OE-PCR

Introduction

OE-PCR can be used to assemble DNA fragemnts based on homologous areas. The homologous areas between separate fractions will anneal and function like primers in a regular PCR. OE-PCR can be done to produce a linear insert instead of a full plasmid.

Materials

- 80 fmol of each fragment
- KAPA PCR protocol

Procedure

1. 25 µl PCR reaction

   X µl H2O (Calculate the amount of water based on how much DNA you use to bring the total volume to 25µl)
   5µl 5 x Buffer
   0,75 µl 10mM dNTP mix
   0,5 µl KAPA HiFi HotStart DNA Polymerase
   80 fmol of each fragment

2. PCR Program:

   95° *C - 3 min*
   98° *C - 30 sec*
   65° *C - 30 sec*
   72° C - 4 min
   72° *C - 10 min*
   4° *C - forever*
   Repeat the underlined steps 15 times

3. Check the product size on gel or transform into competent cells (if backbone was included in the reaction mix)