Using Stratagene Single Site-Directed Mutagenesis Kit:

Dilutions:

Primers – 1:100dilution of a 500µM stock
Primers can be designed using PrimerX

Reaction:

1.5µl	5’ Primer
1.5µl	3’ Primer
1.0µl	Plasmid [100ng/µl]
5.0µl	10x PFU Buffer
38µl	ddH2O
2.0µl	5mM dNTP
1.0µl	PFU Polymerase

50µl Total

PCR Cycle:

Temperature (°C)	Time	Cycles
95	30 seconds	1
95 52 (~10° below Tm of primers) 67	30 seconds 1 minute ~2 minutes/kb[a]	16
67	5-10 minutes	1
4	15-O/N	

Transformation:
Add 1µl of Reaction to 50µl XL1-Blue cells
Put on ice for 20 minutes
Heat shock at 42° for 45 seconds
Put back on ice for 1 minute
Directly plate the 50µl reaction on LB + antibiotic plate
Incubate O/N at 37°

[a]12 minutes extension for SXL-RRM12 in pGEX-6p