L1151-2	Expression and purification of the multi-disulfide bonded FIPV-RBD in *Escherichia coli* (*E. coli*) expression system using solubility enhancing peptide tag (SCP-tag)				
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[Introduction] Feline coronavirus (FCoV) is a prevalent feline virus that infects cells in the gastrointestinal tract. There are thought to be two serotypes of FCoV: type I and type II. Type I is the most common FCoV in naturally infected cats globally, and each FCoV serotype consists of two biotypes: nonlethal feline enteric coronavirus (FECV) and lethal feline infectious peritonitis virus (FIPV). The difficulty of isolating and propagating serotype I CoV in vitro for molecular-level pathogenesis, urgently needed for developing vaccines and therapeutics, has largely hindered progress in FIP research. The difficulty of isolating and propagating serotype I CoV in vitro for pathogenesis at the molecular level, urgently needed for developing vaccines and therapeutics, has largely impeded progress in FIP research. Coronaviruses have three envelope glycoproteins, S, E, and M. The S protein includes two subunits which are S1 and S2. In the S1 subunit, the receptor binding domain (RBD) is responsible for viral entry. Therefore, RBD is a key part of a virus located on its 'spike' domain, allowing it to dock to body receptors to gain entry into cells and lead to infection.

In this study, we try to purify the RBD protein in *E.coli* and analyze the RBD biophysical results.

[Materials and methods] We use RBD wildtype and RBD-C9R two kinds of protein. The C9R is a kind of amino acid tag affixed to the C- terminus. In the previous study, the C9R-tag can effectively increase protein solubility and promote protein yield. The competent cell we choose is the T7 shuffle because it is a suitable *E. coli* for the production of polysulfide bonds.

[Conclusion] In this study, RBD-C9R was successfully purified in the T7 shuffle. However, for some unknown reasons, the RBD wild-type aggregated during dialysis, and therefore the RBD wild-type could not be recovered. That proved the C9R-tag can increase protein solubility and help protein purification. The HPLC results (Figure 1) showed no single peak, implying that part of the protein was misfolded. Although the main peak was recovered, most of the protein was lost due to misfolding. Therefore, the yield was low even if the protein could be recovered eventually. So, we changed some conditions to increase the yield and finally determined the best conditions for RBD-C9R purification. We also test the protein biophysical characterization. We use BeSt sel to recognize the secondary structure (Figure 2). The BeSt sel is a method for determining the secondary structure and identifying folding from circular dichroism spectra of proteins. The result shows that our protein is similar to the native RBD structure.

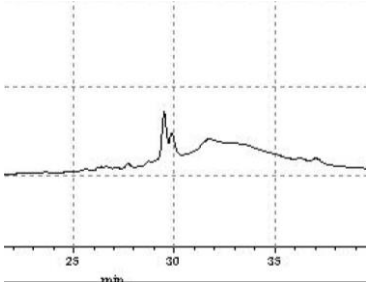

Figure.1. HPL.

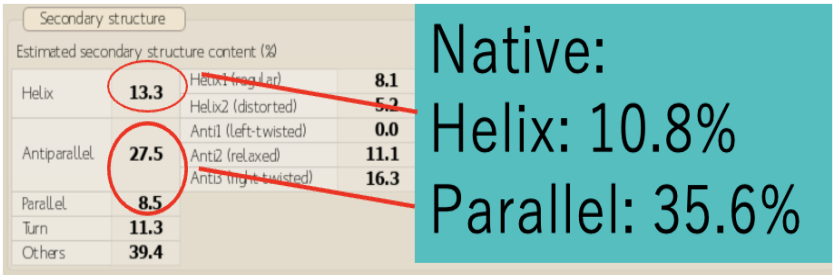

Figure.2. BeSt sel