Overlap extension PCR (M0273)

Introduction

Overlap extension PCR allows us to amplify the two primary fragments using inner primers that carry complementary 5′ tails, and to gel-purify and quantify each so that they can be combined in equimolar amounts; to splice them into a single full-length product in a primer-free reaction, in which the shared overlap serves as both primer and template; and to amplify that spliced product with the two outermost primers, yielding a gel-purified, quantified fusion fragment ready for downstream cloning and sequence verification.

Reference

1. PCR Protocol for *Taq* DNA Polymerase with Standard *Taq* Buffer (NEB #M0273) https://www.neb.com/en/protocols/taq-dna-polymerase-with-standard-taq-buffer-m0273

Materials

- 10X Standard Taq Reaction Buffer
- 10 mM dNTPs 200 µM
- 10 µM Forward Primer 0.2 µM
- 10 µM Reverse Primer 0.2 µM
- Template DNA < 1,000 ng
- Taq DNA Polymerase 1.25 units/50 µl PCR
- Nuclease-Free Water add up to total 25 µL or 50 µL

Procedure

Procedure Overlap extension PCR

1. Assemble all reaction components on ice. Each component should be gently mixed before adding to the reaction in a sterile thin-walled PCR tube.

2. The Taq DNA Polymerase may be added the last

   The entire reaction should be mixed again to ensure homogeneous, consistent mixture.
   Collect all liquid to the bottom of the tube with a quick centrifuge spin if necessary.

Reaction

	Component	25 µl REACTION	50 µl REACTION
1	10X Standard Taq Reaction Buffer	2.5 µl	5 µl
2	10 mM dNTPs	0.5 µl	1 µl
3	10 µM Forward Primer	0.5 µl	1 µl
4	10 µM Reverse Primer	0.5 µl	1 µl
5	Template DNA	variable	variable
6	Taq DNA Polymerase	0.25 µl	0.5 µl
7	Nuclease-Free Water	to 25 µl	to 50 µl

3. Quickly transfer the reactions to a thermocycler preheated to the denaturation temperature (98°C) and begin thermocycling.

Thermocycling Conditions for a Routine PCR:

	STEP	TEMP	TIME
1	Initial denaturation	95 °C	30 seconds
2	25-30 Cycles	95 °C 45-68 °C* 68 °C	15-30 seconds 15-60 seconds 1 minute/kb
3	Final Extension	68 °C	5 minutes
4	Hold	4-10 °C	

Thermocycling Conditions for a routine 2-step PCR:

	A	B	C
1	Initial denaturation	95 °C	30 seconds
2	30 Cycles	95 °C 65-68 °C	15-30 seconds 1 minute/kb
3	Final Extension	65-68 °C	5 minutes
4	Hold	4-10 °C	