Q5 site-directed mutagenesis (NEB)

Materials:

- Q5 hot start high-fidelity 2x master mix (12.5 µl)
- 10 µM forward primer (1.25 µl)
- 10 µM reverse primer (1.25 µl)
- 1-25 ng μl^{-1} template DNA (1 µl)
- 2x KLD reaction buffer (5 µl)
- 10x KLD enzyme mix (1 µl)
- Nuclease-free water

Protocol:

1. Assemble the following reaction in a PCR tube:

Q5 hot start high-fidelity 2x master mix	12.5 µl
10 µM forward primer	1.25 µl
10 µM reverse primer	1.25 µl
Template DNA	1 µl
Nuclease-free water	n µl
Total volume	25 µl

2. Mix reagents completely.
3. Perform the following cycling conditions:

Initial denaturation	98C	30 seconds
25 cycles	98C	10 seconds
	50-72C (Ta for primer set)	10-30 seconds
	72C	20-30 seconds per kb
Final extension	72C	2 mins
Hold	4-10C	Infinity

4. Use the PCR product to assemble the following reaction to allow kinase, ligase, and DpnI treatment:

PCR product	1 µl
2x KLD reaction buffer	5 µl
10x KLD enzyme mix	1 µl
Nuclease-free water	3 µl

5. Mix well with a pipette and incubate at room temperature for 5 mins. Use 5 µl of the product to transform.