Thermus thermophilus HB8 のシステム生物学へ向けて：タンパク質発現

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Protein expression

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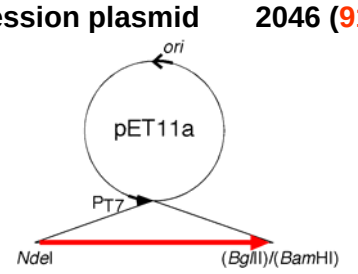
Abstract

- The success rate for protein expression was about 81%.
- Most proteins were expressed without IPTG induction in *E. coli*.
- *E. coli* B834(DE3) strain transformed by pRARE plasmid is useful to express selenomethionine-substituted proteins.
- Co-expression system is useful to produce proteins that are supposed to form a protein complex.

① Plasmid — Current Status

Total ORF	2238
Expression plasmid	2046 (91%)



The plasmid is pET11a.

- Expression the protein without a tag
- Expression induced with IPTG

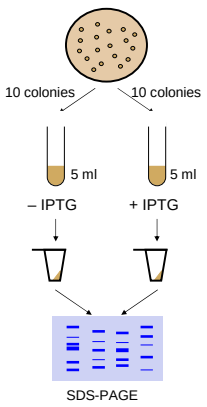
② Expression — Success Rate

	expression/total	success rate	soluble
・BL21(DE3) without IPTG	1068/1482	72.1%	(55.5%)
↓ add IPTG			
・BL21(DE3) with IPTG	22/1482	+1.5%	(+0.7%)
↓ add tRNAs for rare codons			
・Rosetta(DE3)	100/1482	+6.7%	(+4.5%)
↓ change host strain			
・HMS174(DE3)	8/1482	+0.5%	(+0.3%)
↓ repress basal expression			
・BL21(DE3) + glucose	3/1482	+0.2%	(+0.1%)

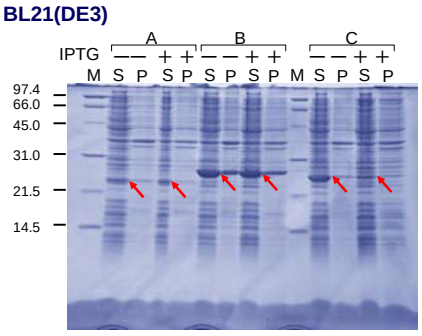
Total success rate was 81% (soluble 61%).

③ Procedure — Expression trial

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 to 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



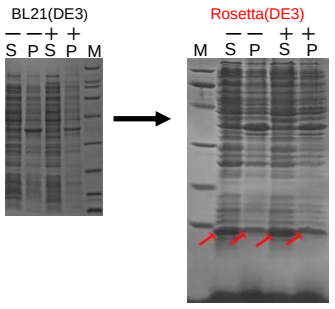
◆ BL21(DE3)



Many target proteins were produced without IPTG induction.

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

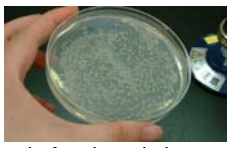
◆ Rosetta(DE3) — Supply of rare tRNAs

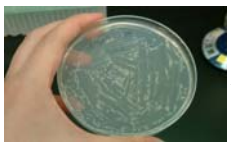


The tRNAs for six codons used rarely in *E. coli* (AUA, AGG, AGA, CUA, CCC, GGA) were supplemented by pRARE plasmid in Rosetta(DE3).

④ 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 – 20 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.

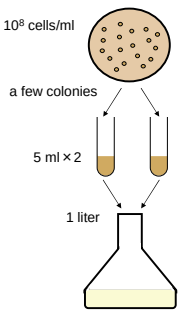
⑤ Procedure — Large-scale culture with IPTG

1st day
・transform *E. coli* cells
↓
2nd day
・transfer 20-30 colonies into 1 liter of medium
incubate at 37℃ until the density of the culture reached to 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℃

2nd day (in the case of necessary for preculture)
・transfer 10-15 colonies into 5 ml of medium
↓
・inoculate the preculture into 1 liter of medium
when the density of the preculture reached to 1–4 x 10^8 cells/ml

⑥ Procedure —Se-Met protein without IPTG

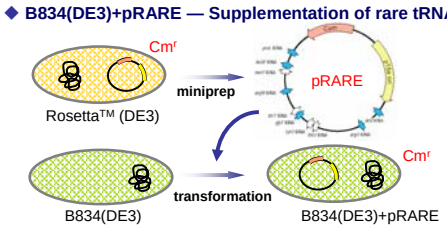
1st day
・transform methionine auxotroph strain
E. coli B834(DE3)
E. coli B834(DE3)+pRARE
E. coli BL21-CodonPlus(DE3)-RIL-X
↓
2nd day
・transfer 10-15 colonies into 5 ml of LB broth.
incubate at 37℃ until the density of the culture reached to 1–4 x 10^8 cells/ml
↓
・inoculate the 10 ml preculture into 1 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℃



⑦ Procedure —Se-Met protein with IPTG

1st day
・transform methionine auxotroph strain *E. coli* B834(DE3)pLysS
↓
2nd day
・transfer 10-15 colonies into 100 ml of LB broth.
incubate at 37℃ for over night
↓
3rd day
・inoculate the 10 ml preculture into 1 liter of the modified LeMaster medium with lactose
incubate for 20–24 hours at 37℃
↓
4th day
・harvest the cells
↓
・store the cells at -80℃

◆ B834(DE3)+pRARE — Supplementation of rare tRNAs

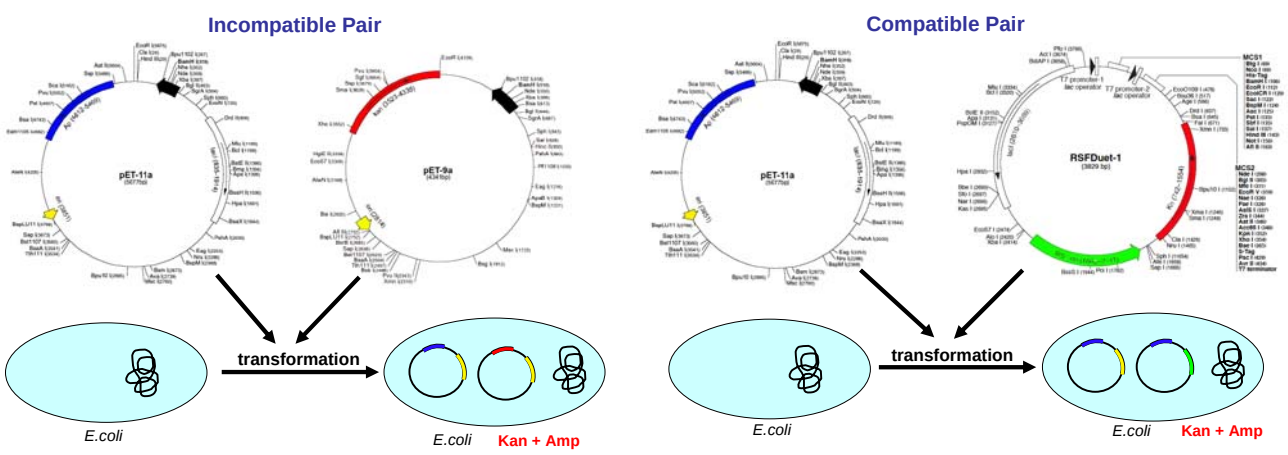


◆ Modified LeMaster medium (*Biochemistry*, **24**, 7263-7268 (1985))

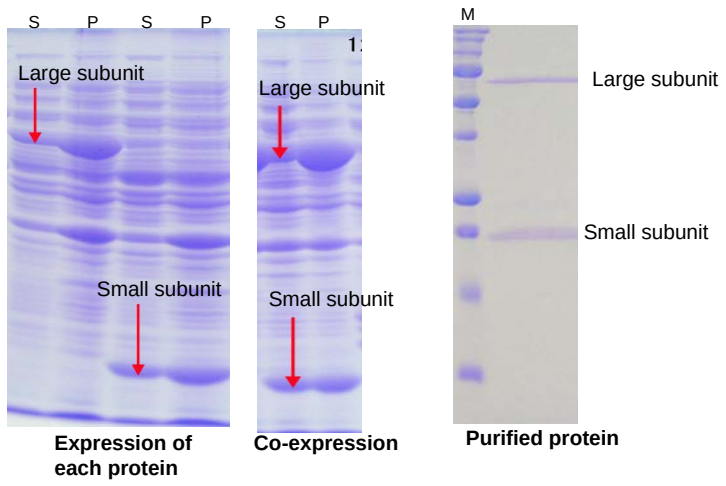
- 50 μg/ml selenomethionine
- 1% Lactose
- 1% Kao and Michayluk Vitamin Solution (Sigma)
- 50 μg/ml ampicillin

⑧ Procedure — Co-expression

Co-expression System



Expression trial



We co-expressed a pair of proteins using incompatible two plasmids (pET9a (Kan^r) and pET11a (Amp^r)) or compatible two plasmids (pET 11a (Amp^r), pRSFDuet-1 (Kan^r)). We constructed a pair of plasmid with different antibiotics-resistance and *E. coli* cells transformed by the pair of plasmids were selected in a medium containing two antibiotics.