MOD1 – DNA ENGINEERING

Fall 2010

Day 1

- **About this Module**
  - Goals for this Module
  - Brief background: Homologous recombination is not just for meiosis!
  - Overview of the Experiment
- **Today's Experiment: Design Primers and Perform PCR**
  - Brief Review of DNA Replication
  - Chemistry of nucleotide addition (5' vs 3' end)
  - PCR - Cycling
  - How you can use PCR to add a restriction site to your PCR product

Module Objectives

•Experimental Design

-Quantitative Measurements
-Controls
-Experimental Variability

•Data Interpretation and Presentation Skills

-Statistics
-Data Interpretation
-Written & Oral Communication

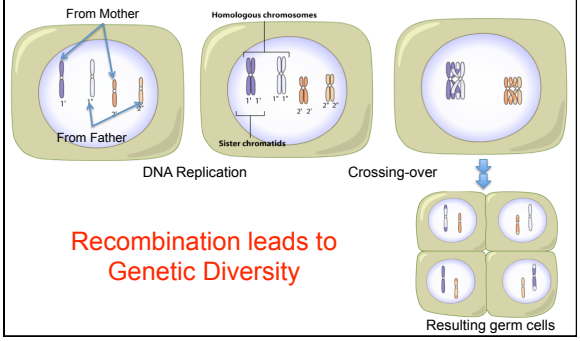
•Basic Laboratory Skills

-Record Keeping
-Sources of Error
-Basic Laboratory Equipment
-Manipulation of enzymes, DNA and mammalian cells

Homologous Recombination and You

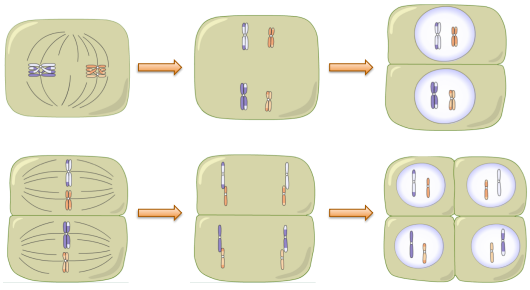
Recombination in Meiosis

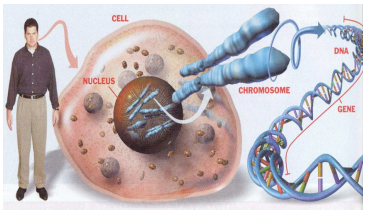
(generation of egg and sperm cells)



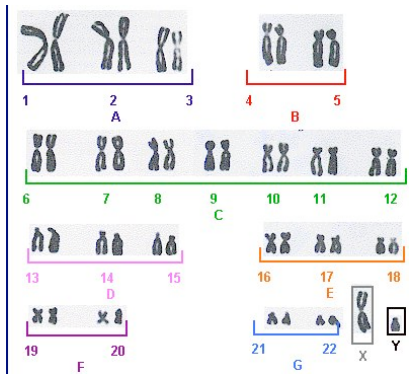
Recombination leads to Genetic Diversity

Further Meiosis - FYI



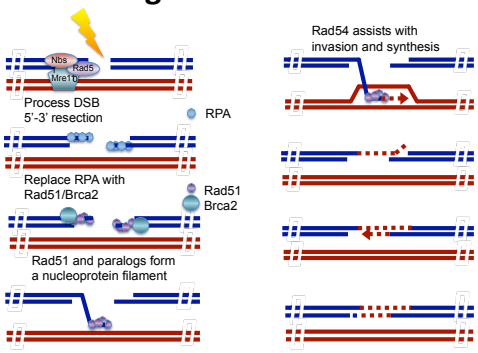


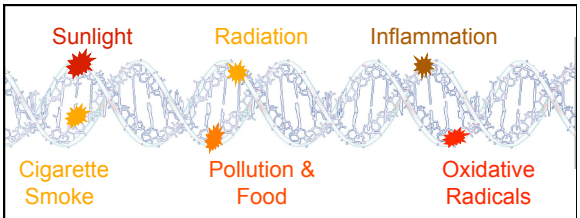
"*TIME*" Nov., 1999



http://homepages.uel.ac.uk/V.K.Sieber/human.htm

Homologous Recombination



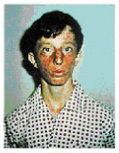


Mutations, Toxicity, Cellular Defects

Cancer, Aging, Heritable Diseases

Homologous Recombination Repairs DNA

Bloom Syndrome (BS)

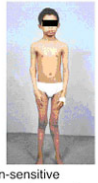


* sun-sensitive skin
* dwarfism
* immune deficiencies
* male infertility
* female subfertile
* cancer as primary cause of death before age of 30

Werner Syndrome (WS)

* develop normally in early age
* premature aging starting at puberty
* short stature
* leg ulceration
* soft-tissue calcification
* average life span = 47
* cancer and cardiovascular diseases are primary cause of death

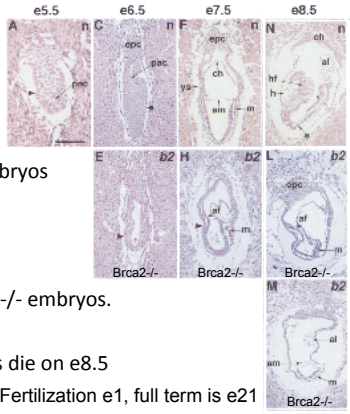
Rothmund-Thomson Syndrome (RTS)



* sun-sensitive
* hyper-pigmentation of skin
* short stature
* bone abnormality
* cancer predisposition, especially osteosarcoma

Defects in HR Promote Aging, Cancer, & Diseases

Brca2 is Necessary for Embryonic Development



Top Row: All WT embryos at different days of development.

Bottom Rows: Brca2-/- embryos.

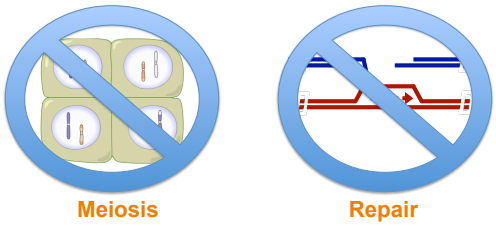
All Brca2-/- embryos die on e8.5

Fertilization e1, full term is e21

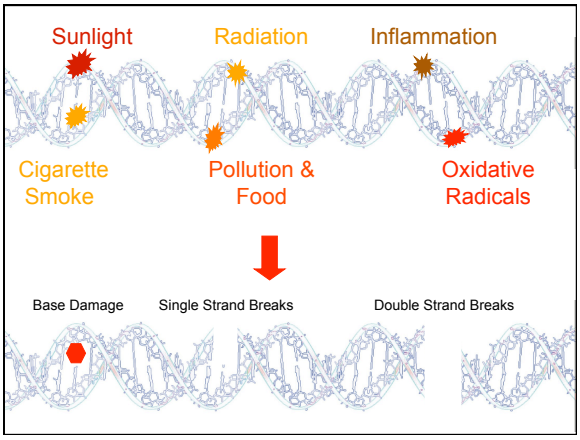
FYI

Species	Formal Name	Gene Symbol	Protein Symbol
Human	Homo sapiens	*BRCA2*	BRCA2
Mouse/ Rat	Mus musculus, Rattus norvegicus	*Brca2*	BRCA2
Frog	Xenopus laevis	*brca2*	brca2
Zebrafish	Ddanio rerio	*brca2*	Brca2

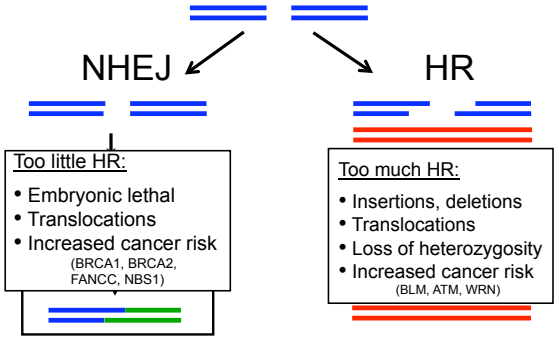
HR is Essential



Life Cannot Exist without Homologous Recombination



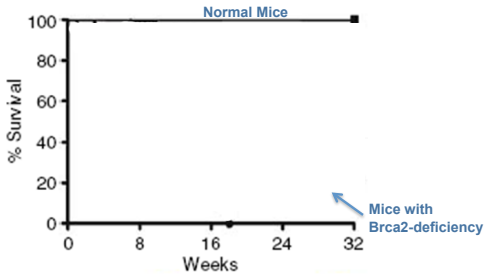
Double Strand Break Repair



Homologous Recombination Protects Cells from the Lethal Effects of DNA Damage

BRCA2 is required for neurogenesis and suppression of medulloblastoma

Pierre-Olivier Frappart, Youngsoo Lee, Jayne Lamont and Peter J McKinnon*



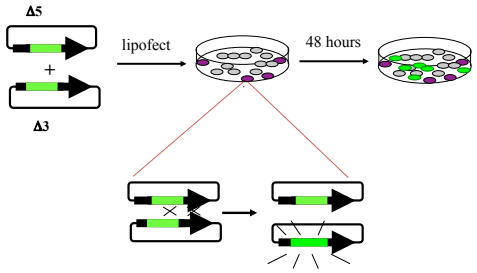
Your Experiment:

Create a plasmid that will be part of a homologous recombination assay.

Measure the frequency of cells in which homologous recombination between two plasmids gives rise to a fluorescent cell.

Test conditions that might affect the frequency of green cells!

A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells



Roadmap: Blueprint of Plasmid Construction Plan

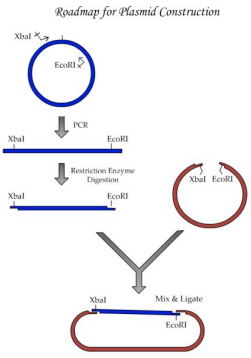
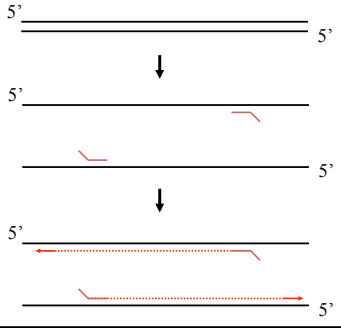


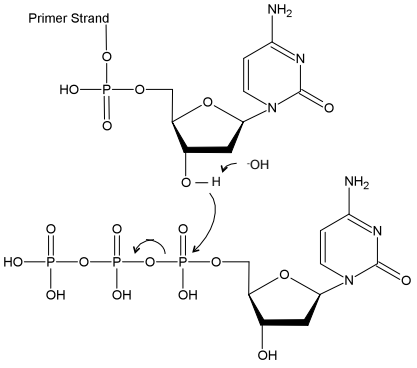
Figure by Justin Lo

PCR

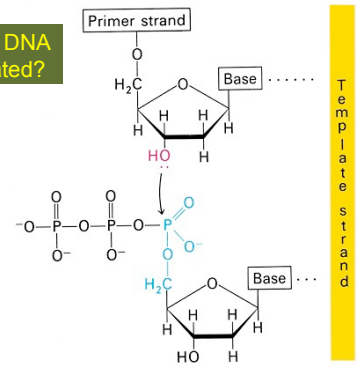
PCR

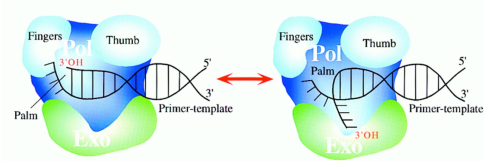
Basic Principles:





How is DNA replicated?





Proofreading prevents extension from a mismatch

Baker and Bell, Cell 1998

Error Rates

Taq

2.1 x 10^{-4} errors/bp
(Keohavang and Thilly, 1989)

Pfu

1.6 x 10^{-6} errors/base
(Lundberg et al., 1991)

What are the components of a PCR reaction?

- Polymerase
- Template
- Primers
- dNTPs
- Mg++
- Correct buffer conditions

Primer Design

1. 17-28 bases
3. 50-60% (GC)
4. Melting Temps should be ~65-80°C
5. 3'-ends of primers should not be complementary to each other (why?)
9. Hairpins should be avoided (why?)
11. Check for 'accidental' annealing elsewhere in your target.

Why is it recommended that primers be about 50% GCs?

What would happen if there was a mismatch at the 5' end of the primer? ...the 3' end of the primer?

What would happen if the annealing temperature was too low? To high?