Characterizing the Binding Efficiency of NR5A1's G35E Mutation in a Synthetic System

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Motivation

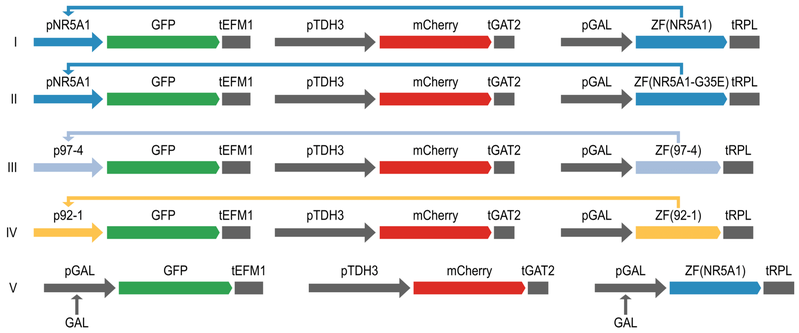
The NR5A1 gene, also referred to as Steroidogenic Factor-1, is heavily implicated in adrenal and reproductive function due to its role in steroidogenesis.

Mutations in NR5A1, such as the G35E mutation, have been implicated in adrenal insufficiency and other endocrine disorders, so by understanding its impact on a synthetic reporter, insights may be made about the gene's native function.

Objectives and Hypothesis

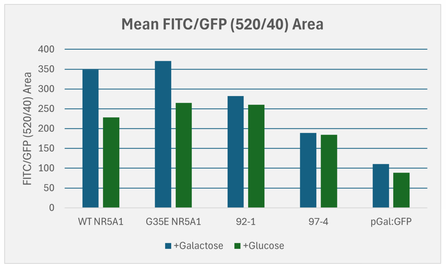
The objective of this project was to determine how specific single nucleotide polymorphs would impact the binding efficiency of NR5A1 to a reporter construct using Green Fluorescent Protein (GFP). It was hypothesized that the synthetic architecture containing the mutant ZF's would bind with less efficiency, and this would be measured with a slight decrease in GFP expression.

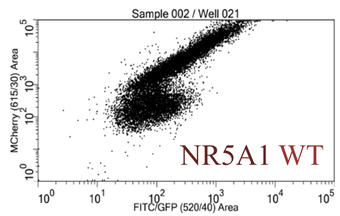
Circuit Topology



*All circuit topologies are induced by Galactose

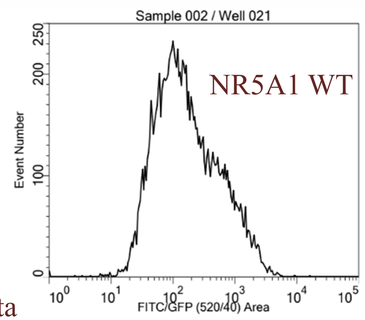
Results





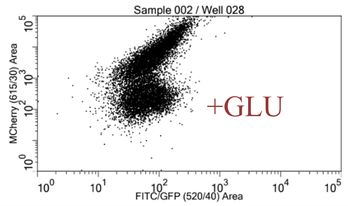
NR5A1 WT Example Flow Data

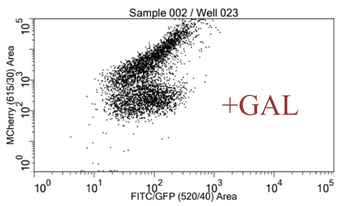
Flow cytometry analysis of the desired topologies under both Glucose and Galactose induction



Discussion and Future Work

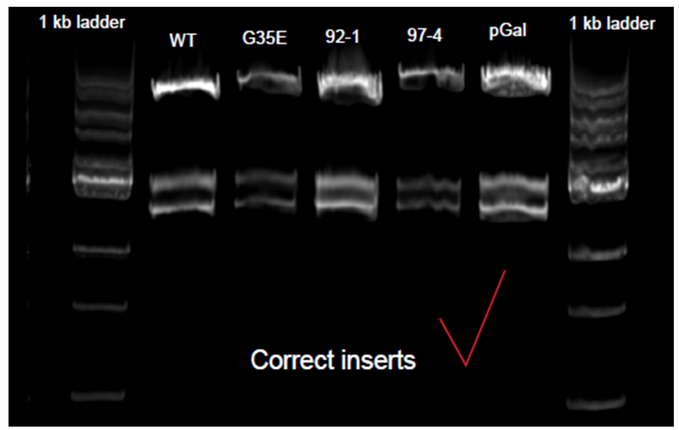
It was expected that G35E would fluoresce at about 75% as much as the WT. The actual expression of the G35E was greater, but not by a large margin. Considering the expression seen in the pGal:GFP vector, it can be inferred that the modified pGal promoter was not truly functional and that any ZF expression was due to basal machinery leakage. Therefore, to produce more meaningful results, pGal will be modified to include its full bidirectional sequence, and this data will be used for accurate comparisons of the synthetic ZF architectures.





Methods

Golden Gate Cloning using BSA-I and SAP-I



Gel of Restriction of complete vectors, confirming accurate parts

For preparing DNA:
- Grew Inserts on E Coli with C+/K+ and Red/White screening

For preparing Yeast (prior to data collection):
- Grew colonies on plates with U- and utilized mCherry screening
- Grew liquid media colonies in Glucose and Galactose for 24h

Flow Cytometry was used for measuring GFP and mCherry fluorescence. mCherry was constitutively expressed, while GFP was only expected to be expressed conditionally under Galactose induction.

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