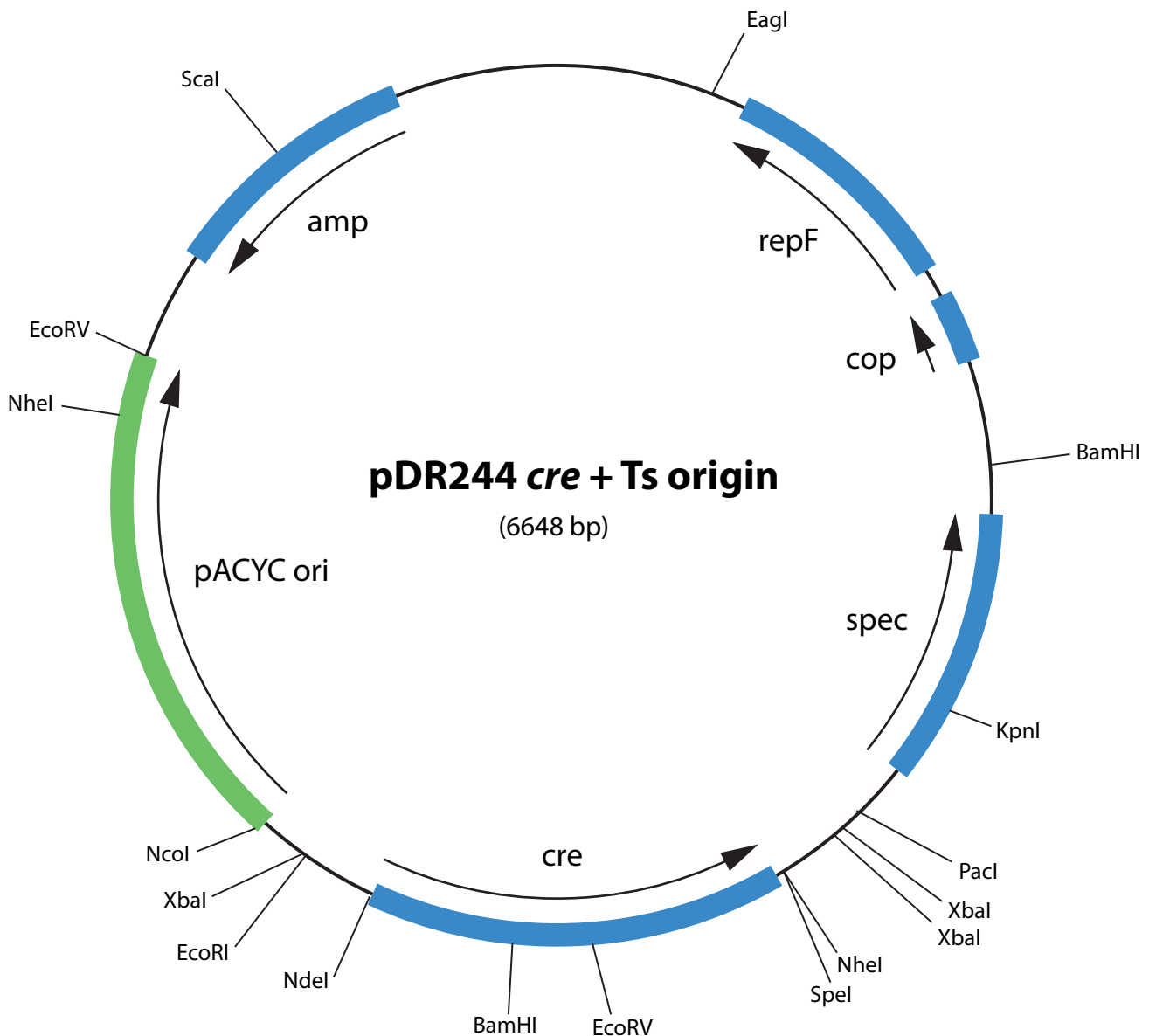


# Loop-out Plasmid for Generating Markerless Deletions in the *Bacillus subtilis* BKE Knockout Collection



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

- (1) Transform pDR244 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