Watson & Crick

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MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

●●●

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Model of DNA replication

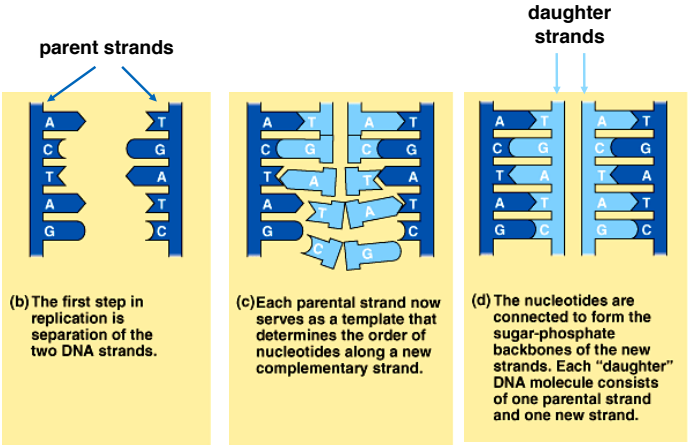
a) The parent molecule has 2 complementary strands of DNA. Each base is hydrogen bonding with its specific partner (A & T, G&C).

b) The first step in replication is the separation of the two DNA strands.

c) Each parental strand now serves as a template that determines the order of nucleotides along a new complementary strand.

d) The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each "daughter" DNA molecule consists of one parental strand and one new strand.

Model of DNA replication



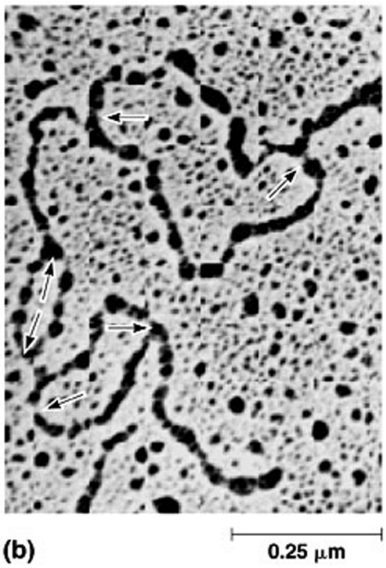
Origins of Replication (Replication Bubbles)

Replication begins at an origin of replication.

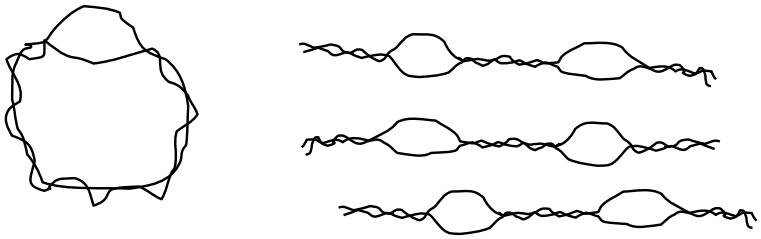
Prokaryotes have ONE origin of replication identified by specific sequence of bases.

Eukaryotes have thousands of origins on each chromosome.

At each origin, DNA double helix is unwound by a helicase enzyme to form replication bubbles.



Prokaryotes vs. Eukaryote: Single vs. Multiple origins of replication



Prokaryote
One circular chromosome,
One replication bubble

Eukaryote
Multiple linear chromosomes
Mutiple replication bubbles on each

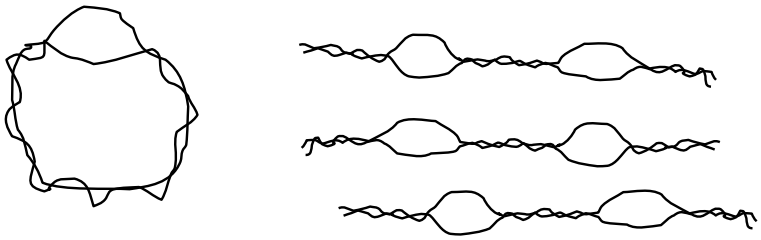
Why do Eukaryotes have multiple origins of replication?

DNA replicates at rate of 50-500 bp / s at each origin.

E. coli genome
5 million bp in 1 chromosome
- 5×10^6 bp divided by 5×10^2 bp / s
- $= 10^4$ s
- = 2.5 h *per* cell division

H. sapiens genome
6 billion b.p. in 46 chromosomes
- 6×10^9 bp divided by 5×10^2 bp / s
- $= 10^7$ s
- = 115 days / cell division

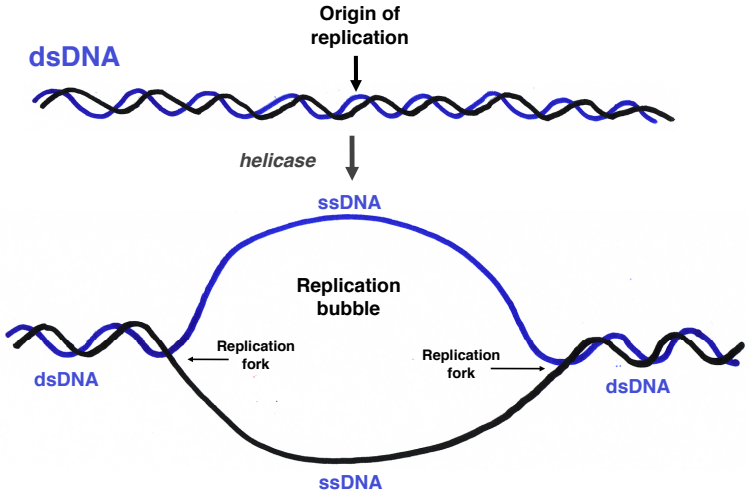
Prokaryotes vs. Eukaryote: Single vs. Multiple origins of replication

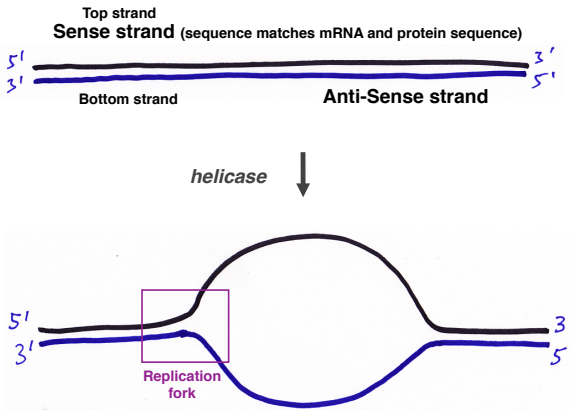


Prokaryote
One circular chromosome,
One replication bubble

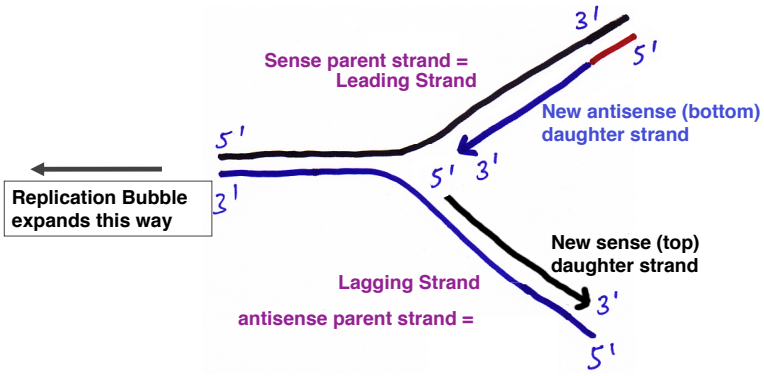
Eukaryote
Multiple linear chromosomes
Mutiple replication bubbles on each

Helicases and the replication bubble

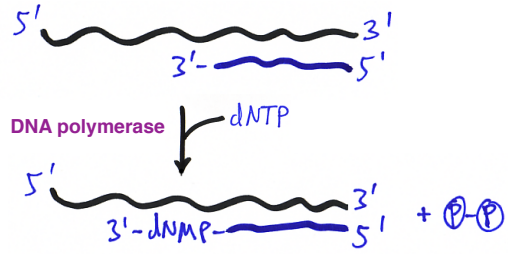


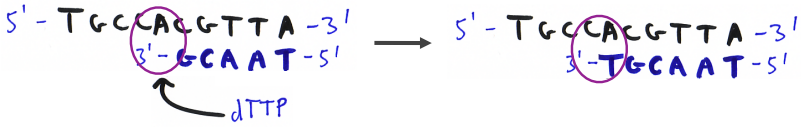


At the Replication Fork:

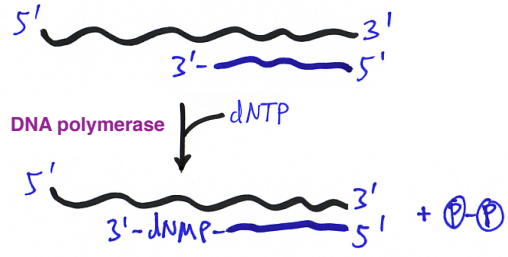


DNA Extension





DNA Extension



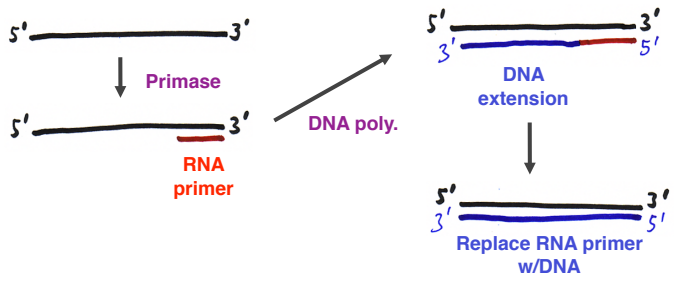
1. Nucleotide triphosphate is used for new base (provides energy, just like ATP).
2. The nucleotide is only added to 3' end of growing DNA strand (daughter strand).
3. Which nucleotide gets added depends on template (parent strand).

Priming

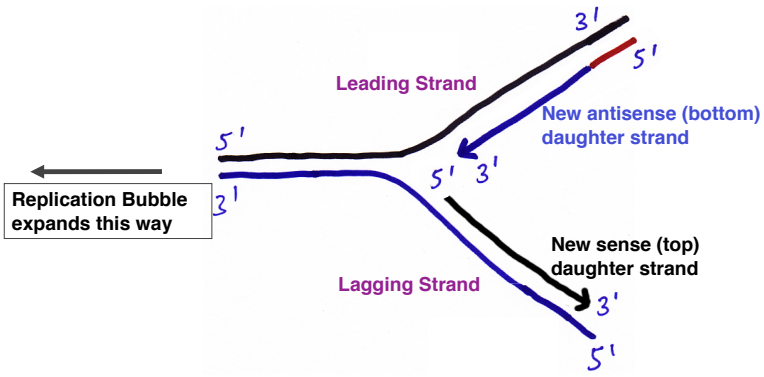
DNA polymerase can't just jump on a parent ssDNA strand and start making a new daughter strand.

DNA polymerase can only extend a short double-stranded segment.

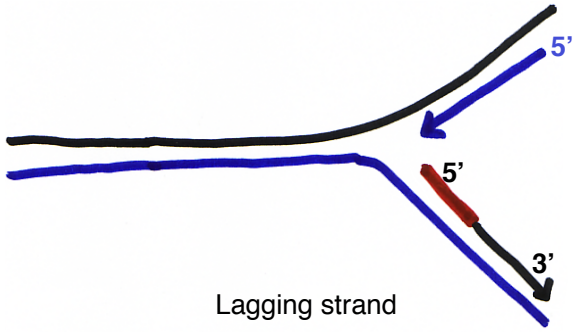
So, parent strand needs to be primed with a short piece of RNA by a primase enzyme.



At the Replication Fork:

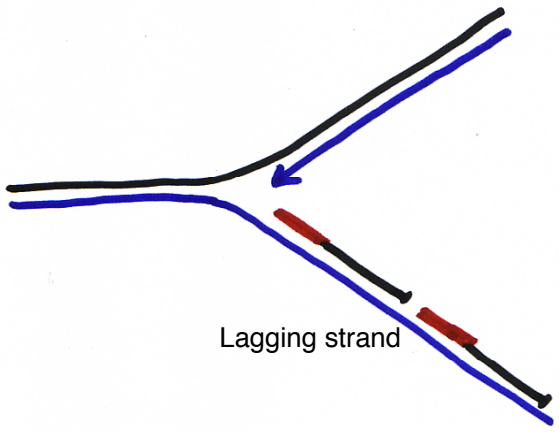


Extension of Lagging Strand

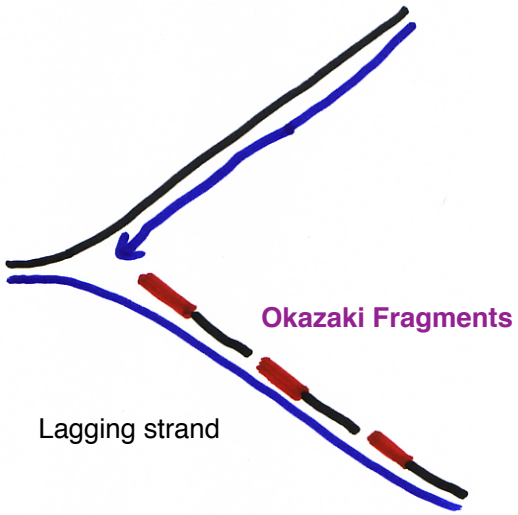


Lagging strand

Extension of Lagging Strand



Extension of Lagging Strand



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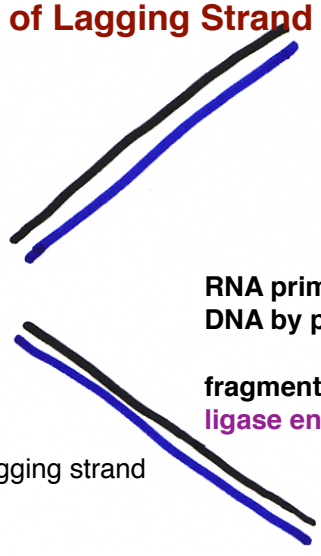
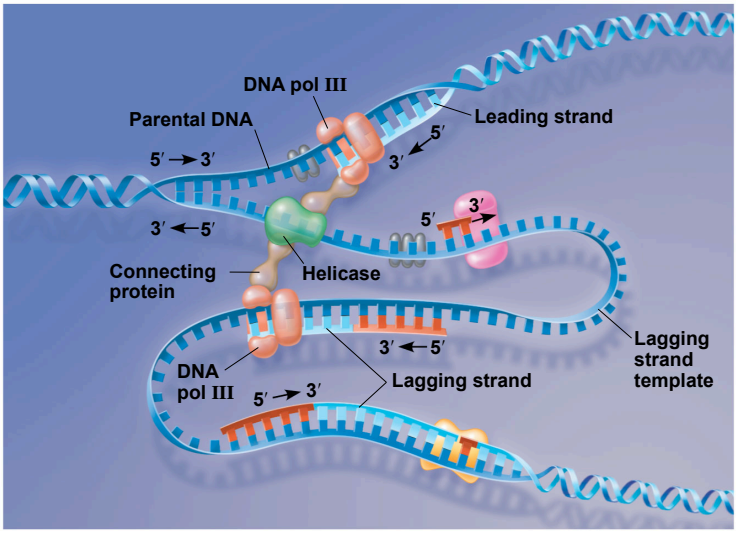


Figure 16.18



DNA repair enzymes

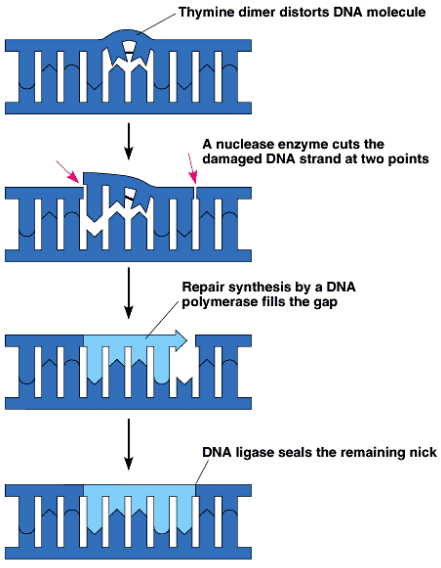
1. Mismatch: 1 error every 10,000 base-pairs
   (e.g. C:T instead of A:T)

   DNA polymerase has proofreading activity.

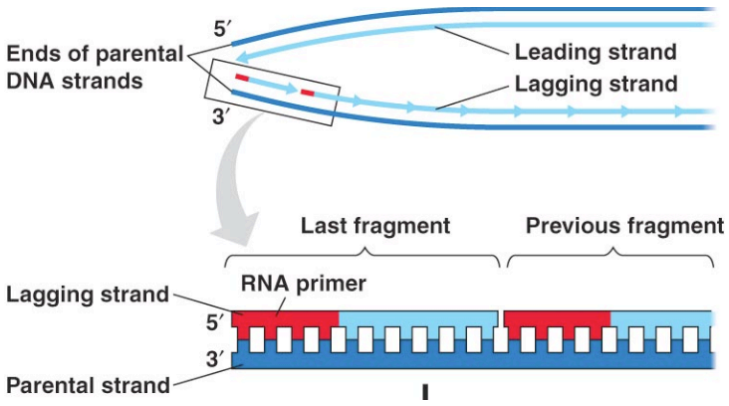
2. Excision repair: damage to non-replicating DNA that needs to be cut out and replaced. (e.g. ultraviolet radiation causes two adjacent thymines to be covalently cross-linked.)

   Nuclease excises damaged DNA; polymerase and ligase fill in the gap.

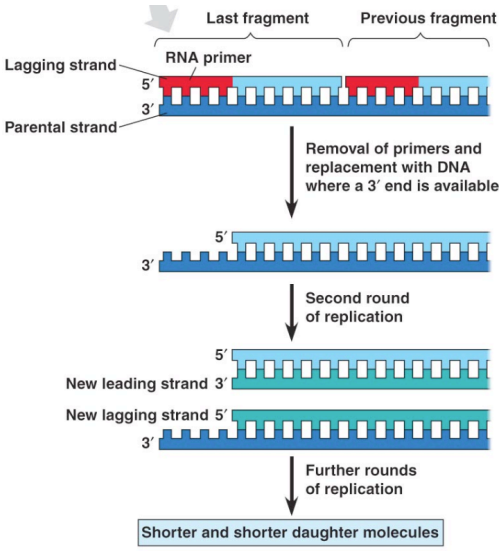
DNA repair



Shortening of Linear DNA

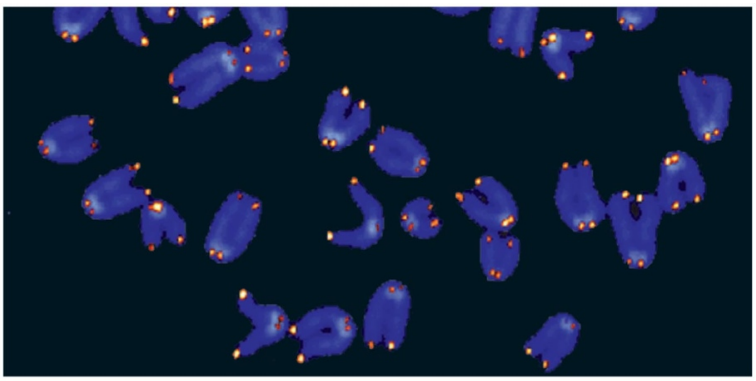


Shortening of Linear DNA



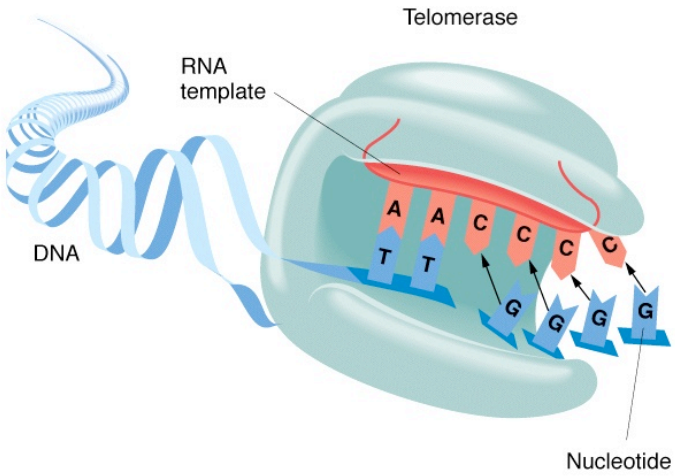
Telomers at end of chromosomes

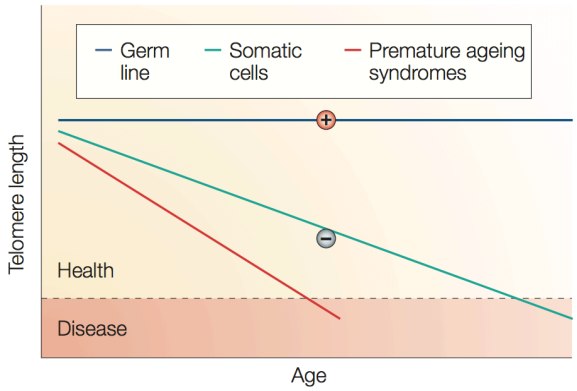
100-1000x repetitions of short DNA sequence (in humans = TTAGGG)



Telomerase

Adds additional telomers to end of chromosome to prevent age-related shortening





Germ cells (eggs and sperm) have high telomerase activity (indicated by the plus symbol on the graph) and maintain telomere length with age.

Most **somatic cells** show progressive telomere shortening owing to low or absent telomerase activity (indicated by the minus symbol on the graph).

Several human **premature ageing syndromes** show an accelerated rate of telomere shortening, therefore resulting in an early onset of ageing-related pathologies.

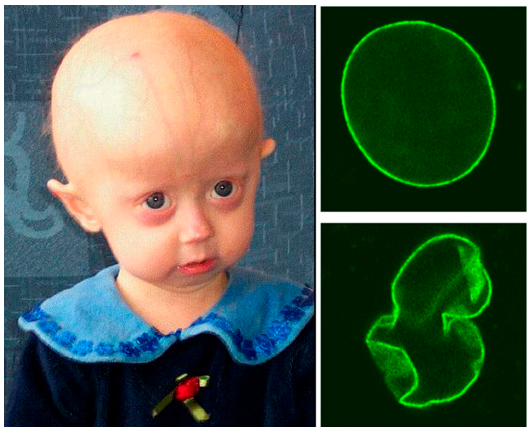
Progeria

Premature aging due to faulty teleomerase and DNA repair enzymes: limits number of high-quality cell replications

Sam Berns, 15, who has the very rare premature-aging disease progeria, plays the drums in his high school's marching band.

Hutchinson-Gilford Progeria Syndrome

HGPS is a childhood disorder caused by mutations in one of the major architectural proteins of the cell nucleus. In HGPS patients the cell nucleus has dramatically aberrant morphology (bottom, right) rather than the uniform shape typically found in healthy individuals (top, right).



doi:10.1371/journal.pbio.0030395.g002

Summary of DNA Replication

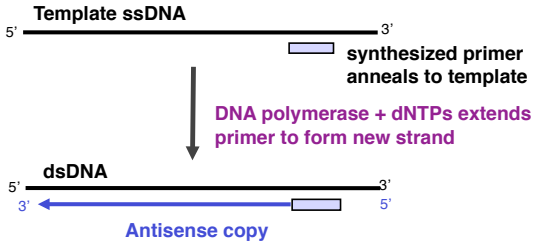
1. Beginning at origin, dsDNA is unwound by helicase to make replication bubble.
2. Replication proceeds away from origin in both directions at the 2 replication forks.
3. New DNA strands are primed by a short piece of RNA primer constructed by primase.
4. Leading strand is synthesized continuously by DNA polymerase, growing 5' -> 3'.
5. Lagging strand is synthesized discontinuously by DNA polymerase as Okazaki fragments, which are stitched together by DNA ligase.
6. Errors in the DNA is corrected by proof-reading by DNA polymerase, and other repair enzymes.

DNA replication

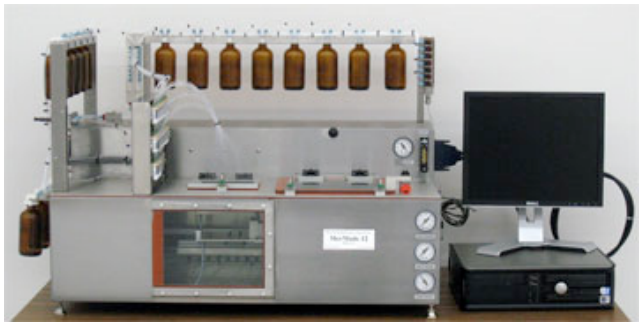
All organisms (prokaryotes and eukaryotes) use essentially the same enzymes and reactions to replicate their DNA.

DNA replication can be carried out in a test-tube just by adding DNA, primers, and DNA polymerase.

DNA Replication in a test-tube: The Polymerase Reaction

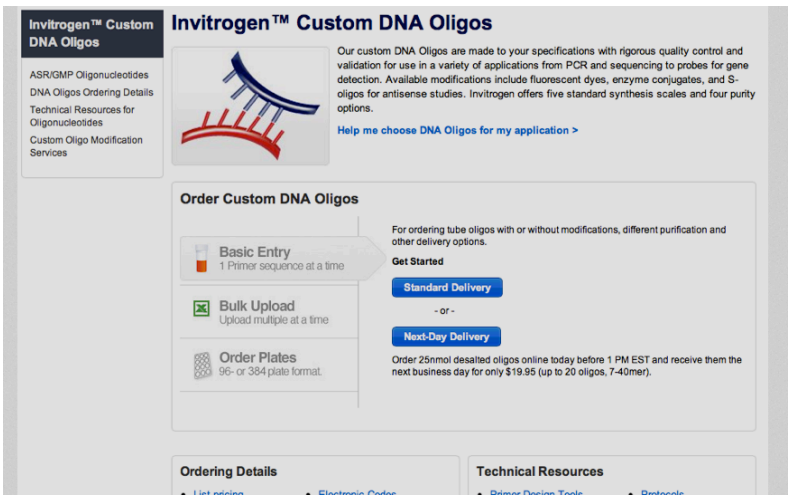


DNA Synthesis machine

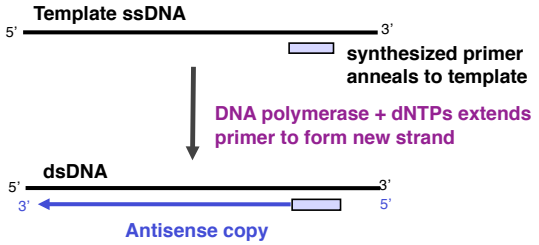


The MerMade-6 Oligonucleotide synthesizer is designed and priced for low throughput synthesis at a low cost. Based on the proven MM12 design the MM6 is rugged, reliable, and easy to service. This 6 column machine offers a wide scale range (50nmole to 200micromole) and On Line Trityl Monitoring (up to 6 columns) at about the same price as competing 4 column machines on the market. Offering much more flexibility and speed this DNA/RNA synthesizer allows the operator to add and remove columns at any point during a run and to synthesize different scales and chemistries on each column, while not wasting reagents or time.

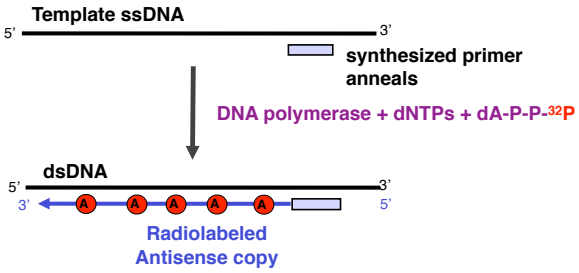
http://www.bioautomation.com



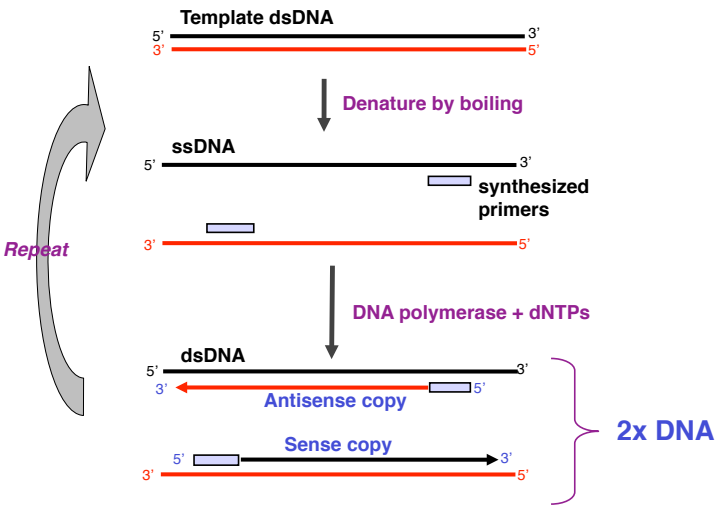
DNA Replication in a test-tube: The Polymerase Reaction



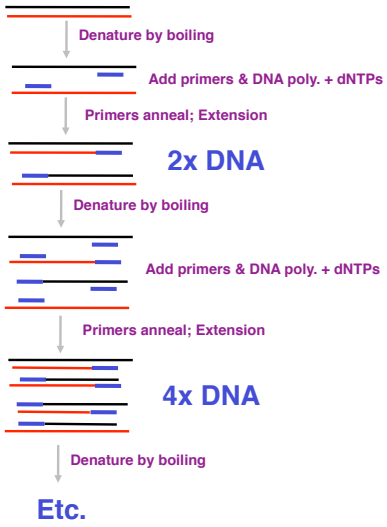
Radioactive DNA Labeling: The Polymerase Reaction



Polymerase Reaction



Polymerase Chain Reaction



Polymerase Chain Reaction (PCR)

One Round of PCR:

Denature Template

Anneal Primers

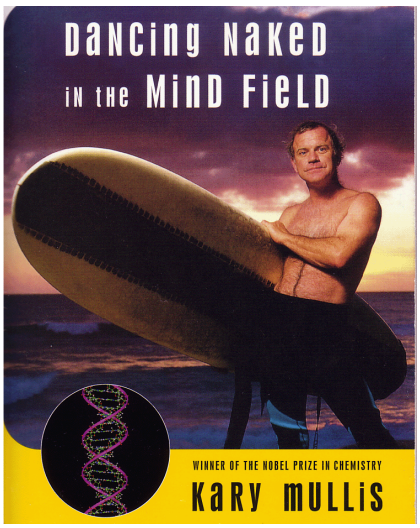
Extend New Strand

24 rounds = 2^{24} = 16.7 million copies

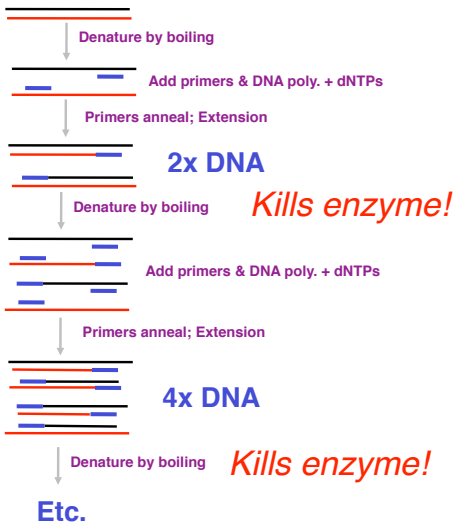
30 rounds = 2^{30} = 1 billion copies

1. Denature Template, Anneal Primers, Extend New Strand — 2x
2. Denature Template, Anneal Primers, Extend New Strand — 4x
3. Denature Template, Anneal Primers, Extend New Strand — 8x
4. ~~Denature Template~~, Anneal Primers, Extend New Strand, ~~Denature Template~~ — 16x
5. Anneal Primers, Extend New Strand, Denature Template — 32x
6. Anneal Primers, Extend New Strand — 64x

Inventor of PCR



Polymerase Chain Reaction (PCR)



Thermus aquaticus (Taq) polymerase

Bacteria from a hot spring near Great Fountain Geyser, Yellowstone Park

Thermal range is 50-80° C (122-176° F), and its optimum is around 70° C (158° F). So it will survive repeated boiling at 100° C.

Applications of PCR

1. **Generate large amounts of specific DNA on demand.**
2. **Gene discovery.**
3. **Forensic identification from blood, skin, or semen.**
4. **Rapidly identify species or strains of organisms.**
5. **Identify polymorphisms or mutations that cause disease.**