Bio Interlude

DNA Replication
DNA Replication: Basics
Issues & Complications, 1

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.
Issue 3: Fragments

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
Uncopied lagging stranded loop (ss)

newly synthesized leading strand (ds)

newly synthesized lagging strand (ds)

parental DNA helix (ds)
Very Nice DNA Repl. Animation

https://www.youtube.com/watch?v=yqESR7E4b_8

(Replication at about 1:41 – 2:50)
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, detangling it.
Topoisomerase I + DNA
Issue 6: Proofreading

Error rate of pol itself is $\sim 10^{-4}$, but overall rate is $\approx 10^{-8} – 10^{-9}$, 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
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*