# Module 1 overview

| *lecture* | *lab* |
|---|---|
| 1. Introduction to the module | 1. Start-up protein eng. |
| 2. Rational protein design | 2. Site-directed mutagenesis |
| 3. Fluorescence and sensors | 3. DNA amplification |
| **PRESIDENT'S DAY** | |
| 4. Review & gene analysis | 4. Prepare expression system |
| 5. Protein expression | 5. Gene analysis & induction |
| 6. Purification and protein analysis | 6. Characterize expression |
| 7. Binding & affinity measurements | 7. Assay protein behavior |
| 8. High throughput engineering | 8. Data analysis |

**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

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Protein engineering research article

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   - 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?
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# Restriction enzymes digest specific DNA sequences

| | | |
|---|---|---|
| *Eco*RI | *Escherichia coli* | 5'GAATTC<br>3'CTTAAG<br><br>5'---G AATTC---3'<br>3'---CTTAA G---5' |
| *Eco*RII | *Escherichia coli* | 5'CCWGG<br>3'GGWCC<br><br>5'--- CCWGG---3'<br>3'---GGWCC ---5' |
| *Bam*HI | *Bacillus amyloliquefaciens* | 5'GGATCC<br>3'CCTAGG<br><br>5'---G GATCC---3'<br>3'---CCTAG G---5' |
| *Hin*dIII | *Haemophilus influenzae* | 5'AAGCTT<br>3'TTCGAA<br><br>5'---A AGCTT---3'<br>3'---TTCGA A---5' |

www.wikipedia.com

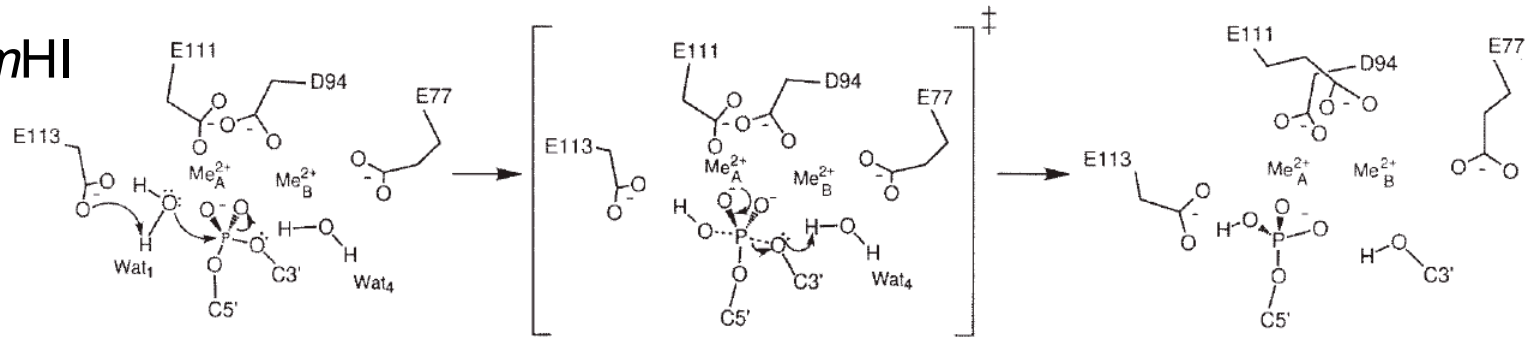
you designed mutations that can be assessed by restriction mapping:

```
...TACATCAGCGCTGCTCAG...        ...TACATCCTCGCTGCGCAG...
...ATGTAGTCGCGACGAGTC...        ...ATGTAGGAGCGACGCGTC...
    Y  I  S  A  A  Q                Y  I  L  A  A  Q
```

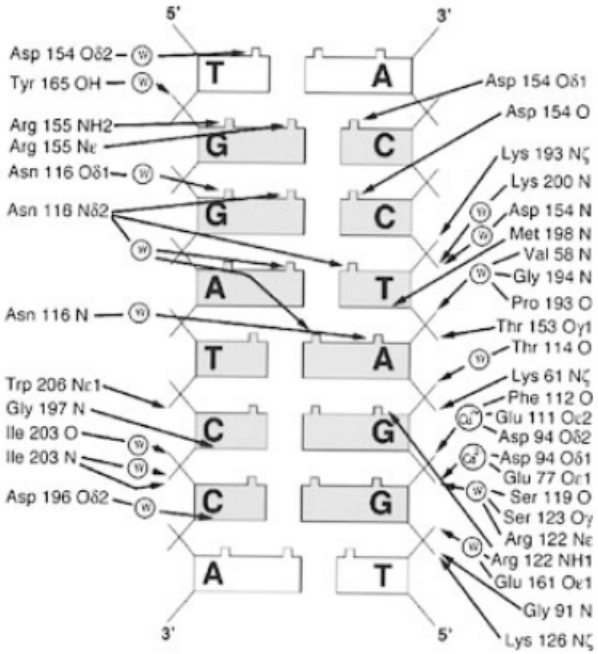
Viadiu & Aggarwal (1998, 2000)

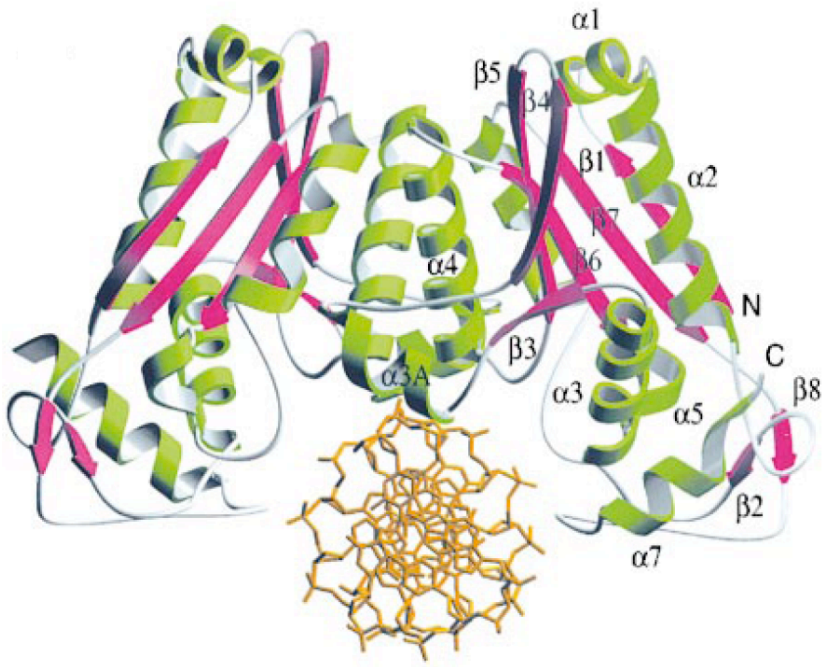
# How do restriction endonucleases work?

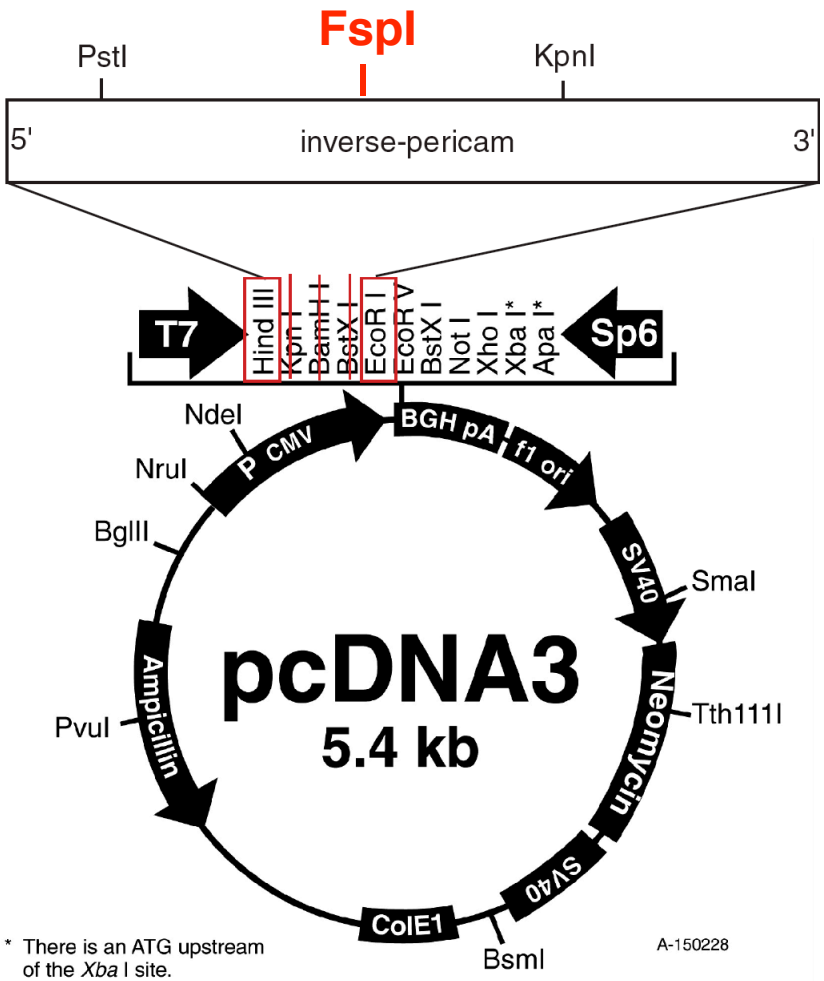
*Bam*HI



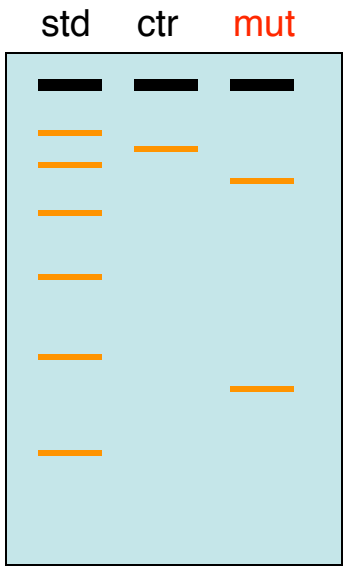

**Pre-reactive state** | **Transition state** | **Post-reactive state**



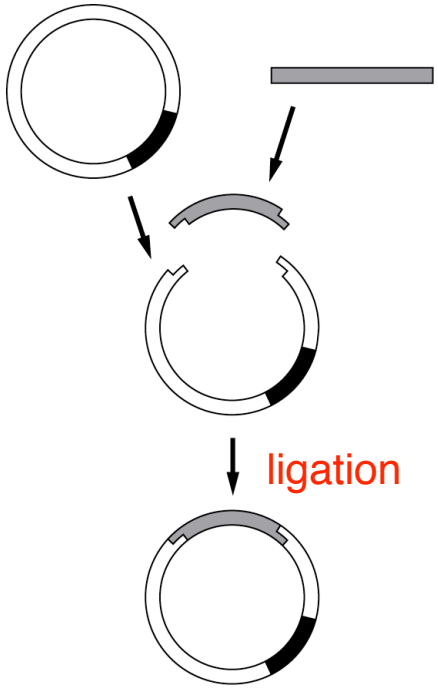





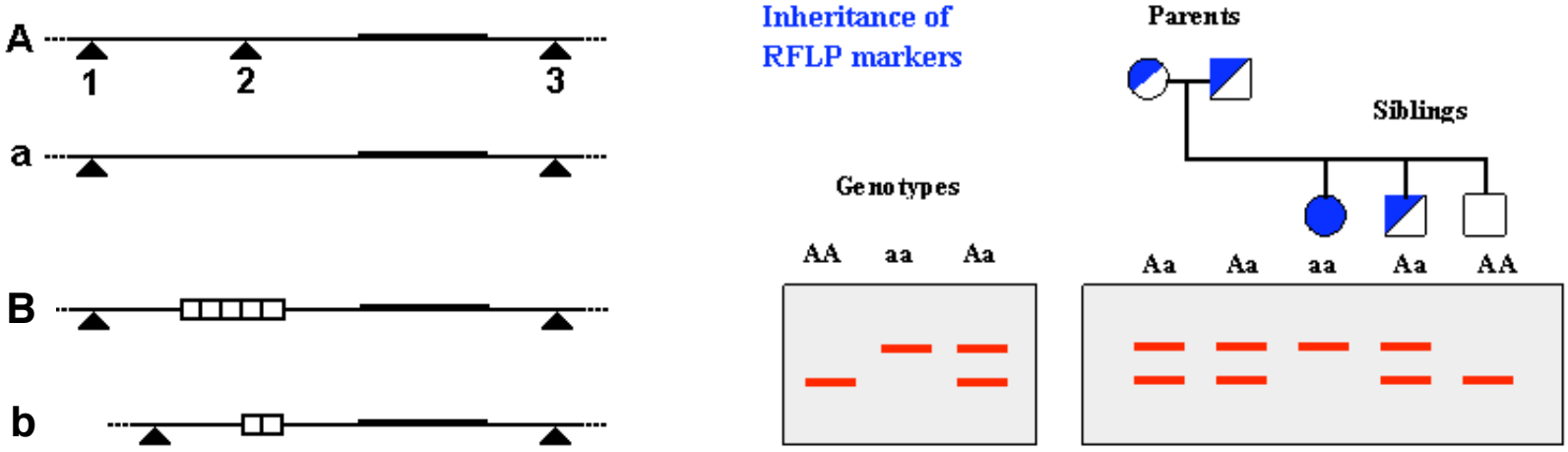

## diagnostic digest




## restriction endonucleases in cloning



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

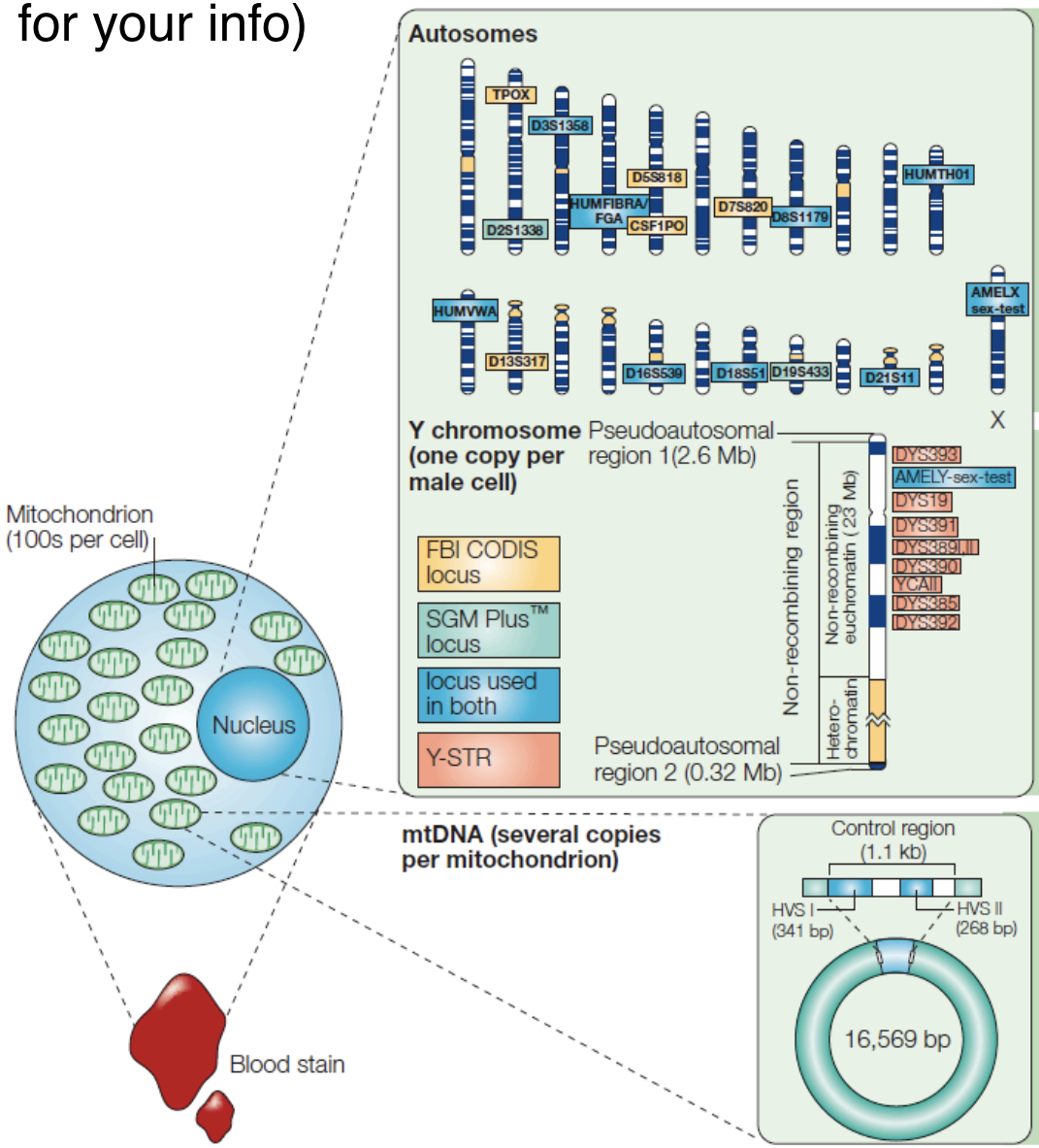



www.wikipedia.com

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)




| Marker types | Source of variation | Advantages | Disadvantages |
|---|---|---|---|
| STRs | Independent chromosomal assortment; recombination; mutation | Extremely high discrimination power | Very degraded DNA difficult to type |
| SNPs | Independent chromosomal assortment; recombination; mutation - but low rate | Usable on very degraded DNA | Mostly biallelic, so relatively low discrimination power; mixtures difficult to resolve |
| STRs | Mutation only | Male-specificity; useful in male-female mixtures | Relatively low discrimination power; sharing within patriline; possible population structure problems |
| SNPs, usually in control region | Mutation only | High copy number, therefore good survival in old/damaged samples | Heteroplasmy; low discrimination power; sharing within matriline; possible population structure problems |

Jobling (2004) Nat. Rev. Genetics