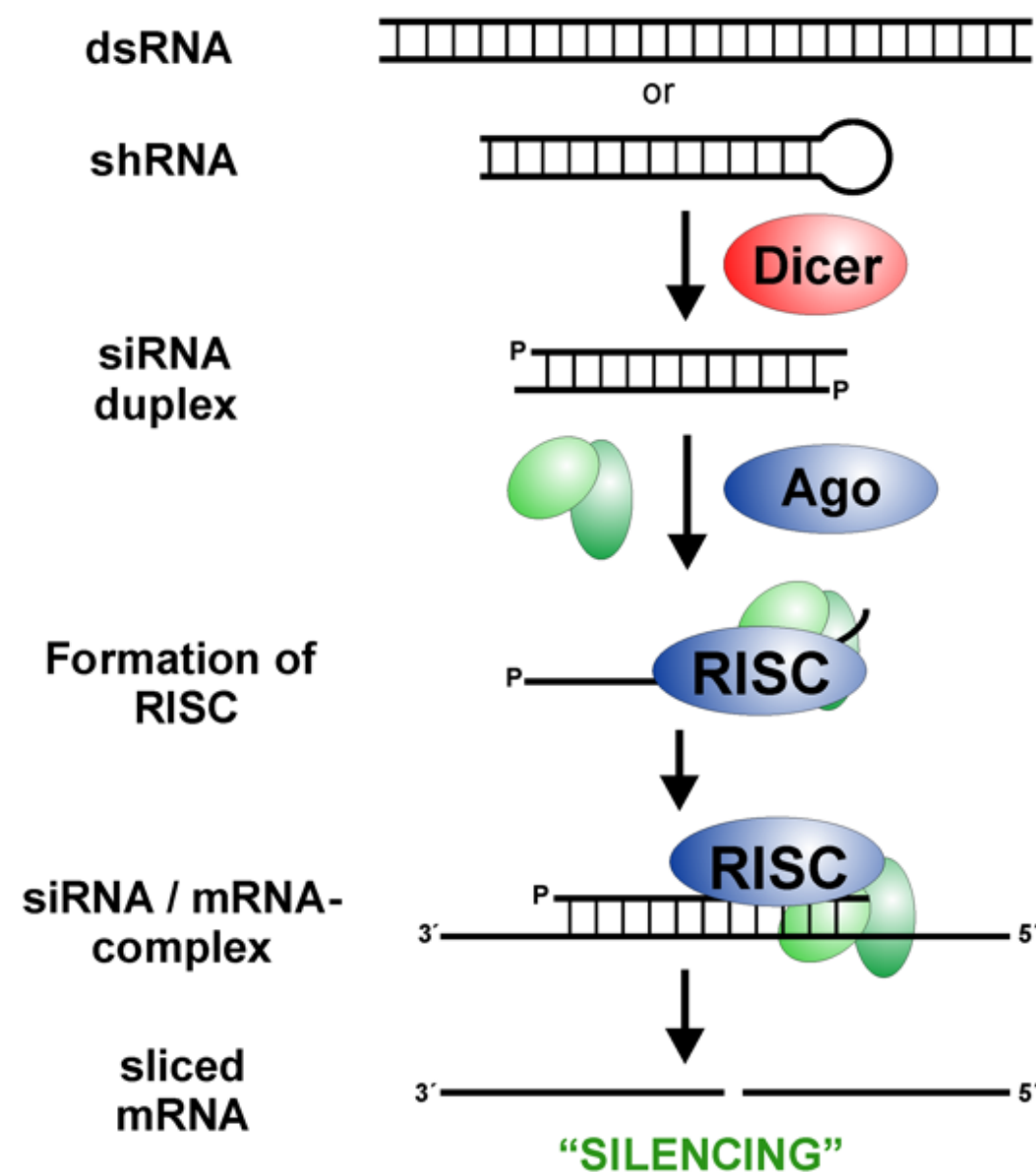
Caveat: Delivery is problematic, highly variable effects, not heritable, not loss-of-function, not specific

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

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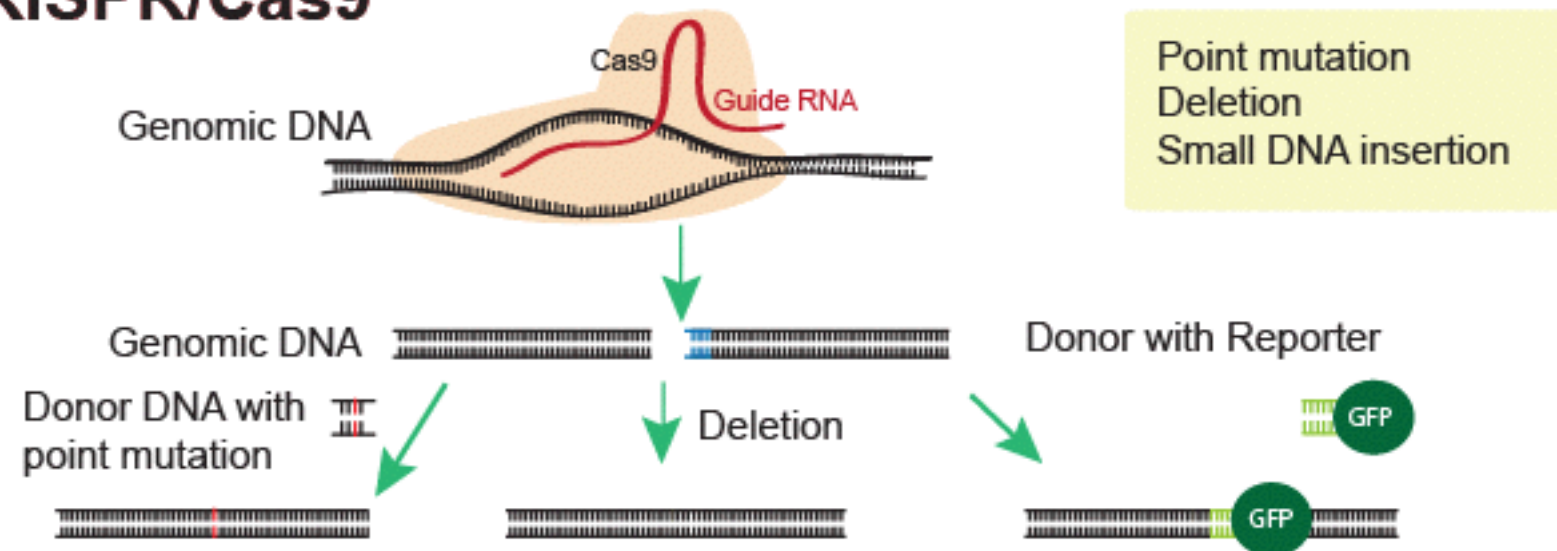

Caveat: Sometimes not specific (off-target effects)

Generate "mutations" by reverse genetic RNAi or CRISPR for "all" genes in an organism

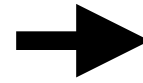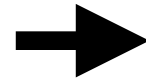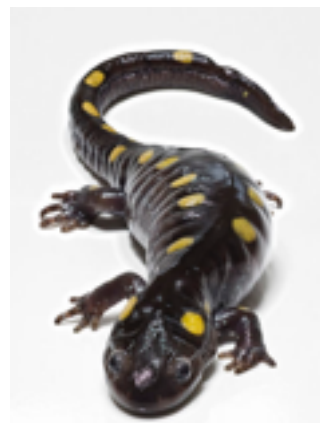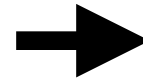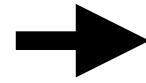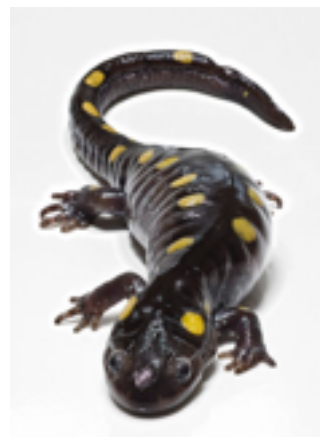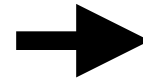| Organism	Approx. # of genes
Yeast (*S. cerevisiae*)	6,000
Fly (*D. melanogaster*)	15,000
Worm (*C. elegans*)	21,000
Zebrafish (*D. rerio*)	26,000
Chicken (*G. gattus*)	17,000
Mouse (*M. musculus*)	23,000
Mustard plant (*A. thaliana*)	28,000
Human (*H. sapiens*)	30,000

Clone by sequencing
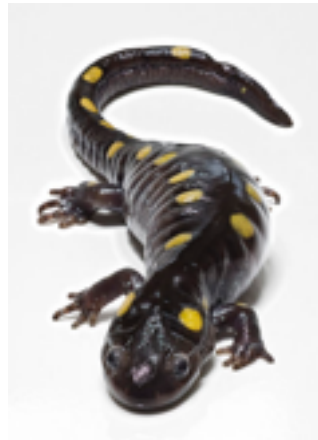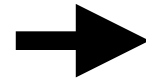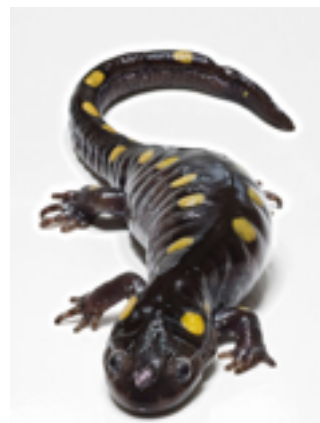
mutant

Clone by sequencing

mutant

Clone by sequencing

mutant

Clone by sequencing
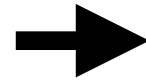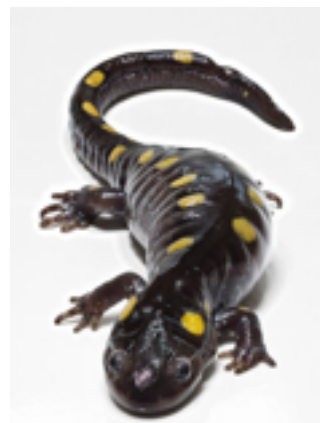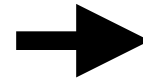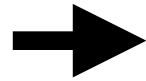

mutant

wild-type GATTACTGC
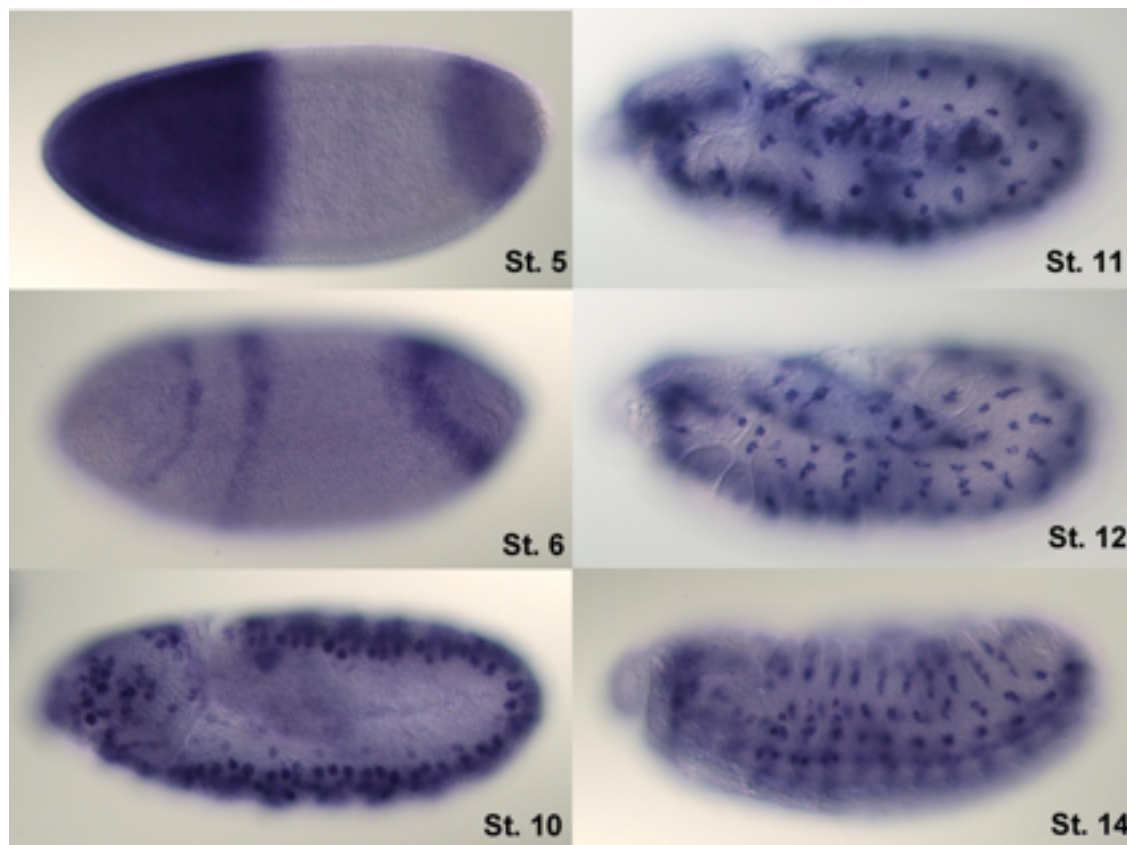mutant   GATTA**T**TGC

Clone by sequencing

mutant

wild-type GATTACTGC

mutant GATTATTGC

Need multiple non-complementing alleles and mapping

16. Determine where gene is expressed
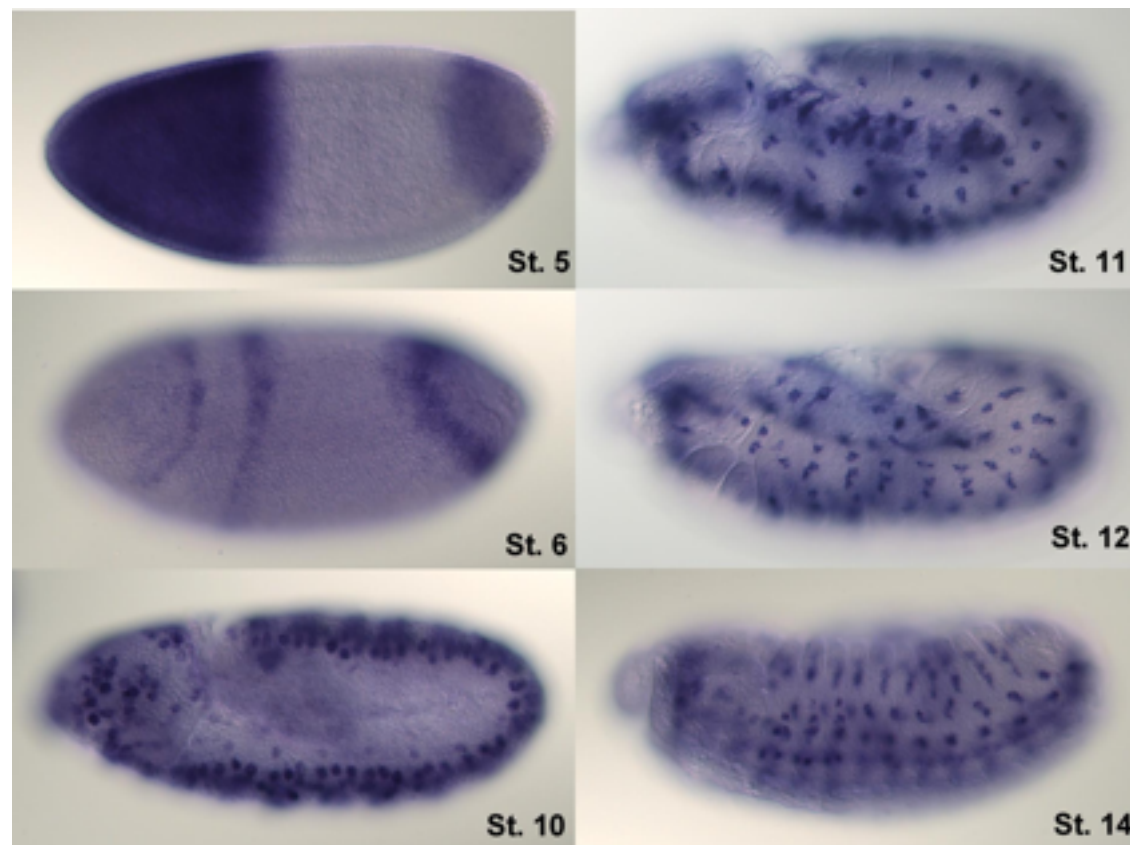
With no transgenesis:

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

16. 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?

16. Determine where gene is expressed

With transgenesis:

1. Fluorescent reporters (GFP, mCherry)

16. Determine where gene is expressed

With transgenesis:
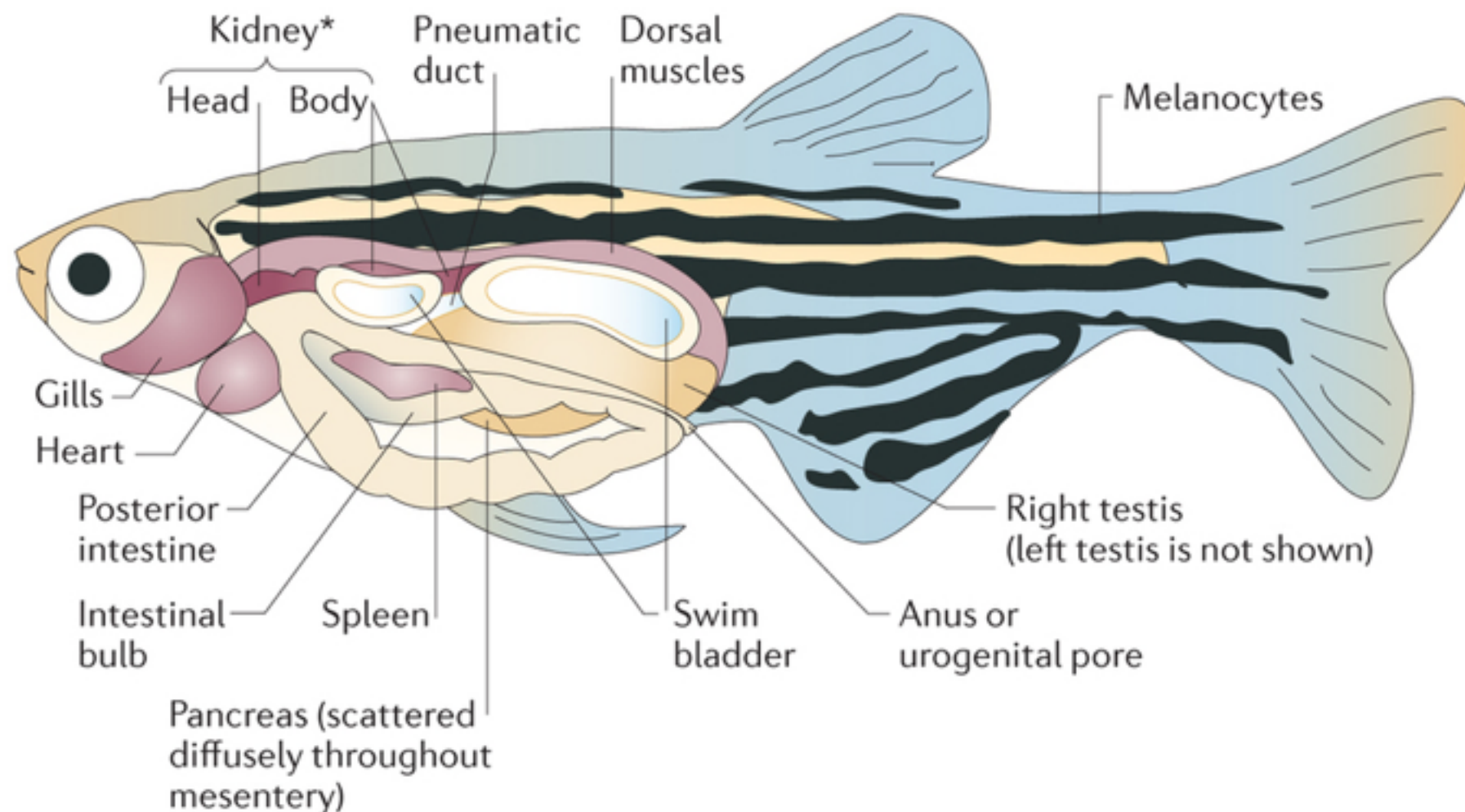
1. Fluorescent reporters (GFP, mCherry)

How do we know we have the right expression pattern?

17. Determine site of gene action

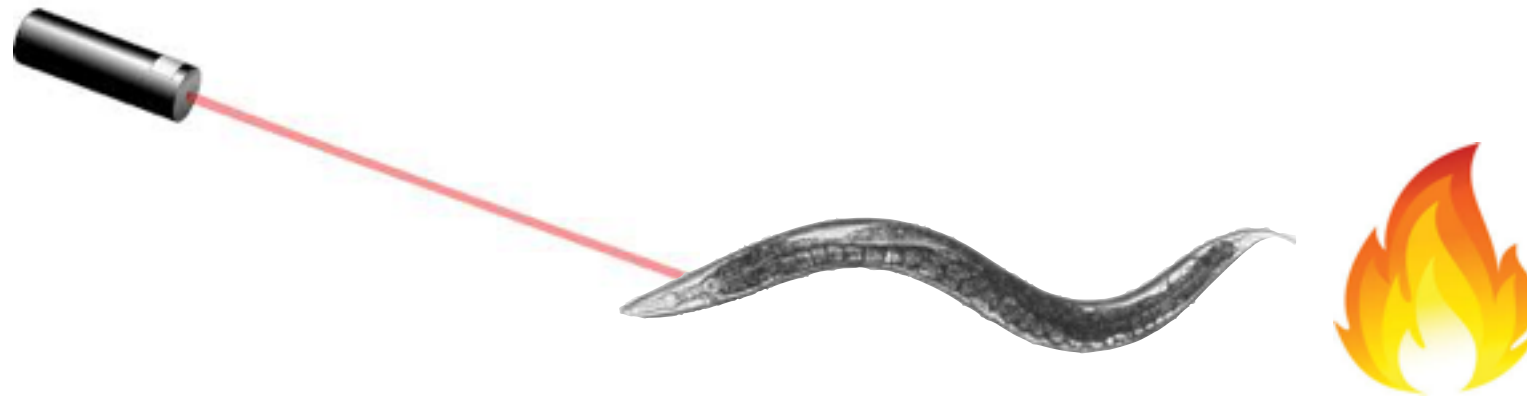What is the cell, organ, and/or tissue where the gene functions?

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

18. Determine time of gene action

When does the gene function?

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

18. Determine time of gene action

When does the gene function?

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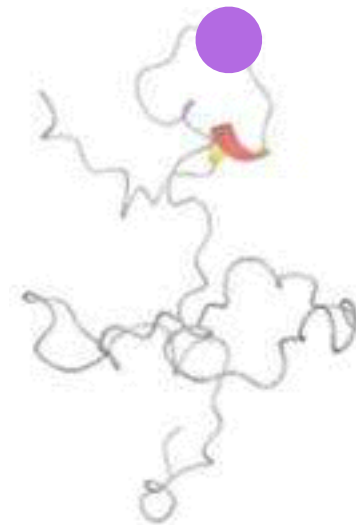2. Use temperature-labile mutants to define the temperature-sensitive period

WT **Mutant**

Permissive temperature

WT **Mutant**

Restrictive temperature

18. Determine time of gene action

Temperature-sensitive period