

Partially complementary overhang:

TCGAGGAACGCGATG **CGCGTCCGGCAA**
AGCTCCTTGC **ACAGCGCAGGCCGTT**

Sequence Number	Number of Clones	Joint Sequence	Fragment Length
1	3	TCGAGGAACGCGAT <u>GT</u> CGCGTCCGGCAA	43
2	2	TCGAGGAACGCGATGCGCGTCCGGCAA	42
3	4	TCGAGGAACGCGTGTTCGCGTCCGGCAA	42
4	1	TCGAGGAACGCGACGCGTCCGGCAA	40
5	1	TCGAGG <u>TGT</u> CGCGTCCGGCAA	36
6	1	TCGAGGAAC <u>CGCG</u> TCCGGCAA	35
7	1	TCG <u>TGT</u> TCGCGTCCGGCAA	35
8	1	TCGATGTCGCGTCCGGCAA	34
9	1	<u>TCG</u> CGTCCGGCAA	28
10	1	CATCACAT <u>TGT</u> TCGCGTCCGGCAA	-

Supplementary Table I. Sequences of clones derived from joining of ends with two complementary bases. Sequences are expressed in terms of the radiolabeled top strand in the experiments. Black and blue letters indicate sequences originating from the left and right ends of the break respectively. Underlined nucleotides could have originated from either end and may have resulted from microhomology annealing and splicing. Overlines indicate apparent preservation of 3' overhangs. Sequence 1 corresponds to annealing of the two terminal nucleotides in the overhangs, followed by gap filling of both strands and ligation (accurate repair). Sequences 2 and 3 correspond to excision of the entire right-hand or left-hand overhang, respectively, followed by fill-in of the remaining 3' overhang primed from the resulting blunt end (see Fig. 3B). Sequence 6 is consistent with annealing of the self-complementary CGCG at the 5'-terminus of the double-strand portion of both ends, presumably preceded by 3' resection. Orange letters in sequence 7 indicate an insertion of unknown origin. Green letters in sequence 10 indicate linkage to a sequence not found anywhere in the plasmid. It may be noted that sequencing results indicate a lower frequency of accurate repair than would be expected from the gel assays with radiolabeled substrates (Fig. 3A). This is likely due in part to substrate molecules in which the labeled oligomer failed to ligate into the plasmid; such substrates can only yield inaccurate repair products, but these unlabeled products would not be detected in the gel assay. Conversely, gel assays detect both recircularization and intermolecular joining, but only recircularized products will form colonies for sequencing; it is possible that recircularization occurs with lower fidelity than intermolecular joining.