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ly ligated; (iv) in the ExSite kit protocol, there is a high probability of cloning non-specific DNA fragments, as the PCR product is directly ligated without purification; (v) the ExSite kit requires the use of *Epicurian Coli* XL1-Blue Supercompetent bacterial cells, which are expensive, sensitive to freezing and thawing, and require particular conditions for transformation; and (vi) unlike our protocol, the ExSite kit does not provide specific criteria for selecting primers that efficiently amplify PCR products from long plasmid templates to generate deletion mutants.

In conclusion, we provide in this report a highly efficient (90%–100%) and convenient protocol for the deletion of large fragments from either small or large plasmids by one-step cloning. To our knowledge, this is the first report of a site-specific deletion method that can be performed on large (12-kb) plasmids in a single step.

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Swapping of Functional Domains Using a Chimeric Monster Deletion Mutagenesis Strategy

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Site-directed mutagenesis is an effective method to determine the function of amino acid and nucleotide residues within protein and RNA molecules. Systematic site-directed mutagenesis for scanning of larger regions has proven useful when only limited structural information is available (2). A variation of the site-directed mutagenesis approach is the swapping of entire domains between two related molecules to determine their unique functional differences. For domains that correspond up to several hundred base pairs, it can be difficult to introduce mutations over these larger regions for the generation of chimeric molecules, as it requires *de novo* gene synthesis or multiple mutagenesis steps followed by sequence verification at each step. Common restriction enzyme cutting sites for swapping domains between two related genes are rarely available and always predetermined. A relatively time-consuming method for changing large DNA segments involves preparation of uracil-containing ssDNA templates and asymmetric PCRs (7). PCR of two overlapping fragments and extension after annealing at the overlap (3) is another widely used approach to generate hybrid genes, but it is critically dependent on primer design and annealing temperatures used in amplifications of the desired DNA fragments. Here, we describe a robust strategy for generating chimeric molecules effectively and precisely. As in PCR of overlapping fragments (3), this approach is effective in the absence of convenient restriction enzyme cutting sites.

In the example described here, our aim was to construct chimeric signal recognition particle (SRP) RNA molecules in which the large domains (LDs) were exchanged precisely between human and *Methanococcus jannaschii* genes (Figure 1). Previous studies demonstrated that *M. jannaschii* SRP RNA bound two polypeptides, proteins

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SRP19 and SRP54, independently (1). In contrast, binding of SRP54 to human SRP RNA relied on a conformational change in RNA, which was induced by an interaction between RNA and protein SRP19 (4). Chimeric DNAs could be used to transcribe the corresponding RNA molecules and identify the assembly control region by measuring the binding activities of SRP19 and SRP54.

Attempts to replace a 150-bp region of the human SRP RNA gene with the equivalent *M. jannaschii* sequence using conventional site-directed mutagenesis steps proved unsuccessful. Compli-

cations were caused most likely by the extensive base-pairing possibilities within SRP RNA (8), which introduce inverted repeats into the DNA and allow the primers to form hairpin structures. To avoid these difficulties, we decided to create first a "monster chimera" (MC) that contained the large domains of both human and *M. jannaschii* SRP RNA in a single molecule. From this larger composite, we could then generate any desired chimera by deleting unwanted portions using a standard site-directed mutagenesis approach (5).

The human SRP RNA gene was pre-

pared for the insertion of *M. jannaschii* DNA by introducing a unique *KpnI* restriction site at the region that corresponded to helix 7 (Figure 1A) using a single 24-mer mutagenic oligonucleotide (5,9) to obtain phSR-K. Template DNA was phSR, which contained the complete human SRP RNA gene under T7 RNA polymerase promoter control (8). The large domain of the *M. jannaschii* SRP RNA gene was amplified from the full-length clone pMjSR (1) with primers a (26-mer) and b (23-mer) to generate *KpnI* sites near the termini (labeled R in Figure 1A, right panel). Amplified DNA was restricted with *KpnI* and ligated to *KpnI*-digested phSR-K to generate pMC containing both human and *M. jannaschii* SRP RNA sequences. The proper orientation of the insert was verified by restriction mapping and sequencing.

From pMC, two intermediate deletions, I-1 and I-2, were generated using PCR site-directed mutagenesis in which a single mutagenic primer and an upstream primer were used, followed by the extension of the PCR product on template DNA to include downstream sequences and amplification of mutant DNA as described previously (5). I-1 was constructed using mutagenic primer m1 (a 27-mer), and I-2 was synthesized with mutagenic primer m2 (a 28-mer) (Figure 1B). Other common primers were c (a 41-mer), d (a 20-mer, identical in sequence to the 5' portion of primer c), and e (25-mer). For example, to obtain I-1, a PCR was carried out with mutagenic primer m1 and primer c, and the PCR product was extended in a single step, followed by the amplification with primers d and e. The deleted region of I-1 (shown as dotted lines in Figure 1B) included a portion of human SRP RNA helix 5 and helix 6. The previously introduced *KpnI* site was also deleted. In I-2, a part of human SRP RNA helix 5 and helix 8 was removed. Not unexpectedly (6), because of the inverted repeat structure of the SRP RNA gene, a mixture containing larger than desired fragments was obtained. However, since the deletions varied in size from 82 to 94 bp, the desired PCR products were distinguished clearly from incorrect fragments during their electrophoretic separation on 1.5% agarose gels. DNA fragments of the correct size were ex-

Figure 1. Schematic overview of steps involved in swapping functional domains between two SRP RNA genes. LDs of human and archaeal (*M. jannaschii*) were exchanged. Regions that correspond to human SRP RNA are in gray; black represents *M. jannaschii* sequences. Panel A (top) shows the construction of pMC. On the left, secondary structure cartoons are depicted with RNA helices 5–8 and 5' and 3' ends labeled as such. The same molecules are shown in linear presentations on the right. Using conventional PCR site-directed mutagenesis (5), a unique restriction site (labeled R) was introduced at helix 7 of the human SRP RNA gene to yield phSR-K. The same sites were present in a fragment amplified with primers a and b from the *M. jannaschii* SRP RNA gene. pMC was derived by inserting the *M. jannaschii*-derived fragment into the human gene. Thin lines correspond to plasmid-derived sequence that contains flanking restriction sites S and T. Panel B (center) shows the generation of two intermediates, pI-1 and pI-2, from pMC with primers c to e and one mutagenic primer (m1 and m2, respectively) using procedures described in Reference 5. Deleted regions are indicated by the dotted lines. Panel C (bottom) shows the desired chimera (pCh) derived by PCR site-directed mutagenesis or assembled from the S/Z-fragment of pI-1 and the Z/T-fragment of pI-2 as described in the text.

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Figure 2. Restriction mapping of the various human and *M. jannaschii* SRP RNA gene constructs. Approximately 300 ng plasmid DNA were digested with restriction enzymes *Eco*RI and *Ban*HI, separated by electrophoresis on a 1% agarose gel, and stained with ethidium bromide. Lanes are labeled as follows: M, DNA markers with numbers of base pairs indicated on the left; HU, human SRP RNA; MJ, *M. jannaschii* SRP RNA; MC, monster chimera; I-1, intermediate construct 1; I-2, intermediate construct 2; and Ch, chimera. Mobilities of fragments of vector (v) and insert (i) DNAs are marked on the right.

tracted, digested at flanking restriction sites (labeled S and T in Figure 1B, right panel), and cloned. Finally, pCh was constructed using the Nelson and Long method (5) with mutagenic primer m2 and pI-1 template DNA or, alternatively, with mutagenic primer m1 and pI-2 DNA. Also, pCh could be obtained directly by isolating DNA fragments derived from digestion with restriction enzymes S and Z of pI-1 and enzymes Z and T of pI-2, followed by their ligation to S/T-digested vector DNA (Figure 1, B and C, right panel). Constructs were selected and verified by restriction mapping (Figure 2) and sequencing.

Our mutagenesis scheme required the straightforward construction of a single "monster chimera" plasmid (pMC). The chimera was obtained by deleting undesired regions from pMC using conventional site-directed mutagenesis procedures. In contrast to other methods for generating hybrid molecules, errors that might be caused by multiple amplification reactions (3) were avoided because only deletions were introduced. Furthermore, deletion

mutagenesis allowed one to separate readily any incorrect PCR products from the proper DNA fragment. We have applied this technique to obtain efficiently and accurately four different chimeras of human and *M. jannaschii* SRP RNA (not shown). As demonstrated here, our approach was successful with SRP RNA genes, which appeared challenging because of their high degree of similarity, potential for extensive base pairing, elevated GC content, and possibility to form inverted repeats. The same strategy can be adopted easily to study the structure and function of other genes, in particular those with similar problematic properties.

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