OE-PCR

Introduction

OE-PCR can be used to assemble DNA fragments 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)