

## PROTOCOL FOR DESIGNING PRIMERS

- I. Go to NCBI (National Center for Biotechnology Information) webpage:  
<http://www.ncbi.nlm.nih.gov/>.
- II. Select **BLAST** search engine found at the top of the webpage.
- III. Select what type of BLAST you want to do (e.g., **Nucleotide BLAST**, Protein BLAST, etc.).
- IV. I did a Nucleotide BLAST and searched for **short nearly exact matches** (This is important to select for microsatellite searches.)
- V. In the “**Search**” window, type what you are interested in finding. (I was interested in the following repeats (at least 10-30 repeats):
  1. AAT (i.e., AATAATAATAATAATAATAATAATAATAAT)
  2. AAG
  3. CAC
  4. CAG
  5. CCG
  6. TAG
  7. CAT
  8. AAC
  9. GAG
  10. GAC
- VI. In the “**Options**” and “**Format**” drop windows, select genome of interest (e.g., “**Dictyostelium discoideum**”).
- VII. Click on “**BLAST**”.
- VIII. Click on “**Format**” to check results.
- IX. Check sequences producing different alignments. (**E value** [Expectation Value] represents the # of alignments with scores equal to or better than the given score that are expected to occur by chance. The lower the E value, the more significant the match.)
- X. Click on first one, for example. Select all, copy, and save as a Word document.
- XI. Go to Primer3 Software:  
[http://www-genome.wi.mit.edu/cgi-bin/primer/primer3\\_www.cgi](http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi)
- XII. Copy and paste just the source sequence in the first blank window.
- XIII. In the “**Sequence ID**” box, provide a name to ID output. [e.g., AAT1(nameof gene)]
- XIV. Click on “**Pick Primers**”
- XV. Find your repeat with “apple F”, and type in repeat (e.g., AATAATAATAATAATAATAATAATAATAAT).
- XVI. Remember position of where repeat begins (numbers provided at beginning of each row).
- XVII. Click on the “**Back**” button
- XVIII. In the “**Targets**” window, type position #, followed by a “,” and then the number of bases to be surrounded by the primer. (e.g., 5701,40)
- XIX. Pick appropriate “**Product Size Ranges**” (e.g., I kept up through 400 and deleted the rest; however, Joan suggests to keep up through 300.).
- XX. Click on “**Pick Primers**”
- XXI. “\*” should be under target sequences and “>> <<” should be before and after the target sequences, denoting the left and right primers, respectively.
- XXII. Select all, copy, and save as a Word document.