Site-Directed Mutagenesis (Stratagene protocol)

This is the protocol for site-directed mutagenesis based on the Stratagene kit.
Materials:

* Pfu turbo
* 10X Pfu turbo buffer
* dNTPs (10mM)
* Forward and reverse primers (0.1ug/uL, see methods section for design tips)
* dH2O
* Dpn1
* competent cells

Methods:
Primer Design

* Forward primer should be between 25 and 45 bases in length and contain the desired mutation in the center with correct sequences on both sides; the reverse primer is the reverse complement of this
* Primers should have a minimum GC content of 40% and terminate in one or more C's or G's
* Tm should be greater or equal to 78 degrees Celsius and can be calculated as follows:
    - Tm = 81.5 + 0.41(%GC) - 675/(length in bases) - %mismatch

Reaction

Set up as follows:

Components	Amount
Template DNA (50ng/uL)	1 uL
10X Buffer	5 uL
Forward Primer (0.1 ug/uL)	1 uL
Reverse Primer (0.1 ug/uL)	1 uL
dNTPs (10mM)	1 uL
Pfu turbo	1 uL
dH2O	40 uL

PCR Program

Depending on the length of the plasmid, this program can become very long, so it may be best to run overnight.

1. 95 degrees for 1 minute
2. 95 degrees for 50 seconds, 60 degrees for 50 seconds, 68 degrees for 1 minute/kb of plasmid length -- repeat this step 17 times, or 18 cycles total
3. 68 degrees for 7 minutes
4. 4 degree hold

Following PCR

Add 1uL of Dpn1 to PCR reaction. Incubate at 37 degrees Celsius for 1-2 hours to digest parental DNA. Run 5uL of the digested reaction on a gel and compare to the undigested parental plasmid - there should be some difference in band pattern. Transform into competent cells.