Module 2 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
4. Protein expression	4. Prepare expression system
SPRING BREAK	
5. Review & gene analysis	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 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

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

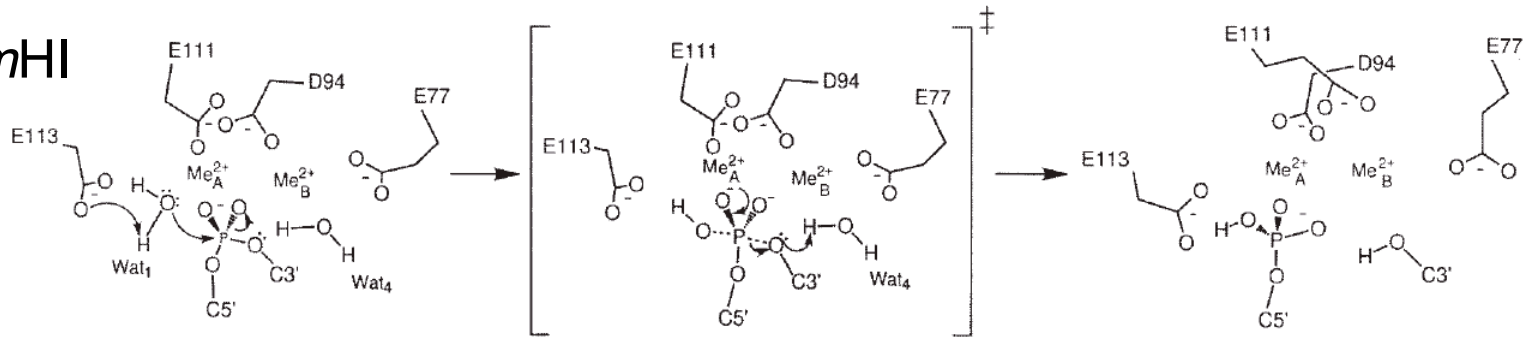
www.wikipedia.com

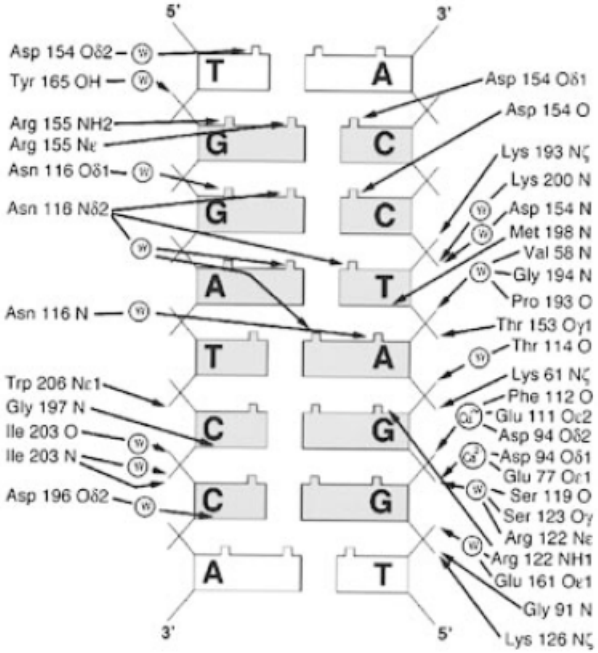
you designed mutations that can be assessed by restriction mapping:

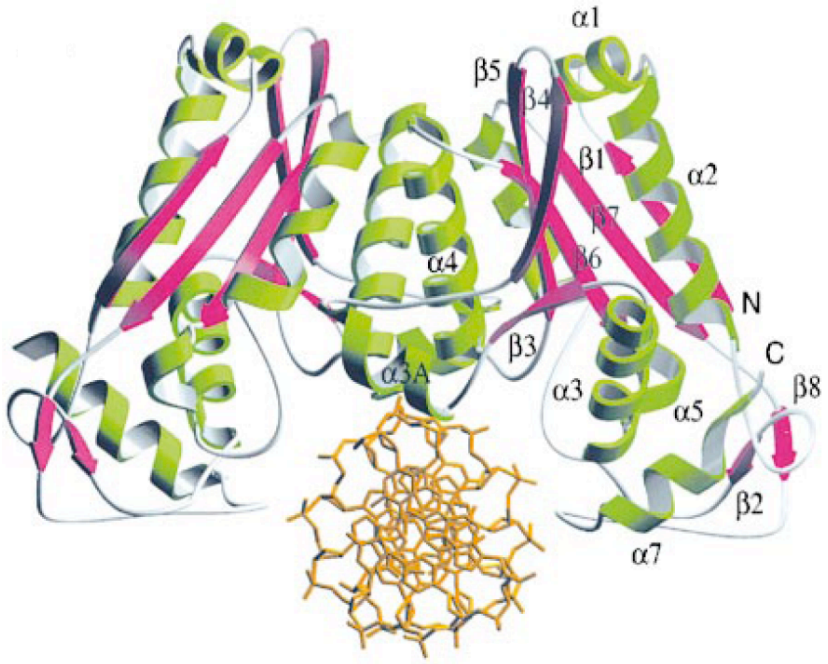
```
...TACATCAGCGCTGCTCAG...        ...TACATCCTCGCTGCGCAG...
...ATGTAGTCGCGACGAGTC...        ...ATGTAGGAGCGACGCGTC...
    Y  I  S  A  A  Q                Y  I  L  A  A  Q
```

How do restriction endonucleases work?

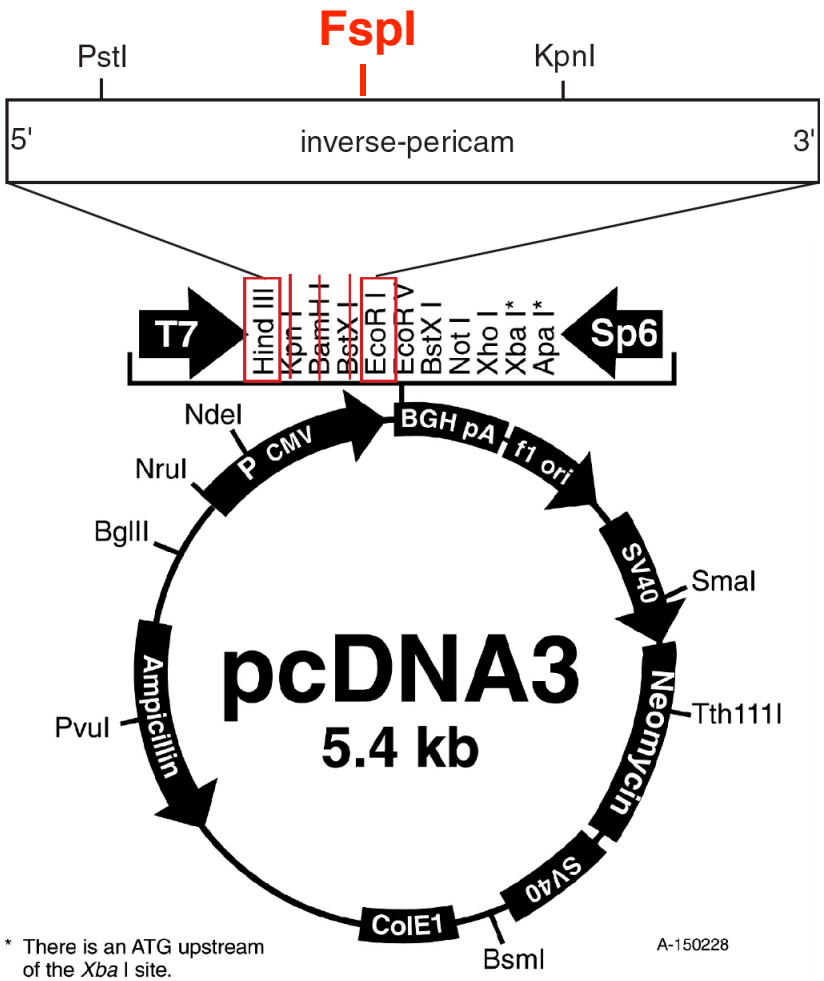
*Bam*HI



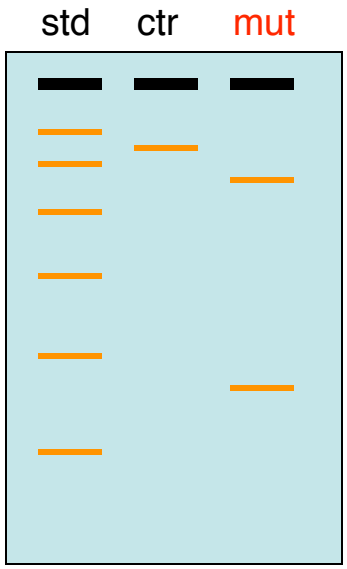




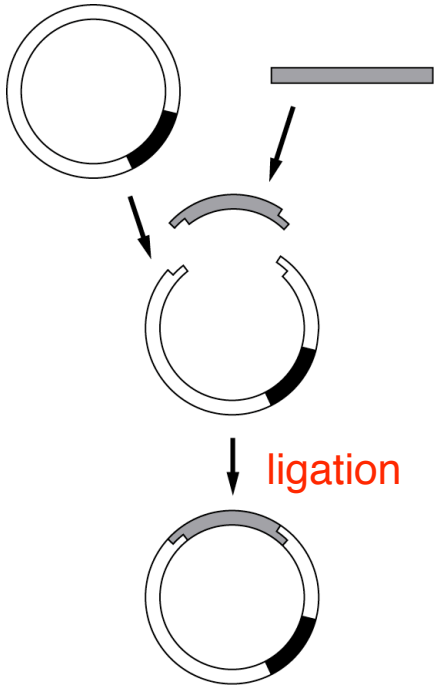
Viadiu & Aggarwal (1998, 2000)



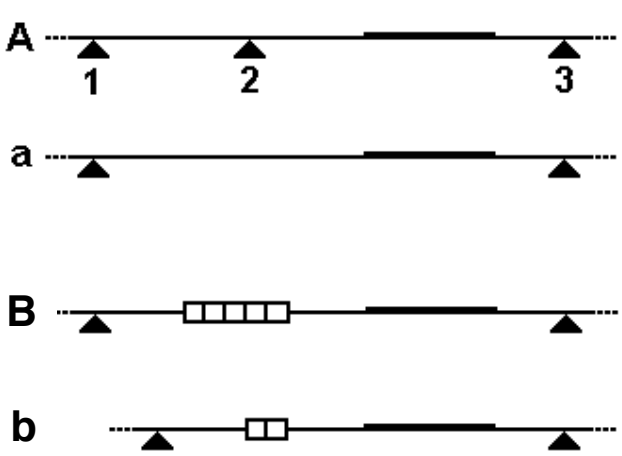
diagnostic digest

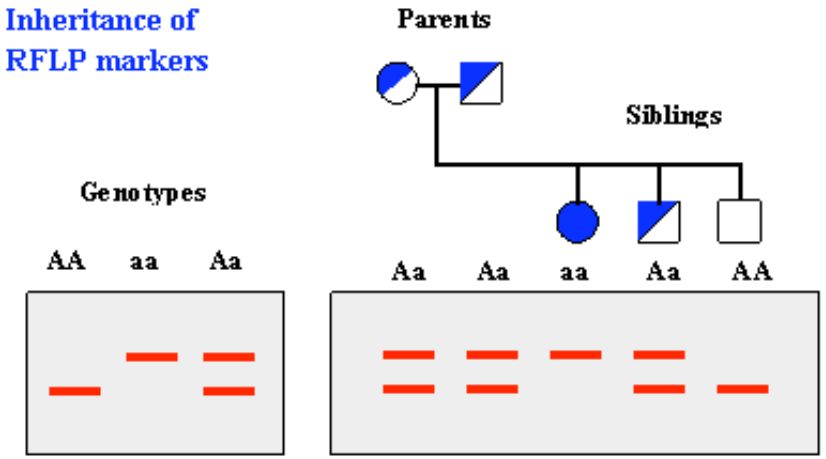


restriction endonucleases in cloning



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

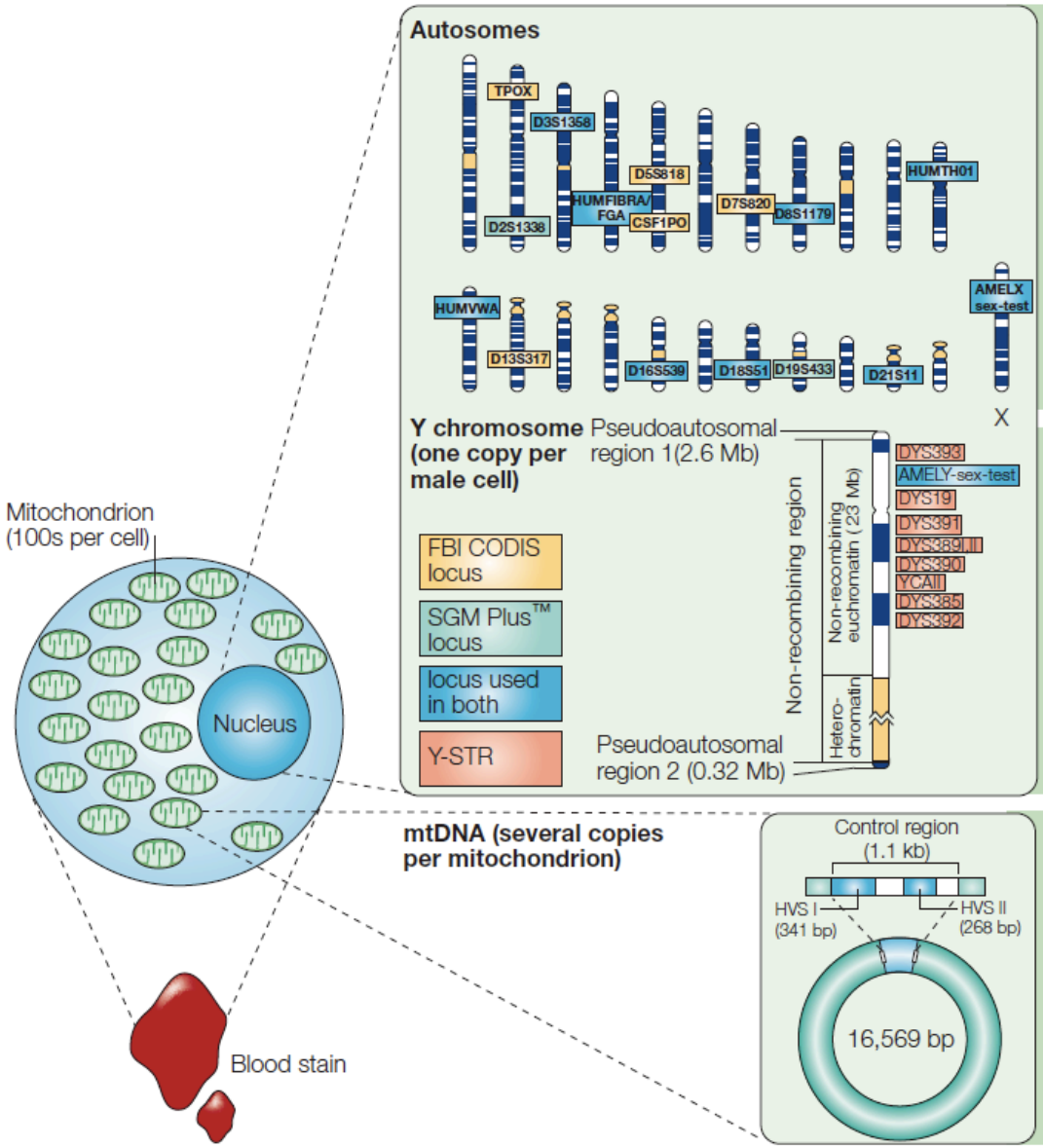




www.wikipedia.com

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?

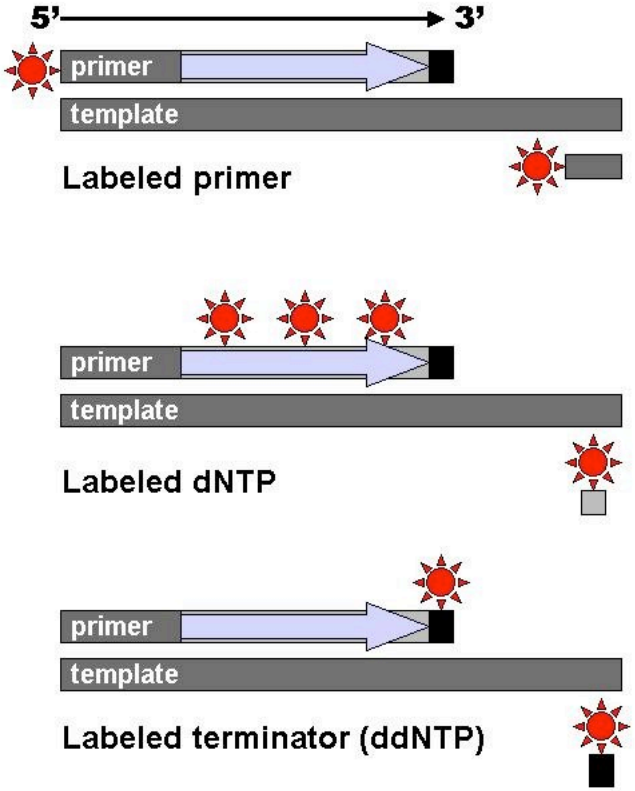


Marker types	Source of variation	Advantages	Disadvantages
STRs	Independent chromosomal assortment; recombination; mutation	Extremely high discrimination power	Very degraded DNA difficult to type
SNPs	Independent chromosomal assortment; recombination; mutation - but low rate	Usable on very degraded DNA	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

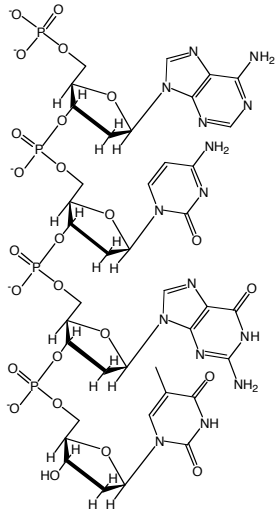
Jobling (2004) Nat. Rev. Genetics

How does sequencing work?

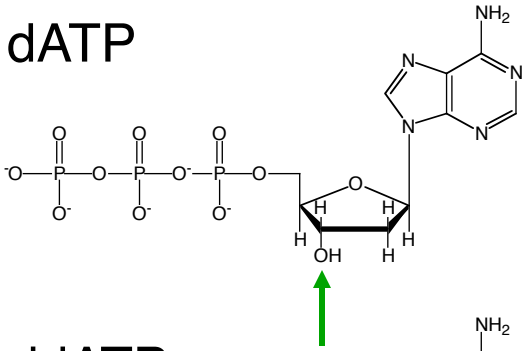
Perform PCR on template to be sequences; each PCR reaction is terminated by a nucleotide analog that can be incorporated, but not added to. Terminated PCR products must be labeled in some way.



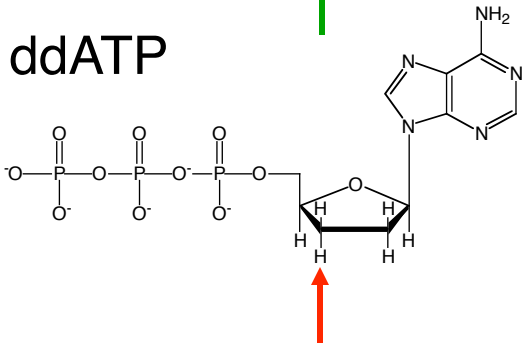
nucleotides linked by phosphodiester bonds



dATP

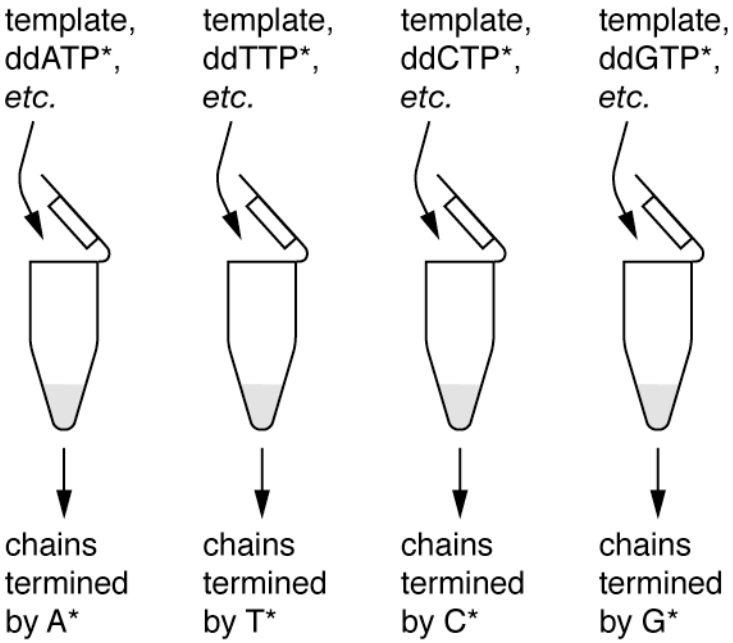


ddATP

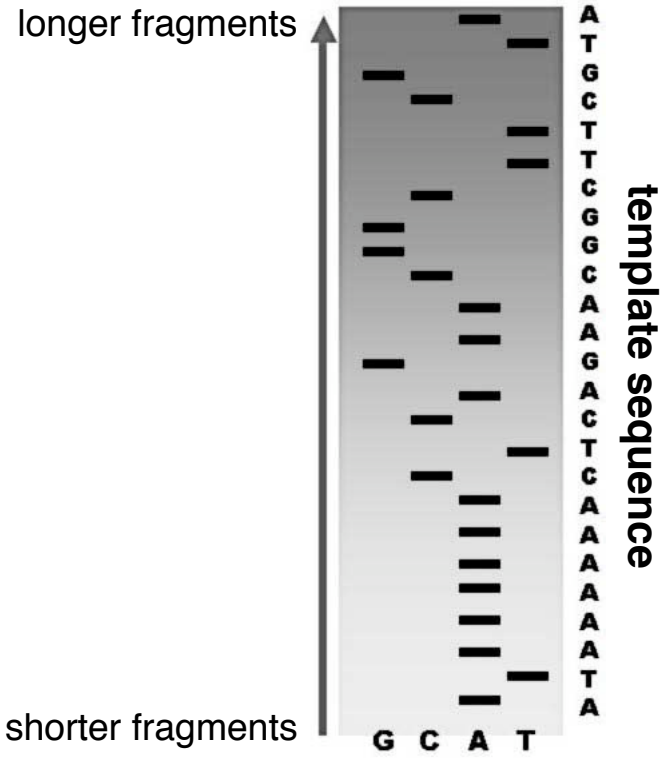


www.wikipedia.com

sequencing with radioactive ddNTPs

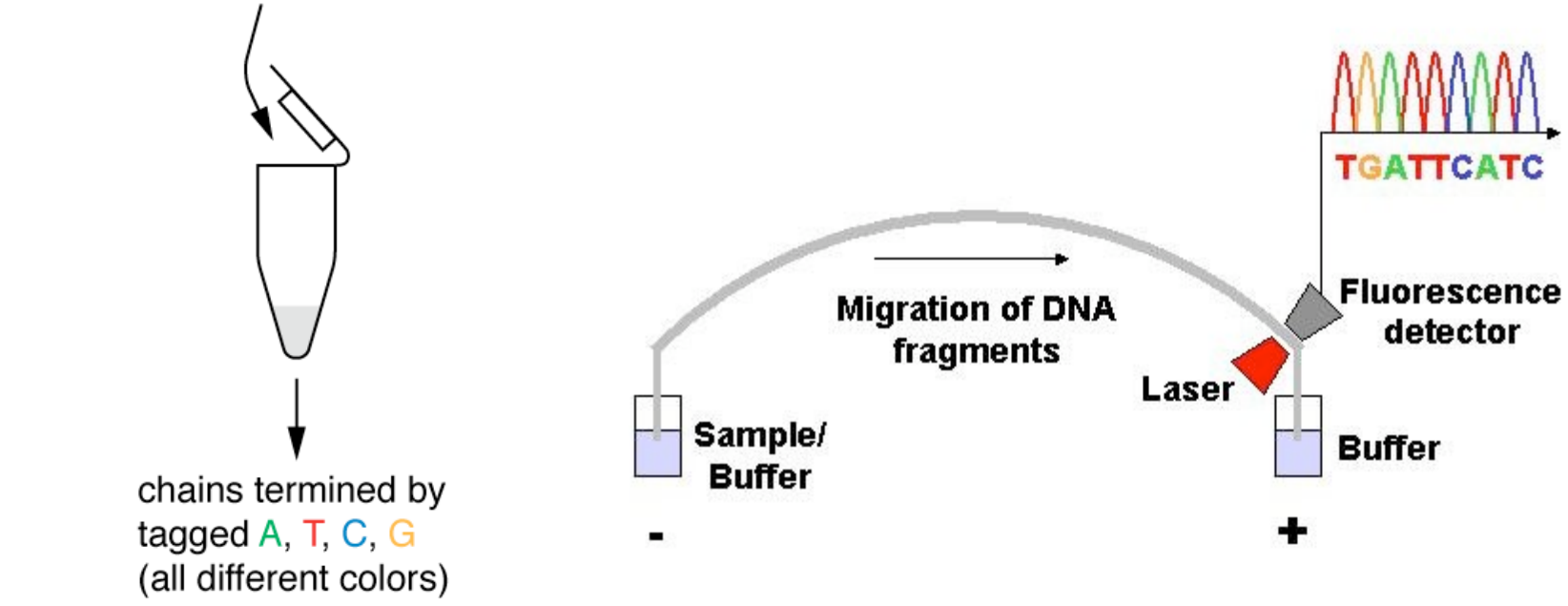


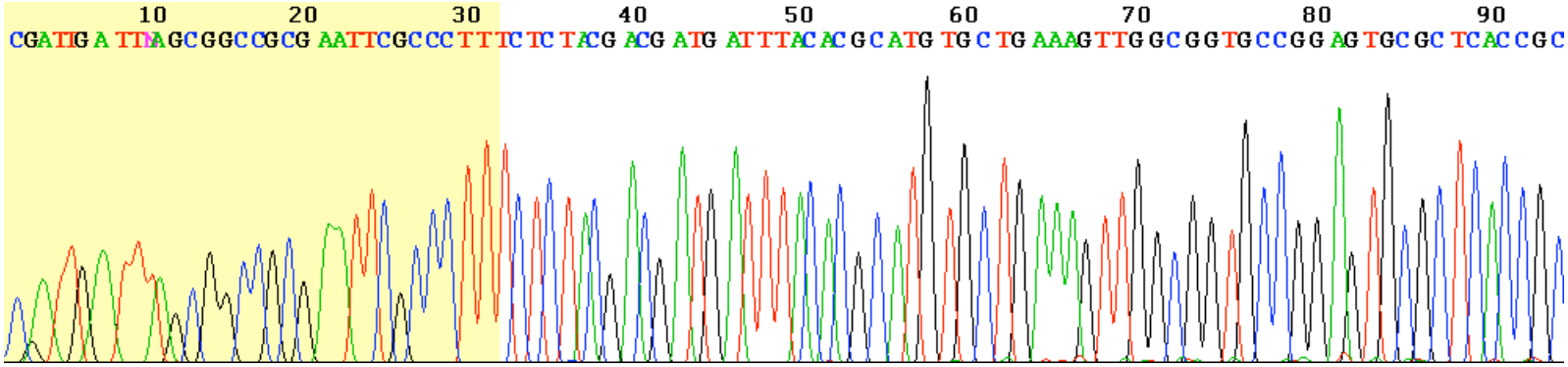
run products in four separate lanes on gel, expose X-ray film



“one pot” sequencing more common today:

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





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