CRISPR/Cas9 gene editing and TSHR knock-out to understand its correlation with Graves' Disease (GD).

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Background

- Graves' Disease - autoimmune hyperthyroidism
  - Body's immune system releases abnormal antibodies that acts like the TSHR and attacks the immune system
  - Overactive thyroid gland - Increased T4 levels and T3 levels

TSHR Genetics

- Gene present on chromosome 14q31
- 10 exons on this gene
- 10th exon codes for the G protein complex which allows for the cascade of reactions leading to TSH production

Deletion (Gene Knockout) of TSHR Protein

- **Biol. question:** TSHR under expression is pertinent for Graves' Disease (GD)
- **Hypothesis:** TSHR gene mutations can cause disorders associated with Graves' Disease (GD). Knocking out the gene can potentially improve pathological phenotype in patients.

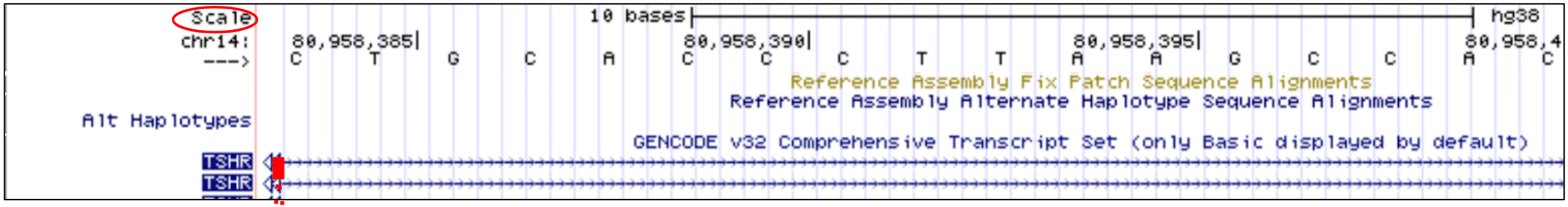
How to test:

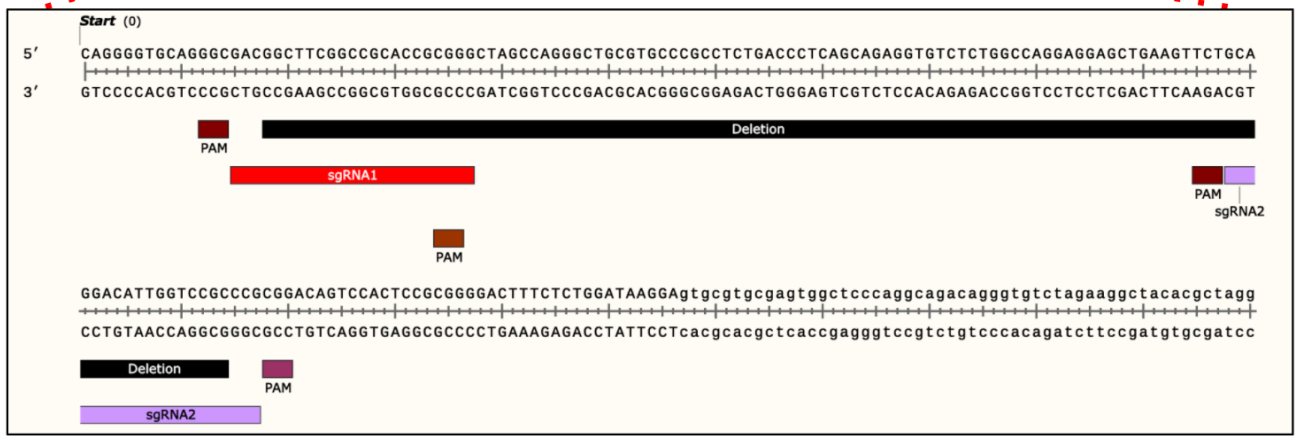
- CRISPR/Cas9
  - TSHR deletion Nonsense-polypeptide

<u>K-562 cell line</u>

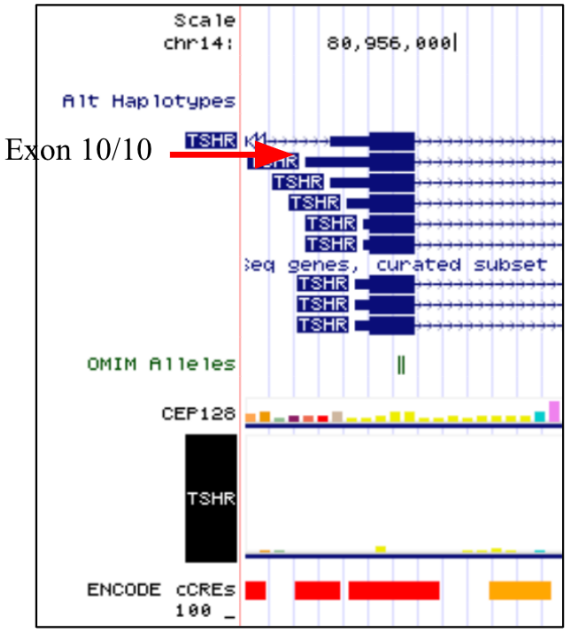
- Lymphoblasts isolated from bone marrow of chronic myelogenous leukemia patient

Deletion (Gene Knockout) of TSHR Protein





- Deletion → 107 bp
- 5 different isoforms
- sgRNA 1 → 23 bp



Procedure

- Protocol:
  - K-562 cell line and would create a CRISPR/Cas-cell-suspension with the guide RNA and Cas9 protein
  - The cells would then be electroporated; this would result in a semi-permeable cell membrane, allowing the sgRNA and Cas9 protein into the cell
  - If cell pool results in no positive clones, the transfection process would be repeated, and the Cas9/gRNA master mix would be adjusted
  - Check for TSHR expression
- Next steps for this knockout protocol would be to conduct it in a sterile, laboratory environment.

gRNA Efficiency

- Used a publicly available package CRISPRseek
- Calculates gRNA Efficiency for a given set of sequences and feature weight matrix
- Arguments
  - Sequence containing gRNA plus PAM (size of gRNA)
  - Indicates parallel processing abilities
  - Data frame (feature weight matrix) to calculate GC content

Pseudocode

- GC content
  - Finding the length of the gRNA, filtering to the G and C content
  - GC content = G content + C content
- Feature weight matrix
  - First column containing significant features and the second column containing the weight of corresponding features
  - Features include low/high GC content in the gRNA sequence, G02 - G at second position of the sequence and GT02 - GT di-nucleotide at 2nd position of the sequence
- The output of the function is is the form of a data frame where the efficiency of the gRNA is this data frame multiplied with the feature weight

Data Analysis

- Data when laboratory experimentation is conducted
  - Genomic DNA would be evaluated using PCR
  - To check for TSHR expression - immunostaining and western blot
    - Look up available antibodies for TSHR and do a protein analysis
- Data obtained from computation analysis
  - To calculate gRNA efficiency, a R package, CRISPRseek was used
    - All online datasets and other resources used are publicly available

Results

- The predicted accuracy for the TSHR knockout was 32.9%
  - In comparison to the currently predicted industry average efficiency (knockout score) of 34%
  - The success rate of CRISPR experiments is dependent on the efficiency of the gRNA

Discussion

- Future research can be conducted that studies therapeutic methods for potentially curing GD
  - Partial knockout of TSHR gene
  - CRISPR/Cas9 on Car-T cells

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