**For primer design of gene expression primers:**

1. Make sure your java is updated and make sure your security settings allow it to run.
2. Find mRNA sequences of selected gene from human, mouse, and rat.
3. Paste all three into a word document.
4. Paste all the sequences into MUSCLE (<https://www.ebi.ac.uk/Tools/msa/muscle/> ) separated with the designations “>human”, “>mouse”, and “>rat”
5. Click submit.
6. Once results are generated, click on the “Result Summary” tab.
7. Click the “Start JalView” button.
8. Once JalView opens, go to the “Format” menu and make sure “Wrap” has a check next to it.
9. Find regions where all three sequences are perfectly aligned.
   1. If you can find regions towards the 3’ end of mRNA, then that is better.
10. Find two regions that are separated by about 200 nt.
    1. 200 nt is sort of an ideal max distance between primer regions. If you can’t find any regions that are this exact distance, try to find something close to 200 nt.
11. Within the regions, try to find sequences of >=16nt with a Tm of approximately 60**°**C. Use the Operon Oligo Toolkit to determine your Tm. <http://www.operon.com/tools/oligo-analysis-tool.aspx>
12. These sequences will be your primer sequences.
13. For the 3’ end sequence, generate the reverse complement using the oligo toolkit.
14. Double check your primer sequences on the primer BLAST web site. <http://www.ncbi.nlm.nih.gov/tools/primer-blast/>

**For primer design of cloning primers:**

1. Find mRNA for gene of interest from organism of interest.
2. Paste into word document.
3. Find and highlight (bold, underline, whatever) start and stop codons.
4. Copy and paste the coding region (part between the start and stop codons) into webcutter. <http://rna.lundberg.gu.se/cutter2/>
5. Find the array of restriction enzymes present in the multiple cloning site (MCS) of pEGFP-N1.
6. Find restriction sites that are in the MCS of pEGFP-N1 but do not cut your sequence of interest.
7. Starting at the ATG, go forward to find >=16nt with a Tm of approximately 60**°**C.
8. Do the same thing upstream of the stop codon. Do not include the stop codon in your primer sequence if you want to make a GFP fusion. Make sure to maintain reading frame with GFP.
9. Decide on your 5’ and 3’ restriction sites. Add these sites on to the 5’ and 3’ ends of your primers. Add the sequence “gcgaa” to the 5’ end of each of your primers.
10. For the 3’ end sequence, generate the reverse complement using the oligo toolkit.