

## **Development of conditionally integrative thermosensitive vectors for efficient gene targeting in *Bacillus* species**

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The pE194 plasmid is naturally thermosensitive and is rescued by integration after cultivation of the host *Bacillus* species at a restrictive high temperature. The plasmid is spontaneously excised from the genomic DNA at a low frequency by precise or imprecise excision. We developed shuttle vectors that contain the thermosensitive replication origin of pE194 plasmid with the aim of using them as delivery and rescue tools for genetic markers, targeted gene disruption and replacement, and for the generation of random mutants of gram-positive hosts. We report in this study the thermosensitivity, integration and excision frequencies of the constructed plasmids.