DNA Synthesis via Splicing for Overlap Extension Polymerase Chain Reaction (SOE PCR)

Introduction

SOE PCR technique enables progressif elongation of the starting DNA sequence with the set of overlapping primers while performing a PCR cycle until getting the sequence of interest.

Reagents :

- Reverse and Forward primers (20ng/µL)
- Template DNA (100-200ng/ul)
- NEB® Hot Start Taq 2X master mix
- Nuclease Free Water

Procedure

1- add in a sterile thin-walled PCR tube :

Reagents	50 µl reaction
Nuclease Free Water	20 µl
NEB® Hot Start Taq 2X master mix	25 µl
Template DNA	2 µl
Forward Primer 20µM	1.5 µl
Reverse Primer 20µM	1.5 µl

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2- Mix all the agents slowly to homogenise the solution and make it flow down to the bottom of the tube

3- Load the mix into a PCR thermocycler and launch it with the the settings below :

Step	Temperature (°C)	time
Initial denaturation	95°C	30 secondes
32 cycles	95 °C 55°C 72°c	30 secondes 30 secondes 50 secondes
Final extension	72°c	5 minutes
Hold	10°C	-

4- Verify the right molecular weight of the product through electrophoresis (1.3% agarose gel, 30 minutes migration at 50V). For that use the correct ladder (1kb in our casse) and control samples.

5- If the product weight seems to be correct, purify the resting PCR product with a kit you prefer (Promega Wizard® SV Gel and PCR Clean-Up System in our case).

If you are using Promega Wizard® SV Gel and PCR Clean-Up System: Add 50 µL of Membrane Binding Solution to your PCR product and follow the 5.A. DNA Purification by Centrifugation part of the protocol given in the kit.

6- Check the concentration and the absorbance curve of the solution on Nanodrop

7A - if the results obtained in Nanodrop seem to be relevant, pursue whether with the next PCR mix preparation (if the sequence is not fully synthesized) whether with any other experiments using the product (if the sequence synthesis is complete)

7B - if the Nanodrop showed that that the step has failed, try to perform all the steps again and/or redesign the primers