Protein Expression Technical Service Report

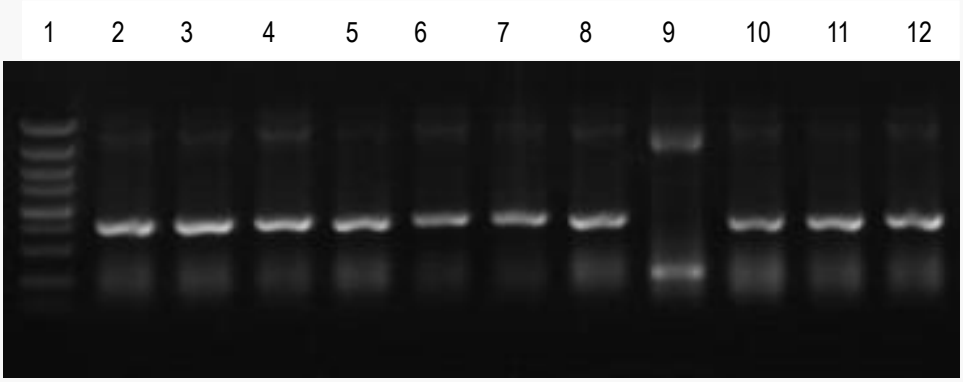
Project Name	Recombinant Human Cytochrome b-245 heavy chain(CYBB),partial
Cat No.	AAA18493
Company Name	aaabiotech
Project Starting Date	28th June 2019
Report date	2th Aug 2019

E. coli expression system has the advantages of simple structure, clear genetic background, high expression level of target gene, short culture period, etc. It is considered as the most extensive and economical classical expression system. In recent decades, E. coli expression system has been continuously developed and improved. It is widely used by scientific research and industrial users for the expression of various recombinant proteins. We have rich experience in expression and purification and professional techniques. Through continuous optimization of experimental conditions, our technical team can solve various difficult problems in the process of protein expression and purification. So far, we have developed more than 7,000 proteins and provide more than 5,000 proteins for customers around the world.

1. Subcloning

1.1 Results of spot selection in recombinant screening map

The gel was run on a 1% agarose gel with a target fragment length of 813 bp, which was consistent with expectations.

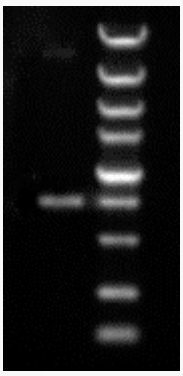


(**Marker: From the Top to bottom** 5000, 3000, 2000, 1500, 1000, 750, 500, 250, 100(bp))

Lane 1: Control

Lane 2-8: The positive rate of spotting is: 9/10

PCR Amplification Image



(**Marker: From the Top to bottom** 5000, 3000, 2000, 1500, 1000, 750, 500, 250, 100(bp))

1.2 Sequencing result

Forward:

AGAGCGCGGGGTACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGG
GCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCAT
GACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCCGGACCGTTGTTACCCATCCGTTTAAAAC
CATTGAACTGCAGATGAAGAAAAAAGGCTTCAAAATGGAAGTGGGCCAGTACATTTTTGTGAAATG
TCCGAAAGTTAGCAAGCTGGAATGGCATCCTTTTACACTGACCAGCGCACCGGAAGAAGATTTTTT
CAGCATTCATATTCGCATCGTTGGTGATTGGACCGAAGGTCTGTTTAATGCATGTGGTTGTGATAAAC
AAGAATTCCAGGATGCATGGAAACTGCCGAAAATTGCAGTTGATGGTCCGTTTGGCACCGCAAGCG
AAGATGTTTTTGATTATGAAGTTGTGATGCTGGTTGGTGCAGGTATTGGTGTTACCCCGTTTGCAAG
CATTCTGAAAAGCGTTTGGTATAAATACTGCAACAATGCCACCAACCTGAAGCTGAAGAAAATCTAT
TTCTATTGGCTGTGCCGTGATACCCATGCATTTGAATGGTTTGCCGATCTGCTGCAGCTGCTGGAAA
GCCAGATGCAAGAACGTAATAATGCAGGTTTTCTGAGCTACAACATTTATCTGACCGGTTGGGATGA
AAGCCAGTGTAATCATTTTGCAGTTCACCACGATGAAGAGAAAGATGTTATTACCGGTCTGAAACA
GAAAACCCTGTATGGTCGTCCGTGTTGGGATAATGAATTCAAAACAATTGCAAGCCAGCATCCGAAT
ACACGCATTGGTGTTTTTCTGTGTGGTCCGGAAGCACTGGCAGAAACCCTGAGCAAACAGAGCATT
AGCAATAGCGAAAGCGGTCCGCGTGGTGTTCATTTTATCTTTAACAAAGAAAATTTTTAACTCGAGC
ACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGAAGCTGAGTGCTGCTGCA
CGCTGAGCCAATAACTAGCATAACCCTTGGGGCTCTAACGGGTCTTGAGGATTTGCTGAAAGAGAAC
TATTATCGATGCGAATGGAACGCGCCTGTTAACG

Reverse:

GCGTCCCGGGCGTAGAGAGGAATCGAGGATCTCCGATCCCGCGAATTAATACGACTCCACTATTAGG
GATGTGAGCGGATACAATTCCCCTCTAGAAATAATTTGTTTACTTTAGAGGAGATATACATGGGCAGC
AGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACTG
GTGGACAGCAAATGGGTCGCGGATCCGAATTCCGGACCGTTGTTACCCATCCGTTTAAAACCATTG
AACTGCAGATGAAGAAAAAAGGCTTCAAAATGGAAGTGGGCCAGTACATTTTTGTGAAATGTCCG
AAAGTTAGCAAGCTGGAATGGCATCCTTTTACACTGACCAGCGCACCGGAAGAAGATTTTTTCAGC
ATTCATATTCGCATCGTTGGTGATTGGACCGAAGGTCTGTTTAATGCATGTGGTTGTGATAAACAAG
AATTCCAGGATGCATGGAAACTGCCGAAAATTGCAGTTGATGGTCCGTTTGGCACCGCAAGCGAAG
ATGTTTTTGATTATGAAGTTGTGATGCTGGTTGGTGCAGGTATTGGTGTTACCCCGTTTGCAAGCATT
CTGAAAAGCGTTTGGTATAAATACTGCAACAATGCCACCAACCTGAAGCTGAAGAAAATCTATTTCT

ATTGGCTGTGCCGTGATACCCATGCATTTGAATGGTTTGCCGATCTGCTGCAGCTGCTGGAAAGCCA
GATGCAAGAACGTAATAATGCAGGTTTTCTGAGCTACAACATTTATCTGACCGGTTGGGATGAAAGC
CAGTGTAATCATTTTGCAGTTCACCACGATGAAGAGAAAGATGTTATTACCGGTCTGAAACAGAAA
ACCCTGTATGGTCGTCCGTGTTGGGATAATGAATTCAAAACAATTGCAAGCCAGCATCCGAATACAC
GCATTGGTGTTTTTCTGTGTGGTCCGGAAGCACTGGCAGAAACCCTGAGCAAACAGAGCATTAGCA
ATAGCGAAAGCGGTCCGCGTGGTGTTCATTTTATCTTTAACAAAGAAAATTTTTAACTCGAGCACCA
CCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCA
CCGCTGAGCCAAATAAACTAAGGCCAA

Post-splicing bases sequence:

ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCT
AGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCCGGACCGTTGTTACCCATCCGTTT
AAAACCATTGAACTGCAGATGAAGAAAAAAGGCTTCAAAATGGAAGTGGGCCAGTACATTTTTGT
GAAATGTCCGAAAGTTAGCAAGCTGGAATGGCATCCTTTTACACTGACCAGCGCACCGGAAGAAG
ATTTTTTCAGCATTCATATTCGCATCGTTGGTGATTGGACCGAAGGTCTGTTTAATGCATGTGGTTGT
GATAAACAAGAATTCCAGGATGCATGGAAACTGCCGAAAATTGCAGTTGATGGTCCGTTTGGCACC
GCAAGCGAAGATGTTTTTGATTATGAAGTTGTGATGCTGGTTGGTGCAGGTATTGGTGTTACCCCGT
TTGCAAGCATTCTGAAAAGCGTTTGGTATAAATACTGCAACAATGCCACCAACCTGAAGCTGAAGA
AAATCTATTTCTATTGGCTGTGCCGTGATACCCATGCATTTGAATGGTTTGCCGATCTGCTGCAGCTG
CTGGAAAGCCAGATGCAAGAACGTAATAATGCAGGTTTTCTGAGCTACAACATTTATCTGACCGGTT
GGGATGAAAGCCAGTGTAATCATTTTGCAGTTCACCACGATGAAGAGAAAGATGTTATTACCGGTCT
GAAACAGAAAACCCTGTATGGTCGTCCGTGTTGGGATAATGAATTCAAAACAATTGCAAGCCAGCA
TCCGAATACACGCATTGGTGTTTTTCTGTGTGGTCCGGAAGCACTGGCAGAAACCCTGAGCAAACA
GAGCATTAGCAATAGCGAAAGCGGTCCGCGTGGTGTTCATTTTATCTTTAACAAAGAAAATTTTTAA

Post-splicing protein sequence:

MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSEFRTVVTHPFKTIELQMKKKGFKMEVGQYIFV
KCPKVSKLEWHPFTLTSAPEEDFFSIHIRIVGDWTEGLFNACGCDKQEFQDAWKLPKIAVDGPFGTASE
DVFDYEVVMLVGAGIGVTPFASILKSVWYKYCNNATNLKLKKIYFYWLCRDTHAFEWFADLLQLLES
QMQERNNAGFLSYNIYLTGWDESQCNHFAVHHDEEKDVITGLKQKTLYGRPCWDNEFKTIASQHPNT
RIGVFLCGPEALAETLSKQSISNSESGPRGVHFIFNKENF-

Remark: The sequence in purple highlight is the pET28a vector sequence.

2. Protein Expression and Purification:

- **Expression Vector:**

  The expression vector used for protein-induced expression is pET28a-JT (hereinafter referred to as His tag), and the fusion protein has a molecular weight of 35.2 kD.

  Molecular weight calculation website: https://web.expasy.org/compute_pi/

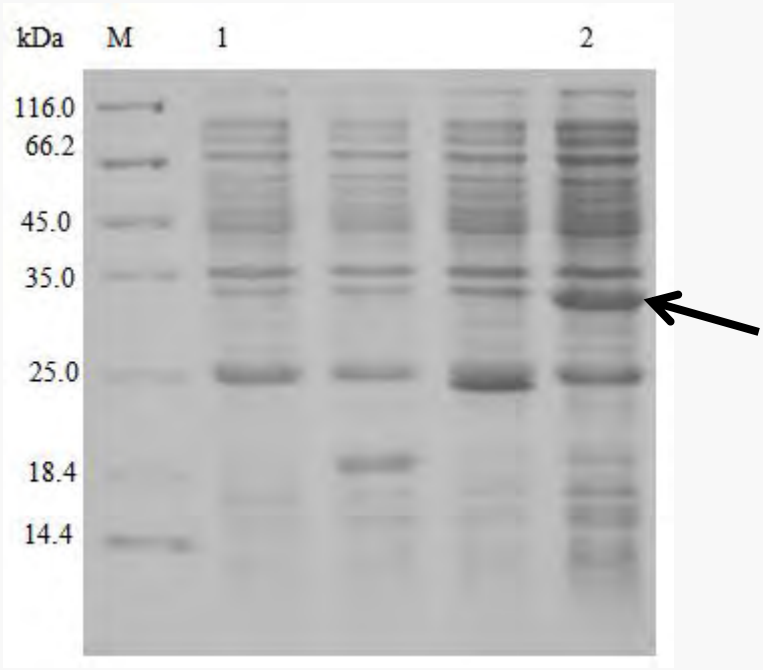
- **Expression Host:**

  Choose Rosetta (DE3) as host expression strain.

◆ **Expression Condition:**

37 ° C, 220 rpm shaker shaking culture, when OD600 = 0.4-0.6, add IPTG at a final concentration of 1 mM, induced expression at 30 ° C for 2.5-3h, resistance: Kan +.

◆ **Small Scale Expression Result:**

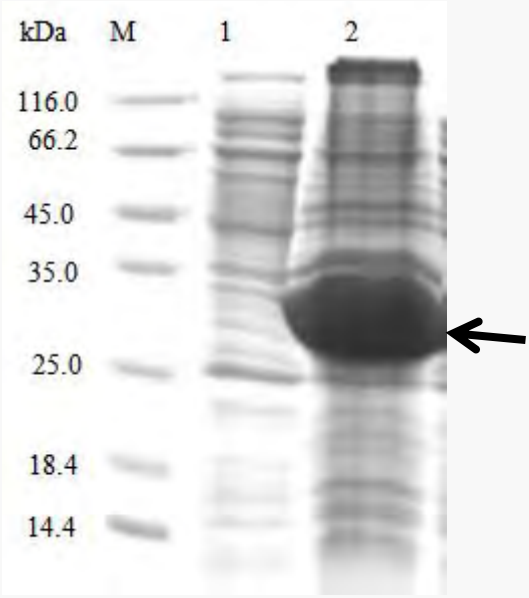


Lane 1: Control

Lane 2: After induction

Note: The arrow indicates the size of the target protein

◆ **Protein SDS-PAGE Detection Image:**



Lane 1: Supernatant

Lane 2: Precipitation

Note: The arrow indicates the size of the target protein.

Note: Proteins are all expressed in the precipitation and we performed inclusion body purification.

◆ **Purification of Fusion Protein:**

The inclusion body was washed by ultrasonication and was renatured. The renaturation method is listed as follows:

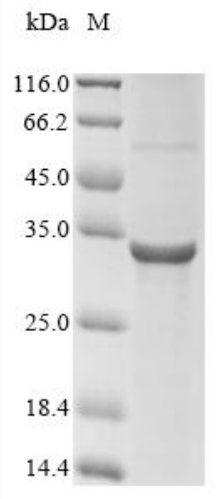
1) The washed inclusion bodies were dissolved in 20 mM NaHCO3, 50 mM NaCl, 0.5 mM EDTA, 0.5% SKL, 5 mM DTT, and shaken at 37 ° C for about 1-2 hours.

2) Centrifuge and take the supernatant.

3) Add 0.2% PEG 4000, 1 mM oxidized glutathione, 2 mM reduced glutathione.

4) The above buffer was added to the protein and placed in a dialysis bag, 10 mM Tris-HCl, 1 mM EDTA, without stirring at 4 ° C, the dialysis time was 72 h, and changed the dialysate one time during this process.

5) After refolding, the protein was obtained and the SDS-PAGE detection is listed as follows:



According to the final purification results, the yield of this protein was 3.8mg/L.