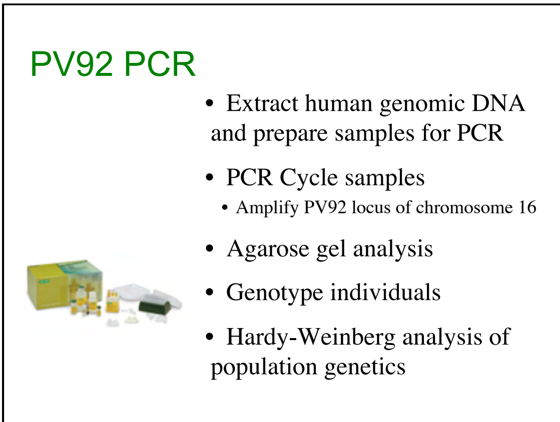
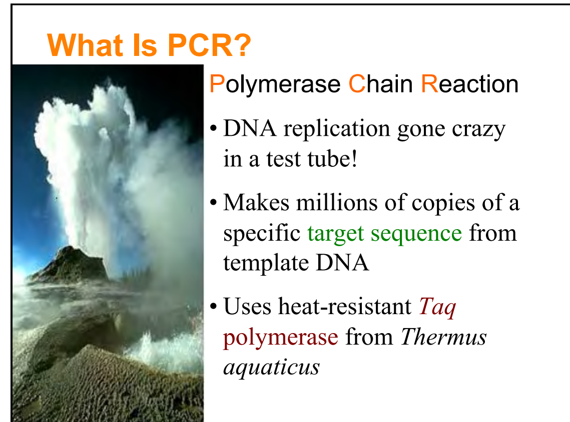
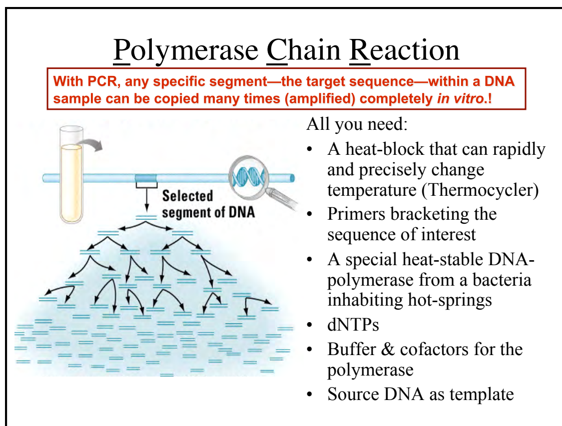
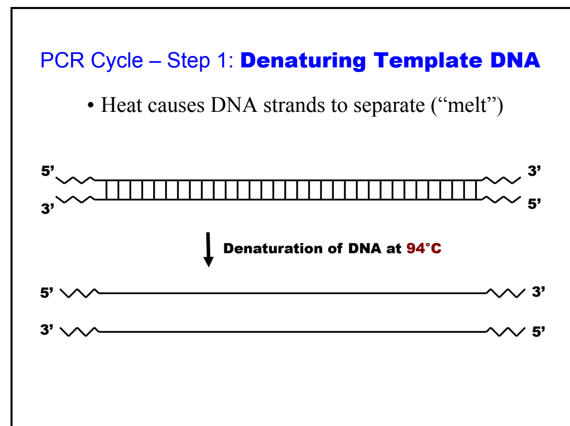
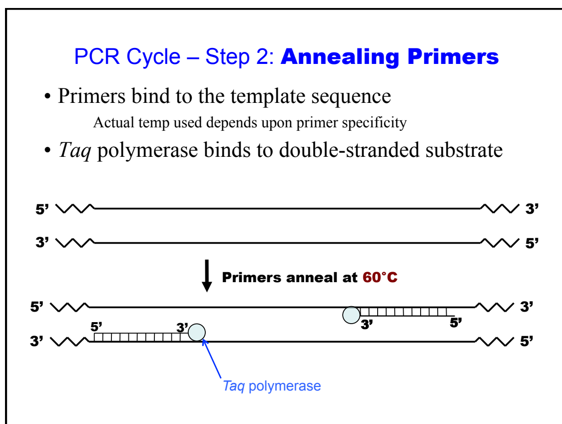
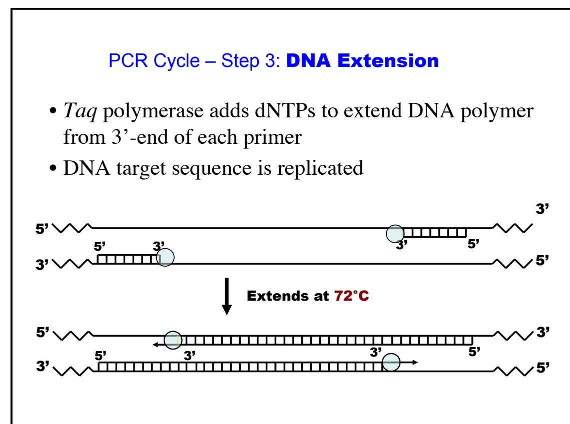

PV92 PCR
• Extract human genomic DNA and prepare samples for PCR
• PCR Cycle samples
• Amplify PV92 locus of chromosome 16
• Agarose gel analysis
• Genotype individuals
• Hardy-Weinberg analysis of population genetics


What Is PCR?
Polymerase Chain Reaction
• DNA replication gone crazy in a test tube!
• Makes millions of copies of a specific target sequence from template DNA
• Uses heat-resistant Taq polymerase from Thermus aquaticus


Polymerase Chain Reaction
With PCR, any specific segment—the target sequence—within a DNA sample can be copied many times (amplified) completely in vitro.!
Selected segment of DNA
All you need:
• A heat-block that can rapidly and precisely change temperature (Thermocycler)
• Primers bracketing the sequence of interest
• A special heat-stable DNA-polymerase from a bacteria inhabiting hot-springs
• dNTPs
• Buffer & cofactors for the polymerase
• Source DNA as template


PCR Cycle – Step 1: Denaturing Template DNA
• Heat causes DNA strands to separate ("melt")
5'
3'
3'
5'
Denaturation of DNA at 94°C
5'
3'
3'
5'


PCR Cycle – Step 2: Annealing Primers
• Primers bind to the template sequence
Actual temp used depends upon primer specificity
• Taq polymerase binds to double-stranded substrate
5'
3'
3'
5'
Primers anneal at 60°C
Taq polymerase


PCR Cycle – Step 3: DNA Extension
• Taq polymerase adds dNTPs to extend DNA polymer from 3'-end of each primer
• DNA target sequence is replicated
Extends at 72°C

Polymerase Chain Reaction

- The PCR procedure

TECHNIQUE

Each temperature cycle:

1. High heat (94°C)
   - "melt" DNA
   - dsDNA→ssDNA
2. Low heat (45–60°C)
   - Allow primers to anneal to DNA
3. Medium heat (72°C)
   - Reduce nonspecific binding
   - Taq polymerase polymerizes new DNA on template DNA
4. Return to Step 1.
   - New DNA from Step 3 also used as template.

Genomic DNA
Target sequence
1 Denaturation: Heat briefly to separate DNA strands
2 Annealing: Cool to allow primers to hydrogen-bond.
3 Extension: DNA polymerase adds nucleotides to the 3′ end of each primer
Primers
New nucleotides
Cycle 1 yields 2 molecules
Cycle 2 yields 4 molecules
Cycle 3 yields 8 molecules; 2 molecules (in white boxes) match target sequence

Polymerase Chain Reaction

- The PCR procedure

TECHNIQUE The starting materials for PCR are double-stranded DNA containing the target nucleotide sequence to be copied, a heat-resistant DNA polymerase, all four nucleotides, and two short, single-stranded DNA molecules that serve as primers. One primer is complementary to one strand at one end of the target sequence; the second is complementary to the other strand at the other end of the sequence.

RESULTS During each PCR cycle, the target DNA sequence is doubled.

- **Theoretical yield: 2^n-fold! (n= # of cycles)**
- By the end of the third cycle, one-fourth of the molecules correspond exactly to the target sequence, with both strands of the correct length. (See white boxes in Cycle 3.)
- After 20 or so cycles, the target sequence molecules outnumber all others by a billionfold or more.

Figure 20.7

POLYMERASE CHAIN REACTION

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DNA region of interest.

1. DNA is denatured. Primers attach to each strand. A new DNA strand is synthesized behind primers on each template strand.

primer

2. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

3. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

4. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

5. Continued rounds of amplification swiftly produce large numbers of identical fragments. Each fragment contains the DNA region of interest.

Polymerase Chain Reaction

- Animation from Harvey Lodish, *et al* Molecular Cell Biology
- http://bcs.whfreeman.com/lodish6e/pages/bcs-main.asp?v=category&s=00010&n=05000&i=05010.06&o=|00510|00520|00530|00540|00550|00560|00570|00580|00590|00600|00610|00010|00020|00030|00040|00050|00060|00070|01000|02000|03000|04000|05000|06000|07000|08000|09000|10000|11000|12000|13000|14000|15000|16000|17000|18000|19000|20000|21000|22000|23000|24000|25000|99000|&ns=293

MOLECULAR CELL BIOLOGY
SIXTH EDITION

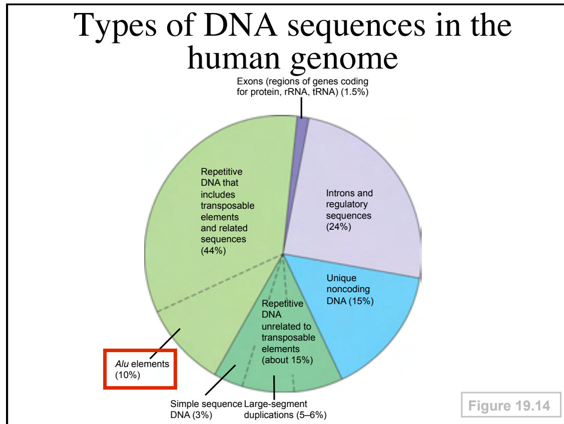
Chromosome 16 PV92 PCR Procedure Overview

DNA template preparation
DNA amplification

Protocol Highlights: Genomic DNA Extraction

Cell membrane
Genomic DNA
Heat disrupts membranes
InstaGene Extraction: InstaGene matrix binds released cellular Mg^{++}

- InstaGene™ - Chelex® cation exchange resin; binds cellular magnesium ions
- 56°C - loosens connective tissue and inactivates DNAses
- 100°C - ruptures cell membranes and denatures proteins



Alu Repeats

- Classified as SINEs (Short Interspersed Repetitive Element) — ~300 bp; highly conserved
- Mobilized by an RNA polymerase-derived intermediate (retroposition)
- Approx. 500,000 *Alu* copies per haploid genome, representing about 5% of the genome
- Named for the *Alu* I restriction site within the element