

The present study used a novel recombinant protein, namely flagellin A (FlaA) N/C, an independent intellectual property biological agent of our project team. FLaAN/C was developed in an earlier study and is derived from the flagellin protein of *Legionella pneumophila*. The FlaAN/C protein was obtained by our group after constructing plasmids through genetic recombination, sequencing to verify the sequence, expressing the fusion protein, and purifying the protein. At present, the invention patent has been granted (ZL 2014 10075339.8)

1.The gene sequence of FlaAN/C in this experiment is as follows:

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ATTAACACCAACGTGGCGAGCCTGACCGCGCAGCGTAACCTGGGCGTGAGCGGCAAC
ATGATGCAGACCAGCATTTCAGCGTCTGAGCAGCGGCCTGCGTATTAACAGCGCGAAAG
ATGATGCGGCGGGCCTGGCGATTAGCCAGCGTATGACCGCGCAGATTTCGTGGCATGAA
CCAGGCGGTGCGTAACGCGAACGATGGCATTAGCCTGGCGCAGGTGGCGGAAGGCGC
GATGCAGGAAACCACCAACATTCTGCAGCGTATGCGTGAAGTGAAGCGTGCAGGCGGC
GAACAGCACCAACAACAGCAGCGATCGTAGCAGCATTTCAGAGCGAAATTAGCCAGCT
GAAAAGCGAACTGGAACGTATTGCGCAGAACACCGAATTTAACGGCCAGCGTATTCTG
GATGGCAGCTTTAGCGGTGGTGGTGGTAGCGGCGGCGGCGGCAGCATTAAACGTATTG
ATGCGGCGCTGAACAGCGTGAACAGCAACCGTGCGAACATGGGCGCGCTGCAGAACC
GTTTTGAAAGCACCATTTGCGAACCTGCAGAACGTGAGCGATAACCTGAGCGCGGCGC
GTAGCCGTATTCAGGATGCGGATTATGCGGCGGAAATGGCGAGCCTGACCAAAAACCA
GATTCTGCAGCAGGCGGGCACCGCGATGCTGGCGCAGGCGAACAGCCTGCCGCAGAG
CGTGCTGAGCCTGCTG

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2.The fusion plasmid was constructed by DNA recombination technology (enzyme digestion, ligation, transformation, screening and other steps). After sequencing and verifying the sequence, the FLaAN/C protein was induced to be expressed by IPTG. The results are shown in Figure 1

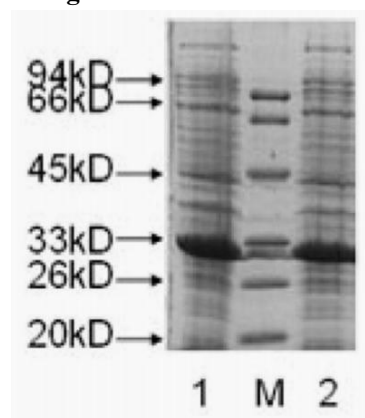


Figure 1: The expression map of FlaAN/C fusion protein (30KD)

3. FlaAN/C fusion protein expression assay and the results are shown in Figure 2

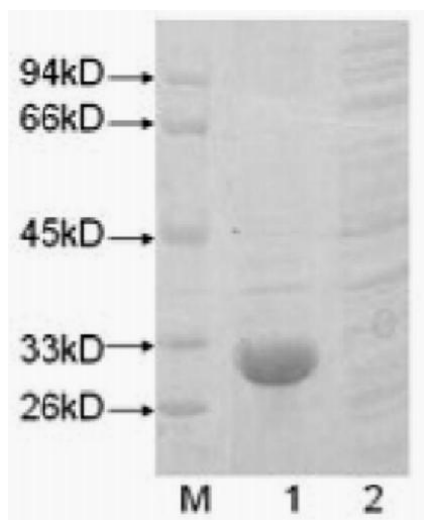


Figure 2: The expression gel map of FlaAN/C

4. The purification results of the FlaAN/C fusion protein (denaturation and purification of inclusion body proteins, and protein denaturation) are shown in Figure 3

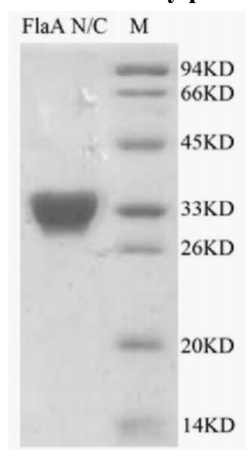


Figure 3: The electrophoresis profile of FlaAN/C purified protein

5. The amino acid sequence of FlaAN/C in this experiment is as follows:

Ile	Asn	Thr	Asn	Val	Ala	Ser	Leu	Thr	Ala	Gln	Arg	Asn	Leu	Gly	Val	Ser	Gly	Asn	Met
1				5					10					15					20
Met	Gln	Thr	Ser	Ile	Gln	Arg	Leu	Ser	Ser	Gly	Leu	Arg	Ile	Asn	Ser	Ala	Lys	Asp	Asp
			25				30						35					40	
Ala	Ala	Gly	Leu	Ala	Ile	Ser	Gln	Arg	Met	Thr	Ala	Gln	Ile	Arg	Gly	Met	Asn	Gln	Ala
			45				50						55					60	
Val	Arg	Asn	Ala	Asn	Asp	Gly	Ile	Ser	Leu	Ala	Gln	Val	Ala	Glu	Gly	Ala	Met	Gln	Glu
			65				70						75					80	
Thr	Thr	Asn	Ile	Leu	Gln	Arg	Met	Arg	Glu	Leu	Ser	Val	Gln	Ala	Ala	Asn	Ser	Thr	Asn
			85				90						95					100	
Asn	Ser	Ser	Asp	Arg	Ser	Ser	Ile	Gln	Ser	Glu	Ile	Ser	Gln	Leu	Lys	Ser	Glu	Leu	Glu
			105				110						115					120	

Arg Ile Ala Gln Asn Thr Glu Phe Asn Gly Gln Arg Ile Leu Asp Gly Ser Phe Ser Gly			
125	130	135	140
Gly Gly Gly Ser Gly Gly Gly Gly Ser Ile Lys Arg Ile Asp Ala Ala Leu Asn Ser Val			
145	150	155	160
Asn Ser Asn Arg Ala Asn Met Gly Ala Leu Gln Asn Arg Phe Glu Ser Thr Ile Ala Asn			
165	170	175	180
Leu Gln Asn Val Ser Asp Asn Leu Ser Ala Ala Arg Ser Arg Ile Gln Asp Ala Asp Tyr			
185	190	195	200
Ala Ala Glu Met Ala Ser Leu Thr Lys Asn Gln Ile Leu Gln Gln Ala Gly Thr Ala Met			
205	210	215	220
Leu Ala Gln Ala Asn Ser Leu Pro Gln Ser Val Leu Ser Leu Leu			
225	230	235	