20.109 MOD1 – DNA ENGINEERING
Fall 2010

Measuring HR in mice, using HR in mouse genome engineering

Orsi Kiraly
Engelward lab

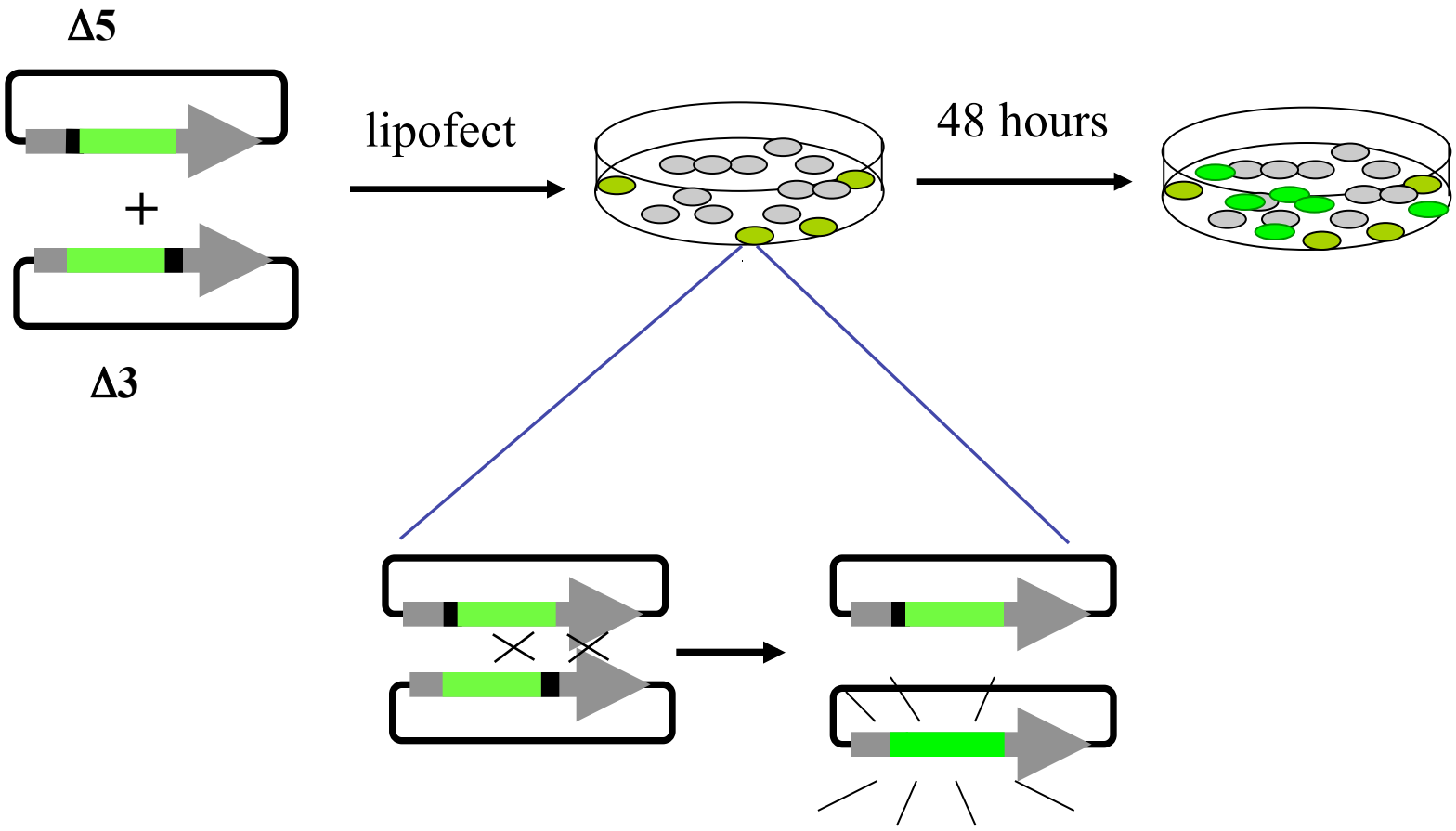
Going from Understanding to Solutions

-Exploiting Understanding of HR for genetic engineering

Mod1 Overview: Methods and Logic

-Strategy for analysis

A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells

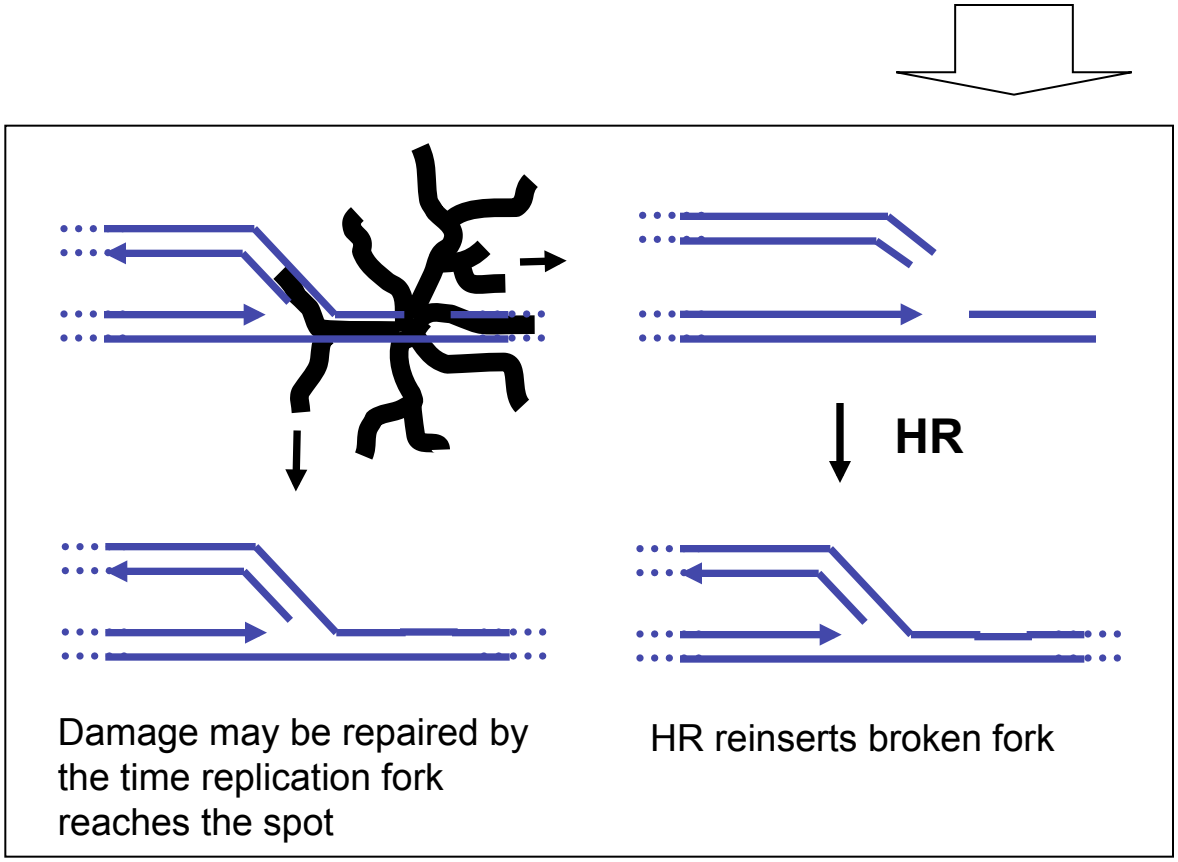


What is the role of poly(ADP-ribosylation)?

Inhibit it, see what happens:

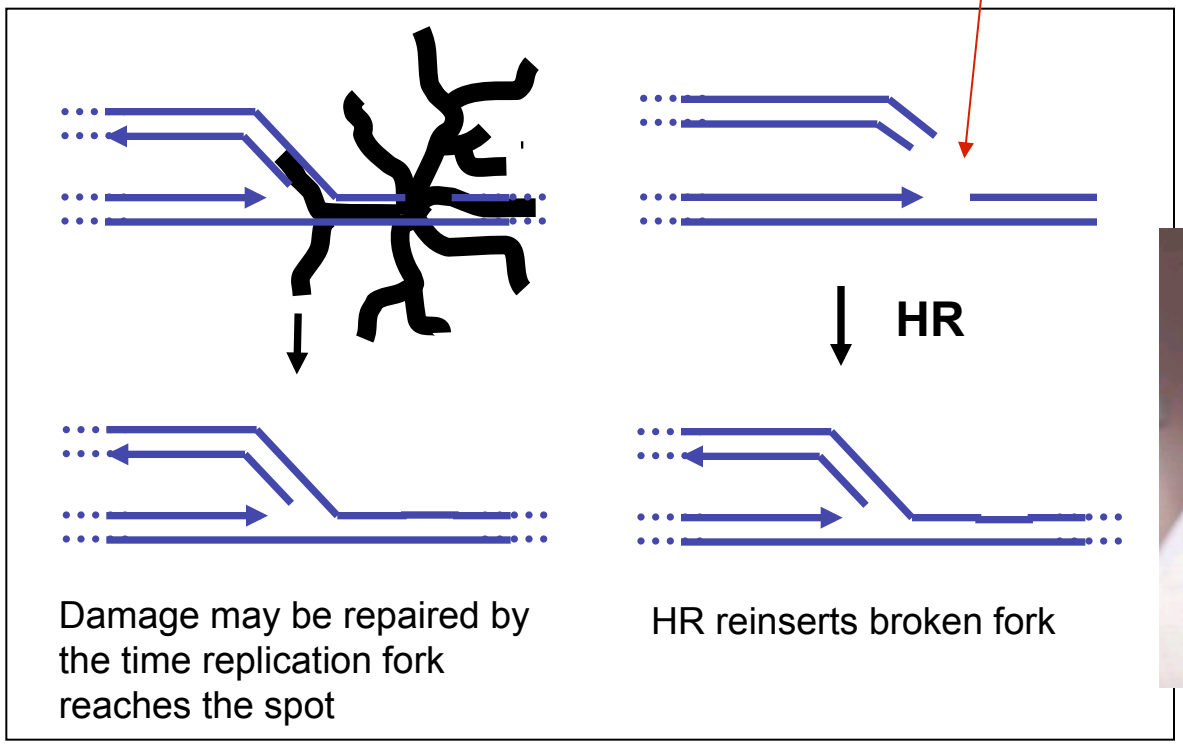
More HR

More single strand breaks



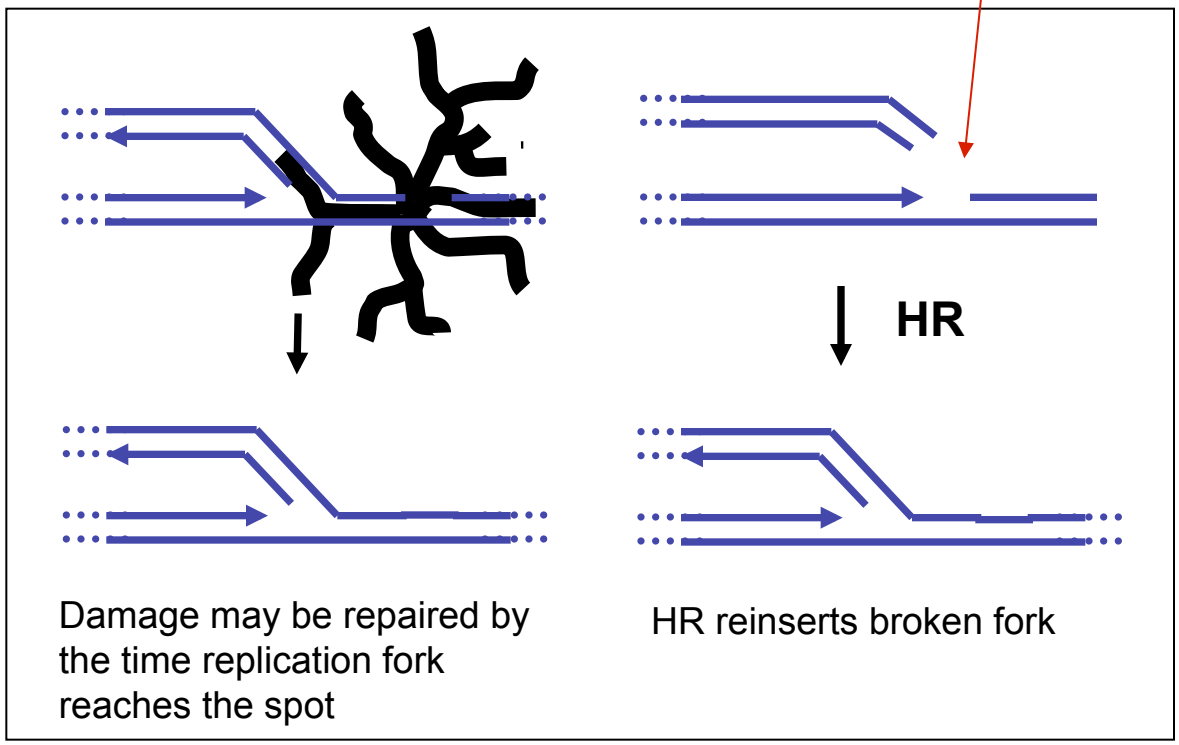
DNA damage can block replication fork progression

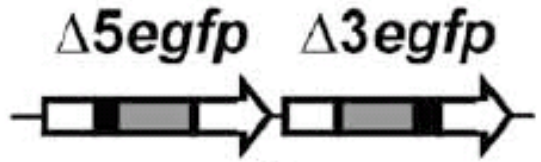
- Single strand break
- Bulky adduct
- Interstrand crosslink

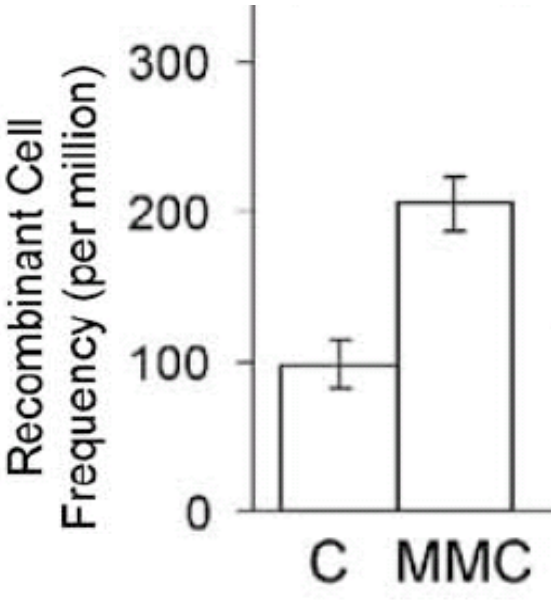


DNA damage can block replication fork progression and induce HR in cells

- **Interstrand crosslink**





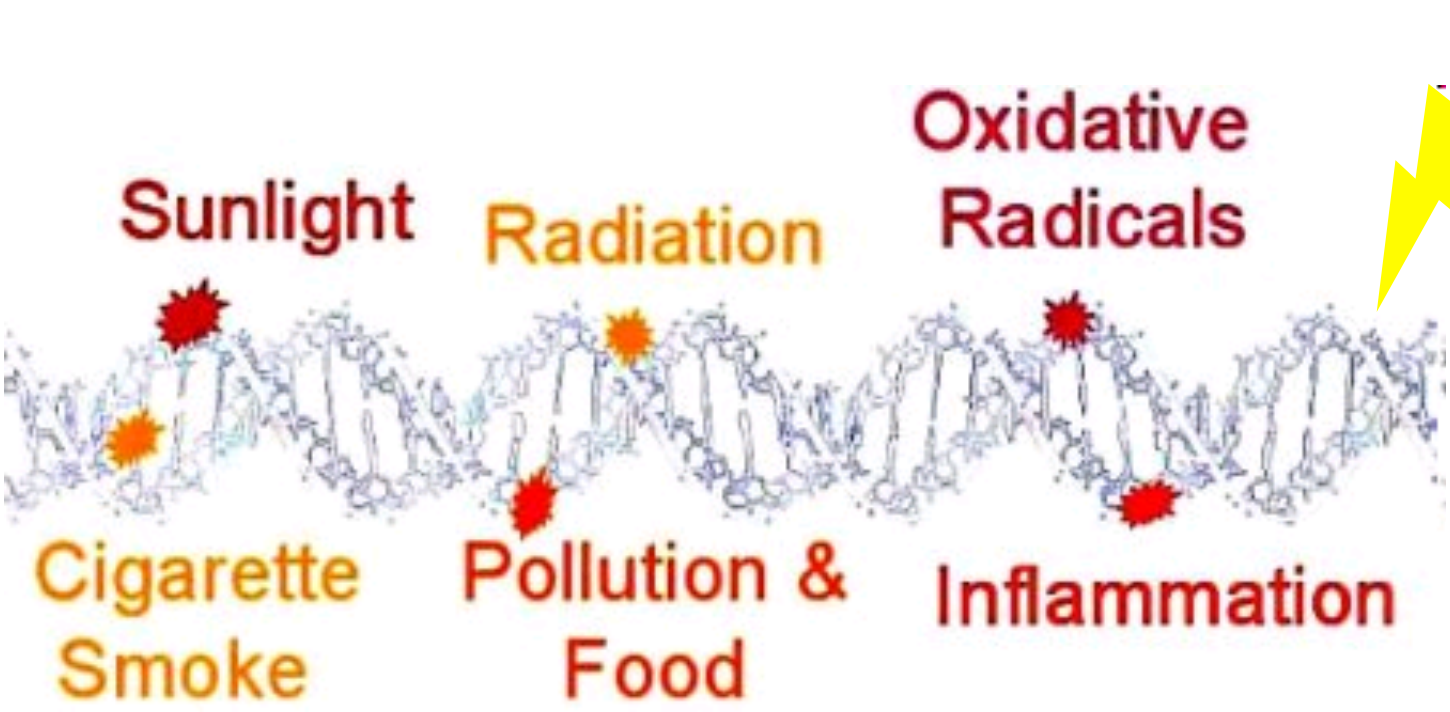


Jonnalagadda 2005

DNA damage comes from many sources

How do we know if these chemicals are safe?

83,000 new chemicals since WWII
+ 2000 new chemicals added each year



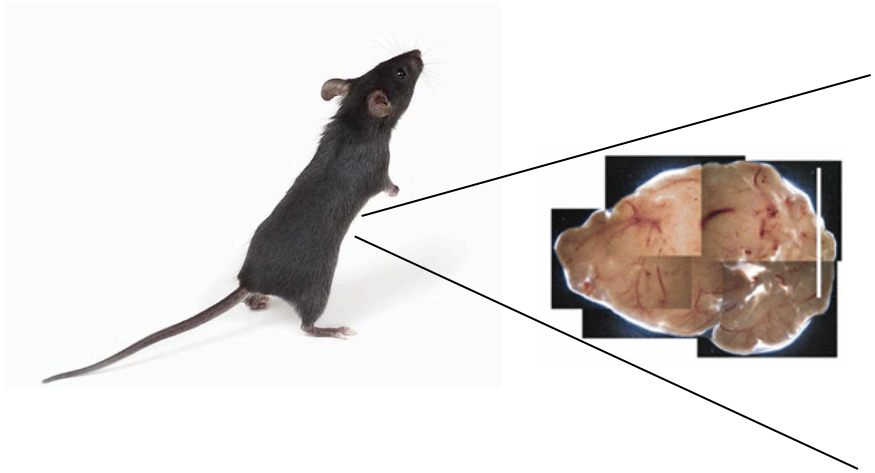
Cancer test in mice

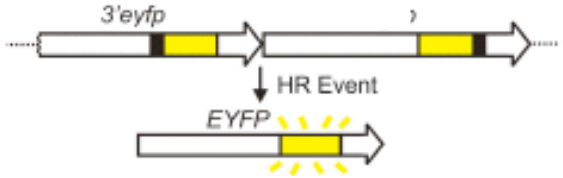
x50 ≤ x50

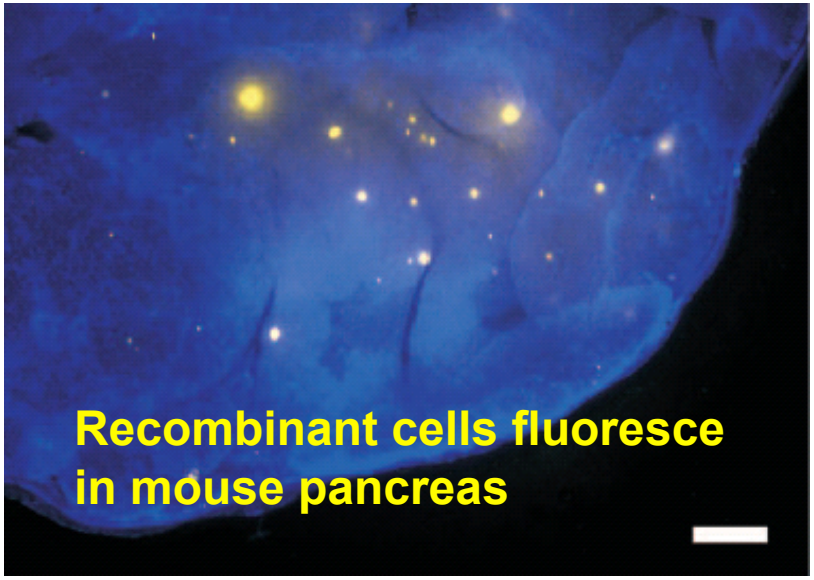
100 mice x 2000 new compounds = 200,000 mice per year !!

FYDR mice are engineered to detect HR

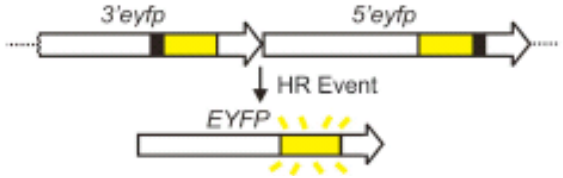
FYDR mouse



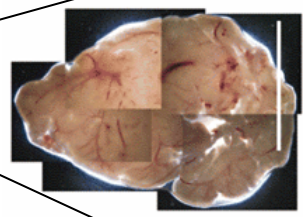




FYDR mice are engineered to detect HR

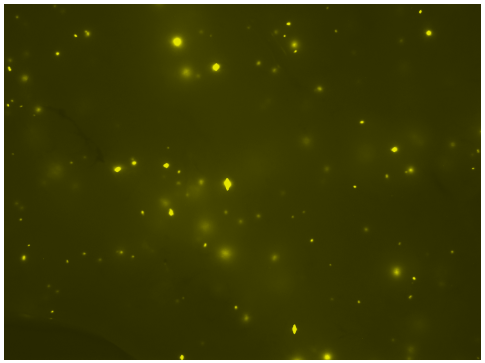


FYDR mouse



control

alkylating agent



Genetic Engineering in Mice:

1) Gene Targeting

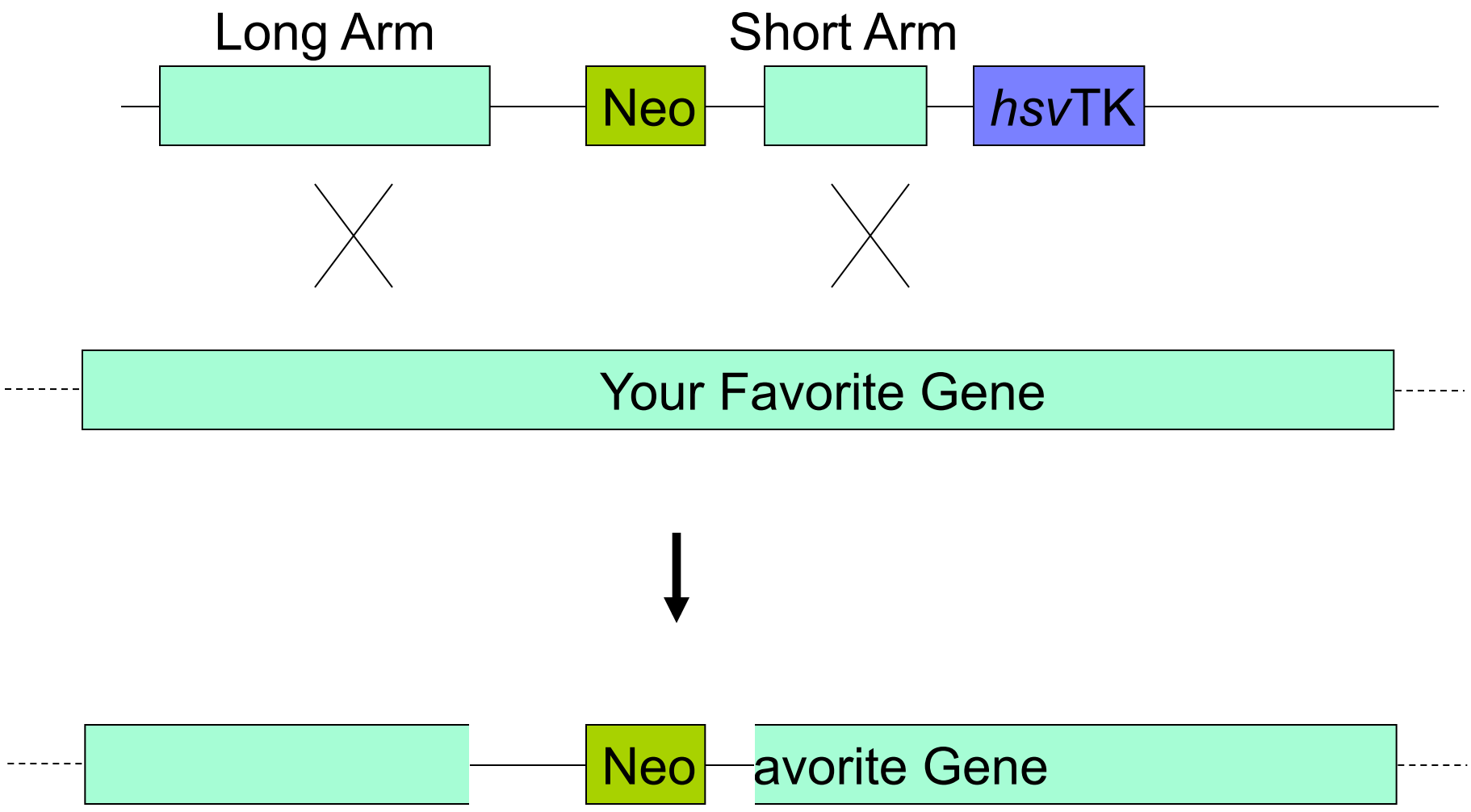
    -Turning genes on and off

2) Transgenics

    -Inserting genes

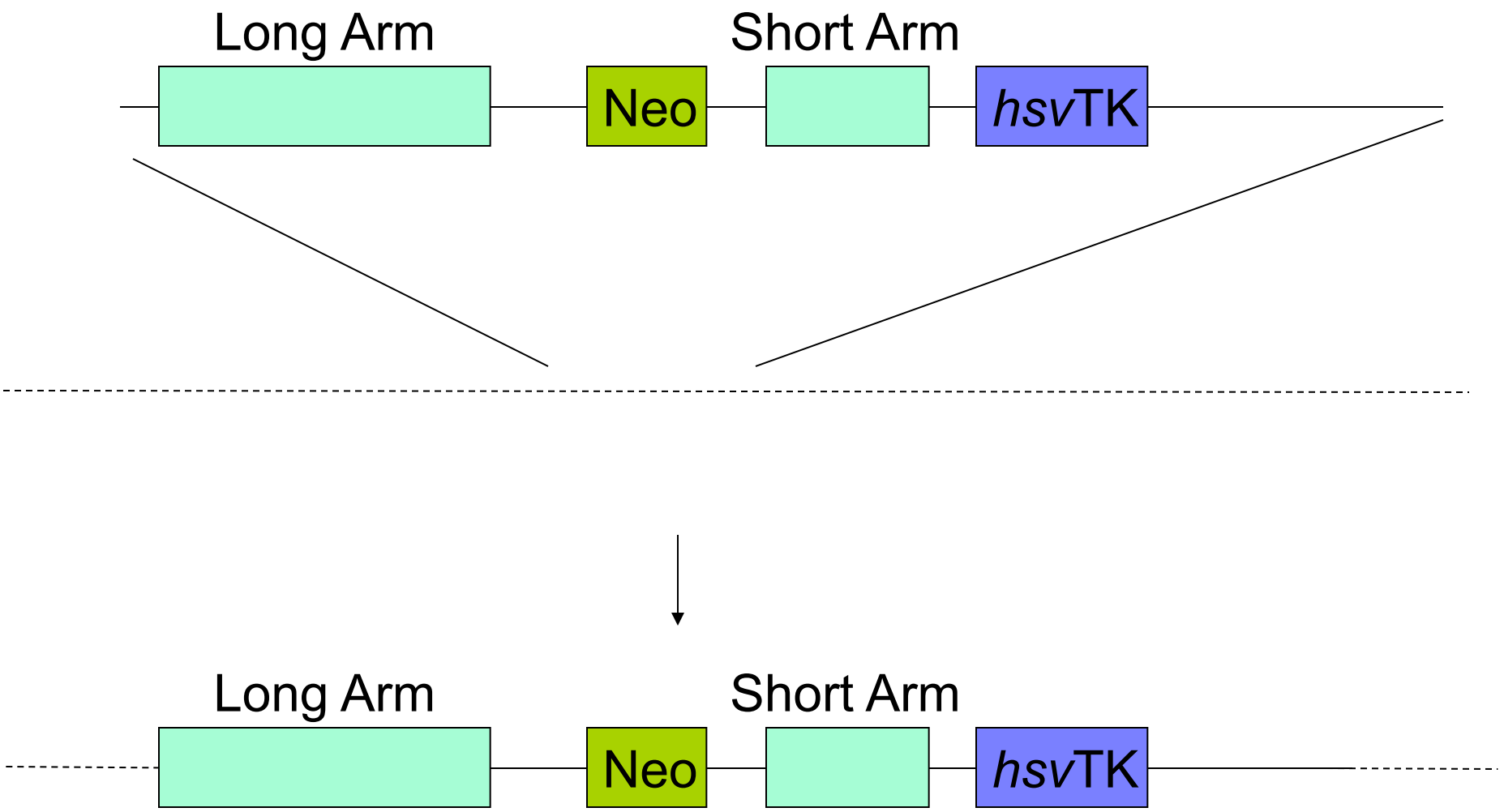
Traditional Knock-Out Technology

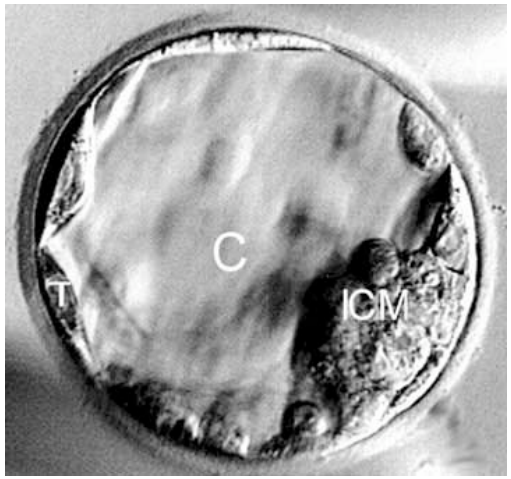
Targeted Homologous Recombination

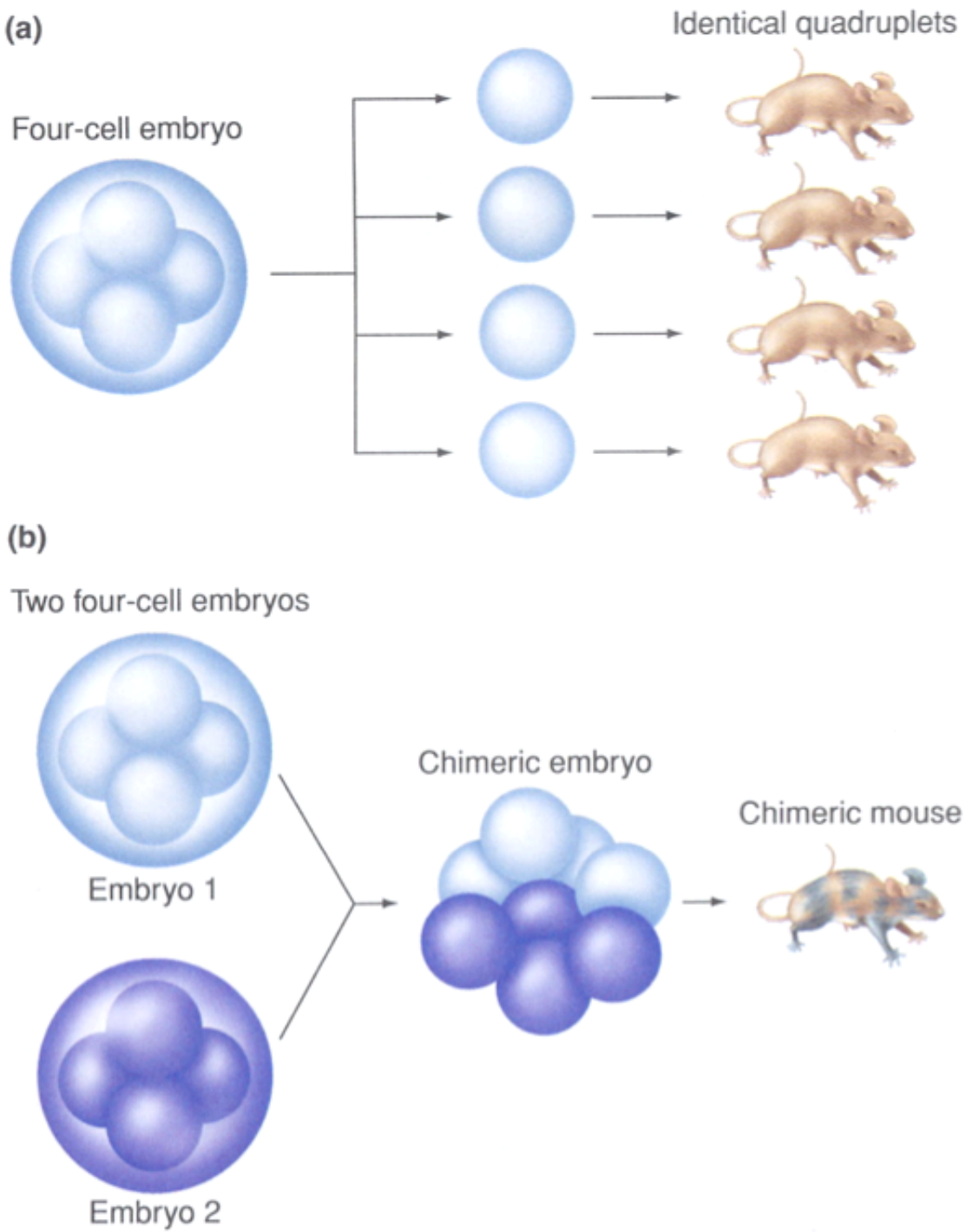


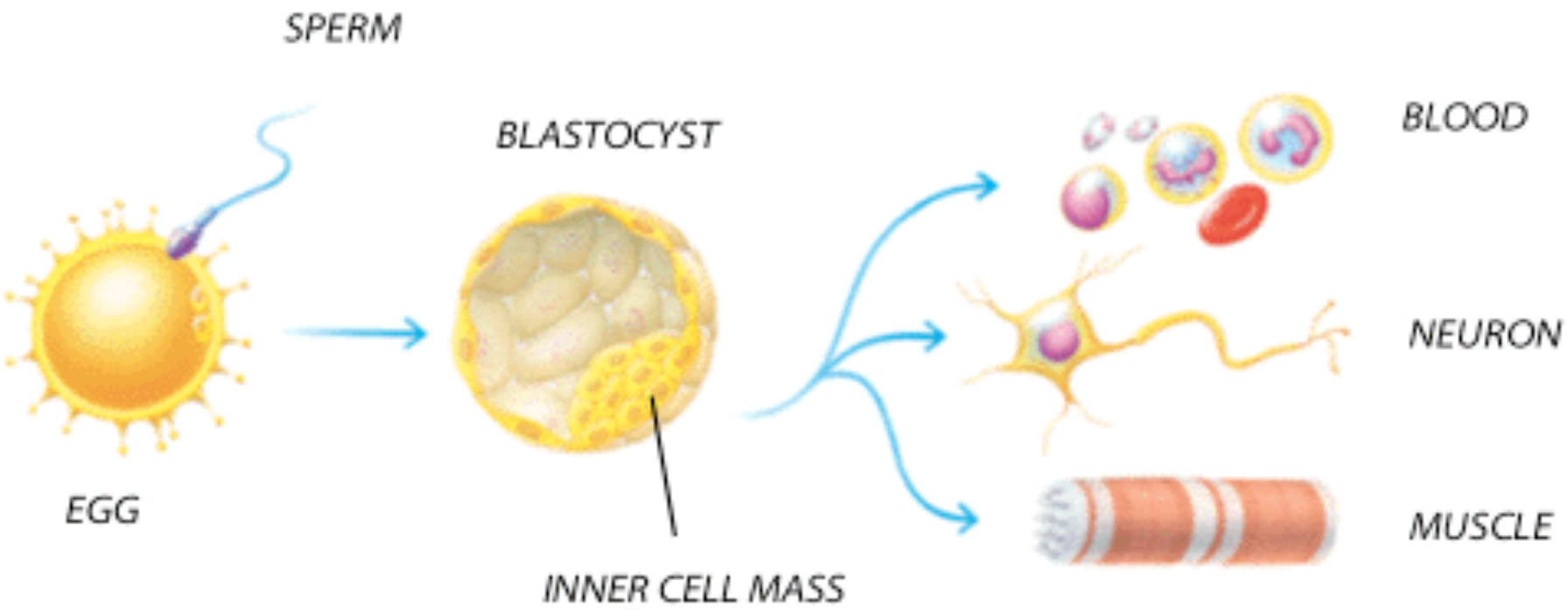
Traditional Knock-Out Technology

Random Integration

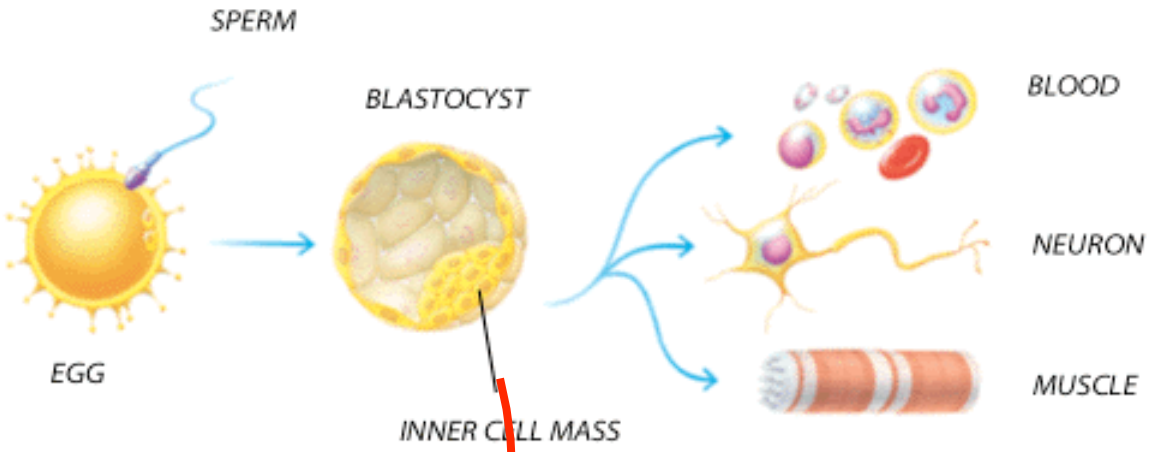


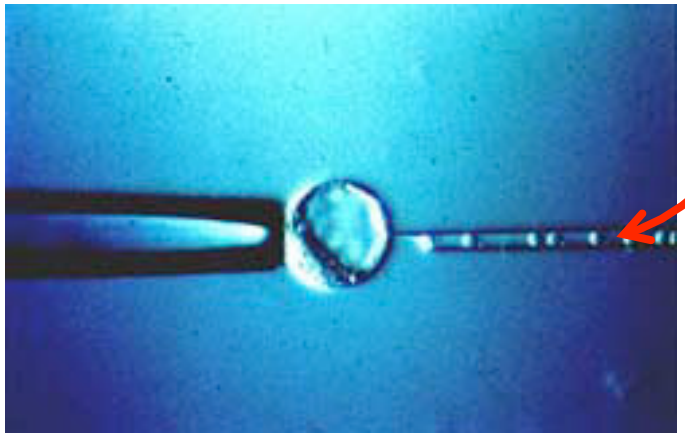

C
T
ICM


(a)
Identical quadruplets
Four-cell embryo
(b)
Two four-cell embryos
Chimeric embryo
Chimeric mouse
Embryo 1
Embryo 2

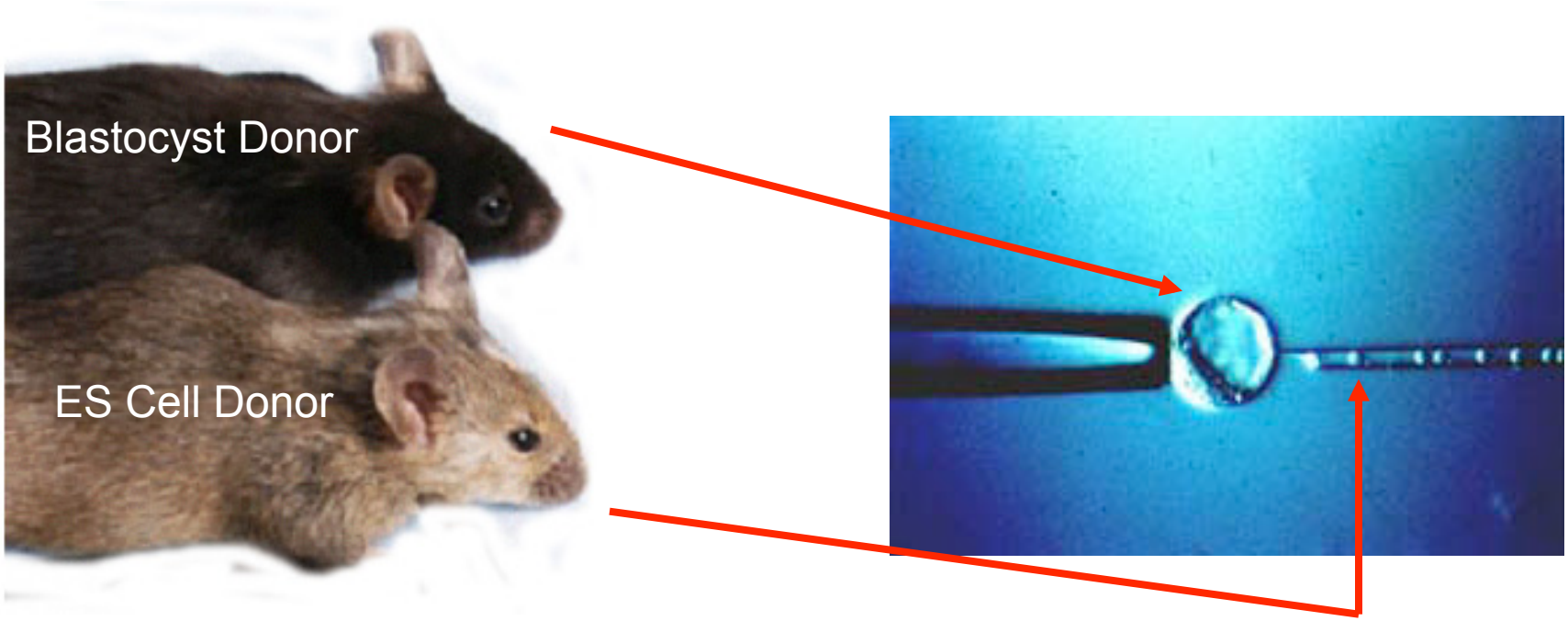

SPERM
BLASTOCYST
BLOOD
NEURON
MUSCLE
EGG
INNER CELL MASS

Traditional ES Knock-Out Technology

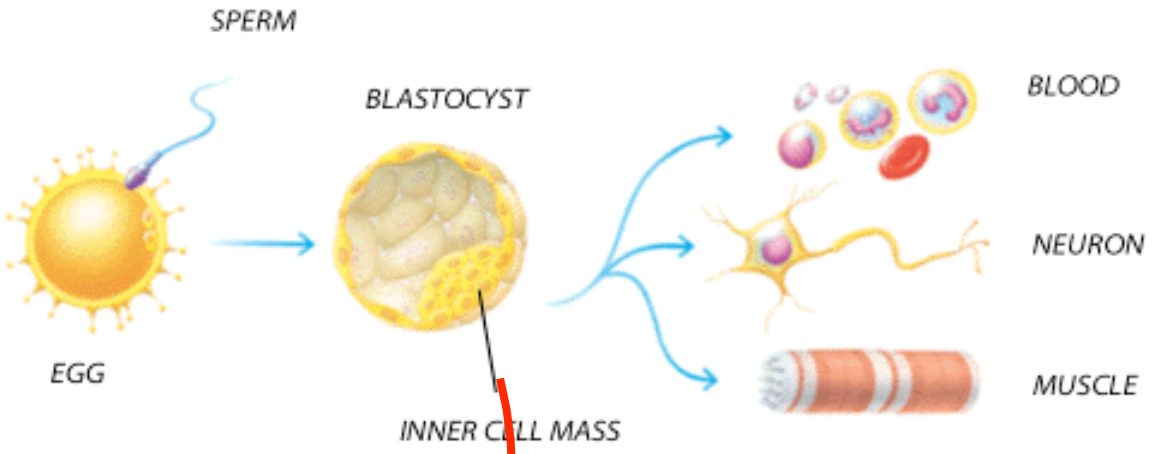


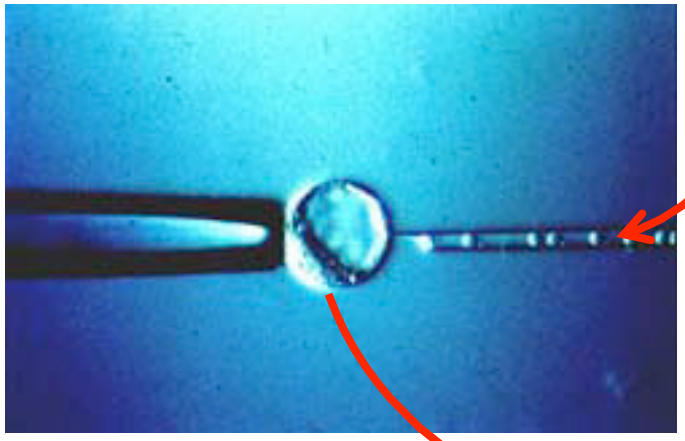


Engineered cells mix with recipient blastocyst to make a chimeric mouse


Blastocyst Donor
ES Cell Donor

Traditional ES Knock-Out Technology

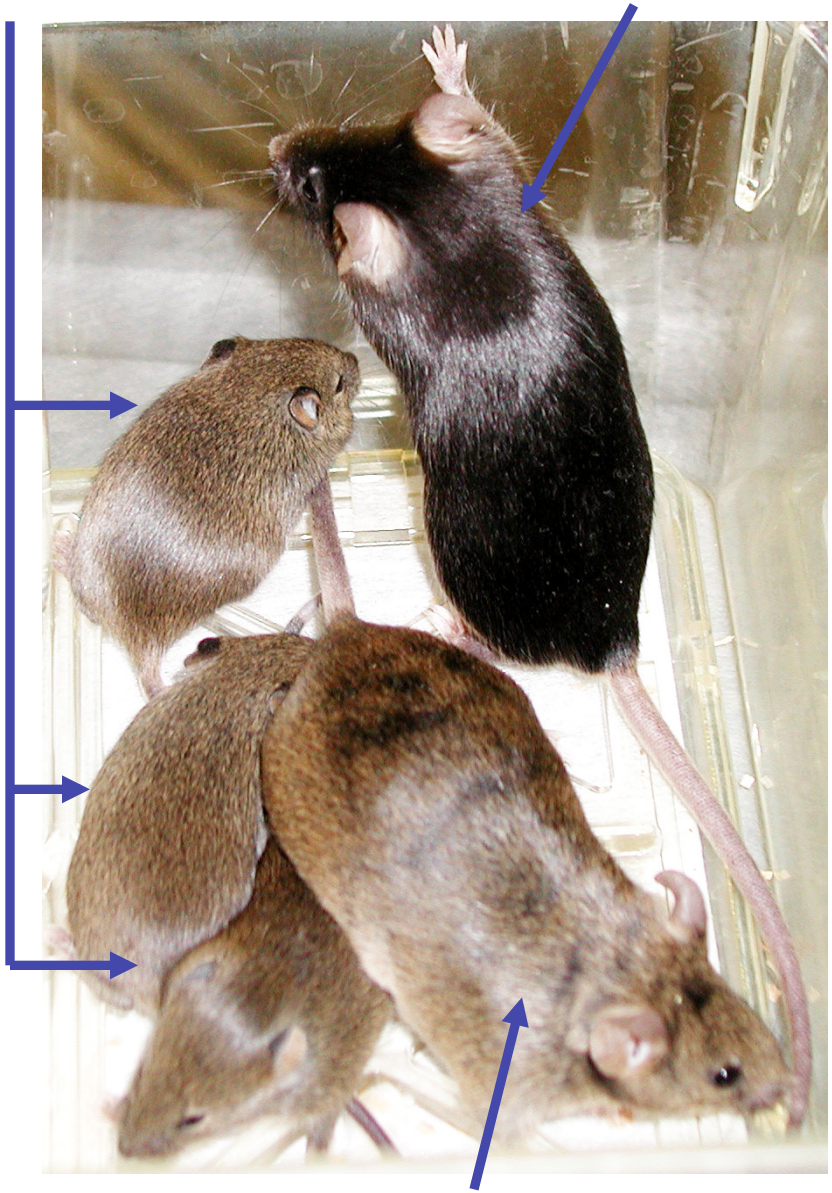




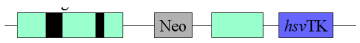
Engineered cells mix with recipient blastocyst to make a chimeric mouse

Germline Offspring

C57Bl Male

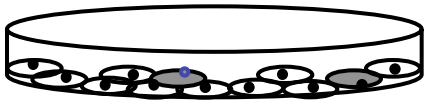


Germline Chimeric Female

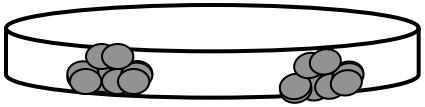


Targeting Vector

Electroporate

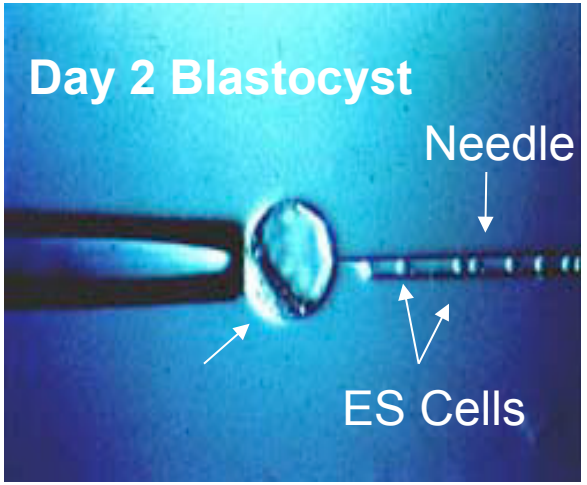


G418
FIAU

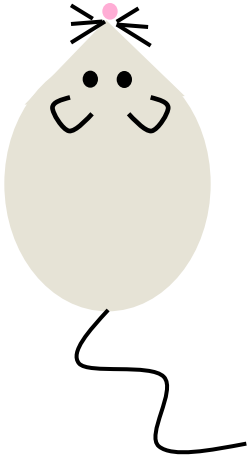


Drug Resistant Colonies

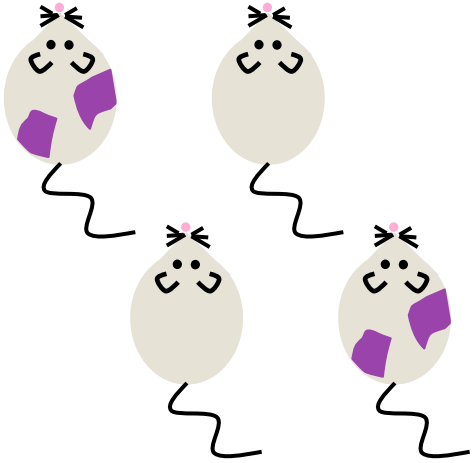
Characterize Clones
Inject Three Clones



Inject ES cells from selected clones into blastocysts

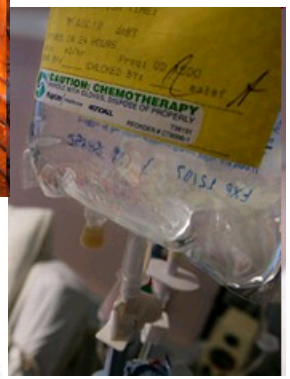


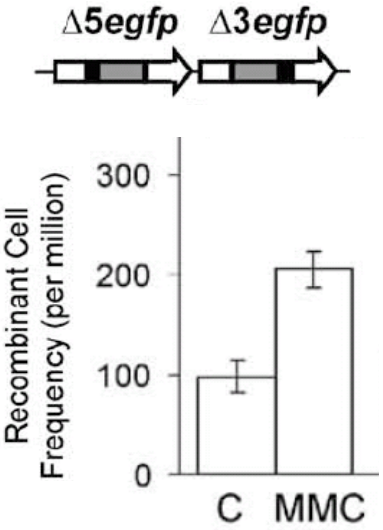
Implant into surrogate



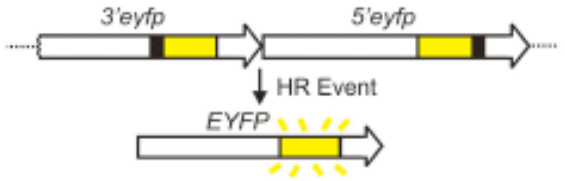
Chimeras

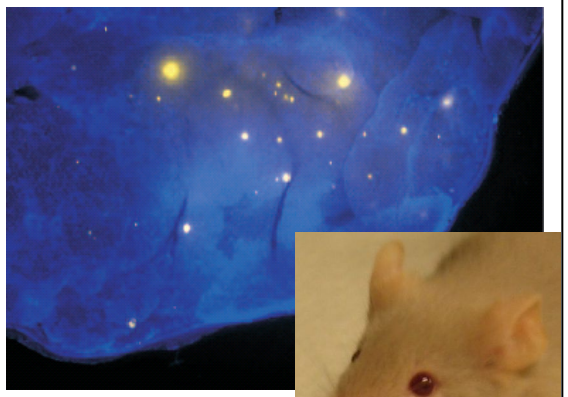
HR: important to measure

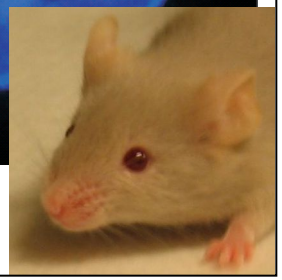




Jonnalagadda 2005

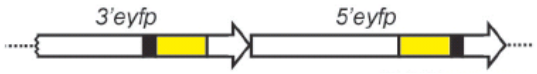


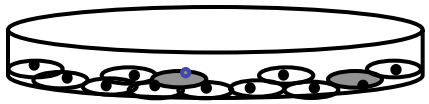


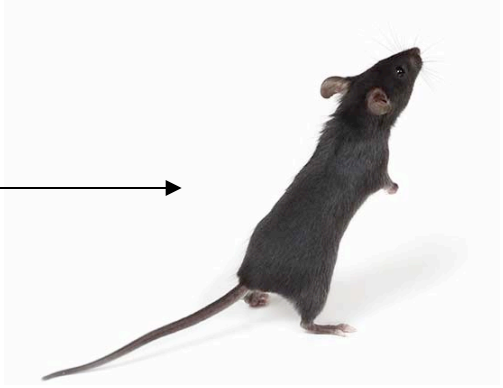


Wiktor-Brown 2006

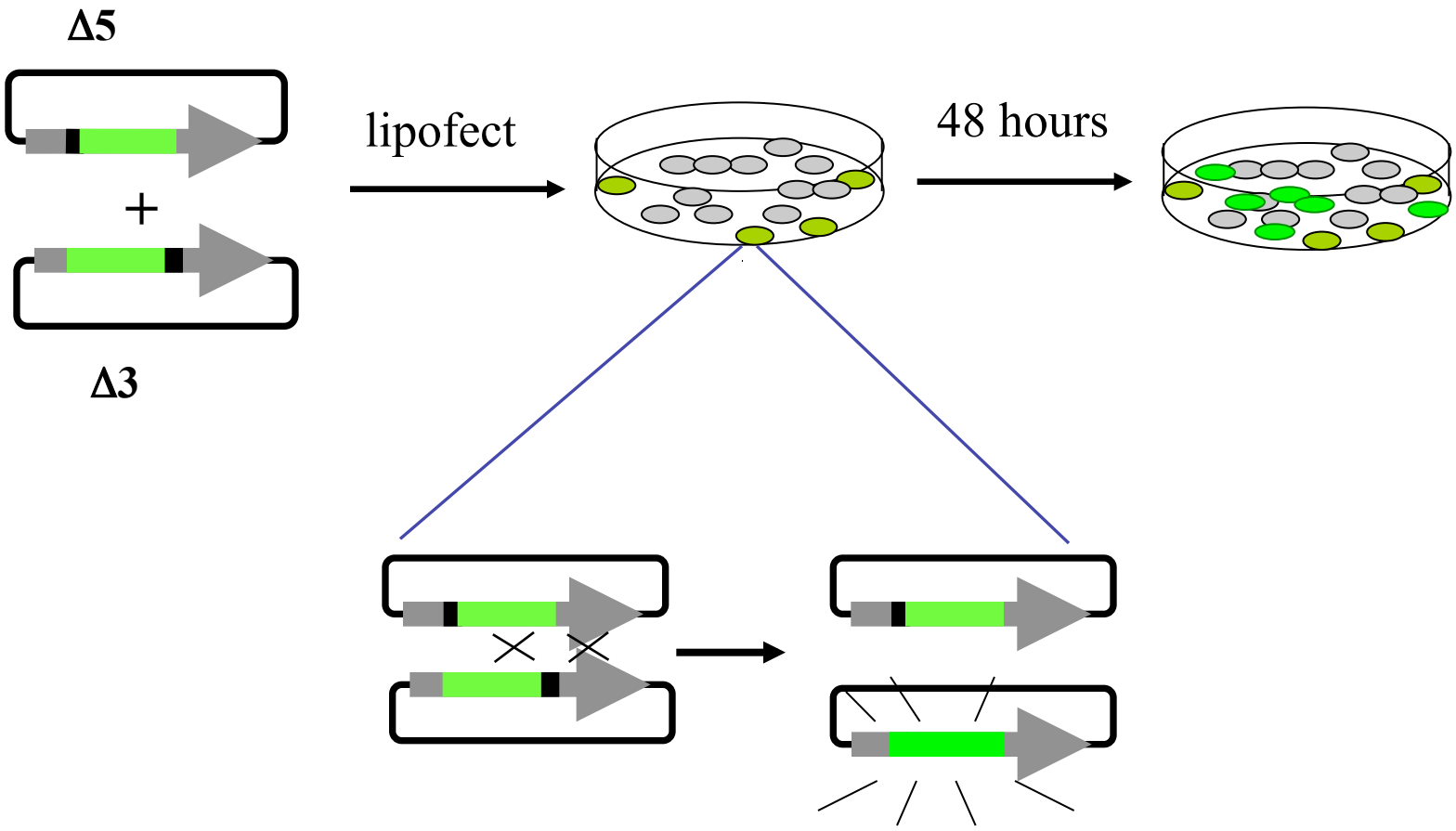
HR = tool



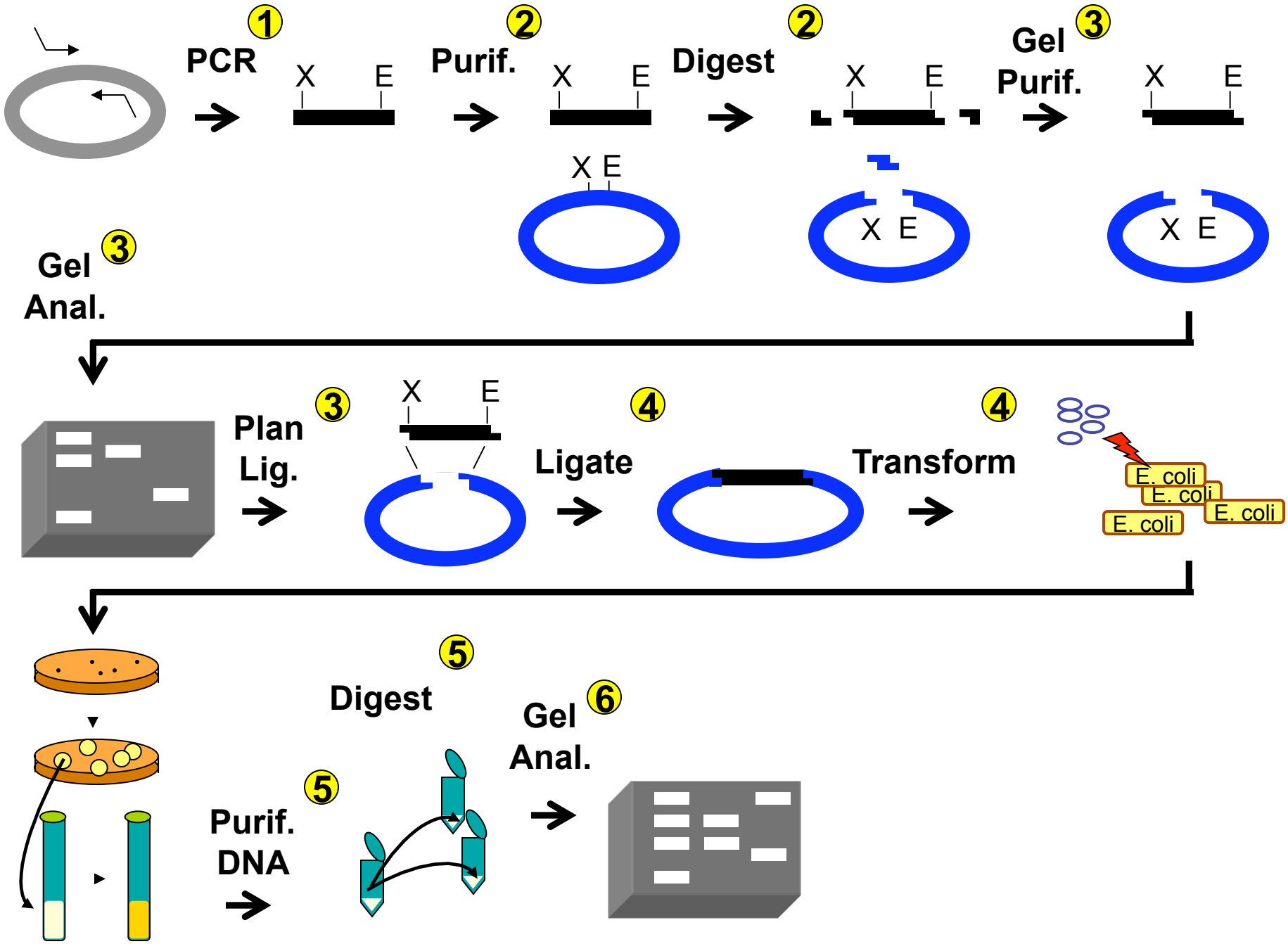




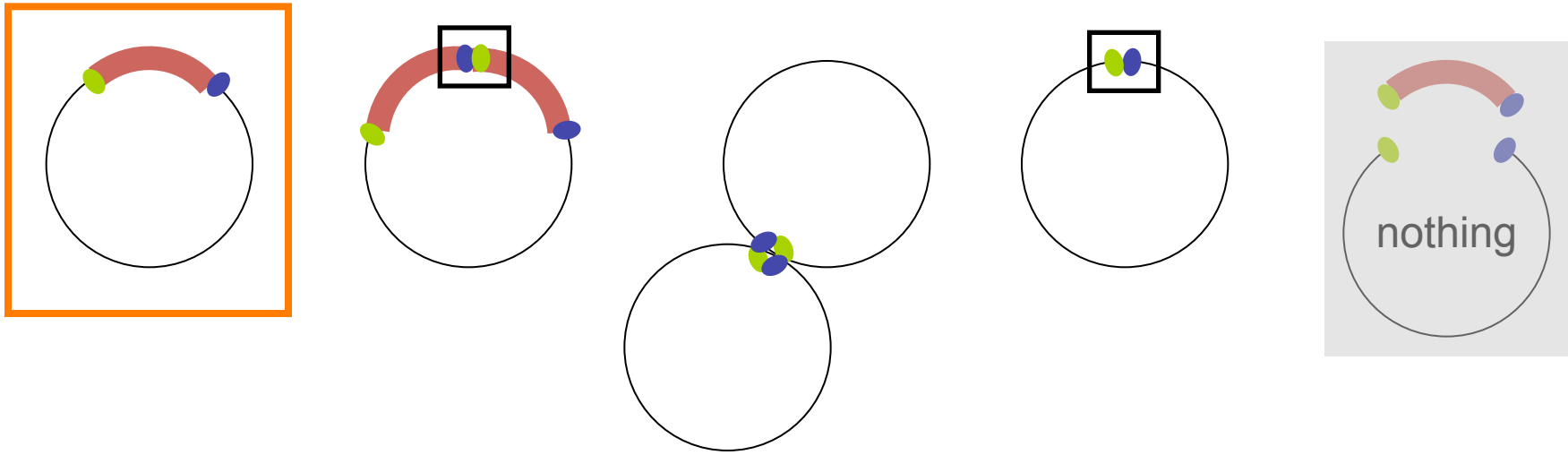
A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells



Methods & Logic
For Mod1


1
PCR
X
E
2
Purif.
X
E
2
Digest
X
E
3
Gel
Purif.
X
E
X E
X E
X E
3
Gel
Anal.
3
Plan
Lig.
X
E
4
Ligate
4
Transform
E. coli
E. coli
E. coli
E. coli
5
Digest
6
Gel
Anal.
5
Purif.
DNA

Your ligation reaction: possible outcomes



Population of different products

Need to separate and amplify individual products to analyze and select correct one

Transforming bacteria with ligation reaction

MIX

SHOCK

↓

PLASMID TAKEN UP

electric

(10-20 kV/cm)

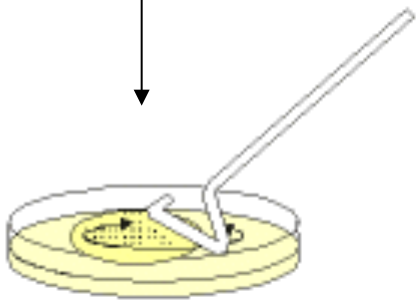
temperature

(42°C → ice)

RECOVER

grow in rich media

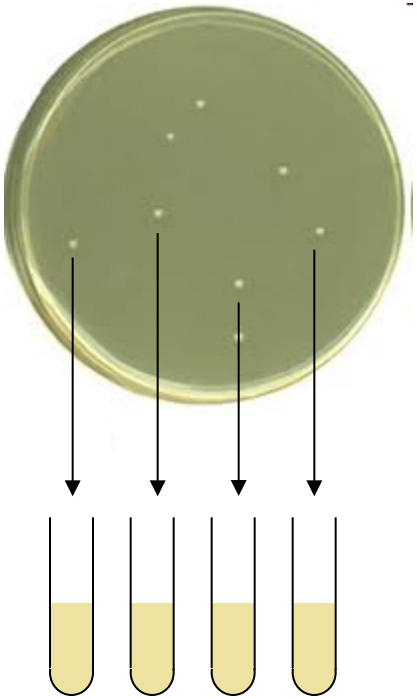
SEPARATE
SELECT
AMPLIFY



separate by spreading

Proceed to grow up individual colonies and analyze ligation products

SEPARATE
SELECT
AMPLIFY



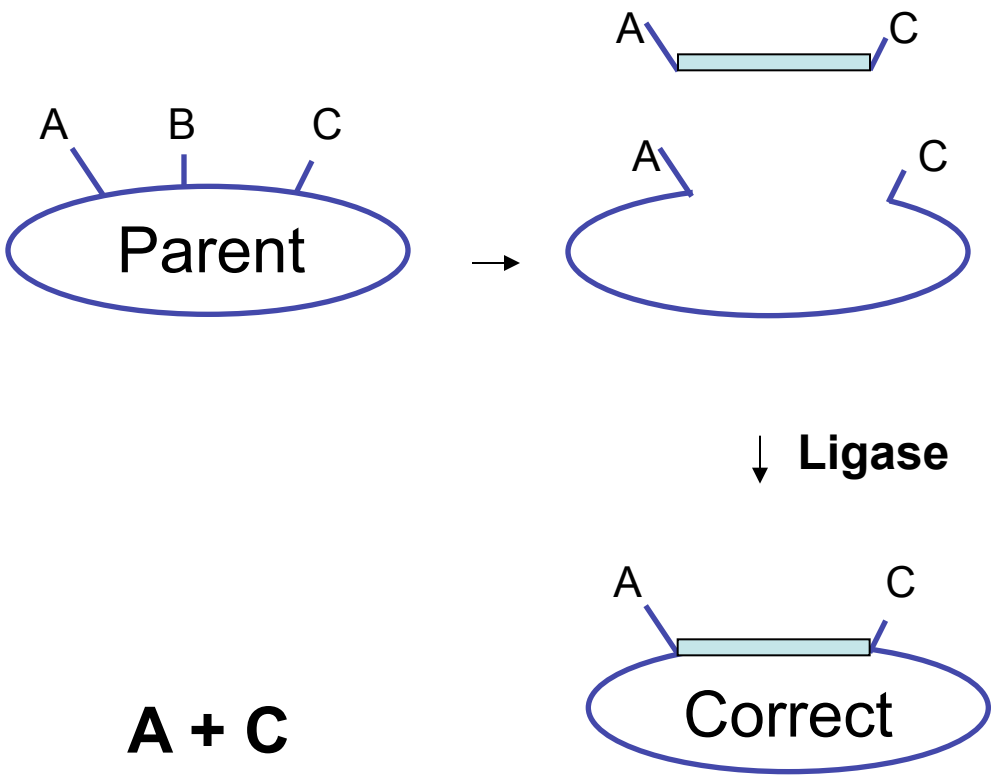
GROW UP
INDIVIDUAL
COLONIES

ISOLATE
PLASMID DNA

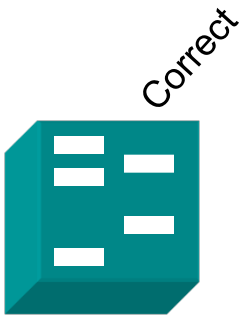
ANALYZE

How can you test to make sure your vector is correct?

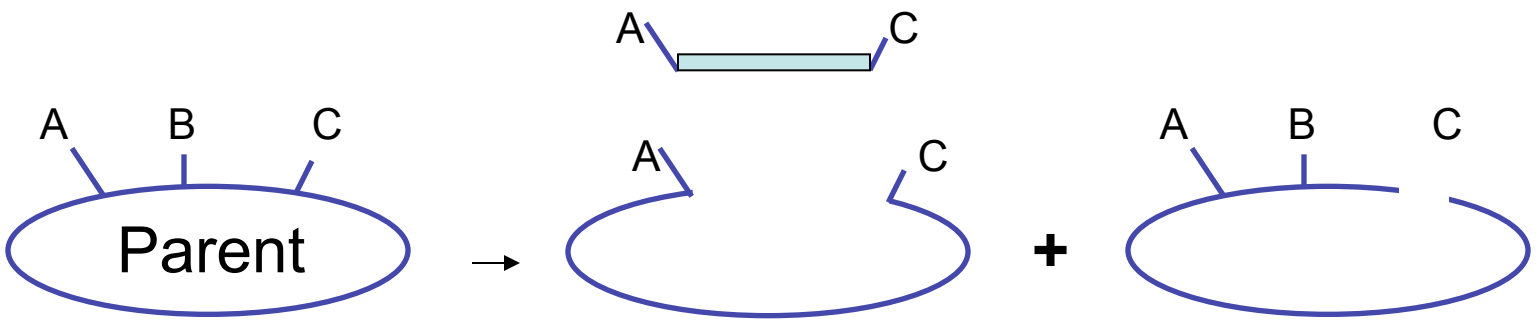
How to test for correct product



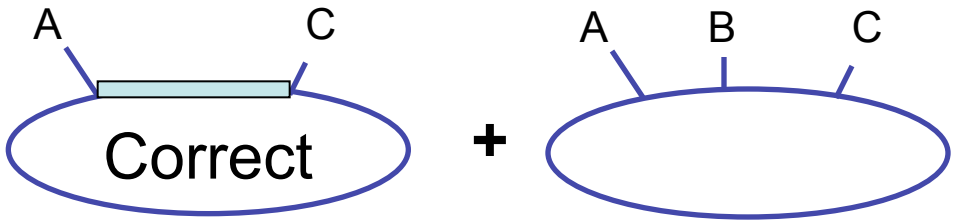
A + C



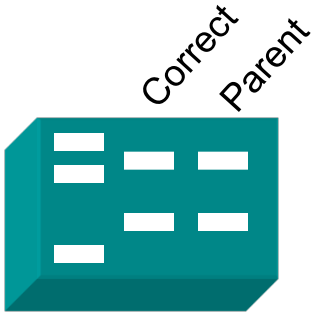
How to test for correct product



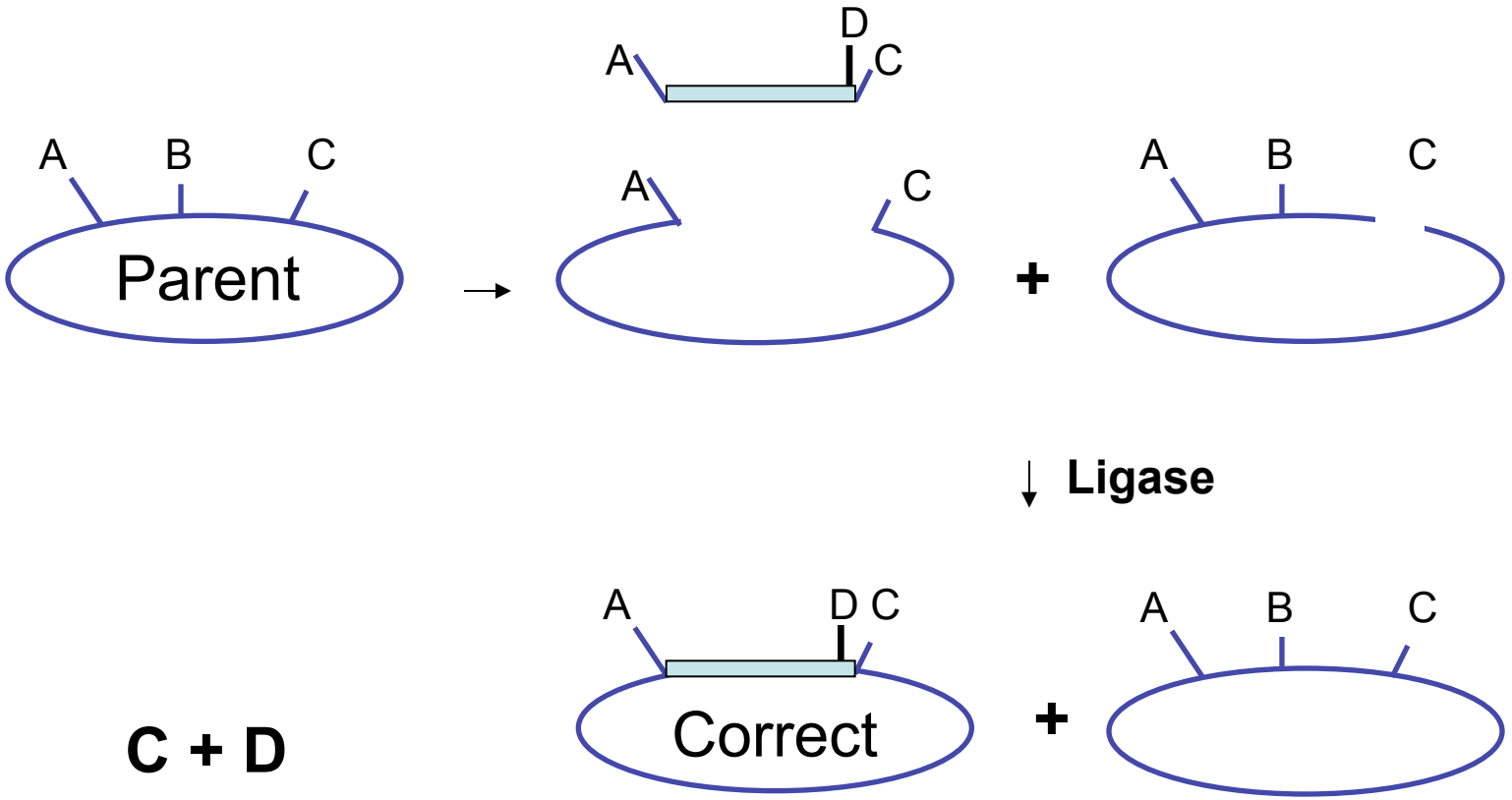
↓ **Ligase**



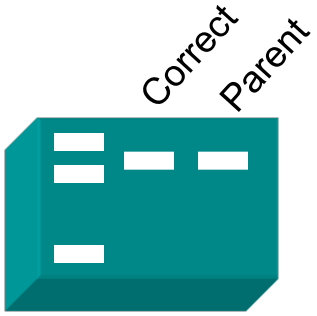
A + C



How to test for correct product

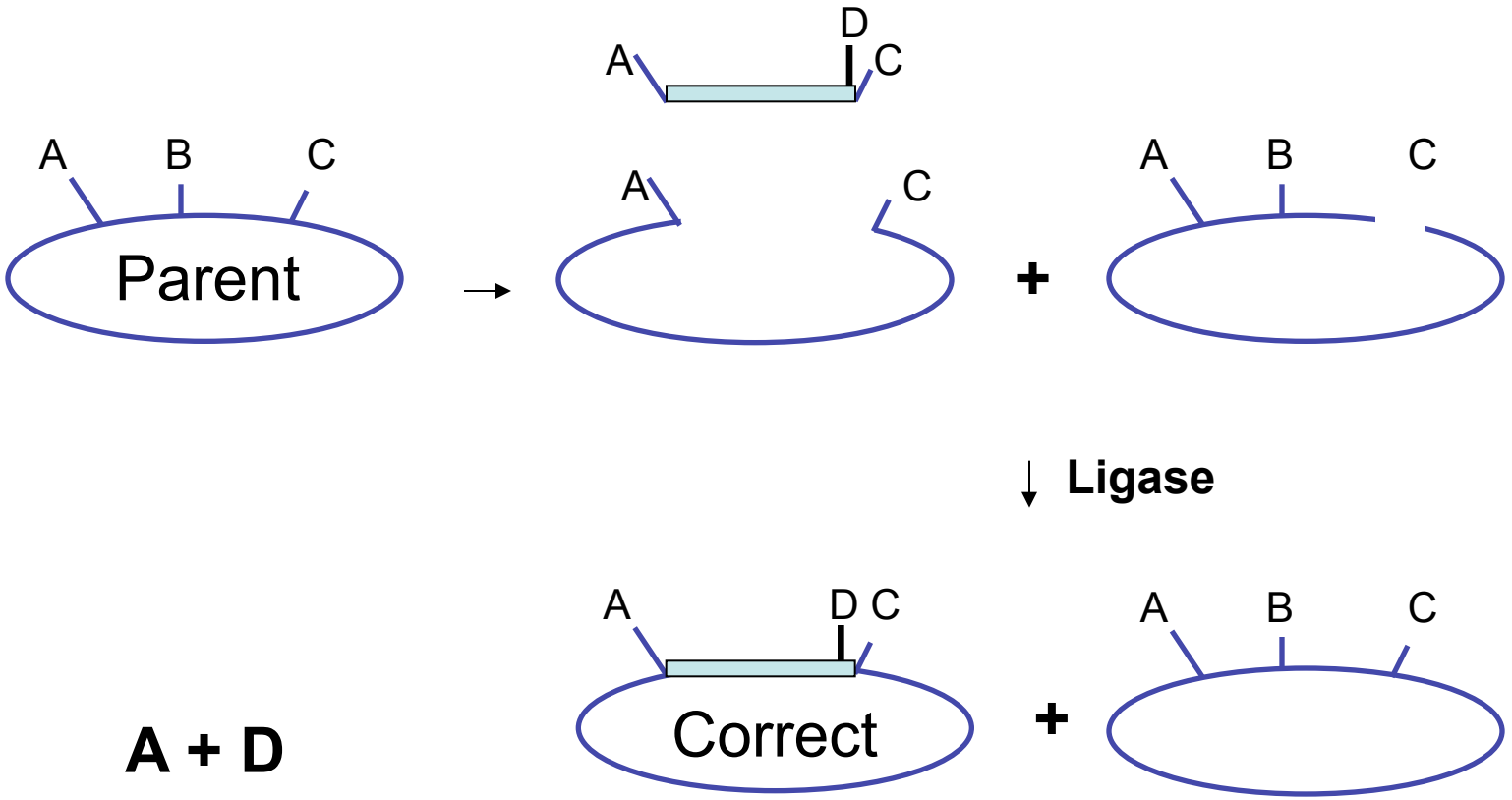


C + D



Be sure the expected fragment is easy to see and evaluate (*e.g.*, 0.3 to 2 kb)

How to test for correct product



A + D