Qualitative Comparison of BIG TREE Gene Editing with respect to the creation of Alzheimer's Disease -relevant Isogenic Lines

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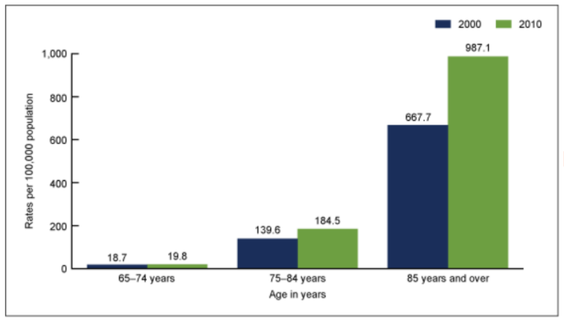
Research Inspiration

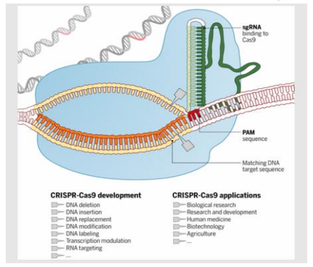
Alzheimer's disease is one of the Sixth leading causes of death in the United States[1]. Currently there is known cure or treatment for Alzheimer's Disease (AD). Since most AD cases are sporadic, it makes this disease difficult to study in vitro. Thus, there is a need for the ability to create isogenic lines to study single genes and their roles in neurodegenerative diseases. Genomic engineering can be used to create edits in the genome to generate isogenic lines to study genes.

Figure 2. Age-adjusted death rates for Alzheimer's disease: United States, 2000 and 2010



SOURCE: National Vital Statistics System, Mortality.

Objective

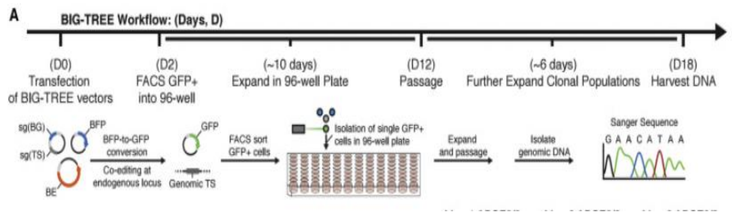


The figure above displays Cas9 enzyme creating double stranded DNA breaks [2].

The goal of this research project was to use genomic engineering to create edits in the genome to generate isogenic lines to study genes specific to Alzheimer's Disease. Due to the pandemic, research has been limited to examining papers with methods that use a genome editing tool such as the clustered regularly interspaced short palindromic repeat (CRISPR/cas9).

Research Methods

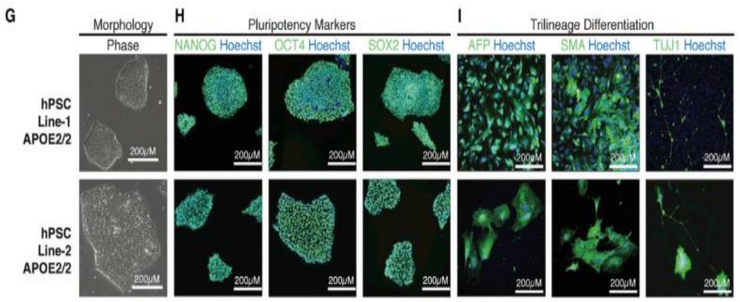
The research methods used in the papers examined involve gene editing tools such as CRISPR and BIG TREE.



The figure above displays a schematic for the generation of clonal isogenic hPCS lines using BIG-TREE[3].

Preliminary Findings

A gene editing method known as BIG TREE has resembled the needed methods in order to create isogenic lines. BIG-TREE adopts the method of TREE by co-transfecting a blue fluorescent protein (BFP) variant that converts to a green fluorescent protein (GFP) with a C to T nucleotide change with CRISPR/Cas9 systems. This BFP plasmid acts as a visual indicator that base-editing is occurring within the cell. By single-cell sorting for GFP(+) cells using a fluorescence activated cell sorter (FACS), it was found that edited cells could be enriched for up to 80% of editing efficiency. These cells can then be sorted into 96-well plates and grown into isogenic lines with specific genomic edits.

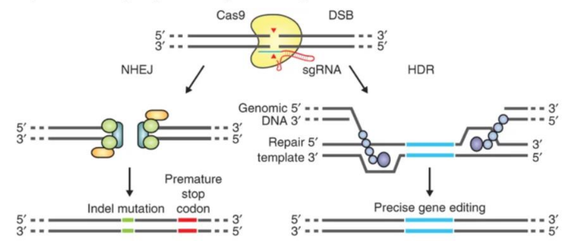


The figure above displays clones edited at the APOE locus using the method BIG TREE[1].

Study Expansion/ Further Investigation

Further investigations could involve using genomic editing tools such as CRISPR/Cas9 to introduce double-stranded breaks in DNA at targeted sites by utilizing archaeal and bacterial Cas9 nucleases. These breaks can be used to remove, replace, or add pieces of DNA. This allows for the possible creations of genomic edits to the DNA sequence in order to generate induced pluripotent stem cell (iPSC) lines to study genes relating to neurodegenerative diseases.

Figure 2: DSB repair promotes gene editing.



The figure above displays the one of two ways that the DSBs introduced by Cas9 (yellow) can be repaired [4].

Acknowledgements

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References:
[1] Brookhouser, Nicholas, et al. "BIG-TREE: Base-Edited Isogenic HPSC Line Generation Using a Transient Reporter for Editing Enrichment." *Stem Cell Reports*, Cell Press, 30 Jan. 2020, www.sciencedirect.com/science/article/pii/S2213671119304527.
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[3] "Products - Data Briefs - Number 116 - March 2013." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 6 Nov. 2015, www.cdc.gov/nchs/products/databriefs/db116.htm.
[4] Ran, F Ann, et al. "Genome Engineering Using the CRISPR-Cas9 System." *Nature News*, Nature Publishing Group, 24 Oct. 2013, www.nature.com/articles/nprot.2013.143.

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