Induction of I-SceI and λ-Red, from pACBSCE, with arabinose

I-SceI cleaves pACBSCE and generates a linear DNA fragment from the pDOC plasmid.

The λ-Red proteins facilitate recombination between the linear DNA fragment and homologous regions on the chromosome.

Only cells that have lost the sacB gene and have retained the kanamycin resistance cassette can survive and grow on media supplemented with kanamycin and sucrose.

Gene coupling or gene deletions are verified by PCR amplification of the modified chromosomal region.