Abstract: <strong>Introduction: </strong>Antibodies are powerful tools to study protein functions, protein localization and protein-protein interactions. Antibodies are usually conjugated to fluorescent molecules for research and diagnostic purposes which are termed fluobody.<br />
In this study, an anti-human CD4 single-chain antibody fragment (scFV) was cloned and genetically linked to the C terminus of the enhanced green fluorescent protein (EGFP) at cDNA level to generate an EGFP/scFV fusion protein. Different sets of expression vectors were constructed that permitted the efficient fusion and expression of CD4 scFV to EGFP. The effects of different temperatures were also assessed in expression of the fusion protein and fluorescent emission of EGFP.<br />

<strong>Method: </strong>Different vectors including pAB1 and pCANTAB5E were used to clone the CD4 scFV EGFP. <em>Escherichia coli </em>strain, Origami, were transformed by these vectors and the level of expression in four different temperature was assessed using flow cytometry, fluorescent microscopy, SDS PAGE, dot blot and western blotting.<br />

<strong>Results: </strong>Several constructs of fluobody containing EGFP and CD4 scFV were cloned and expressed in <em>E.coli. </em>In lower temperatures and in pCANTAB5E vector, expression of fluobody was higher than those conditions with high culture temperatures and pAB1 vector.<br />

<strong>Conclusions: </strong>The present recombinant fluobody is a bifunctional antibody which retains both antigen binding activity and its fluorescence simultaneously. Thus lower temperature culture condition for high production of this fluobody seems to be required for appropriate purification of this fluobody for therapeutic and diagnostic applications.<br />

floubody, green fluorescent protein, single-chain antibody fragment(scFV),Escherichia coli.

Presentation: Poster