MOD1 – DNA ENGINEERING

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Spring 2008

Day 4

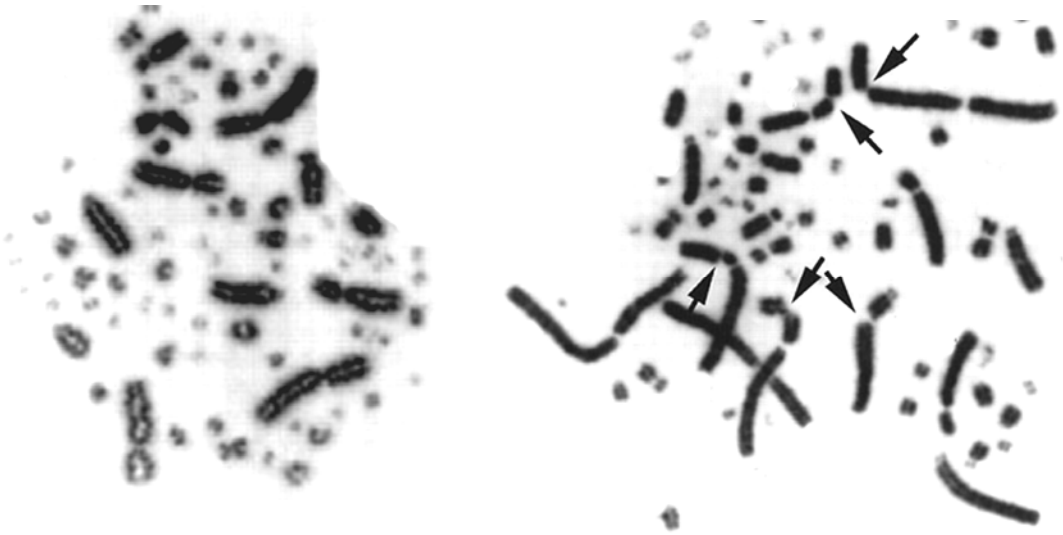
Using Homologous Recombination to Engineer Mice

Gene Cloning – Tips and Tricks!

Your Data – Controls & Interpretation

Why you owe Your Life to Homologous Recombination…

Normal



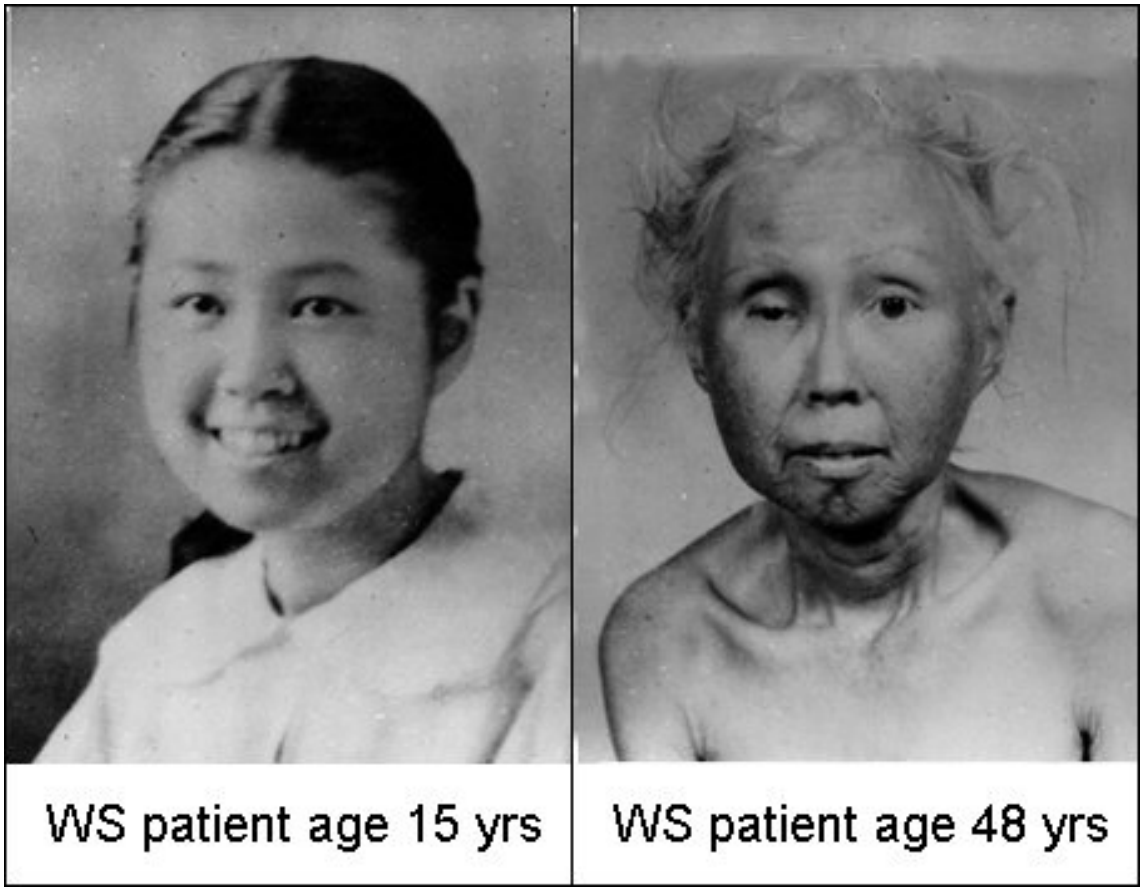
Rad51-/-

Turn Off Homologous Recombination
→ Chromosomes Fall Apart

Sonada *et al*., *EMBO J.* **17,** 598–608 (1998).

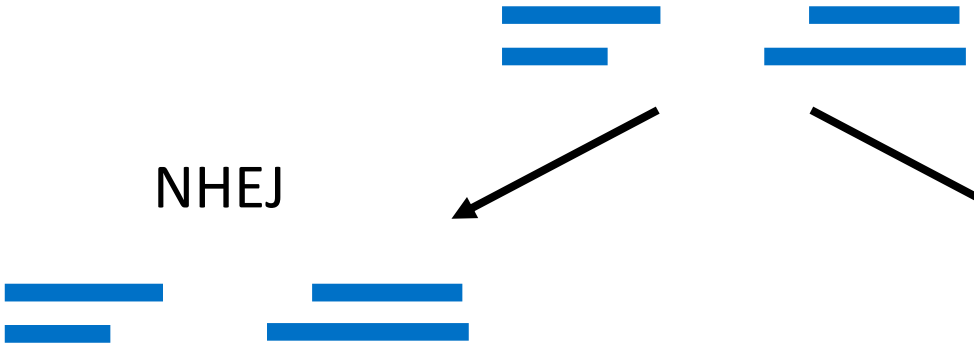
Why you owe Your Youthfulness to Homologous Recombination…

Loss of Helicase $\rightarrow$ Faulty Recomb.



Werner's Syndrome

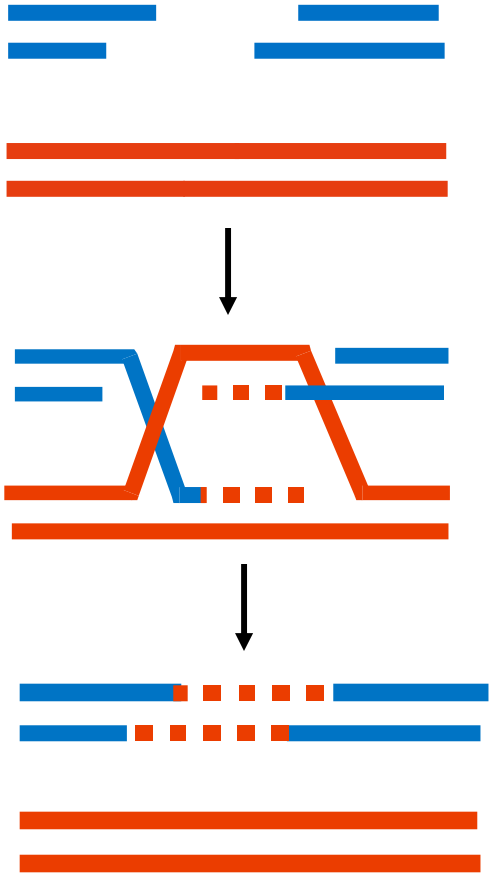
Double Strand Breaks

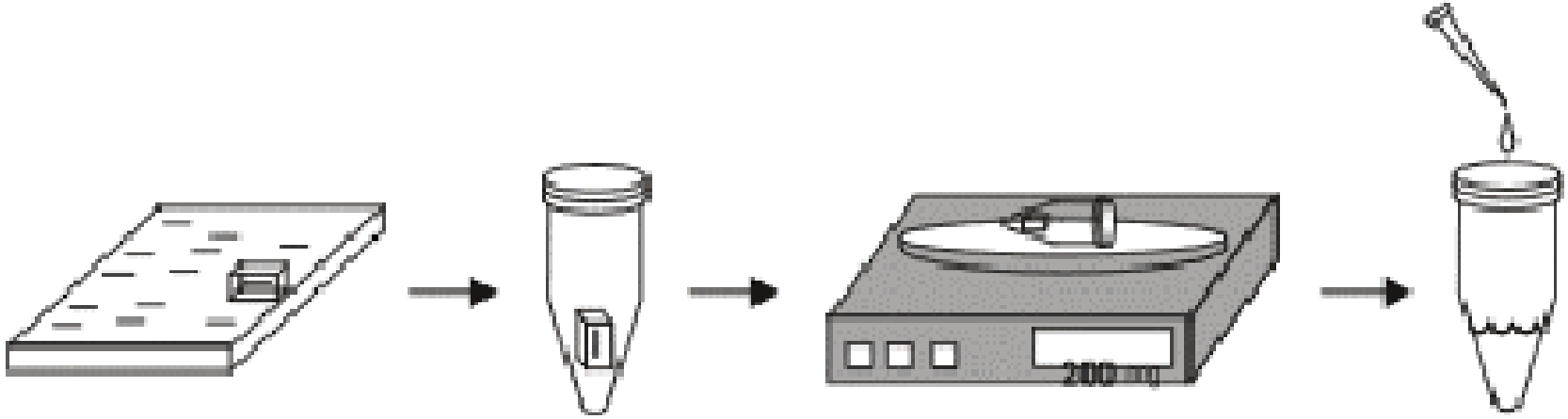


NHEJ

HR

KU heterodimer
(Ku70&80)
DNA-PKcs
DNA ligase IV
XRCC4





Exploiting HR for Mouse Engineering

You need to understand gene-targeting in order to understand the paper you will be reading.

Genetic Engineering in Mice:

1) Transgenic Mice

-putting genes in

2) Knock-Out Mice

-turning genes off

Genetic Engineering in Mice:

1) **Transgenic Mice**

-putting genes in

2) Knock-Out Mice

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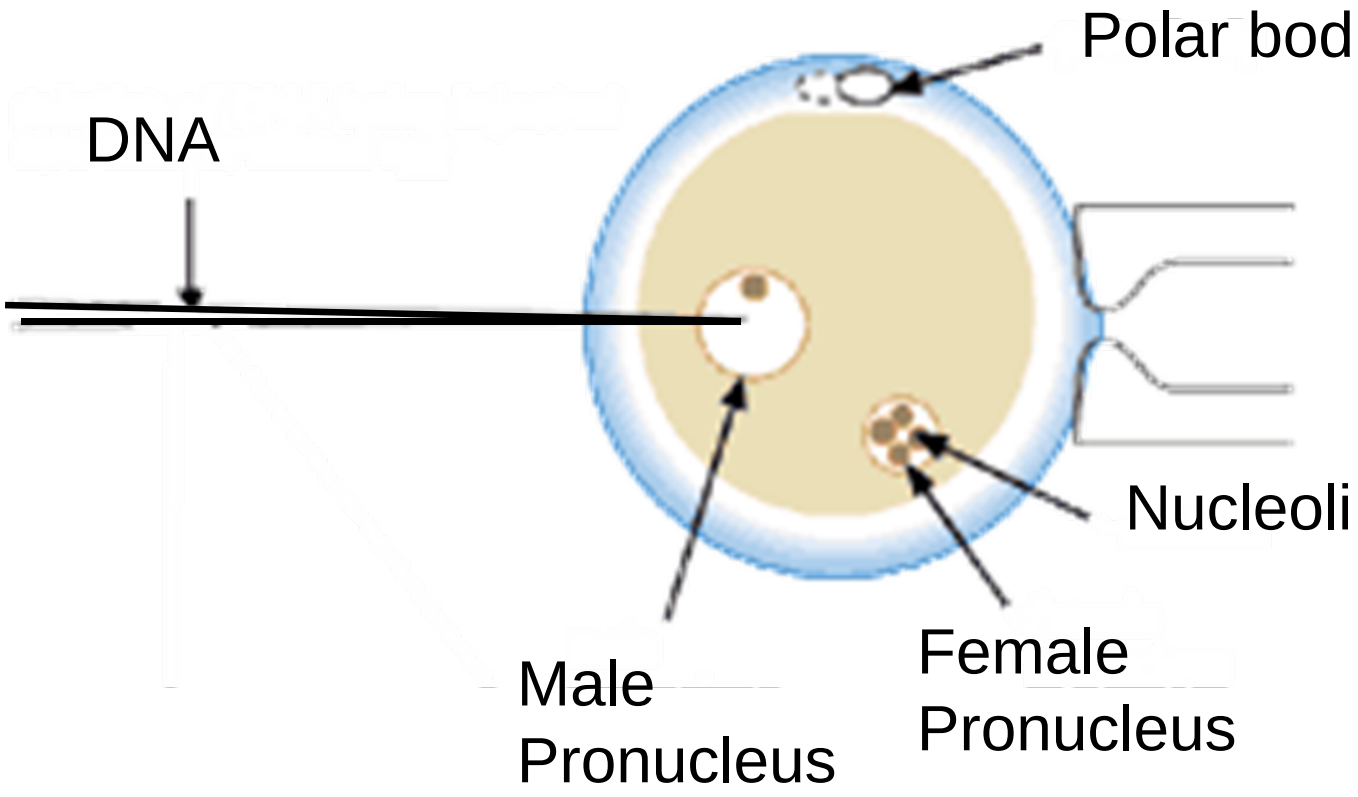
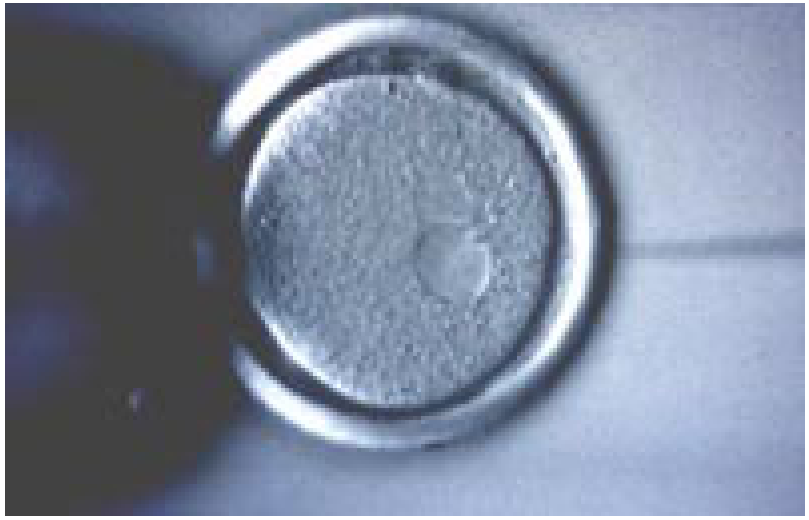
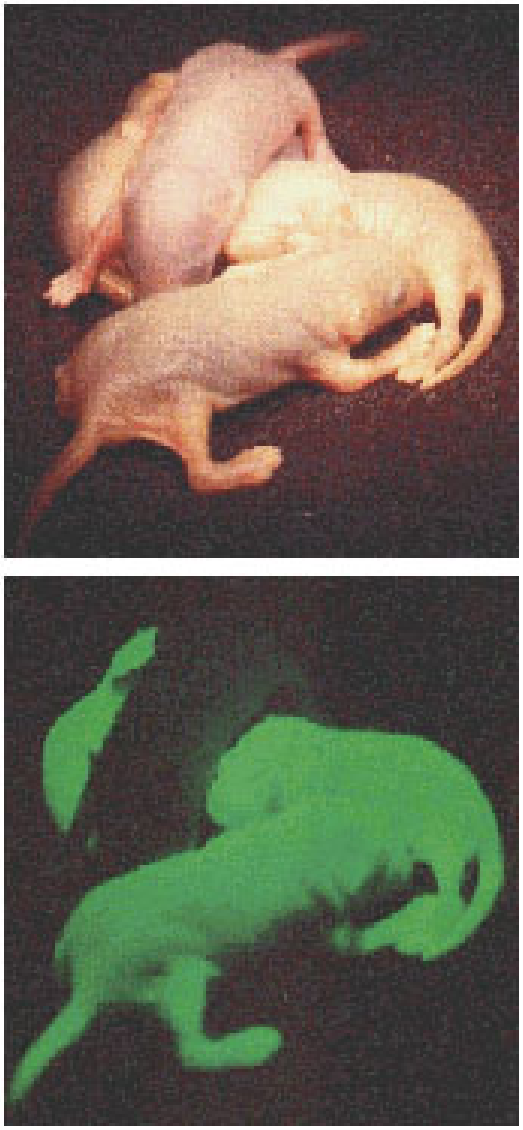

Polar bodies
DNA
Nucleoli
Male Pronucleus
Female Pronucleus





Okabe *et al.*

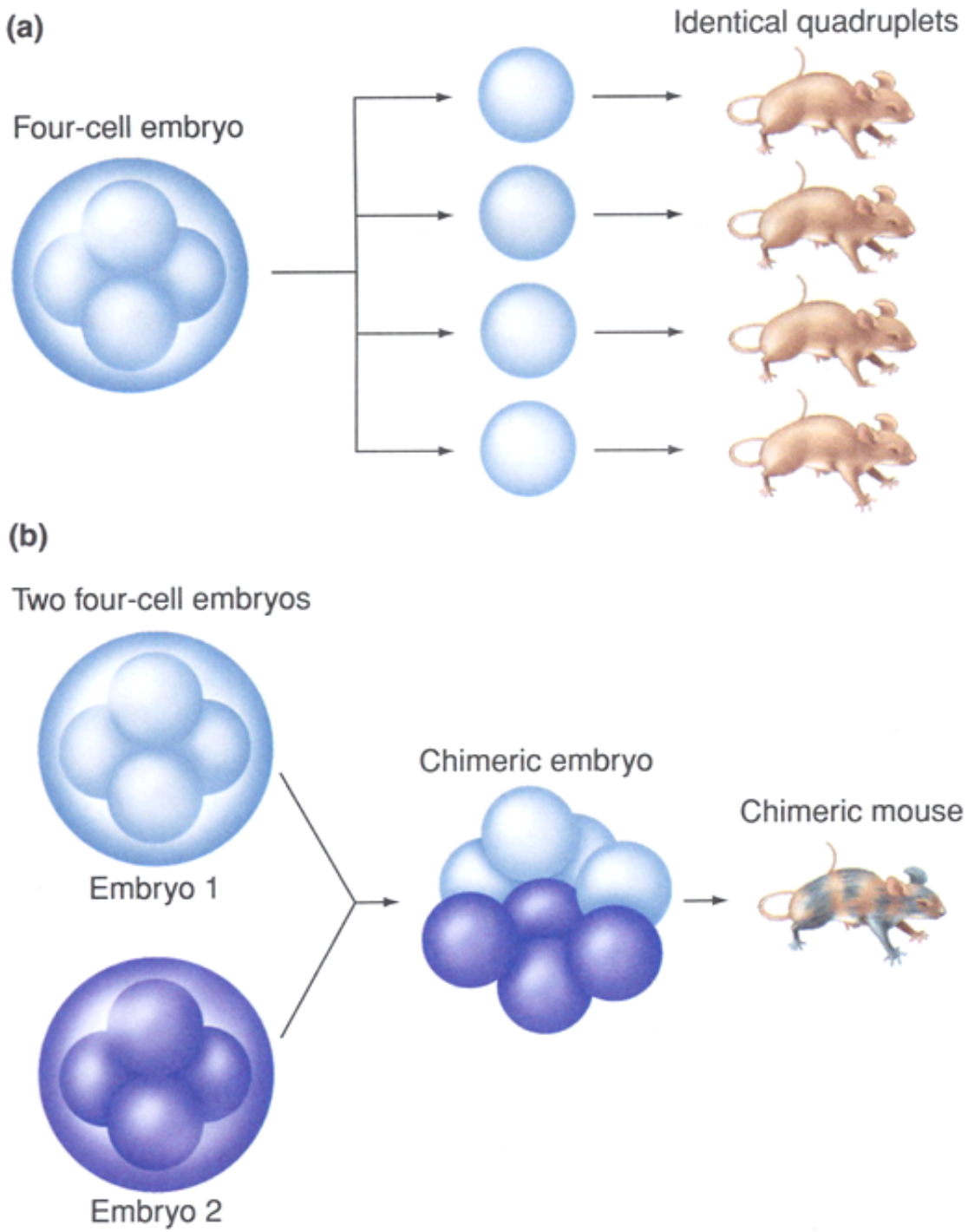
Genetic Engineering in Mice:

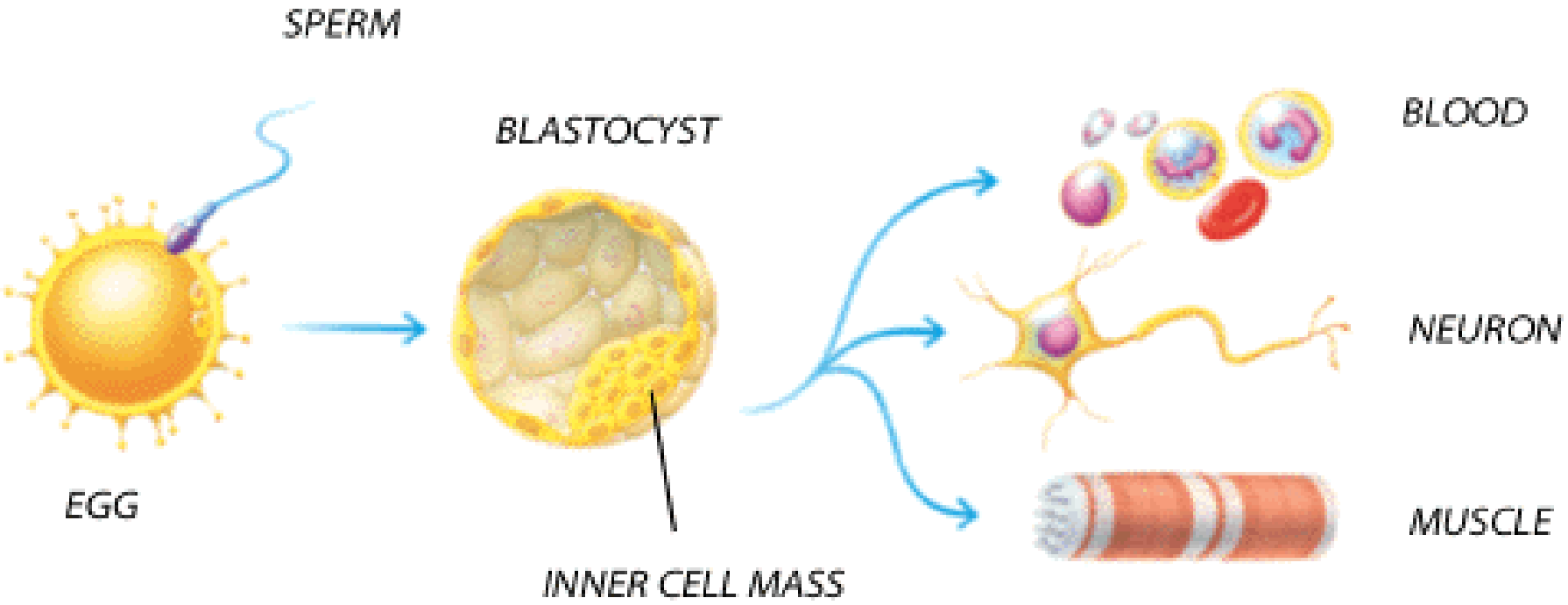
1) Transgenic Mice

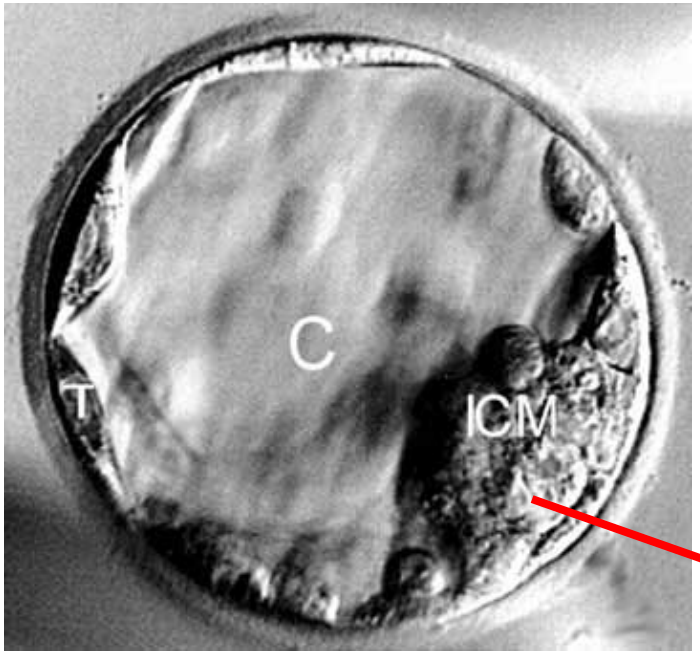
-putting genes in

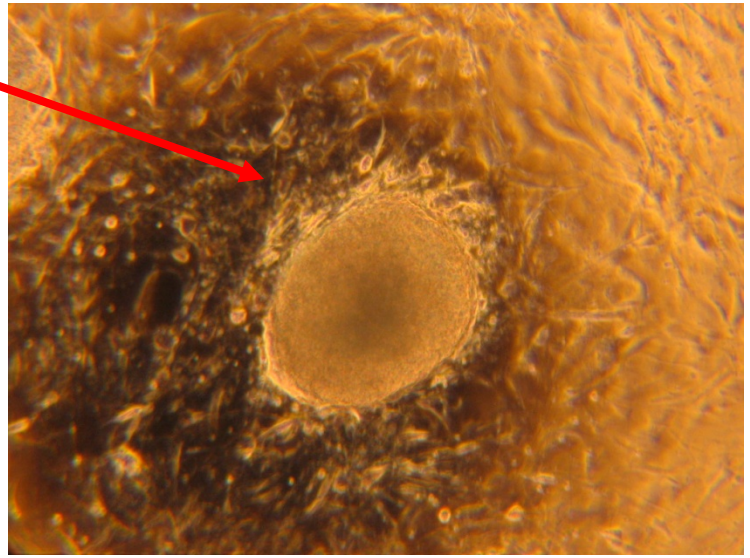
2) Knock-Out Mice

-turning genes off

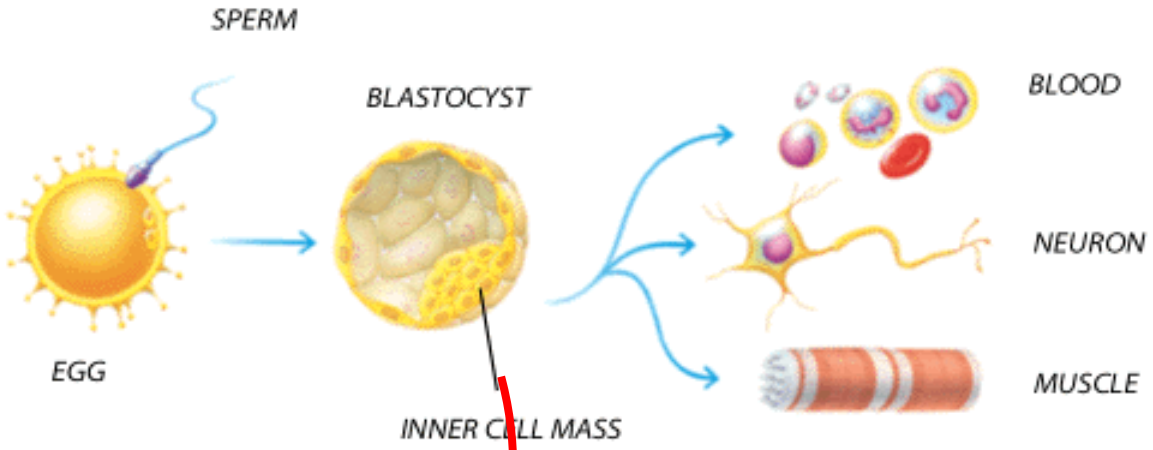

(a)
Identical quadruplets
Four-cell embryo
(b)
Two four-cell embryos
Chimeric embryo
Chimeric mouse
Embryo 1
Embryo 2

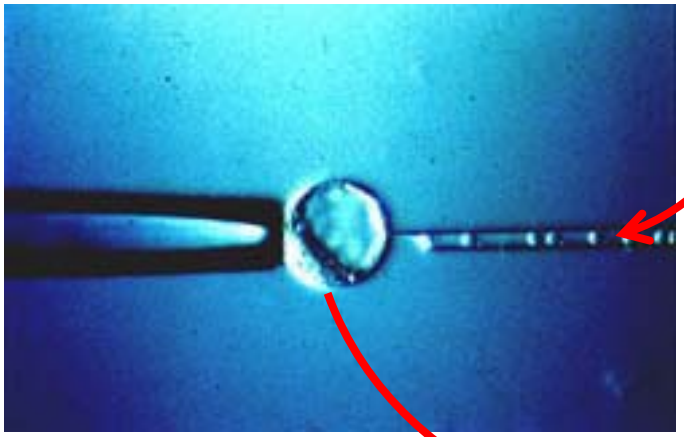

SPERM
BLASTOCYST
BLOOD
NEURON
MUSCLE
EGG
INNER CELL MASS


C
T
ICM

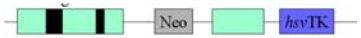


Traditional ES Knock-Out Technology



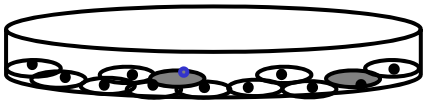


Engineered cells mix with recipient blastocyst to make a chimeric mouse

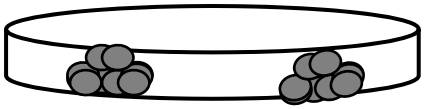


Targeting Vector

Electroporate

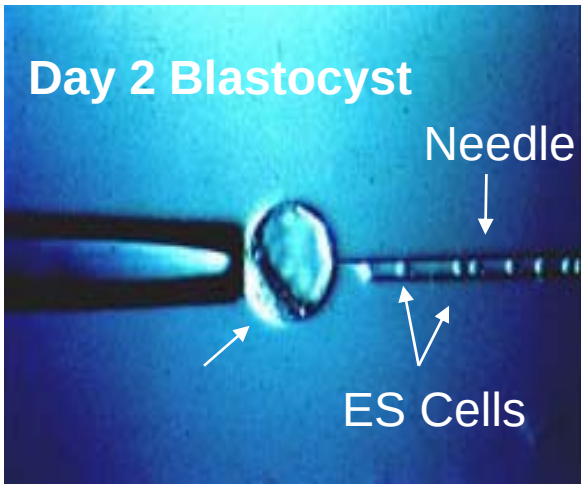


G418
FIAU

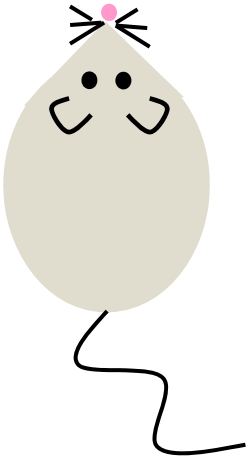


Drug Resistant Colonies

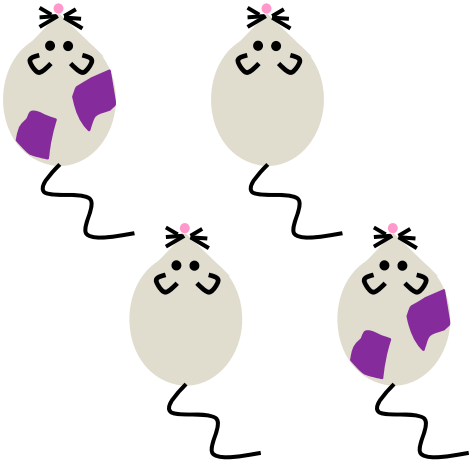
Characterize Clones
Karyotype
Inject Three Clones



Inject ES cells from selected clones into blastocysts



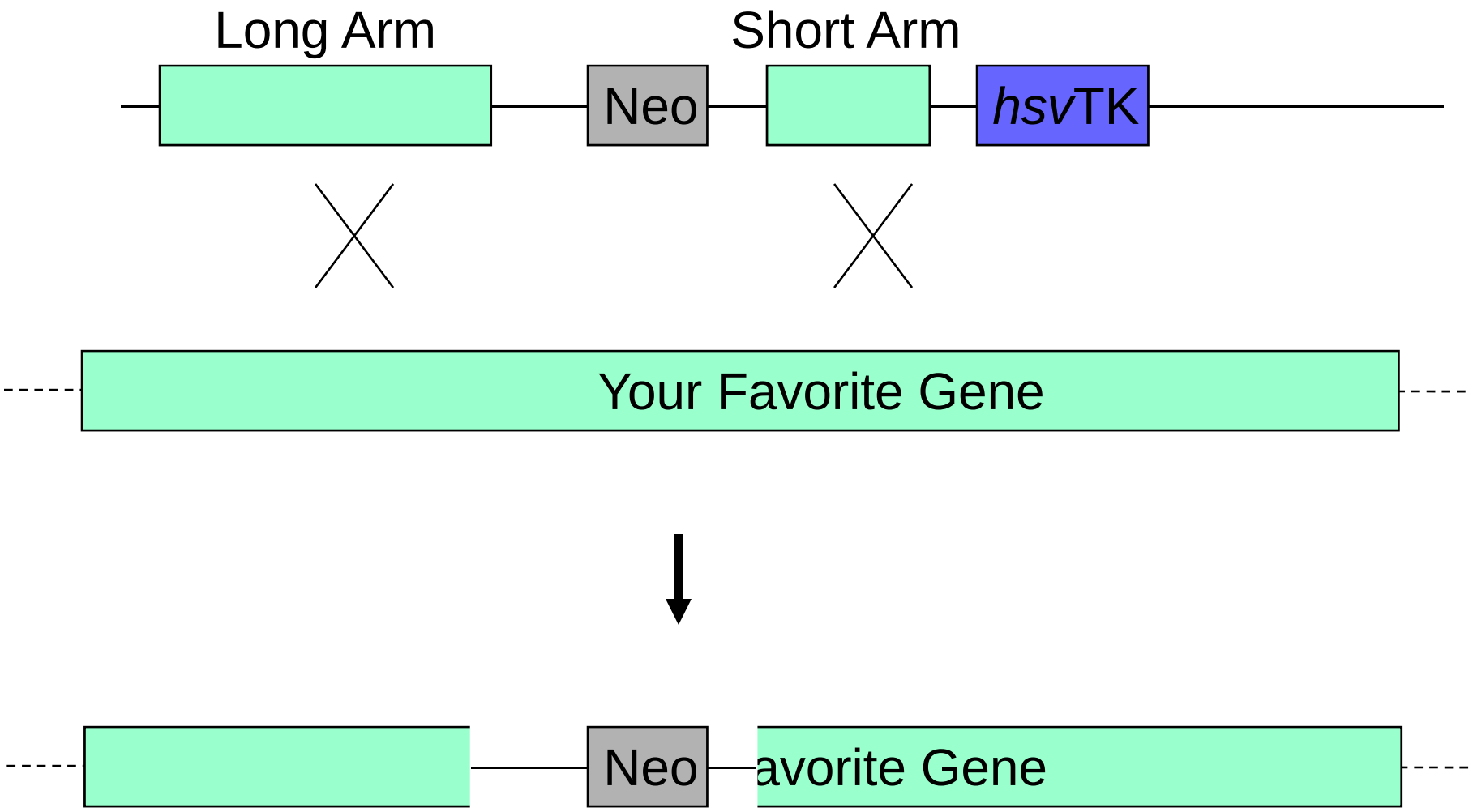
Implant into surrogate



Chimeras

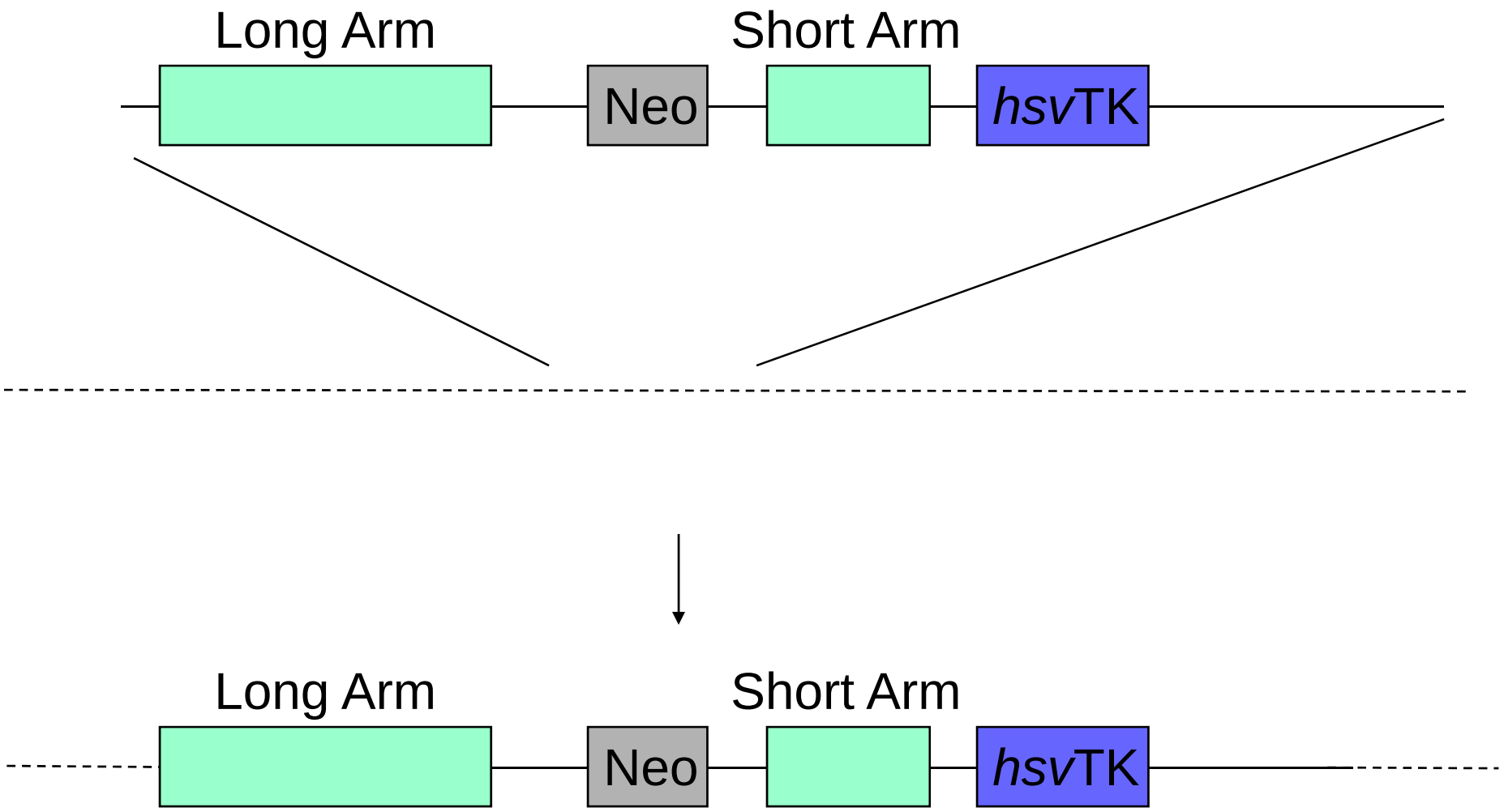
Traditional ES Knock-Out Technology

Targeted Homologous Recombination



Traditional ES Knock-Out Technology

Random Integration



Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking *Brca2*

Shyam K. Sharan*†, Masami Morimatsu‡§‖, Urs Albrecht¶, Dae-Sik Lim‡☆, Eva Regel†, Christopher Dinh*†, Arthur Sands‡, Gregor Eichele¶, Paul Hasty‡ & Allan Bradley*†

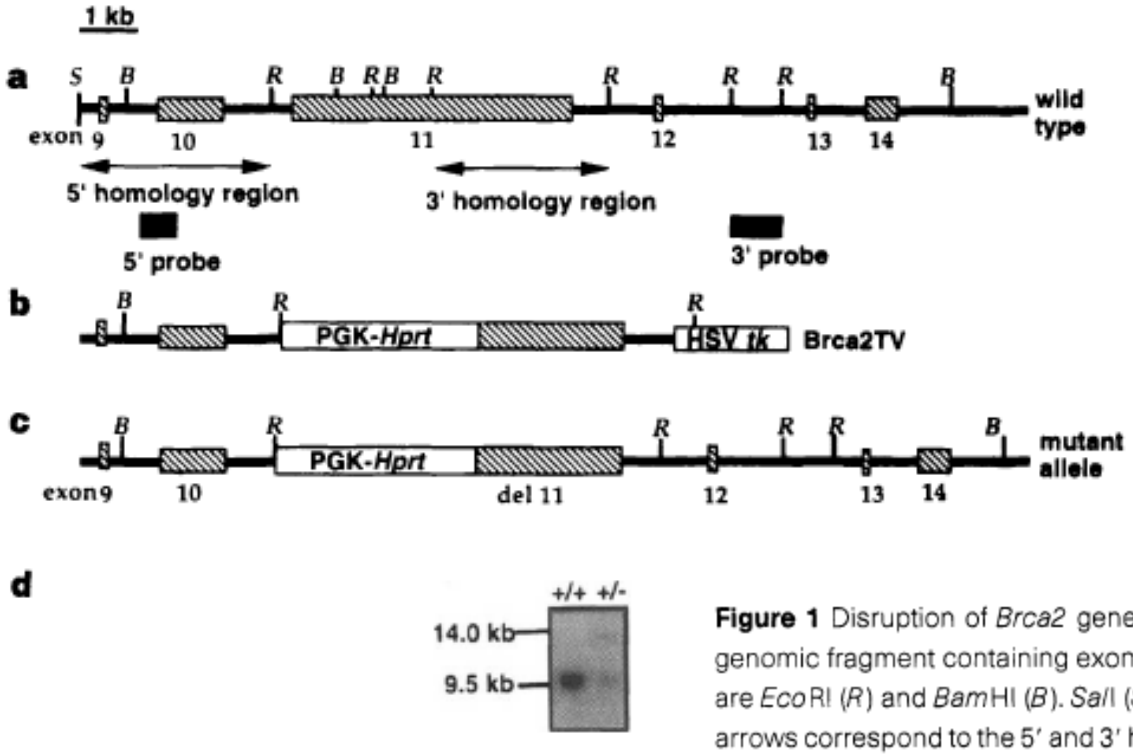
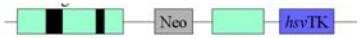


Figure 1 Disruption of *Brca2* gene in ES cells. **a**, Restriction map of the *Brca2* genomic fragment containing exon 9–15 is shown. Restriction sites shown here are *Eco*RI (*R*) and *Bam*HI (*B*). *Sal*I (*S*) is from the cloning vector. Double-headed arrows correspond to the 5′ and 3′ homology regions and the dark shaded boxes show the probes used. **b**, Restriction map of the targeting vector pBrca2TV. **c**, Expected restriction map of the mutated *Brca2* locus. A 2.8-kb genomic region is deleted and replaced by the 3.6-kb *Hprt* gene. **d**, Southern analysis to identify heterozygous ES cells by digesting genomic DNA with *Bam*HI. The 3′ probe detects a 9.5-kb wild-type band and a 14.0-kb mutant band. **e**, Restriction map of



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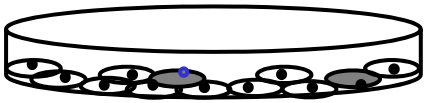

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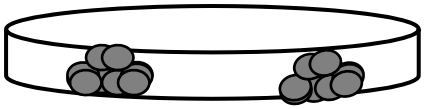

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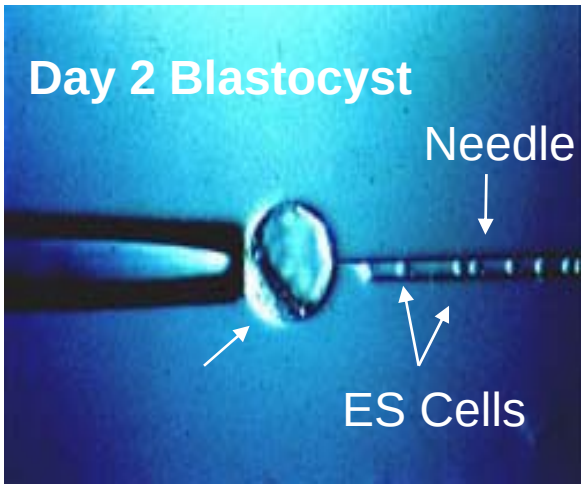

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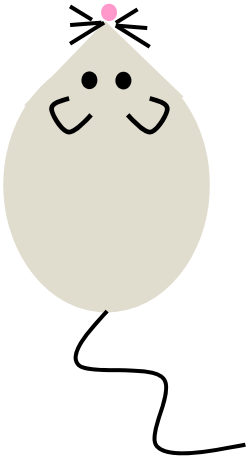

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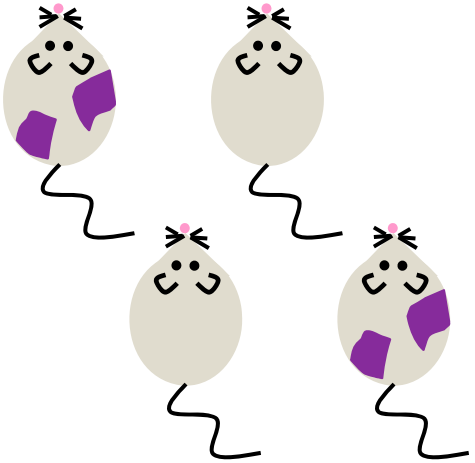
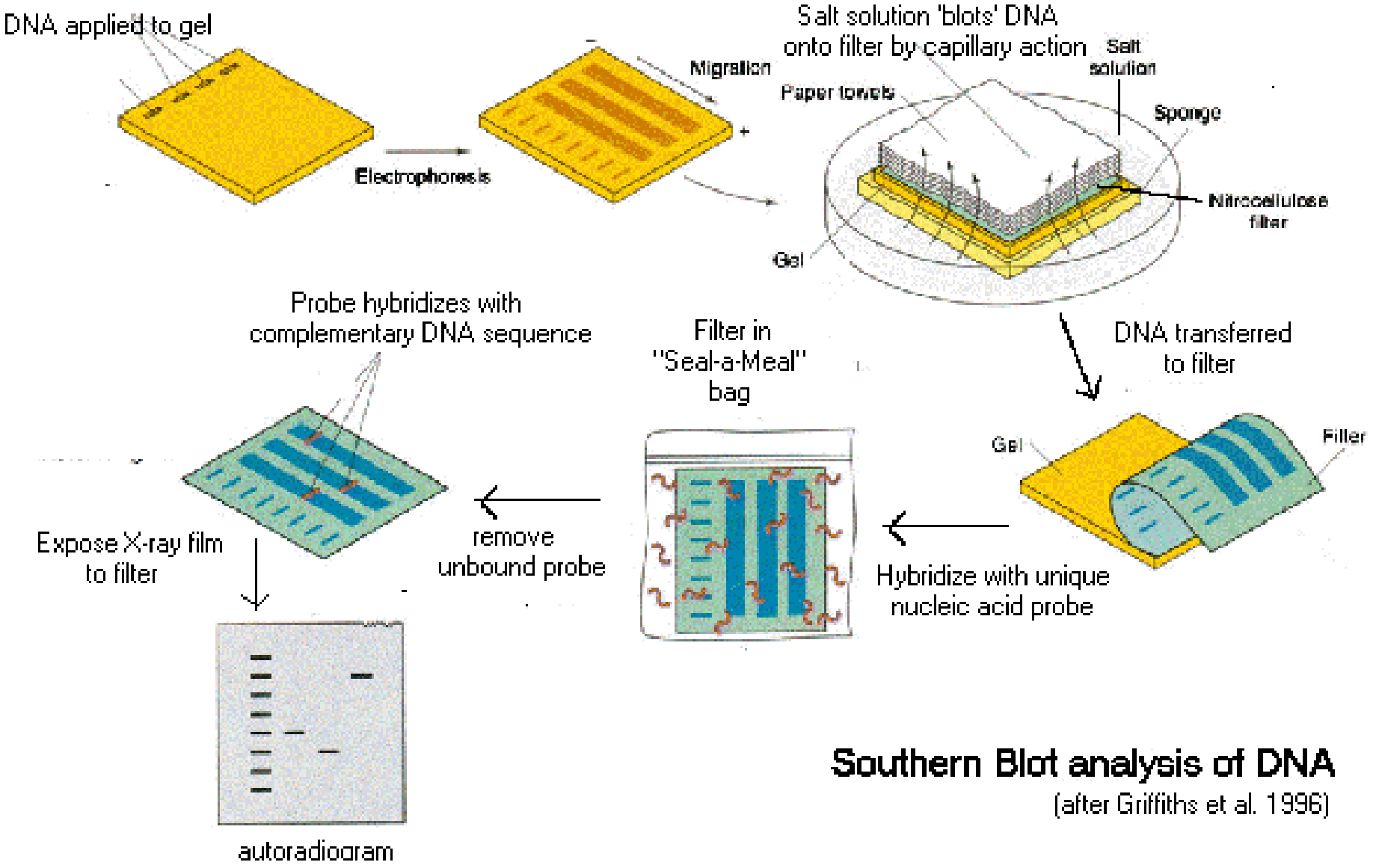


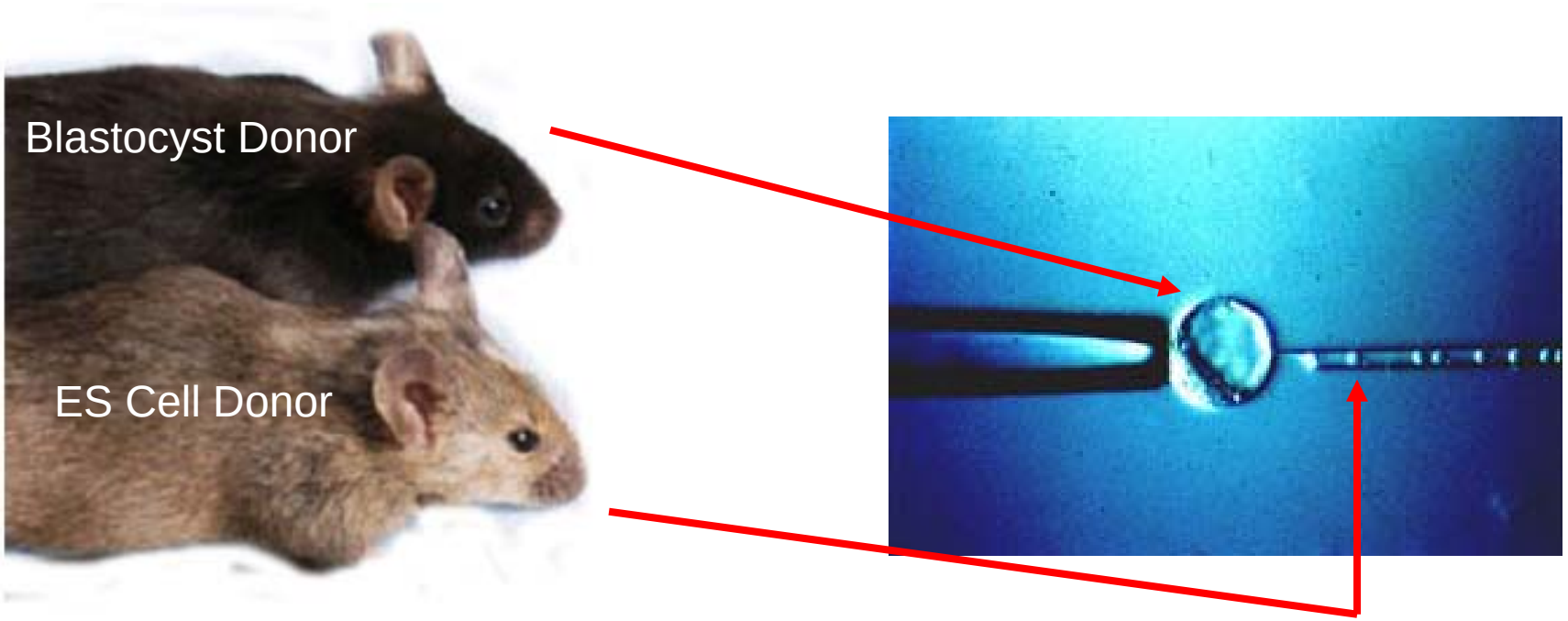
Chimeras

Southern Blot Analysis



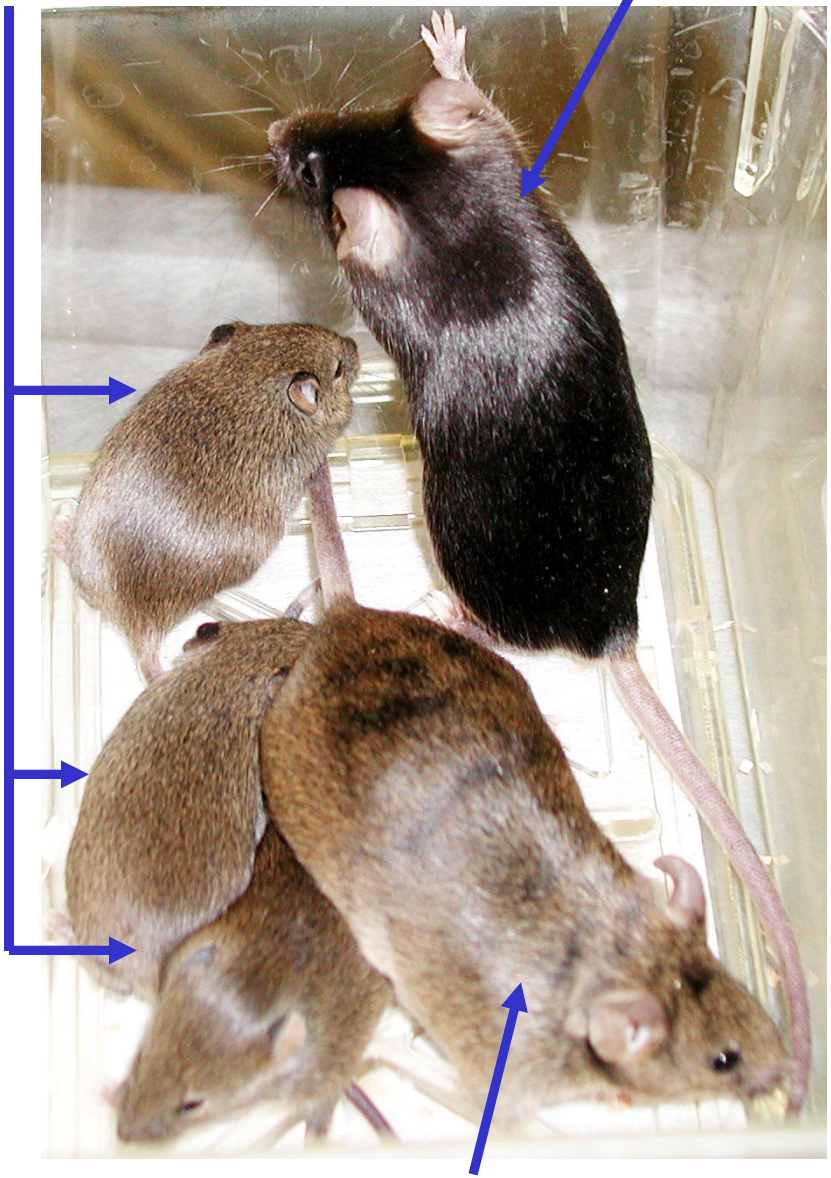
Southern Blot analysis of DNA

(after Griffiths et al. 1996)


Blastocyst Donor
ES Cell Donor

Germline Offspring

C57Bl Male



Germline Chimeric Female

Gene Cloning: Tips and Tricks!

Not the same as cloning!

Know Your Vector!

- Find out as much as you can about your vector

  How do you know if your promoter will work?
  What antibiotic is used?
  What is the origin of replication?

- Watch out for incorrect maps (!)

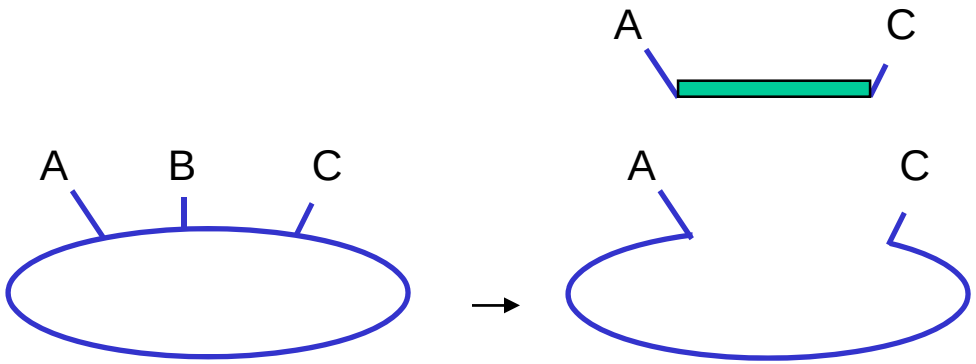
- Watch out for methylation-sensitive sites

Picking Restriction Enzyme Sites for PCR Product Insert

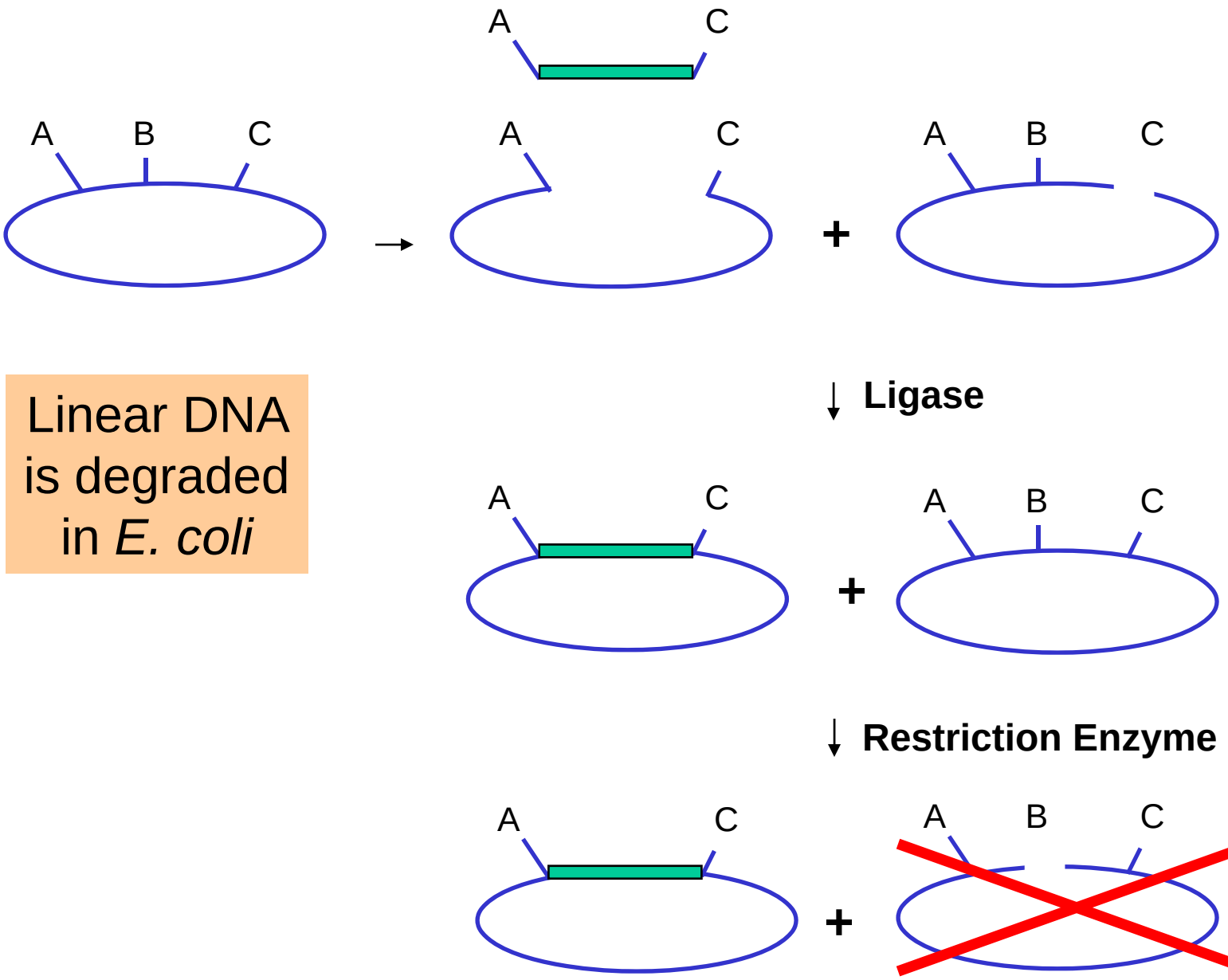
-They must not be in the insert

-Each must be unique in the vector

-Ideally they cut in the same buffer (check in catalog)



When possible, use a “Kill” site to get rid of parent-vector



Common Pitfalls in Preparing Vector

-DNA is not completely digested....

Why might incomplete cutting cause a problem?

What can cause incomplete digestion?

-EDTA

-contamination with salts

-ethanol

-phenol

-not adding enough enzyme

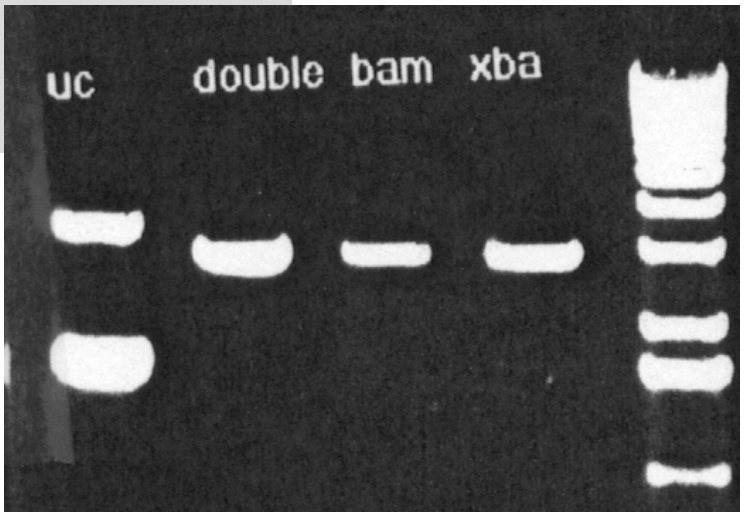
-not waiting long enough

-incorrect buffer or temp.

Preparing the Backbone for Ligation

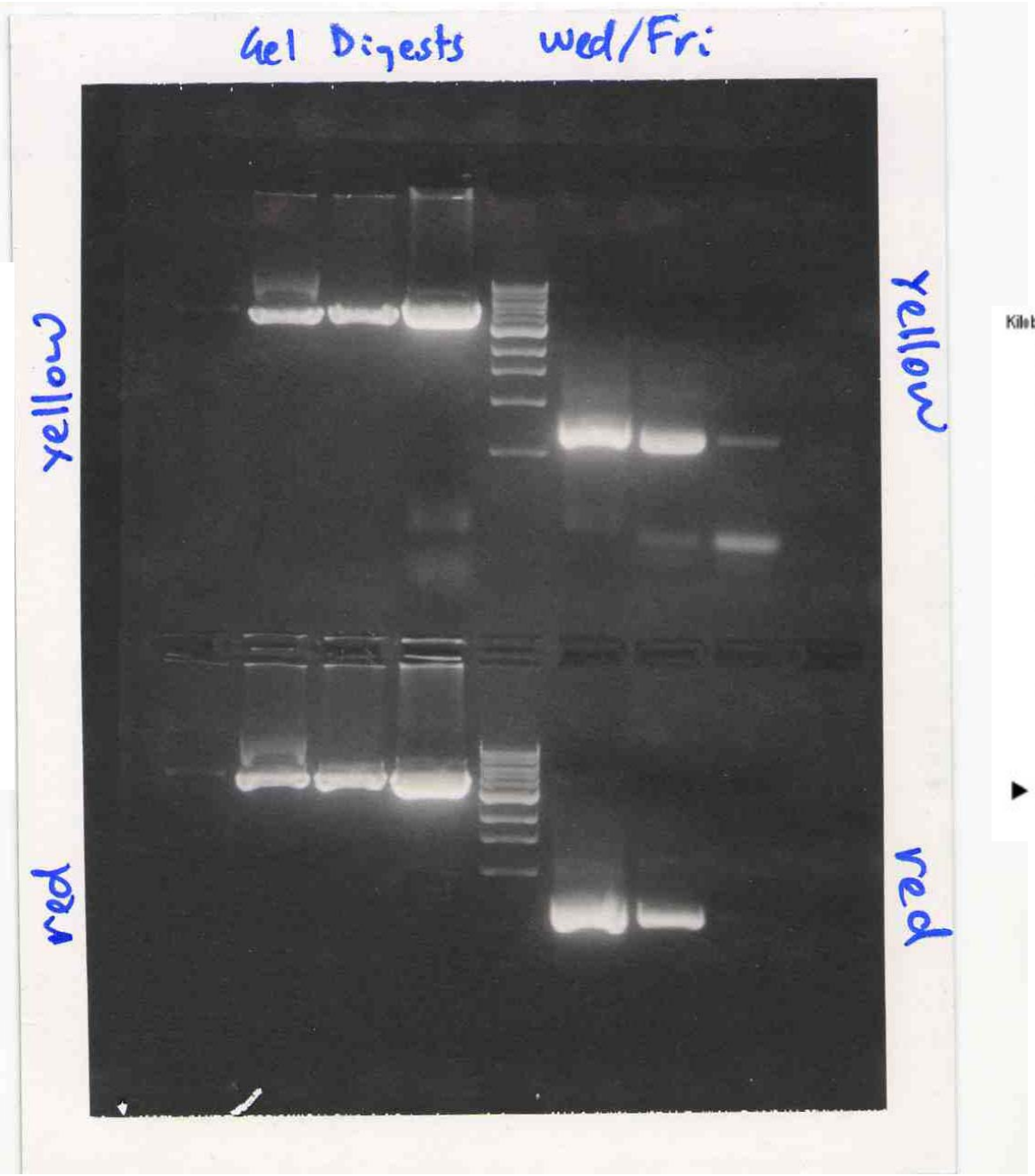
When double digesting, set up four reactions:

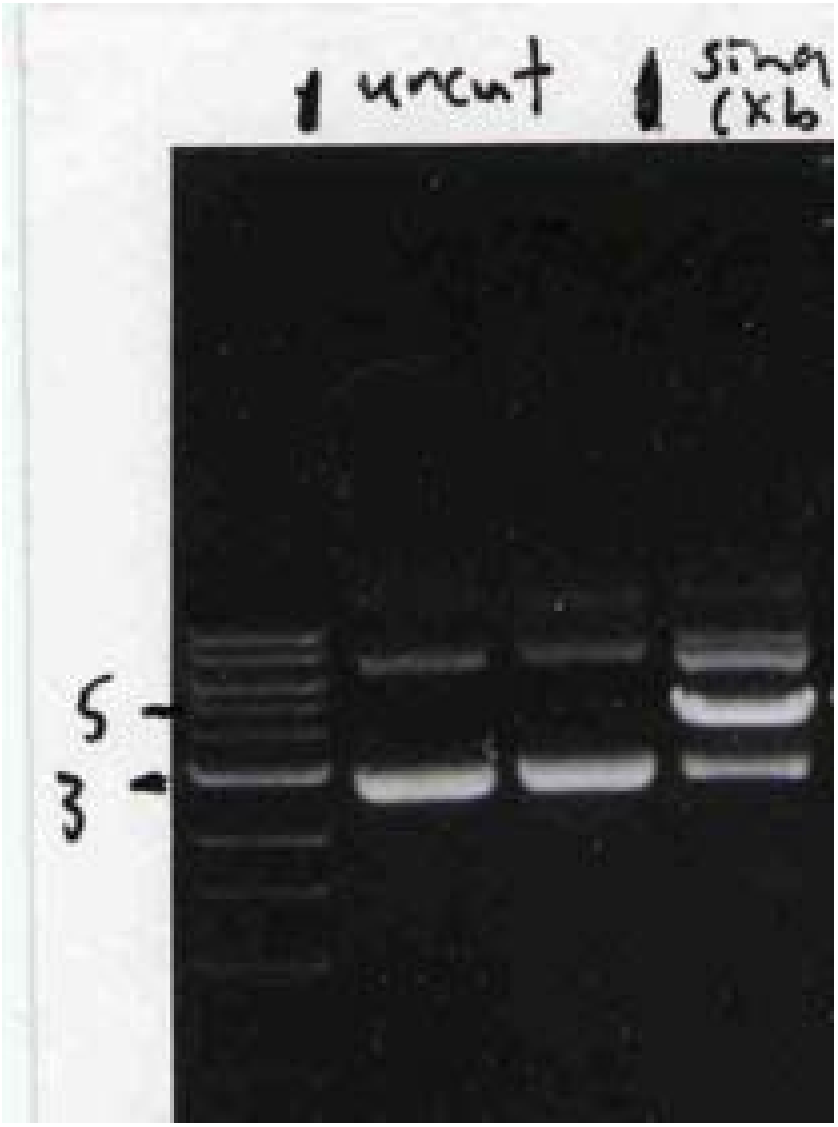
- vector with enzyme 1
- vector with enzyme 2
- vector with enzymes 1+2
- vector with *no* enzymes



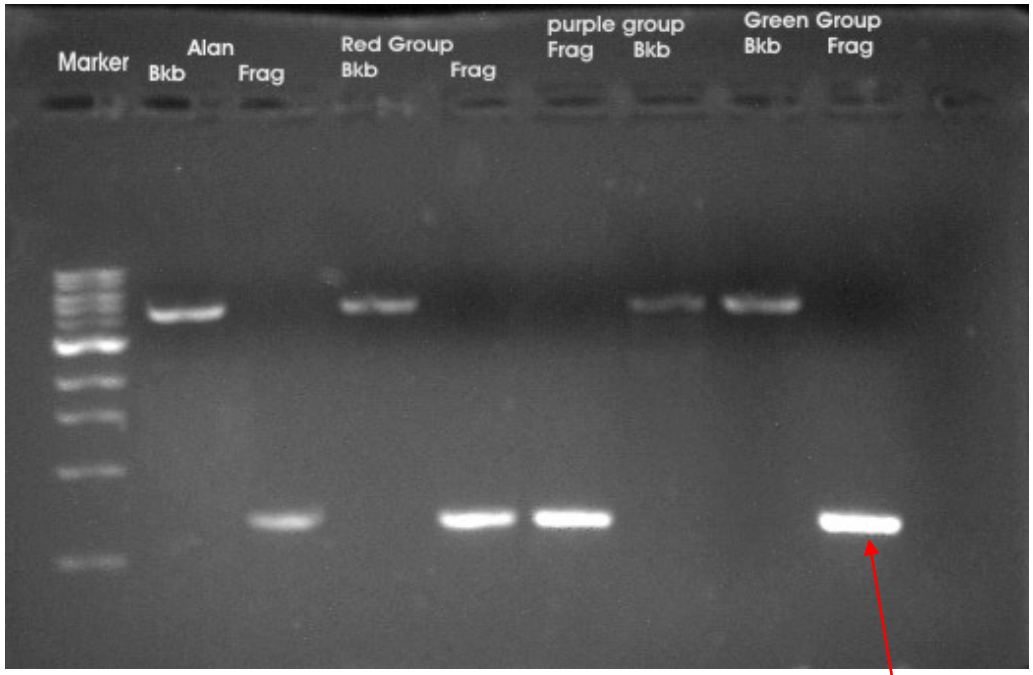
Your Data

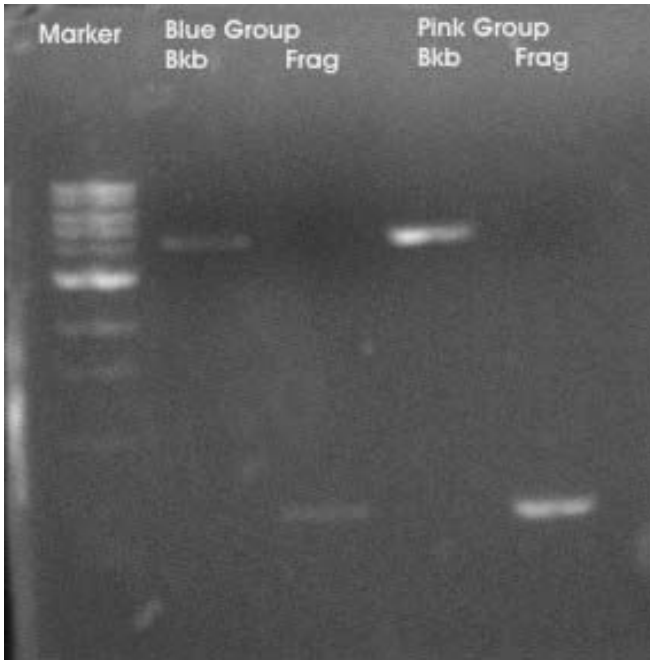
1 Uncut pCX-NNX
2 pCX-NNX XbaI
3 pCX-NNX EcoRI
4 pCX-NNX XbaI + EcoRI
5 DNA Ladder
6 PCR Product XbaI + EcoRI
7 PCR Product Uncut
8 PCR no-template-control




uncut
5
3

Data from a previous year….





Why is it difficult to assess the quantity here?

Expecting the Unexpected

What might go wrong in your ligation? What controls do you want to have?

Need to be sure your plates are actually selecting for cells that carry the drug resistance marker.

May have uncut plasmid and get original vector back.

May have singly cut vector that recloses.

May get strange products, like multiple inserts.

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