Exercise 19

Your task is to produce primers to genotype the following region. The bolded sequence indicates a poly-T repetitive region that can vary in size in different individuals. You need to design primers to amplify and sequence the poly-T region along with ~50bp of flanking DNA to ensure you get high quality sequence information at the poly T position. It is also known that the italicized region is deleted in some individuals, so you do not want to use that location to choose primers. Your advisor would like you to design 12 different primer pairs with different three different product sizes (200-300, 300-400, and 400-500), two different melting temperatures (57 and 60 degrees Celsius), and two different primer lengths (19 and 21 bp) long.

>DNA sequence
cgaattttgtacctgaaaattctcaatttaaaaacaaattcagatgcgacgccatcgact
cgaacataataaaatgccgcaattttggaatgactagaatgatccaaaaacgaacgtatt
gcgggccatcgaaaatagcaggcaatggacttttcttgttggaacccgccgaaaaagacg
agttcattacggaatacactggtgaacgaatttccgatgatgaagccgaacgacgcggtg
cgatatatgatcgatatcagtgcagctacatttttagtatgtacttttttagatcacgg
tgtcgcagaaaaatgttttaaatttaaatttttttttgggtttttagagacaacatttcga
ttttttgaaaatcaaataaataagaaaaaattatttcaaaaaattaacaaaatatcgaaa
aaaaaaaccatcaaaa**gttttctttttttttg**aaaattcaatttttcggaacaaaaaaaa
atatttttgcagacattgaaaccgggggtgcgatcgattcgtacaaaattggaaacttgg
cacgatttgccaatcatgacagcaagaatccgacgtgctacgcgagaacgatggttgtcg
ccggagagcaccgaattgggttctatgcgaagagacggcttgagattagtgaagagctca
catttgactacagttattctggagaacatcagatagcgttcaggtagattttgcaaaaag
cttttcgggtctcgagacgactgcagctaccgtaccctctagttttttccactcaatttt
taaaaatttataacacaaagatttttttggatcaaaaaaatcatgataagccgtagaaaa
ttgtggttggttacggtagctgcaaattttcggtcgcttcgagaaccagttttgtgtaga
ctttcaacttgacttttttgaataaacttttttccaggatggtccaaaccaaggagagatcc
gagaagcccagcaggcccaaaagccagaaactctccaaaccaatgacttctgaataatct

Identify the 12 primer pairs first using 1) the web based version of primer3 and then 2) the command line version of primer3.