Abstract

- The success rate for protein expression was as high as 77%.
- Most proteins were expressed easily and economically without IPTG induction in *E. coli*.
- The B834(DE3)+pRARE constructed by us generally improved expression level of selenomethionine substituted proteins.

Plasmid — Current Status

Total ORF number	**2236**
Plasmids for overexpression	**2059**

The vector we selected was **pET11a**.

- Overexpressing the protein without tag
- Regulable for induction with IPTG

Expression — Success Rate

	expression/total	success rate	soluble
·BL21(DE3) without IPTG	867/1227	70.7%	(53.9%)
↓ add IPTG			
·BL21(DE3) with IPTG	8/1227	+0.7%	(+0.2%)
↓ tRNAs for rare codons			
·Rosetta(DE3)	61/1227	+5.0%	(+3.2%)
↓ change host strain add			
·HMS174(DE3)	7/1227	+0.6%	(+0.2%)
↓ repress basal expression			
·BL21(DE3) + glucose	3/1227	+0.2%	(+0.2%)

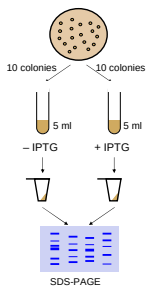
Total success rate was 77% (soluble 58%).

Procedure — Expression test

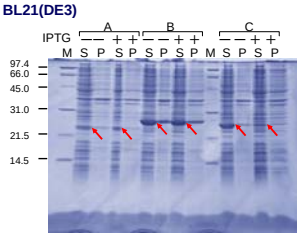
1st day
·transform *E. coli* cells

2nd day
·transfer 10 colonies into two tubes of media
incubate at 37℃ until the density of the culture reached 1–4 x 10^8 cells/ml
·add IPTG into one of the tubes
incubate for 4 hours at 37℃
·harvest the cells
·store the cells at -80℃

3rd day
·confirm expression pattern by SDS-PAGE



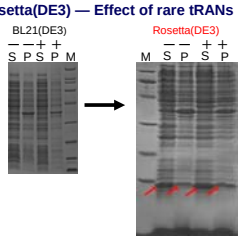
◆ **BL21(DE3)**



Many target proteins were produced without IPTG induction.

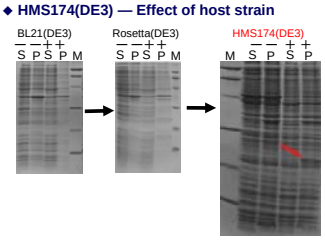
M : marker
S : supernatant
P : precipitate
— : without IPTG
+ : with IPTG

◆ **Rosetta(DE3) — Effect of rare tRANs**



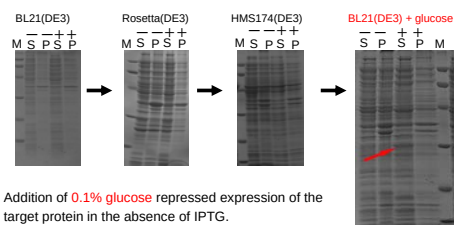
The tRNAs for six codons used rarely in *E. coli* (AUA, AGG, AGA, CUA, CCC, GGA) was supplemented in Rosetta(DE3).

◆ **HMS174(DE3) — Effect of host strain**



Some target proteins were produced in HMS174(DE3), K-12 derivative.

◆ **BL21(DE3) + glucose — Effect of growth media**



Addition of 0.1% glucose repressed expression of the target protein in the absence of IPTG.

Procedure — Large scale culture without IPTG

1st day
·transform *E. coli* cells

2nd day
·transfer 20-30 colonies into 1 liter of medium
incubate for 12 – 18 hours at 37℃

3rd day
·harvest the cells
·store the cells at -80℃


before inoculation


after inoculation

It is important to inoculate many colonies into 1 liter of medium.

Procedure — Large Scale Culture with IPTG

1st day
·transform *E. coli* cells

2nd day (in the case of necessary for preculture)
·transfer 10-15 colonies into 5 ml of medium
incubate at 37℃ until the density of the culture reached 1–4 x 10^8 cells/ml
·inoculate the preculture into 1 liter of medium

2nd day
·transfer 20-30 colonies into 1 liter of medium
incubate at 37℃ until the density of the culture reached 1–4 x 10^8 cells/ml
·add IPTG (final concentration 1 mM)
incubate for 4 –5 hours at 37℃
·harvest the cells
·store the cells at -80℃

Procedure —Se-Met protein

1st day
·transform methionine auxotroph strain — *E. coli* B834(DE3), *E. coli* B834(DE3)+pRARE

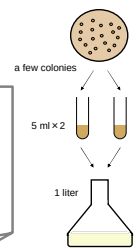
2nd day
·transfer 10-15 colonies into 5 ml of LB broth.
incubate at 37℃ until the density of the culture reached 1–4 x 10^8 cells/ml
·inoculate the 10 ml preculture into 1 liter of the modified LeMaster medium with lactose
incubate for 20–24 hours at 37℃

3rd day
·harvest the cells
·store the cells at -80℃

modified LeMaster medium[1)]

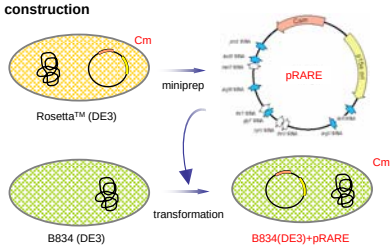
· 50 μg/ml	selenomethionine
· 1%	carbon source
· 1x	vitamin mixture[2)]
· 50 μg/ml	ampicillin

[1)] LeMaster, D. M. and Richards, F. M. (1985) *Biochemistry*, **24**, 7263-7268
[2)] KAO AND MICHAYLUK VITAMIN SOLUTION, Sigma

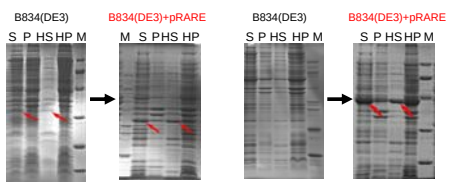


◆ **B834+pRARE** — Effect of rare tRANs

construction



Rosetta(DE3) supplies the tRNAs for six codons used rarely in *E. coli* (AUA, AGG, AGA, CUA, CCC, GGA) on pRARE (pACYC184-derived plasmid).

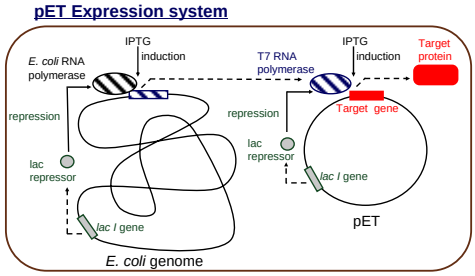


Expression level was increased using B834(DE3)+pRARE.

Some target proteins were produced in BL21(DE3)+pRARE.

M : marker
S : supernatant
P : precipitate
HS : supernatant of heat treatment (70℃, 10 min)
HP : precipitate of heat treatment (70℃, 10 min)

pET Expression system



Members