

Module 2 overview

lecture

1. Introduction to the module
2. Rational protein design
3. Fluorescence and sensors
4. Protein expression

lab

1. Start-up protein eng.
2. Site-directed mutagenesis
3. DNA amplification
4. Prepare expression system

SPRING BREAK

5. Review & gene analysis
6. Purification and protein analysis
7. Binding & affinity measurements
8. High throughput engineering

5. Gene analysis & induction
6. Characterize expression
7. Assay protein behavior
8. Data analysis

Lecture 5: 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 2 assignment

Protein engineering research article

1. Abstract
2. Introduction
3. Materials and Methods
4. Results
5. Discussion
6. References
7. Figures

Module 2 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

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7. Figures

Module 2 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
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7. Figures

Restriction enzymes digest specific DNA sequences

EcoRI *Escherichia coli*



EcoRII *Escherichia coli*



BamHI *Bacillus amyloliquefaciens*



HindIII *Haemophilus influenzae*



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www.wikipedia.com

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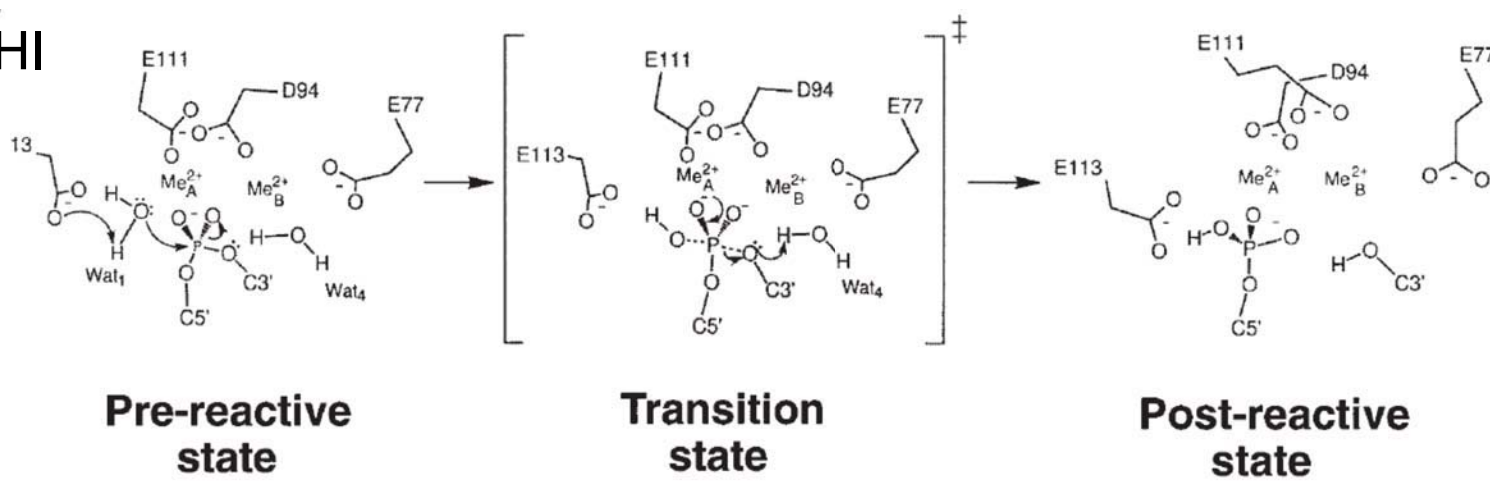
you designed mutations that can be assessed by restriction mapping:

...TACATCAGCGCTGCTCAG...
 ...ATGTAGTCGCGACGAGTC...
 Y I S A A Q

...TACATCCTCGCTGCGCAG...
 ...ATGTAGGAGCGACGCGTC...
 Y I L A A Q

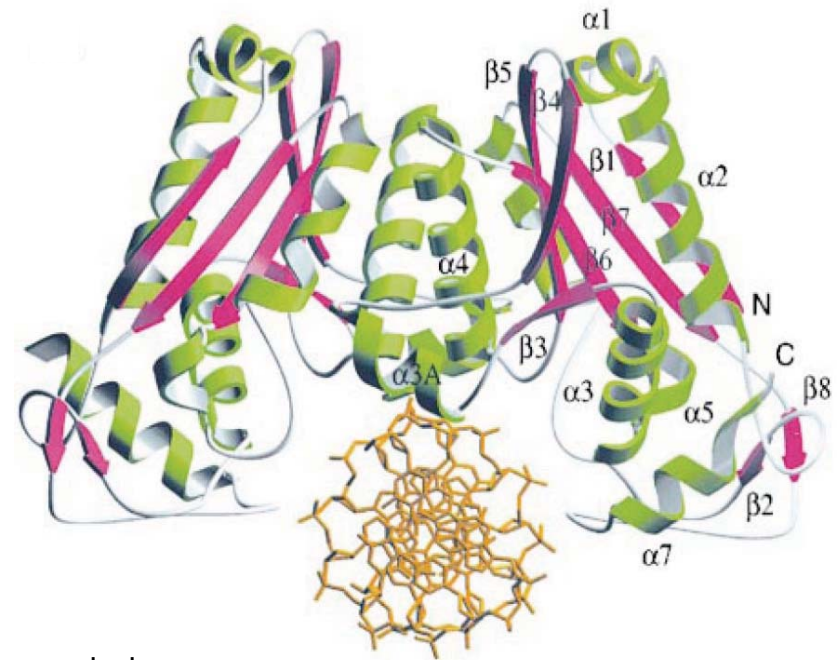
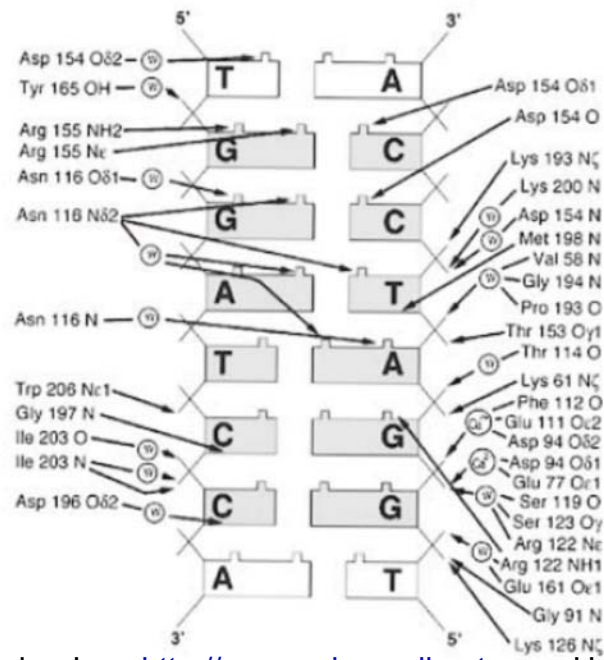
How do restriction endonucleases work?

*Bam*HI



7

Reprinted by permission from Macmillan Publishers Ltd: Nature Structural Biology. Source: Viadiu, H., and A. K., Aggarwal. "The Role of Metals in Catalysis by the Restriction Endonuclease Bam HI." *Nature Structural Biology* 5 (1998): 910-916. © 1998.



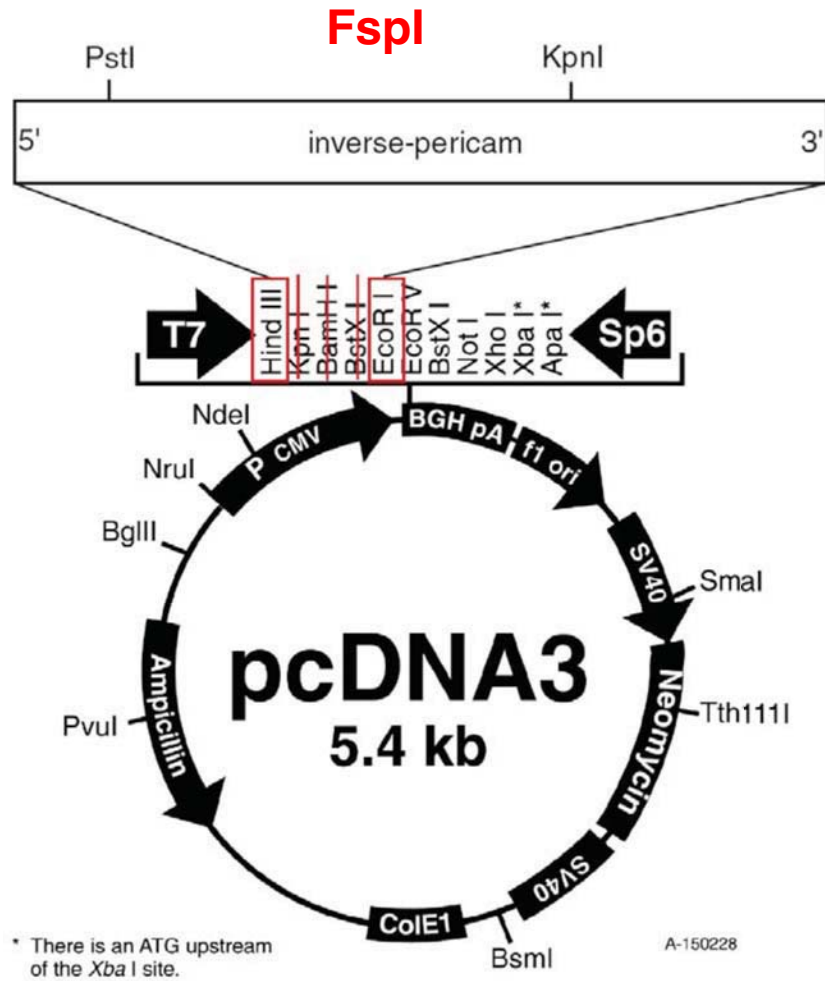
Courtesy of Elsevier, Inc., <http://www.sciencedirect.com>. Used with permission.

Source: Viadiu, H., and A. K., Aggarwal.

"Structure of BamHI Bound to Nonspecific DNA: A Model for DNA Sliding."

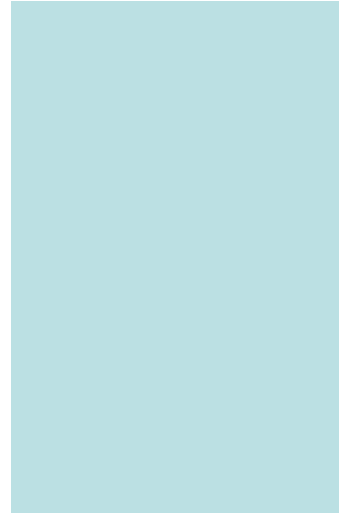
Molecular Cell 5, no. 5 (2000): 889-895.

Viadiu & Aggarwal (1998, 2000)

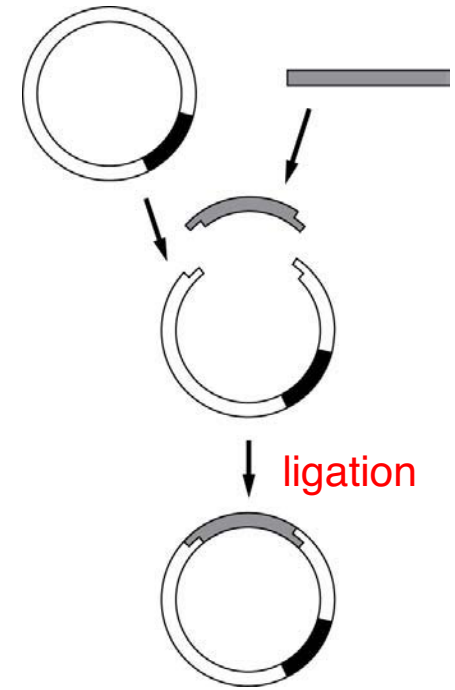


diagnostic digest

std ctr mut



restriction endonucleases in cloning



Courtesy of Life Technologies. Used with permission.

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

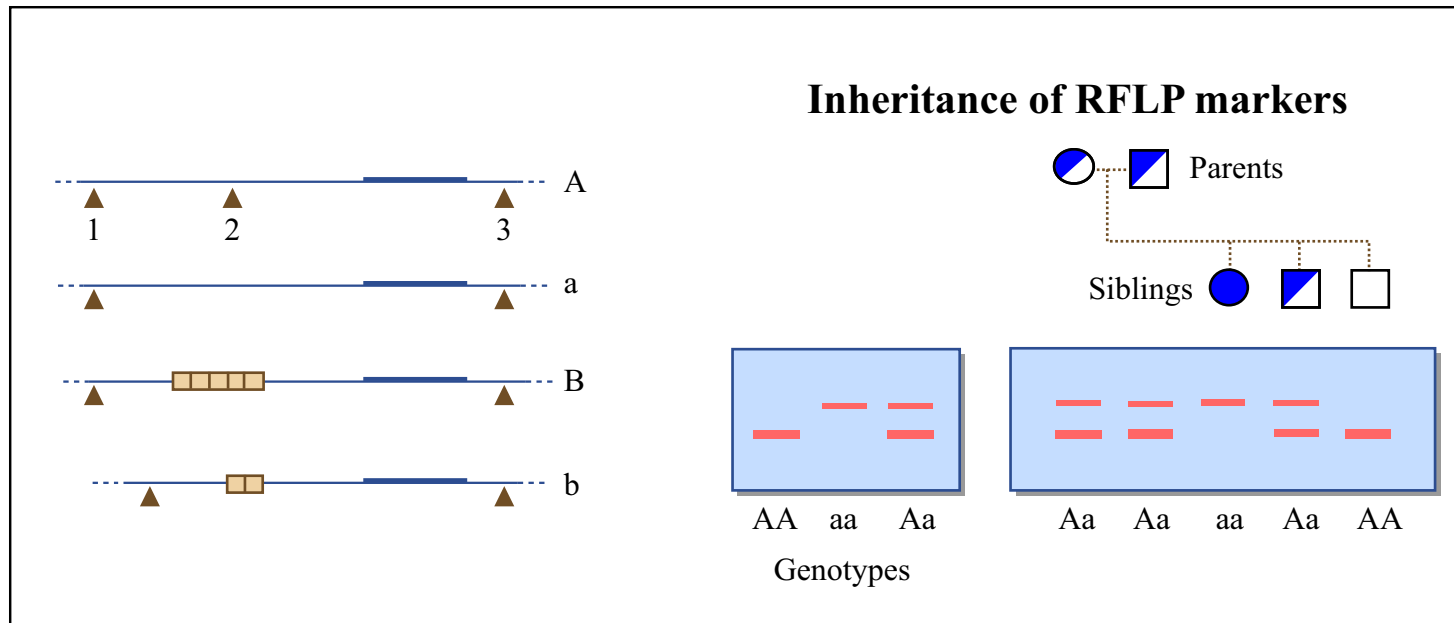
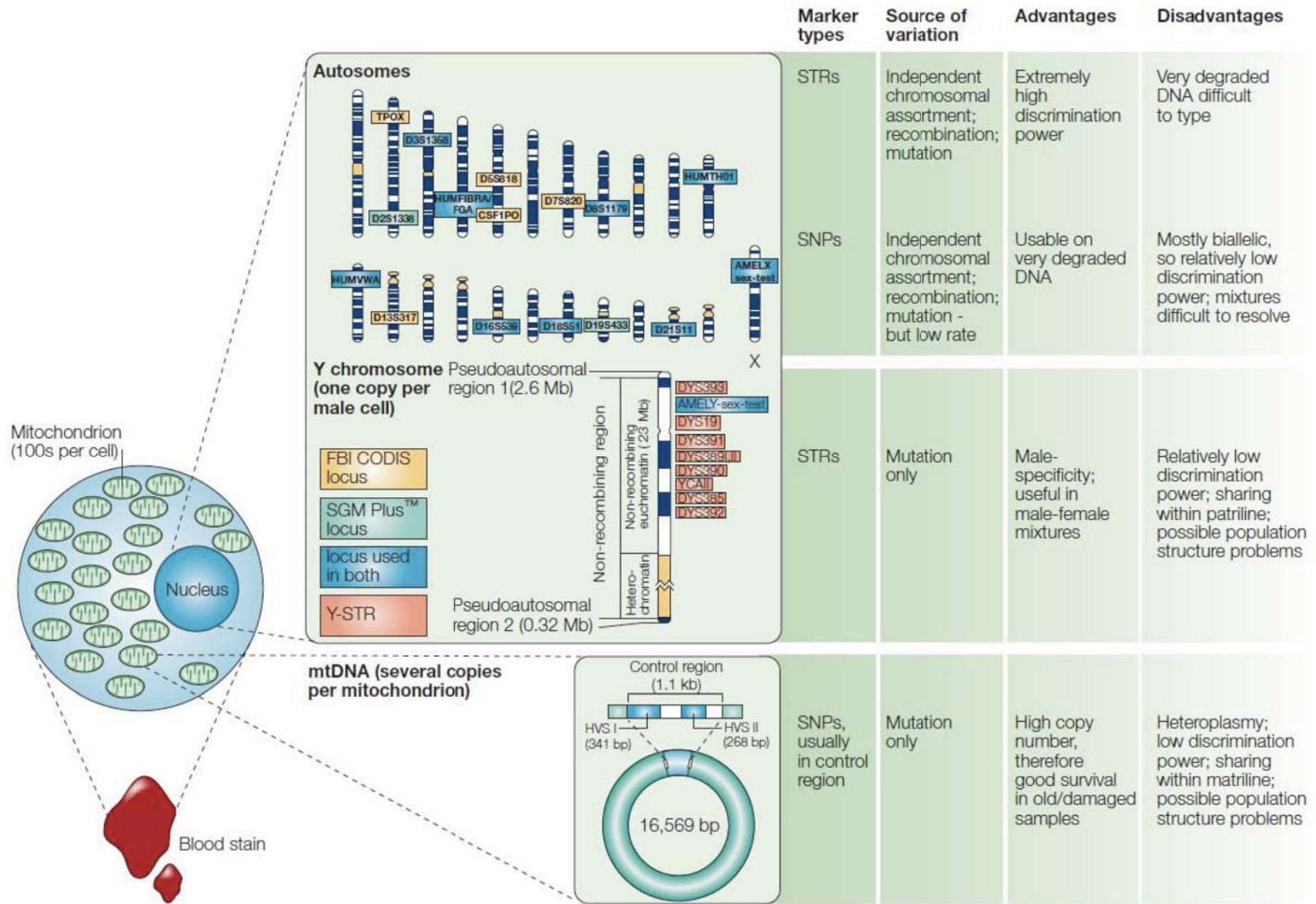


Figure by MIT OpenCourseWare.

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?

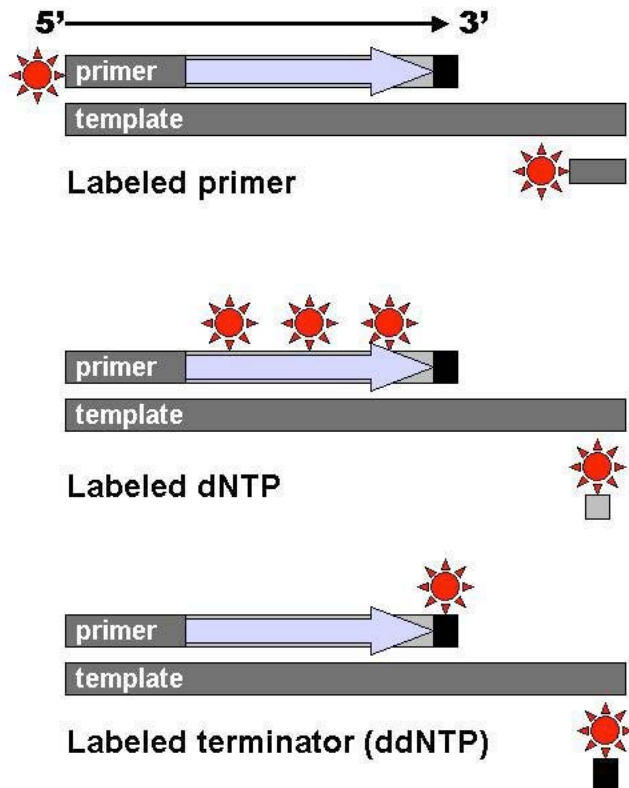


Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Genetics. Source: Jobling, M. A., and P. Gill. "Encoded evidence: DNA in Forensic Analysis." *Nature Reviews Genetics* 5, (October 2004): 739-751. © 2004.

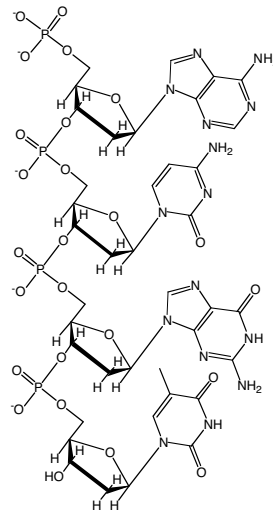
Jobling (2004) *Nat. Rev. Genetics*

How does sequencing work?

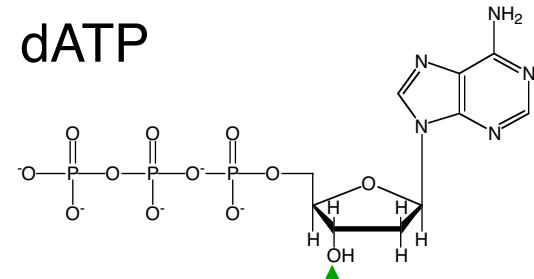
Perform PCR on template to be sequences; each PCR reaction is terminated by a nucleotide analog that can be incorporated, but not added to. Terminated PCR products must be labeled in some way.



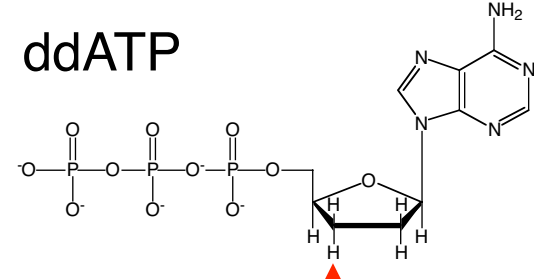
nucleotides linked
by phosphodiester
bonds



dATP

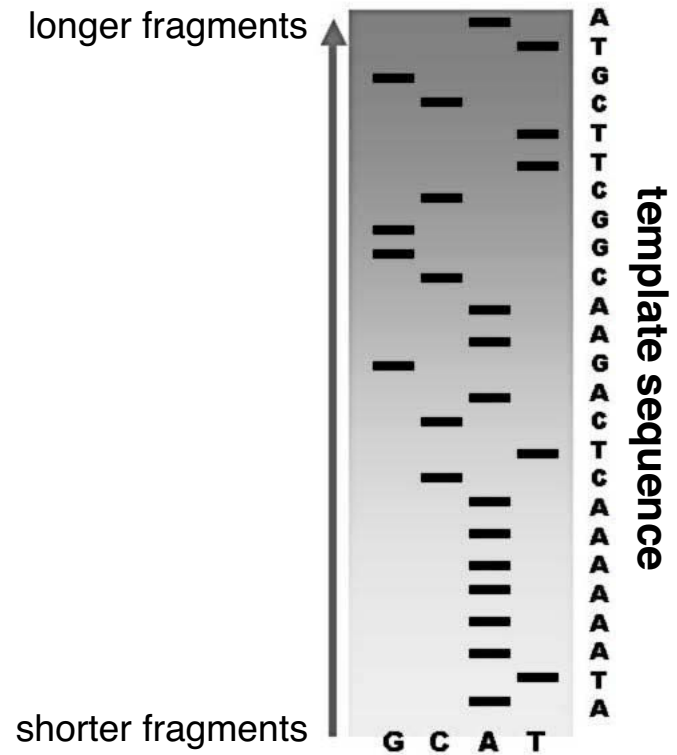
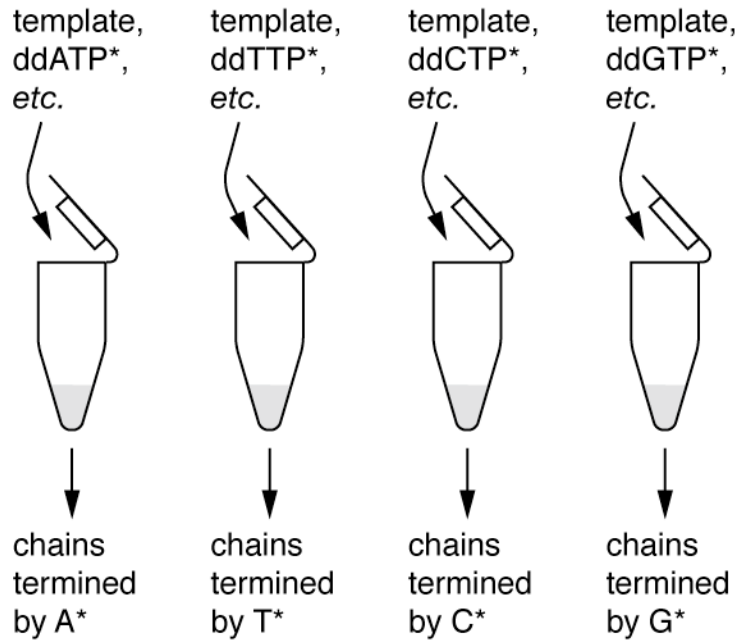


ddATP



sequencing with radioactive ddNTPs

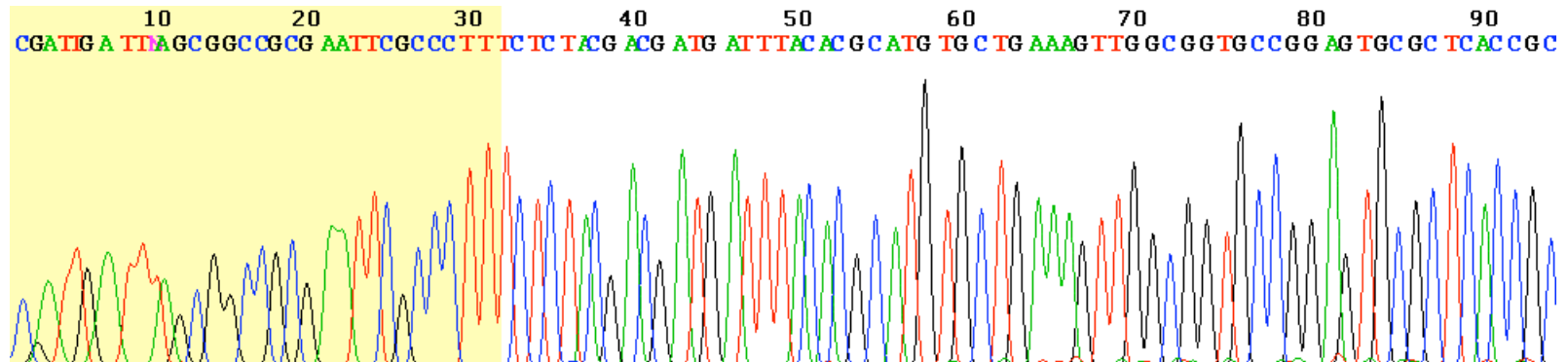
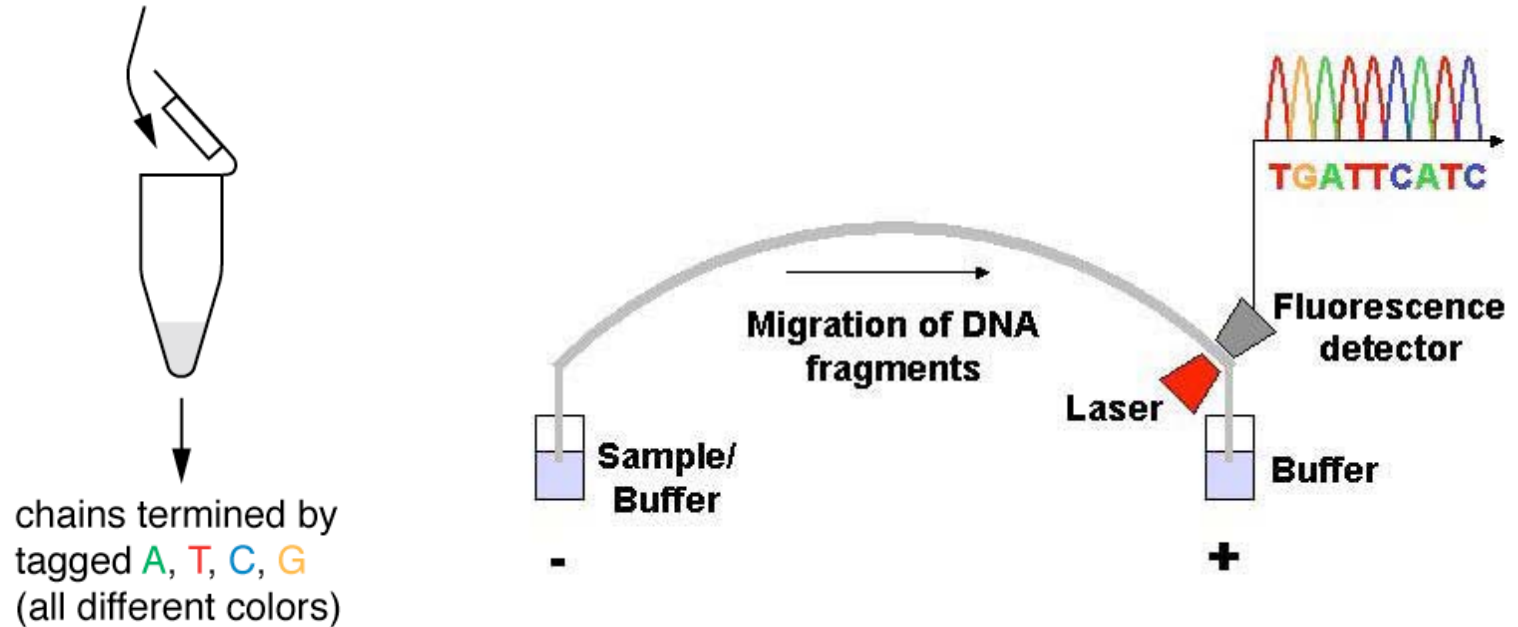
run products in four separate lanes on gel, expose X-ray film



Wikipedia (Public domain image)

“one pot” sequencing more common today:

template, fluorescently tagged
 ddATP, ddTTP, ddCTP, ddGTP, etc.



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