

Site-directed Mutagenesis

1. Mutant Strand Synthesis

The sample reaction

10 µL of 5XKAPA HiFi Buffer

1.5 µL of dNTP mix

X µL (125 ng) of Forward Primer

X µL (125 ng) of Reverse Primer

X µL (5-50 ng) of DNA template

ddH₂O to final concentration 50 µL

Then add 1 µL of KAPA HiFi Polymerase

Cycling Parameters

Segment	Cycles	Temperature	Time
1	1	95 °C	2 min
2	12-18	98 °C	20 sec
		55 °C	45 sec
		72 °C	15-30 sec/kb of plasmid length

Number of cycles

Type of mutation	Number of cycles
Point mutation	12
Single amino acid change	16
Multiple amino acid indels	18

Following temperature cycling, place the reaction on ice for 2 min

2. Dpn I Digestion

Add 1 µL of the DpnI restriction enzyme to the reaction mixture; gently and thoroughly mix the reaction mixture by pipetting, spin down for 1 minute in micro centrifuge and immediately incubate the reaction at 37 °C for 1 hour.

3. Transformation of Supercompetent Cells

Use 0.5 (up to 1) µL of DpnI-treated DNA for electroporation OR 1 (up to 4) µL – for CaCl₂-transformation.

Primer parameters:

1. Both of the primers must contain the desired mutation and anneal to the same sequence on opposite strands of the template plasmid
2. Length should be 25-45 bases
3. T_m ≥ 78° C
4. The desired mutation should be in the middle of the primer
5. GC content ≥ 40 %