Project 18049 - Humanization of ~ 2kb region of mouse *Clu* locus to introduce LOAD SNP risk variant *rs2279590*

TMF 1304 - MegaMer used to generate the “hClu-2kb” allele.

*CGAGATAGTTTTGCTCGGAATTCTCTAGGCATCCAGAAATGACAGATAACCCGCAGATCCATCGTCTAGATGTATAAGAAGCATGGGGTCAGCTCTCTAGGTTTCCTTGGAGgAGCAGGAGGACTTCCTTAcCAGAAGCCTGACTTCTGTTGCAGAGCGCATGCATTTTGACCACAGTGTTTCAGCTCTTCCCTTTTCTCTTGTTCCATTTAG***GTGGCTTCCCACACTTCTGACTCGGACGTTCCTTCCGGTGTCACTGAGGTGGTCGTGAAGCTCTTTGACTCTGATCCCATCACTGTGACGGTCCCTGTAGAAGTCTCCAGGAAGAACCCTAAATTTATGGAGACCGTGGCGGAGAAAGCGCTGCAGGAATACCGCAAAAAGCACCG***GTAAGCA*G*GCGGGCCTTTCCTGCGGGCCTGCAGGGCCCAGTGAGTCTCTGGGAGCCACAAAAAAACAAACAAAGTGCAGACTCTATAGCCTGGTGGGAACGACTCCGCCCGGAGCCAGAGCCCAAGAACAAAGCCAGGAAGTTACGGGGGAATTTTATTTTTCCTTTGGAGGATGTTTTACTTTGGAGGATAACTGTTTTTTATTTCAG***GGAGGAGTGAGATGTGGATGTTGCTTTTGCACCTACGGGGGCATCTGAGTCCAGCTCCCCCCAAGATGAGCTGCAGCCCCCCAGAGAGAGCTCTGCACGTCACCAAGTAACCAGGCCCCAGCCTCCAGGCCCCCAACTCCGCCCAGCCTCTCCCCGCTCTGGATCCTGCACTCTAACACTCGACTCTGCTGCTCATGGGAAGAACAGAATTGCTCCTGCATGCAACTAATTCAATAAAACTGTCTTGTGAGCTGATCGCTTGGAGGGTCCTCTTTTTATGTTGAGTTGCTGCTTCCCGGCATGCCTTCATTTTGCTATGGGGGGCAGGCAGGGGGGATGGAAAATAAGTAGAAACAAAAAAGCAGTGGCTAAGATGGTATAGGGACTGTCATACCAgtgaagaataaaagggtgaagaataaaagggATATGATGACAAGGTTGATCCACTTCAAGAATTGCTTGCTTTCAGGAAGAGAGATGTGTTTCAACAAGCCAACTAAAATATATTGCTGCAAATGGAAGCTTTTCTGTTCTATTATAAAACTGTCGATGTATTCTGACCAAGGTGCGACAATCTCCTAAAGGAATACACTGAAAGTTAAGGAGAAGAATCAGTAAGTGTAAGGTGTACTTGGTATTATAATGCATAATTGATGTTTTCGTTATGAAAACATTTGGTGCCCAGAAGTCCAAATTATCAGTTTTATTTGTAAGAGCTATTGCTTTTGCAGCGGTTTTATTTGTAAAAGCTGTTGATTTCGAGTTGTAAGAGCTCAGCATCCCAGGGGCATCTTCTTGACTGTGGCATTTCCTGTCCACCGCCGGTTTATATGATCTTCATACCTTTCCCTGGACCACAGGCGTTTCTCGGCTTTTAGTCTGAACCATAGCTGGGCTGCAGTACCCTACGCTGCCAGCAGGTGGCCATGACTACCCGTGGTACCAATCTCAGTCTTAAAGCTCAGGCTTTTCGTTCATTAACATTCTCTGATAGAATTCTGGTCATCAGATGTACTGCAATGGAACAAAACTCATCTGGCTGCATCCCAGGTGTGTAGCAAAGTCCACATGTAAATTTATAGCTTAGAATATTCTTAAGTCACTGTCCCTTGTCTCTCTTTGAAGTTATAAACAACAAACTTAAAGCTTAGCTTATGTCCAAGGTAAGTATTTTAGCATGGCTGTCAAGGAAATTCAGAGTAAAGTCAGTGTGATTCACTTAATGATATACATTAATTAGAATTATGGGGTCAGAGGTATTTGCTTAAGTGATCATAATTGTAAAGTATATGTCACATTGTCACATTAATGTCACACTGTTTCAAAAGTTA**CTGTACAGGCACTCAAGAGAACAAAGATGGTTGTTAGTTGGTACCAGGCACCTCTGTGACTCCACCTT

Key

Mouse flanking sequence

Humanized sequence

**EXON**

*INTRON*

**TGA** - seems to be the most commonly used site for addition of poly A (after the ‘A’)

*Mouse intron 7* - human intron 7 - **human exon 8 -** *human intron 8 -* **human exon 9 - human longest 3’ utr -** mouse intergenic region

*rs2279590* is located within an enhancer element in intron 7 of human *CLU*. G is risk allele. A is non-risk allele. Ideally, we would also have mice with the non-risk allele for comparison as control for humanization of this region. If the expression (i.e. splicing, polyadenylation etc) of this allele looks OK, we could look at whether we could switch the risk allele SNP (G) to the non-risk allele SNP (A) via CRISPR.

SNPs found in hClu2kb rs mouse ID 16848

G = G/T

C = C/A

Question - Which bases are in the mouse line being used ? Check Bulk-RNA-seq files to see if the SNP is segregating in these mice.

Structure of 3’ end of *ApoJ* / *Clusterin* locus in mouse and human Showing exons 7, 8 and 9.

Mouse (BLAST search shows mouse sequence in MegaMer)

Timeline

Description automatically generated with low confidence

Human (BLAST search shows human sequence in MegaMer) Note extended exon 9 in some transcripts.

A picture containing timeline

Description automatically generated