QuickChange PCR (multi-site mutation)

Materials

- HiFi Phusion polimerase + buffer (Biolabs)
- Nuclease-free water
- Primers forward and reverse (containing your mutation)
- DNA template
- dNTP mix
- PCR tubes
- DpnI enzyme

Apparatus

- Thermocycler

Method

Obs: The first and most important part is the primer designing. To facilitate this task check this design tool:

http://www.genomics.agilent.com/primerDesignProgram.jsp

1. Prepare the following mixture:

10x Buffer HiFi Phusion Polymerase	2,5 µL
10 mM dNTP mix	1 µL
100 ng Forward primer	1 µL
100 ng Reverse primer	1 µL
DNA template Plasmid DNA	~ 50 ng
HiFi Phusion DNA Polymerase	0.5 (2.5U)
Water, nuclease free	to 25 µL

2. Two different approaches were used for the thermalcycling. One with initial cycles done separately with the forward and reverse primer, which were combined for another PCR. The other method was a simple PCR run with both primers.

Cycles

Method 1

Step	Temperature (°C)	Time	Number of cycles
Initial denaturation	94-95	2 min	1
Denaturation	94-95	20 s	30
Annealing	55	30 s	
Extension	65	2,5 min	
Final Extension	65	5 min	1

For primers F and R separately

After these cycles mix both of the PCR reactions and perform another PCR following the same cycles above.

Method 2

Step	Temperature (°C)	Time	Number of cycles
Initial denaturation	94-95	2 min	1
Denaturation	94-95	20 s	30
Annealing	55	30 s	
Extension	65	2,5 min	
Final Extension	65	5 min	1

3. Remove the tube from the thermocycler and add 1 µL of DpnI to your reaction.
4. Incubate at 37°C for 1 hour.
5. Heat kill the DpnI at 80°C for 20 min (use the thermocycler or the waterbath)
6. Transform 4 µL of your reaction.