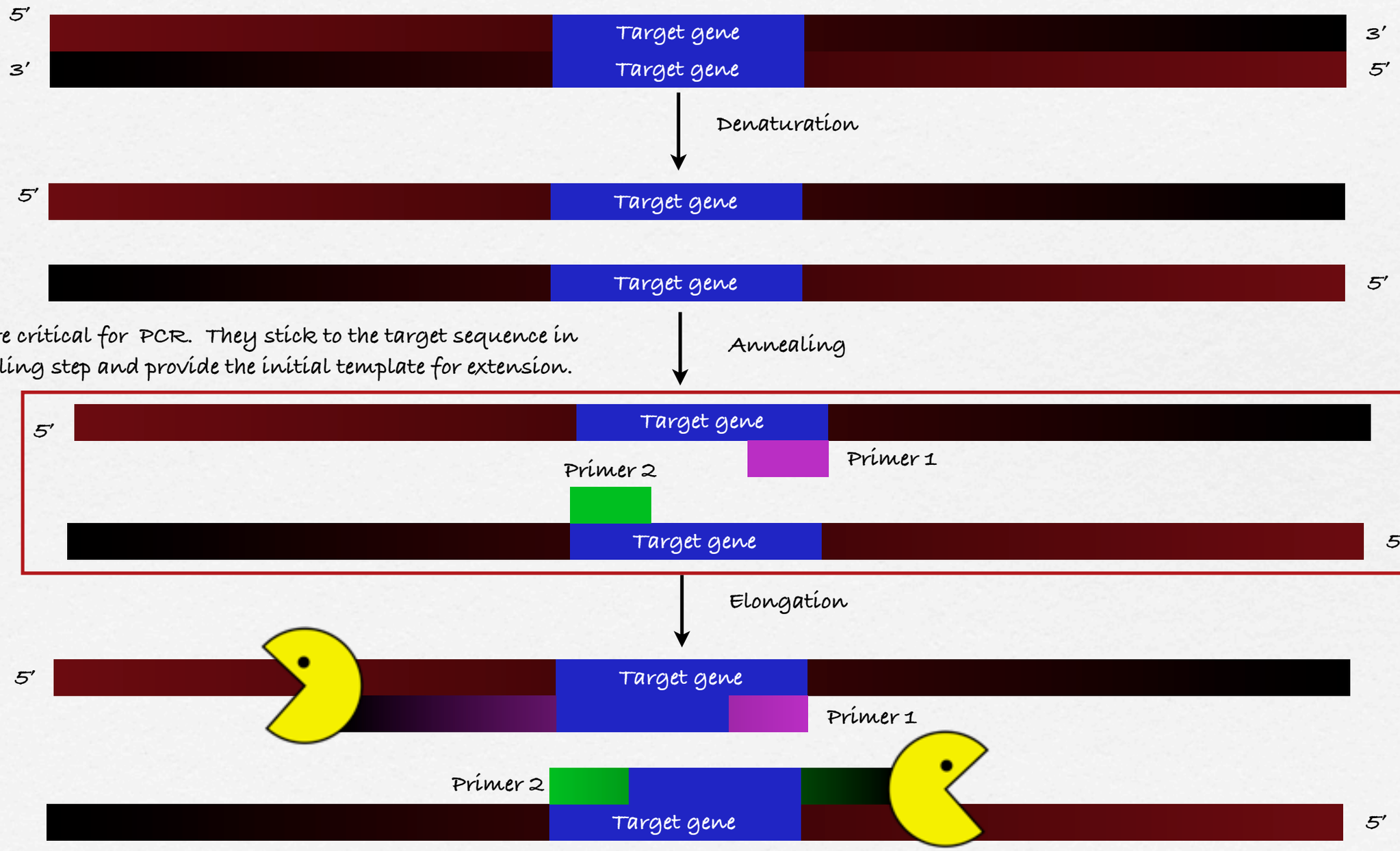
Synthetic Biology for

Primer Design

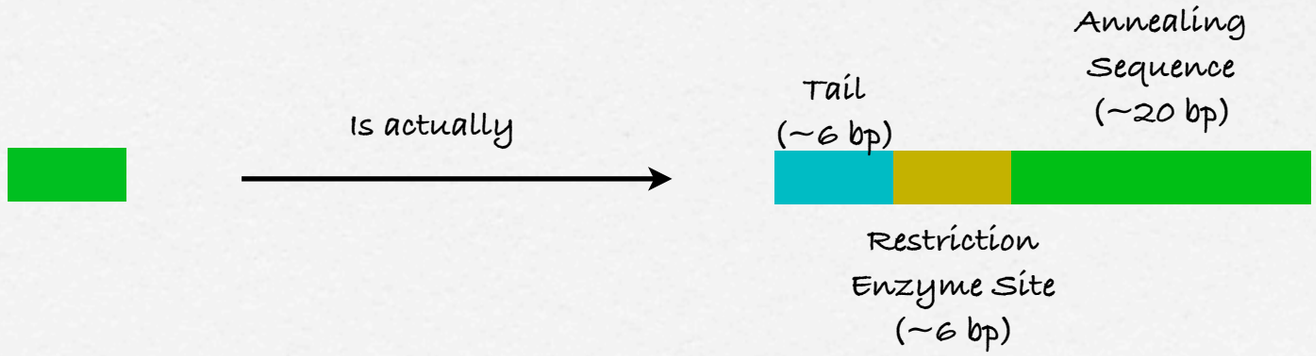
Review: PCR



Primers are critical for PCR. They stick to the target sequence in the annealing step and provide the initial template for extension.

Primer Composition

- Annealing Sequence: 20 or so bp of recognition that are the reverse complement of genomic DNA
- Restriction Enzyme Site: To effectively cut and paste our target amplification sequence, we add in restriction enzyme sites
- Tail: Restriction enzymes need a few bases on either end to work properly



Review: Reverse Complimentary Sequences

- Since DNA has direction (5' to 3'), matching sequences must have reverse complementarity

So the reverse compliment of
5' ATGTTCAGAT 3'
is
3' ATCTGAACAT 5'

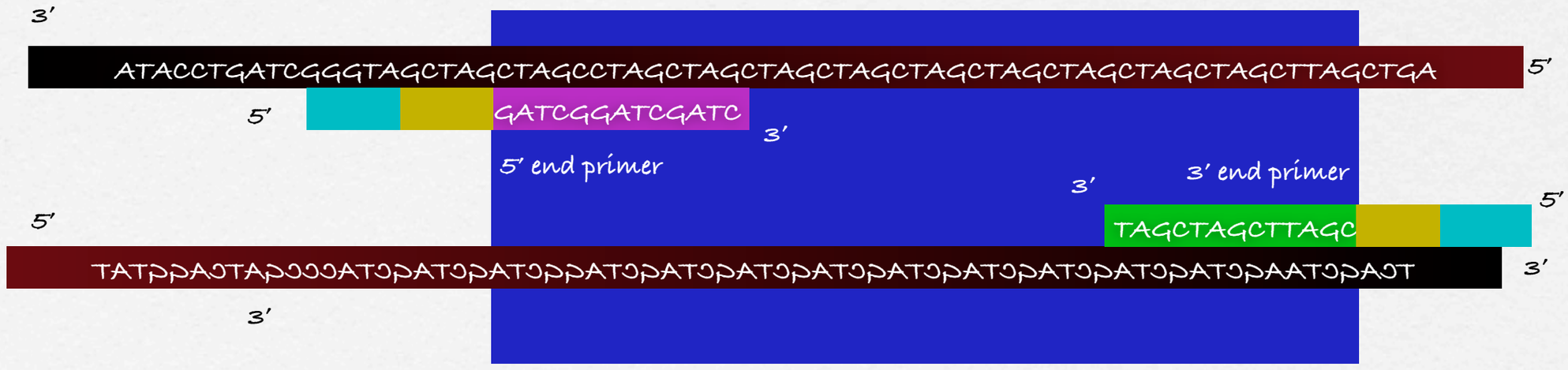
Reverse Compliment Nature of Primers

- PCR uses 2 primers: One on the 5' end of the target and one on the 3' end

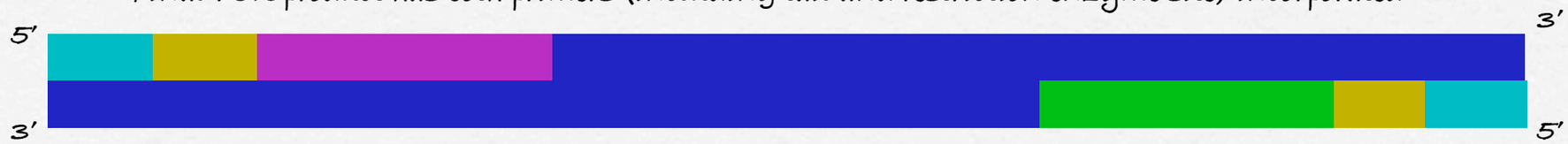
Target

- Note that the annealing sequence, restriction enzyme site, and tail can (and often will) be different for both primers

One primer binds to the 5' region of the target gene and the other primer to the 3' end. Note that the primers anneal to opposite strands to promote synthesis (synthesis always occurs 5' to 3')



Final PCR product has both primers (including tail and restriction enzyme site) incorporated



Designing the Annealing Sequence

- Usually the annealing sequence can be the first 20 bases of your target sequence
- However, sometimes it will not be, and the following list has good points to keep in mind

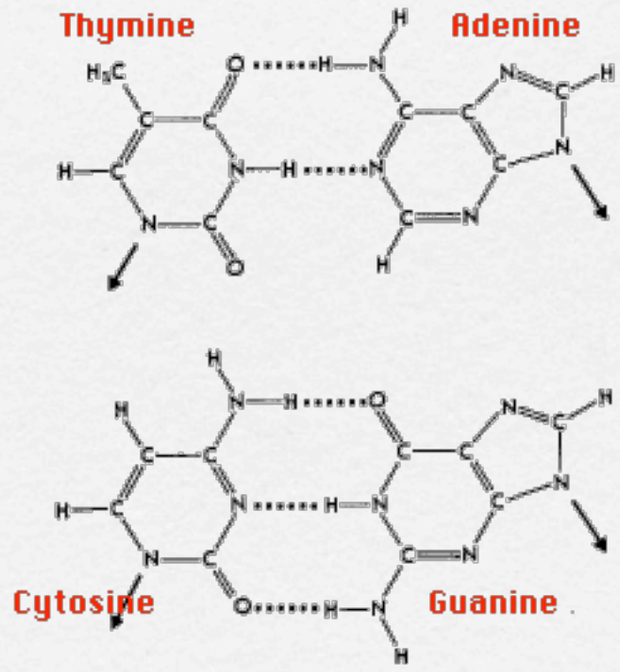
Considerations for Annealing Sequence Design

- In general, the 3' base of your oligos should be a G or C
- The overall G/C content of your annealing region should be between 50 and 65%
- The overall base composition of the sequences should be balanced (no missing bases, no excesses of one particular base)
- The length of your sequence can be modified to be around 18 and 25 bp
- The sequence should appear random. There shouldn't be long stretches of a single base, or large regions of G/C rich sequence and all A/T in other regions
- There should be little secondary structure. Ideally the self Tm for the oligo should be under 40 degrees.

List courtesy of Open Wet Ware.org, http://openwetware.org/wiki/Arking:JCAOligoTutorial1b

Melting Temperature (Tm)

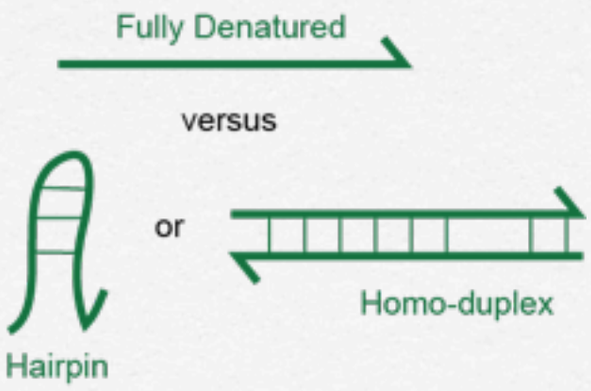
- Tm Definition: Temperature at which half the DNA strands are in a single stranded state (ssDNA)
- C-G base pairing is stronger than A-T base pairing, because C-G base pair with three hydrogen bonds, and A-T base pair with 2
- DNA with higher GC content (greater percentage of C-G bonds) has higher melting temperature
- When doing PCR, the annealing step must be below the annealing temperature, or primers will not bind



Different Tms: What They Mean

- Self Tm: Temperature below which the primer will anneal to itself
- Initial Round Tm: Annealing temperature for round 1; only the annealing sequence will bind the genomic DNA
- Later Rounds Tm: Annealing temperature for the entire primer; later rounds will have reverse complement of primers included, so primer will fully anneal; temperature reported by ApE

Self Tm



Initial Round Tm

In the initial round, the primer only matches the annealing sequence

Late Rounds Tm

In the later rounds most of the DNA is target gene only, and includes the previous primer sequences. Remember that primers are incorporated into the amplified genes!

Designing Tms

- Self Tm: Avoiding long sequences of single base repeats or pair repeats (ex: AATATA) should keep the Tm very low. You want the self Tm low because the primer should be binding other DNA, not itself; 40 C is a safe number
- Other Tms: Though the Late Rounds Tm is reported by ApE, it is actually higher than the Initial Round Tm. Therefore, you should use the Initial Round Tm if you can, as it will be easier for primers to bind in the early rounds

Review

- Primers have 3 usual regions: annealing regions, restriction enzyme sites, and tails
- Melting Temperature (Tm) is a critical design point of primers