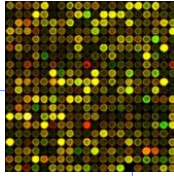


# AP Biology

## Advanced Techniques

Electrophoresis, PCR,  
Southern Blot, Sequencing,  
RFLPs, Microarrays

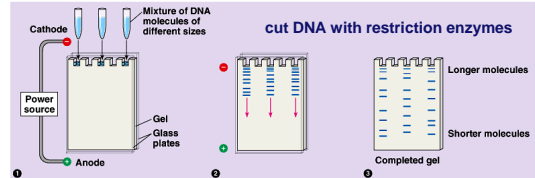


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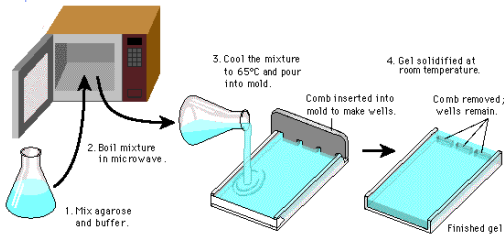
2004-2005

## Gel Electrophoresis

- Separation of DNA fragments by size
  - ◆ DNA is negatively charged
    - moves toward + charge in electrical field
  - ◆ agarose gel
    - "swimming through Jello"
    - smaller fragments move faster



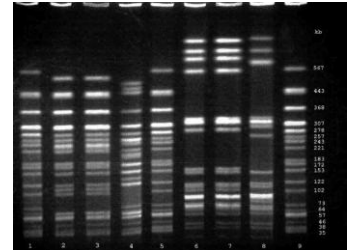
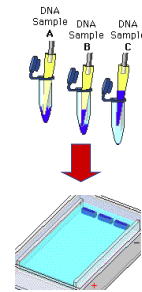
## Gel Electrophoresis



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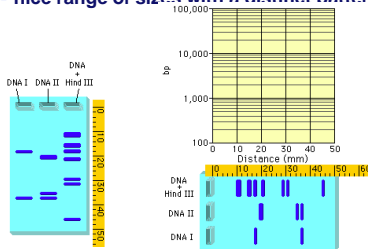
## Gel Electrophoresis



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## Measuring fragment size

- compare bands to a known "standard"
  - ◆ usually lambda phage cut with HindIII
    - nice range of sizes with a distinct pattern

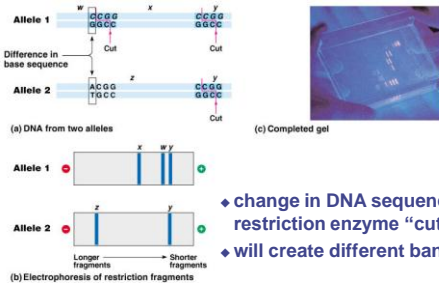


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2005

## RFLP

- Restriction Fragment Length Polymorphism



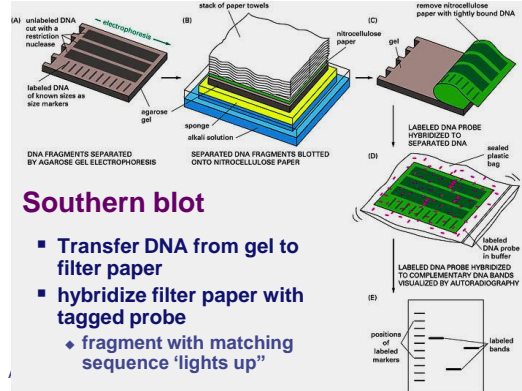
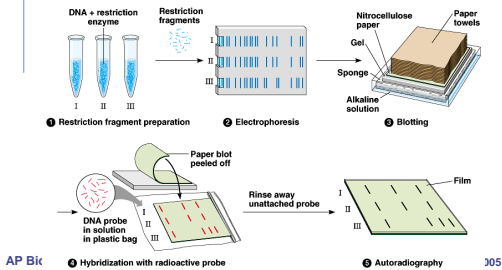
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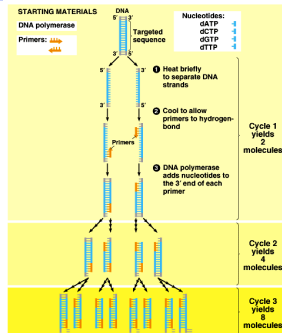
## Southern Blot

- Want to locate a sequence on a gel?



## Polymerase Chain Reaction (PCR)

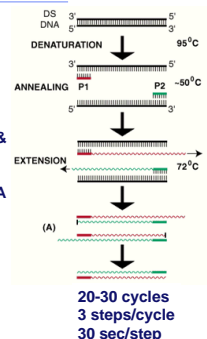
- What if you have too little DNA to work with?
  - PCR is a method for making many copies of a specific segment of DNA
  - ~only need 1 cell of DNA to start



copying DNA without bacteria or plasmids!

## PCR primers

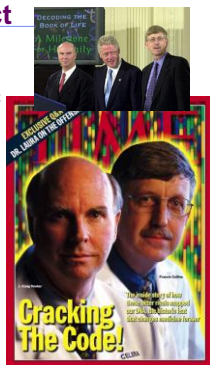
- The primers are critical!
  - need to know sequence to make proper primers
  - primers flank target sequence
    - start with long piece of DNA & copy a specified shorter segment
    - primers define section of DNA to be cloned
- Taq polymerase
  - from hot springs bacteria
  - why do we use it?



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## Human Genome Project

- U.S government project
  - begun in 1990
    - estimated to be a 15 year project
  - DOE & NIH
    - initiated by Jim Watson
    - led by Francis Collins
  - goal was to sequence entire human genome
    - 3 billion base pairs
- Celera Genomics
  - Craig Venter challenged gov't
  - would do it faster, cheaper
  - private company



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## Human Genome Project

On June 26, 2001, HGP published the "working draft" of the DNA sequence of the human genome.

### Historic Event!

- blueprint of a human
- the potential to change science & medicine



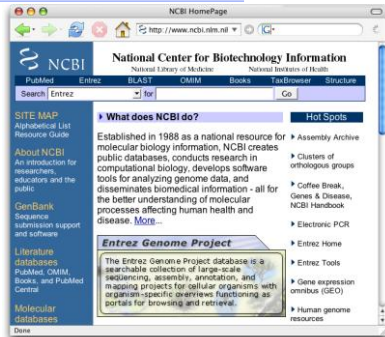
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## GenBank

Database of genetic sequences gathered from research

Publicly available!



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## Other genomes

Table 20.1 Genome Sizes and Numbers of Genes

Organism	Genome Size	Estimated Number of Genes	Genes per Mb*
<i>H. influenzae</i> (bacterium)	1.8 Mb*	1,700	950
<i>S. cerevisiae</i> (yeast)	12 Mb	6,000	500
<i>C. elegans</i> (nematode)	97 Mb	19,000	200
<i>A. thaliana</i> (plant)	100 Mb	25,000	200
<i>D. melanogaster</i> (fruit fly)	180 Mb	13,000	100
<i>H. sapiens</i> (human)	3,200 Mb	30,000–40,000	10

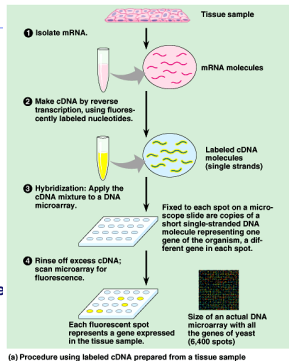
\*Mb = million base pairs

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## Microarrays

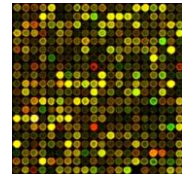
- Measuring expression of genes in a tissue sample
  - samples of mRNA from cells
    - make cDNA
    - 2-color fluorescent tagging
  - hybridize to spots of genes
    - colored spots = gene expression
    - red, green, yellow



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## When to use microarrays?

- Research tool
  - it's all about comparisons
    - gene expression...
      - before vs. after treatment
      - cancer vs. normal cells
      - wound healing vs. scarring
      - stages of development
    - color coding
      - red = expression in 1 sample
      - green = expression in other sample
      - yellow = expression in both samples
      - dark = low expression in both



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## Biotechnology today: Applications

- Application of DNA technologies
  - basic biological research
  - medical diagnostics
  - medical treatment (gene therapy)
  - pharmaceutical production
  - forensics
  - environmental cleanup
  - agricultural applications

...and then there's the **ethics issues!**

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## Application of recombinant DNA

- Combining sequences of DNA from 2 different sources into 1 DNA molecule
  - often from different species
    - human insulin gene in *E. coli* (humulin)
    - frost resistant gene from Arctic fish in strawberries
    - "Roundup-ready" bacterial gene in soybeans
    - BT bacterial gene in corn
    - jellyfish glow gene in Zebra "Glofish"



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## What next?

- **After you have cloned & amplified DNA (genes), you can then tackle more interesting questions**
  - ◆ how does gene differ from person to person?
    - ...or species to species
  - ◆ is a certain allele associated with a hereditary disorder
  - ◆ in which cells is gene expressed?
  - ◆ where is gene in genome?