GPR15L-GFP: Cloning A Novel Fusion Protein for Immune Cell Migration and Visualization Studies

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Introduction

Tagging a protein with Green Fluorescent Protein (GFP) is an effective method for studying the protein's properties, as it allows for visualization within living cells[4]. By cloning the human GPR15L (hGPR15L) with a GFP tag, we can provide insights into the protein's behaviors and functions.

Cloning:

- Cloning is a molecular biology technique used to create copies of a specific DNA sequence, allowing for the manipulation and study of genes and proteins in various systems[6].
- This process often involves the use of restriction enzymes to cut DNA at specific sequences and plasmids as vectors to insert the desired DNA into host cells for replication and expression[6].

Green Fluorescent Protein (GFP):

- GFP is a protein with a sequence of amino acid residues which, in response to UV light, will generate a vivid, green fluorescent signal[4,5].

GPR15L:

- GPR15L is expressed and found in a variety of epithelia, including (but not limited to) the colon, skin, cervix, and stomach, indicating that GPR15L may play a part in the protection of various external body surfaces[2,3].
- GPR15L is a natural ligand of the GPR15 receptor. GPR15 is a G protein-coupled receptor (GPCR) that manages regulatory T cell migration to the gut, indicating a larger role in the pathogenesis of chronic inflammatory and autoimmune diseases[1,2].
- However, the physiological implication of GPR15L remains largely unexplored and the GPR15L-GPR15 interaction has not been well studied.

Objective: Clone the GPR15L protein with a GFP tag to be able to understand the mechanisms with which it interacts with its receptor (GPR15) in autoimmune diseases and skin conditions.

Methodology

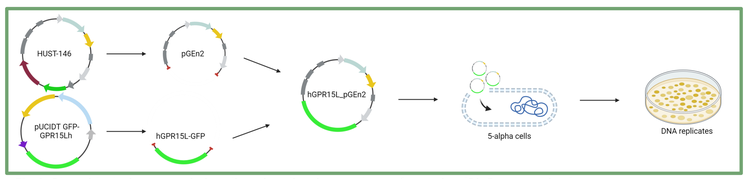

Fig 1: *Plasmids during a restriction digestion, ligation, and transformation [BioRender object]*

- **Restriction Digest:** Using the restriction enzymes SalI and NotI, specific sites of our given plasmids were cleaved. From the HUST-146 cells, we cut out the pGEn2 vector. We cleaved the insert hGPR15L-GFP from a plasmid with an IDT vector.
- **Agarose Gel Electrophoresis:** An electric current ran through the gels containing the vector/insert solutions, separating the DNA fragments.
- **Gel Extraction:** The insert/vector was removed from the gel by cutting out the desired bands under UV light.
- **Ligation:** An enzyme, DNA ligase, joined the pGEn2 vector with the hGPR15L-GFP insert permanently. This created hGPR15L_pGEn2 plasmid.
- **Transformation:** The new, foreign DNA was introduced to 5-alpha cells (cells with high transformation efficiency) which allowed the DNA to make multiple copies of itself.
- **Sequencing:** The cloned DNA was sent for sequencing to determine the nucleotides were in the correct place.

Results

pGEn2 Vector Cleaving

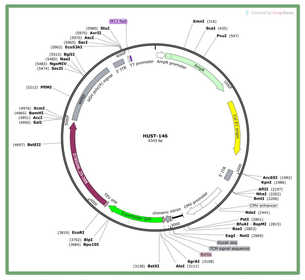

Fig 2.0: *SnapGene plasmid map sequence of the pGEn2 vector*

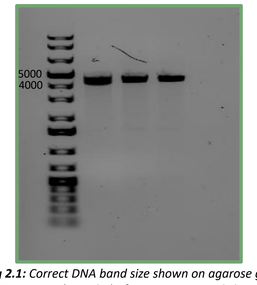

Fig 2.1: *Correct DNA band size shown on agarose gel for pGEn2 vector (4362 bp) after extraction and cleanup*

hGPR15L-GFP Insert Cleaving

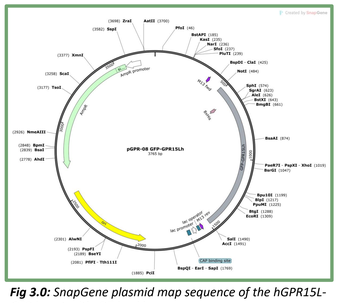

Fig 3.0: *SnapGene plasmid map sequence of the hGPR15L-GFP insert with IDT backbone*

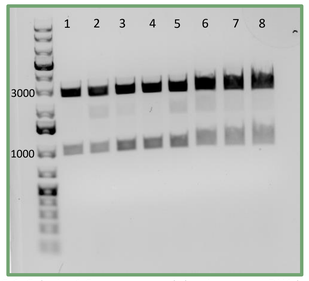

Fig 3.1: *Colonies 1-8 on agarose gel showing correct DNA band sizes for hGPR15L-GFP insert (1006 bp) with IDT backbone (2759)*

Ligation of pGEn2 Vector with hGPR15L-GFP Insert

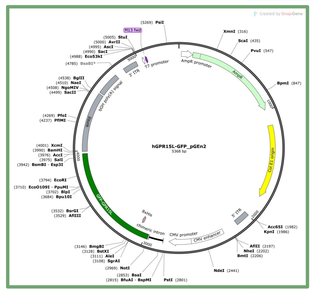

Fig 4.0: *SnapGene plasmid map sequence of the new hGPR15L_pGEn2 protein*

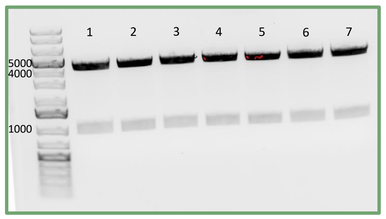

Fig 4.1: *Colonies 1-7 on agarose gel after ligation and transformation in 5 alpha cells showing correct band sizes, indicating successful transformation*

Sequencing Results

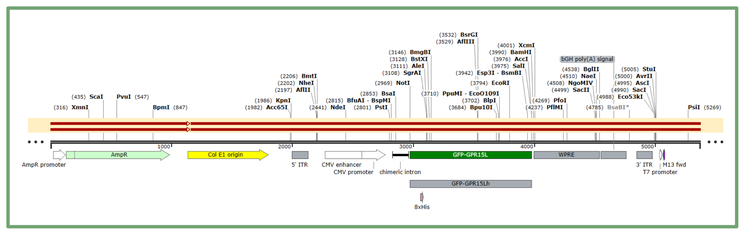

Fig 5: *DNA sequencing results confirming the cloning was successful*

Conclusion

- Using SalI and NotI restriction enzymes, the vector pGEn2 and insert hGPR15L-GFP were successfully cleaved from their plasmids.
- pGEn2 and hGPR15L-GFP were successfully ligated into hGPR15L_pGEn2, as confirmed by the agarose gel (Fig. 4.1).
- After transforming hGPR15L_pGEn2 in 5-alpha cells, colony growth was observed.
- The sequencing results confirmed the GFP tag was correctly inserted, and the protein construct was as intended (Fig. 5).

Future Directions

The successful cloning of the hGPR15L-GFP fusion protein open several promising avenues for further research:

- Investigate the functional role of GPR15L-GFP in immune cell migration using various in vitro and in vivo models.
- Utilize advanced microscopy techniques to conduct live cell imaging to observe the real-time dynamics of GPR15L-GFP in different cellular environments.
- Better understand the structure-function relationship of GPR15L and its interaction with GPR15 and other proteins.
- Investigate the therapeutic potential of targeting the GPR15-GPR15L axis in immune-related diseases by exploring how modulating this pathway can influence disease progression.

Acknowledgements

- I would like to thank Dr. Lisa Willis, Adura Etuk, and the rest of the Willis Lab for their endless support and help this summer.
- Special thanks to Ehlam Iftikhar for introducing me to the program and guiding me through the whole process.
- Thank you to my sponsors for allowing this project to be possible.
- Great thanks to my family and friends for supporting me throughout this whole journey.
- Finally, I would like to thank WISEST for creating this program and allowing me to participate in this remarkable opportunity.

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