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Title: Construction of a high-efficiency cloning system using the Golden Gate method and I-SceI endonuclease for targeted gene replacement in *Bacillus anthracis*

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Running title: Constructing high-efficiency cloning and gene replacement system
in *B. anthracis*

Construction of a high-efficiency cloning system using the Golden Gate method and I-SceI endonuclease for targeted gene replacement in *Bacillus anthracis*

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Highlights

- The efficiency of using Golden Gate method to build knockout vector is more than 85%.
- 2.The expression of I-SceI endonuclease promoted homologous recombination. The allelic-exchange schemes driven by I-SceI nuclease at rates of 85%–100%.

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