Power is nothing without control: Efficient recombinant protein production in E.coli using Staby™Express.

Delphi Genetics Inc.
Sharpened Tools for Lifescience Discoveries

Technical Note

Technical notes provide unique applications, innovative methods, and clear protocols designed specifically for Delphi Genetics products.

INTRODUCTION

Plasmid instability is a significant concern in recombinant protein production. Typically protein-production processes in prokaryotes require the use of bacterial plasmids as vectors carrying the gene of interest to be over-expressed. It has been demonstrated that the growth of plasmid bearing cells is significantly reduced relative to plasmid free hosts, simply because protein production (corresponding to the gene of interest overproduction) represents a significant burden on cellular metabolism. Antibiotic resistance genes are the most common selectable markers used in fermentation processes to avoid plasmid free cells to overgrow the culture. However this technique has several severe drawbacks especially when the antibiotic ampicllin is used. The new stabilization system developed by Delphi Genetics is based on the use of antidote/poison (the ccd operon in this case) genes naturally found in plasmids bacterial chromosomes and bacteriophages. The ccdB poison gene codes for a small stable protein whereas the ccdA antidote gene codes for a small unstable protein that neutralizes the poison protein both transcriptionaly and via protein-protein interactions.

The StabyExpress™ technology has been extensively tested and validated in the Laboratories of Experimental Allergology (Dr A. Jacquet) and Molecular Virology (Prof C. Van Lint) at the Institute of Molecular Biology and Medicine of the Université Libre de Bruxelles. In these laboratories, StabyExpress has consistently outperformed protein expression kits from other leading suppliers.

During a particular project the overexpression of HIV-1 Reverse Transcriptase was attempted. In order to assess new inhibitors of this enzyme, the researchers had to produce two different forms of the recombinant enzyme, the complete form (P66) and a truncated version (P51). A bacterial system was considered to be the best choice for producing this protein.

PROTOCOL:

The experiments using the products from the Novagen range have been realized following the manufacturers instructions.

For the StabyExpress system:

- The gene of interest was cloned in the expression plasmid pStaby1 using restriction digestion (Nde1 and Not1) and ligation.
- The recombinant plasmid was transformed in the cloning host CYS21 and the desired construction was selected.
- Positive recombinant plasmids were transformed in the expression host SE1.
- Cells were grown to an OD_{600} of 0.8.

- Expression was induced by adding IPTG (1mM) during 3 hours at 37°C.
- Total cellular proteic extracts were obtained.
- The expression of the desired protein was assessed using SDS-PAGE electrophoresis followed by staining with Coomasie blue.
- After Western-Blotting, the presence of the recombinant proteins were assessed using an anti-polyHis antibody.

RESULTS

In a first attempt, the genes of interest have been cloned into a pET15b vector from Novagen. The expression plasmid has been integrated in several distinct expression hosts including BL21, Origami B and Rosetta-Gami B. Different expression temperatures (37°C and 30°C) IPTG concentrations (0.1, 0,5 and 1mM) and induction times (2, 4 and 20 hours) have been tested. Unfortunately none of the aforementioned conditions provided satisfying results as no trace of the recombinant protein could be detected (Fig1 and 2).

Based upon these disappointing results, the research team decided to comparatively evaluate the StabyExpress system from Delphi Genetics, (Fig 1 and 2). Figure 1 illustrates the results obtained for the complete and the truncated form of the recombinant protein (P60 and P51 resp.) on SDS-Page, Figure 2 is a Western blot of the aforementioned gel.

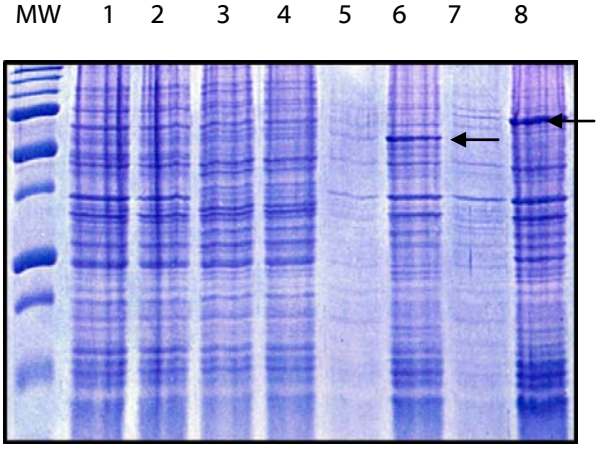

Figure 1

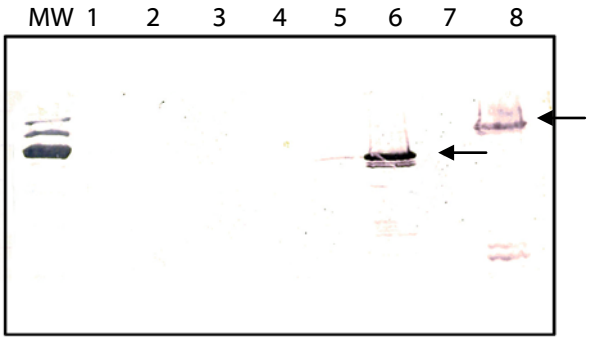

Figure 2

Lane1: P51 Origami, Lane 2 P66 Origami, Lane3: P51 Rosetta-Gami, Lane 4: P66 Rosetta Gami, Lane 5 : P51, StabyExpress Supernatant, Lane 6: P51 StabyExpress Cellular Extract, Lane 7 : P66 StabyExpress Supernatant, Lane 8 : P66 StabyExpress Cellular Extract.

These results clearly indicate that the Staby™Express system is better suited to express both forms of the HIV-RT protein than the other kits tested by the Laboratory of Experimental Allergology. A visible band is obtained when using the Staby™Express kit whereas no expression is detected using Novagen's pET system whichever expression host is being used. This indicates that the most likely reason why this gene fails to express in conventional systems is the lack of stability of the expression plasmid. The pET15b plasmid is selected using ampicillin and it is more than likely that the lack of expression is due to the depletion of the vast majority of plasmid bearing cells at the time of induction. This is a common pitfall using ampicillin-based systems (see below). Another explanation might be that the recombinant protein is somewhat toxic to the host, which will induce a heightened pressure for "loosing the plasmid".

It is noticeable that a unique band is visible after purification of both forms of the HIV-RT protein indicating the high degree of purity of the overexpressed protein. Likewise, the overall background is reduced even in the supernatant indicating that the protein of interest clearly represents a major band of the total cellular proteins. The combination of the overexpressing power of the T7 system with the Staby™ technology thus allows the efficient and reliable expression of recombinant proteins in E. coli with high yields and a high degree of purity.

In fact the stabilisation technology of the StabyExpress system allows for the efficient overexpression of recombinant proteins, which have been impossible to produce before. Another example is shown hereafter (Figure 3):

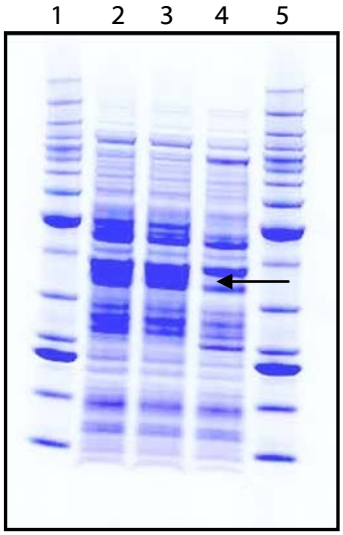

Figure 3

These experiments have been carried out by a contract manufacturer (CMO). The overexpression of a 22.5 kD human protein was attempted. The fermentation was performed in 50 liters. Using StabyExpress, the plasmid was perfectly stabilized before and after the induction period; 100% of the induced bacteria harboured the expression plasmid. The expression was visible on gel. After purification, the yield of protein of interest was estimated to 600mg/L. On the contrary, using a conventional system, the protein is not visible using SDS-PAGE analysis and the yield after purification remained very low (even after several trials). Consequently, the production of the protein is higher (3 to 5 times) with StabyExpress (lanes 2 and 3) than with a conventional system (lane 4). Lanes 1 and 5 are molecular weight markers.

Generally speaking, overall yields are consistently higher when using the Staby™ technology for plasmid stabilization. The system ensures that only plasmid-bearing clones can grow whereas every clone losing the plasmid will inevitably die. This unique feature guarantees the highest possible yields from a given amount of bacteria. Note that the Staby™ system can be used in any culture medium.

ABOUT Staby™Express.

The stabilization system is based on the use of bacterial antidote/poison genes naturally found in the F plasmid of E. coli (ccdA and ccdB). In the StabyExpress system, the antidote gene ccdA is introduced in the plasmid DNA under the control of a constitutive promoter. The bacterial toxin gene ccdB is introduced in the chromosome of the cloning or expression bacteria (CYS21 and SE1 respectively) (See figure 4).

Expression of the poison gene is under the control of a strongly repressed promoter in the presence of the plasmid. When the plasmid is lost, the antidote protein is degraded and the production of the toxin is induced causing cell death. This unique system allows for the perfect stabilization of the plasmid without the use of antibiotics. Furthermore this system guarantees that during protein expression every bacterium is carrying the expression plasmid thus enhancing the overall yield of any given protein expression experiment. If some bacteria lose the vector, they will not obtain a selective (growth speed) advantage but will die. In practice, this additional stabilization technology solves the problem of plasmid instability and insures that upon induction 100% of the bacteria will produce the recombinant protein leading to higher yields of the target protein and less background caused by unwanted proteins (proteins from bacteria lacking the plasmid). Thus, the production of the protein of interest is higher and purer. This is a tremendous improvement over conventional expression systems in E. coli.

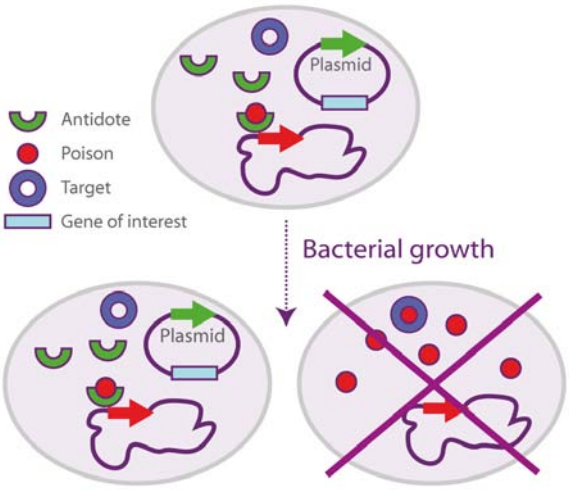

Figure 4

For manufacturers of recombinant proteins this system offers a great benefit because it is an antibiotic free expression system. Therefore the manufactured protein will also be free of traces of antibiotics and antibiotic resistance genes, which can be important from a regulatory point of view (cf. FDA recommendations)

BENEFITS OF THE Staby™Express SYSTEM

- Higher yields of heterologous protein expression, even with otherwise toxic proteins.
- Reduced background of non-target proteins.
- The plasmid is perfectly stabilized
- No need to transform the plasmid before each expression
- No need to use antibiotics
- System can be used in any culture medium.

KIT COMPONENTS

- pStaby 1.2 vector DNA
- CYS21 bacteria (for cloning)
- SE1 bacteria (for expression)
- Regeneration medium
- Fw and Rv sequencing primer
- Expression control

The Staby™ Range of Products is available with either electro or chemically – competent cells.

ORDER REFERENCES

- SET7-0505: electro-competent cells, 5 reactions
- SET7-0707: chemically-competent cells, 5 reactions
- SET7-1010: electro-competent cells, 10 reactions
- SET7-1212: chemically-competent cells, 10 reactions
- SET7-0020: additional bacteria for expression (SE1), electrocompetent cells, 20 reactions
- SET7-0022: additional bacteria for expression (SE1), chemically-competent cells, 20 reactions

RELATED PRODUCTS

Please visit www.delphigenetics.com to obtain information about our newest products

LEGAL

ACKNOWLEDGMENTS

The following scientists: M. El Bakkoury, S. De Walque, A. Jacquet and Prof. C. Van Lint of, the IBMM, Université Libre de Bruxelles, have contributed valuable data to this technical Note.

Delphi Genetics SA
Rue C. Ader, 16
B-6041 Charleroi
Belgium
Tel: +32.71.37.85.25
Fax: +32.71.37.60.57
www.delphigenetics.com
delphigenetics@delphigenetics.com
order@delphigenetics.com