

## MOD1 – DNA ENGINEERING

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Engelward, Fall 2009

**Day 2**

### **About the experiments in Mod1**

- how does DNA damage cause mutations
- how is recombination used to fix double strand breaks
- how your two-plasmid assay works
- overview of the experiments you will be doing

### **Restriction Enzymes**

- basics restriction enzymes

### **Anticipating Potential Problems & Pitfalls**

- what controls are needed and why?

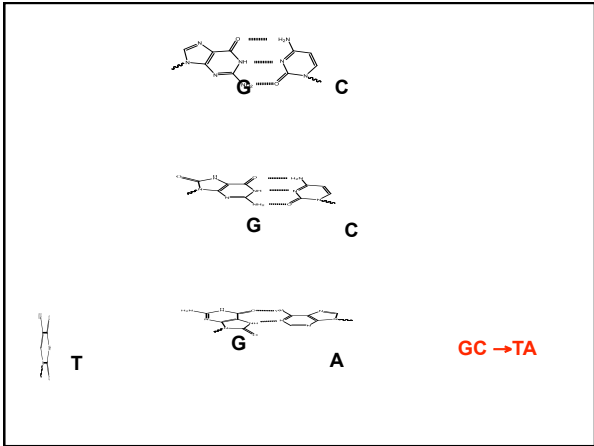
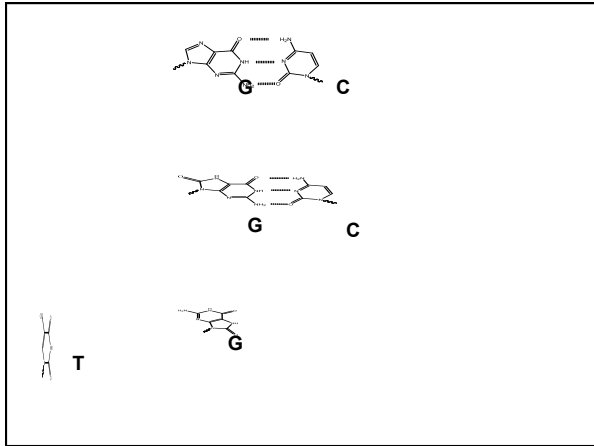
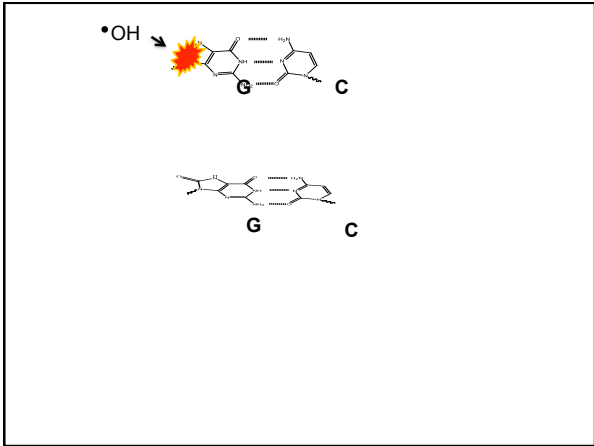
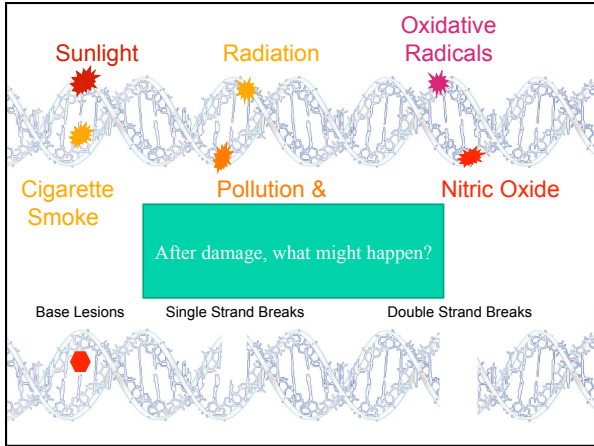
### **Mod1 - What you will do:**

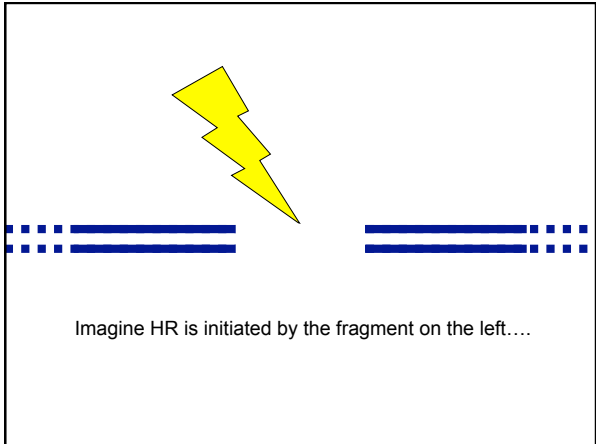
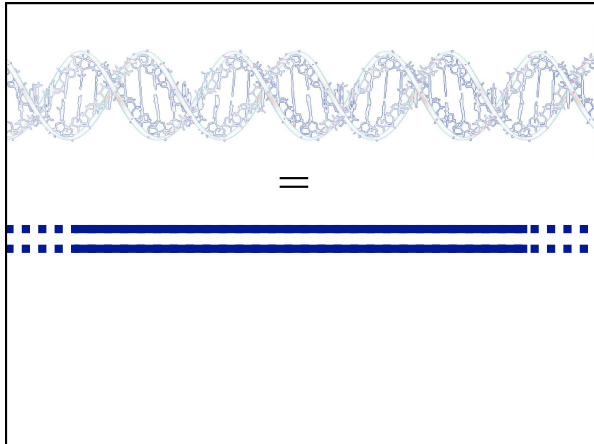
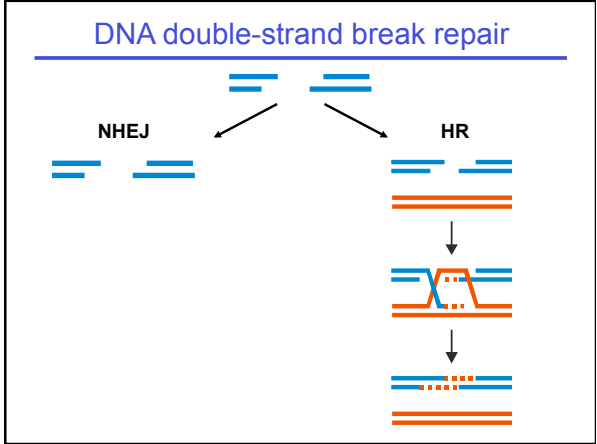
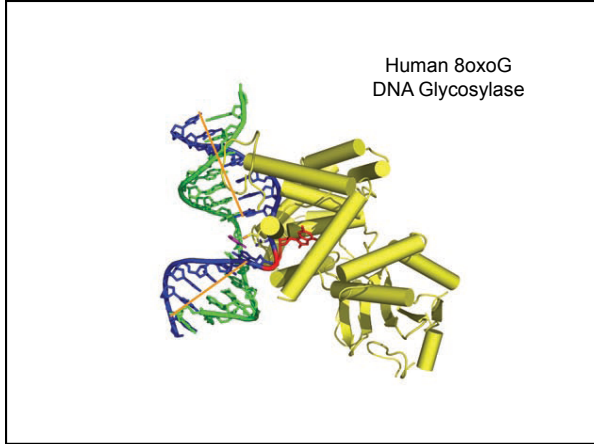
**In this module, you will create a plasmid that will be used in an assay to measure homologous recombination activity in mammalian cells.**

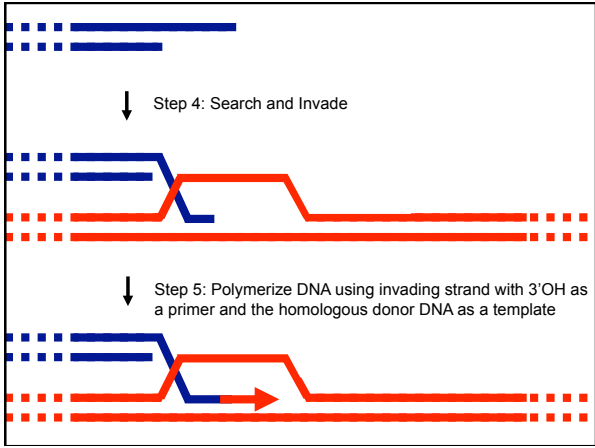
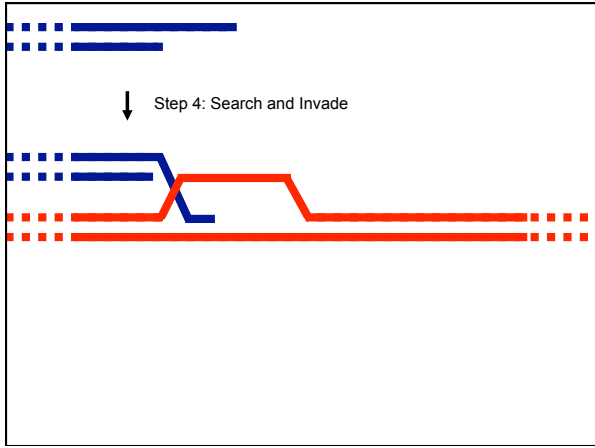
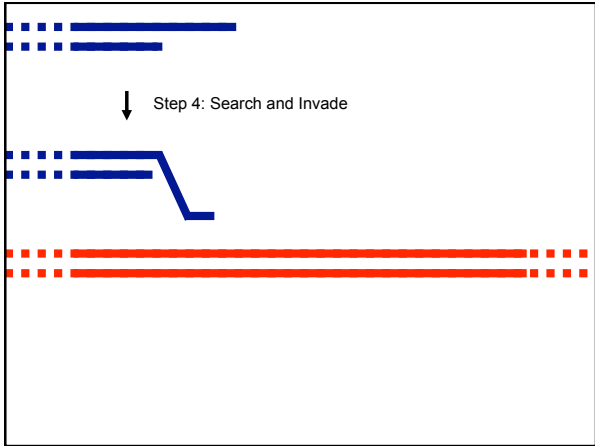
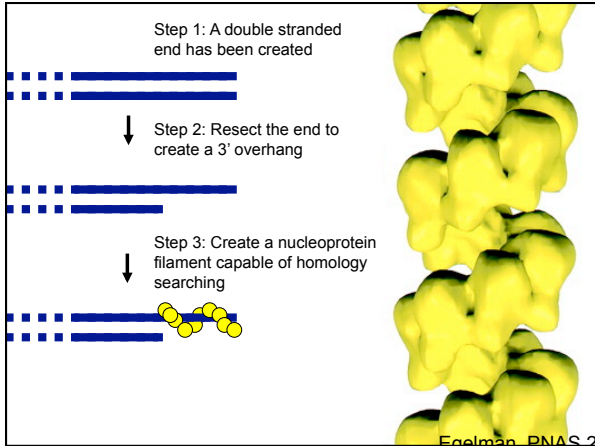
### **Background & Significance:**

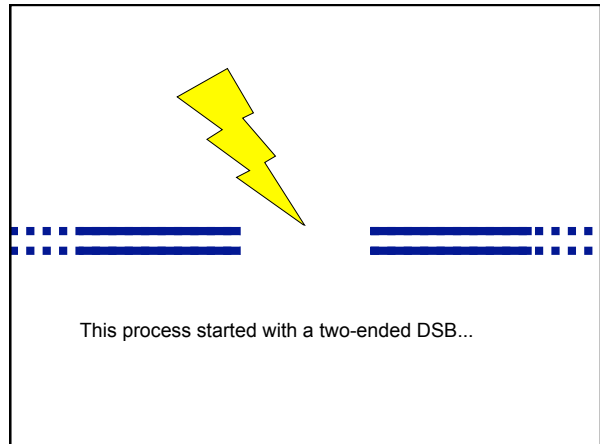
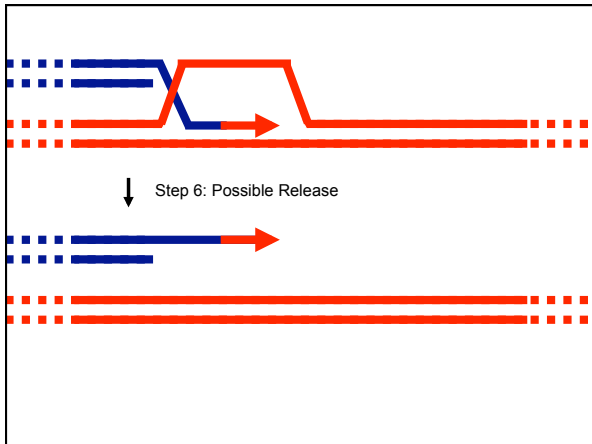
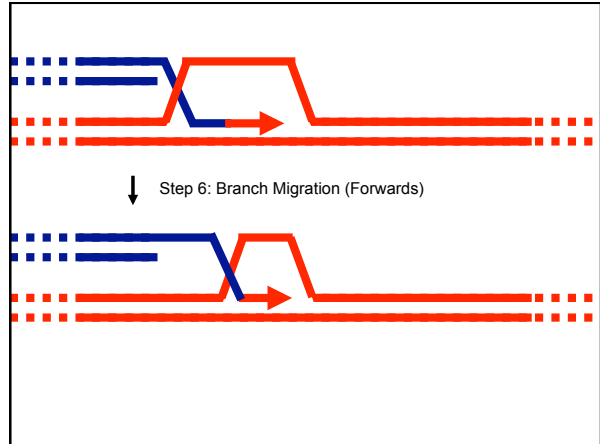
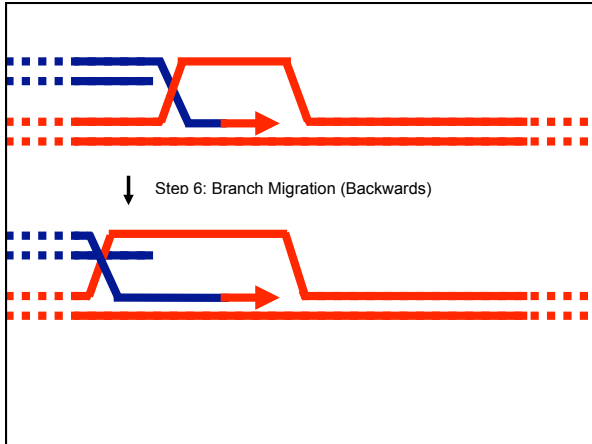
“Homology-Directed Repair” for double strand breaks

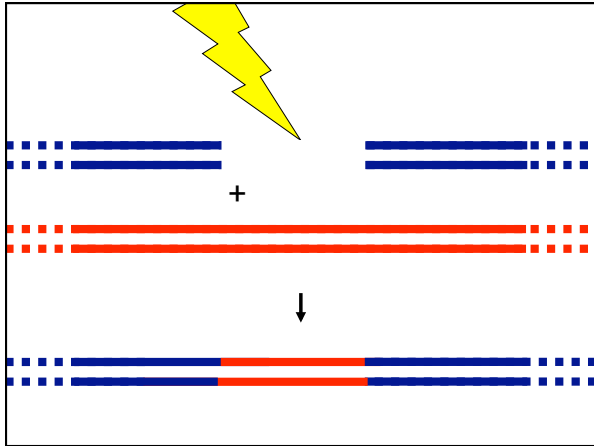
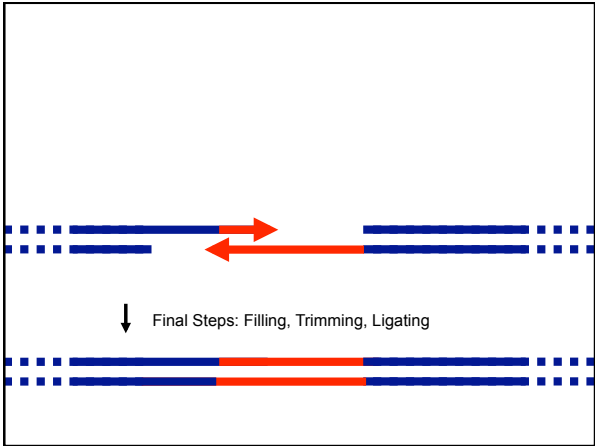
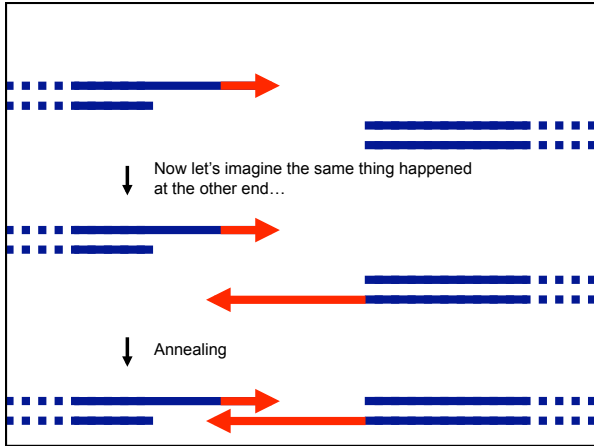
You will need to understand this material in order to analyze your data.



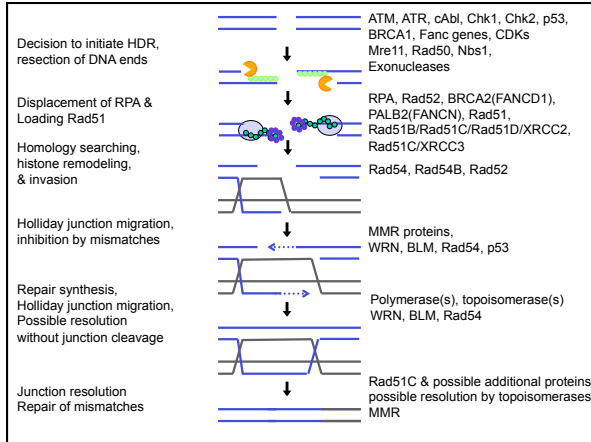




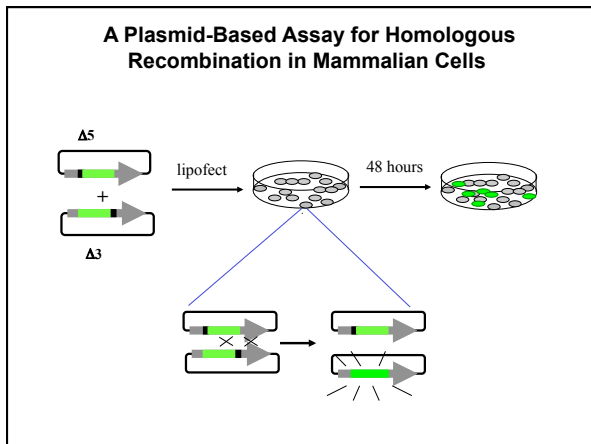




See SDSA  
 Prototypic Model  
 & Replication Fork  
 Animations by Justin Lo



## Your Assay for Homologous Recombination



- About the experiments in Mod1**
- how does DNA damage cause mutations
  - how is recombination used to fix double strand breaks
  - how your two-plasmid assay works
  - overview of the experiments you will be doing
- Restriction Enzymes**
- basics restriction enzymes
- Anticipating Potential Problems & Pitfalls**
- what controls are needed and why?

## Overview of the Experiments in Mod1

Where you are,  
and where you are going

### Construction of the $\Delta 5$ Plasmid

*Roadmap for Plasmid Construction*

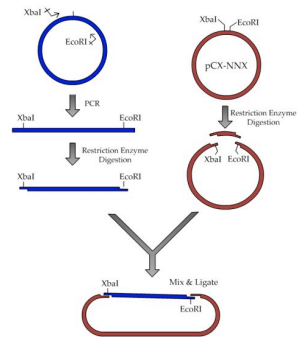


Figure by Justin Lo

**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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**Anticipating Potential Problems & Pitfalls**

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## Restriction Enzymes

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

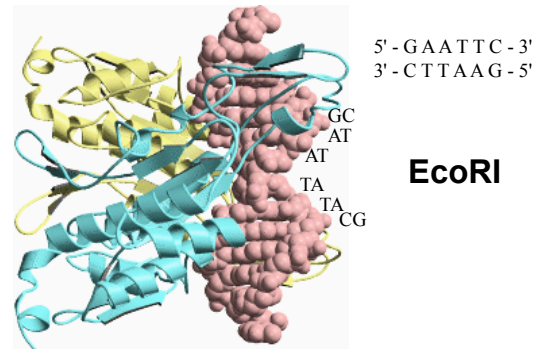
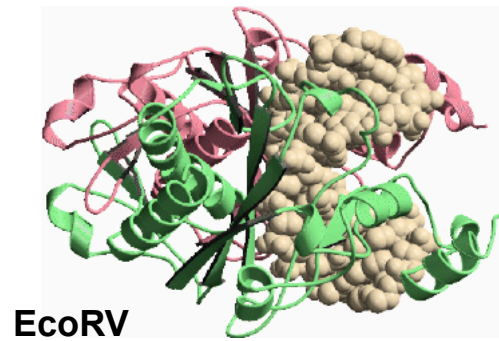
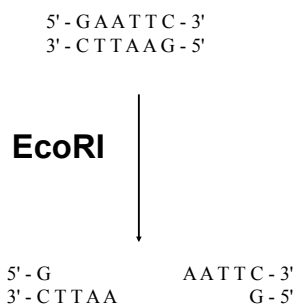


Image from: Rosenberg, J. M. *Curr. Opin. Struct. Biol.* 1: 104-110 (1991)



Structure from: Winkler *et al.*, *EMBO J.*, 12, 1781-1795 (1993)

How do bugs keep from chopping themselves up?

“Cognate Methyltransferases”

*M. HaeIII*

5' -GGCC-3'  
3' -CCGG-5'

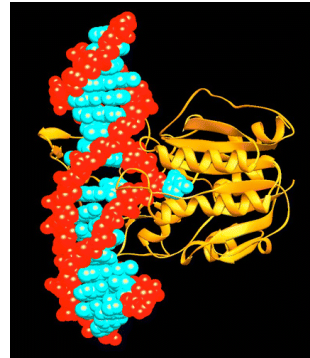
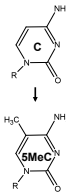
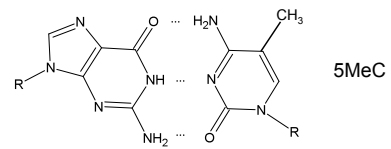
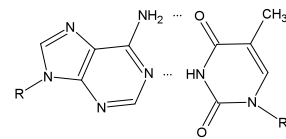
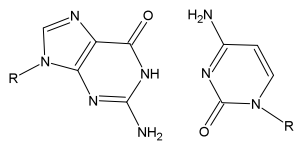
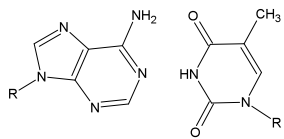
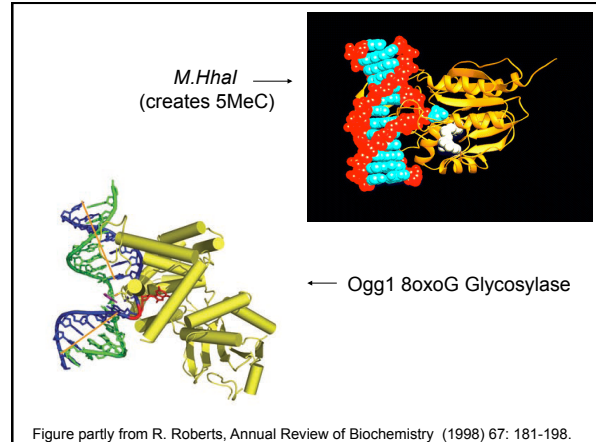
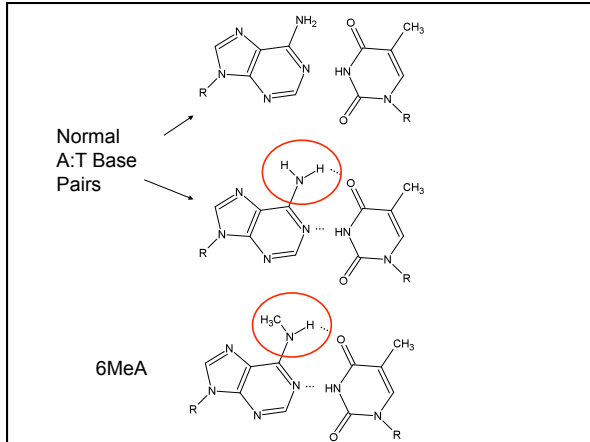


Figure from R. Roberts, Annual Review of Biochemistry (1998) 67: 181-198.





### 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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