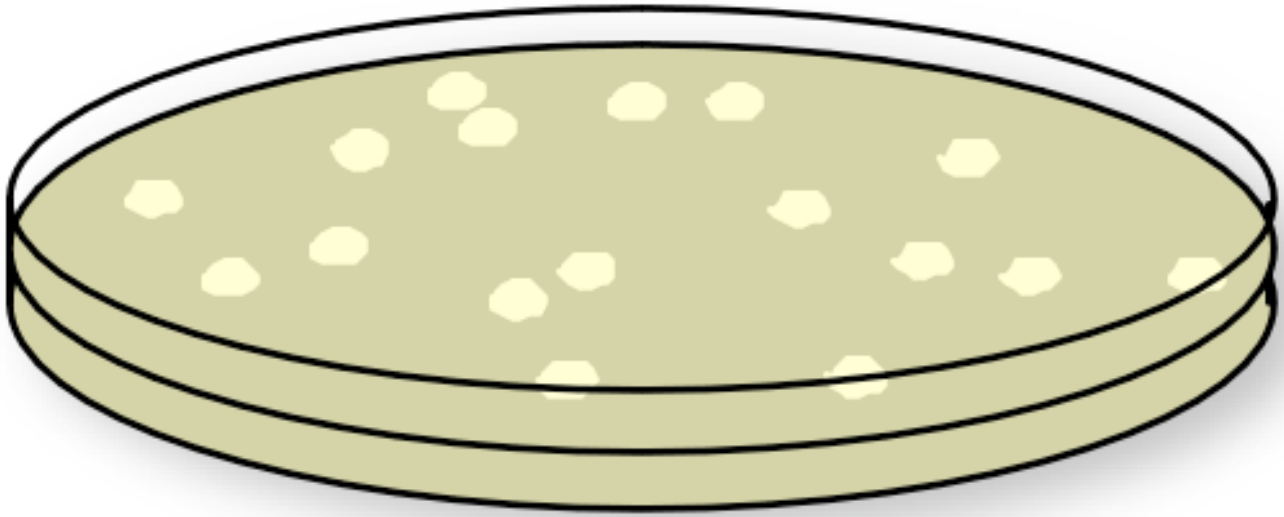
Analyzing mutants

Drosophila transposon mutagenesis

Genetics of cancer

Cloning genes



Suppose you are setting up a transposon mutagenesis screen in fruit flies starting with the following flies:

$\frac{X^w}{X^w}$; $\frac{+}{+}$; $\frac{+}{P[w^+]}$ females and $\frac{X^w}{Y}$; $\frac{CyO\ \ P[TNase]}{+}$; $\frac{Ubx}{+}$ males

$P[w^+]$ = P-element that can "hop";

CyO = dominant Curly-wing marker;

w^+ = dominant red-eye marker

P[TNase] = *Jump-starter* providing the transposase;

Ubx = dominant Ultrabithorax marker

Note that the allele arrangement is different here from the ones you have seen in quiz section!

(a) From the cross between the animals above, what progeny **phenotype** (including sex) would you pick as the animals **in whose germ cells*** the transposon hop would occur? Briefly justify your answer. (*What do these progeny correspond to, in the time line of "4 weeks ago"/"2 weeks ago", etc.?)

Phenotype of animal in whose germ cells transposition will occur:

Eye color: Red eyes
Wing type: Curly wing
Haltares: Enlarged haltere
Sex: Male

Why you would pick that phenotype?

Red-eye: so we know it has the transposon P[w+]

Curly-wing: linked to Jump-starter, so we know the fly has the transposase gene

Enlarged haltere: to track the chromosome III homologue that does NOT contain P[w+]--so we can identify progeny flies that have a transposon jump.

Male: so there'll be no recombination

For homework:

Proceed with the next cross, using an animal that was a progeny of the cross in (a); at least one member of this next cross must match your answer in (a).

Female **genotype**:

X

Male **genotype**:

(b) How would you detect mutant progeny resulting from a transposon hop?

(Begin by providing the **phenotype** of mutant progeny, and indicate why this phenotype tells you that a transposon hop has occurred.)

"Big picture" reality check

Why would one mess around with the fly transposon, anyway?

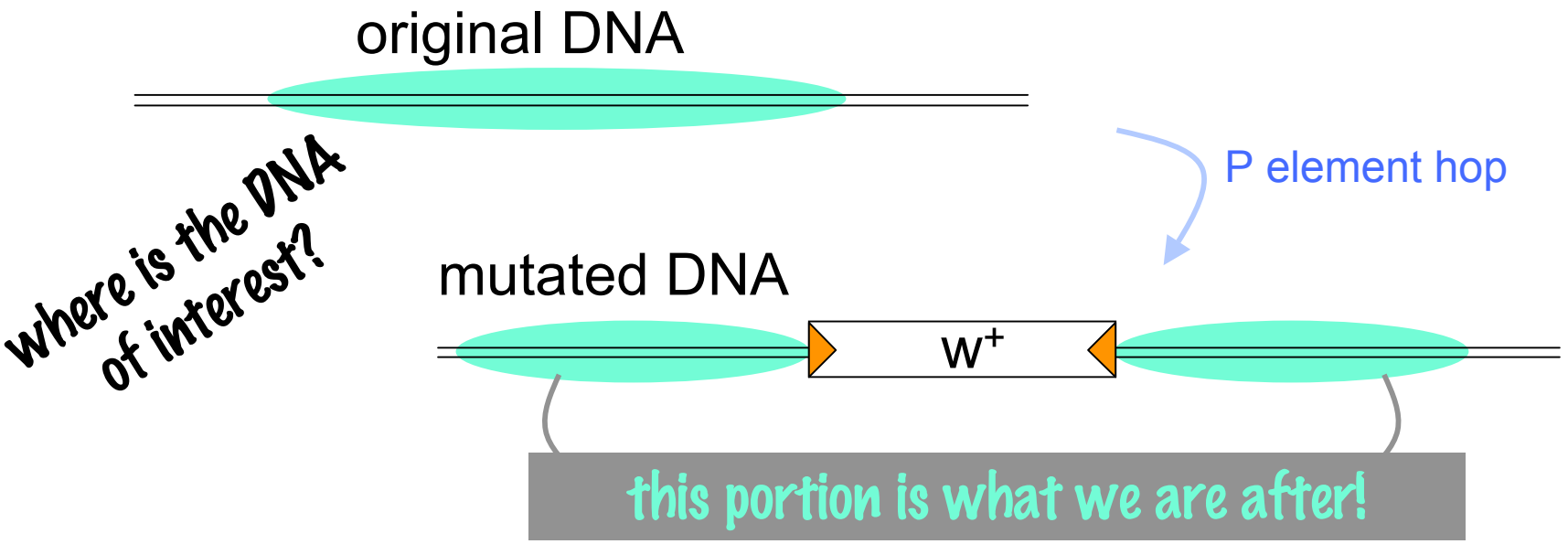
In the cross (= CROSS 3) you set up this week... what progeny phenotype would you want to pick for further analysis? Why?

white-eyed female x red-eye, curly-wing male

...because those flies have a hopped transposon

...and therefore may have an interesting mutation where the transposon landed

“Big picture” reality check (cont’d)



- Curly, TNase, Ubx, etc. let us deliver the mutagenic “blow” and identify when the mutagenic event has happened
- P[w+] causes the mutation; the w+ portion lets us track where the mutation is (which fly has it)

Where did the P-element land when it hopped?

In the quiz section:

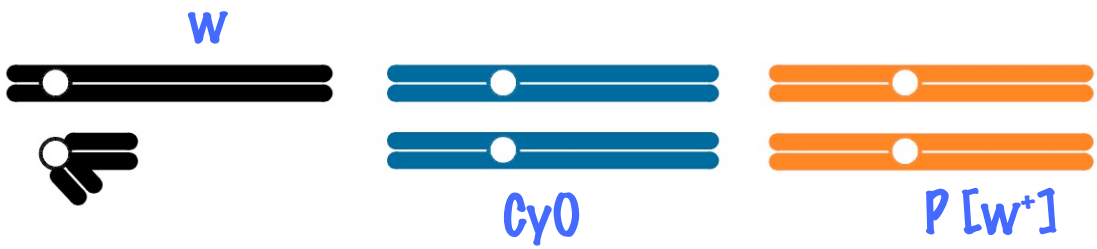
You picked a mutant with a hop…

which chromosome had the P element hopped into?

Suppose your mutant had a hop into chromosome III

Mate these again to white-eyed females... CROSS 3

$$\frac{X^w}{X^w};\frac{+}{+};\frac{+}{+} \quad x$$



pick red-eye progeny (male or female)

Genotype?

$$\frac{X^w}{X^w \text{ or } Y};\frac{+}{+};\frac{+}{P[w^+]}$$

…and suppose they don't show any abnormal phenotype!

Where's the phenotype?

Genotype—same as previous slide

$$\frac{X^{w}}{X^{w}\text{ or }Y}\;;\;\frac{+}{+}\;;\;\frac{+}{P[w^{+}]}$$

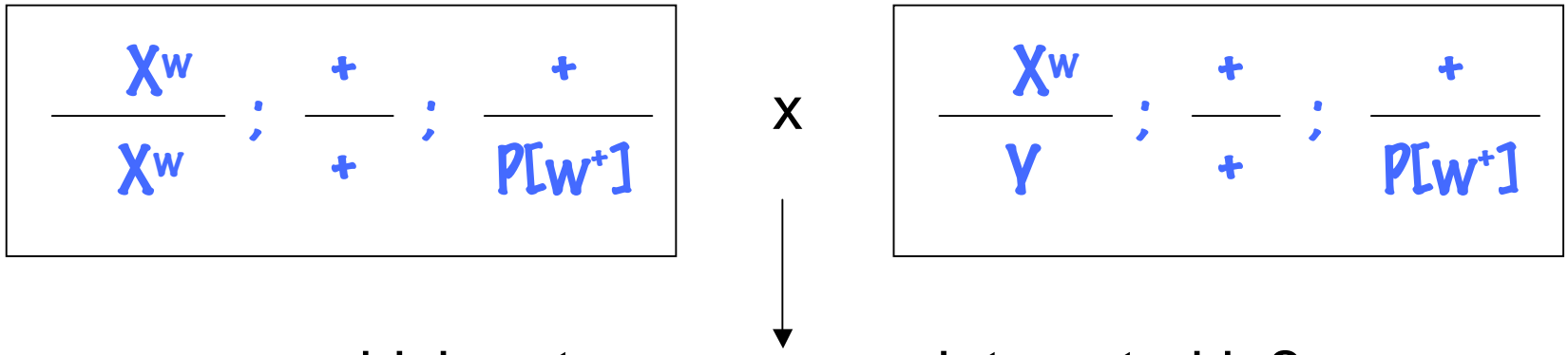
We *know* it has a "hopped" transposon…

so why might it not show any abnormal phenotype?

1. Insertion site
   - between genes/in introns → may have no phenotypes
   - near promoters → may have weak phenotypes
2. Function of the mutated gene not revealed in lab conditions
3. Most mutations are recessive, LOF!

We've got to make a homozygote of the mutation!

Looking for a phenotype



which outcome are we interested in?

Looking for a phenotype (cont'd)

The problem:

How do we identify homozygotes? What would they look like?

Making the mutant homozygous

The problem:

How do we identify homozygotes? What would they look like?

Can we say for sure that

$$\frac{X^w}{X^w \text{ or } Y} ; \frac{+}{+} ; \frac{+}{P[w^+]}$$

red eyes

will look any different from

$$\frac{X^w}{X^w \text{ or } Y} ; \frac{+}{+} ; \frac{P[w^+]}{P[w^+]}$$

also red eyes!

Making the mutant homozygous

Drosophila geneticists have established strains with special chromosomes that allow the construction of homozygous mutants

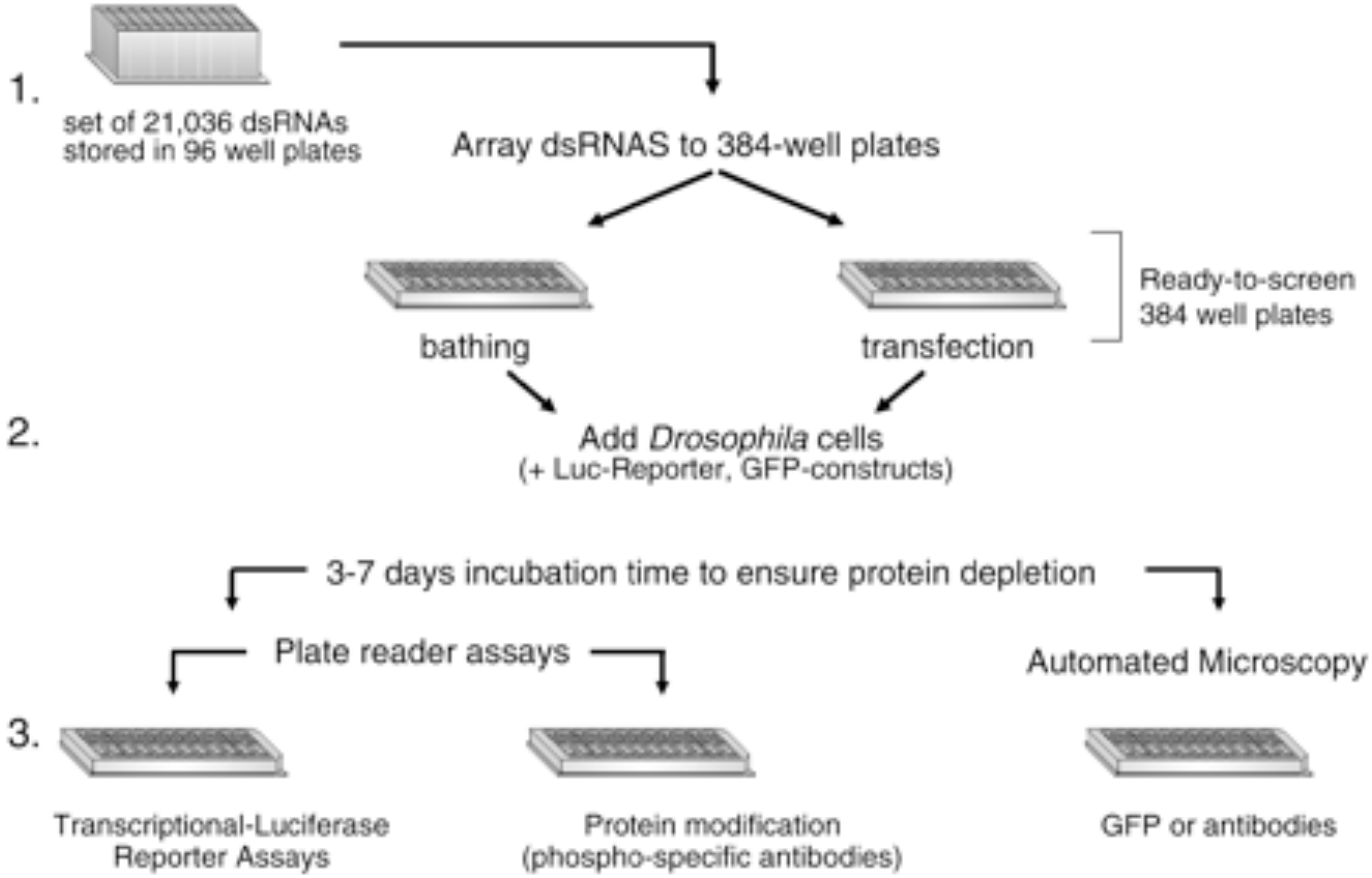
You need to know the chromosome that the mutation is on in order to use the correct strain

Beyond the Basics

A simpler mutagenesis strategy?

RNA interference (RNAi) uses double-stranded RNA to silence a gene by specific destruction of its mRNA

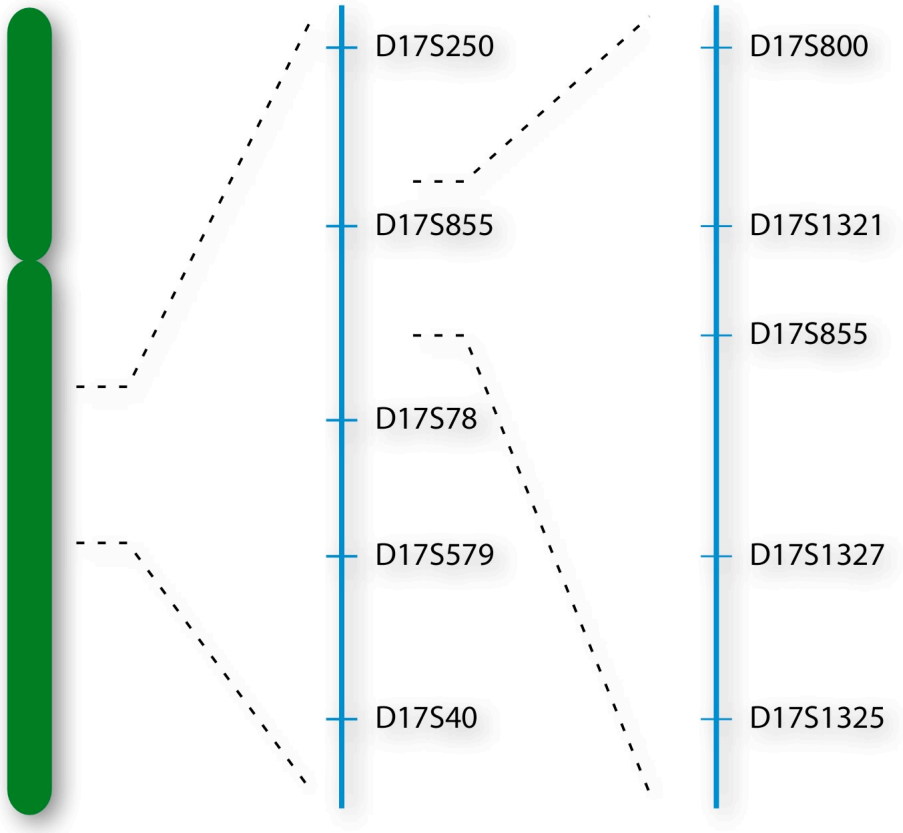
A genomewide collection of RNAi reagents (>20,000) exists that targets each predicted gene in Drosophila



Genetic analysis of cancer

Analysis of gene function

Case study—BRCA1



Cancer summary

Cancer: second-leading cause of death in US

Breast cancer: leading form of cancer among women

~ 1 million cases each year worldwide

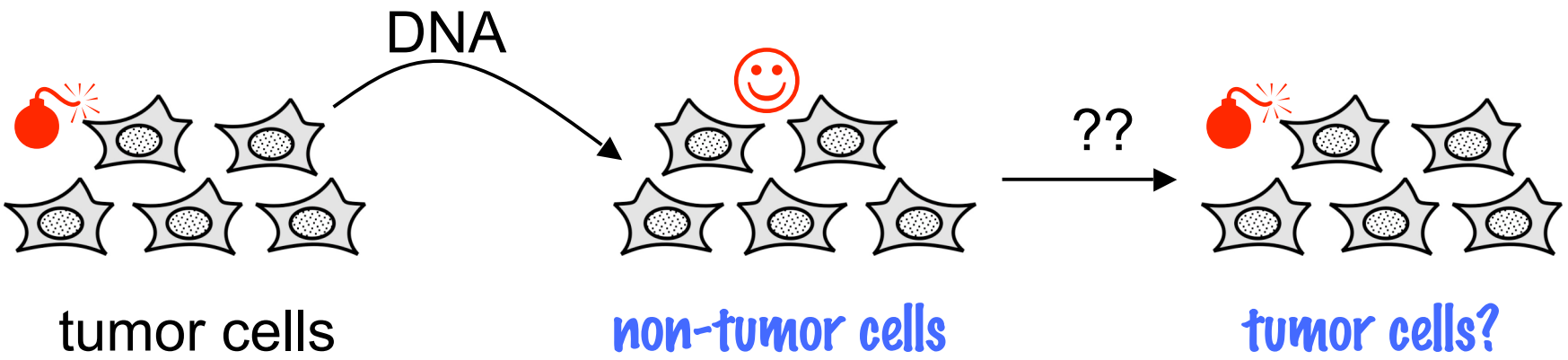
~ 5% of cases due to inherited susceptibility

Properties of cancer cells

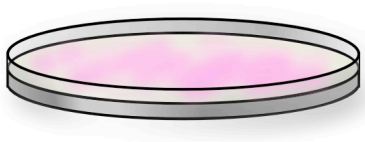
Properties: Proliferation and metastasis

Demonstration of cancer as a genetic disorder… early 1980s

Experiment: Can DNA from cancer cells transform non-cancer cells?



Demonstrating the genetic basis of cancer



Normal mouse cells:
monolayer in culture

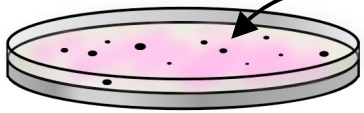
"contact inhibition"

DNA from human bladder cancer

cell foci

loss of contact inhibition!

Demonstrating the genetic basis of cancer



cell foci

injected into mice → tumors

compare transforming DNA with normal DNA…

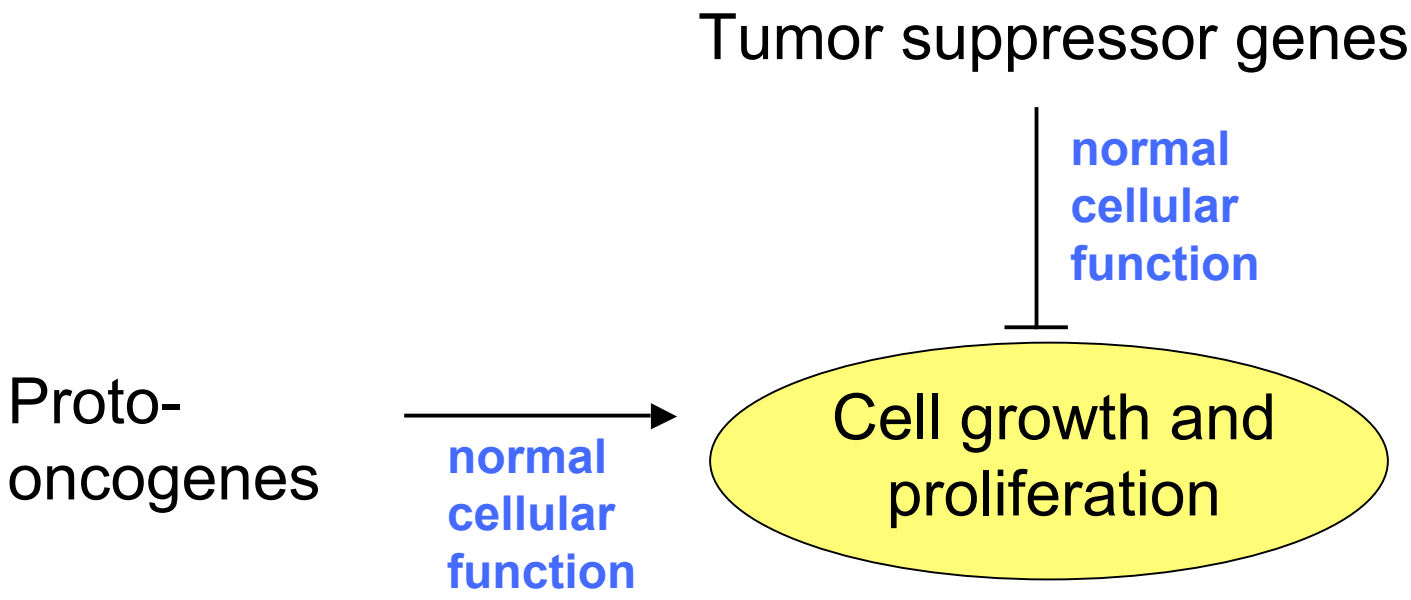
a single base change (G → T)
glycine → valine

The cancer-causing version: an oncogene

The original (normal) version: a “proto-oncogene”

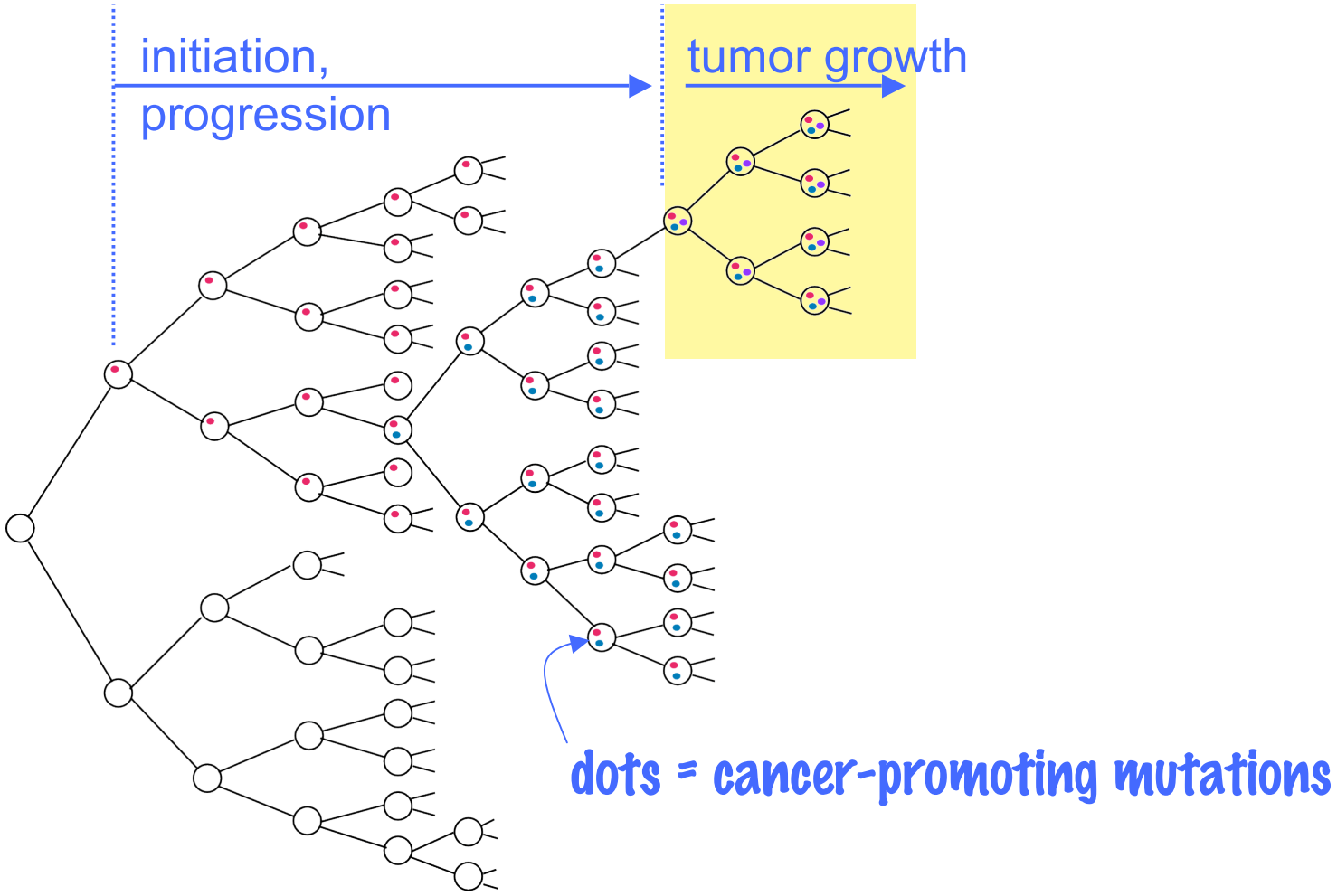
in this example: *c-ras* proto-oncogene

Two types of cancer-related genes



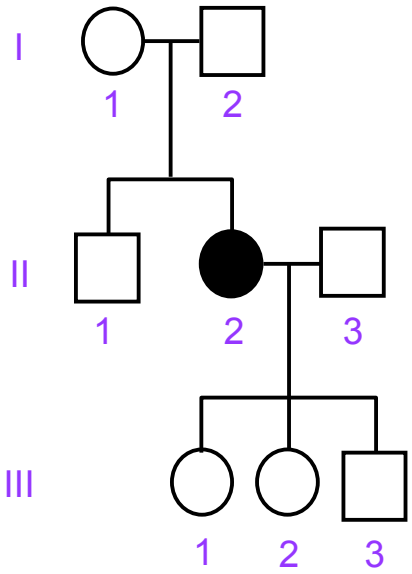
Interpreting the transformation results

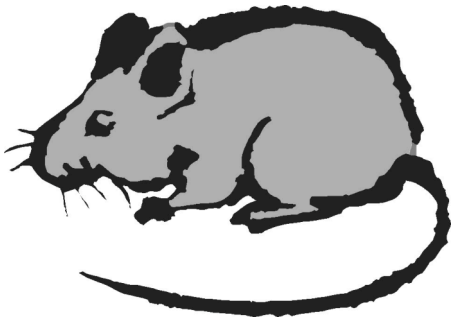
Does it take only a single base change to cause cancer?
…No. From kinetics of transformation: "multiple-hit" model



Quiz section next week

BRCA1 mutations: responsible for familial breast and ovarian cancer

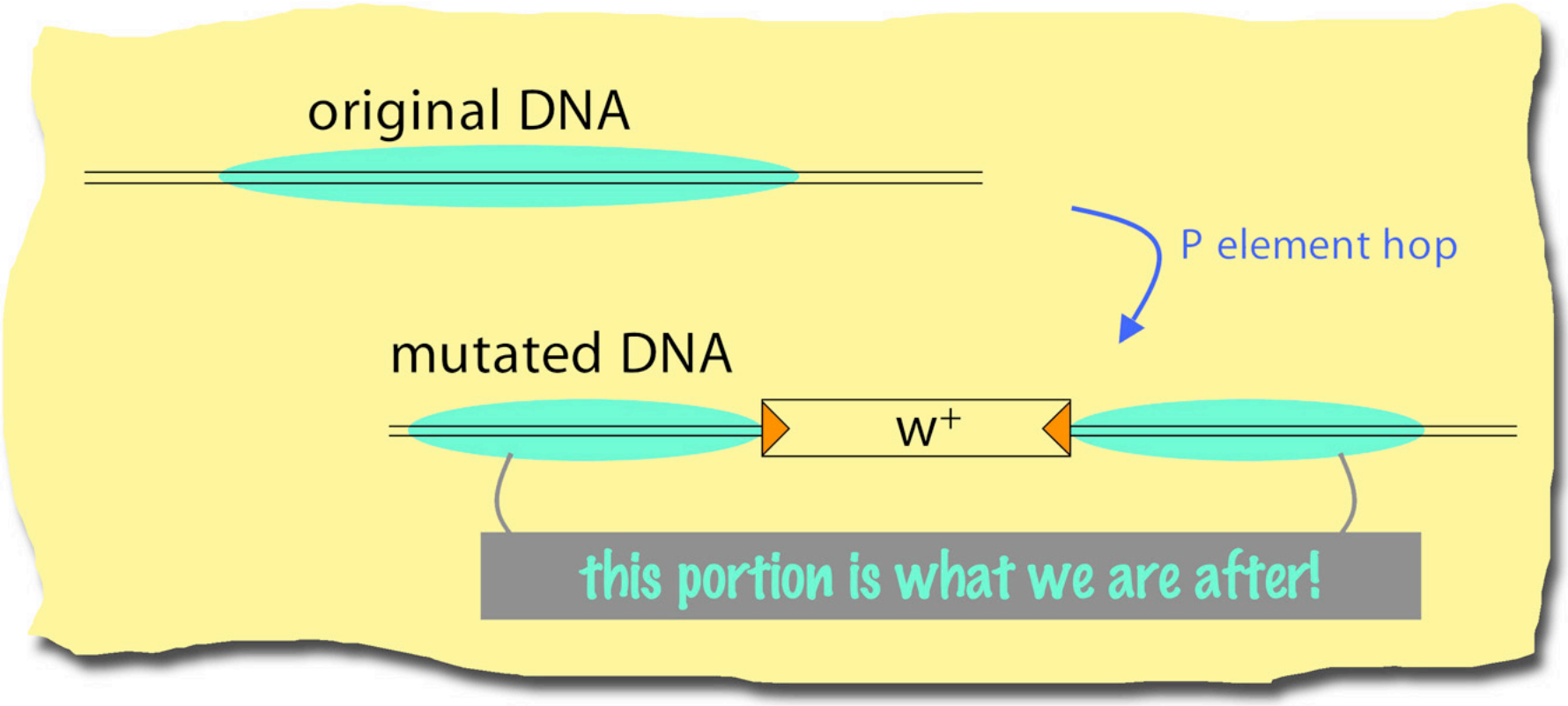




Distinguish oncogenes vs. tumor suppressor genes

Use pedigree, polymorphism, mutation and mouse model data to understand how inheritance of BRCA1 mutations and how they lead to cancer

“Big picture” reminder



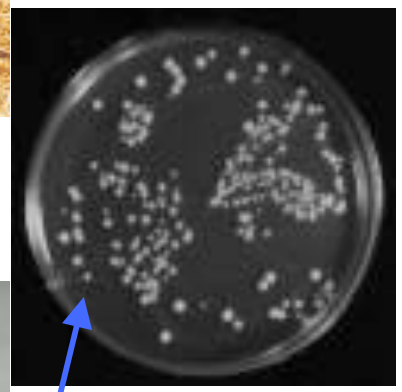
Coming up… cloning the DNA of interest

DNA Cloning

Clone: cells or organisms that are genetically identical because they are related by non-sexual reproduction—i.e., by mitosis not meiosis.

DNA clone: identical copies of a DNA fragment, usually generated by propagation in bacteria

Dolly



yeast

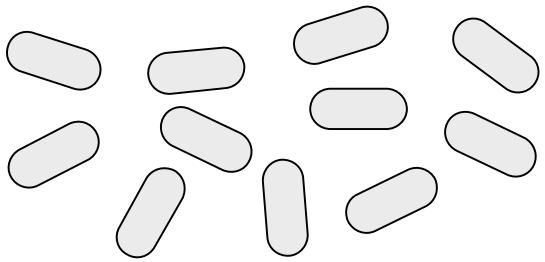
Copy Cat

Each colony is >10^6 identical cells.

DNA cloning—making libraries

We want to:

» maintain the genome of interest as separate pieces in bacteria…

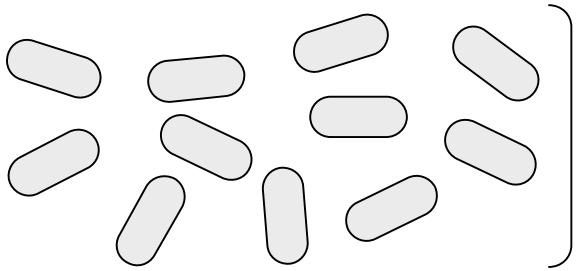


» …while preserving some information on how those pieces fit together

DNA cloning—Propagating DNA in bacteria

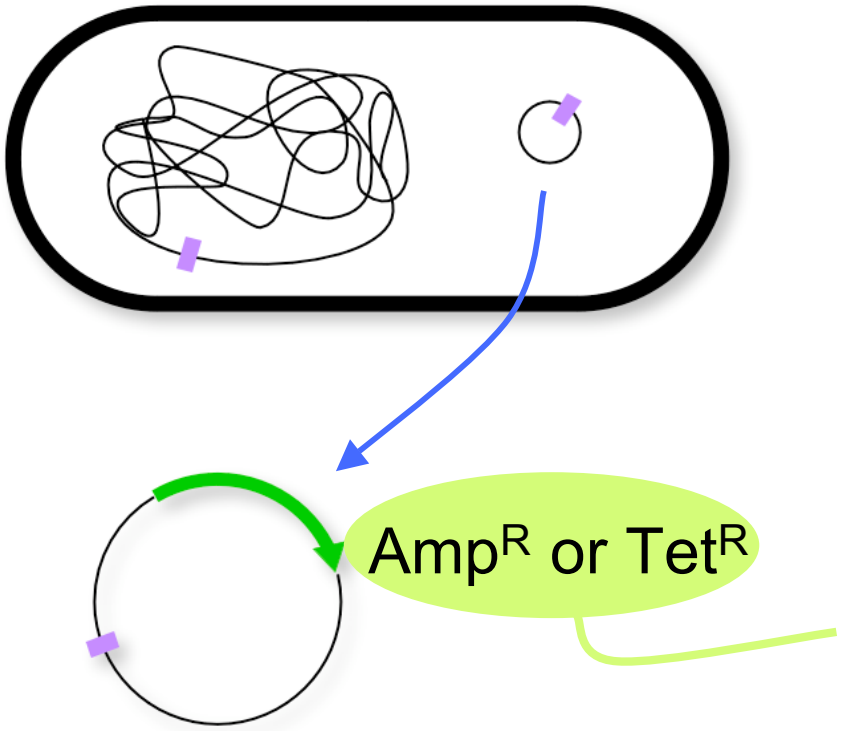
Problems to solve

» How can the new DNA survive and replicate?
» How can we “select” for bacteria with the new DNA?



Which bacterial cells have “our” DNA and which ones don’t?

Plasmids as vectors for transformation



Non-essential DNA molecules that can replicate independently of the chromosome

because they have an origin of replication

and a gene that confers resistance to a drug such as ampicillin or tetracycline.

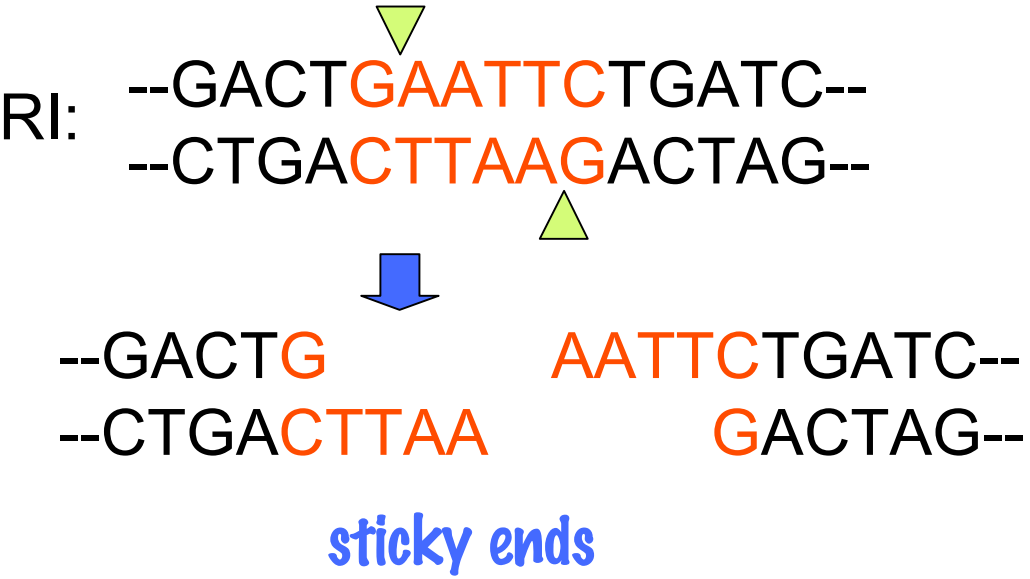
For cloning: "recombine" the foreign DNA into a plasmid.

Solves both problems for the transforming DNA—
maintenance and selection.

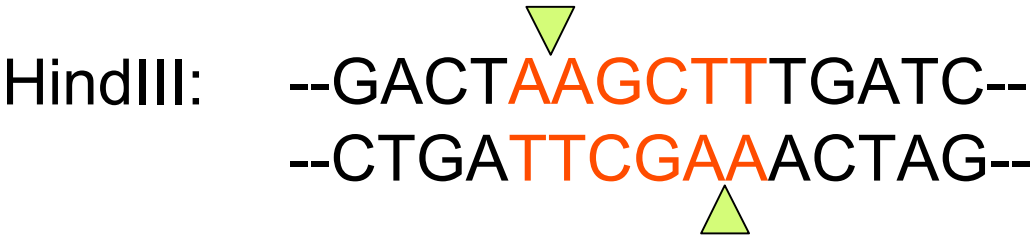
Restriction endonucleases

Recognize and cut specific sequences in DNA

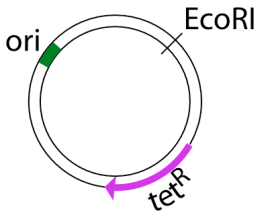
Restriction enzyme EcoRI:
(for example)



HindIII:

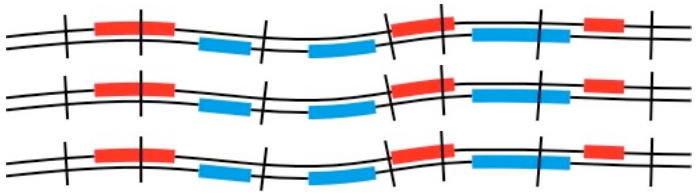


Making recombinant DNA



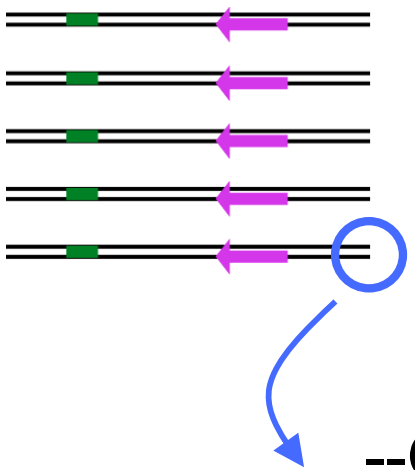
(Watson & Crick strands shown)

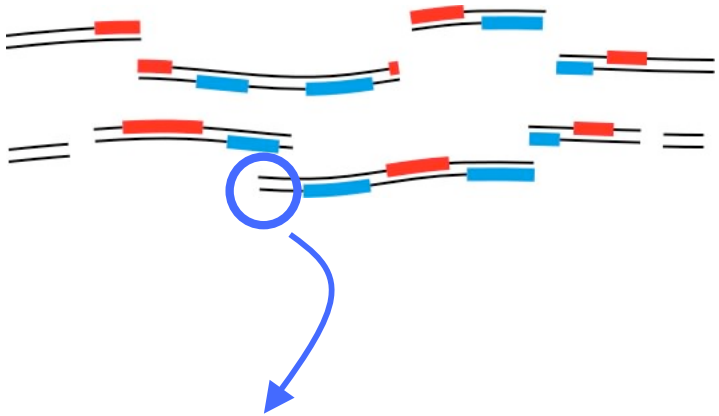
"foreign" genome which we want to clone in a library



cut with EcoRI

partial digest with EcoRI





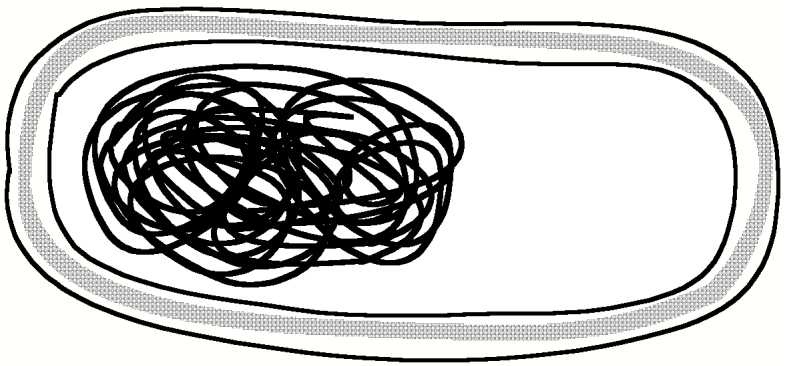
--GACTG
--CTGACTTAA

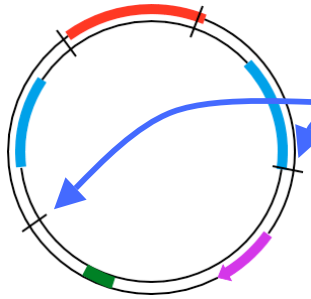
AATTCTGATC--
GACTAG--

sticky ends

Making a DNA library

Tetracycline-sensitive E. coli

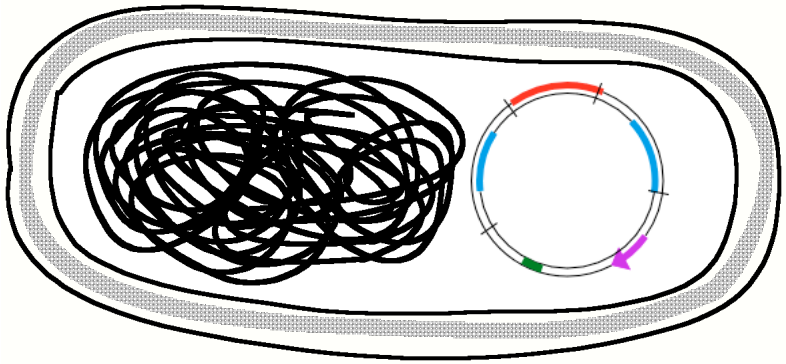




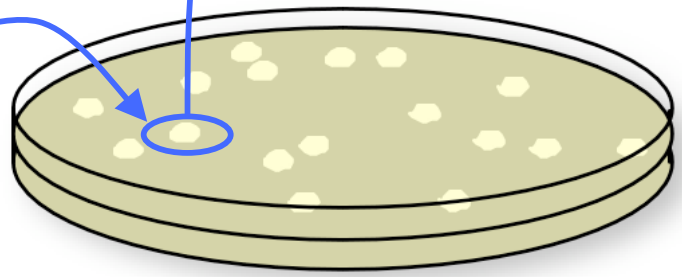
DNA ligase to repair phosphodiester backbone

transformation

($CaCl_2$, heat shock)



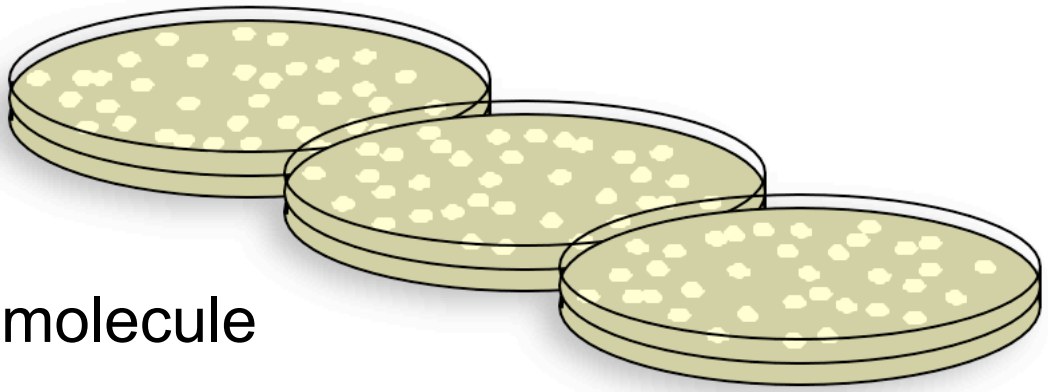
This is a clone



tetracycline plate

A DNA library

Each clone may have
a different piece of the
"foreign" genome in it in the
form of a recombinant DNA molecule



"Genomic DNA library"—

- start with genomic DNA
- expect all the chromosomal DNA to be represented
- about equal representation of all sequences

Practice question: Working with genomic DNA libraries

Yeast genome ≈ 12,000 kb

If average insert size is 12 kb, would need

_____

colonies to get 1x coverage (i.e., each sequence in the genome represented on average once in the library)?

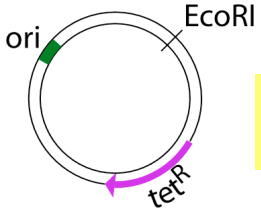
For 10x coverage would need _____ colonies.

At 200 colonies per plate, would need _____ plates.

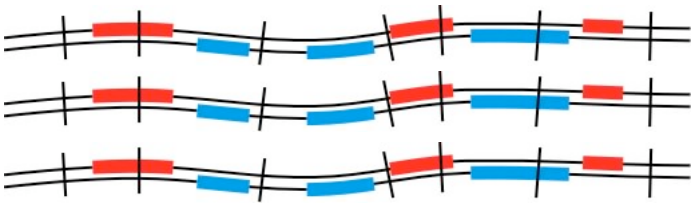
Exercise: Suggest a reason why some fragments may not be represented in the library even at 10x coverage.

Does the plasmid really have an insert?

With the original vector…

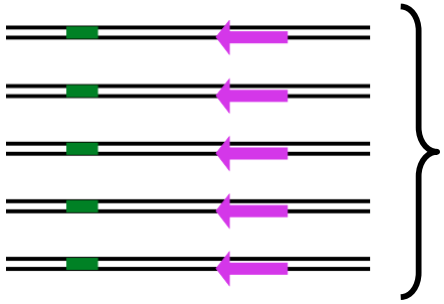


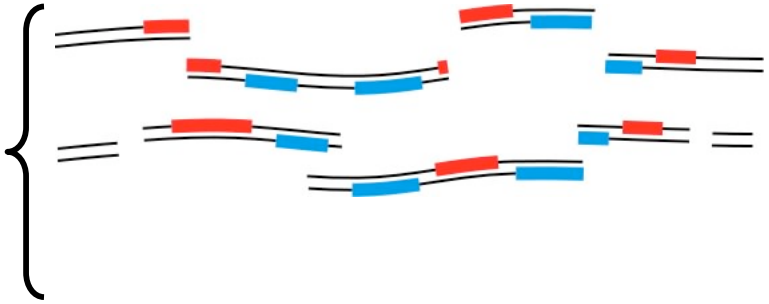
vector



cut with EcoRI

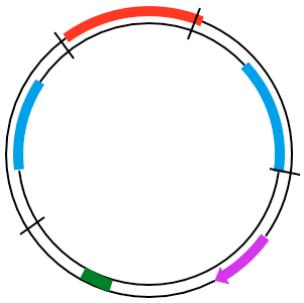
partial digest with EcoRI



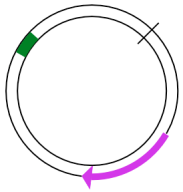


ligate

vector with insert



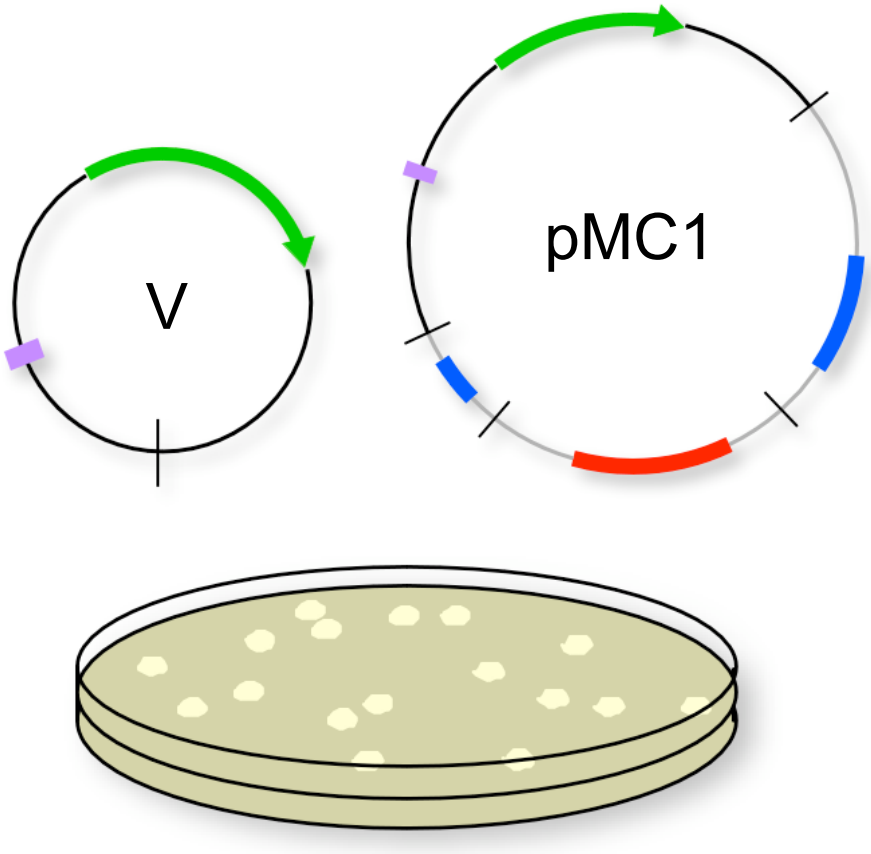
and



re-closed vector (no insert)

Does the plasmid really have an insert?

Purify plasmid DNA and check its size and number of EcoRI sites.



Predictions:

If the plasmid does NOT have an insert…

one EcoRI fragment the size of the vector

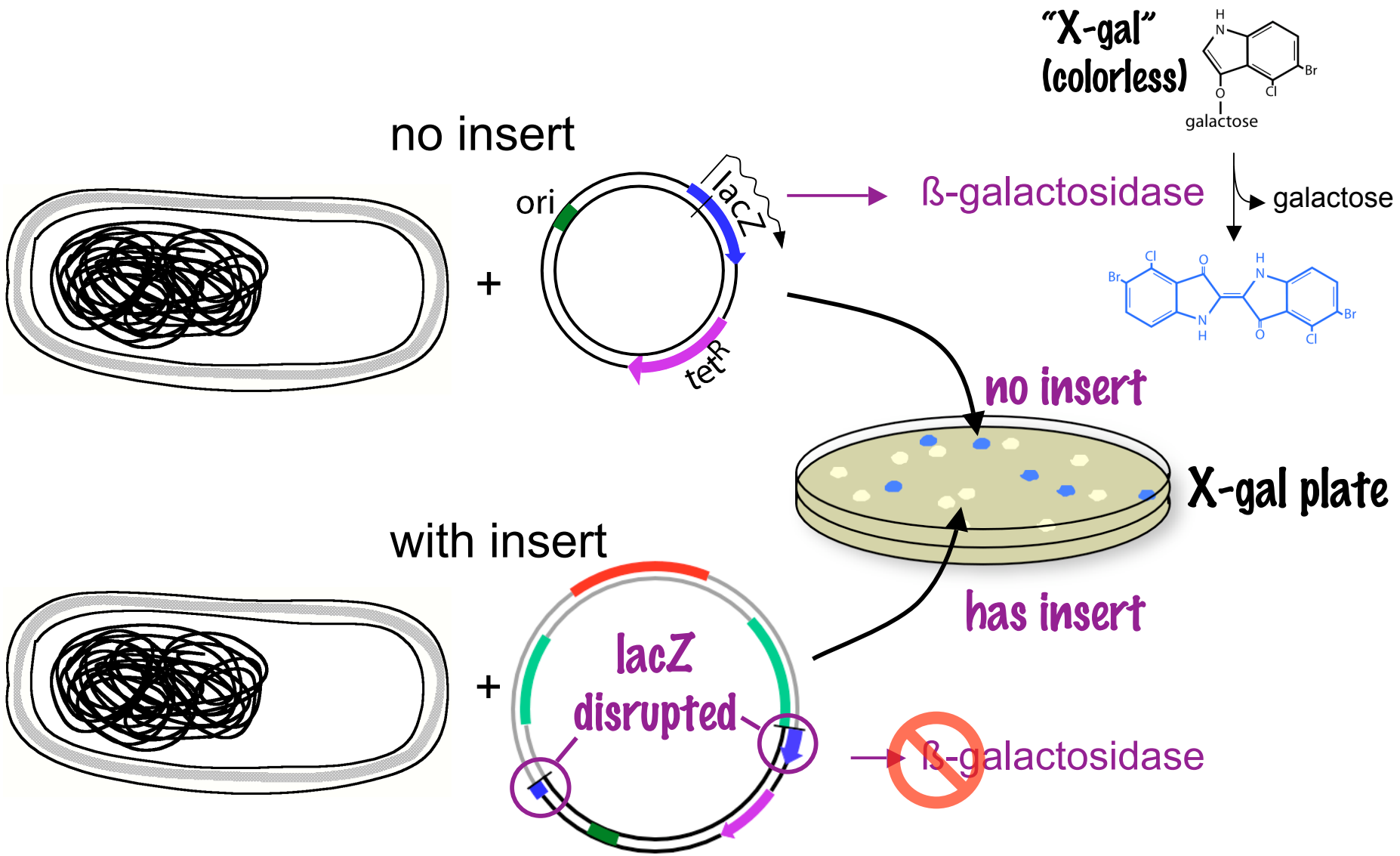
If the plasmid DOES have an insert…

more than one EcoRI fragment

total size > size of the vector

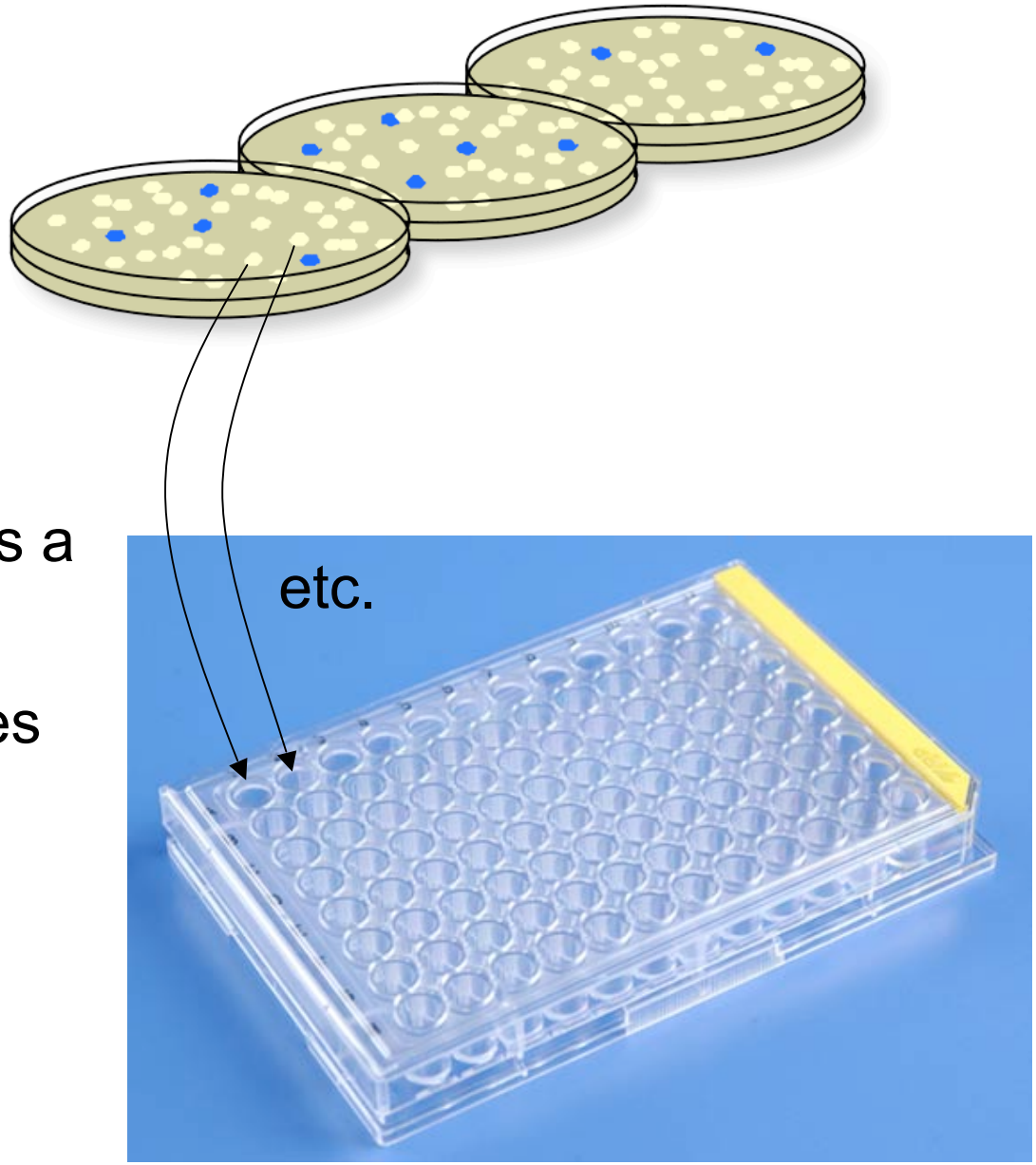
Isn't there an easier (genetic) way to determine whether a given clone has an insert or not?

lacZ disruption screen for successful insertion



In a genomic DNA library made with this improved vector…

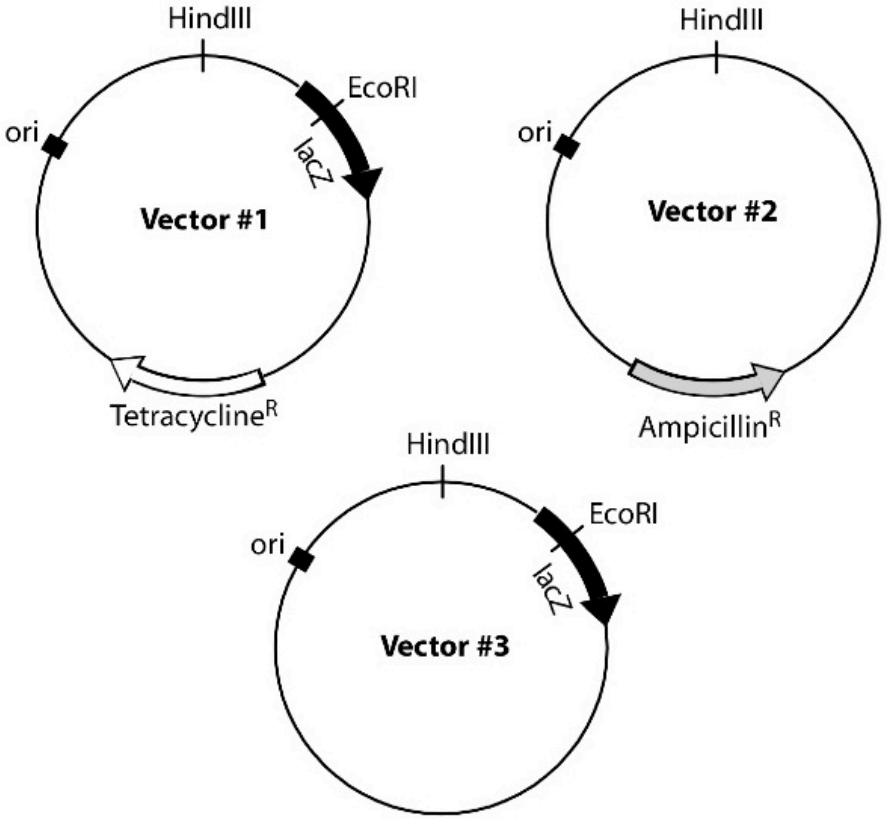
- pick the white colonies, freeze the cells away. This collection of cells is a genomic DNA library.
- discard the blue colonies



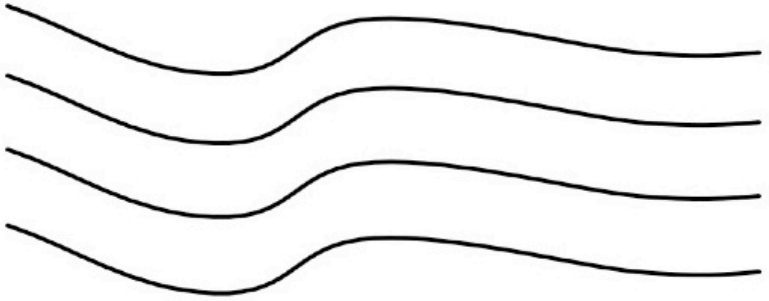
Practice question

Your goal is to construct a mouse genome DNA library, choosing appropriate elements from the reagents/material shown below. Note: you are just asked to do the best you can given these constraints.

Possible vectors:



Mouse genomic DNA (assume that it has lots of EcoRI as well as HindIII cut sites)



E. coli strains available:

- ampicillin- and tetracycline-sensitive, lacZ$^+$
- tetracycline-sensitive, lacZ$^-$

Plates available:

- Ampicillin
- Ampicillin + X-gal
- X-gal

Other reagents:

EcoRI, HindIII, DNA ligase, $CaCl_2$, appropriate reaction conditions