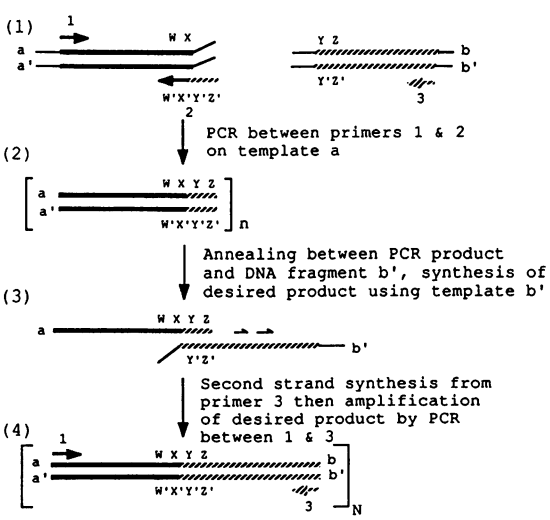
Volume 17 Number 12 1989 Nucleic Acids Research

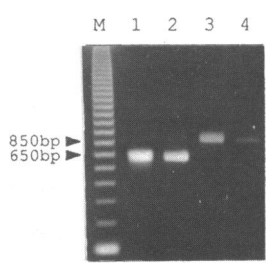
Precise gene fusion by PCR

Jeff Yon and Mike Fried

Eukaryotic Genome Organisation and Expression Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK
Submitted May 15, 1989

There are numerous instances when the need to construct hybrid fusion genes arises. Examples include constructs where 5' sequences of one gene are fused to the coding region of a second gene (to determine which sequences are involved in gene expression or to express the second gene efficiently), fusion genes specifying hybrid proteins, and precise deletion mutants. The construction of such fused or deleted genes usually involves ligating different restriction fragments together (a tedious and time-consuming process) and the join between the sequences derived from different DNA fragments is governed by the availability of restriction sites and is not always ideal. Here we describe a simple method for the construction of fusion genes using the polymerase chain reaction (PCR) (1) in which the desired join between the genes or deletions within a gene can be at any chosen location. The PCR is carried out with reagents of the Perkin Elmer Cetus Gene-Amp kit and contains two cloned DNA fragments (1ng each) carrying the sequences to be fused and three oligonucleotides as shown in the figure. The 'outer oligos' (1 and 3) are present at 1μM and anneal to different fragments of DNA. The third oligo (the 'linking oligo', a 40mer) is present at much lower concentration (the optimum is about 0.01μM, see photo) and carries sequences homologous to both DNA fragments around the desired join. Initially PCR produces the intermediate shown in step 2, until the linking oligo is exhausted from the reaction mixture. Then the PCR can only proceed once interstrand annealing between strands a and b', elongation (step 3) and second strand synthesis (step 4) have occurred. After PCR for 30 cycles of 2min at 94°C, 2min at 59°C, 2min at 70°C, the desired fusion product predominates (see photo). We have sequenced this PCR product and the join is created according to the sequence specified by the linking oligo. This fast and simple method has proved useful in creating gene fusions and deletions, and additional changes can be introduced at the join by modifying the linking oligo.





Concentration of linking oligo:lane 1-1μM, lane 2-0.1μM, lane 3-0.01μM, lane 4-0.001μM

Step (2) product 650bp
Step (4) product 850bp

References:
1. Saiki, R.K et al (1988) Science 239, 487-491