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CRISPR/Cas9 系統於大腸桿菌進行無痕之基因組編輯:動力學研究

CRISPR/Cas9 nuclease cleavage enables marker-free genome editing in *Escherichia coli* : A sequential study

國立成功大學　化學工程學系　碩士班二年級　高培勛
國立成功大學　化學系　學士班四年級　洪迎馨
指導教授：吳意珣 教授

創意重點

In recent years, CRISPR/Cas9 is an adaptive defense system existed in bacteria. The *E. coli*, most important genetic strain, can be more easily manipulated with its chromosome by the assistance of lambda Red recombinase that relies on the insertion of antibiotic resistance for screening or selection. The aim of this study is to explore the possibility of using CRISPR/Cas9 only for the genome editing in *E. coli*. The results showed that the performance can be controlled by transforming pCRISPR with a dual-spacer and followed up by transforming pCas9 with donor DNA. This sequential strategy can allow marker-free in genomic editing of *E. coli*. Moreover, the efficiency of genomic editing is found over 90% at the optimal conditions, which are using a larger length (i.e. , > 3000 bp) of donor DNA at 500 ng in CRISPR/Cas9 system with lambda Red assistance. Due to the energy crisis, in the future, it not only accelerates the evolution of metabolic regulation and the development of modified strains, but also adds lots of applications for microalgae biomass.

創意成果

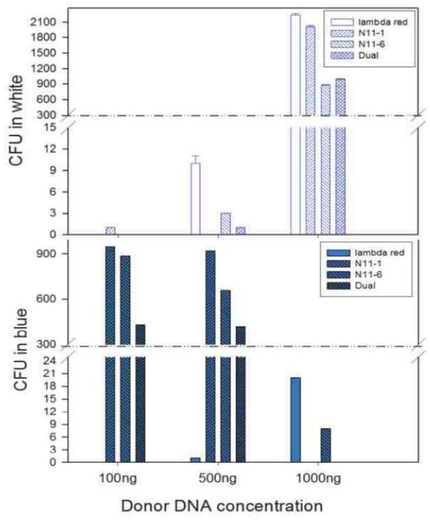
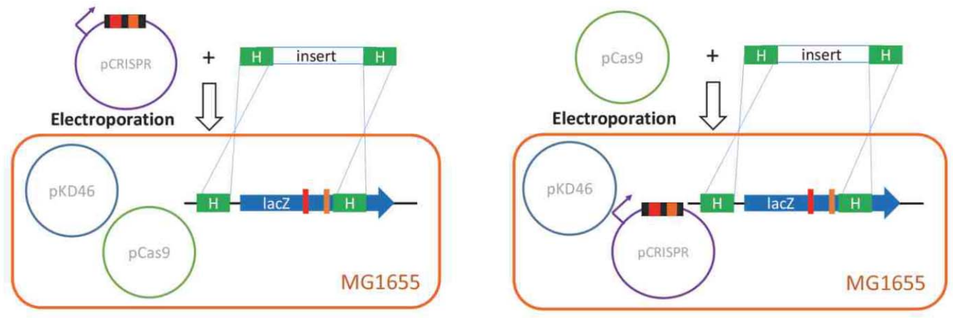
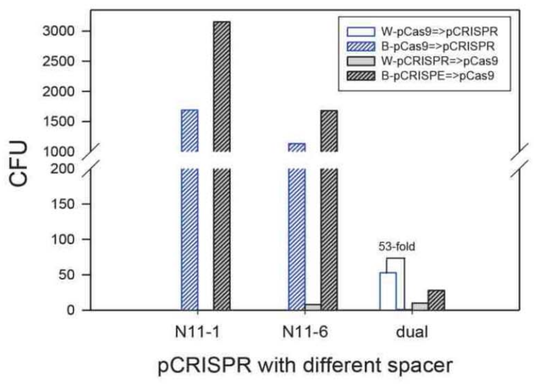


FIG.1 CRISPR/Cas9 coupled with lambda Red recombineering for using three kinds of pCRISPR under selection by antibiotic resistance.

The recombination efficiency decreases when CRISPR/Cas9 introduced into the system. This effect is assumed to be due to the obstruction on pCas9 and pCRISPR, which causes the cell to interrupt the lambda Red when it is rescuing the cell.

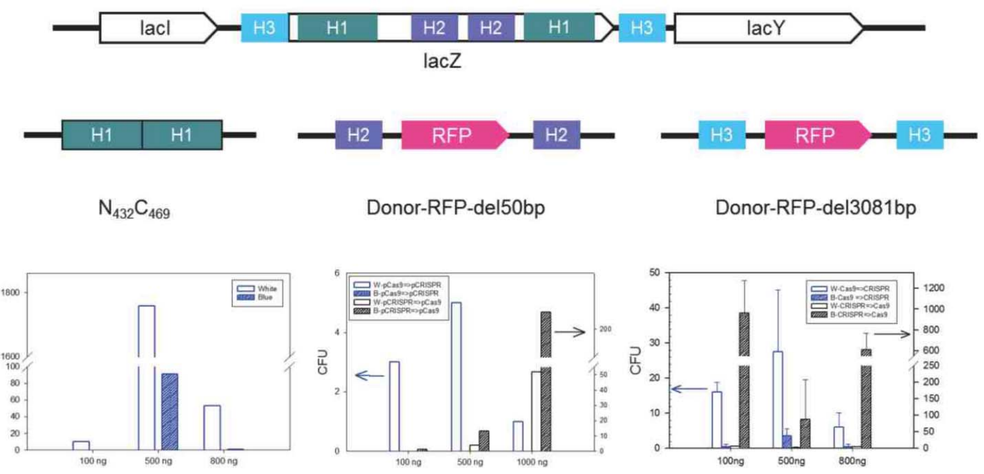
FIG.2 Two approaches of CRISPR/Cas9 system under marker-free recombination





The recombination efficiencies are up to 100% with pCRISPR::lacZ-dual and followed by using pCas9, while 26% is obtained with the reverse transforming order of both vectors under marker-free recombination.

FIG.3 Different concentrations of marker-free donor DNA used in the dual spacer CRISPR/Cas9 recombination system.



The great improvement in pCRISPR transformation followed by using pCas9 has thus been confirmed once again. Besides, the optimal donor concentration is 500 ng, which is much less than that with only lambda Red recombination. Remarkably, the recombination efficiencies are 92% for $N_{432}C_{469}$, 100% for Donor-RFP-del50bp, and 88% for Donor-RFP-del3081bp at 500 ng of donor DNA.

創意心得

首先感謝中技社評委的肯定，讓我們的努力能夠被看見，也感謝指導老師吳意珣教授的指導與栽培。全心投入並發揮創意而獲得一般人無法獲致的成果，可能是這次得獎者的共同經驗，堅持完美與追求卓越，絕對是所有學術研究者的共同目標，掌握最新的世界趨勢，希望有朝一日能為地球環境盡一份心力，為提升人類福祉做出努力。

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