MOD1 – DNA ENGINEERING

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Day 7
Overview of this Module’s Experiments

Restriction Analysis – Assuring your DNA is correct

New Cancer Treatments – how your assay might be used

Thoughts about variables that might affect HR frequency
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 is it important to excise the DNA from the gel relatively quickly?
Why run this gel?
Your objective is a 1:4 vector:insert ratio – Why?

What is it was 1:100?
What if it was 100:1?
How do you figure out how to get a 1:4 molar ratio?
In your experiment, you will heat-shock E. coli to get the DNA in, and then immediately plate onto amp plates… is it always OK to plate immediately after transformation?
1. PCR
2. Purif.
3. Gel Purif.
4. Digest
5. Gel Anal.
6. DNA
7. Transfect
8. Flow
Analysis of Restriction Digestions:

How can you be certain your construct is what you think it is?
How to test for correct product...

Parent → A + C

↓ Ligase

Correct
How to test for correct product...

Parent → A + C → Correct + Parent

↓ Ligase
How to test for correct product...

**Parent**

$$A \quad B \quad C$$

$$\rightarrow$$

$$A \quad C$$

$$\rightarrow$$

$$A \quad B \quad C$$

$$\downarrow$$ **Ligase**

$$A \quad D \quad C$$

$$\rightarrow$$

$$A \quad B \quad C$$

Be sure the expected fragment is easy to see and evaluate (e.g., 0.3 to 2 kb)
How to test for correct product...

Parent

A + D

Correct

↓ Ligase

A + D Correct
How to test for correct product...

What will E+F find that A+D misses?

Why not use E + D?
Restriction Analysis: Raw Data
Blue (BamHI, XhoI) 1250 (SalI, EcoRV) 2.3 + 2.7
Pink (XbaI, XhoI) 1250 (EcoRV, NotI) 625
Gold (EcoRV, NotI) 625 (BamHI, SalI) 1623
Purple (BamHI, NotI) 1250 (EcoRV, NsiI) 625
Green (BamHI, XhoI) 1250 (EcoRV, NotI) 625
Red (EcoRV, NotI) 625 (BamHI, XhoI) 1200

Xba to Bam = 728 in D3
Xba to Bam = 625 in D5
Blue (BamHI,XhoI) ~1200 (SalI,EcoRV) 2.3 + 2.7
Pink (XbaI,XhoI) 1250 (EcoRV,NotI) 625
Gold (EcoRV,NotI) 625 (BamHI,SalI) 1623
Purple (BamHI,NotI) 1250 (EcoRV,NsiI) 625
Green (BamHI,XhoI) 1250 (EcoRV,NotI) 625
Red (EcoRV,NotI) 625 (BamHI,XhoI) 1200
Cutting Edge Approaches to Cancer Chemotherapy: How an HR assay might be used
Cancer Treatment today: Why researchers care about Homologous Recombination

Limitations of today’s treatments

What we know we want to do

Combinatorial Therapy

- two different drugs at the same time
- a drug plus an siRNA to knock down expression
What might affect the frequency of recombination between two plasmids?
What kind of cells will you be using in your experiment?
Where do mammalian cells come from?
Normal lung fibroblasts

Glial cells

Hela cells

Lung cancer cells
Terminology you need to know:

Primary
Transformed
Undifferentiated
Cell Line (vs ‘strain’)
Isogenic
Genomic Stability
Mixed populations
What might affect the frequency of recombination between two plasmids?
Roles of Homologous Recombination:

Preventing Cancer

& Affecting Treatment
BRCA2 Loads Rad51
Normal Human Chromosomes

\[ BRCA2 \sim\sim \]

Chromosomes


www.rctradiology.com
Homologous Recombination

Toxicity

Base Damage

Single Strand Break

Double Strand Break

Interstrand Crosslink

Radiation & Chemotherapy
Replication Fork Animation
By Tet Matsuguchi
Why you owe Your Life to Homologous Recombination...

Turn Off Homologous Recombination → Chromosomes Fall Apart

How can you test to make sure your vector is correct?
How to test for correct product...

Parent → Correct

A + C

↓ Ligase
How to test for correct product...

Parent → A + C + Correct

↓ Ligase

Correct + Parent → A + B + C
How to test for correct product...

Be sure the expected fragment is easy to see and evaluate (e.g., 0.3 to 2 kb)
How to test for correct product...

A + D

Correct

Ligase

Correct

Parent
How to test for correct product...

What will E+F find that A+D misses?

Why not use E + D?
MOD 3 – GENETIC ENGINEERING

Day 4

1) Roadmap Review
2) Restriction Enzymes
3) Ligase
4) Tips on getting your experiments to work
5) Restriction Mapping
1. PCR
2. Purif. → Digest
4. Ligate → Transform
5. Digest
6. Gel Anal.
7. Transfect
8. Flow

Steps:
- PCR
- Purification
- Digestion
- Gel Purification
- Gel Analysis
- Ligation
- Transformation
- Digestion
- Gel Analysis
- Transfection
- Flow
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 is it important to excise the DNA from the gel relatively quickly?
Why run this gel?
Ran out of time here.. Will cover some in during lab time and rest during lecture.
Your objective is a 1:4 vector:insert ratio – Why?

What is it was 1:100?
What if it was 100:1?
How do you figure out how to get a 1:4 molar ratio?
The rest should go into the next lecture.
Why is the DNA ‘cleaned up’ prior to transformation?
In your experiment, you will heat-shock E. coli to get the DNA in, and then immediately plate onto amp plates… is it always OK to plate immediately after transformation?
Sometimes instead of seeing nice isolated colonies, people see the following:

What you hope to see….

What you sometimes see….

Why? How would you avoid this problem?
Imagine you left your DNA in the fridge for weeks before setting up a 16°C ligation. What might happen?
What you will know by the end of this module:

- PCR – Primer design
- Plasmids – Purification & Restriction Analysis
- Mammalian Cell Culture & Transfection
- Basic Statistics
- Flow Cytometry
- Basic Vector Design Know-How

Meta-Level Goal: Avoid assumptions about what is in your test tube and what will happen when you set up reactions.
Non-Homologous End-Joining

Double Strand Break

\[ \downarrow \]

\[ \text{DNA} \]

Homology-Directed Repair

Double Strand Break

\[ \downarrow \]

\[ \text{DNA} \]

Why do cells have two different ways to repair a DSB??
1. Inject foreign DNA into one of the pronuclei

Fertilized mouse egg prior to fusion of male and female pronuclei

2. Transfer injected eggs into foster mother

About 10–30% of offspring will contain foreign DNA in chromosomes of all their tissues and germ line

3. Breed mice expressing foreign DNA to propagate DNA in germ line