MOD1 – DNA ENGINEERING
Engelward, Spring 2009
Day 4

Background & Significance:
“Homology-Directed Repair” for double strand breaks

Why you owe Your Youthfulness to Homologous Recombination...
Loss of Helicase → Faulty Recomb.

Werner’s Syndrome

Going from Understanding to Solutions
- Exploiting Understanding for Drug Design
- Engineered Approaches for Drug Delivery

DNA Ligase

Mod1 Overview: Methods and Logic
- Logic for steps so far
- Your data
- Looking ahead...
Why you owe Your Life to Homologous Recombination...

Turn Off Homologous Recombination → Chromosomes Fall Apart


Why you owe Your Health to Homologous Recombination...

Defective Homologous Recombination → Cancer

Why you owe better Cancer Treatments to HR...

Cloning BRCA2

Step 2: Create Pedigrees
- Identify families with multiple cases of early onset breast cancer
- Create a record of the family history of disease
- Obtain DNA samples
Cloning BRCA2

mRNA = 11386 nts

Characterizing BRCA2

BRCA2 mutant cells fail to form Rad51 foci in response to DNA damage

Normal BRCA2

Yuan et al., Cancer Res. 1999

BRCA2 Loads Rad51 to create a nucleoprotein filament

Moynahan et al., Mol. Cell 2001

Characterizing BRCA2

BRCA2 mutant cells have reduced levels of damage-induced homologous recombination

The Jasin Lab uses assays just like yours!
BRCA2 is critical for repair of broken forks

Misrepair & Toxicity

BRCA2-/-

BRCA2 -/- Chromosomes

Normal Human Chromosomes


Rational Cancer Chemotherapy

How can we use our understanding to come up with better cancer treatments?

Science → Engineering Opportunities

What is the Achilles heel of a BRCA2 null tumor cell???
**Characterizing BRCA2**

Can we think of a way to induce fork breakdown events?

BRCA2-/-

Potentially TOXIC

**PARP Shields SSBs from Forks**

Poly(ADP-Ribose) Polymerase (PARP)

Single Strand Break (SSB)

SSBs Induce PARP Automodification

*J. Jung et al., J. Mol. Biol., 1994*

**PARP Automodification Creates Branched Structure**
PARP Shields SSBs from Forks

PARP

STOP

PARP

BRCA2-/-

NHEJ

Potentially TOXIC

Inhibition of Poly(ADP-Ribose) Polymerase in Tumors from BRCA Mutation Carriers

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Normal  
Rad51−/−

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→ Chromosomes Fall Apart


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Defective Homologous Recombination  
→ Cancer

Why you owe better Cancer Treatments to HR...

Understanding  Engineered Solutions
Nanocell for Drug Delivery

Our major goals are:

To teach strong fundamentals in laboratory science

&

To inspire

DNA Ligase
Human DNA Ligase I


Human DNA Ligase I


Methods & Logic
For Mod1

A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells

Δ5 → lipofect → 48 hours → Δ3

How do you know that your restriction enzymes actually cut the DNA?
What else is in the reaction with the digested PCR product? What effect could it have?

Why is it important to excise the DNA from the gel relatively quickly?

Why run this gel?

Your objective is a 1:4 vector:insert ratio – Why?

What if it was 1:100?
What if it was 100:1?
How do you figure out how to get a 1:4 molar ratio?
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