DNA Replication

Basic Reaction -- Components Needed

- Free Nucleotide Base (Needed for basepairing).
- 3’ End on the Backbone (with an Oxygen).
- Nucleoside TriPhosphate (NTP). Must be correct nucleotide base for basepairing. The reaction frees two of the phosphates from the nucleotide.
- DNA Polymerase to catalyze the 3’-5’ Phosphodiester bond.
DNA Replication

**Polymerase**

- DNA Dependent DNA Polymerase
- DNA Dependent RNA Polymerase
- RNA Dependent DNA Polymerase
- RNA Dependent RNA Polymerase

**DNA Dependent DNA Polymerase (DNA Polymerase)**

Requirements:
1. 3’ end with Oxygen on backbone
2. Unimpeded Nucleotide base on the Template Strand
3. Correct NTP (based on 2)
4. Energy requirement is met by NTP → NMP + DP

Direction:
Replication goes in the 5’ to 3’ direction -- called 3’ Elongation.

Proofreading
DNA Polymerases can have **exonuclease** activity. Some can backup and redo incorrect base matches. Some are specialized to look for errors.
DNA Replication

Details for *E. coli*

1. Unwinding the DNA double helix -- Unwinding Proteins (DNA helicase); Helix Destabilizing Proteins (HDP); DNA Gyrase (DNA topoisomerase).
2. Replication Bubble. Replication takes place in replication forks on either side of the Bubble. Some replication is unidirectional (such as with Conjugation).
DNA Replication

Details for *E. coli* (cont.)

3. Replication Fork
   1. DNA Polymerase I, II, III
      - DNA Polymerase I -- Error Correcting
      - DNA Polymerase II -- Unknown Function
      - DNA Polymerase III -- Main Replication Enzyme
   2. Synthesis of the new chromosome is bidirectional.
      a. Leading Strand -- 5’ to 3’
      b. Lagging Strand -- 5’ to 3’ in sections (Okazaki Fragments).
         i) RNA Primase initiates the primer.
         ii) DNA Polymerase III attaches to primers and synthesizes DNA.
         iii) Synthesis continues through the RNA primers of the next fragment (DNA Polymerase I).
         iv) DNA ligase attaches the backbones of the separate fragments.
DNA Replication

Relationship Between Leading & Lagging Strands

• Polymerases form a dimer.
• Lagging strand loops around polymerase core units
• Leading and Lagging Strands are replicated together (different polymerases, but attached to each other).