

# **Study on the Framework of ATP Energy Cycle System in**

## ***Escherichia coli***

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## Supplementary Information

### 1. Statistical table of protein expression

Table S1 Analysis of gene expression of ATP synthesis pathway in different species

| No. | Sequence ID    | Length (AA) | Weight (KDa) | Protein expression level in supernatant | Strain name    |
|-----|----------------|-------------|--------------|-----------------------------------------|----------------|
| 1   | AJR61575.1     | 340         | 36.4         | Not obvious                             | 1-1;1-2;1-3    |
| 2   | NP_006712.2    | 362         | 40.5         | Not obvious                             | 2-1;2-2;2-3    |
| 3   | WP_011729690.1 | 325         | 34.9         | ++                                      | 3-1;3-2;3-3    |
| 4   | NP_001287054.1 | 345         | 37.7         | +                                       | 4-1;4-2;4-3    |
| 5   | WP_001220237.1 | 214         | 23.5         | +++                                     | 5-1;5-2;5-3    |
| 6   | WP_178927831.1 | 475         | 55.8         | +++                                     | 6-1;6-2;6-3    |
| 7   | WP_003091935.1 | 496         | 58.3         | +++                                     | 7-1;7-2;7-3    |
| 8   | MDI9519035.1   | 298         | 35.5         | ++++                                    | 8-1;8-2;8-3    |
| 9   | WP_003416928.1 | 295         | 34.1         | +++                                     | 9-1;9-2;9-3    |
| 10  | WP_096053282.1 | 253         | 29.3         | +++                                     | 10-1;10-2;10-3 |
| 11  | WP_011338472.1 | 336         | 38.1         | ++                                      | 11-1;11-2;11-3 |
| 12  | WP_000427319.1 | 267         | 32.2         | ++                                      | 12-1;12-2;12-3 |
| 13  | WP_003112634.1 | 357         | 40.8         | ++                                      | 13-1;13-2;13-3 |

The protein weight is predicted by snappene software.

“+” representing different expression levels. The SDS-PAGE gel electrophoresis bands were analyzed by using Image J to quantify the band strength. The maximum expression level “++++”, almost 20%; “+++” for 10%-19%; “++” for 5-10%; “+” for (-5%).

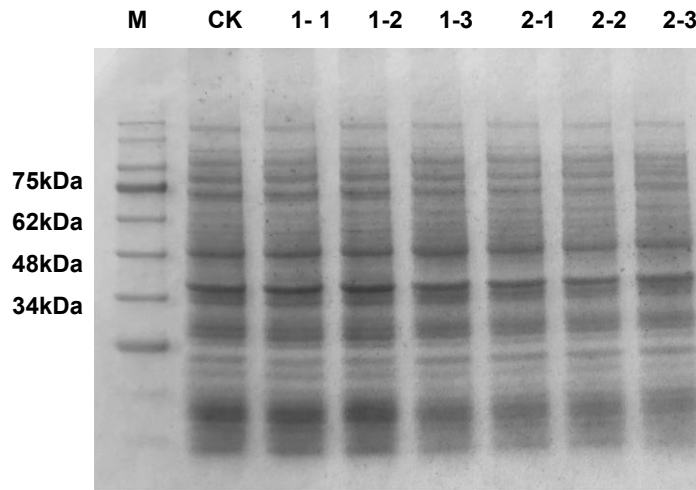


Fig.S1 Gene expression situation of 1-1,1-2,1-3,2-1,2-2,2-3  
CK, the control blank strain BL21 (DE3)

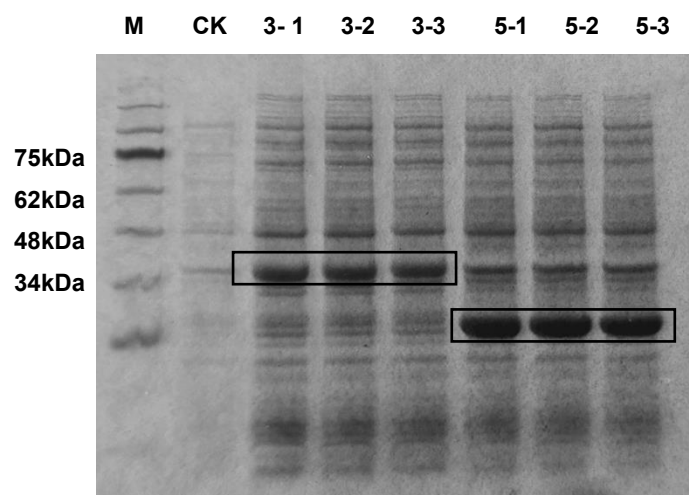


Fig. S2 Gene expression situation of 3-1,3-2,3-3,5-1,5-2,5-3 CK, the control blank strain BL21 (DE3)

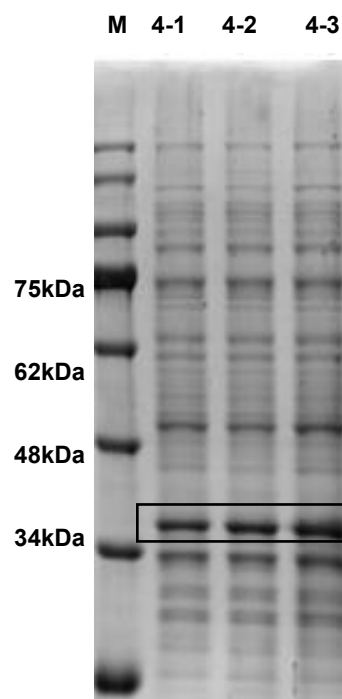


Fig.S3 Gene expression situation of 4-1,4-2,4-3

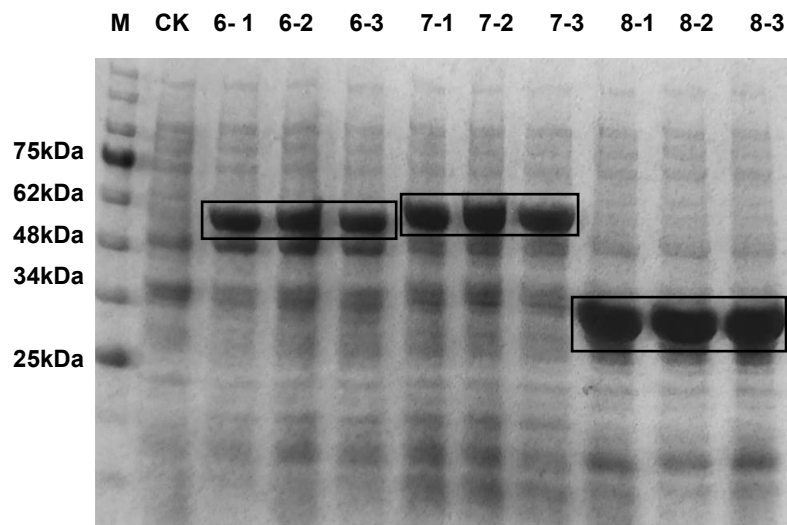


Fig. S4 Gene expression situation of 6-1,6-2,6-3,7-1,7-2,7-3,8-1,8-2,8-3 CK, the control blank strain BL21 (DE3)

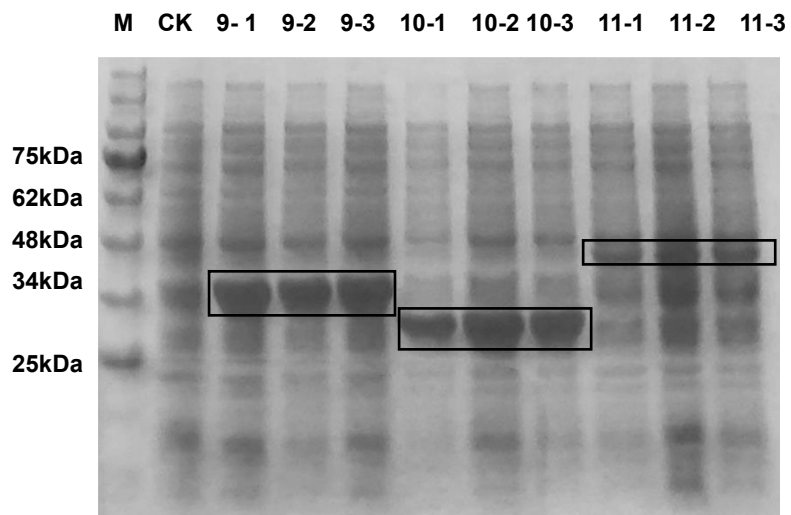


Fig. S5 Gene expression situation of 9-1,9-2,9-3,10-1,10-2,10-3,11-1,11-2,11-3 CK, the control blank strain BL21 (DE3)

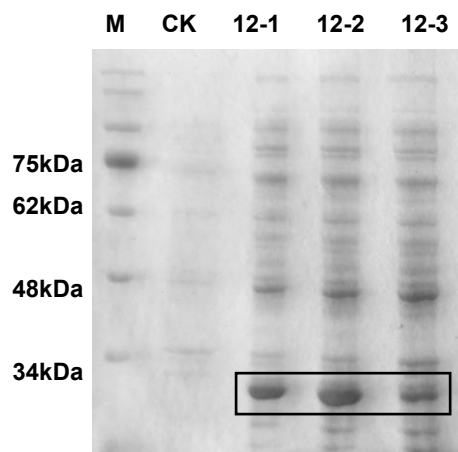


Fig.S6 Gene expression situation of 12-1,12-2,12-3  
CK, the control blank strain BL21 (DE3)

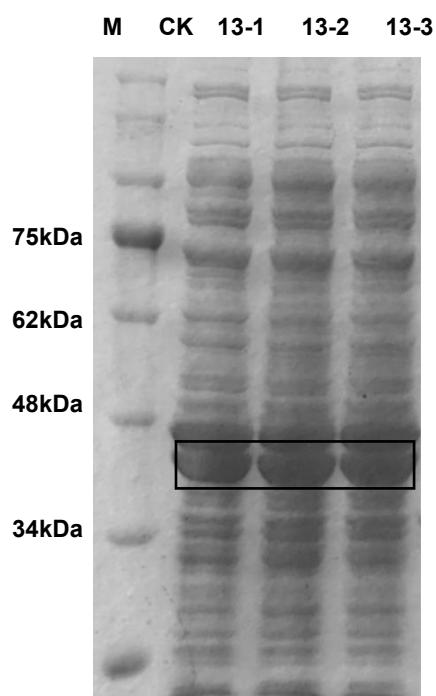


Fig. S7 Gene expression situation of 13-1,13-2,13-3  
CK, the control blank strain BL21 (DE3)

2 Identification primers sequence (5' → 3')

2.1 IS1 (Universal primer)

IS1fw: GGCCTCAACACGATTTTACG

IS1rev: GGTAATGACTCCAACCTTATTG

2.2 IS186(1 forward primer, 5 different reverse primers)

IS186F: CACTGTAATGATAGGCAATTCAGG

IS186R1: ATGAAGCAGCATAAGGCGATG

IS186R2: GAAGCGCAACAGGCATCTG

IS186R3: ATCGTGAGAATGAGATGTTTGCTG

IS186R4: GGATGCTGCAAATCCCACAG

IS186R5: CA ACCTGAGGCTTACGTTTCG

### 2.3 8array (8 different pairs of special primers)

cr1UF: CAACTGGTCACGCTGATCG

cr1DR: CTGTTCAACCTGGTCCAGCC

cr2UF: GCCATGCTAACTCGTCCAAAC

cr2DR: GCGTATGAAGAGCGGTGAGC

cr3UF: GTGGAATTACTGGCAGCGAC

cr3DR: CGGTGGTATATTCCCTTCTGATG

cr4UF: CTGCATGTACGTGCCTTCC

cr4DR: GCTACCGAAGGGACTATTCTC

cr5UF: CAGAGATGGAAATTACCCTGCAAG

cr5DR: CGTTTGCCGTTCAACAACG

cr6UF: GGTGCAAGTATCATCACCCTTC

cr6DR: GTCGATTACGAGACGTTGATGAAG

cr7UF: CTGGTATCGTAAAATGGTTCAACG

cr7DR: CCATGTCCTGACCTGGGTTC

cr8UF: TCTTCCGCATTACCCTGGTG

cr8DR: TACTGAACTCTCTGGCTCAC

## 3 Gene copy number analysis

### 3.1 IS186 Locus

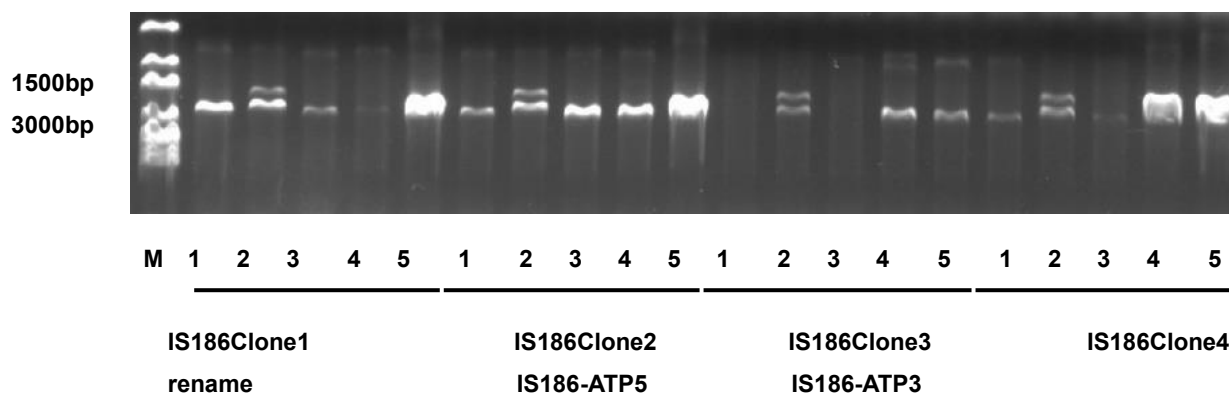


Fig.S8 Identification of IS186 locus insertion of 4 strains

In the initial screening, there were a total of 4 monoclonal clones, all of which were fully inserted at all 5 loci except for the third clone, which was inserted at 3 copies of 2,4,5.

3.2 IS1 Locus

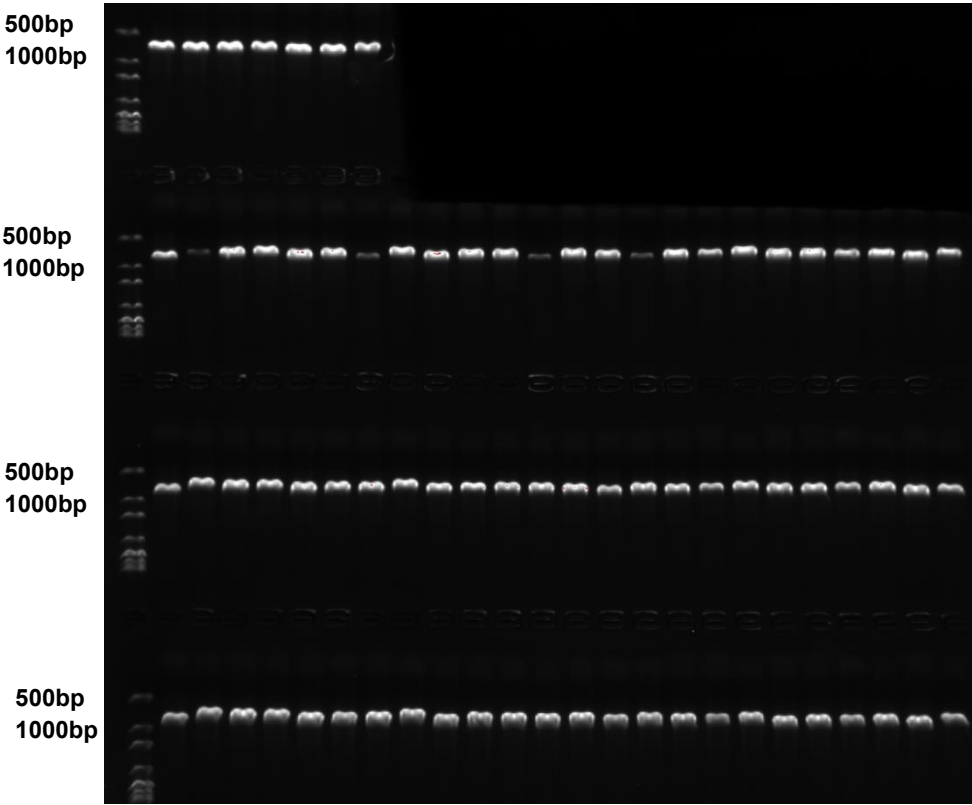


Fig.S9 Identification of IS1 locus insertion  
After identification with universal primers, multiple monoclonal strains showed no gene insertion.

4. Protein expression after genome editing

4.1 IS186 Locus

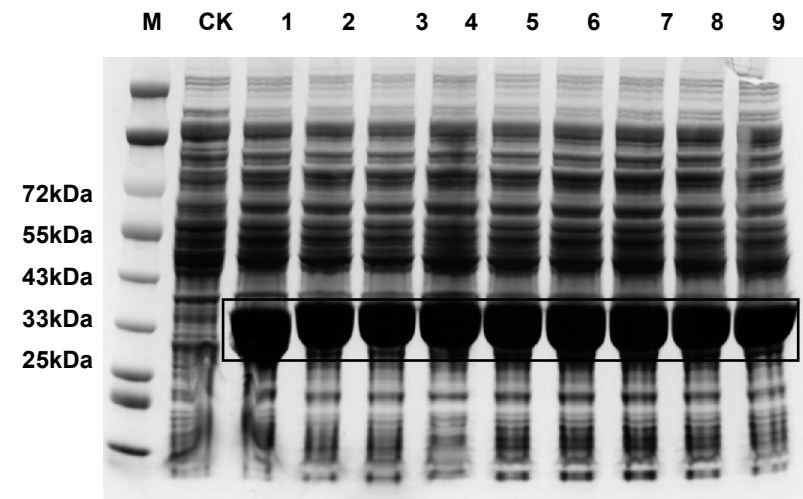


Fig. S10 Expression of four different monoclonal clones at IS186  
CK, the control blank strain BL21 (DE3)  
1,pET29a-ATP; 2-3,IS186Clone1; 4-5,IS186Clone2 rename IS186-ATP5; 6-7,S186Clone3 rename IS186-ATP3; 7-8, IS186Clone4; No significant expression difference observed.

3.2 8Array Locus

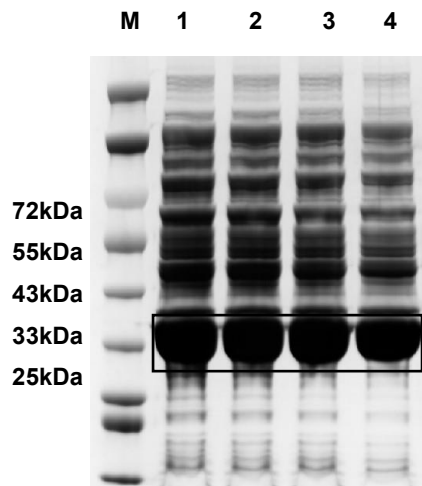


Fig.S11 Expression of four monoclonal clones at 8array (all inserted at cr5)  
4.3 IS1 Locus and IS186+8array

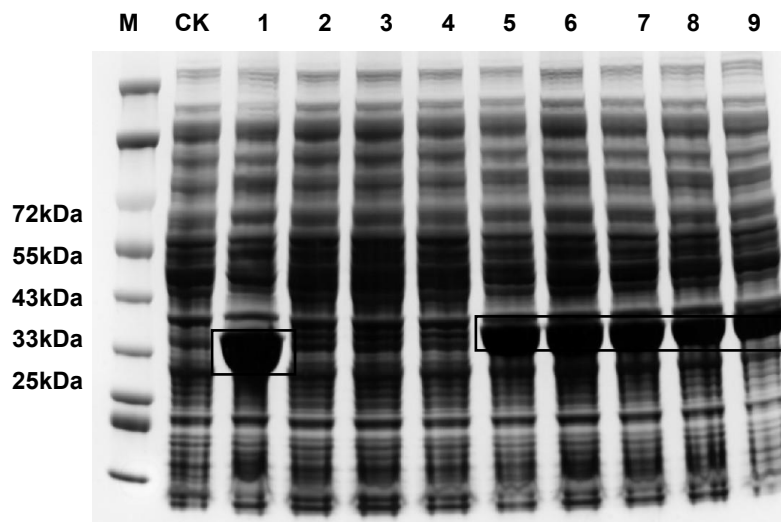


Fig. S12 Expression of different monoclonal at IS1 and IS186+8array  
CK, the control blank strain BL21 (DE3)  
1,pET29a-ATP; 2-4, IS1Clone1-3; 5-9, IS186+8array Clone1-5;  
IS186+8array strains showed significant decrease in expression compared to pET29a-ATP.

#### 4.4 Promoter conflict and expression of one bacterium and two enzymes

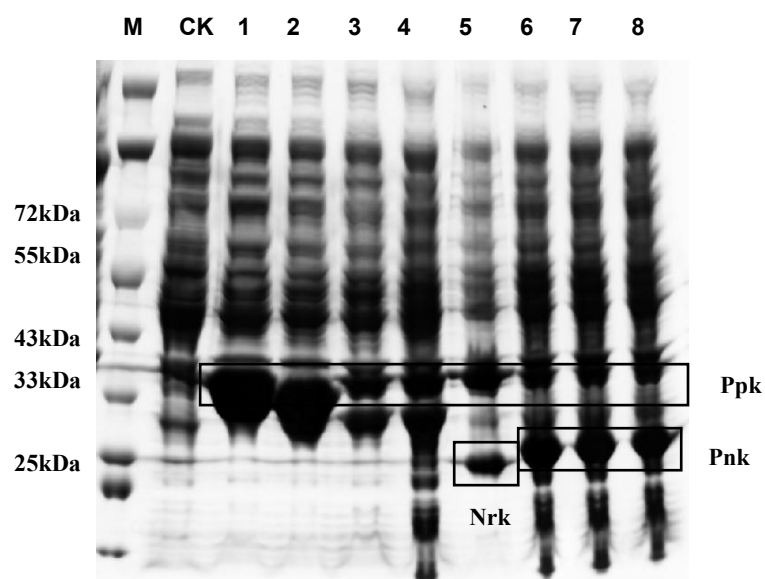


Fig. S13 The changes of expression levels of one plasmid and two genes

CK, the control blank strain BL21 (DE3)

1, IS186-ATP5;2, pET29a-Pnk-RBS-ATP; 3, pET29a-Pnk (IS186-ATP5) Clone1;4, pET29a-Pnk (IS186-ATP5) Clone2;5, pET29a-Nrk-RBS-ATP;6-8, pET29a-Nrk (IS186-ATP5) Clone1-3;

The conflict phenomenon is severe when the same promoter plasmid is imported after genome editing.

The expression level is directly proportional to enzyme activity.