

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

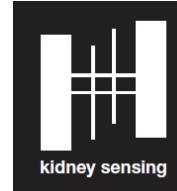
Method

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



- 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

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