Generally, primers should have 18-20 nucleotides of overlap with template stands.

5’ primer (forward primer) should align to the first 18-20 nucleotides of the sense strand of the gene ('normal' strand, complement to antisense strand). It should also include the recognition site of the desired restriction enzyme at the 5' end (upstream) of the first nucleotide of overlap.

3’ primer (reverse primer) should align to the last 18-20 nucleotides of the anti-sense strand (complement to antisense strand). Make sure a stop codon (TAA (recommended), TAG, TGA) is present if needed. The primer should also include the recognition site of the desired restriction enzyme at the 3' end (downstream) of the last nucleotide of overlap and downstream of the stop codon. Take the reverse-complement of the sequence to make it align to the anti-sense strand.

Quik-Change mutagenesis primers are recommended to be 25-45 bases in length. Remember that the primer should contain the desired mutation.

Once primer sequences have been double-checked, order through EPIC (IU's ordering software) using the IDT vendor. Make sure the primer DNA sequence is put in from 5' to 3'.

To calculate the melting temperature of a primer:
Tm (°C) =(G+C)*4+(A+T)*2-5