Special Commentary

A quarter century of reaping what we SOE

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Gene Splicing by Overlap Extension (SOE), described in a *BioTechniques* Research Report 23 years ago, is still widely used today in spite of changes in technology and scientific fashion. The history of SOE offers practical lessons for those who seek the best techniques, and for those who strive to develop them.

Molecular biologists have long striven to create or modify DNA sequences at will. The best methods evolve in response to technological advances and demands of the marketplace. Few graduate students today know how their elders created site-directed mutants prior to the invention of the Polymerase Chain Reaction (PCR) in 1985 (11). By 1989, the pages of *BioTechniques* were replete with articles describing new applications of PCR. Higuchi et al. (5) and Ho et al. (6) independently invented gene splicing by overlap extension (SOE) as a site-directed mutation method. Horton et al. then demonstrated how SOE could also be applied to recombine parts of homologous genes (8).

Horton et al. subsequently described the SOE method in even greater detail in an influential article published in *BioTechniques* (7), the foundation of which (Figure 1) is still widely employed today. Four primers are designed to amplify two DNA segments (adjacent parts of the same gene for site-directed mutagenesis, different genes for fusions). Two of the primers (b and c, Figure 1) are chimeric, such that the 3' ends are complementary to the gene segments that are initially amplified (primers a and b complement Gene I; primers c and d complement Gene II) while their 5' ends encode the joining sequences of the other segment. The resulting PCR products are separated from the primers in an agarose gel and purified by some form of silica gel chromatography. The two products are then combined and amplified in a second PCR; the top strand of one product (AB) can anneal to the bottom strand of the other (CD), and this primer dimer can then be extended by the thermostable polymerase.

To their credit, Horton et al. acknowledged that their SOE technique was limited in three ways (7, 8). In retrospect, they were ahead of their time as subsequent technical innovations have mostly addressed these limitations. The cost of oligonucleotide synthesis was once prohibitive, but the per nucleotide prices have decreased more than ten-fold between 1994 and 2008 (9). Taq DNA polymerase is error-prone, and is not reliable for the amplification of long DNA sequences (>4 kb). These problems have been reduced, at least in part, with more accurate and processive thermostable DNA polymerases, such as Phusion (16). The SOE technique has also evolved to meet new demands. DNA shuffling, the SOE of many DNA fragments that contain overlapping sequences (13), galvanized the development of directed evolution methods. Gene synthesis, the SOE of oligonucleotides designed to encode DNA fragments (≤3 kb), is transformative because it obviates the need for template DNA and enables the facile construction of codon-optimized genes that don't exist in nature. Overlap extension PCR cloning, in which one of the two fragments is a plasmid, has enabled some molecular biologists to meet the rising demand for cloned genes.

Overlap extension PCR cloning was first described in 2001 (4) and subsequently refined, and sometimes re-named (Restriction Free Cloning or Circular Polymerase Extension Cloning), over the next decade (1-3, 10, 12, 14, 15, 17, 18). The history of this methodological

variation of SOE offers some general lessons for the readers of *BioTechniques*. This method has always competed for users against a wide variety of other PCR cloning techniques. The number and diversity of these techniques suggest that none are perfectly reliable, and that further innovation can be expected. Unfortunately, it is already difficult for novices to make informed decisions about cloning methods. Virtually

Figure 1. Gene Splicing by Overlap Extension. Four primers (a-d) are designed such that a and b amplify Gene I, while c and d amplify Gene II (for site-directed mutagenesis, Genes I and II are adjoining parts of the same gene). Primers b and c are chimeric, with 3' ends that complement Genes I and II respectively and 5' ends that encode the joining sequences of the other gene. The two PCR products (AB and CD) are gel purified, then reacted together in a third PCR (the SOE step) with primers a and d. The top strand of AB and the bottom strand of CD should anneal to one another, forming a mega-primer dimer that is extended by the thermostable DNA polymerase. The resulting recombinant product is then PCR amplified by primers a and d.

every innovator claims, generally without empirical support, that their method is quicker, easier, more versatile, less expensive and more efficient, at least under ideal reaction conditions, than every competing method. The confusion is exacerbated by commercial vendors of cloning kits, who generally don't disclose the composition of their reagents. Novices thus have less opportunity to master the art of PCR cloning at a time when expectations for operational efficiency are increasing.

BioTechniques articles are more transparent and informative than mix-and-pray commercial kits, and therefore offer better long-term value. In addition, methods articles that reveal the limitations of a technique, like that of Horton et al. (7), are more useful and therefore more widely read than those that only show positive results. Such articles guide new readers towards a mechanistic understanding of the reactions, thereby providing mastery over their own experiments. The competitive culture of research fosters the invention of new techniques and the extinction of those that are obsolete. In the early 1990s, some might have predicted that PCR technologies would obviate gene cloning. Others might have supposed that the era of PCR methods development would long be over by now. What happened instead is that the best ideas, including gene SOEing, adapted to changing technology and marketplace demands. Those who seek reliable techniques should therefore

consider the longevity and adaptability of each one under consideration. The most influential methods papers are not necessarily those most viewed recently, but rather those that continue to attract attention decades after they are first published.

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