

Engineering EGFP reporter constructs into a 210 kb Alphoid BAC clone using GET recombination

By

Wong Sie Chuong

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Abstract

Alphoid BAC with α 21-I alpha satellite DNA inserts has been shown to be competent in seeding *de novo* centromere formation in mammalian artificial chromosome (MAC) research. However, the alphoid DNA inserts with tandemly repetitive DNA are prone to rearrangement even during routine maintenance and propagation, thus limiting the alphoid DNA manipulation. In this work, the possibility of alphoid BAC modification using GET recombination has been explored by retrofitting the EGFP reporter construct into the vector backbone of an alphoid BAC. GET recombination is a simple inducible homologous recombination system in *E. coli* DH10B and is tightly regulated by the L-arabinose inducible promoter. By using selected relatively stable alphoid BAC clones (210 α BAC), it has been demonstrated that the GET recombination system can be used to modify the alphoid BAC of up to 210 kb with the alphoid inserts remaining intact. However, during the process of recombination, some rearrangement in the alphoid region of the BAC occurred, producing a series of retrofitted alphoid BACs ranging from 15 kb to 210 kb. This series of alphoid BACs will be valuable to ascertain the optimal size requirement for *de novo* centromere formation in MAC.