PRIMER DESIGN

1. Go to ensembl.org. Search for the Gene of interest
2. Click on the transcript that has all/most of the exons
3. Amplicon should span exons to avoid amplifying genomicDNA
4. Copy and paste the 2 exons into Primer3Plus
5. Put bracket at the 3’ of first exon and before 5’ of second exon to delineate between the exons
   1. To make sure the primers are not on the same exon
6. Go to Settings > Product Size and set to 60-120bp
7. Checking w UCSC BLAT
   1. Copy and paste the Forward Primer.
   2. Make sure the Forward Primer doesn’t align with other genes or even non-coding DNA
   3. If it does, check Reverse Primer to see if amplification could happen at this off-target location.