Module 1 overview

<table>
<thead>
<tr>
<th>Lecture</th>
<th>Lab</th>
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<tbody>
<tr>
<td>1. Introduction to the module</td>
<td>1. Start-up protein eng.</td>
</tr>
<tr>
<td>2. Rational protein design</td>
<td>2. Site-directed mutagenesis</td>
</tr>
<tr>
<td>3. Fluorescence and sensors</td>
<td>3. DNA amplification</td>
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**PRESIDENT’S DAY**

<table>
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<tr>
<th>Lecture</th>
<th>Lab</th>
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<tbody>
<tr>
<td>4. Review &amp; gene analysis</td>
<td>4. Prepare expression system</td>
</tr>
<tr>
<td>5. Protein expression</td>
<td>5. Gene analysis &amp; induction</td>
</tr>
<tr>
<td>6. Purification and protein analysis</td>
<td>6. Characterize expression</td>
</tr>
<tr>
<td>8. High throughput engineering</td>
<td>8. Data analysis</td>
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Lecture 4: Review & gene analysis

I. Review of the project
   A. Project aims and rationale
   B. Methods, work completed so far

II. Analysis of mutant genes
   A. Restriction digests
   B. DNA sequencing
Module 1 assignment

Protein engineering research article
1. Abstract
2. Introduction
3. Materials and Methods
4. Results
5. Discussion
6. References
7. Figures
Module 1 assignment

Protein engineering research article

1. Abstract
2. Introduction
   - Why are calcium sensors important?
   - What is protein engineering; how does it relate?
   - What is inverse pericam?
   - Why is it useful/interesting to tune pericam?
   - Why did you choose your mutations?
3. Materials and Methods
4. Results
5. Discussion
6. References
7. Figures
Module 1 assignment

Protein engineering research article

1. Abstract
2. Introduction
   Why are calcium sensors important?
   What is protein engineering; how does it relate?
   What is inverse pericam?
   Why is it useful/interesting to tune pericam?
   Why did you choose your mutations?

3. Materials and Methods
4. Results
5. Discussion
6. References
7. Figures
### Restriction enzymes digest specific DNA sequences

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Species</th>
<th>Sequence 5' → 3'</th>
<th>Sequence 3' → 5'</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoRI</td>
<td><em>Escherichia coli</em></td>
<td>TACATCAGCGCTGCTCAG</td>
<td>TACATCCTTAAG</td>
</tr>
<tr>
<td>EcoRII</td>
<td><em>Escherichia coli</em></td>
<td>CCWGGGTGCAGCGCTGCTGCA</td>
<td>CCWGGGGWCC</td>
</tr>
<tr>
<td>BamHI</td>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>GGATCCCTTAGG</td>
<td>GATCCCTAG</td>
</tr>
<tr>
<td>HindIII</td>
<td><em>Haemophilus influenzae</em></td>
<td>AAGCTTTTCGA</td>
<td>AGCTTTTCGA</td>
</tr>
</tbody>
</table>

You designed mutations that can be assessed by restriction mapping:

```
...TACATCAGCGCTGCTCAG...    ...TACATCCTCGCTGCGCAG...
...ATGTAGTCGCGACGAGTC...    ...ATGTAGGAGCGCGACGACGTC...
Y I S A A Q                  Y I L A A Q
```
How do restriction endonucleases work?

**Restriction Endonucleases in Cloning**

**pcDNA3**

5.4 kb

- There is an ATG upstream of the Xba I site.

**Diagnostic Digest**

- std
- ctr
- mut

**Ligation**
Genetic polymorphisms can be associated with different distributions of restriction sites—restriction fragment length polymorphisms (RFLPs) used for genotyping.

Suppose alleles A and B each occur in 50% of the population and segregated independently, what are the chances that a randomly chosen individual displays the AB phenotype?

How many biallelic polymorphisms would have to be considered for each genotype to have a 1:1,000,000 chance of occurring, assuming equal prevalence of each?
(This slide not covered in lecture, just for your info)

<table>
<thead>
<tr>
<th>Markers</th>
<th>Source of Variation</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>STRs</td>
<td>Independent chromosomal assortment; recombination; mutation</td>
<td>Extremely high discrimination power</td>
<td>Very degraded DNA difficult to type</td>
</tr>
<tr>
<td>SNPs</td>
<td>Independent chromosomal assortment; recombination; mutation - but low rate</td>
<td>Usable on very degraded DNA</td>
<td>Mostly biallelic, so relatively low discrimination power; mixtures difficult to resolve</td>
</tr>
<tr>
<td>STRs</td>
<td>Mutation only</td>
<td>Male-specificity; useful in male-female mixtures</td>
<td>Relatively low discrimination power; sharing within patriline; possible population structure problems</td>
</tr>
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