# Development of a Rapid Method for Genome Engineering in a Quest Towards Customized Protein Expression Strains

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## INTRODUCTION

The ability to produce high levels of recombinant protein has become one of the cornerstones of biological sciences. Purified proteins allow us to obtain information about their specificities and even structures. To date, many proteins have been expressed and purified from engineered host cells, such as E. coli, due to the relative simplicity of the process. Unfortunately, many other proteins of interest are not readily expressed or expressed as insoluble aggregates in bacterial hosts. Many advances have been made over the past several decades to understand and improve folding and solubility of recombinant proteins within their new hosts, however, there are still many enzymes that do not fold well.

Each protein is unique and we believe that creating customized expression strains for each difficult protein is a strategy that is currently under-utilized, in part because of the time and effort required for extensive genome modification in E. coli. Here, we present the development of a novel, rapid method for E. coli genome engineering, allowing us to make markerless genome modifications in 48 hours. As an example of such modification, we restored wild-type lon allele in a B strain and demonstrate its beneficial effect on production of a membrane protein.

## METHODS

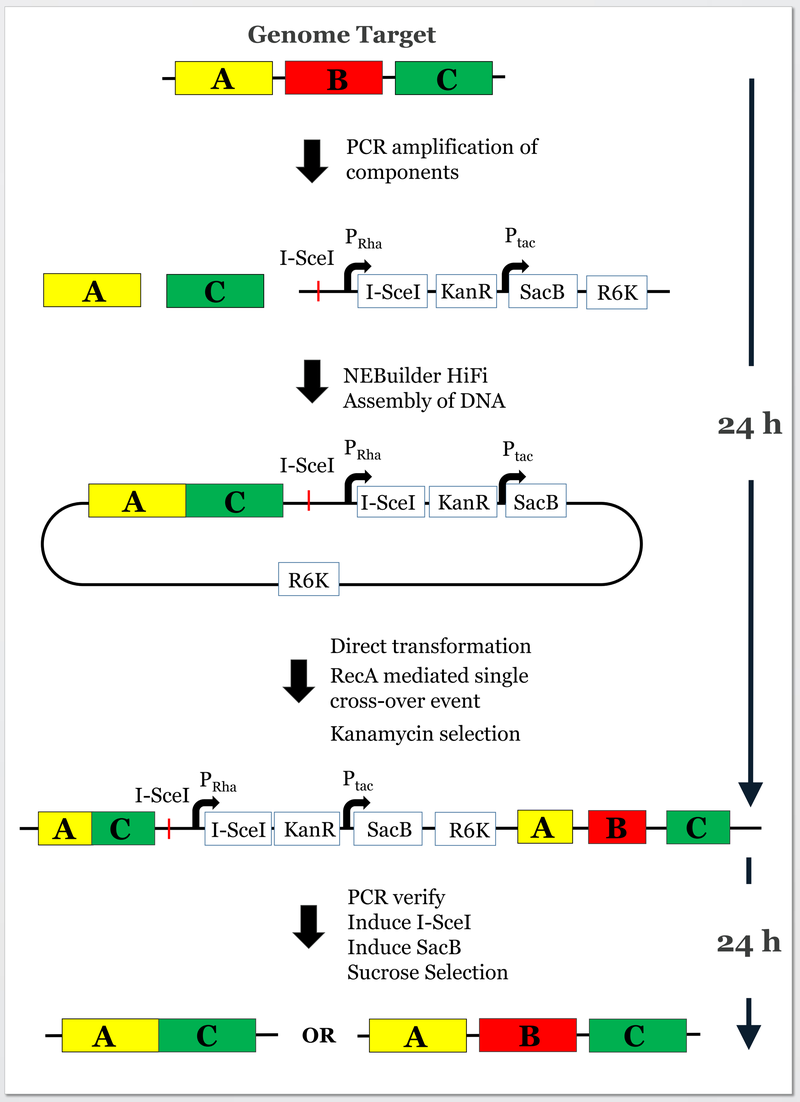


***Figure 1:*** Overview of the rapid genome modification method

## MODIFICATION EFFICIENCY

| Locus | Integration efficiency | Resolution efficiency | Desired change |
|---|---|---|---|
| lacZ E462A | 7/8 | 31/32 | 1/31 |
| Δlac | 8/8 | 22/32 | 7/22 |
| lon | 4/8 | 15/16 | 6/15 |
| rplI-Dasher | 8/8 | 13/32 | 0/13* |

* Resolution of all integrants to WT strongly suggests that rplI-Dasher fusion is lethal to the cells

## SEC PATHWAY JAMMING

We believe that one of the reasons for the toxicity of membrane proteins is the jamming of the translocation machinery. Specifically, high level expression of foreign membrane proteins clogs up the co-translational translocation machinery of the secYEG complex, excluding the translocation of native proteins destined for the inner membrane or the periplasm. Lon protease is known to play a role in easing some of the jamming, however, it is not expressed in the majority of B strains (1).

To confirm the theory that presence of Lon protease can be beneficial for the expression of recombinant membrane proteins, we utilized the newly developed genome engineering method to restore the intergenic region from K12 strains in a B strain, thus eliminating the repression of lon by the IS element. We then tested the new strain for expression of a reporter membrane protein.

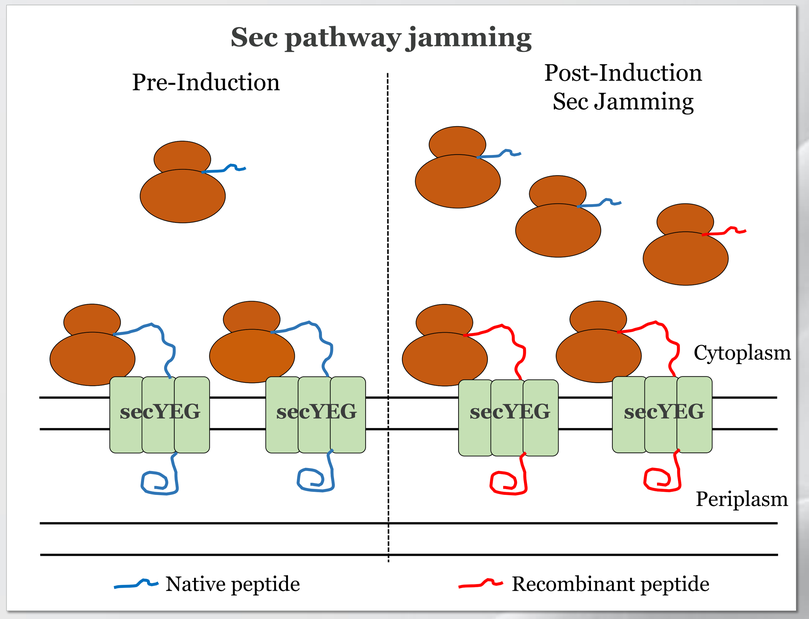


***Figure 2:*** Illustration of jamming by recombinant proteins (red) during co-translational translocation excluding native peptides (blue) from the translocation machinery.

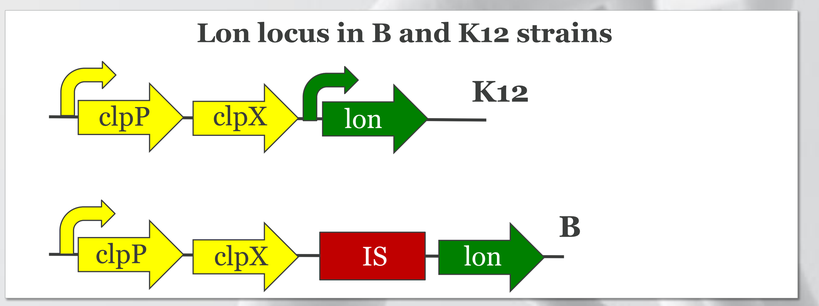


***Figure 3:*** Genomic context of lon in K12 and B strains. Red block is the IS element which disrupts the native lon promoter.

## EFFECT OF LON

Testing effect of Lon on production of a recombinant membrane protein using an Oxa-PhoA reporter and measuring alkaline phosphatase activity

Assays were done 3h post induction at 37°C

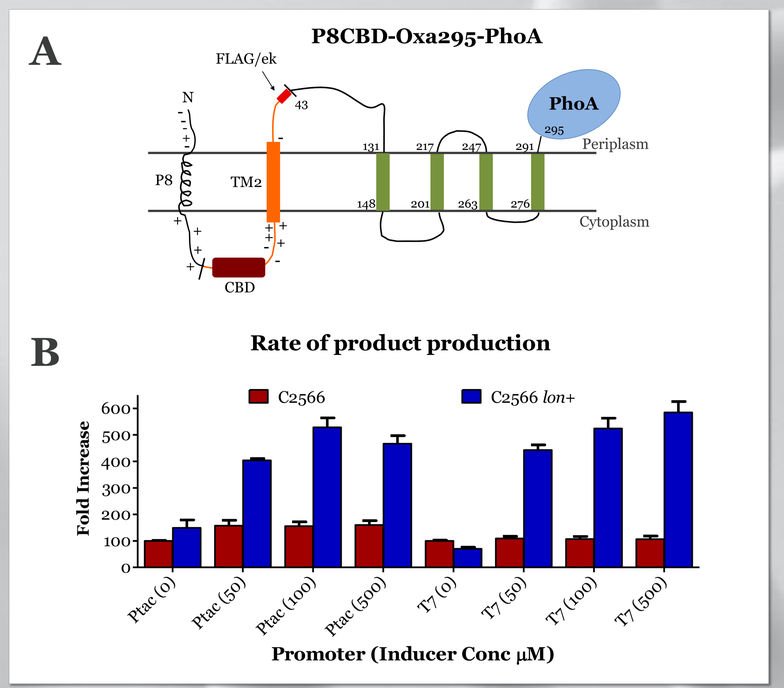


***Figure 4:*** **(A)** Graphic showing the makeup of the test membrane protein-phoA fusion. **(B)** Rate of product production in a PhoA assay. The test protein was expressed from either a $P_{tac}$ or $P_{T7}$ promoter, with various concentrations of inducers in a B or a B *lon*$^+$ strain.

## CONCLUSIONS

We present an effective, rapid method for genome engineering

Our method is highly effective in quickly determining permissive modifications of essential genes

We demonstrate the positive effect of the presence of Lon protease on production of test membrane proteins

This is the first step towards being able to design and make *E. coli* strains customized for the project at hand

## REFERENCES

Constructs containing the P8CBD-oxa295-phoA fusion were made by Dr. Carine Robichon

1) Snyder, W.B. and Silhavy, T.J. (1992) Enhanced export of beta-galactosidase fusion proteins in prlF mutants is Lon dependent. *J. Bacteriol.*, 174, 5661–8