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Biomedical Engineering

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Site Directed Mutagenesis

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Title
Site Directed Mutagenesis

1 PCR

Component	Quantity/mass/final concentration	Volume (µL)
dH_2O	Fill up to 25 µL	..
10× QuikChange Lightning Multi reaction buffer	1x	2.5
Plasmid DNA	100 ng (stock … ng/µL)	..
Forward primer	100 ng (10 µM stock)	
dNTP mix	1x	1
QuikChange Lightning Multi enzyme blend	1x	1
Total		25

Segment	Cycles	Temperature	Time
1	1	95°C	2 min
2	30	95°C	20 sec
		55°C	30
		65°C	30 seconds/kb of plasmid length -> 3 min
3	1	65°C	5 min

2 Dpn I Digestion of the Amplification Products

- Add 1 µL of Dpn I restriction enzyme directly to each amplification reaction.
- Gently and thoroughly mix each reaction mixture by pipetting the solution up and down several times. Spin down the reaction mixtures in a microcentrifuge for 1 minute, then immediately incubate each reaction at 37°C for 5 minutes to digest the parental (nonmutated) ds-DNA.

3 Transformation of XL10-Gold Ultracompetent Cells

- Gently thaw the XL10-Gold ultracompetent cells on ice. For each mutagenesis reaction, aliquot 45 µL of the ultracompetent cells to an Eppendorf tube
- Add 2 µL of the β -ME mix provided with the kit to the 45 µL of cells. Using an alternative source of β-ME may reduce transformation efficiency.
- Swirl the contents of the tube gently. Incubate the cells on ice for 10 minutes, swirling gently every 2 minutes
- Transfer 4 µL of the Dpn I-treated DNA from each mutagenesis reaction to a separate aliquot of the ultracompetent cells

- Swirl the transformation reactions gently to mix, then incubate the reactions on ice for 30 minutes
- Heat-pulse the tubes in a 42°C water bath for 30 seconds exactly
- Incubate the tubes on ice for 2 minutes
- Add 0.5 ml SOC medium to each tube and incubate the tubes at 37°C for 1 hour with shaking at 225–250 rpm
- Plate 100 µL of each transformation reaction onto agar plates
- Incubate the transformation plates at 37°C for >16 hours