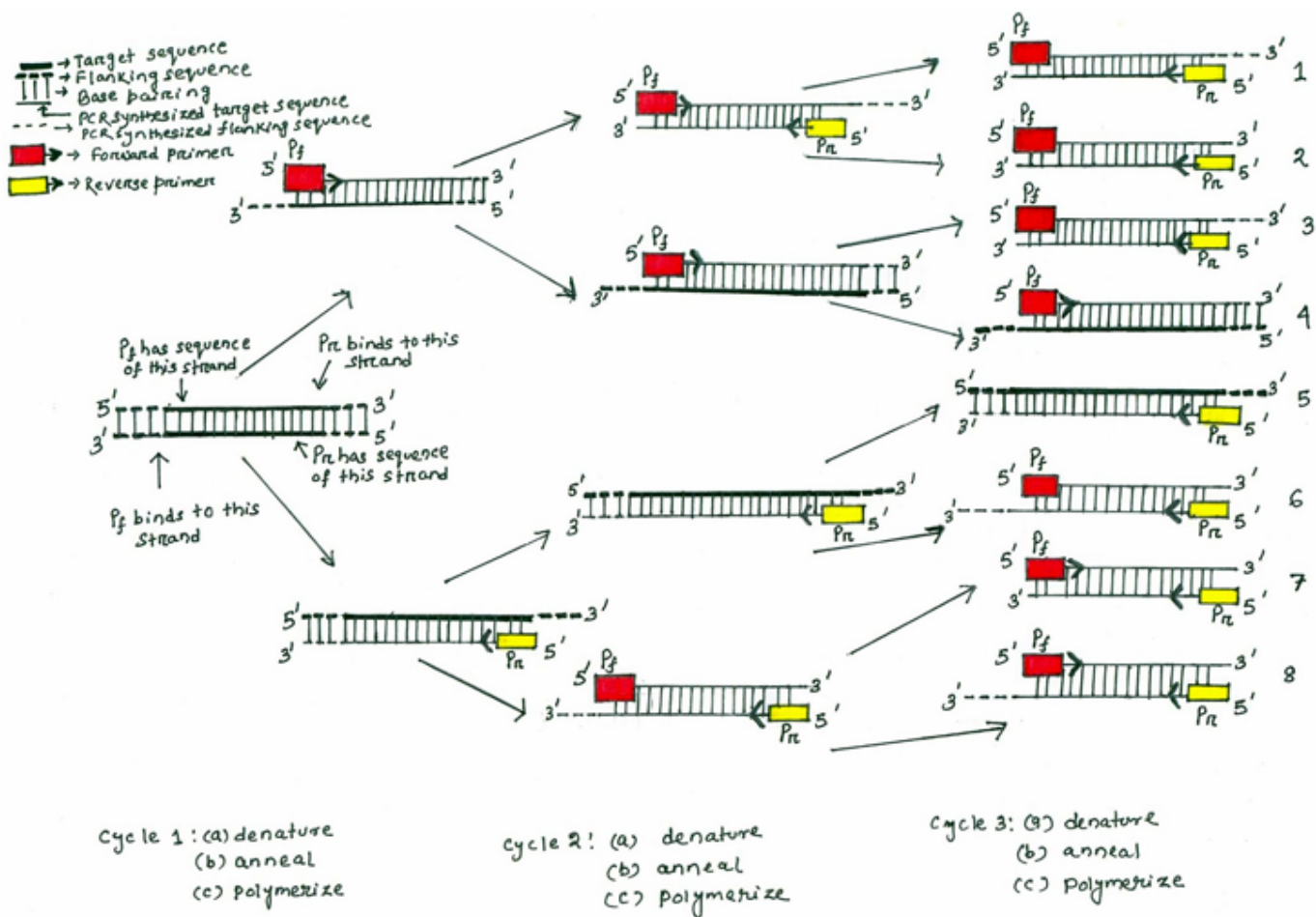


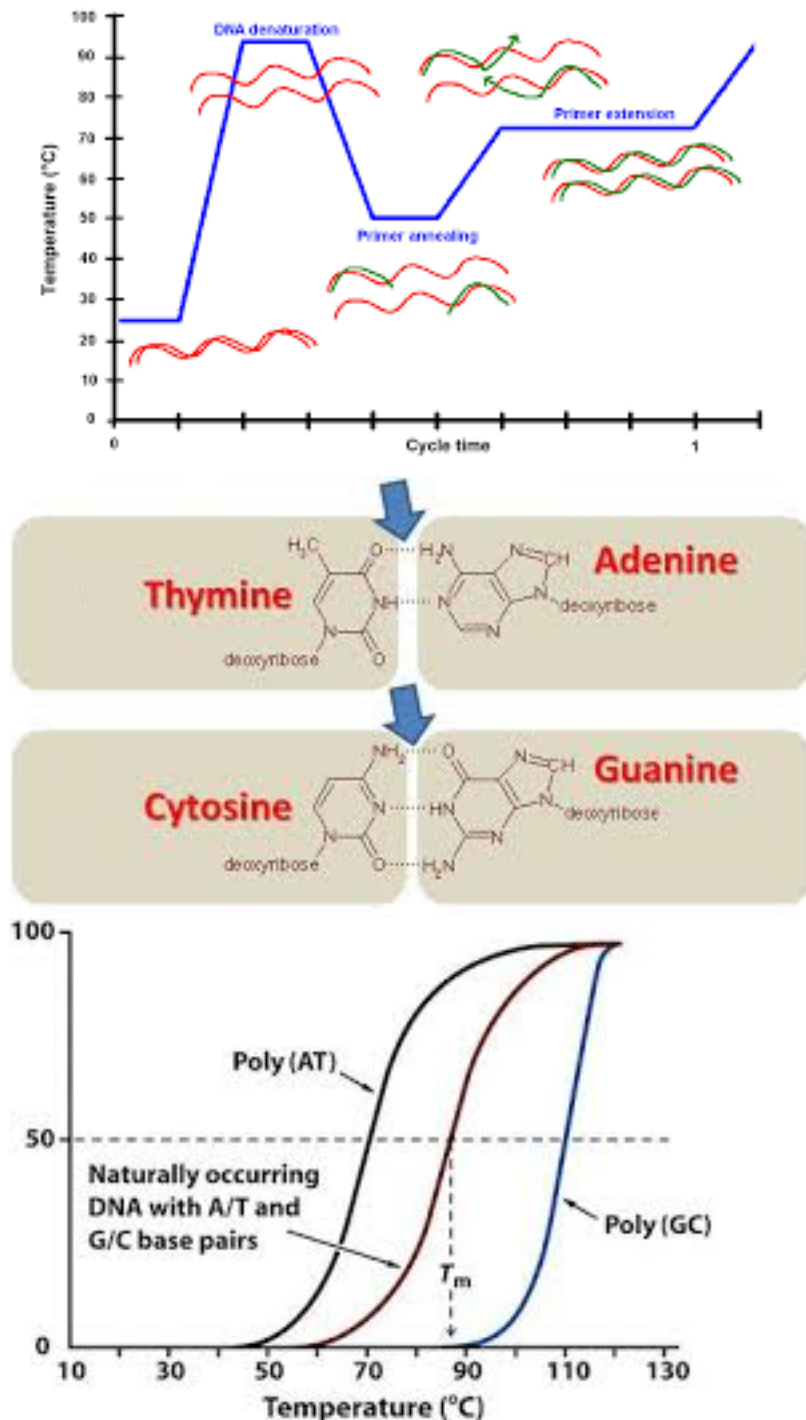
Lecture 19: Command line

PCR

The polymerase chain reaction (**PCR**) is a technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. PCR requires the design and synthesis of two DNA primers, which specify the region of DNA that is amplified.



In order to successfully amplify a piece of DNA, you need to ensure that both DNA primers only binds to the target site at the annealing temperature. The melting temperature depends on both the sequence and the length of the primer. Other issues that can affect primer binding are the ability of the primer to bind to itself or the second primer.



Primer3

The program primer3 is a ubiquitous program to identify good primers for amplifying a region of choice. Some DNA sequence is pasted below. A web interface to use primer3 can be found at:

<http://primer3plus.com/cgi-bin/dev/primer3plus.cgi>

>DNA sequence

```
cgaattttgtacctgaaaattctcaattttaaaaacaaatttcagatgcgacgccatcgact
cgaacataataaaaatgccgcaatttttggaatgactagaatgatccaaaaacgaacgtatt
gcggggccatcgaaaatagcaggcaatggacttttctgttggaacccgccgaaaaagacg
agttcattacggaatacactgggtgaacgaatttccgatgatgaagccgaacgacgcggtg
cgatatatgatcgatatcagtgcagctacatttttagtatgtacttttttagatcacgg
tgtcgcagaaaaatgttttaaatTTAAATTTTTTTTgggttttagagacaacatttcga
TTTTTTgaaaatcaaataaataagaaaaaattatttcaaaaaattaacaaaatatcgaaa
aaaaaaaccatcaaaaagttttcttttttttgaaaattcaatttttcggaacaaaaaaa
atatTTTTgcagacattgaaaccgggggtgcgatcgattcgtacaaaattggaaacttgg
cacgatttgccaatcatgacagcaagaatccgacgtgctacgcgagaacgatggttgctg
ccggagagcaccgaattgggttctatgcgaagagacggccttgagattagtgaagagctca
catttgactacagttattctggagaacatcagatagcgttcaggtagattttgcaaaaag
cttttcgggtctcgcagacgactgcagctaccgtaccctctagtTTTTTccactcaatttt
taaaaatttataacacaaagatttttttgatcaaaaaaatcatgataagccgtagaaaa
ttgtggttggttacggtagctgcaaatTTTcggtcgcttcgagaaccagttttgtgtaga
ctttcaacttgactTTTTgaataaaactTTTTccaggatggtccaaaccaaggagagatcc
gagaagcccagcaggcccaaaagccagaaactctccaaaccaatgacttctgaataatct
```

Primer3 has a large number of options. In general, the settings that will be most useful to you are: Product Size Ranges (under General Settings tab) and Primer Size (you might need to adjust the min and max if it can't find a good primer).

Primer3 also has an extremely useful way to force it to pick primers that amplify a certain region. Just put brackets around the sequence you want amplified:

>DNA sequence

```
cgaattttgtacctgaaaattctcaattttaaaaacaaatttcagatgcgacgccatcgact
cgaacataataaaaatgccgcaatttttggaatgactagaatgatccaaaaacgaacgtatt
gcggggccatcgaaaatagcaggcaatggacttttctgttggaacccgccgaaaaagacg
agttcattacggaatacactgggtgaacgaatttccgatgatgaagccgaacgacgcggtg
cgatatatgatcgatatcagtgcagctacatttttagtatgtacttttttagatcacgg
tgtcgcagaaaaatgttttaaatTTAAATTTTTTTgggttttagagacaacatttcga
TTTTTTgaaaatcaaataaataagaaaaaattatttcaaaaaattaacaaaatatcgaaa
aaaaaaaccatcaaaaagttttcttttttttgaaaattcaatttttcggaacaaaaaaa
atatTTTTgcagacattgaaaccgggggtgcgatcgattcgtacaaaattggaaacttgg
cacgatttgccaatcatgacagcaagaatccgacgtgctacgcgagaacgatggttgctg
ccggagagcaccgaattgggttctatgcgaagagacggccttgagattagtgaagagctca
catttgactacagttattctggagaa[catcagatagcgttcaggtagattttgcaaaaag
cttttcgggtctcgcagacgactgcagctaccgtaccctctagtTTTTTccactcaatttt
taaaaatttataacacaaagatttttttgatcaaaaaaatcatgataagccgtagaaaa
ttgtggttggttacggtagctgcaaatTTTcggtcg]cttcgagaaccagttttgtgtaga
ctttcaacttgactTTTTgaataaaactTTTTccaggatggtccaaaccaaggagagatcc
gagaagcccagcaggcccaaaagccagaaactctccaaaccaatgacttctgaataatct
```

Primer3 command line

While the web interface is extremely useful for picking single primers. But sometimes you want to pick a large number of primers. For example, if you want to genotype a large number of mutations or a polymorphisms in a strain. In this case, it is useful to run this on the command line. You can using conda:

(base) → [ExampleData](#) conda install -c bioconda primer3

<https://primer3.org/manual.html#invokingPrimer3>

In order to run this, it is easiest to place all of the arguments into a single file:

```
SEQUENCE_ID=example
SEQUENCE_TEMPLATE=tgaagaaagcaactcacatttggtgtacctgacattaactatcgatttaatttttttcaatttg
aagagattaagaaatgctg$
PRIMER_PICK_LEFT_PRIMER=1
PRIMER_PICK_RIGHT_PRIMER=1
PRIMER_OPT_SIZE=18
PRIMER_MIN_SIZE=15
PRIMER_MAX_SIZE=21
PRIMER_PRODUCT_SIZE_RANGE=75-100
SEQUENCE_INTERNAL_EXCLUDED_REGION=37,21
=
```

(base) → [ExampleData](#) primer3_core primer3_example.txt