Efficient production, detection and solubilisation of recombinant proteins using Cherry™codon kit

Delphi Genetics SA.

Sharpened Tools for Lifescience Discoveries

Technical Note

Technical notes provide customers with innovative applications and clear protocols specifically designed for Delphi Genetics products.

INTRODUCTION

This document is based on the following web-page written by Dr. Ayako Uchiyama from Ochanomizu University, Tokyo, Japan (http://www.researchtool.jp/modules/tinyd0/index.php?id=15). Protein production using E. coli is a useful technique to produce large quantities at a low cost. However, when the number of samples increases, it is difficult to manage IPTG induction (involving OD measurements), protein solubility check and protein solubilisation if necessary. Several expression systems exist but the Cherry™Express and Cherry™Codon kits (products made by Delphi Genetics and distributed by Cosmo Bio in Japan) have a unique property: it is possible to see by eyes protein expression and to evaluate protein solubility. To see the protein, the Cherry™ tag is fused to the protein of interest and bacteria expressing the fusion protein are red when this protein is soluble. The Cherry codon kit combines several technologies: T7 expression, plasmid stabilization, efficient supply of rare tRNAs and protein visualization using the Cherry tag. Dr Ayako Uchiyama evaluated the usability of this kit for two proteins: Lipocortin and Rluc proteins. Lipocortin is a calcium- and phospholipid-binding protein from the annexin class (protein mostly associated to cell membrane). This protein is involved in anti-inflammatory response. Rluc protein is the *Renilla* luciferase frequently used as a fluorescent marker. Induction was done automatically for both proteins using **Staby™Switch** auto-inducible medium.

PROTOCOL

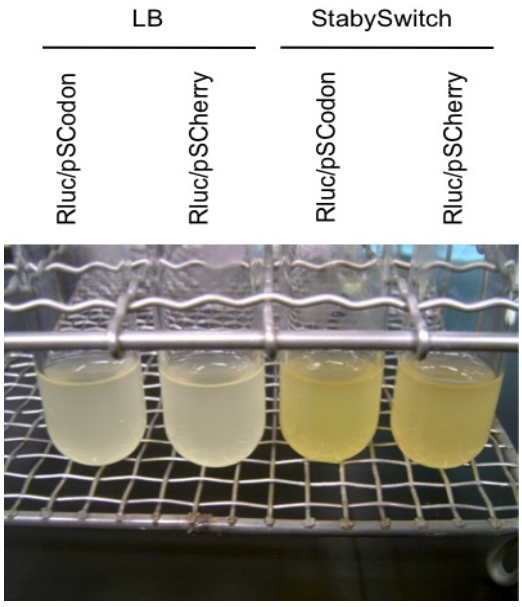
1) The genes of interest were amplified by PCR. The primers contained restriction sites for gene cloning into pSCherry2 and pSCodon1.2 vectors. The pSCodon1.2 is a control vector included in the kit. This vector does not encode the Cherry tag. PCR fragments were restricted and cloned into the vectors. Ligation mixes were transformed in CYS21 cloning bacteria (competent bacteria included in the kit). The bacteria were spread on LB plates and incubated at 37°C for one night.

2) Recombinant clones were screened by PCR. Single colonies of bacteria containing vector with insert were grown in few millilitres of LB medium at 37°C for plasmid purification. Plasmids with insert were purified and transformed in SE1 expression bacteria (competent bacteria included in the kit). Bacteria were spread on LB plates and incubated at 37°C overnight.

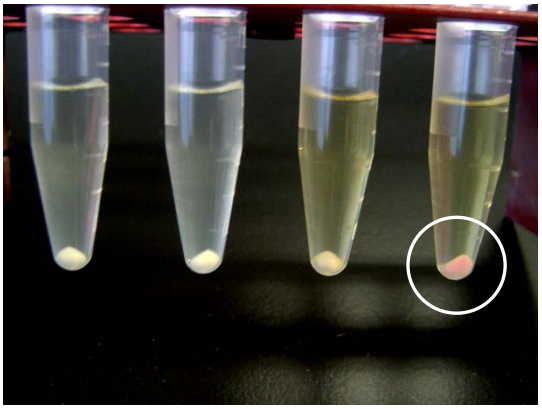
3) Single colonies of SE1 bacteria were picked on plates and cultured in few millilitres of LB medium. 1ml was used for glycerol stock.

4) 30µl of each culture were inoculated in 3ml of Staby™Switch medium (an auto-inducible medium developed by Delphi Genetics). As uninduced control, 30µl were also inoculated in 3ml of LB medium containing 1% glucose. Tubes were incubated in a water bath at 37°C, 160 rpm, and overnight.

5) After incubation, culture turbidity was measured: OD_{600}= 3.2 to 3.4 in LB medium and 3.9 to 4.3 in Staby™Switch medium.

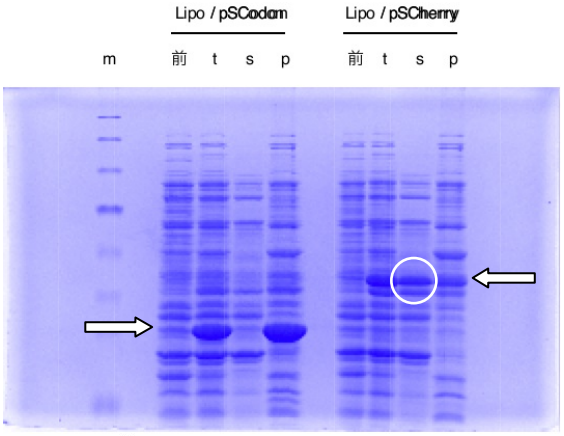


6) Bacteria were centrifuged; a red pellet was visible only with samples from bacteria expressing the target protein fused to the Cherry™ tag.



7) Supernatant was discarded and total proteins were extracted using BugBuster protein extraction reagent (Novagen). Bacterial DNA was digested using Benzonase nuclease (Novagen). After centrifugation, soluble proteins were present in supernatant (sup) and the pellet contained insoluble proteins (ppt). Red colour was visible in supernatant (soluble fraction) for the protein expressed with Cherry™Codon. All samples and fractions (total extract, sup and ppt) were analyzed on SDS-PAGE. After electrophoresis, proteins were detected using Coomassie blue staining.

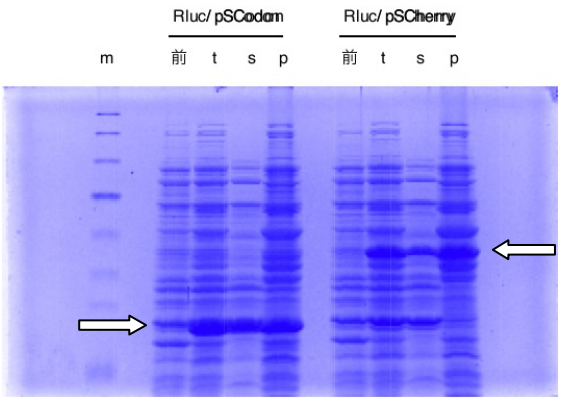
8) a) Expression of Lipocortin:



(m: marker, 前 uninduced control, t : total extract, s : supernatant, p : pellet)

The lipocortin protein is expressed using both systems (pSCodon or pSCherry2) but the protein is **soluble** only when fused to the Cherry™ tag: half amount of the fused protein is soluble while lipocortin is completely insoluble without Cherry™ tag.

b) Expression of Rluc



Rluc protein is well expressed using pSCodon and pSCherry vectors. Since the protein is partially soluble and co-migrates with an E. coli protein when using pSCodon, it is difficult to evaluate the solubilisation due to the use of pSCherry. However, it was possible to see and to track protein expression in E. coli using the Cherry™ tag.

BENEFITS OF THE Cherry™Codon KIT FOR EXPRESSION OF RECOMBINANT PROTEINS IN *E. coli*.

- **Protein solubility:** The pSCherry vectors encode the Cherry™ tag (heme binding part of cytochrome, 11kDa) which is fused to the protein of interest. This tag is visible only when the protein is soluble. The tag itself being highly soluble, it can increase the solubility of the target protein. As observed here with Lipocortin, the protein was completely insoluble when expressed as a non-tagged protein and about the half amount of the protein is soluble when fused to the Cherry™ tag.

- **Visibility and tracking of protein expression:** Expression of both proteins (Lipocortin and Rluc) was visible in E. coli: the bacterial pellet was coloured only for bacteria encoding the Cherry™ tag fused to the target protein and when expression was induced. The intensity of the red colour is **directly proportional** to the expression level and solubility of the protein. No special reagent or apparatus is necessary to see the Cherry™ protein. Using Cherry™, it is easy to screen different conditions for protein expression and solubility.

- **Easy protein quantification:** When using the Cherry™ tag, it is possible to quantify the protein concentration at any step from protein production (total protein extract) to the end of purification (protein binding on column, elution): a simple absorbance measurement at 413nm allows specific and accurate calculation of the target protein concentration.

- **Compatibility with Staby™Switch auto-inducible medium:** the pSCherry vectors express proteins under the control of the T7 promoter. As shown here for Lipocortin and Rluc proteins, induction was performed automatically using Staby™Switch auto-inducible medium. As observed by Dr. Ayako Uchiyama, turbidity of the cultures was always higher using Staby™Switch compare to LB medium. Moreover, it is neither necessary to add IPTG (isopropyl-β-D-thiogalactoside) nor to monitor optical density during bacterial growth. Here, the researcher used pre-culture as a starter but picking a single colony or a few micro-litres of glycerol stock can be used for culture and expression.

- **Efficient supply of rare t-RNAs:** when a heterologous gene is expressed in *E. coli*, this gene might exhibit some codons that are common in the original host but are rarely used in *E. coli*. The presence of clusters of and/or numerous rare codons generates a demand for one or more rare tRNAs leading to very low expression. The pSCherry2 vector encodes the tRNA genes of the six rare codons in *E. coli* allowing high expression of genes containing rare codons.

- **Antibiotic-free expression system:** The pSCherry vectors contain the Staby™ technology for plasmid stabilization even without antibiotics. Using standard expression systems, antibiotics are necessary to select for bacteria containing the plasmid encoding the target protein and even in the presence of antibiotics, some bacteria without plasmid can appear. These bacteria without plasmid are able to grow faster than bacteria containing the plasmid but do not produce the protein of interest. As observed here for Lipocortin and RLuc proteins, using Staby™ technology, it is not necessary to add

antibiotics for high level of protein expression. For more information about the Staby™ technology, please, visit our website www.delphigenetics.com.

KIT COMPONENTS

- pSCherry2 DNA (expression vector)
- pSCodon1.2 DNA (expression vector)
- CYS21 competent bacteria (for cloning)
- SE1 competent bacteria (for expression)
- Regeneration medium
- Forward and Reverse sequencing primers
- Cherry™ booster
- Manual

Cherry™ kits are available with either electro- or chemically- competent cells.

ORDER REFERENCES

- CCT7-05: electro-competent cells, 5 reactions
- CCT7-07: chemically-competent cells, 5 reactions
- CCT7-10: electro-competent cells, 10 reactions
- CCT7-12: chemically-competent cells, 10 reactions

RELATED PRODUCTS

- AIME-04: Staby™Switch auto-inducible medium (2 X 1L, liquid, sterile, ready-to-use)

- CET7-05: Cherry™Express kit, electro-competent, 5 reactions
- CET7-07: Cherry™Express kit, chemically-competent, 5 reactions
- CET7-10: Cherry™Express kit, electro-competent, 10 reactions
- CET7-12: Cherry™Express kit, chemically-competent, 10 reactions

LEGAL

ACKNOWLEDGEMENTS

This application note is based on the results of Ayako Uchiyama from Ochanomizu University, Tokyo, Japan. The results were first published on the web (http://www.researchtool.jp/modules/tinyd0/index.php?id=15). We thank Cosmo Bio, our distributor in Japan.

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