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Notes & Tips

Recombination between linear double-stranded DNA substrates *in vivo*

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ABSTRACT

Recombineering technology in *Escherichia coli* enables targeting of linear donor DNA to circular recipient DNA using short shared homology sequences. In this work, we demonstrate that recombineering is also able to support recombination between a pair of linear DNA substrates (linear/linear recombineering) *in vivo* in *E. coli*. Linear DNA up to 100 kb is accurately modified and remains intact without undergoing rearrangements after recombination. This system will be valuable for direct *in vivo* manipulation of large linear DNA including the N15 and PY54 prophages and linear animal viruses, and for assembly of linear constructs as artificial chromosome vectors.

Recombineering technology enables facile modification of large circular DNA using homologous recombination in *Escherichia coli* without dependence on suitably placed restriction sites or *in vitro* enzymatic manipulations [1–3]. In the presence of short 40- to 60-nucleotide homology sequences, expression of the *E. coli recE* and *recT* genes and/or the bacteriophage λ *red* genes facilitates targeting of linear donor DNA (e.g., linear PCR products) to a specific site on circular recipient DNA *in vivo* [1,2].

To date, recombineering has been demonstrated for the following substrates: (1) linear DNA and a circular replicon (including plasmids [2,4], PACs, and BACs [1,2,5], and the *E. coli* chromosome [6,7]), and (2) linear DNA and a linearized plasmid vector backbone that becomes recircularized after gap repair following *in vivo* recombination [8]. However, so far, recombineering between a pair of linear DNA substrates has not been tested *in vivo* because of the lack of a system that could serve as linear recipient plasmids in *E. coli*.

We recently developed a system to assemble BACs up to 100 kb as linear plasmids capped with telomeres derived from the bacteriophage N15 [9]. This linear BAC was resistant to RecBCD, which degrades linear DNA in *E. coli*, and was functional after transfer into human cells and produced correctly spliced β-globin transcript [9]. In this work, using this linear BAC DNA system, we demonstrate recombineering between a pair of linear DNA substrates (linear/linear recombineering) *in vivo* in *E. coli* and discuss its application.

To investigate recombineering between a pair of linear DNA substrates, a linear PCR product (donor DNA) was electroporated into electrocompetent *E. coli* DH10B containing a resident linear 100-kb human β-globin BAC and plasmid pGETrec [strain *telN*$^+$ DH10B (pGETrec, linear BAC)] [9] (Fig. 1a). The linear BAC serves as the recipient DNA (Fig. 1a), while plasmid pGETrec provides the recombineering enzymes, Gam, RecE, and RecT. To initiate linear/linear recombineering the linear donor DNA (Kan60) is electroporated into these cells as described in Fig. 1a.

First, to provide transient expression of the recombineering enzymes in this strain, the cells were grown at 150 rpm at 30 °C and plasmid pGETrec was induced for 10 min with arabinose when the OD_{600} reached 0.55 [3] (Fig. 1a). The linear donor DNA (Kan60) contains the kanamycin resistance gene (Km^r)[1] flanked by 60 bp of homology that directs this 1162 bp DNA to recombine to a sequence centered around the *Bst* 1107I site on the vector backbone of the linear BAC recipient DNA (Fig. 1a). Kan60 was PCR-amplified using primers kan60F (5′-TTC CGG TCA CAC CAC ATA CGT TCC GCC ATT CTT ATG CGA TGC ACA TGC TGT ATG CCG GTA caa gaa atc aca gcc gaa gc-3′) and kan60R (5′-AGA CTT CCG TTG AAC TGA TGG ACT TAT GTC CCA TCA GGC TTT GCA GAA CTT TCA GCG gta gcg tga tct gat cct tca act-3′) from pCyPAC7 (a gift from the late Dr. Panos Ioannou) according to our standard protocol [1] and electroporated into the host cells (Fig. 1a).

After recombineering between the linear DNA substrate pair (linear/linear substrates), 58 Km^r recombinants were obtained (Fig. 1b). In comparison, a parallel set of linear/circular substrates was recombined by electroporation of the same linear Kan60 donor fragment into a strain containing a circular β-globin BAC recipient [DH10B (pGETrec, circular BAC)] [10]. This recombination event produced 211 Km^r recombinants (Fig. 1b). Although linear/circular

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[1] *Abbreviations used:* Km^r, kanamycin resistance gene; PFGE, pulsed field gel electrophoresis.

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