

DNA Amplification by the Polymerase Chain Reaction

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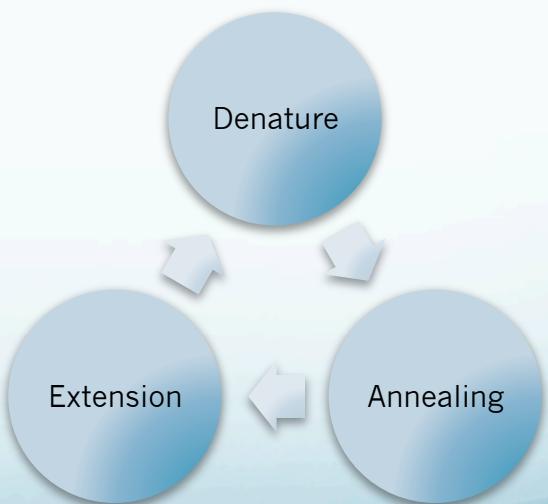
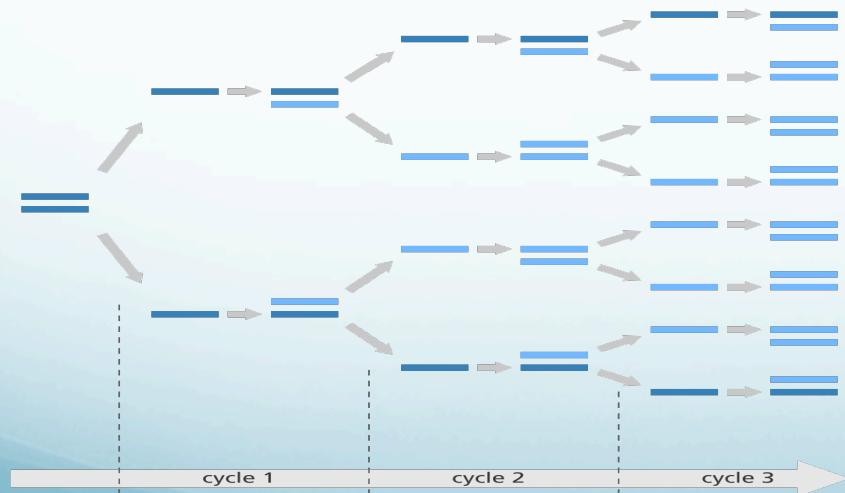
Presented by Jacob Turner

Overview

- Background
- Materials
- General procedure
- Variations in PCR
- Sequencing PCR
- Applications

Background

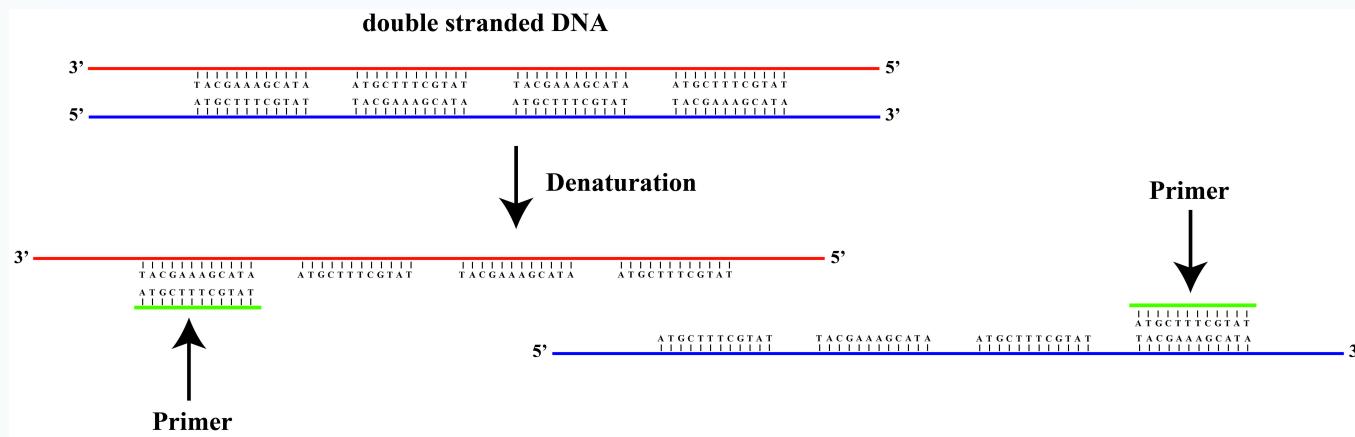
- PCR is DNA amplification
- Isolate/Purify DNA containing gene of interest → PCR → insert **PCR products into plasmids** → transform plasmid into bacterial cell → grow cells on gel



Materials

- DNA containing gene of interest
- Oligonucleotide Primers
- DNA Polymerase
- dNTPs
- Bivalent cations
- Thermal cycler

Primers



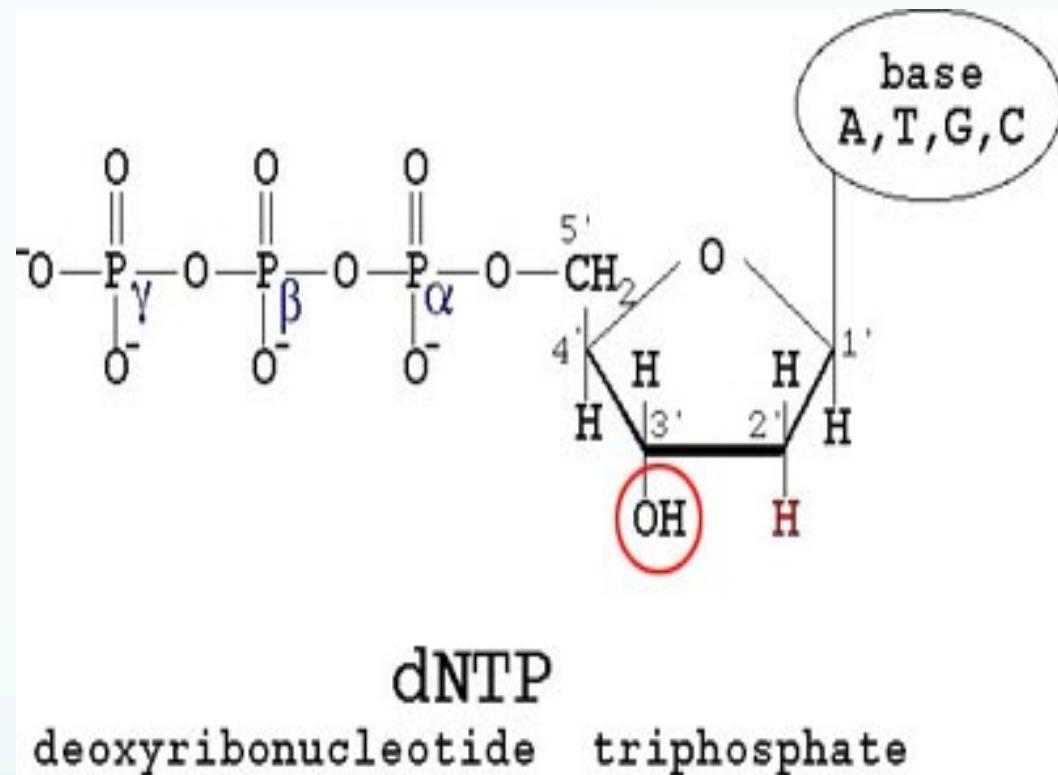
- ssDNA sequence specific to certain regions of the parent DNA.
 - Can be oligo dT, random, or gene specific
- Bind to parent DNA strands at the 3' end, making the marker the 5' end of the soon-to-be synthesized DNA strand.
- Allows for binding of DNA polymerase, which will synthesize in the 5'-3' direction.

Polymerase & Bivalent Cations

- The goal of Polymerase is to synthesize a new stand complementary to the one it is reading.
 - The polymerase will undergo extreme heat, so we want our polymerase from a heat resistant organism (Taq)
- The Taq polymerase will bind to the end of the primers and with the parent strand. The polymerase will then use dNTPs in solution to form the new strand
- Bivalent cations (Mg^{2+}) will act as a catalyst in facilitating the formation of phosphodiester bonds between dNTPs
 - Will also stabilize the repelling (-) charges between backbones

dNTPs

- The building blocks for the new DNA strand
- Form phosphodiester bonds with each other to form backbone of new strand.
- dATP, dTTP, dGTP, dCTP

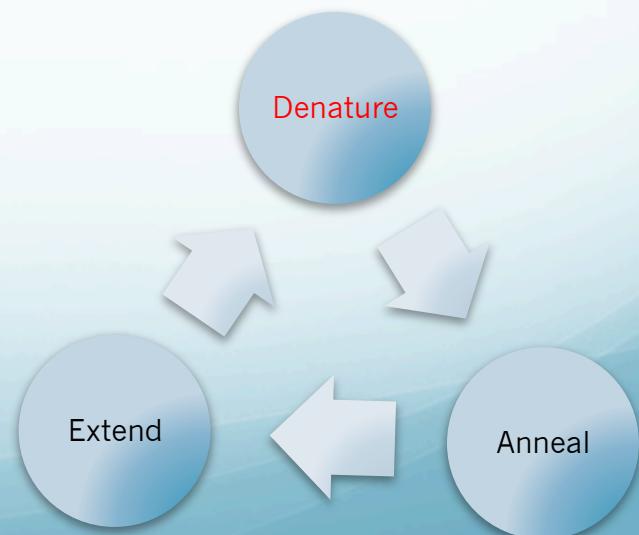


Thermal cycler



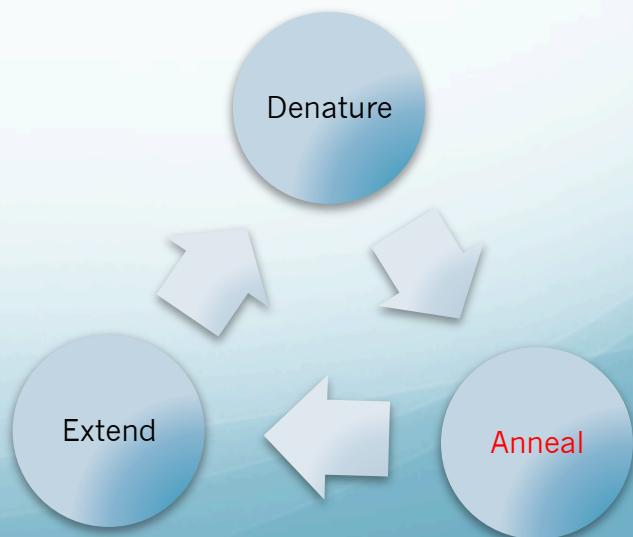
Denaturation

- Initial: 95 degrees C for 5 minutes
 - Heat above the T_m for DNA to break the H bonds between the nitrogenous bases, creating 2 ssDNA
- Initial heating also activates Taq polymerase



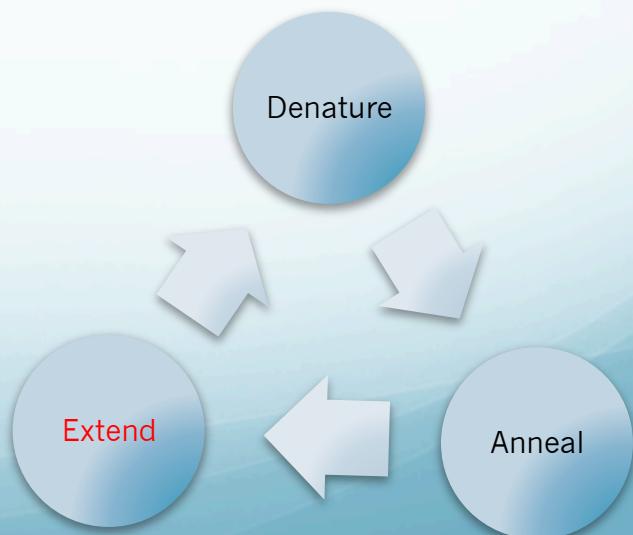
Annealing

- Primers will bind to denatured DNA strands
 - Best annealing temperature = 3-5 degrees lower than lowest primer Tm. (62 C)
 - 30 seconds

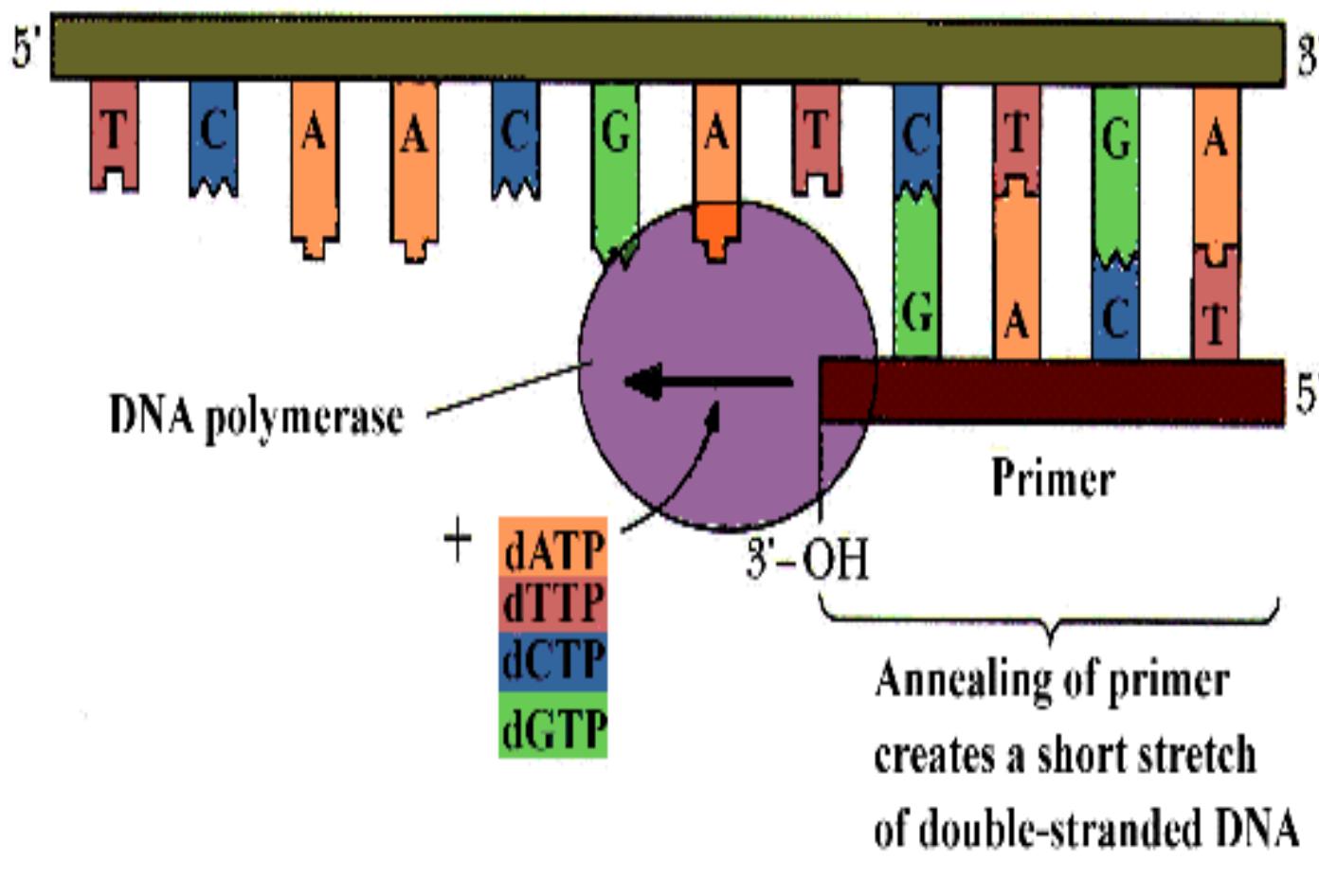


Extension

- Heat to optimal temperature for polymerase activity (72 C) for 30 seconds
- Polymerase collects dNTPs complementary to template strand and forms them together to synthesize new strand



Single-Stranded DNA



Repeat, repeat, repeat

- # of DNA created per thermal cycle = 2^n
 - N = number of cycles

Cycles	# of target DNA
1	2
15	32,738
20	1,048,576
30	1,073,741,764

- Less than half a day to make 1 billion copies of DNA

Variations in PCR

- RNA PCR (RT PCR)
 - Uses mRNA and reverse transcriptase to generate cDNA
 - Good for the study of expressed gene sequences
 - RT enzymes: MMLV, SS3
- Quantitative PCR (qPCR)
 - Focuses on quantitative methods to improve reproducibility
 - Monitors amplification at each cycle of PCR
- Mutagenizing DNA Sequence by PCR
 - Alteration of DNA sequences by PCR in 2 ways
 - Natural error frequency of Taq polymerase ($1/10^4$ – $1/5 \times 10^4$)
 - Use a third oligonucleotide primer

Variations in PCR

- Multiplex PCR and DNA Deletion Detection
 - Multiple primers to allow for multiple sequence amplifications in one reaction
 - No sequence = No PCR
- DMD and Dystrophin gene (2Mb) deletion
 - >50% of DMD arise from partial deletions on locus
 - 9 primers constructed for 9 regions on locus
 - Normal gene has all 9 bands, deletions = loss of bands

Incomplete Sequence PCR

Often the DNA sequence of interest is not entirely known – use sequence info from flanking regions or related DNA templates

- Vector PCR: DNA is in vector – oligonucleotides directed towards sequences adjacent to the cloning sites
- Linker PCR: Synthetic oligonucleotides ligated at each end of restriction endonuclease digested fragments
 - Same priming sequence is at each end of DNA to be amplified
- Redundant Primers: Degenerate oligonucleotide primers provide for better hybridization to DNA
 - Every 3rd position of the primer will have a degenerate NT

Incomplete Sequence PCR

- Repeated Sequences: use species-specific highly reiterated sequences as markers to recover human DNA from a hybrid
 - PCR fingerprints
- Anchor PCR: have a unique primer which functions opposite a redundant priming site (oligo dT on poly-A tail)
- PCR & circularization: DNA mixture is digested & ligated at low concentrations to promote circle formation
 - primers constructed from known sequence info to amplify around the circle

Identifying Subtle Sequence Variation of PCR Products

- Sequencing Products: 2 approaches – cloning and direct sequencing
 - Cloning: transform into bacteria prior to sequencing
 - Direct Sequencing: fluorescent protocols – biotin residues added onto 5' terminus of primer & captured with streptavidin-coated beads
- Scanning products for mutations – when you don't need to sequence the whole thing
 - The length of each fragment on electrophoresis
 - Endonuclease digestion; melting analysis; heteroduplex mapping
- Rapid detection of known sequence differences
 - Restriction endonuclease digestion
 - Allele specific oligonucleotide primers

Applications of PCR

- Human genetic diseases
 - Survey entire genes to determine mutations and then test family for disease or carrier status
- Infectious diseases
 - PCR & HIV – Routine diagnosis, identifying viral sequences and diversity
- Neoplasia
 - Primers positioned to amplify mutated sequences, but not normal DNA

Applications of PCR

- Studies of somatic mutations
 - Monitor the effect of even weak mutagens on DNA sequences using PCR
- Evolutionary studies
 - Sequence analysis of mitochondrial DNA
 - The “Eve” hypothesis
- The Human Genome project
 - Sequence tag segments isolated by PCR
 - Selected verification of interesting regions of sequence

Works Cited

Gibbs, A. R. (1990). DNA Amplification by the Polymerase Chain reaction. *Analytical Chemistry*, 62, 1202-1214