Self-assembly cloning: a rapid construction method for recombinant molecules from multiple fragments

PROTOCOL FOR:

Self-assembly cloning: a rapid construction method for recombinant molecules from multiple fragments

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See full protocol online: <https://benchtalk.biotechniques.com/users/8521-biotechniques-editors/posts/11342-protocol-for-self-assembly-cloning-a-rapid-construction-method-for-recombinant-molecules-from-multiple-fragments>

Protocol Summary: Enzyme-free cloning (EFC) can rapidly produce an in-frame fusion gene with multiple fragments. To practically apply EFC, we investigated the extent and sequence of complementary staggered overhangs necessary to direct self-assembly of multiple fragments as well as a size limitation of the constructed DNA molecule. Six-base pair overhangs with 50% GC content were sufficient to direct self-assembly. A functional plasmid that exceeded 10 kb, which includes an in-frame fusion domain, was efficiently constructed from four PCR fragments in one step by our improved method.

Protocol Schematic: A multifunctional plasmid created by SA cloning that expresses reporter proteins in different subcellular compartments in insect cells. The plasmid, designed to self-assemble with four fragments, has several functional units: a replication origin (represented as an oval); three antibiotic resistance genes for ampicillin, zeocin, and kanamycin (a hexagon); two genes encoding the fluorescent reporter proteins enhanced GFP (EGFP) and DsRed (a double box) are driven by PAc5 and POpie-2 promoters (an arrow) and terminated by the polyA signal sequences (a rounded-edge rectangle), respectively; an NLS (box) fused in-frame to EGFP-Zeo, in which the NLS sequence was also used as a complementary overhang to self-assemble between fragments B and C. Only the sense strand of each fragment with the 5' overhang

region (a numbered box) is illustrated around the designed plasmid. The signal of EGFP is shown as green in nucleus (left panel of insets) and red in cytoplasm (middle). The right panel represents the merge of EGFP and DsRed signals.