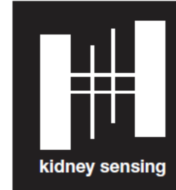


PCR for synthesis

Materials

- Taq polymerase buffer (Biolabs)
- Nuclease-free water
- Primers forward and reverse
- DNA template
- dNTP mix
- PCR tubes
- DpnI enzyme

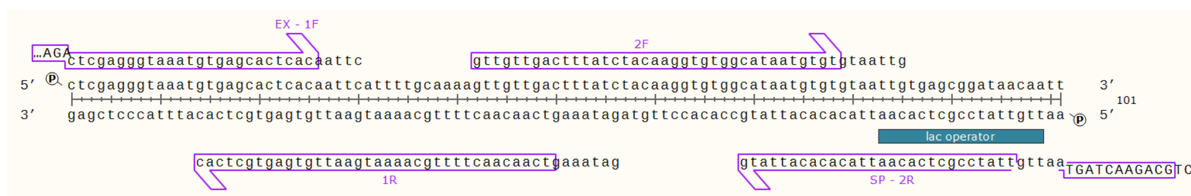
Apparatus

- Thermocycler

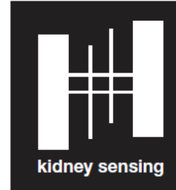
Method

Obs: Primer design

The primers you have to design must cover the whole extension of your sequence of interest, having an overlap of at least 15 bp for this protocol to work properly. Note that the number of primers must be even.



This protocol has two sequential PCR reaction. The first one with all your primers at low concentration. The following PCR will have 2 µL of the first one added to it and only your external primers at a higher concentration.



1. Prepare the following mixture:

10x Buffer Taq Polymerase	2,5 µL
10 mM dNTP mix	1 µL
10 µM primers (for each primer)	1 µL
50 mM $MgCl_2$	0,75
Taq DNA Polymerase (5U/µL)	0.5
Water, nuclease free	to 25 µL

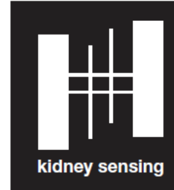
2. Place your tube in the thermocycler and run the following cycle:

Cycle 1

Step	Temperature (°C)	Time	Number of cycles
Initial denaturation	94-95	2 min	1
Denaturation	94-95	30 s	10
Annealing	45	20 s	
Extension	72	15 s	
Final Extension	72	5 min	1

3. Remove your tube from the thermoclycler and prepare another PCR reaction as indicated:

10x Buffer Taq Polymerase	2,5 µL
10 mM dNTP mix	1 µL
100 µM of external primers	1 µL
PCR product of the first reaction	2 µL
50 mM $MgCl_2$	0,75
Taq DNA Polymerase (5U/µL)	0.5
Water, nuclease free	to 25 µL



Cycle 2

Step	Temperature (°C)	Time	Number of cycles
Initial denaturation	94-95	2 min	1
Denaturation	94-95	30 s	15
Annealing	45	20 s	
Extension	72	20 s	
Denaturation	94-95	30 s	15
Annealing	55	20 s	
Extension	72	20 s	
Final Extension	72	10 min	1