1. **Mutagenesis by the QuikChange ligation protocol**
   Whole plasmid is PCR amplified using primers that contain desired mutation/deletion sequences and digested with enzyme DpnI to remove WT plasmid template. The digested PCR product is cleaned up using MinElute PCR Purification kit (Qiagen; 28006) and incubated with the enzymes T4PNK and T4 DNA ligase to circularize the plasmid. The plasmid is transformed into competent bacteria. DNA is purified from bacterial culture using QIAprep® Spin Miniprep Kit (Qiagen; 27106) and sequenced to confirm the mutation.

2. **Subcloning**
   PCR product is synthesized using cDNA specific primers containing unique restriction sites and cleaned up using minElute PCR Purification kit (Qiagen; 28006). In parallel, the PCR product and 2 µg of the vector are digested with restriction enzyme and treated with alkaline phosphatase. Both digested PCR product and vector are gel purified using DE81 paper, and the products are eluted in high temperature (70°C), high salt (1.5M NaCl) conditions, ethanol precipitated, and dissolved in 10 µl of water. Digested PCR product and vector are ligated in 3:1 using T4 DNA ligase. The ligation product is transformed into competent bacteria. DNA is purified from bacterial culture using QIAprep® Spin Miniprep Kit (Qiagen; 27106) and whole insert is sequenced.

3. **Mutagenesis by overlapping PCR and chimeric cDNA cloning**
   Two PCR products are synthesized using cDNA specific primers containing unique restriction sites and two overlapping primers containing the desired mutation or specific for the chimeric cDNA. Both PCR products are gel purified using DE81 paper, eluted in high temperature (70°C), high salt (1.5M NaCl) conditions for 15 min, ethanol precipitated, dissolved in 10 µl of water, mixed together, and subjected to one cycle of overlapping PCR (95°C-1 min; 65°C-1 min; 72°C-3 min). Primers containing unique restriction sites are added to the reaction and 30 cycles of conventional PCR amplification are performed. The resulting PCR product is cleaned up using minElute PCR Purification kit (Qiagen; 28006). In parallel, the PCR product and 2 µg of vector are digested using restriction enzymes and treated with alkaline phosphatase. Both digested PCR product and vector are gel purified using DE81 paper, eluted in high temperature (70°C), high salt (1.5M NaCl) conditions for 15 min, ethanol precipitated, and dissolved in 10 µl of water. Digested PCR product and vector are ligated in 3:1 using T4 DNA ligase. Ligation product is transformed into competent bacteria. DNA is purified from bacterial culture using QIAprep® Spin Miniprep Kit (Qiagen; 27106) and whole insert is sequenced.