

Figure 1

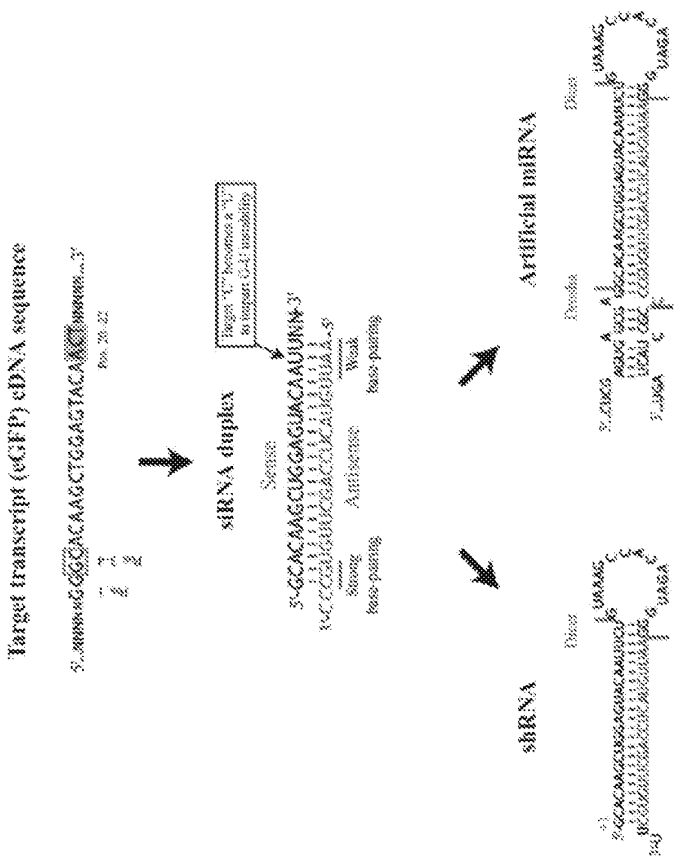


Figure 2. Processing of RNAi Mediators Expressed from DNA

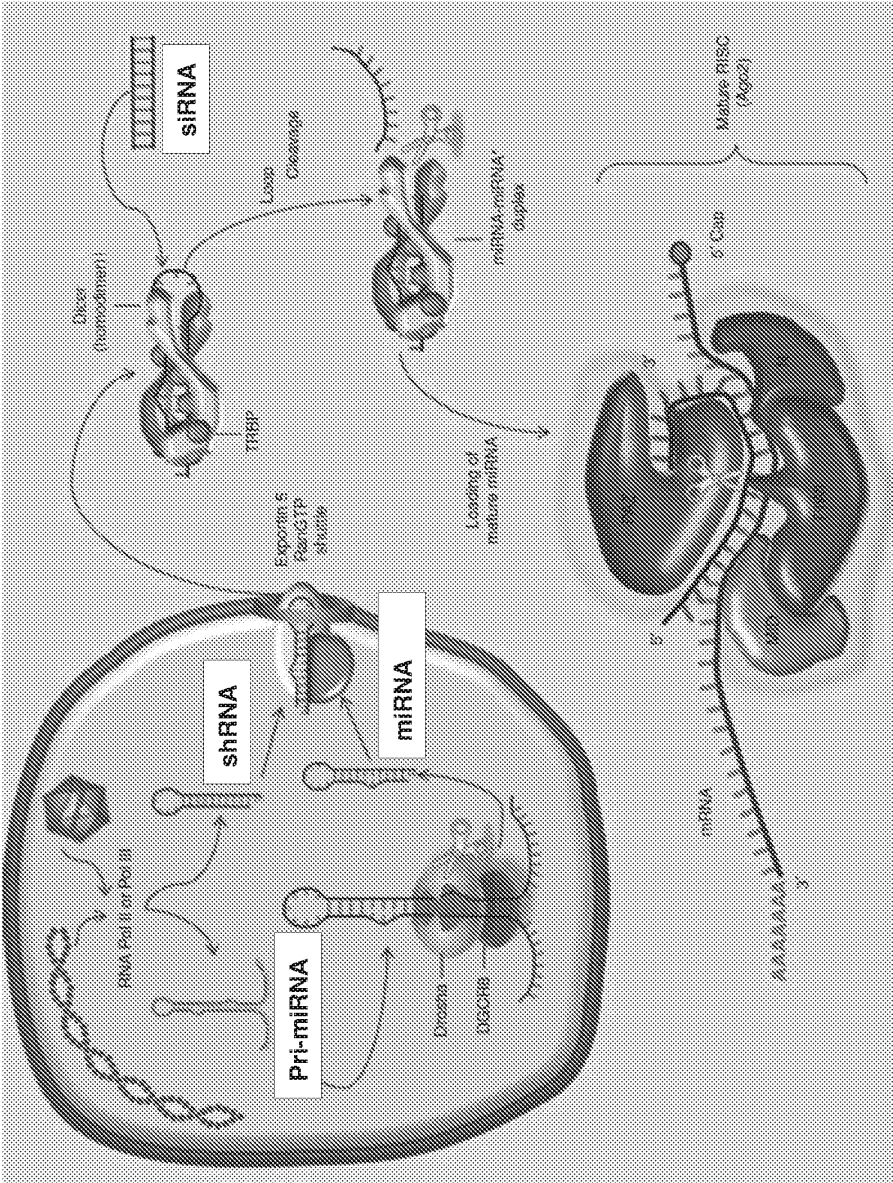


Figure 3. Construct Configuration 1

CAG promoter drives hAPOE2-HA and miRNA

A. Insertion in the promoter intron B. Insertion between transgene and polyA

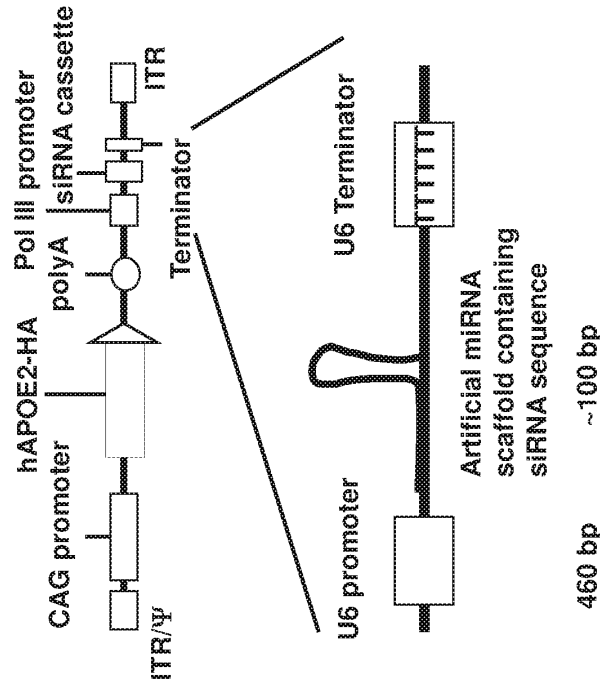


- Multiple miRNAs can be placed in tandem for enhanced silencing of APOE4
- Level of hAPOE2-HA and miRNA expression are similar
- Lower level of miRNA expression (compared with U6 promoter) for fewer off-target effects and lower potential for toxicity

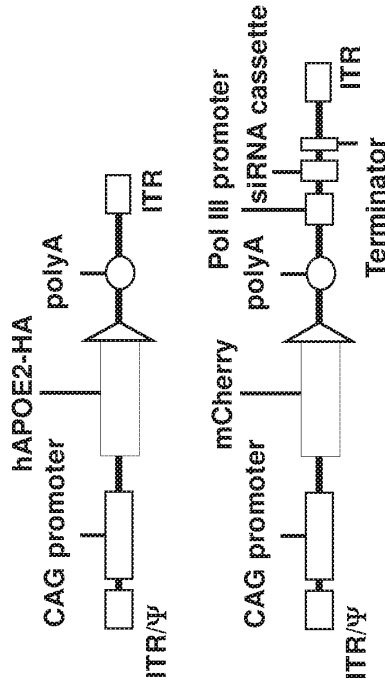
Figure 4. Construct Configurations 2

A constitutive promoter such as CAG drives hAPOE2-HA; U6 (Pol III) promoter drives miRNA

A. One vector



B. Two vectors

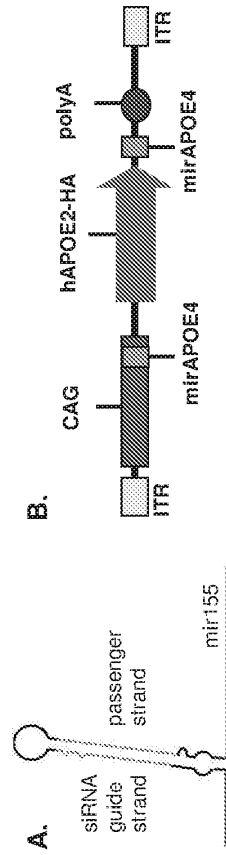


- Multiple miRNAs placed in tandem to enhance silencing of APOE4
- Pol III promoters used for transcription of rRNA, tRNA, miRNA
- Defined terminator, no poly A needed
- U6 (Pol III) drives high levels of miRNA expression
- Two vector system includes reporter gene to maintain length and track expression

Total length: ~4000 bp

Figure 5: Silence ApoE4, Express ApoE2

CAG promoter drives hApoE2-HA and miRNA



*There is room to insert several miRNA in tandem

- miRNA will knockdown all APOE
- Vector derived APOE2 is resistant to miRNA
- By using CAG promoter, level of hApoE2-HA and miRNA expression are similar
- Lower level of miRNA expression (compared with U6 promoter) may mean less silencing, however also fewer off-targets and toxicity
- miRNA can be inserted in the CAG intron or 3' untranslated region

Figure 6. Testing APOE Knockdown Efficiency by siRNAs in U87 Cells

- 4 different siRNAs targeting the coding sequence of APOE were generated based on comparison of multiple siRNA design algorithms
 - siRNAs were transfected into U87 cells (astroglioma cell line), and APOE mRNA copies were quantified by RT-qPCR
- Target sequences:
- GGUGGAGCAAGCGUGGAGuu
 - GGAGUUGAAGGCCUACAAuu *
 - GGAAGACAUGCAGCGCCAGuu
 - GCGCGGGAUGGAGGAGAUuu
- Non-targeting GTAGCGACTAACACATCAuu
- *siRNA targeting same region showed 90% knockdown in Casey 2015 JBC

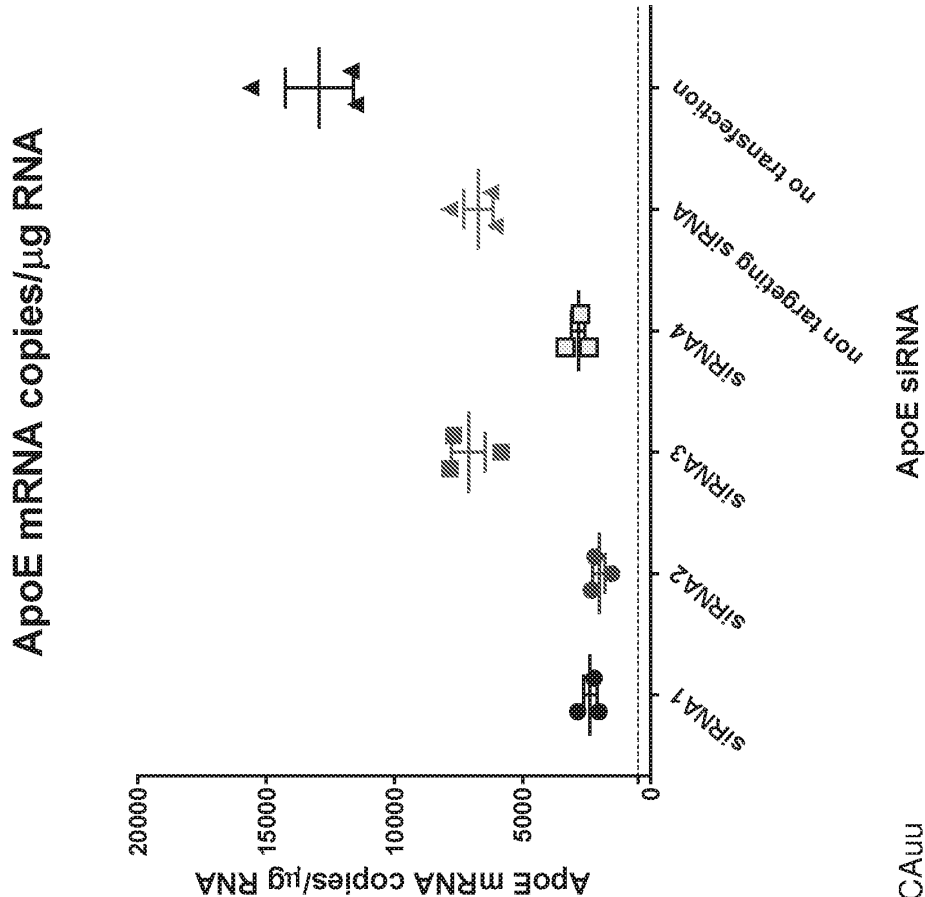
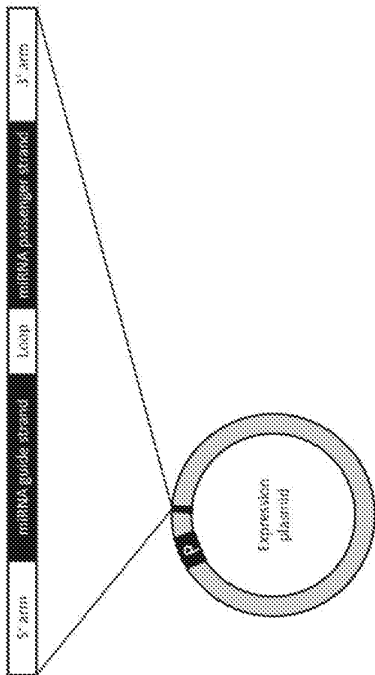


Figure 7. APOE Targeted siRNA Converted to miRNA for Expression in AAV Vector

- The sequence from the best siRNA (#2) was converted into a miRNA
 - Scaffold based on a modified version of mir155 (Fowler et al. 2015 Nuc Acids Res)
 - Mismatches in the passenger strand were chosen for optimal GC content and position
- Two tandem copies of the miRNA were cloned into either the CAG intron or the 3' untranslated region of the pAAV expression cassette

Artificial miRNA cassette in AAV plasmid



Up to four copies of the miRNA can be inserted within the AAV size limits

Figure 8. Gene Therapy for APOE4 Homozygotes at High Risk for Alzheimer's Disease

