

Markerless Genome Editing in *Bacillus subtilis*

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Abstract

Bacillus subtilis is the model organism for studying Gram-positive bacteria as well as cell biology including sporulation, bacterial chromosome replication, cell development and differentiation. It is also one of the most advanced model organisms for systems biology study and host for synthetic modules. However, genetic engineering of *B. subtilis* is very inefficient and time-consuming, relying on *E. coli*-*B. subtilis* shuttle vectors. In addition, a resistance marker is often created for either gene knockin or knockout. The limitation of available resistance markers and long turnover time significantly impede the research related to *B. subtilis*. In this work, we developed an *E. coli* independent method for marker-less genome editing. Four pieces of DNA for deletion or five pieces for insertion are assembled using Gibson assembly and then used to transform *B. subtilis*. Double crossover homologous recombination followed by linearizing the chromosome at a designed I-SceI site will force the removal of the resistance marker. We have demonstrated this method by deleting the *amyE* gene or integrating a *gfp* expression operon into the *amyE* site. The development of the *E. coli* independent marker-less gene replacement method will greatly facilitate the genome editing in *B. subtilis* and expedite relevant research including cell biology study, strain development, and system/synthetic study.

Protein display on *B. subtilis* spore surface

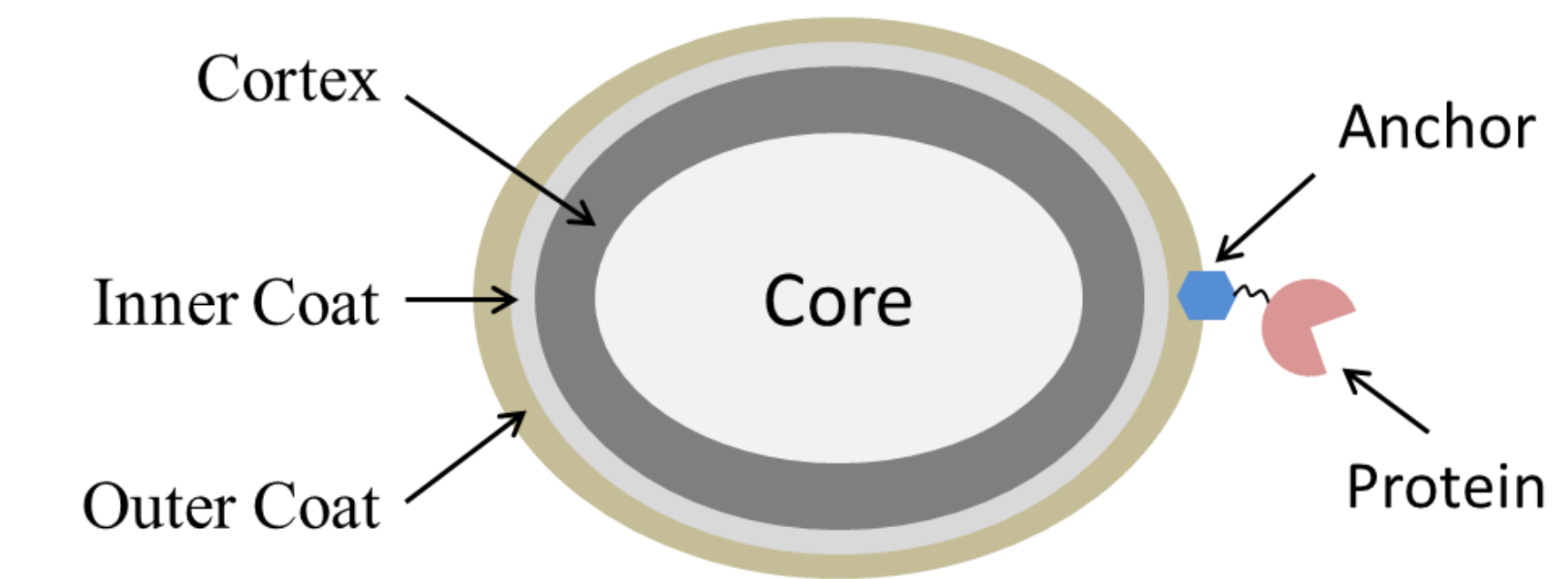


Fig. 1. Sporulating *B. subtilis* and protein display on *B. subtilis* spore surface.

Advantages of displaying proteins on *B. subtilis* spore surface

- GRAS bacterium and safe for food and drug processing.
- Enhanced robustness
- No protein purification is needed.
- Immobilized enzymes allow for multiple-cycle catalysis.

Applications

- Oral Vaccine delivery: (HA2) universal flu vaccine
- Drug delivery: (PAL) treating metabolic diseases
- Bioremediation: (PbrR) heavy metal removal
- Biodegradation: (PETase, MHETase) plastic degradation
- Biosynthesis: *In vitro* metabolic engineering

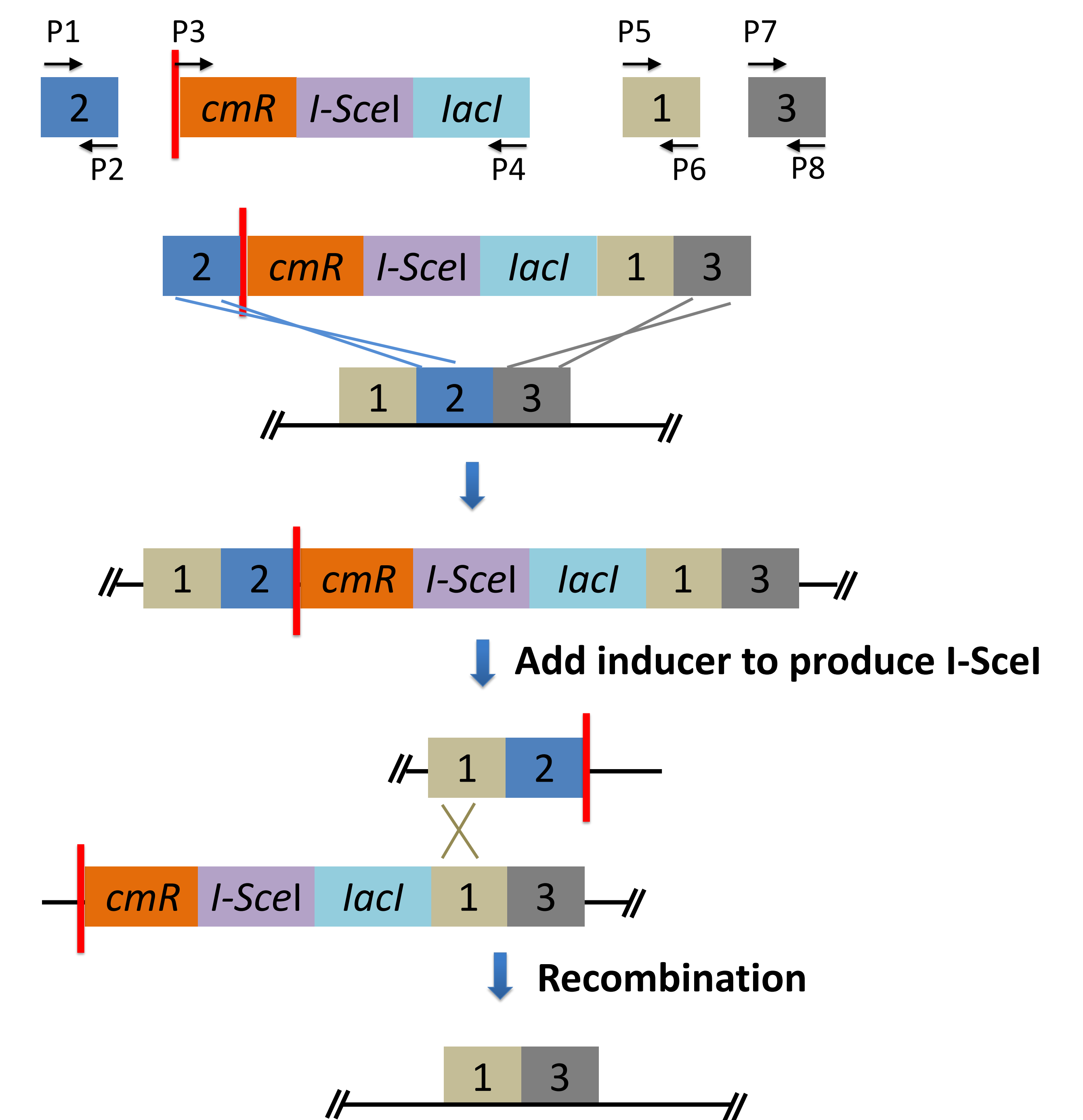
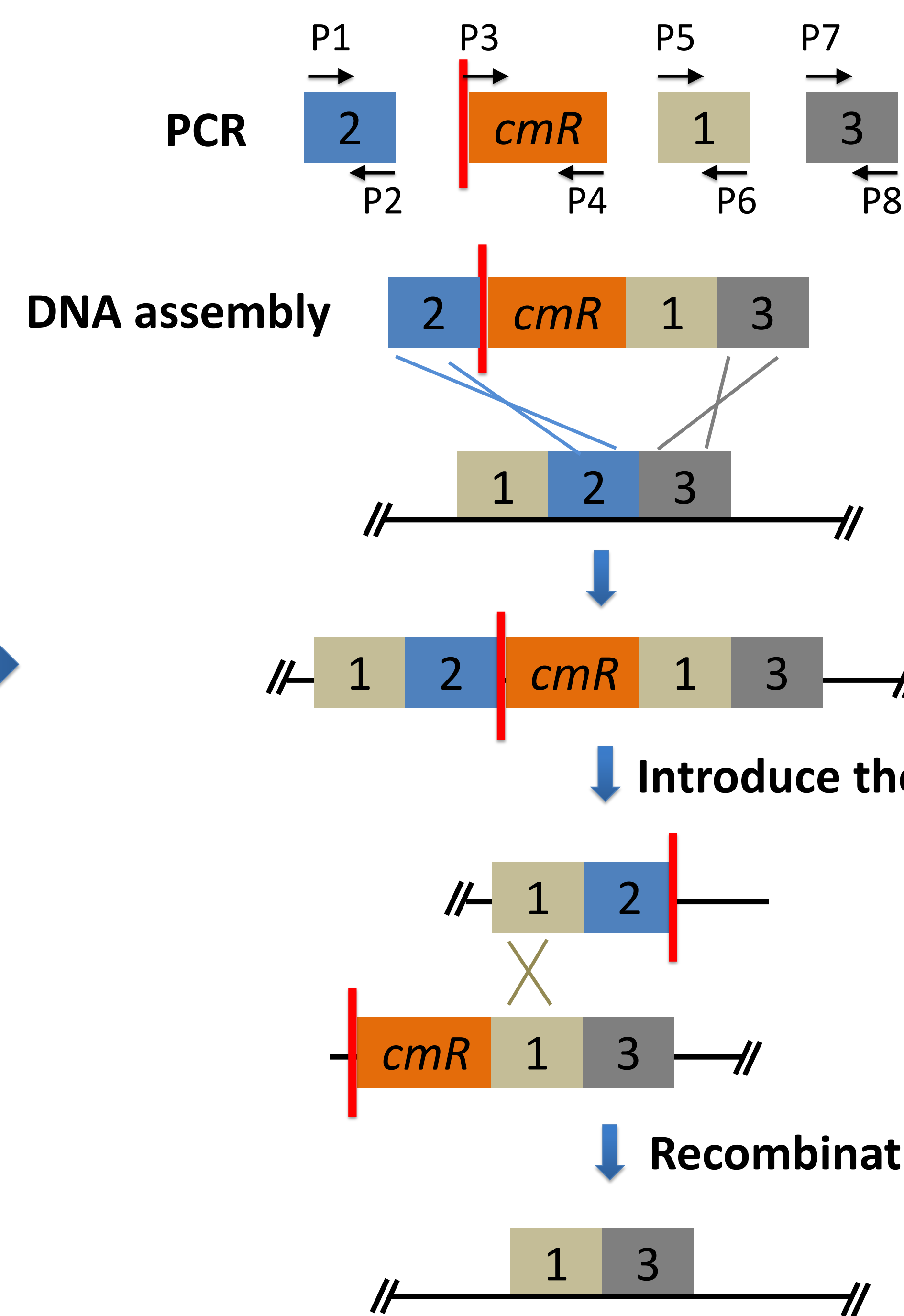
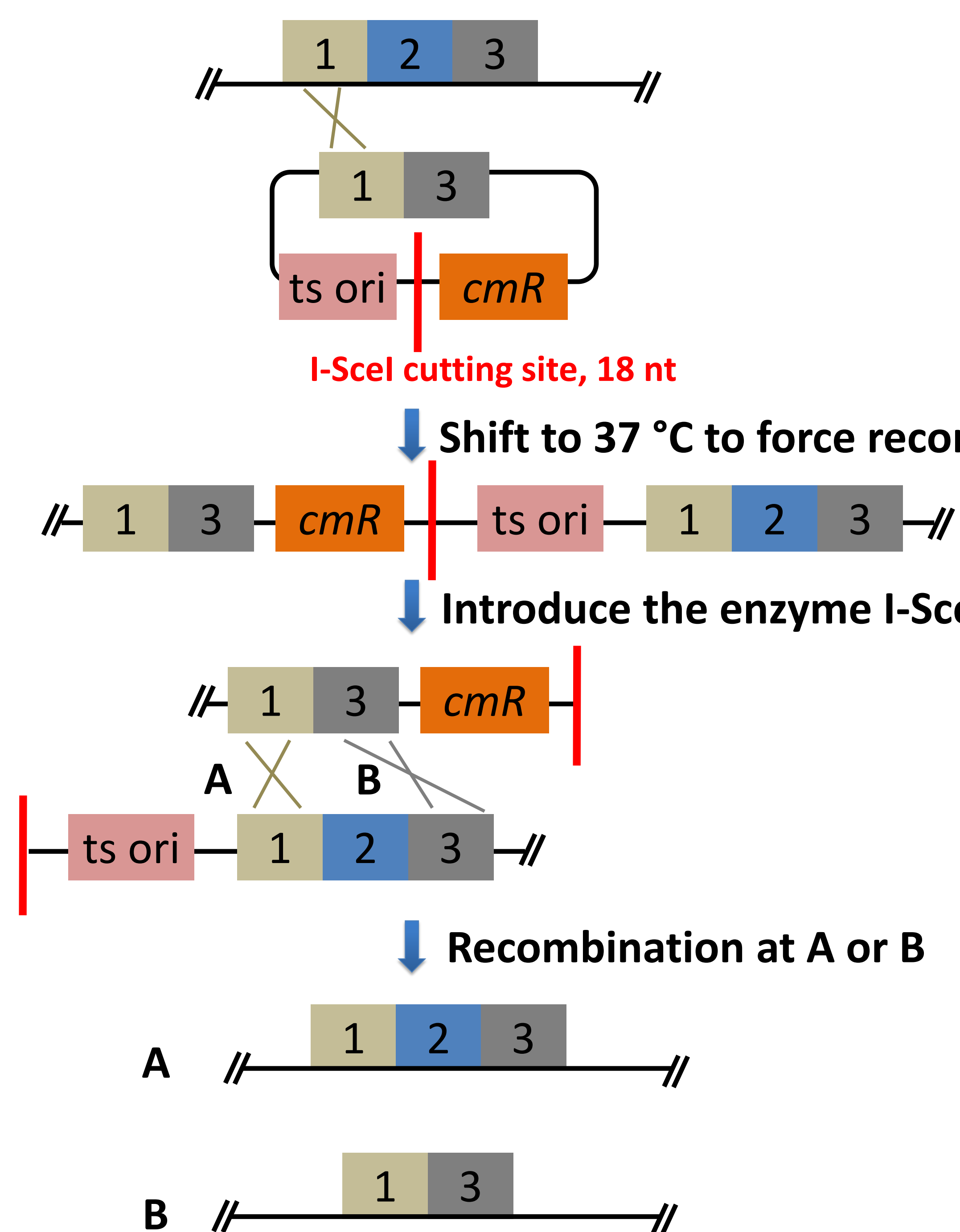
Markerless Gene Knockout:

Design by Janes and Stibitz, 2006



Original design

Improved design



Conclusion

- The success rate increases from 50% to 100%.
- No need to do cloning in *E. coli*.
- Only need one transformation of DNA.

Future Work

- Further increase the efficiency of assembly and transformation.
- Use CRISPR/Cas9 to replace I-SceI