

Physiological Studies of Recombinant Protein Production in *Escherichia coli*

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Introduction

- Recombinant protein expression in *E. coli* is often unpredictable
- Sometimes expression level is unexpectedly low
- A case study in investigating the reason of low expression level
- A metabolic model that enables prediction of protein expression level from easily measurable variables.

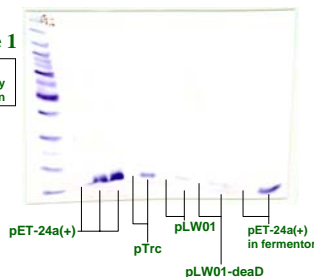
A Case Study

- Target protein: HIV gp41 protein fragment: a.a. 504-657 with His-tag
- Host: *E. coli* BL21(DE3)
- Vector: pET-24a(+) and pLW01
- Phenomenon: pLW01 system has much lower expression level than pET-24a(+)

Western Blot

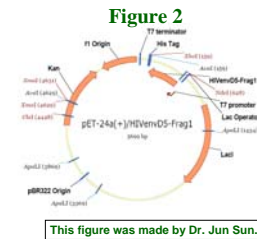
Figure 1

Results obtained by Dr. Jun Sun



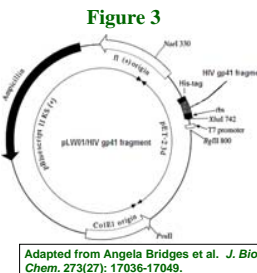
pET-24a(+) Vector

- Kanamycin resistance
- T7-lac promoter
- pBR322 origin of replication (15-20)



pLW01 Vector

- Ligation of *NaeI/PvuII*-digested pET-23d and pBluescript II KS(+)
- Ampicillin resistance
- T7 promoter
- high copy origin of replication ColE1 (300-500)



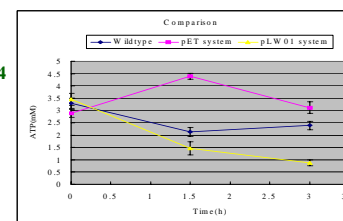
Vector Difference

- Ampicillin vs. Kanamycin resistance
- T7 vs. T7-lac promoter
- Copy number: 300-500 vs. 15-20
- Literature about high copy number vector higher level expression same level expression lower level expression cell viability impaired (with strong promoter)

Intracellular ATP Level

Normal range: 1.3-7.0 mM

Figure 4



Glucose Level in the Medium

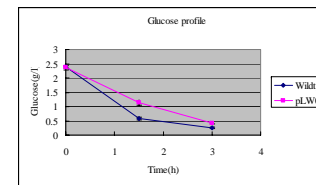


Figure 5

Conclusion: Glucose level is not the reason for low ATP level.

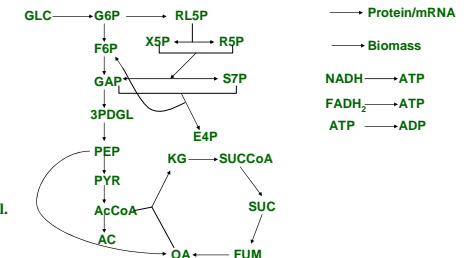
Conclusion

- Glucose is not limiting. Other nutrients are not limiting either, because the same media can support growth to a 10 times higher OD in fermentor.
- Strong promoter and high copy number result in fast transcription which depletes the ATP pool
- The low ATP level results in low protein expression level.

Metabolic Modeling

- $AF=R$, $F=(A^T A)^{-1} A^T R$
- **A**: matrix of stoichiometric coefficients (25X24)
- **F**: flux vector
- **R**: metabolites specific consumption/production rate.
- Pseudo steady-state assumption for intermediates
- Combine the amino acids synthesis and protein synthesis into one flux

Reactions



What does the model do

- Five inputs: biomass growth rate, respiration rates (O_2 , CO_2), glucose consumption rate, acetate production rate
- Output: all the metabolic fluxes
- Calculate target protein expression rate
Experimental value: 0.65g/l
Calculated value: 0.72g/l
Error: 10.8%