MOD1 – DNA ENGINEERING

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Engelward, Fall 2009

Day 2

Mod1 - What you will do:

In this module, you will create a plasmid that will be used in an assay to measure homologous recombination activity in mammalian cells.

Background & Significance:

“Homology-Directed Repair” for double strand breaks

You will need to understand this material in order to analyze your data.

About the experiments in Mod1
- how does DNA damage cause mutations
- how is recombination used to fix double strand breaks
- overview of the experiments you will be doing

Restriction Enzymes
- basics restriction enzymes

Anticipating Potential Problems & Pitfalls
- what controls are needed and why?
After damage, what might happen?

\[ \text{GC} \rightarrow \text{TA} \]
Imagine HR is initiated by the fragment on the left....
Step 1: A double stranded end has been created

Step 2: Resect the end to create a 3’ overhang

Step 3: Create a nucleoprotein filament capable of homology searching

Step 4: Search and Invade

Step 5: Polymerize DNA using invading strand with 3’OH as a primer and the homologous donor DNA as a template
Step 6: Branch Migration (Backwards)

Step 6: Branch Migration (Forwards)

Step 6: Possible Release

This process started with a two-ended DSB...
Now let's imagine the same thing happened at the other end…

Annealing

Final Steps: Filling, Trimming, Ligating

See SDSA Prototypic Model & Replication Fork

Animations by Justin Lo
Your Assay for Homologous Recombination

A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells

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Overview of the Experiments in Mod1

Where you are, and where you are going

Design Cloning Strategy; Create insert by PCR
Restriction digestion of insert and vector
Purification of insert and vector
Ligation and Transformation
Analysis of Ligation Products: DNA Purification (Minipreps) & Restriction Digestions
Learn Tissue Culture
Mammalian Cell Transfection (Lipofection)
Flow Cytometry

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Restriction Enzymes

- where they come from
- what they do
- how cells protect themselves
- how to use them


5' - G A A T T C - 3'
3' - C T T A A G - 5'

EcoRI

Structure from: Winkler et al., EMBO J., 12, 1781-1795 (1993)
How do bugs keep from chopping themselves up?
On a practical level...
Using Restriction Enzymes

- Different lengths of recognition sequences
- Different kinds of restriction enzymes (blunt/distal)
- Shared recognition sequences
- Shared overhangs
- Buffer conditions
- Storing and diluting your restriction enzymes
- Specificity (potential pitfalls!)
- Lack of activity (host cells & potential pitfalls)

Get to know your tool box!

Anticipating Problems & Pitfalls:

What might go wrong in your experiment?

Incomplete Reactions

Controls: How can you tell if your DNA has actually been cut?

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