

MOD1 – DNA ENGINEERING

Engelward, Fall 2009

Day 5

Lecture 1: Intro to importance of HR
Polymerases & PCR

Lecture 2: How HR works
Overview of experiments & discussion of controls (single digests)

Lecture 3: Why understanding matters: HR & BRCA2
Overview; Running an agarose gel; Purification from a gel; Discussion of controls

Lecture 4: Exploiting Scientific Understanding for Engineering:
Achilles heel/Parp Inhibitors & Drug delivery
Ligase and transformation & Data discussion (purified fragments)

Lecture 5: DNA Engineering in Mammals: Gene Targeting & Transgenics (Sonoda prep)
Overview & Strategies for DNA analysis

Lecture 6: DNA Engineering: Conditional Expression (Sonoda Prep)

Lecture 7: DNA Engineering Reveals HR Function: Discussion of Sonoda

Lecture 8: Flow Cytometry: How it works and how to do it

Going from Understanding to Solutions

-Exploiting Understanding of HR
for genetic engineering

Mod1 Overview: Methods and Logic

-Logic for steps so far
-Strategy for analysis

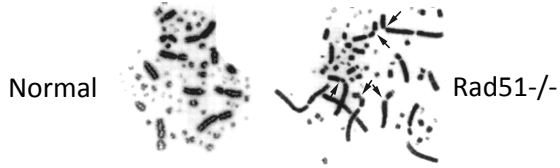
Why you owe Your Youthfulness to Homologous Recombination...

Loss of Helicase → Faulty Recomb.



Werner's
Syndrome

Why you owe Your Life to Homologous Recombination...



Turn Off Homologous Recombination
→ Chromosomes Fall Apart

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

Why you owe Your Health to Homologous Recombination...



Defective Homologous Recombination
→ Cancer

Why you owe better Cancer Treatments to HR...



New drug targets PARP
to kill BRCA2-/- tumor cells

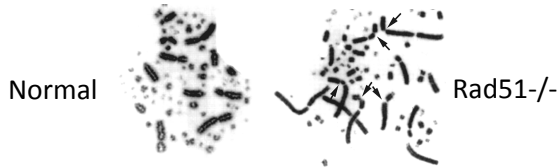
Nanocell for Drug Delivery



CERULEAN Pharma Inc.
Pioneering the Field of Nanobiology



Why you owe Your Life to Homologous Recombination...



But how do we that cells cannot survive without HR?

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

To understand how the experiments were done to show that HR is essential, you need to understand:

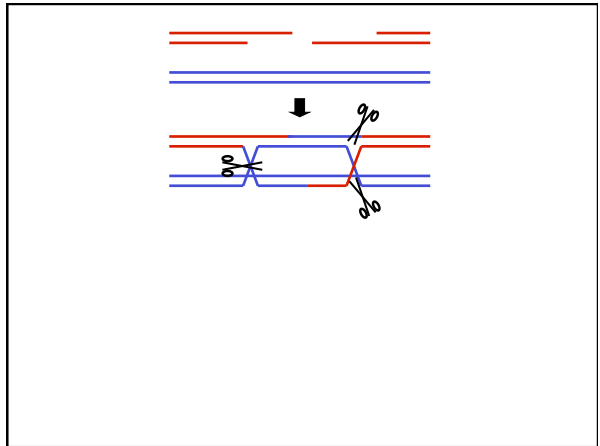
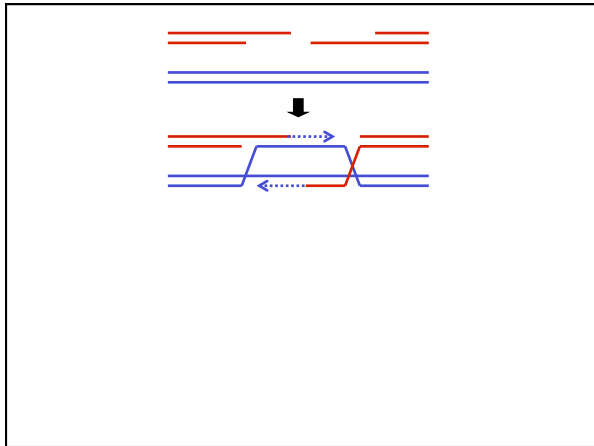
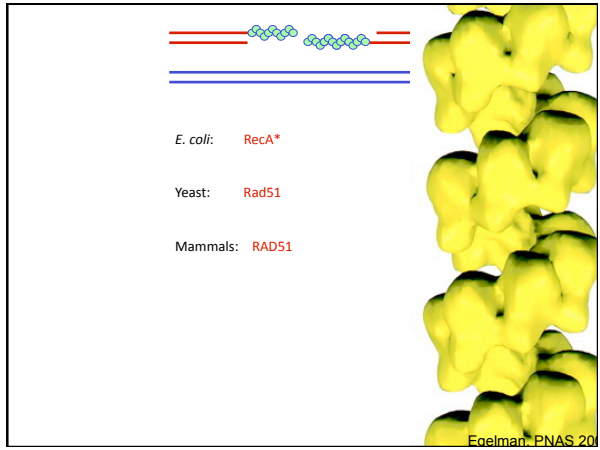
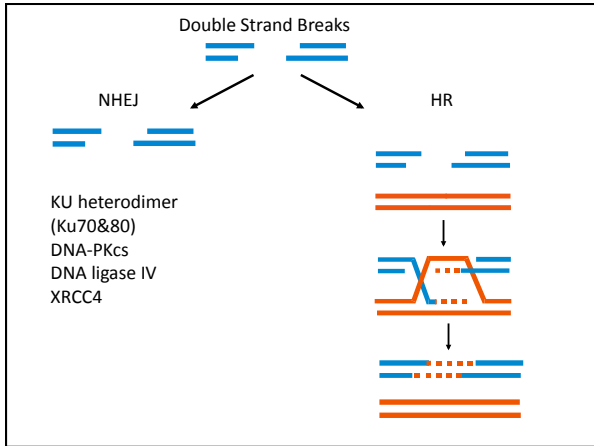
- a) Gene Targeting
- b) Conditional Expression
- c) Cell Cycle Analysis by Flow Cytometry

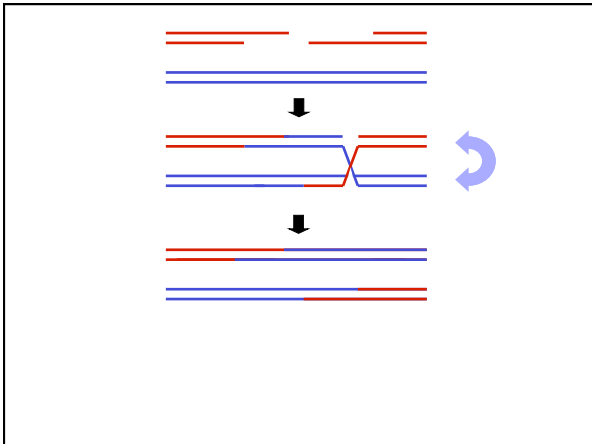
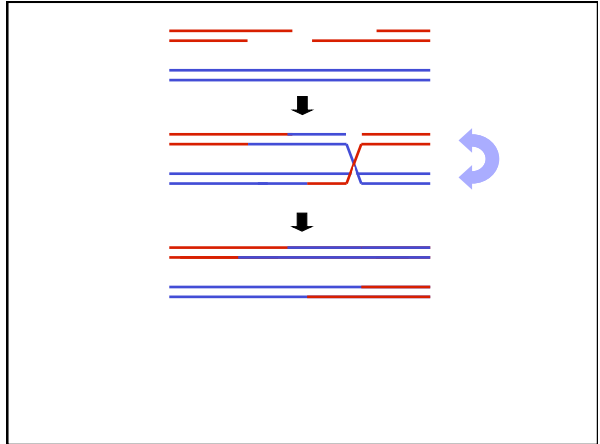
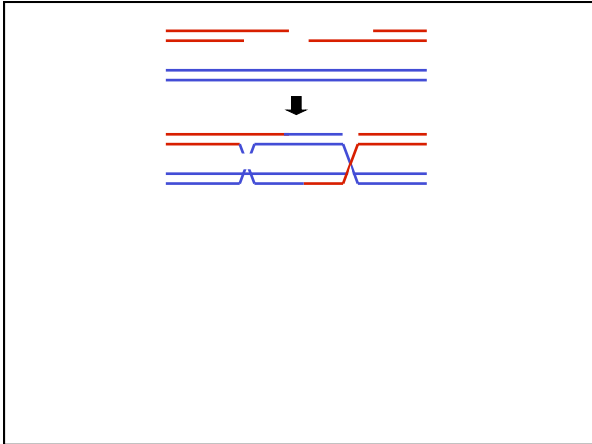
Gene Targeting

Gene Targeting is all about exchanging DNA...

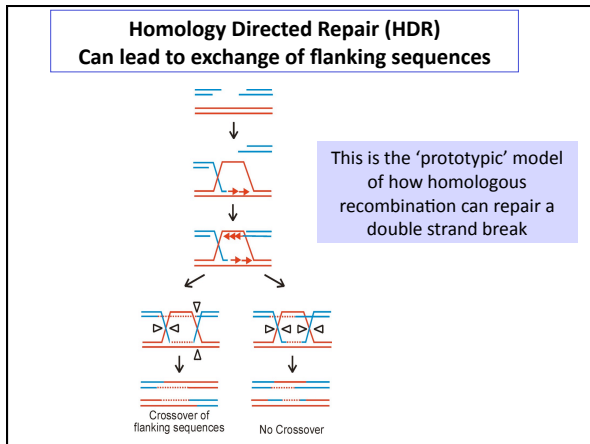
But how do we swap one piece for another?

See SDSA Animation
by Justin Lo





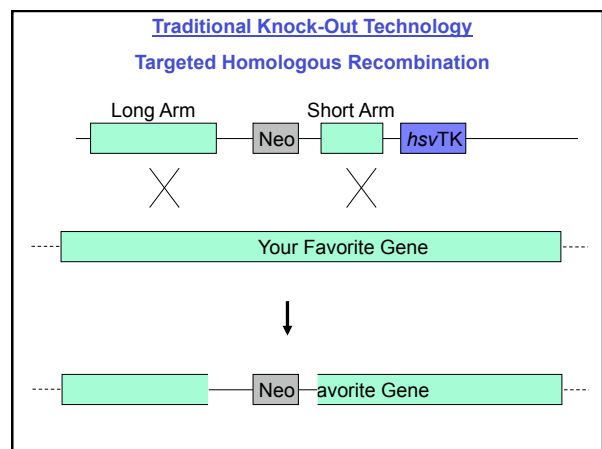
**See Prototypic Model Animation
by Justin Lo**

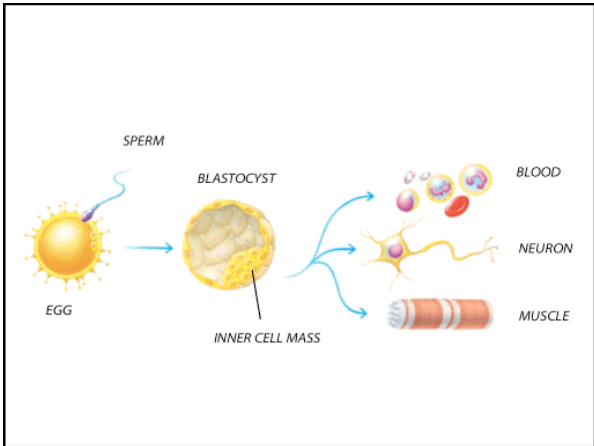
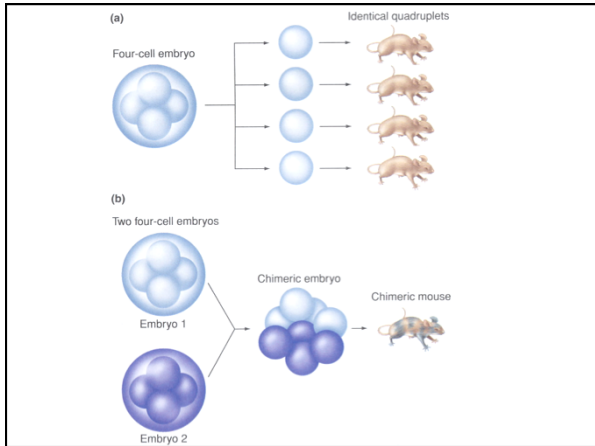
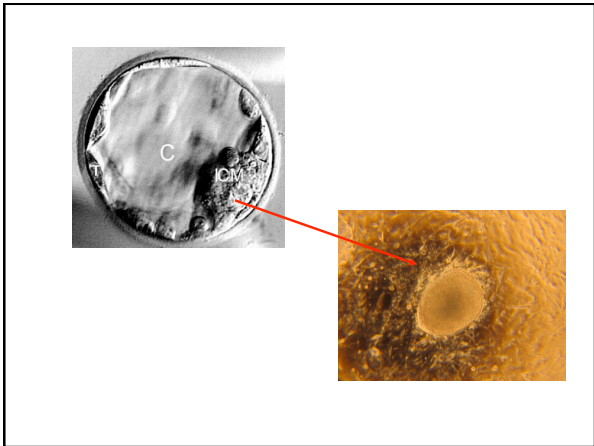
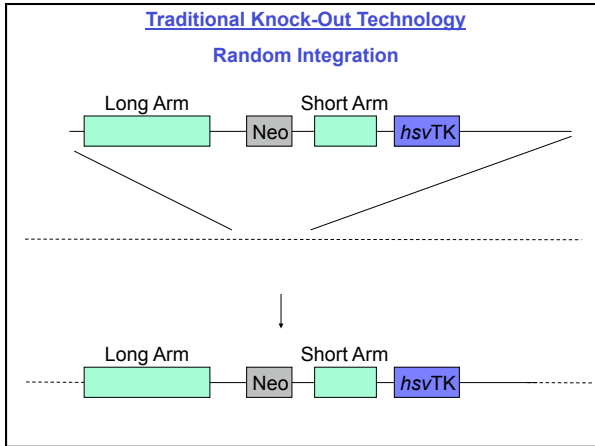


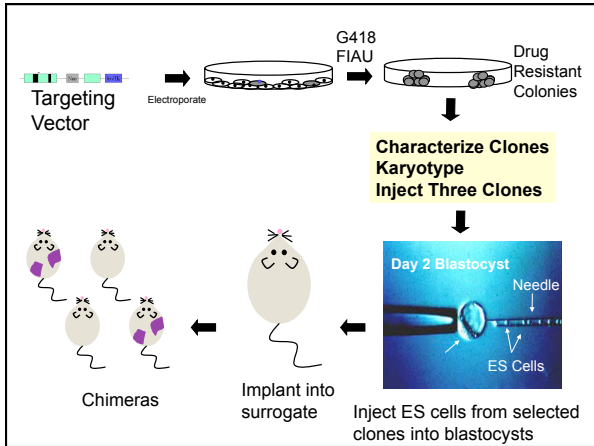
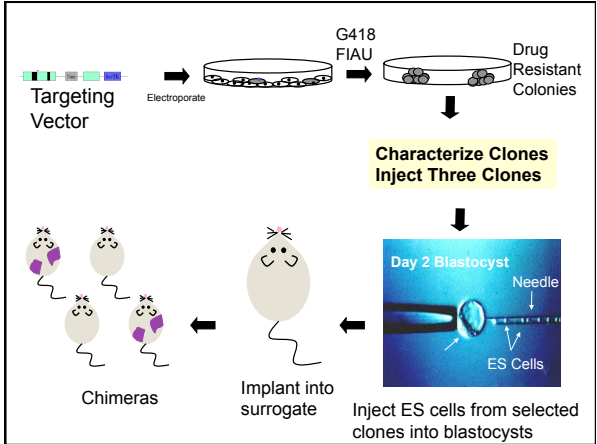
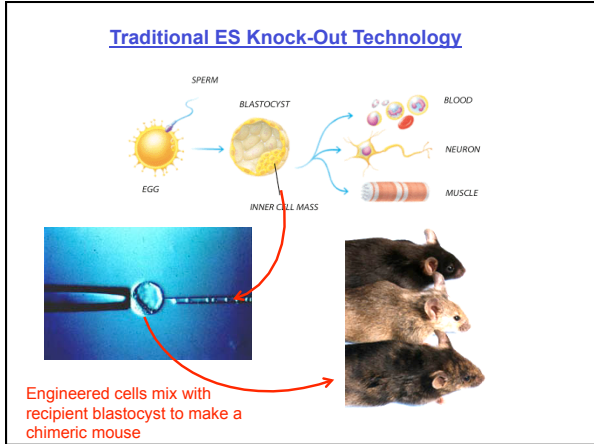
How can we exploit our understanding of HR & NHEJ for Genetic Engineering?

Genetic Engineering in Mice:

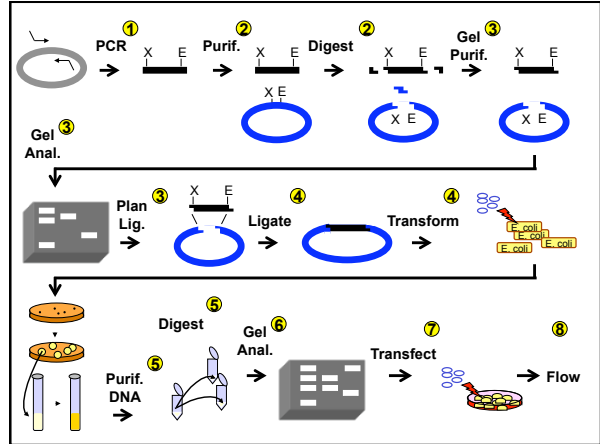
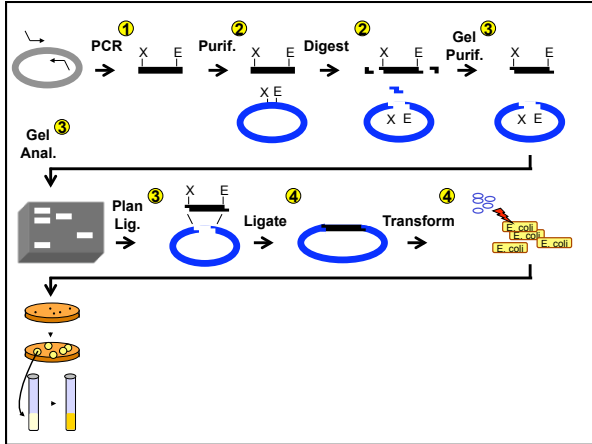
- 1) Gene Targeting
 - Turning genes on and off
- 2) Transgenics
 - Inserting genes



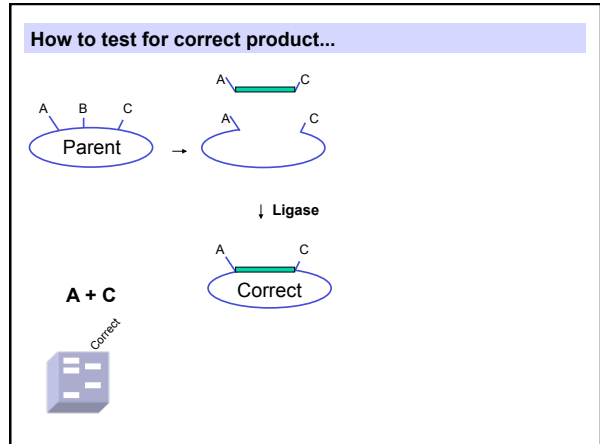




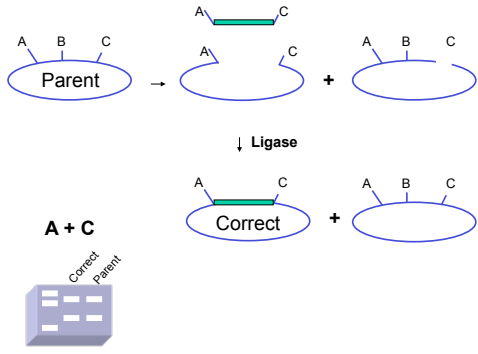
**Methods & Logic
For Mod1**



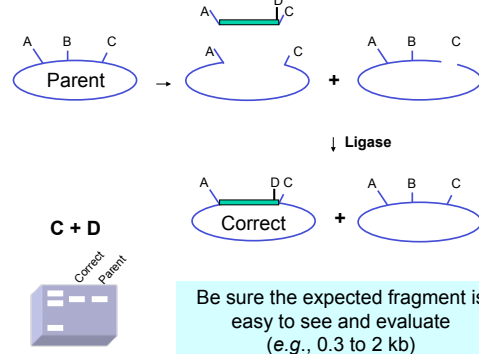
How can you test to make sure your vector is correct?



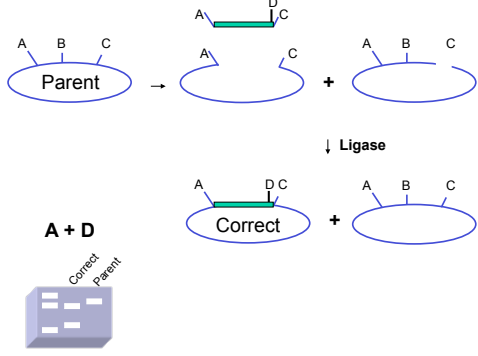
How to test for correct product...



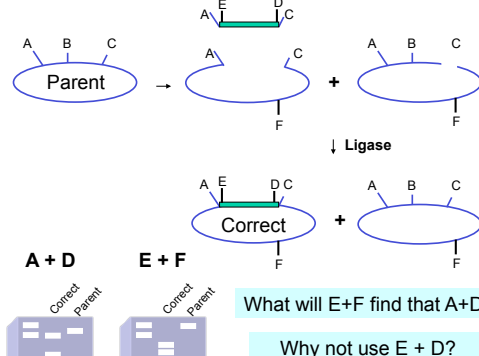
How to test for correct product...



How to test for correct product...



How to test for correct product...



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