

MOD1 – DNA ENGINEERING

Fall 2012

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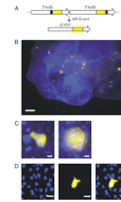
MOD1 – DNA ENGINEERING LECTURE 1

About this Module

Goals for this Module

Brief background: HR is not just for meiosis!

Overview of the Experiment



In preparation for your first experiment: Design Primers and Perform PCR

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

Key Conceptual Objectives for Mod1

- Enzymes and Reaction Conditions
- Engineering Gene Expression Vectors
- Transfection and Transformation
- Cell Culture (mammalian cells)
- Flow Cytometry

Objectives for Research Skills (Mod1)

•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

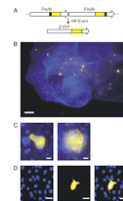
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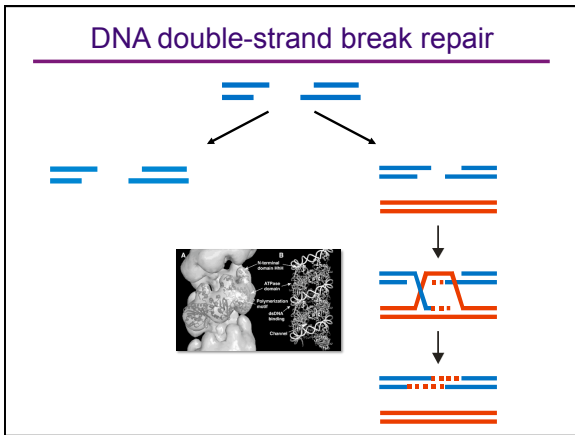
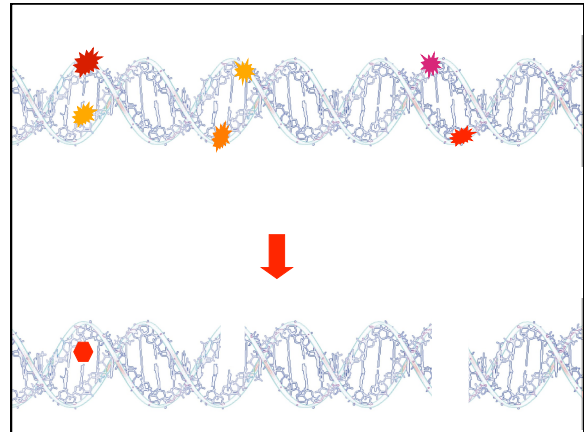
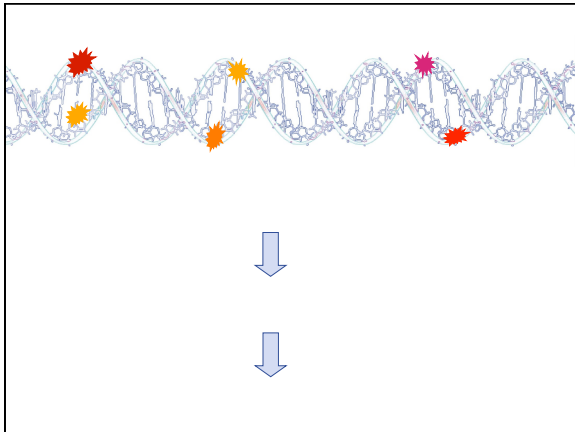
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Homologous Recombination & Why it is important to you



Homologous Recombination Repairs DNA

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

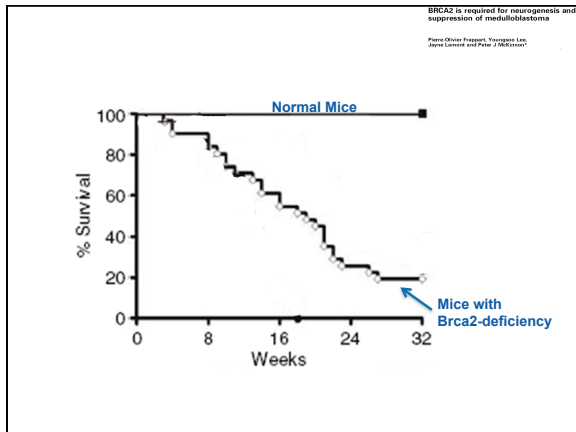
Defects in HR Promote Aging, Cancer, & Diseases

Homologous Recombination Repairs DNA

<p>Bloom Syndrome (BS)</p> <ul style="list-style-type: none"> * sun-sensitive skin * dwarfism * immune deficiencies * male infertility * female subfertile * cancer as primary cause of death before age of 30 	<p>Werner Syndrome (WS)</p> <p style="text-align: center;">15 yrs old 48 yrs old</p> <ul style="list-style-type: none"> * 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 	<p>Rothmund-Thomson Syndrome (RTS)</p> <ul style="list-style-type: none"> * sun-sensitive * hyper-pigmentation of skin * short stature * bone abnormality * cancer predisposition, especially osteosarcoma
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Defects in HR Promote Aging, Cancer, & Diseases





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- How you can use PCR to add a restriction site to your PCR product

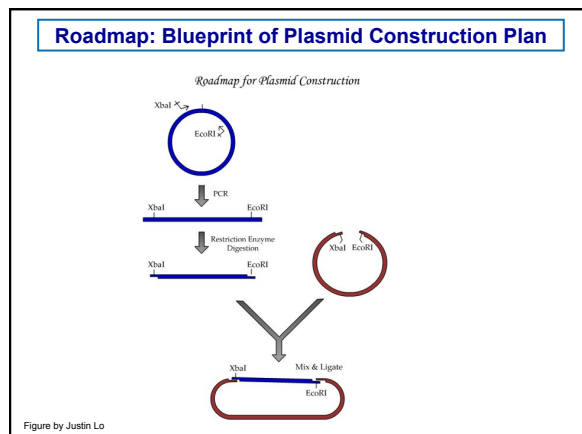
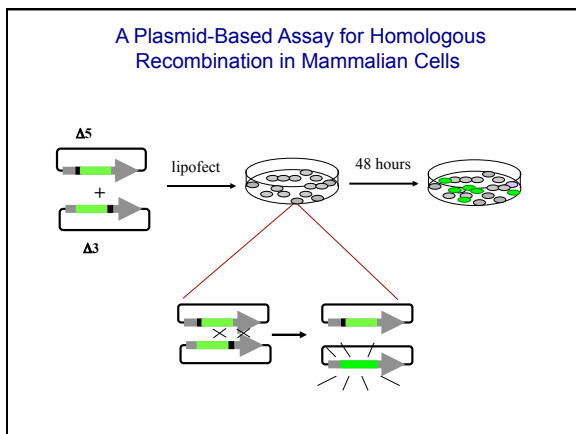
Your Experiment:

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!

Components of an Expression Cassette



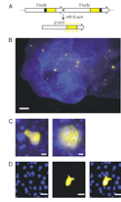
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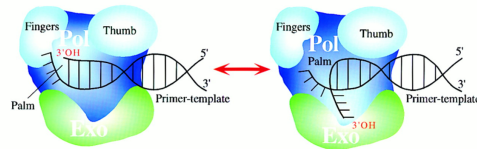
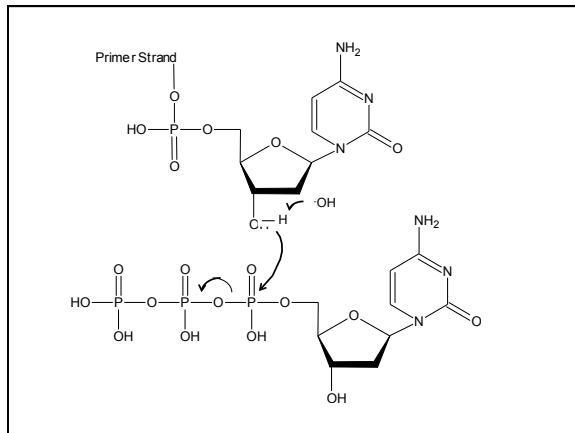
→ Review of DNA Replication

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DNA Polymerases



Proofreading prevents extension from a mismatch

Baker and Bell, Cell 1998

Replication in Action – For you to watch on your own

Howard Hughes Video on DNA Replication

http://www.youtube.com/watch?v=gL3aigv7w4A&feature=player_detailpage



DNA Replication

DNA replication animation in real time. It's amazing to think that millions of you...

CC BY 1 year ago | 122,526 views

by CoolScienceVideos

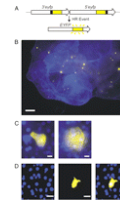
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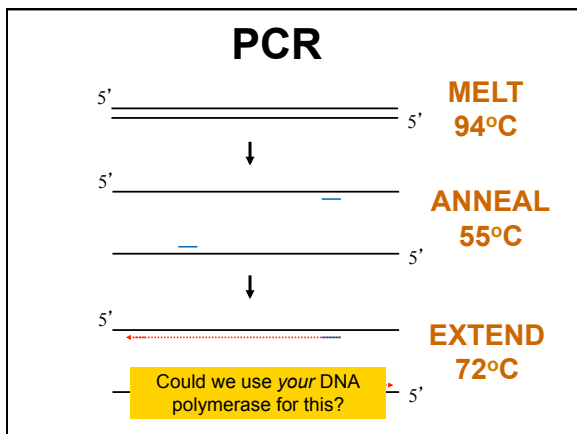
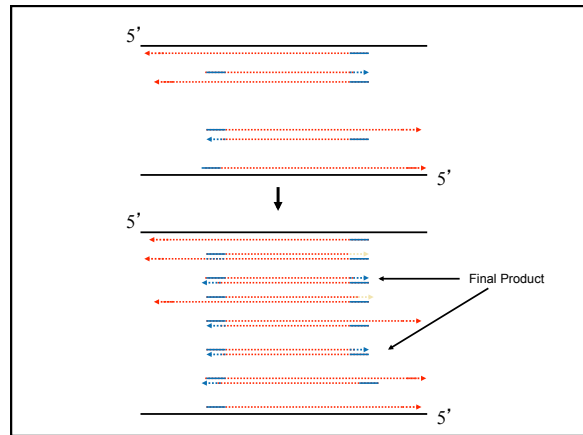
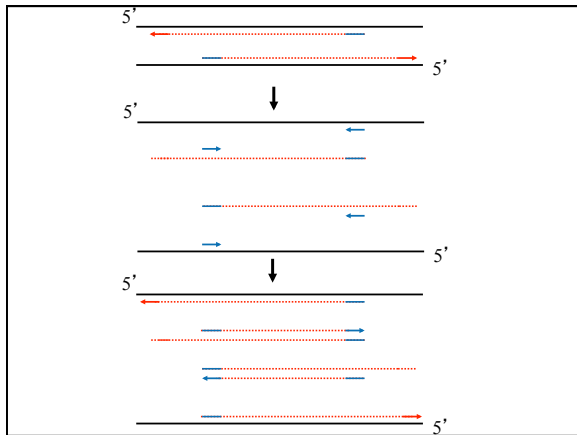
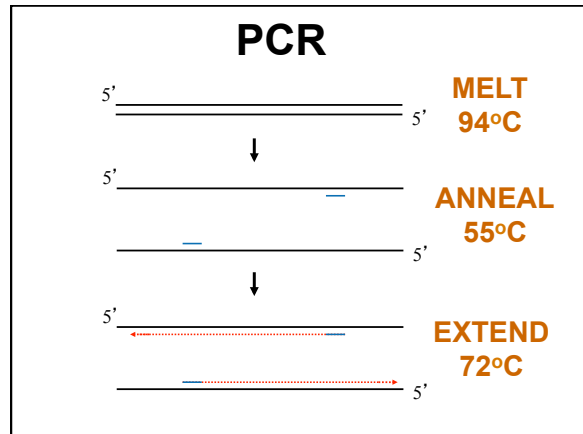
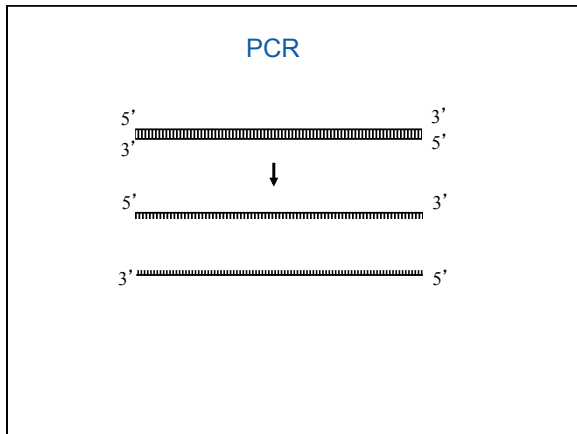
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<http://www.youtube.com/watch?v=2KoLnIwoZKU>

Polymerase Chain Reaction (PCR)
 Polymerase chain reaction (PCR) allows researchers to amplify DNA in a test tu...
HD CC 2 years ago | 149,538 views
 by DNALearningCenter

What are the components of a PCR reaction?

Primer Design

1. 17-28 bases
2. 50-60% (GC)
3. Melting Temps should be ~65-80°C
4. 3'-ends of primers should not be complementary to each other (why?)
5. Hairpins should be avoided (why?)
6. Check for 'accidental' annealing elsewhere in your target.

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

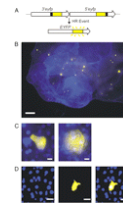
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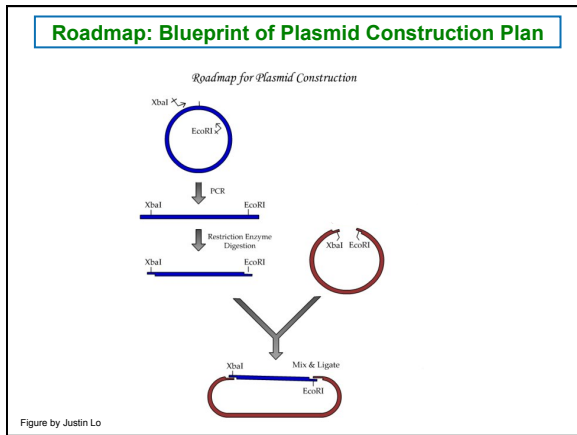
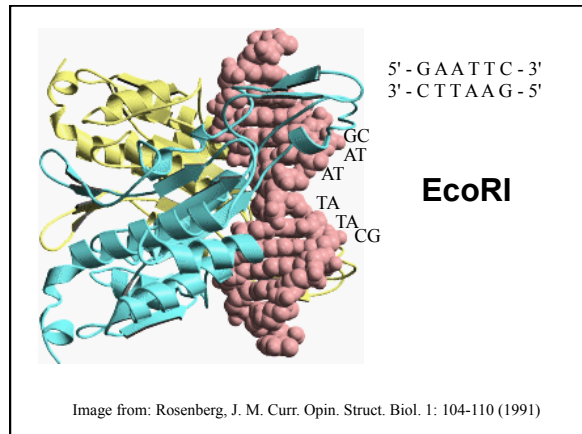
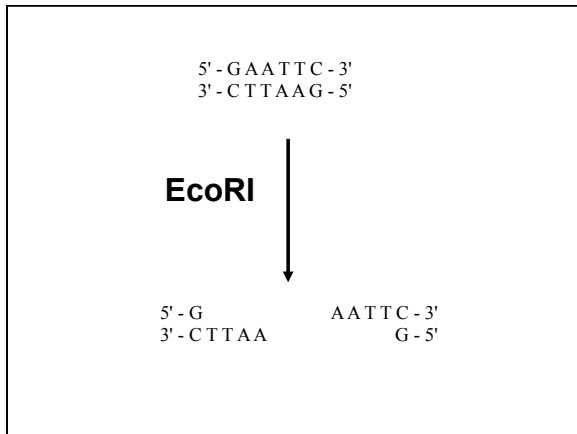
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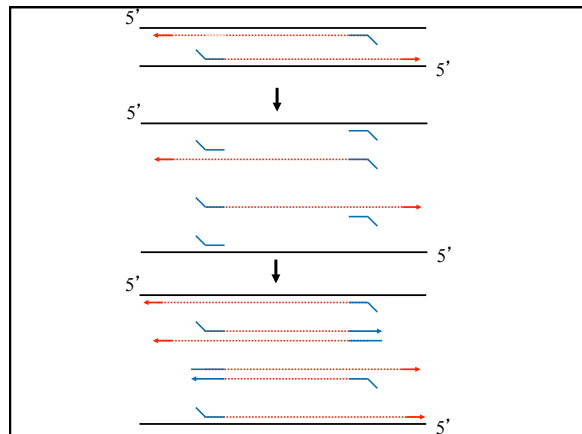
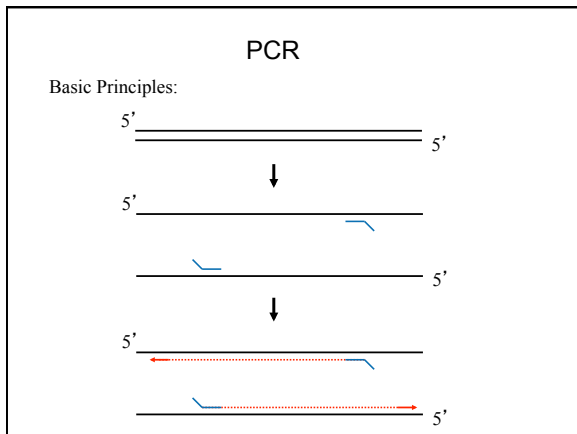
→ How you can use PCR to add a restriction site to your PCR product

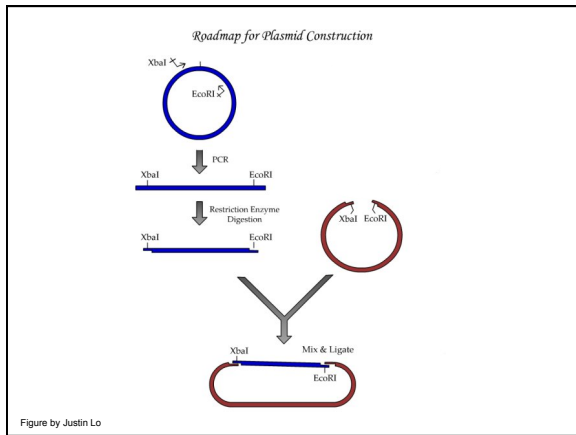
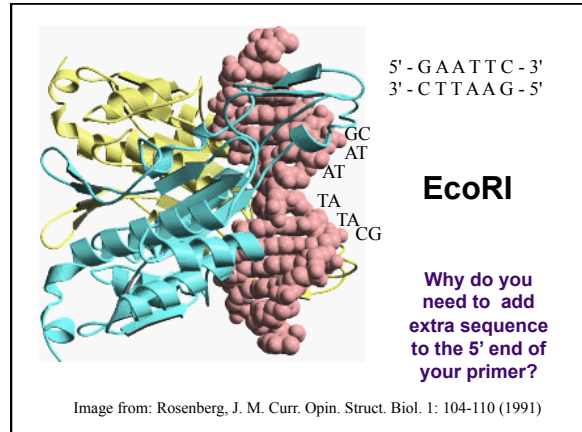
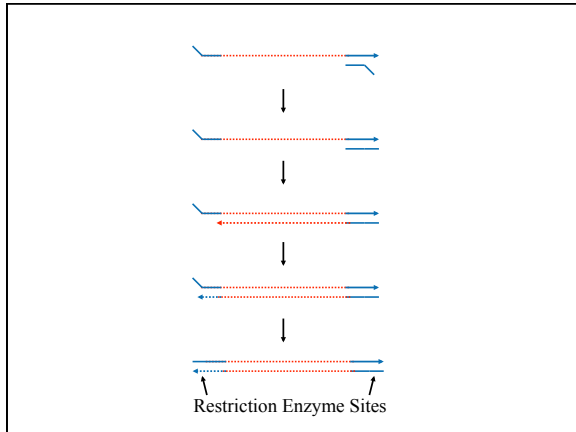
What are sticky ends and why are they useful?

Restriction Enzymes



How do you add sticky ends to your PCR product?





Why do you need a “no template control”?

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Today's Experiment: Design Primers and Perform PCR

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How you can use PCR to add a restriction site to your PCR product