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Generation of Chimeric Antibody Light Chain Plasmid

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ABSTRACT

In order to determine the distance between the antigen-binding site and the crystallizable fragment (Fc) region of an antibody using single-molecule Förster Resonance Energy Transfer, dyes must be attached to these locations. To accomplish this, the variable regions of the light and heavy chains of an antibody can be modified to introduce dye-reactive amino acids. Initial attempts to alter the variable light chain (V\textsubscript{L}) gene included trying to ligate a 350 bp variable gene into an 11,000 bp plasmid. When these attempts were unsuccessful, the variable light chain gene was ligated into a commercially available 3500 bp plasmid.

INTRODUCTION

Antibodies are comprised of four polypeptide chains including two identical light chains and two identical heavy chains (Figure 1).\textsuperscript{1} One end of each of the two heavy chains interacts with the other to form the crystallizable fragment (Fc) region of the antibody, which is the lower lobe and the other ends interact with the light chains to form the upper lobes. The two upper lobes are separated from the lower lobe by an unstructured hinge region. The presence of this hinge region provides segmental flexibility between the lobes and grants antibodies a larger range to search for antigens.\textsuperscript{2} Binding of effector molecules to the Fc region can result in an immune response within the body. The two upper lobes of the antibody are where the fragment antigen-binding (Fab) regions reside. If the two Fab regions engage a single antigen, the possibility of forming an immune complex, or a group of antibodies bound to the antigen, is increased. The formation of immune complexes is necessary for the generation of an immune response and it is affected by antibody flexibility, or the ability of an antibody to take on a variety of structures. Further researching the possible antibody conformations will lead to a better understanding of the variety of structures antibodies exhibit.

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molecules must be attached to those regions. The dyes cannot be attached unless both the variable light (V_L) and variable heavy (V_H) chains are modified and dye-reactive amino acids are introduced in the appropriate locations. Through past experiments the variable heavy chain and the Fc region have been mutated and a dye-reactive cysteine residue was introduced in the Fc region. The goal of this experiment was to add a dye-reactive lysine residue to the Fab region by manipulating the variable light chain gene. In order to introduce the lysine residue, a V_L gene from a mouse antibody was inserted into a plasmid containing the other genes for the light chain through ligation. The process of ligation involved isolating the original plasmid, opening it through the use of enzymes, and adding in the new V_L region (Figure 2). This new region was taken from a mouse antibody because it would produce an antigen-binding site containing a reactive lysine residue, which is necessary for dye attachment.3

Once dye molecules are attached to any location on the antibody, its structure can be examined using FRET. This technique is based on a weak dipole-dipole coupling involving two different dye molecules, a donor and an acceptor, which are attached at two different points on an antibody. The process begins by the excitation of the donor molecule, or having the donor molecule absorb light, by means of a laser. If there is no acceptor molecule present, no transfer of energy occurs and the donor molecule will fluoresce, or give out some of the absorbed energy in the form of light. If energy is transferred to an acceptor molecule, the acceptor will fluoresce. The relative intensity of the fluorescence signals from the donor and acceptor depends on the distance between the two dye molecules, meaning that the closer they are to one another, the higher the energy transfer.
efficiency. Single molecule FRET is beneficial because it allows the distances between the dye molecules on many individual molecules to be examined. A histogram can then be constructed with a distance value for each molecule instead of simply the average distance in the experiment.

RESULTS

Initial attempts in the experiment included amplifying the $V_L$ gene from a plasmid containing a single chain variable fragment of a mouse antibody called 84G3. The $V_L$ gene was amplified using the polymerase chain reaction (PCR). A ligation reaction between the $V_L$ gene and an 11,000 bp plasmid was carried out. To determine if the ligation was successful, restriction enzymes were used to cut the ligation product to see if the expected 350 bp $V_L$ would be observed. When analyzed on an agarose gel, the results indicated that the ligation was unsuccessful. The lack of success was attributed to the difficulty involved in ligating a very small fragment into a much larger one. To avoid this, a commercially available pFuse 3500 bp plasmid was then purchased, and was opened at the EcoRI and BsiWI restriction enzyme cut sites in order to allow for ligation of the $V_L$ gene (Figure 3).

The newly ligated plasmid was then transformed into E. coli cells and the DNA was replicated. The DNA from colonies that grew was then isolated. PCR was performed on the new ligated pFuse plasmid, and the results were run on an agarose gel. A band at the expected 350 bp mark for the $V_L$ gene was observed, showing that the ligation was successful (Figure 4).

![Figure 4: Stained agarose gel of the variable light chain gene from the uncut ligated pFuse plasmid. Lanes 1-4 and 6-9 are ligated PCR products with amplified $V_L$ region at 350 bp. Lane 5 is the ladder for base pair reference.]

CONCLUSION

After many attempts at ligating a $V_L$ gene into an 11,000 bp plasmid, the $V_L$ region of the single chain variable region of a mouse antibody was successfully ligated into the smaller 3500 bp pFuse plasmid. The 350 bp band that appeared on the agarose gel demonstrated this success and the DNA can now be sent out for sequencing. Following this, the antibody DNA will be shipped to a company that will express and purify the mutated antibody. Dyes will then be attached to the antibody, and single-molecule FRET experiments will be performed on the dye-labeled antibodies to determine the Fc-Fab distances present.
REFERENCES

