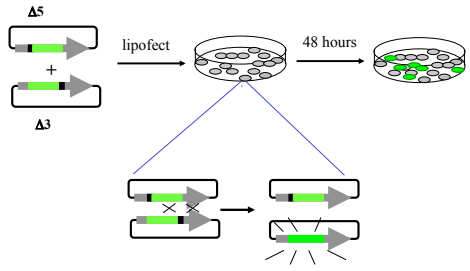
# MOD1 – DNA ENGINEERING

Spring 2010

**Day 3**

## A Plasmid-Based Assay for Homologous Recombination in Mammalian Cells




## Research theme in DNA Engineering Module

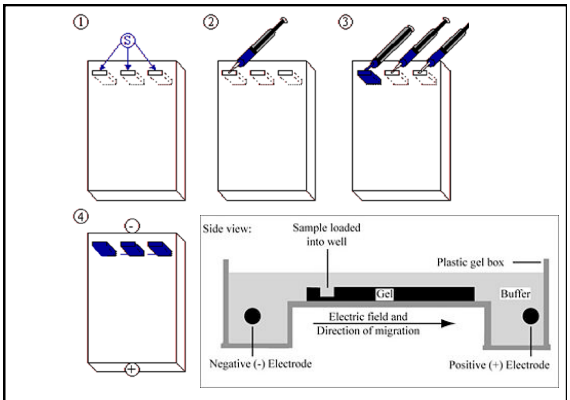
| | |
|---|---|
| Lecture 1: | Intro to importance of HR |
| Lecture 2: | How HR works |
| Lecture 3: | Why understanding matters: BRCA2 and HR |
| Lecture 4: | Exploiting Scientific Understanding for Engineering: BRCA2 targeted therapies |
| Lecture 5: | Measuring HR in genotoxicity testing, using HR in genome engineering of mice |
| Lecture 6: | Journal article discussion |
| Lecture 7: | Statistics |
| Lecture 8: | Flow Cytometry: How it works and how to do it |

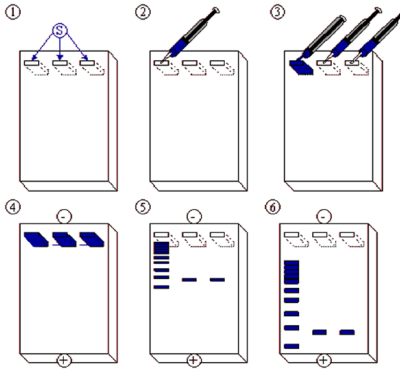
## Experimental techniques in DNA Engineering Module

| | |
|---|---|
| Lecture 1: | PCR |
| Lecture 2: | Restriction digestion |
| Lecture 3: | Agarose gel electrophoresis |
| Lecture 4: | Ligation and transformation |
| Lecture 5: | Diagnostics |
| Lecture 6: | Cell culture |
| Lecture 7: | Transfection |
| Lecture 8: | Flow Cytometry |

## Agarose Gels & Gel Purification

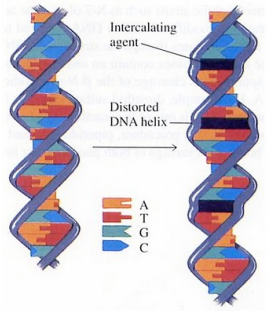
-How do we 'look' at DNA?
-How do we get our DNA out of a gel?
-What will we do with it once we get it?

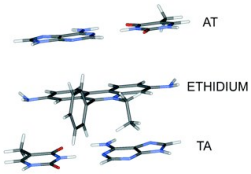




How can you see your DNA?

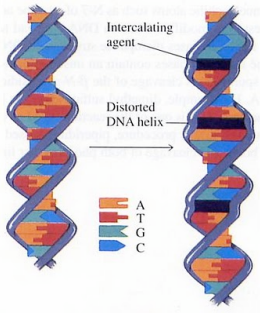
## Ethidium Bromide (EtBr)

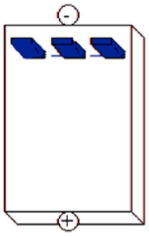




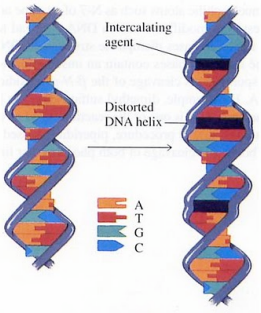
## Ethidium Bromide (EtBr)

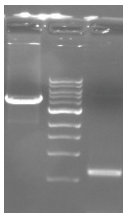


Where is EtBr in this gel?

## Ethidium Bromide (EtBr)



Why do we see "red" only where DNA is?

## Agarose Gels – How do we 'look' at DNA?

-Loading

-Standards

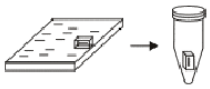
-Parameters that affect migration

- -gel concentration
- -length of DNA
- -tertiary structure
- -effects of overloading

## Agarose Gels & Gel Purification

-How do we 'look' at DNA?
-How do we get our DNA out of a gel?
-What will we do with it once we get it?

## Gel Purification

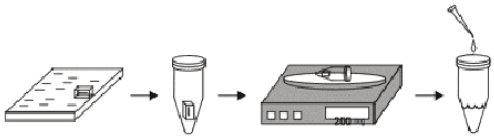


Why do you need to cut out your band fairly quickly?

You will need to dissolve the gel to get the DNA out.. You do this by adding 3 volumes of a gel-dissolving solution.

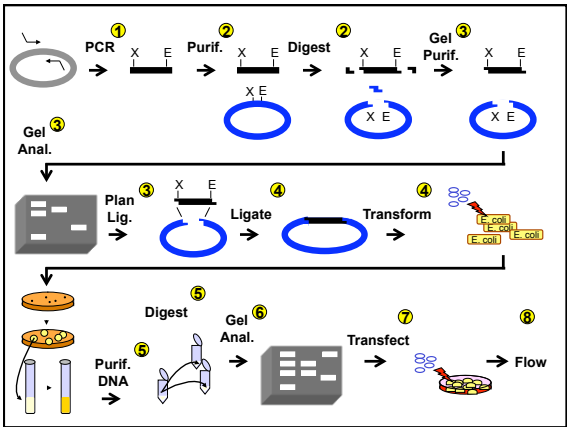
What does it mean to 'add 3 volumes'?

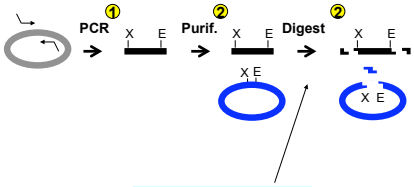
How can you estimate the volume of your gel slice?



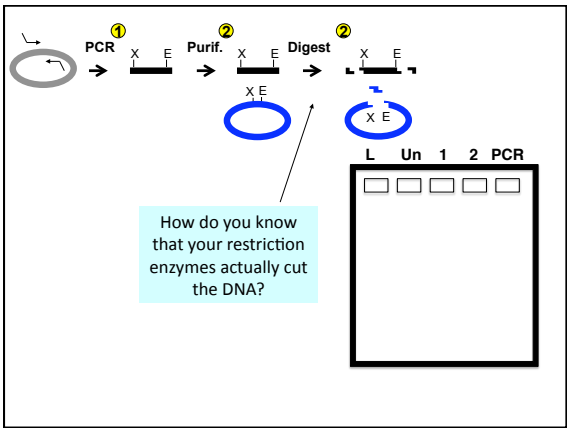
## Agarose Gels & Gel Purification

-How do we 'look' at DNA?
-How do we get our DNA out of a gel?
-**What will we do with it once we get it?**




How do you know that your restriction enzymes actually cut the DNA?


PCR
Purif.
Digest
L Un 1 2 PCR
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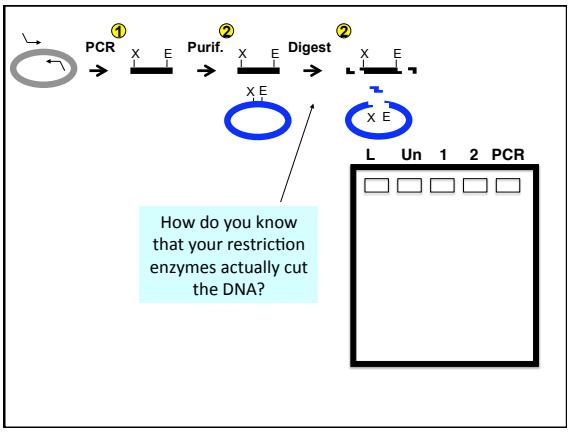
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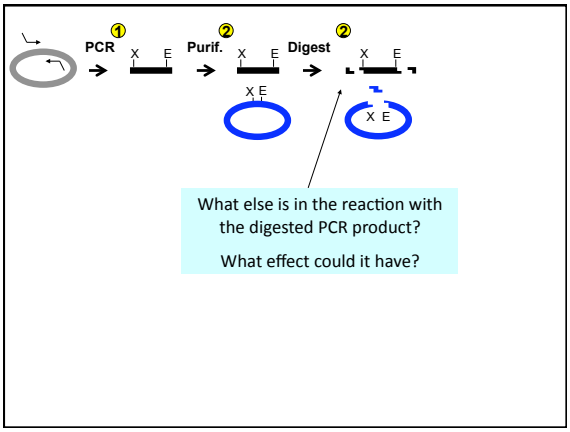
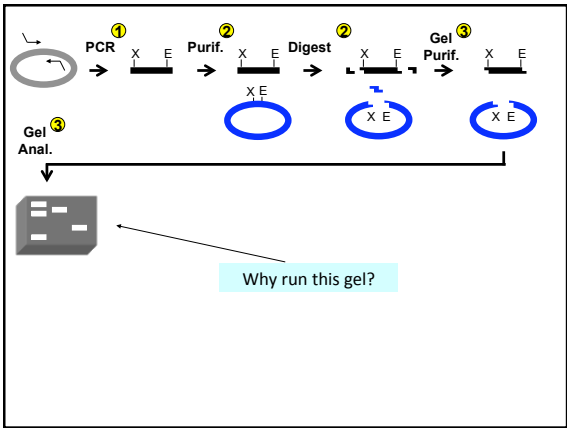
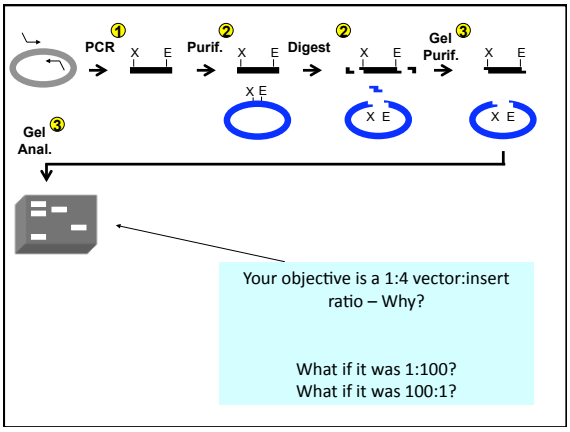
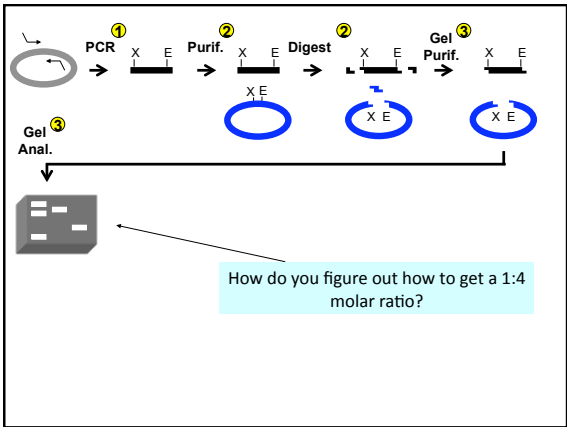

PCR
Purif.
Digest
What else is in the reaction with the digested PCR product?
What effect could it have?


PCR
Purif.
Digest
Gel Purif.
Gel Anal.
Why run this gel?


PCR
Purif.
Digest
Gel Purif.
Gel Anal.
Your objective is a 1:4 vector:insert ratio – Why?
What if it was 1:100?
What if it was 100:1?


PCR
Purif.
Digest
Gel Purif.
Gel Anal.
How do you figure out how to get a 1:4 molar ratio?