Site Directed Mutagenesis Protocol

Based on the QuikChange™ Site Directed Mutagenesis Kit

1. Follow the QuikChange ™ recommendations for primer design:
   a. Both mutagenic primers must contain the desired mutation and anneal to the same sequence on opposite strands of the plasmid
   b. Primers should be 25-45 bases in length
   c. T_m should be greater then or equal to 78°C

      The following formula is commonly used for estimating the T_m of primers:
      T_m=81.5 + 0.41(%GC) - 675/ N - % mismatch, where
      • N is the primer length in bases
      • values for % GC and % mismatch are whole numbers

      For calculating Tm for primers intended to introduce insertions or deletions, use this modified version of the above formula:
      T_m=81.5 + 0.41(%GC) - 675/N, where N does not include the bases, which are being inserted or deleted.

   d. Desired mutation must be in the middle of the primer with >/= 15 bp on both sides of the mutation/insertion/deletion
   e. CG content should be higher then 40%
   f. 3'-end of primers should end in 1 or more G or C
   g. Protocol says they must be HPLC purified but when ordering, desalted have always worked just fine

2. For each 50 µl reaction, use the following protocol for the reactions and add in the order listed below:

Stock	Final	Content	Volume (µl)
		ddH_2O	Bring to 50 µl
Tube at -20	1 M	Ethylene Glycol	2.8
	25 ng	Template DNA	
5X	1X	Phusion Buffer	10
25 mM	0.4 mM	dNTPs	0.8
20 µM	0.2 µM	Forward Primer	0.5
20 µM	0.2 µM	Reverse Primer	0.5
		*Phusion Polymerase	0.5

*Polymerase should not be added to the reaction until the thermocycler has begun heating up to 98C

3. Program the Thermocycler to the following set up for the reaction:

Cycle	Temperature	Time	Repeats
1	98°C	10 sec	1
2	98°C	30 sec	12-18 Times#
	55°C	60 sec	
	72°C	30 sec/kb	
3	4°C	∞	

#Type of mutation desired	Number of cycles
Point mutations	12
Single amino acid changes	16
Multiple amino acid deletions or insertions	18

4. Add 1 ul of DpnI endonuclease to the PCR tube and place at 37°C for at least 2 hours.
5. Transformation.