Loop-out Plasmid for Generating Markerless Deletions in the *Bacillus subtilis* BKE Knockout Collection

**pDR244 cre + Ts origin**

(6648 bp)

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**cre/lox-Mediated Loop-out Protocol for pDR244**

1. Transform pDR224 into one of the *Bacillus subtilis* BKE collection or any other strain harboring a loxP-flanked antibiotic resistance cassette with selection for spectinomycin resistance at 30°C.
2. Transfer several transformant colonies to plain LB plates and incubate overnight at 42°C.
3. Screen resulting colonies for plasmid curing (spectinomycin sensitivity) and the antibiotic resistance cassette (erythromycin sensitivity).
4. Streak for fresh single colonies and then confirm loss of the cassette by PCR.