Bio Interlude

DNA Replication
DNA Replication: Basics
Issues & Complications, I

1st ~10 nt’s added are called the **primer**
In simple model, DNA pol has 2 jobs: prime & extend
Priming is error-prone
So, specialized **primase** does the priming; pol specialized for fast, accurate extension
Still doesn’t solve the accuracy problem (hint: primase makes an RNA primer)
“Replication Fork”: DNA double helix is progressively unwound by a DNA helicase, and both resulting single strands are duplicated.

DNA polymerase synthesizes new strand 5’ -> 3’ (reading its template strand 3’ -> 5’)

That means on one (the “leading”) strand, DNA pol is chasing/pushing the replication fork.

But on the other “lagging” strand, DNA pol is running away from it.
Lagging strand gets a series of “Okazaki fragments” of DNA (~200nt in eukaryotes) following each primer.

The RNA primers are later removed by a nuclease and DNA pol fills gaps (more accurate than primase; primed by DNA from adjacent Okazaki frag).

Fragments joined by ligase.
Issue 4: Coord of Leading/Lagging

Alberts et al., Mol. Biol. of the Cell, 3rd ed, p258
Figure 5-19bc  Molecular Biology of the Cell 5/e (© Garland Science 2008)
https://www.youtube.com/watch?v=yqESR7E4b_8

(Replication starts at about 1:40)
Issue 5: Twirls & Tangles

Unwinding helix (~10 nucleotides per turn) would cause stress. *Topoisomerase I* cuts DNA backbone on *one* strand, allowing it to spin about the remaining bond, relieving stress.

*Topoisomerase II* can cut & rejoin *both* strands, after allowing another double strand to pass through the gap, de-tangling it.
Issue 6: Proofreading

Error rate of pol itself is $\sim 10^{-4}$, but overall rate is $10^{-9}$, due to proofreading & repair, e.g.

- pol itself can back up & cut off a mismatched base if one happens to be inserted
- priming the new strand is hard to do accurately, hence RNA primers, later removed & replaced
- other enzymes scan helix for “bulges” caused by base mismatch, figure out which strand is original, cut away new (faulty) copy; DNA pol fills gap
- which strand is original? Bacteria: “methylate” some A’s, eventually. Euks: strand nicking
• Nobel prize in chem 2015, for DNA damage repair

Replication Summary

Speed: 50 (eukaryotes) to 500 (prokaryotes) bp/sec
Accuracy: 1 error per $10^9$ bp
Complex & highly optimized
Highly similar across all living cells

More info:
Alberts et al., *Mol. Biol. of the Cell*