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) Intercalates into DNA

Where is EtBr in this gel?

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

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

Why run this gel?

Your objective is a 1:4 vector:insert ratio – Why?
What if it was 1:100? What if it was 100:1?