MOD1 – DNA ENGINEERING

Fall 2012

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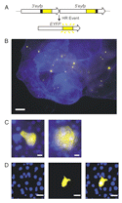
Jennifer Kay (TA)

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

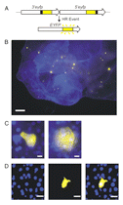
Key Conceptual Objectives for Mod1

- 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

MOD1 – DNA ENGINEERING LECTURE 1



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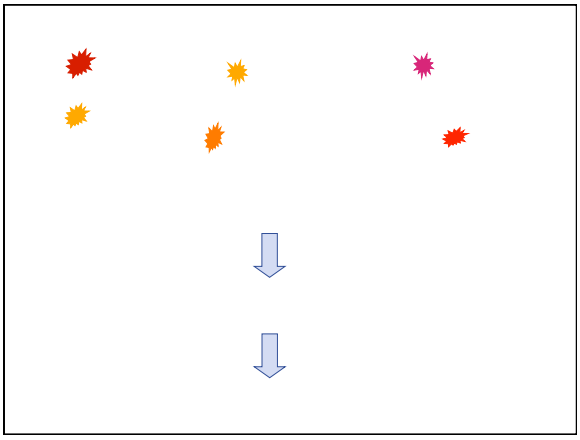
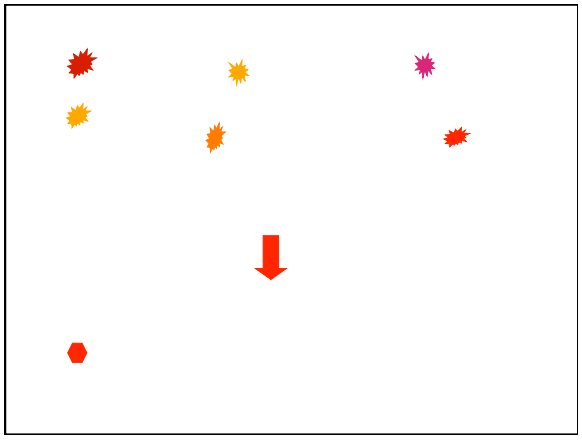
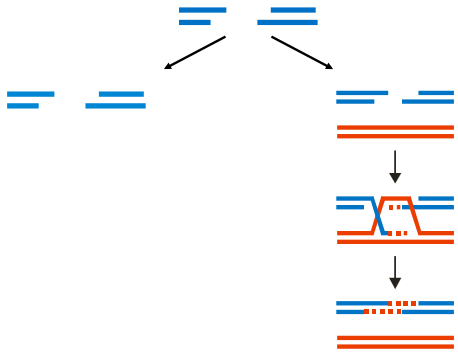
Homologous Recombination & Why it is important to you





DNA double-strand break repair



Homologous Recombination Repairs DNA

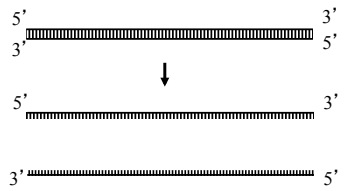
Defects in HR Promote Aging, Cancer, & Diseases

Homologous Recombination Repairs DNA

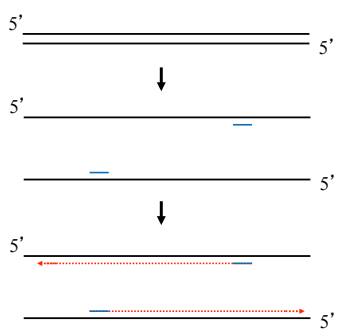
Defects in HR Promote Aging, Cancer, & Diseases

Why else should you care about HR?

PCR



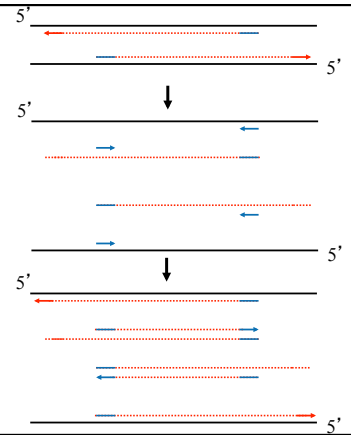
PCR

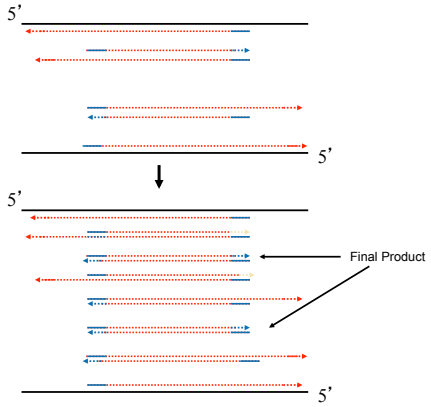


MELT
94°C

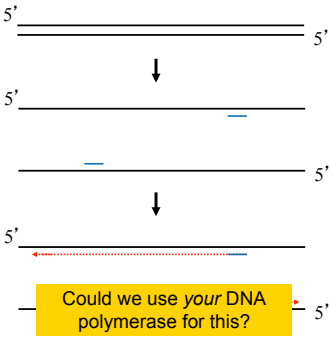
ANNEAL
55°C

EXTEND
72°C





PCR



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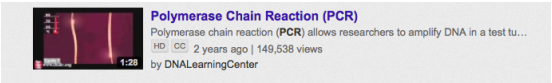
Could we use *your* DNA polymerase for this?

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



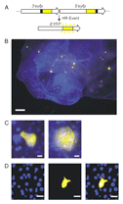
What are the components of a PCR reaction?

Primer Design

1. 17-28 bases
2. 50-60% (GC)
3. Melting Temps should be ~65-80ºC
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?

MOD1 – DNA ENGINEERING
LECTURE 1



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Brief background: HR is not just for meiosis!

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

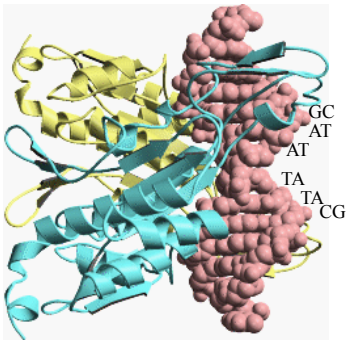
What are sticky ends and why are they useful?

Restriction Enzymes

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

EcoRI

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



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EcoRI

Image from: Rosenberg, J. M. Curr. Opin. Struct. Biol. 1: 104-110 (1991)

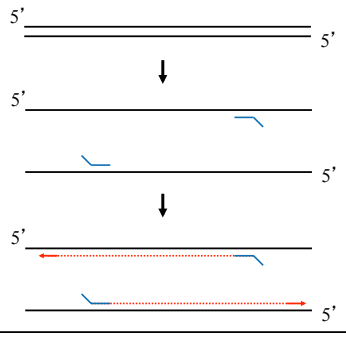
Roadmap: Blueprint of Plasmid Construction Plan

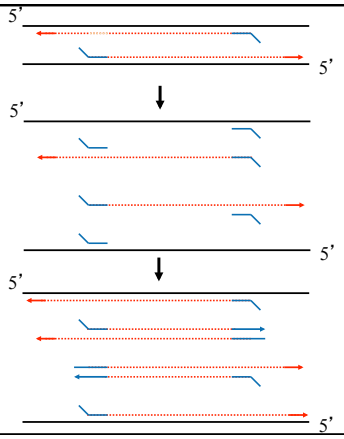
Figure by Justin Lo

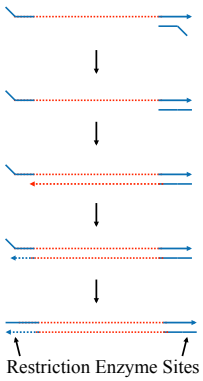
How do you add sticky ends
to your PCR product?

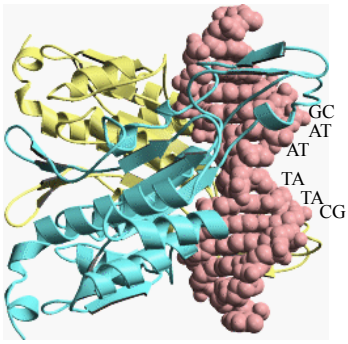
PCR

Basic Principles:









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

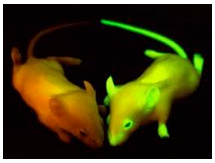
EcoRI

Why do you need to add extra sequence to the 5' end of your primer?

Image from: Rosenberg, J. M. Curr. Opin. Struct. Biol. 1: 104-110 (1991)

Figure by Justin Lo

Why do you need a "no template control"?



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