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SOP-113

Polymerase Chain Reaction (PCR)

Constructing recombinant DNA molecules by using the PCR.

Section 1

Materials and Reagents

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Template DNA (1 ng to 10 ng of plasmid or phage DNA; 20 ng – 300 ng genomic or cDNA)

Oligonucleotide Primers (0.6 – 1.0 mM)

DNA polymerase

TE Buffer, pH 8.0

- 10 mM Tris-Cl, pH 8.0
- 1 mM EDTA, pH 8.0

Equipment: Thermocycler

Section 2

Protocol

Any two segments of DNA can be ligated together into a new recombinant molecule using the polymerase chain reaction (PCR). The DNA can be joined in any configuration with any desired junction point reading frame or restriction site by engineering primers with appropriate sequences. It is not necessary to know the sequence of the template DNA being subcloned other then two short flanking regions. This technique can be used to generate in-frame fusion constructs.

1. Prepare template DNA.
2. Prepare oligonucleotide primers.

   If the PCR product is to be cloned by blunt end ligation phosphorylate the 5 'hydroxyl of the primer. A 5'phosphate on the end of the PCR primer is needed for ligation. This step is essential if the DNA template has been treated with phosphatase.

3. Set up standard amplification reaction. Perform the PCR in a thermocycler for 20 to 25 cycles under the following conditions: denature 1 minute at 94 C, anneal 1 minute at 50 C and extend 1 – 3 minutes at 72 C.

   Note: Polymerase inserts ~1000 bases / minute.

   Note: There are a number of DNA polymerases commercially available that have different features regarding proof reading and thermal stability.

4. Analyze an aliquot of the reaction by agarose gel electrophoresis.

5. For cloning or sequencing, prepare PCR mixture. Clean mixture using spin column from Qyiagen or extract DNA using chloroform and phenol. Then precipitate DNA using 100% EtOH. Resuspend DNA in TE buffer.

6A. For blunt end ligation repair 3'end of amplified product using DAN polymerase I to fill in the overhang. (Called polishing).

6B For primers that contain unique restriction digests. Digest half of the amplified material in 20 ul with appropriate restriction enzymes.

7. Digest recipient DNA with appropriate restriction enzymes. If necessary treat vector DNA with calf intenstinal alkaline phosphatase (CIP) to prevent recircularization. Separate linearized DNA from uncut DNA by agarose gel electrophoresis. Recover linearized DNA from the agarose.

8. Ligate PCR product and linearized DNA.

9. Transform ligation reactions into competent bacteria.

Section 3 References

Short Protocols in Molecular Biology. 4th Edition. Editors Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J.G. Seidman, John A Smith and Kevin Struhl. John Wiley & Sons, Inc. 1999