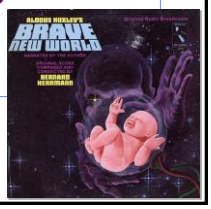
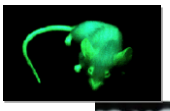
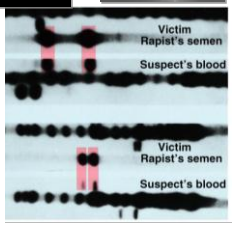
Biotechnology



A Brave New World



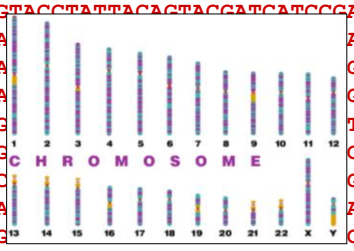


23andMe

TACGCACATTTACGTACGCGGATGCCGCGACTATGATC
ACATAGACATGCTGTCAGCTCTAGTAGACTAGCTGACT
CGACTAGCATGCTGTCAGCTCTAGTAGACTAGCACACYC
GTACATCGATGCTGTCAGCTCTAGTAGACTACATGCTA
CTAGCTACTGACTCATGATCCAGATCACTGAAACCCTA
GATCGGGTACCTATTACACTACGATCATCCGATCAGAT
CATGCTAATCTAGC
TCAATCAGACTAGC
TGACTGAGGTACCT
ATTACAGTAGTACA
TCGATCGCAAACTC
TTTTTGCGATCATG
ACTCTGAAGTACGA
TCATCCGCGATACT

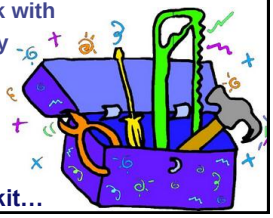
human genome

3.2 billion bases



Biotechnology today

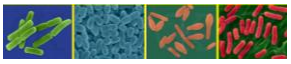
- Genetic Engineering
  - manipulation of DNA
  - if you are going to engineer DNA & genes & organisms, then you need a set of tools to work with
  - this unit is a survey of those tools…

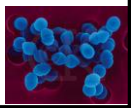


Our tool kit…

Bacteria

- one-celled prokaryotes
- reproduce by mitosis
  - binary fission
- rapid growth
  - generation every ~20 minutes
    - 10^8 (100 million) colony overnight!
  - a dominant form of life on Earth
  - incredibly diverse



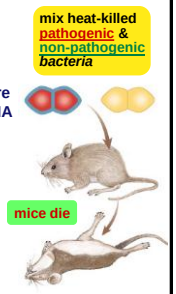


Bacterial genome

- Single circular chromosome
  - haploid
  - naked DNA
    - no histone proteins
  - ~4 million base pairs
    - ~4300 genes
    - 1/1000 DNA in eukaryote

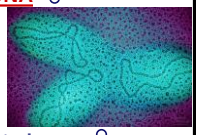
Transformation

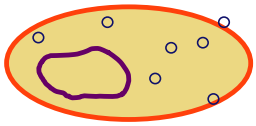
- Bacteria are opportunists
  - pick up naked foreign DNA wherever it may be hanging out
    - have surface transport proteins that are specialized for the uptake of naked DNA
  - import bits of chromosomes from other bacteria
  - incorporate the DNA bits into their own chromosome
    - express new genes
    - transformation
    - form of recombination



Plasmids

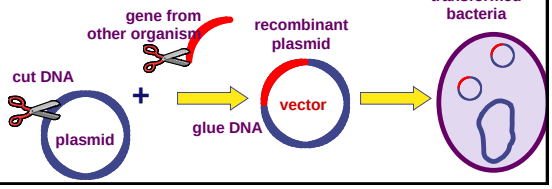
- Small supplemental circles of DNA
  - 5000 - 20,000 base pairs
  - self-replicating
  - carry extra genes
    - 2-30 genes
    - genes for antibiotic resistance
  - can be exchanged between bacteria
    - bacterial sex!!
    - rapid evolution
  - can be imported from environment





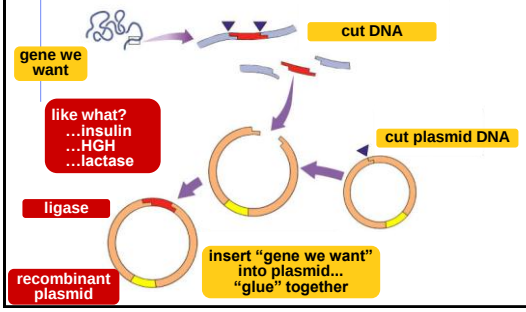
How can plasmids help us?

- A way to get genes into bacteria easily
  - insert new gene into plasmid
  - insert plasmid into bacteria = vector
  - bacteria now expresses new gene
    - bacteria make new protein



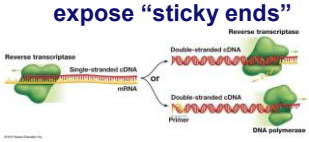
Biotechnology

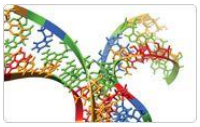
- Plasmids used to insert new genes into bacteria



How do we get the gene we want?

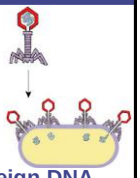
- Individual mRNA codes for specific proteins extracted
- mRNA converted to Complementary DNA (cDNA) using reverse transcriptase
  - "reverses transcription"
- Then cut to acquire exact code and to expose "sticky ends"

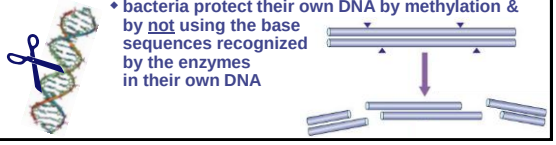




How do we cut DNA?

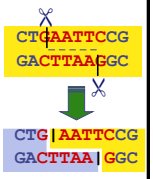
- Restriction enzymes
  - restriction endonucleases
  - discovered in 1960s
  - evolved in bacteria to cut up foreign DNA
    - "restrict" the action of the attacking organism
    - protection against viruses & other bacteria
      - bacteria protect their own DNA by methylation & by not using the base sequences recognized by the enzymes in their own DNA





Restriction enzymes

- Action of enzyme
  - cut DNA at specific sequences
    - restriction site
  - symmetrical "palindrome"
  - produces protruding ends
    - sticky ends
    - will bind to any complementary DNA
- Many different enzymes



1960s | 1978

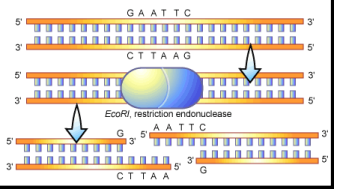
Discovery of restriction enzymes


Werner Arber


Daniel Nathans

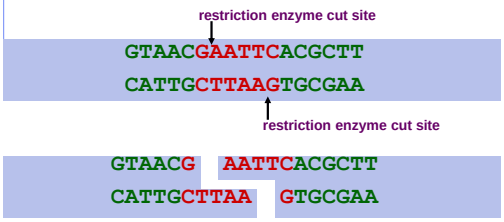

Hamilton O. Smith

Restriction enzymes are named for the organism they come from:
EcoRI = 1st restriction enzyme found in E. coli



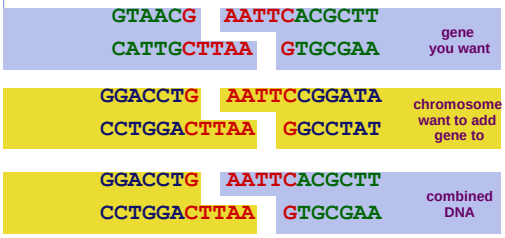
Restriction enzymes

- Cut DNA at specific sites
  - leaves "sticky ends"

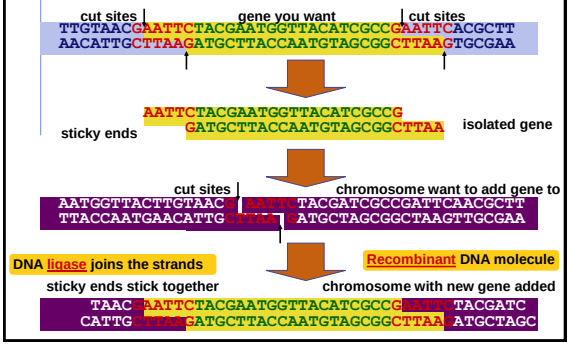


Sticky ends

- Cut other DNA with same enzymes
  - leave "sticky ends" on both
  - can glue DNA together at "sticky ends"

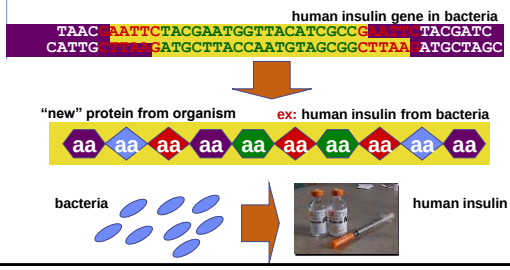


Sticky ends help glue genes together



Why mix genes together?

- Gene produces protein in different organism or different individual

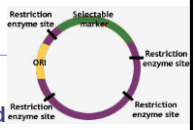


The code is universal

- Since all living organisms…
  - use the same DNA
  - use the same code book
  - read their genes the same way

First base (5' end)	Second base U	Second base C	Second base A	Second base G	Third base (3' end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met or start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Copy (& Read) DNA



- Transformation
  - insert recombinant plasmid into bacteria
  - grow recombinant, transformed bacteria in agar cultures
    - bacteria make lots of copies of plasmid
    - "cloning" the plasmid
  - production of many copies of inserted gene
  - production of "new" protein
    - transformed phenotype

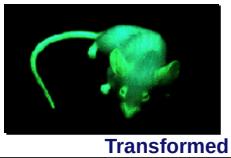
DNA → RNA → protein → trait

Identifying Recombinant Bacteria

- Only a small % of our bacteria will have taken up the recombinant plasmid
- How do we know which?
  - Traditionally have used plasmids with desired gene + antibiotic resistance
  - Issues with this: fear of pathogenic bacteria picking up these genes
  - Alternatives?
- GFP—green fluorescent protein!

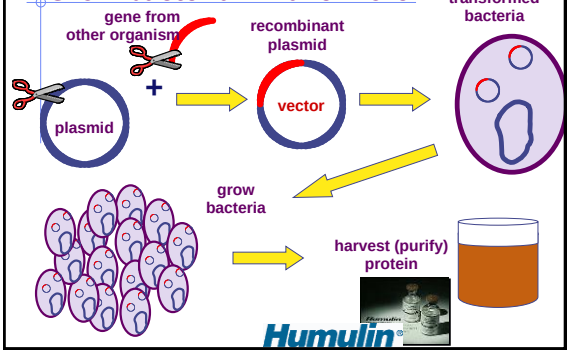
Green with envy??

Jelly fish "GFP"



Transformed vertebrates

Grow bacteria…make more

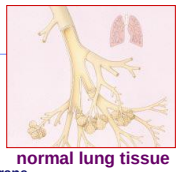


Uses of genetic engineering

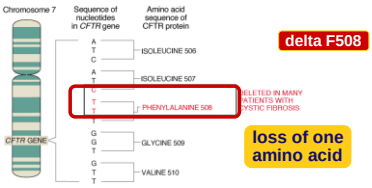
- Genetically modified organisms (GMO)
  - enabling plants to produce new proteins
    - Protect crops from insects: BT corn
      - corn produces a bacterial toxin that kills corn borer (caterpillar pest of corn)
    - Extend growing season: fishberries
      - strawberries with an anti-freezing gene from flounder
    - Improve quality of food: golden rice
      - rice producing vitamin A improves nutritional value

Gene Therapy: Cystic fibrosis

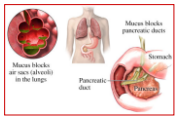
- Remember this?
  - normal allele of the CFTR gene codes for a membrane protein that transports Cl^- across cell membrane
    - defective or absent channels limit transport of Cl^- & H_2O across cell membrane
    - thicker & stickier mucus coats around cells



normal lung tissue



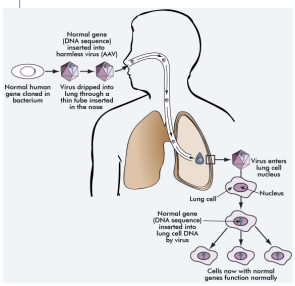
delta F508



loss of one amino acid

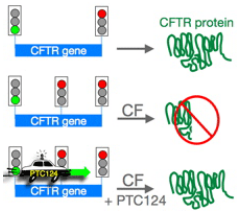
Gene Therapy for CF??

- Caused by TWO copies of defective recessive allele in CFTR gene
  - One properly coding allele confers no symptoms = good candidate for gene therapy
  - Attempts to put the functioning allele into a retrovirus and vector it into human lung cells have had varying degrees of success



Other CF efforts…

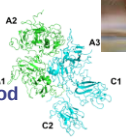
- New mutation discovered is a point mutation that generates a stop codon
- Drug that enables transcription to continue over this stop codon
  - PTC124—more convenient for patients because they just need to take a pill each day!

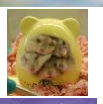


Insulin Production

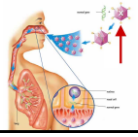
- Beta cells from pancreas extracted, mRNA that codes for human insulin isolated
- mRNA incubated with reverse transcriptase
  - Generates cDNA of insulin genes with sticky ends (cut by restriction enzyme)
- When mixed together, prepared plasmid vector picks up sticky cDNA complementary to its own cut, sticky ends
- Plasmids added to bacteria to form transformed bacteria that make insulin!
  - Much cheaper and more efficient than extracting porcine insulin

Benefits



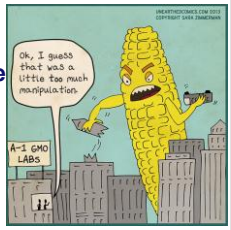


- Can produce proteins necessary for humans
  - Factor VIII (hamster blood clotting) and Insulin
- Can produce food enzymes
  - Rennin (milk clotting) used to be extracted from unweaned calf guts—this is much more humane!
- Gene therapy
  - CF



Hazards?

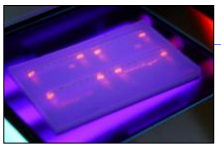
- Gene-jumping?
- Harming non target organisms?
- Antibiotic resistance?
- Largely based on bias against "unnatural" life



Got any Questions?

More Basic Biotechnology Tools

Sorting & Copying DNA

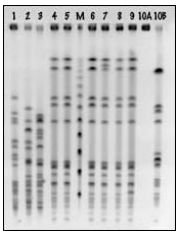


Many uses of restriction enzymes…

- Now that we can cut DNA with restriction enzymes…
  - we can cut up DNA from different people… or different organisms… and compare it
  - why?
    - forensics
    - medical diagnostics
    - paternity
    - evolutionary relationships
    - and more…

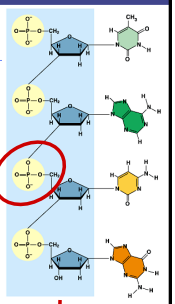
Comparing cut up DNA

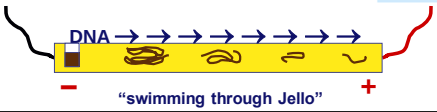
- How do we compare DNA fragments?
  - separate fragments by size
- How do we separate DNA fragments?
  - run it through a gelatin
    - agarose
    - made from algae
  - gel electrophoresis



Gel electrophoresis

- A method of separating DNA in a gelatin-like material using an electrical field
  - DNA is negatively charged
  - when it's in an electrical field it moves toward the positive side

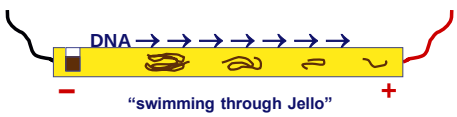




"swimming through Jello"

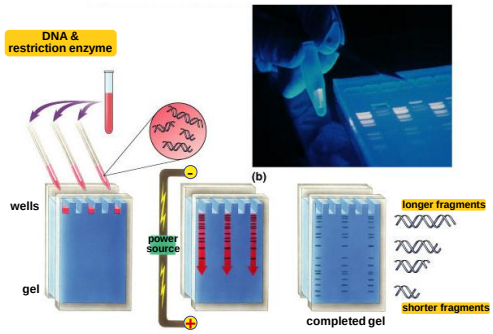
Gel electrophoresis

- DNA moves in an electrical field…
  - so how does that help you compare DNA fragments?
    - size of DNA fragment affects how far it travels
      - small pieces travel farther
      - large pieces travel slower & lag behind



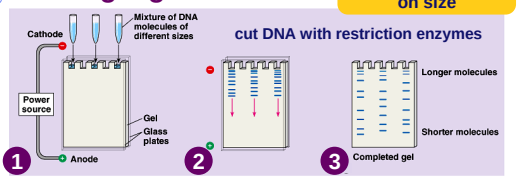
"swimming through Jello"

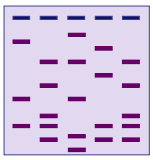
Gel Electrophoresis



Running a gel

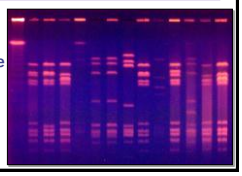
fragments of DNA separate out based on size





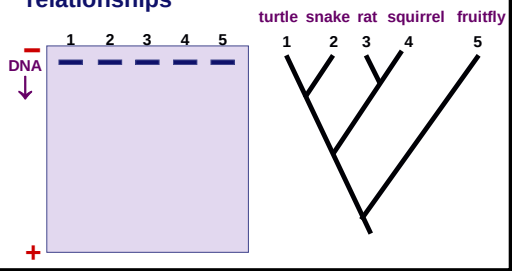
Stain DNA

- ethidium bromide binds to DNA
- fluoresces under UV light



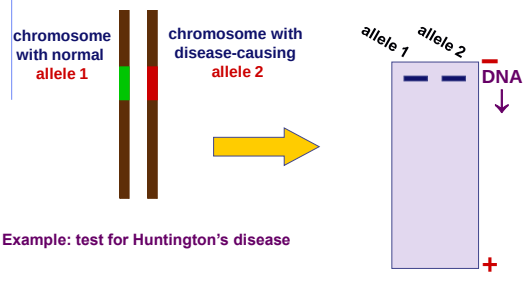
Uses: Evolutionary relationships

- Comparing DNA samples from different organisms to measure evolutionary relationships



Uses: Medical diagnostic

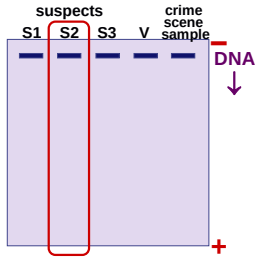
- Comparing normal allele to disease allele



Example: test for Huntington's disease

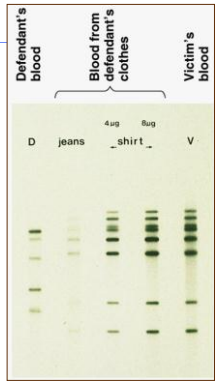
Uses: Forensics

- Comparing DNA sample from crime scene with suspects & victim



DNA fingerprints

- Comparing blood samples on defendant's clothing to determine if it belongs to victim
  - DNA fingerprinting
  - comparing DNA banding pattern between different individuals
  - ~unique patterns



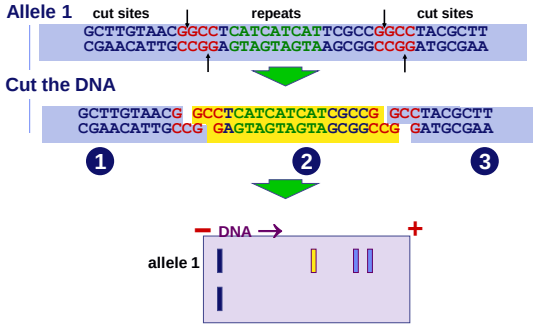
Differences at the DNA level

- Why is each person's DNA pattern different?
  - sections of "junk" DNA
    - doesn't code for proteins
    - made up of repeated patterns: variable number tandem repeats
      - CAT, GCC, and others
      - each person may have different number of repeats
    - many sites on our 23 chromosomes with different repeat patterns

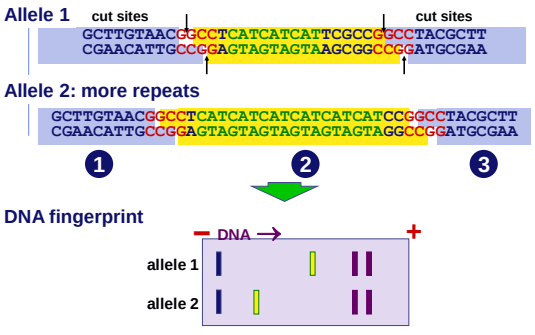
GCTTGTAACGGCCTCATCATCATTCGCCGGCCTACGCTT
CGAACATTGCCGGAGTAGTAGTAAGCGGCCGGATGCGAA

GCTTGTAACGGCATCATCATCATCATCATCCGGCCTACGCTT
CGAACATTGCCGTAGTAGTAGTAGTAGTAGGCCGGATGCGAA

DNA patterns for DNA fingerprints

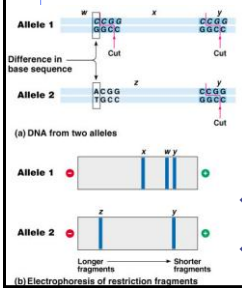


Differences between people

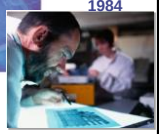


RFLPs

- Restriction Fragment Length Polymorphism
  - differences in DNA between individuals



(c) Completed gel

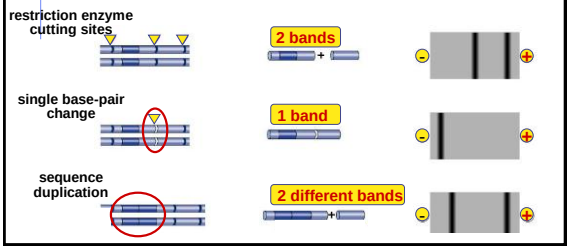


Alec Jeffries
1984

- change in DNA sequence affects restriction enzyme "cut" site
- creates different fragment sizes & different band pattern

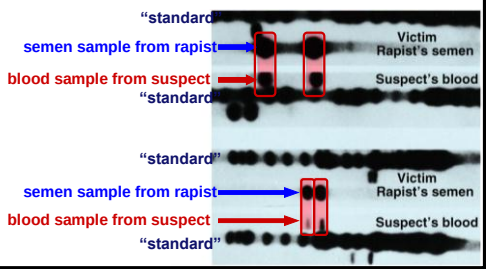
Polymorphisms in populations

- Differences between individuals at the DNA level
  - many differences accumulate in "junk" DNA



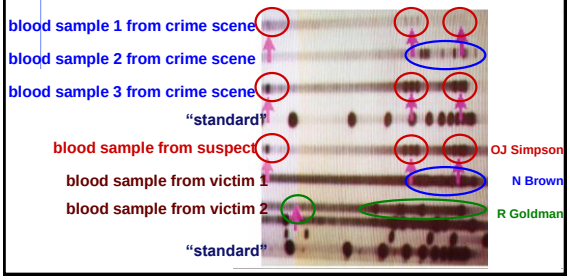
RFLP / electrophoresis use in forensics

- 1st case successfully using DNA evidence
  - 1987 rape case convicting Tommie Lee Andrews



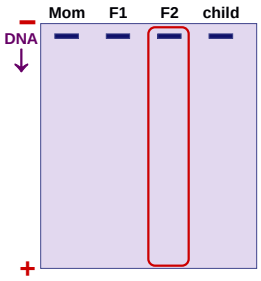
Electrophoresis use in forensics

- Evidence from murder trial
  - Do you think suspect is guilty?

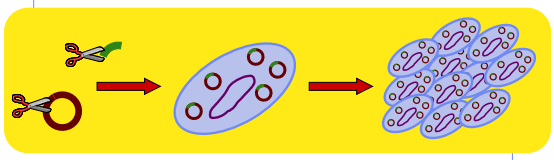


Uses: Paternity

- Who's the father?



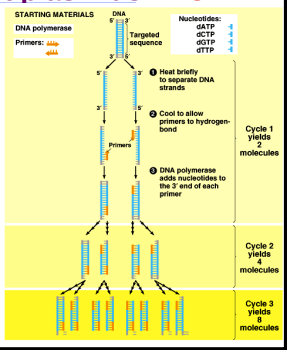
Most DNA analysis requires making lots of copies of DNA



But it would be so much easier if we didn't have to use bacteria every time…

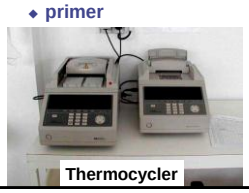
Copy DNA without plasmids? PCR!

- Polymerase Chain Reaction
  - method for making many, many copies of a specific segment of DNA
  - ~only need 1 cell of DNA to start



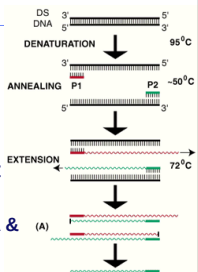
PCR process

- It's copying DNA in a test tube!
- What do you need?
  - template strand
  - DNA polymerase enzyme
  - nucleotides
    - ATP, GTP, CTP, TTP
  - primer


Thermocycler

PCR primers

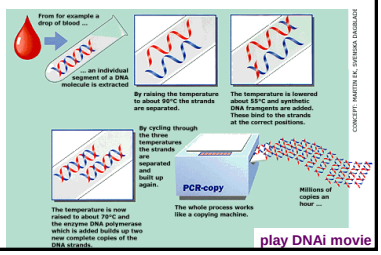
- The primers are critical!
  - need to know a bit of sequence to make proper primers
  - primers can bracket target sequence
    - start with long piece of DNA & copy a specified shorter segment
    - primers define section of DNA to be cloned



20-30 cycles
3 steps/cycle
30 sec/step

PCR process

- What do you need to do?
  - in tube: DNA, DNA polymerase enzyme, primer, nucleotides
  - denature DNA: heat (~90°C) DNA to separate strands
  - anneal DNA: cool to hybridize with primers & build DNA (extension)



play DNAi movie

The polymerase problem

PCR
20-30 cycles
3 steps/cycle
30 sec/step

- Heat DNA to denature (unwind) it
  - 90°C destroys DNA polymerase
  - have to add new enzyme every cycle
    - almost impractical!
- Need enzyme that can withstand 90°C…
  - Tag polymerase
    - from hot springs bacteria
      - *Thermus aquaticus*

Kary Mullis

1985 | 1993

- development of PCR technique
  - a copying machine for DNA