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Creating chimeric molecules by PCR directed homologous DNA recombination

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For many experiments aimed at the elucidation of protein structure-function relationships, two DNA fragments from different sources have to be joined to generate a chimeric molecule. In most cases this can be achieved by using a variety of conventional cloning techniques. More recently *Yon and Fried* (1) introduced the PCR with 'linking oligo' as a generally applicable strategy for any gene fusion project. Here we demonstrate that in those cases where the DNA fragments contain overlapping identical sequences, they can be joined by a simple standard polymerase chain reaction (2). We show that a PCR containing the two templates I and II, whereby the 3'-end of template I has to be identical to the 5'-end of template II, and two oligonucleotide primers, each one being specific for the unique part of only one template, produces a defined chimeric molecule containing the unique sequence elements of both templates. This 'recombination PCR' was applied for generating a full length cDNA for human uteroglobin (hUG). The two templates used were a genomic subclone of the hUG gene containing exon I, 5'-flanking sequences, and part of the first intron (template I, M.W. unpublished) and the 5'-truncated hUG cDNA from *Singh et al.* (3) (template II). Both templates have overlapping identical sequences 62 bp long and could be used in the form of supercoiled plasmids. The primer PI was complementary to the 5'-end of the hUG message of template I, whereas the primer PII was complementary to the 3'-end of the cDNA of template II (see fig., step 1). After 30 cycles of PCR (20 sec at 94°C, 60 sec at 62°C, 15 sec at 72°C) only the reaction containing both templates together produced the expected 384 bp fragment (fig., lanes 3 and 4), whereas the reactions containing template I or II alone failed to amplify any fragment (lanes 5–7). The identity of the amplified fragment with the expected one was verified by subcloning and sequencing. The experiment suggests the occurrence of multiple 'premature' Taq polymerase pause or termination sites during the early cycles of amplification (fig., step 1). This explains that e.g. primer PI extended on template I into the region identical to both templates is now able to anneal to template II (step 2), gets further extended (step 3), and yields the upper strand of a chimeric molecule having at its 5'-end a sequence unique to template I and at its 3'-end a sequence unique to template II. As soon as one strand of the chimeric molecule is synthesized it gets exponentially amplified via primers PI and PII (step 4). Step 2 of the reaction sequence is more complex because alternatively to extended primer PI, extended primer PII can switch from template II to I. In addition not only the input plasmids can serve as templates but also 'complete' extension products from primers PI and PII. In accordance with our proposed reaction sequence the amplification could be made more directed by linearizing template I at a PstI site which defines a short main extension product of primer PI on template I that can efficiently switch to template II for further extension.

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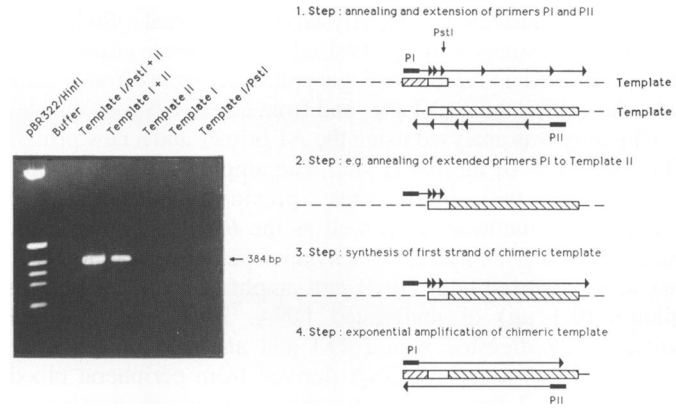

Creating chimeric molecules using 'recombinant PCR'. Broken lines represent promoter, intron or vector sequences. hUG cDNA sequences are boxed. The boxes representing cDNA regions unique to templates I and II are hatched to the right and left respectively. Overlapping cDNA sequences identical to both templates are indicated by a plain box. Primers PI and PII are marked by a short black line. Arrowed lines depict sequences newly synthesized by Taq polymerase, each arrow indicating a possible termination site. For lanes 3 and 7 template I was linearized at a PstI site indicated in step 1. For further explanations see text. PCR conditions were: 10 ng of each template indicated, 200 μM dNTPs, 50 pmoles of primers PI and PII each, 16.6 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl pH 8.8, 6.7 mM $MgCl_2$, 10 mM ß-ME, 2U Taq polymerase, and 2 mg/ml BSA in a 100 μl reaction. For cycle conditions see text.