

## PCR

The polymerase chain reaction

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## Crick and Watson – structure of DNA



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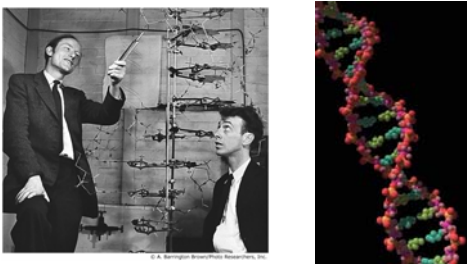
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## A double helix with two antiparallel strands



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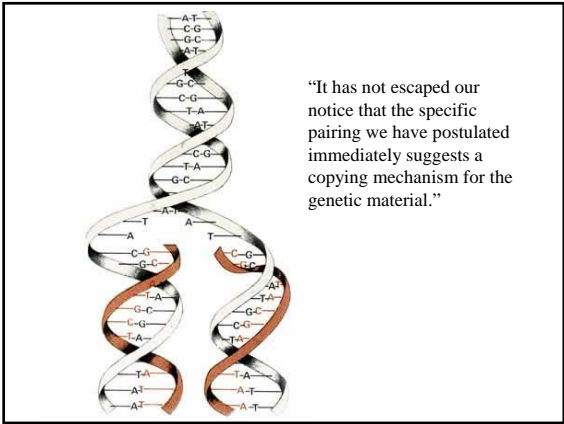
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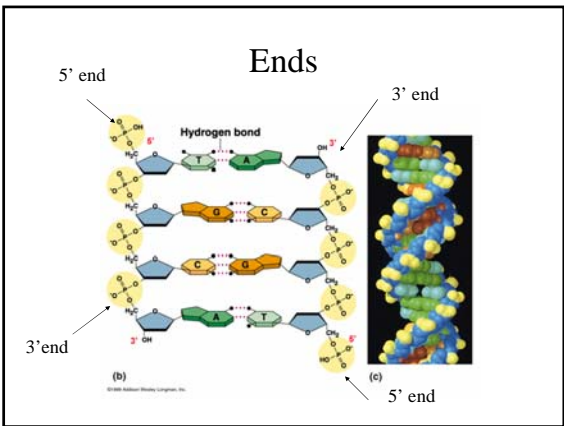
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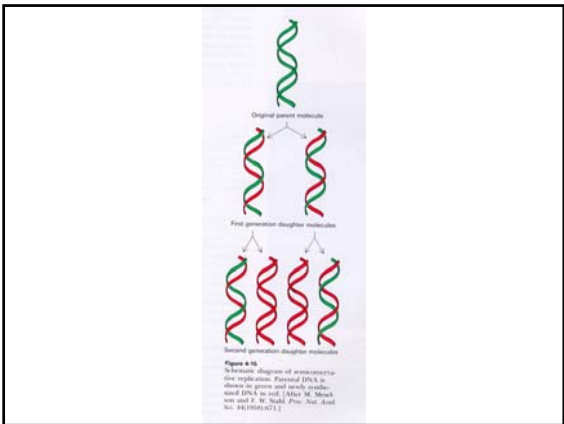
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### DNA-Dependent DNA Polymerases

All DNA polymerases add deoxyribonucleotides to the 3'-hydroxyl terminus of a primed double-stranded DNA molecule (Kornberg, 1980), as shown in Figure 3.5.1.

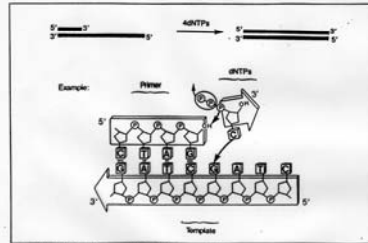


Figure 3.5.1 DNA polymerase 5'→3' polymerase activity.

Synthesis is exclusively in a 5'→3' direction with respect to the synthesized strand. Each nucleotide that is incorporated during polymerization is complementary to the one opposite to it in the template (A pairs with T, G with C). The reaction requires the four deoxyribonucleoside triphosphates (dNTPs) and magnesium ions.

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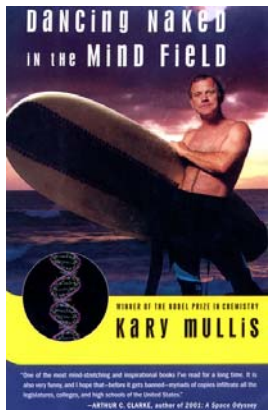
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My gene:

5' TCACGACGTTGTAAAACGACGTCTATACCGAGCTCCTCTCTCTCATAGCTGTTTCTCTGTGAA 3'

I design custom oligonucleotides to the "sense" strand at the beginning and the "antisense" at the end

Sense 5' TCACGACGTTGTAAAACGACGTCTATACCGAGCTCCTCTCTCTCATAGCTGTTTCTCTGTGAA 3'

Anti 3' AGTGCTGCAACATTTGCTGAGATATGGCTCGAGGAGAGAGAGTATCGACAAAGGACACACTT 5'

"forward primer"

5' TCACGACGTTGTAAAACGACG 3'

"reverse primer"

3' GTATCGACAAAGGACACTT 5'

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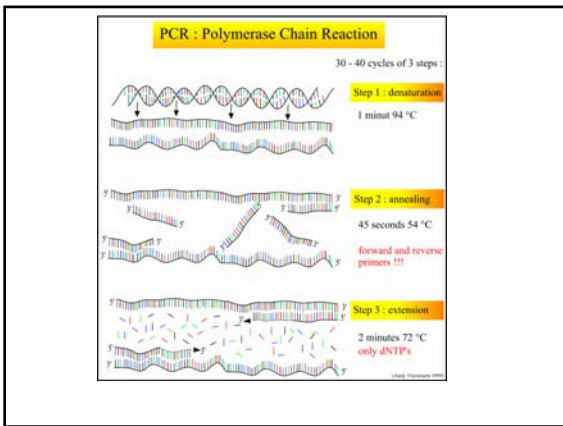
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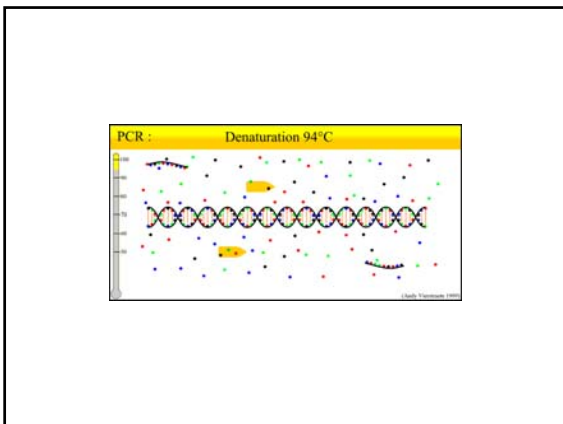
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<http://www.people.virginia.edu/~rjh9u/pcranim.html>

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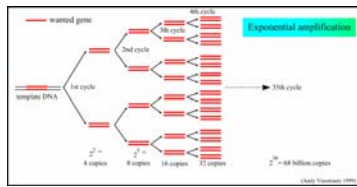
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CYCLE NUMBER	NUMBER OF DOUBLE-STRANDED TARGET MOLECULES
1	0
2	0
3	2
4	4
5	8
6	16
7	32
8	64
9	128
10	256
11	512
12	1024
13	2048
14	4096
15	8192
16	16,384
17	32,768
18	65,536
19	131,072
20	262,144
21	524,288
22	1,048,576
23	2,097,152
24	4,194,304
25	8,388,608
26	16,777,216
27	33,554,432
28	67,108,864
29	134,217,728
30	268,435,456
31	536,870,912
32	1,073,741,824

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**DNA hybridization rate is altered by changing**

Temperature: template dependent

Salt Concentration: can be standardized

DNA concentration: can be standardized

Time: can be standardized

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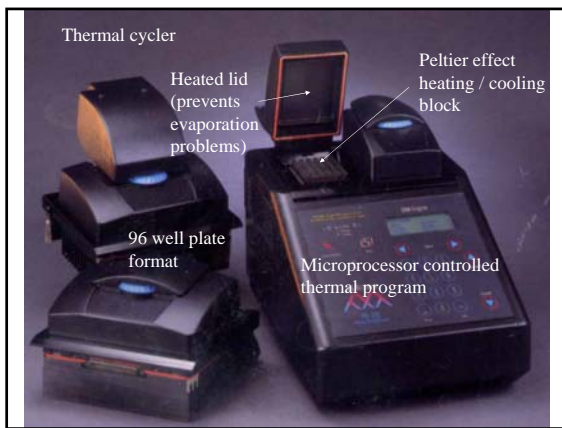
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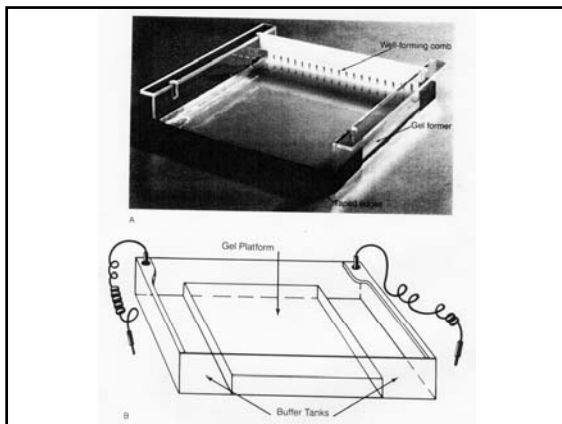
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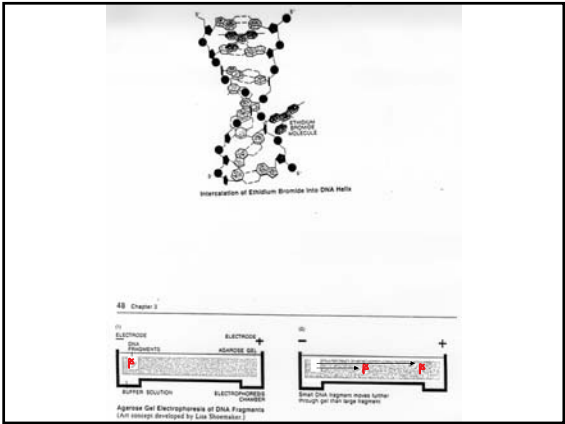
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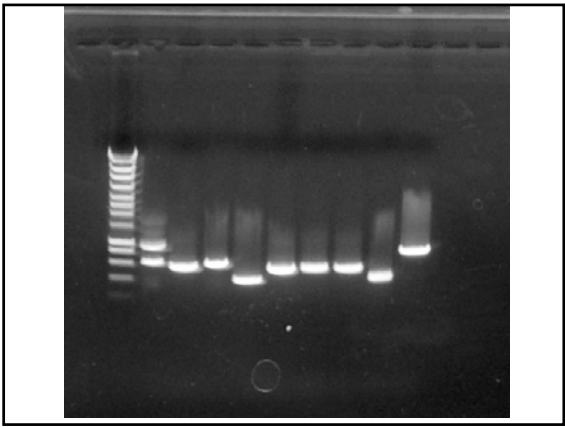
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**Real-time PCR**

Quantitative

Fast

High sample processing speed

Very sensitive

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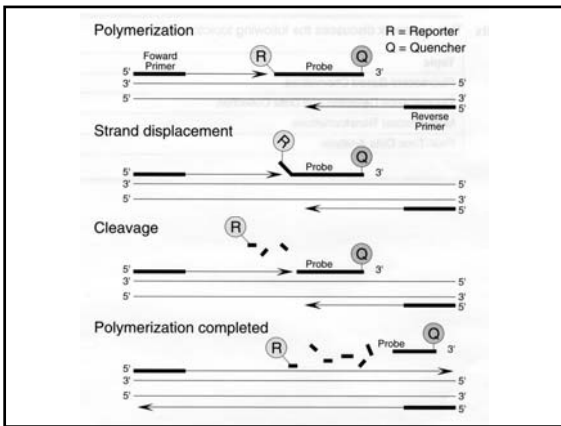
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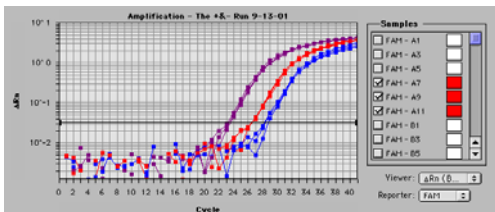
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**Parasitic Worm Inoculation Levels**



**Violet** = 200 worms per root

**Red** = 50 worms per root

**Blue** = 20 worms per root

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