Luciferase-inducible gene expression in E. coli

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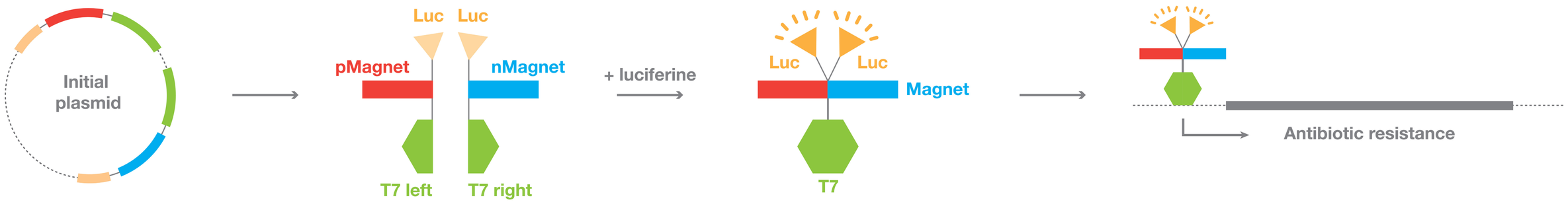
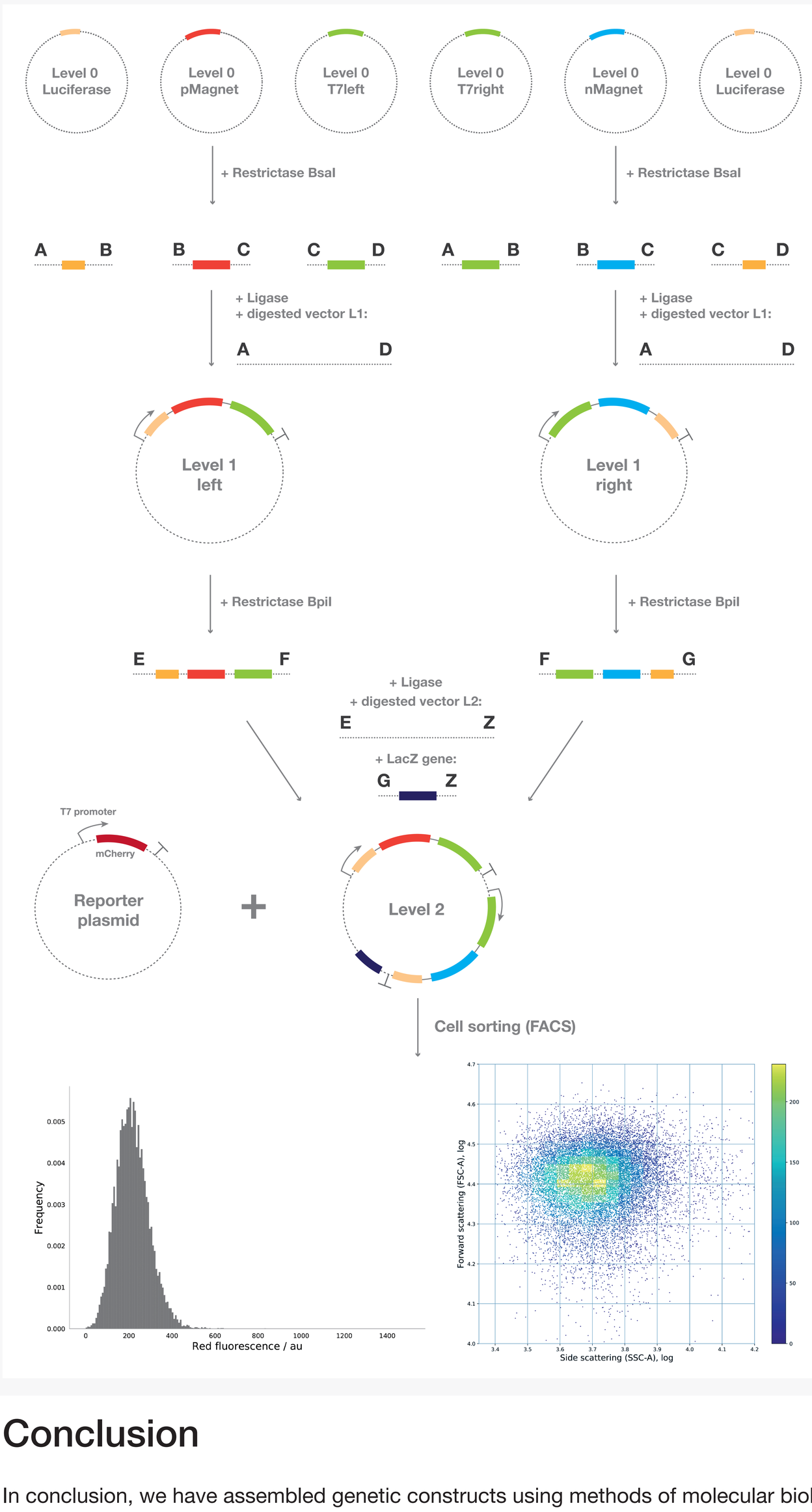
Introduction

Luciferases are widely used as reporter genes in biology, being a base for biosensors, reporter assays and microscopy tags. We designed a genetic construct that allows bacterial cells to sense the intensity of light they emit and to respond with expression of a selectable marker. This technique may help to automatically measure the brightness of different mutants of luciferases and therefore determine the best gene variants to eliminate manual screening. Our approach may assist in further research for natural luciferases.





1. Assembly of combinations of Level-0 genes

We created variants of plasmids with single inserts, Level-0 plasmids that produce compatible overhangs upon digestion. Level-0 plasmids were purified and assembled under an arabinose-inducible promoter to make different variants of left and right pieces of the construct, and transformed into bacteria by heat shock. We checked the length of the constructs by colony PCR.

Results: all 12 possible Level-1 constructs assembled.

2. Assembly of halves into final Level-2 plasmids

12 different Level-1 plasmids were combined to assemble 36 different Level-2 plasmids with a full construct. LacZ was also inserted into Level-2 to differentiate bacteria by colour.

Results: 27 variants out of 36 contained the construct. On the final screening, 9 out of 27 constructs demonstrated an appropriate estimated length.

3. Co-transformation with a reporter plasmid

A reporter plasmid containing mCherry gene under T7 promoter was co-transformed with Level-2 plasmids by electroporation into an applicable bacterial strain.

As an **alternative strategy** (not depicted), we planned to insert GFP gene under T7 promoter into the same plasmid with our construct. To verify the performance of the reporter plasmid we transformed the plasmid into cells constitutively expressing T7 polymerase. However, since no light emission appeared in the bacteria, we rejected this alternative.

4. Fluorescence analysis with cell sorter

We divided cell cultures in 3 groups: group 1 was incubated in darkness, group 2 grew under constant light induction, group 3 grew in darkness in a luciferin-containing medium. We expected group 1 not to emit any light and groups 2 and 3 to produce fluorescence.

Results: FACS revealed no satisfying results: all 3 cell groups did not produce any light, due to inefficiency of the reporter plasmid.

Conclusion

In conclusion, we have assembled genetic constructs using methods of molecular biology and transformed them into an E.coli strain applicable for experiments. In order to test whether our constructs function properly, we analysed fluorescence of bacterial cells by cell sorting, but due to poor performance of the reporter construct we did not get any data. As a future perspective, we are planning to reconstruct the reporter plasmid and repeat our experiment.

* - these authors contributed equally to the work