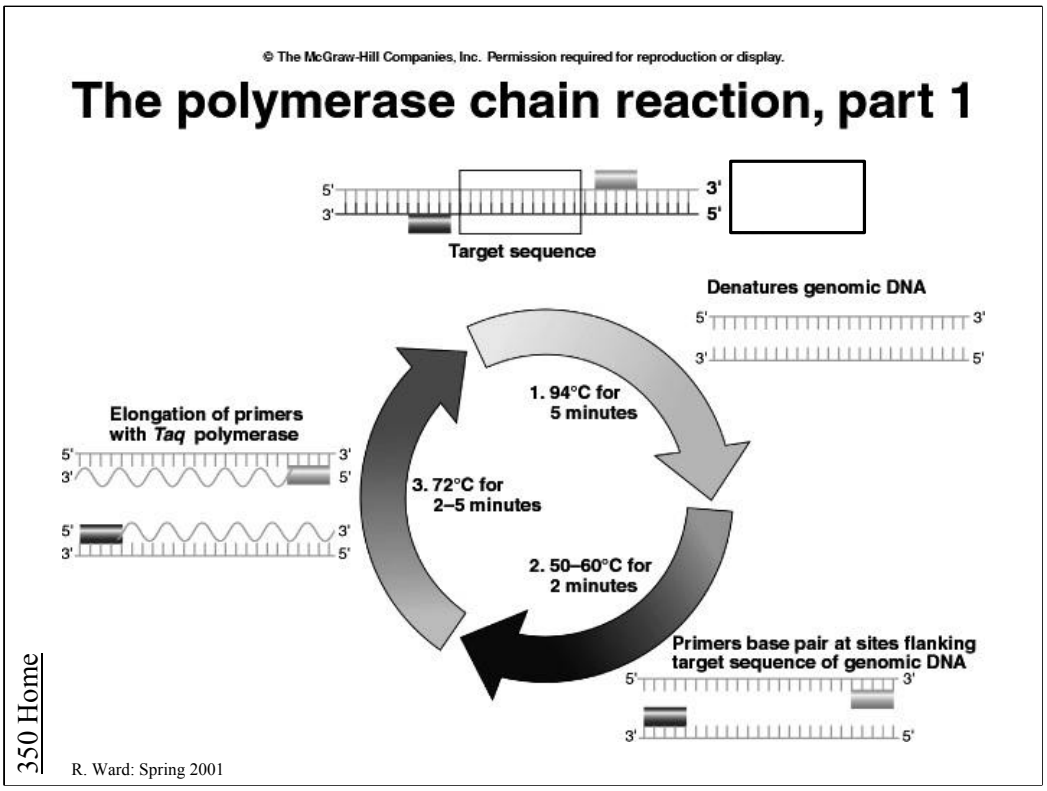


•heat (e.g. 94C) 'denatures' dsDNA by disassociating the two strands

- •hydrogen bonds are broken

•single stranded DNA will 'reaneal' or hybridize with complementary sequences extremely rapidly once temperatures are dropped below 60C or so.

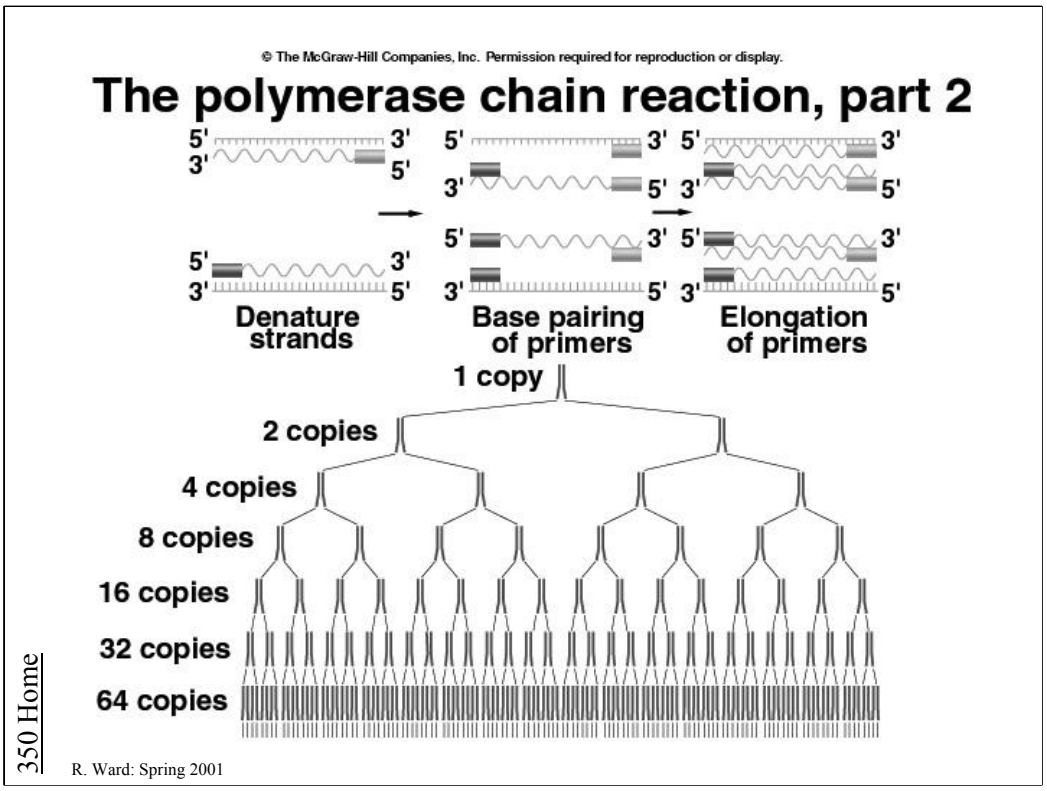
•Primer sequences greatly outnumber the number of original template molecules and will therefore preferentially anneal with target DNA

•TAQ polymerase is a DNA polymerase isolated from a bacteria that lives in very hot water

- •optimum temp for polymerization is around 72C

•Note that the targeted sequence may be imbedded in extremely large fragments of DNA

- •genomic DNA
- •large fragment vectors (YAC-yeast artificial chromosomes, BAC-bacteria artificial chromosomes)



350 Home

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•after the first few cycles, most of the templates molecules are copies created during the previous cycles of PCR.

•all products or amplicons begin and end with primer sequences or their complement

PCR: Polymerase Chain Reaction: in vitro Selective exponential amplification (replication) of a defined sequence of DNA

- Requirements:
  - DNA template that has the targeted sequence
    - genomic DNA (all there is from a plant)
    - small or large fragment clones
  - you know the bp sequence (15 or more bp) of the sequences flanking the subsequence you want to amplify
  - oligonucleotide "primer" sequences complementary to the template terminal sequences
  - Thermal-stable DNA polymerase (Taq)
    - active at 70C, and not denatured at 95C
  - all four deoxy-nucleotides (dNTP)
  - "thermal cycler" unit to cyclically vary temperature with a defined sequence and timing

350 Home

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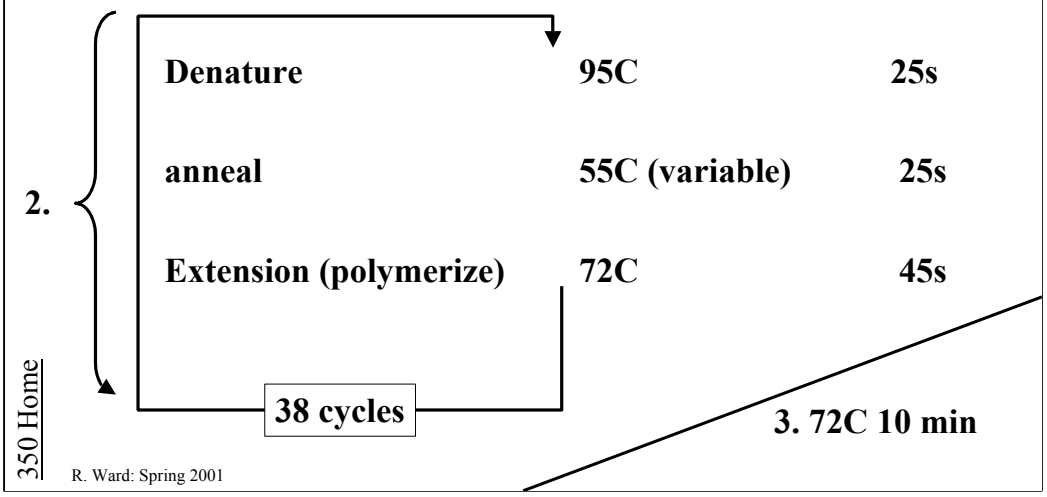
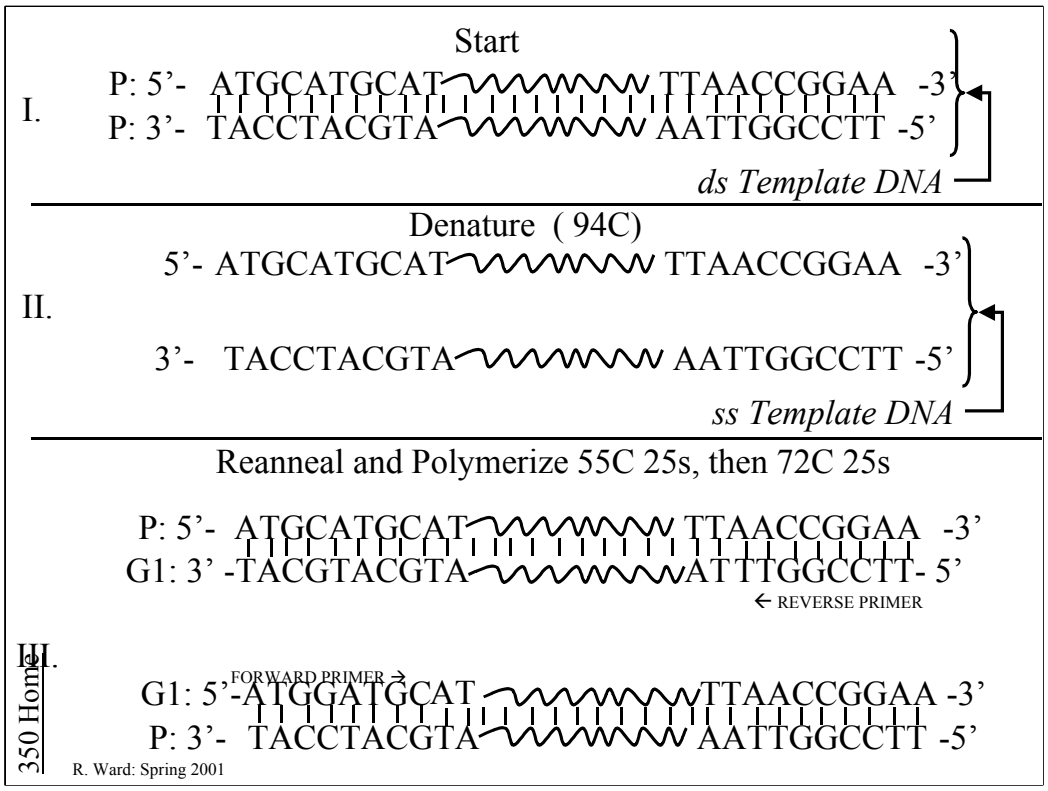
See pages 294 and 295 in your text book.

Notes:

MSU PCR reaction steps for Wheat Microsatellite amplification

1. 95C 3 min.




Start
I.
P: 5'- ATGCATGCAT TTAACCGGAA -3'
P: 3'- TACCTACGTA AATTGGCCTT -5'
ds Template DNA
Denature (94C)
II.
5'- ATGCATGCAT TTAACCGGAA -3'
3'- TACCTACGTA AATTGGCCTT -5'
ss Template DNA
Reanneal and Polymerize 55C 25s, then 72C 25s
P: 5'- ATGCATGCAT TTAACCGGAA -3'
G1: 3' -TACGTACGTA ATTTGGCCTT- 5'
← REVERSE PRIMER
III.
FORWARD PRIMER →
G1: 5'-ATGGATGCAT TTAACCGGAA -3'
P: 3'- TACCTACGTA AATTGGCCTT -5'
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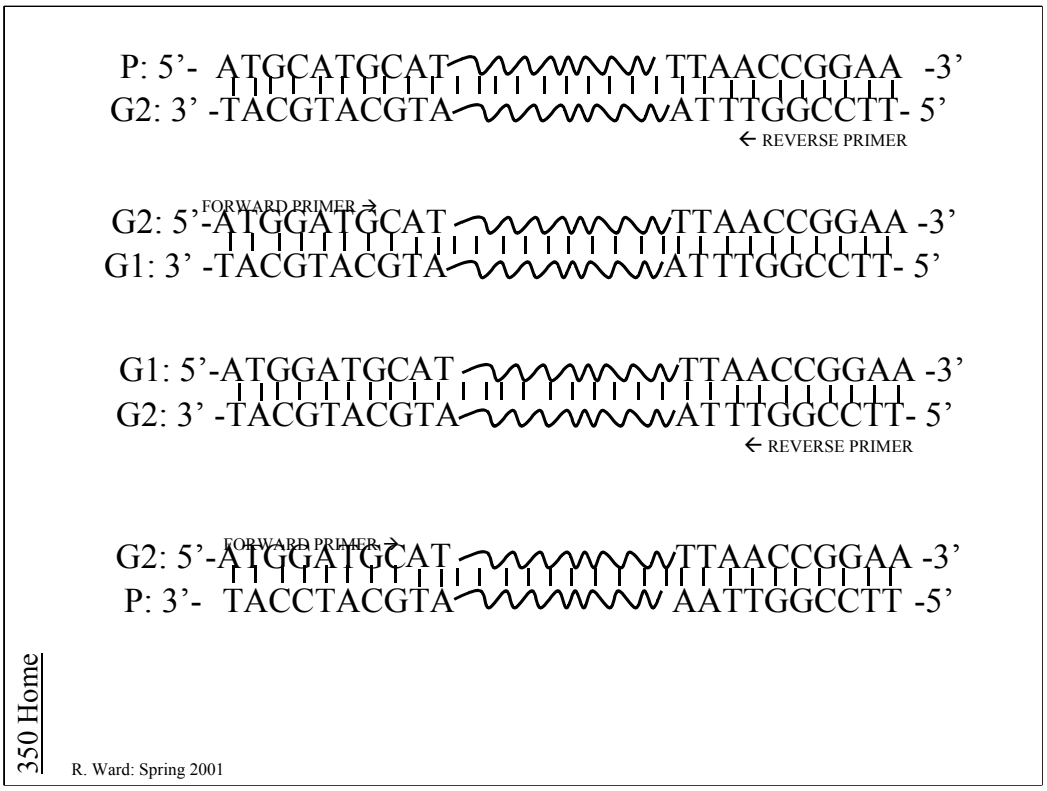

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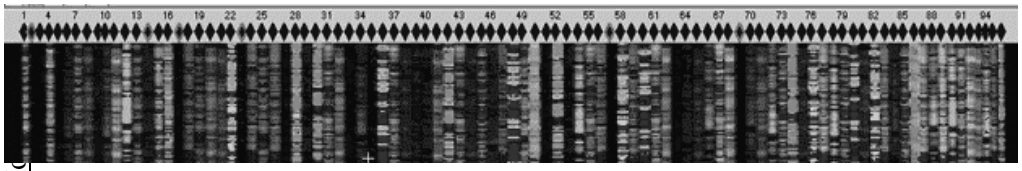
PCR products

- All "amplicons" or amplified fragments are identical in length
  - and start and end with the primer sequences
- # molecules of product vastly outnumbers the number of initial template molecules
- primers or nucleotides can be labeled for subsequent auto-detection



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PCR Products (II)

- different sources of template can be amplified if they are complementary to the 3' ends of both primers- even if they differ in the intervening sequences
- exclusion of one primer results in a linear increase in copies of one strand of a template duplex

PCR: Ingredients

All in a single tube

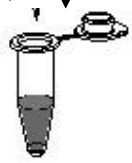
Template DNA

Taq polymerase

Nucleotides (in great excess)

forward and reverse primers (in great excess)

plus buffer solution



"eppendorf tube"

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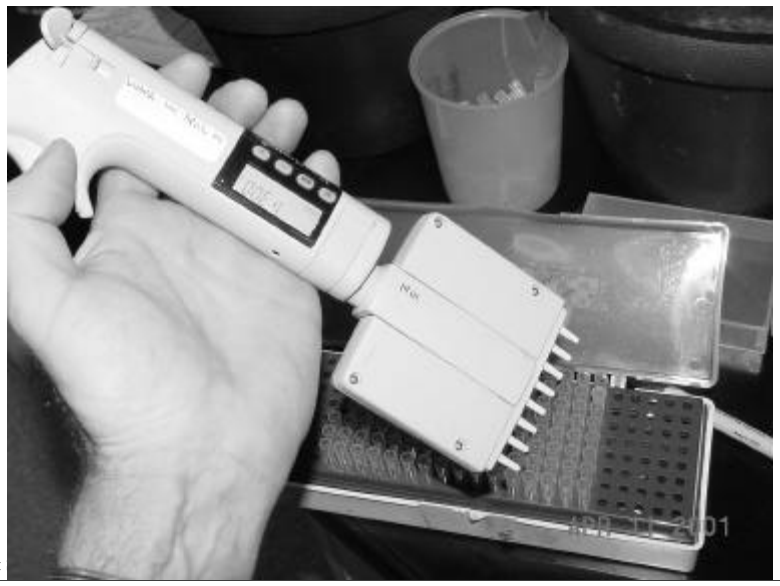
Multi-channel pipetter



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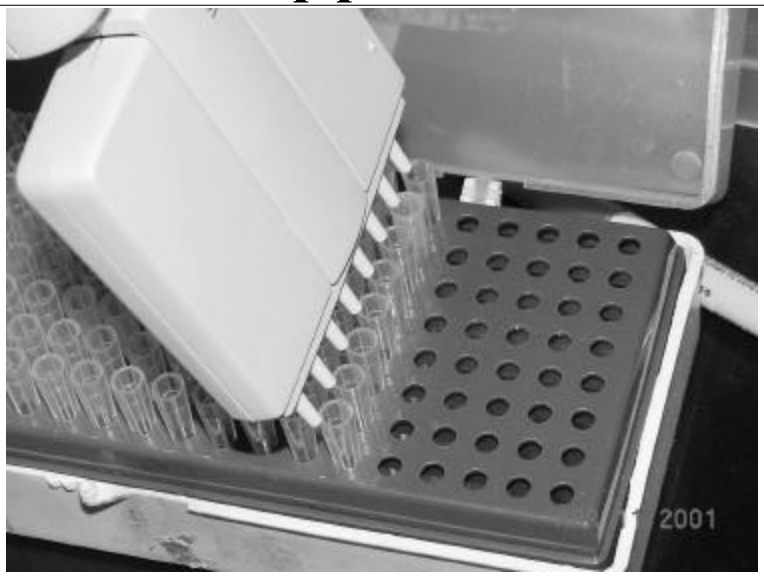
Tip attachment with Multi-chnnel pipetter



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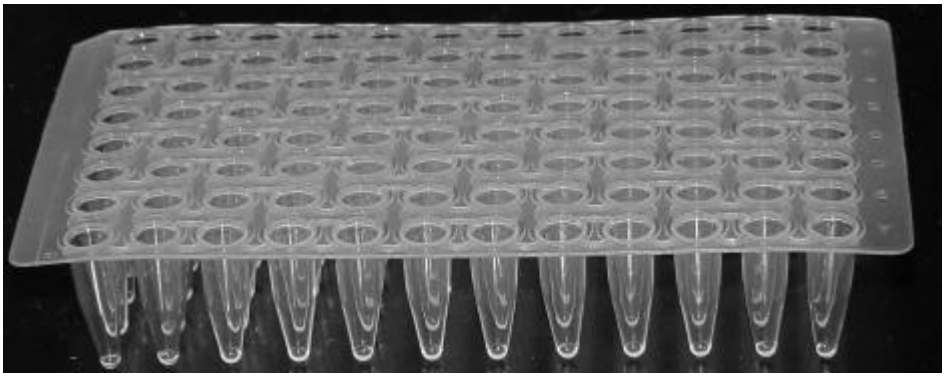
96 well PCR plate



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MJ Quad PCR unit: 4 x 96 reactions

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