Construction of a Ligation Independent Cloning Based Platform for High Throughput Cloning and Expression Screening of Membrane Proteins from *Mycobacterium tuberculosis*

H. Qin, J. Hu, M. Sharma, Y. Hua, S.V. Challa, T.A. Cross, F.P. Gao (NHMFL, FSU, Chemistry & Biochemistry, Institute of Biophysics)

Introduction

One of the major challenges for membrane protein structural genomics is establishing high-throughput cloning and expression screening methods to obtain enough purified protein in a homogeneous preparation for structural and functional studies. A series of ligation independent cloning based vectors were constructed and the feasibility of this platform tested with 41 putative membrane proteins from *Mycobacterium tuberculosis*.

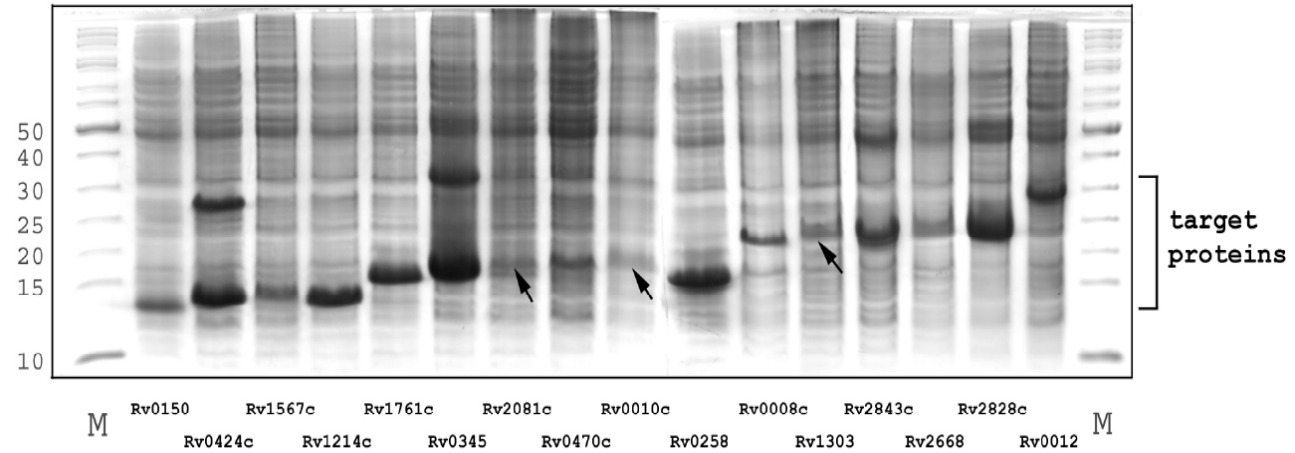

Fig. 1. Overexpression of 16 putative membrane proteins in the first round of expression screening. After induction at 37°C for 4 hours, cells were harvested and lysed by sonication. The whole cell samples were subjected to SDS-PAGE. The gels were stained by Coomassie blue and the expressions were confirmed by western blot using an antibody against the His-tag. The arrows indicate the expressed proteins for lanes where it is not obvious.

Experimental

Ligation Independent cloning has been established for water soluble proteins, but this efficient strategy has not yet been adapted for membrane proteins where expression levels have to be optimized and often there is an effort to shift thee expression from one cell fraction to another.

Results and Discussion

The efficiency for direct cloning of these target genes from PCR products was very high (95%; 39/41). Over 40% of cloned genes were overexpressed in *Escherichia coli* BL21 (DE3)-RP codon plus strain in the first round of expression screening. For those proteins which showed no expression, three protein fusion partners using the same efficient cloning platform were prepared and it was found that each of the target proteins could be overexpressed by at least one of these fusions, resulting in the overexpression of two thirds of the cloned genes. Considering that there were still a number of proteins for which expression was low in the first round, but were not worked on in the second round there is still the opportunity to increase this percentage of overexpression even higher.

Conclusions

This expression platform features high throughput cloning, high flexibility for different constructs, and high efficiency for membrane protein overexpression. The high efficiencies reported here are unique in the membrane protein arena and bode well for membrane protein structural biology efforts.

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References: This work has been submitted for publication