

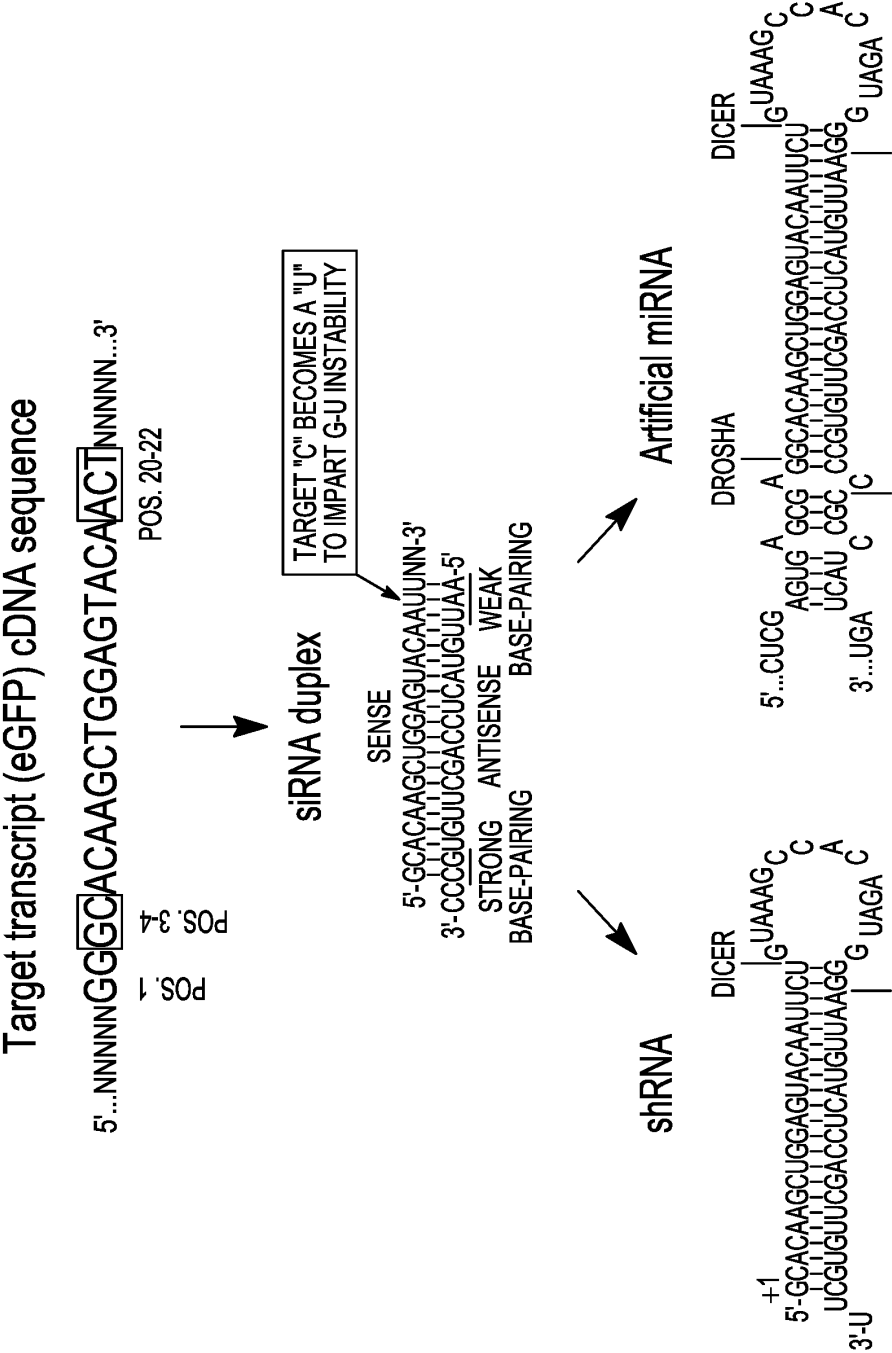


FIG. 1

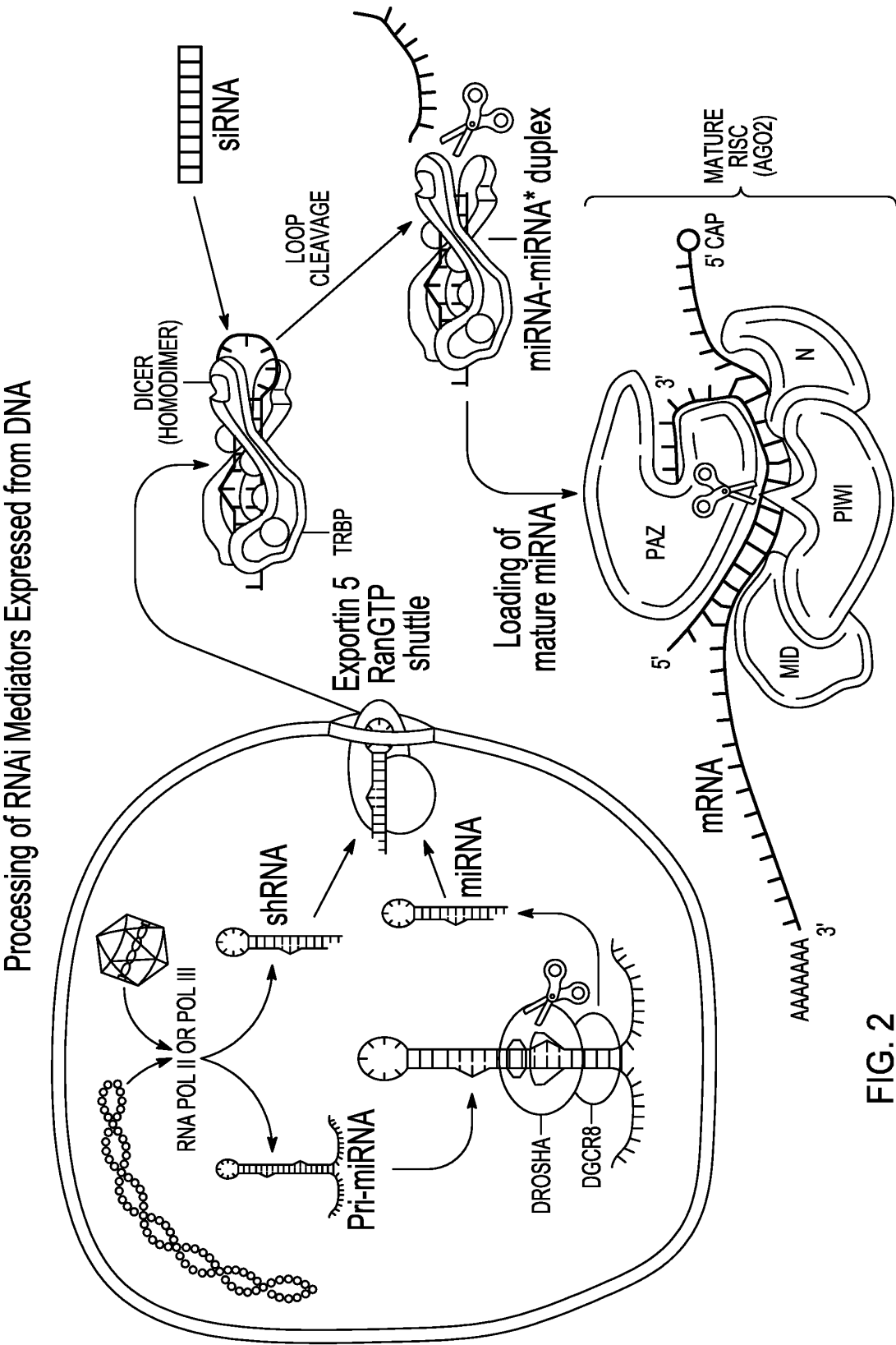


FIG. 2

Figure 3. Construct Configuration 1

CAG promoter drives hAPOE2-HA and miRNA

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

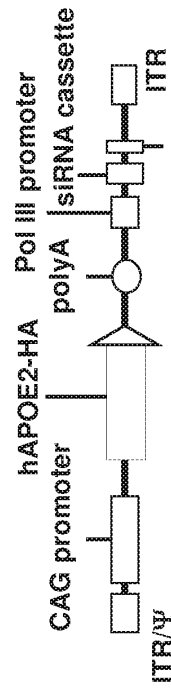


- Multiple miRNAs can be placed in tandem for enhanced silencing of APOE4
- Level of hAPOE2-HA and miRNA expression are similar
- Lower level of miRNA expression (compared with U6 promoter) for fewer off-target effects and lower potential for toxicity

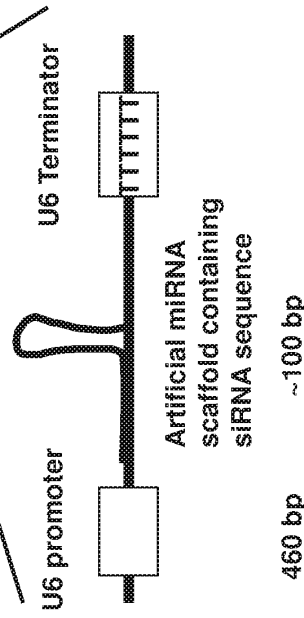
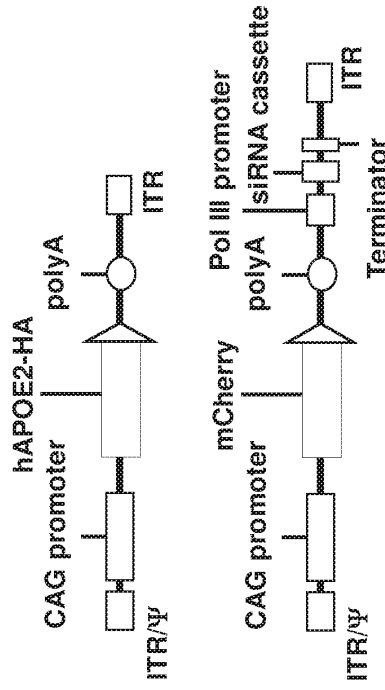
Figure 4. Construct Configurations 2

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

A. One vector



B. Two vectors

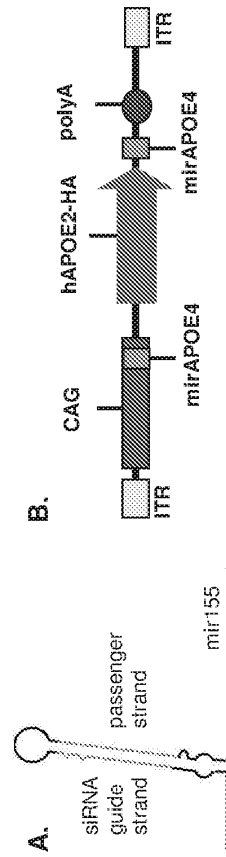


- Multiple miRNAs placed in tandem to enhance silencing of APOE4
- Pol III promoters used for transcription of rRNA, tRNA, miRNA
- Defined terminator, no poly A needed
- U6 (Pol III) drives high levels of miRNA expression
- Two vector system includes reporter gene to maintain length and track expression

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

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:**
1. GGUGGAGCAAGCGUGGAGuu
 2. GGAGUUGAAGGCCUACAAuu *
 3. GGAAGACAUGCAGGCCAGuu
 4. GCGCGCGAUGGAGGAGAUuu
- Non-targeting GTAGCGACTAAACACATCAuu
- *siRNA targeting same region showed 90% knockdown in Casey 2015 JBC

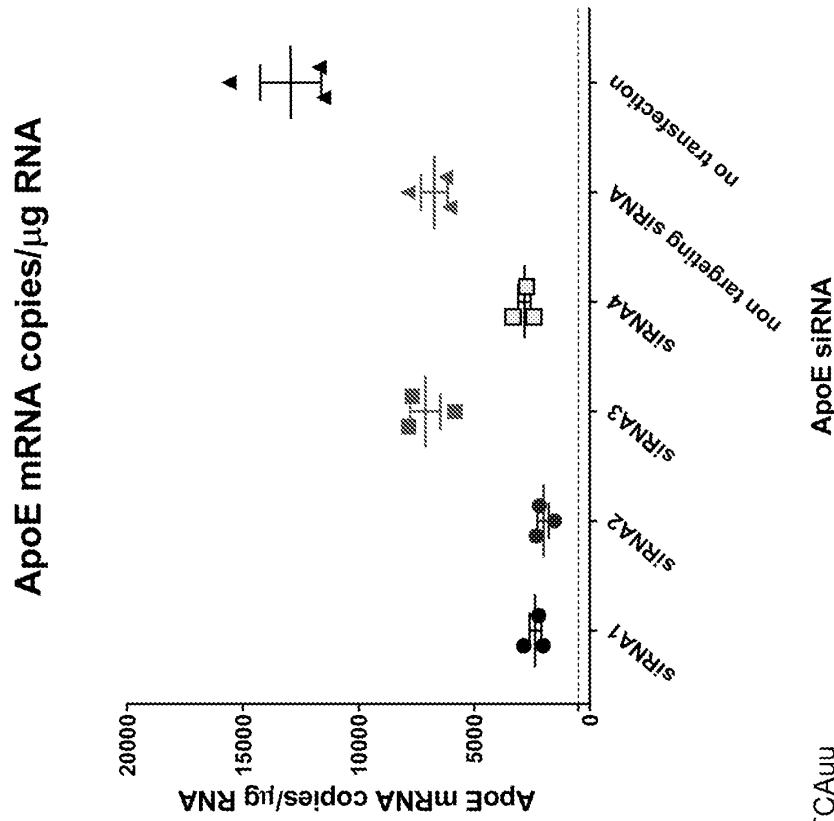


FIG. 6

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 Acids Res)
 - Mismatches in the passenger strand were chosen for optimal GC content and position
 - Mfold used to predict miRNA hairpin secondary structures

- Two tandem copies of the miRNA were cloned into either the CAG intron or the 3' intrantranslated region of the pAAV expression cassette
- Up to four copies of the miRNA can be inserted within the AAV size limits

Artificial miRNA cassette in AAV plasmid

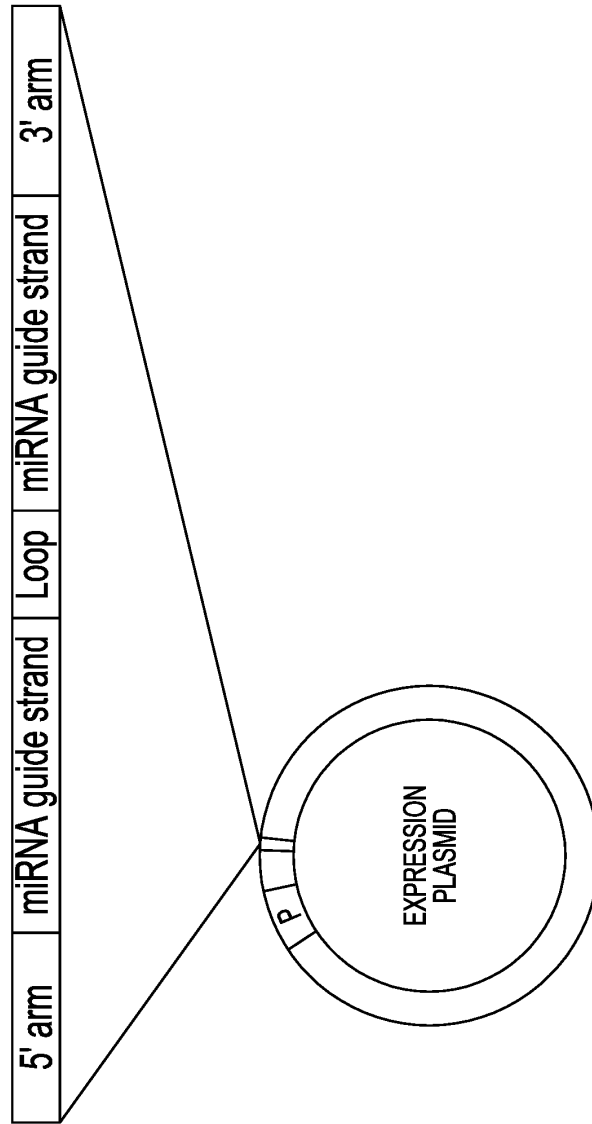
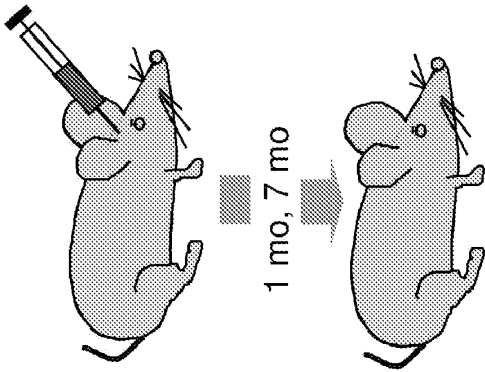


FIG. 7

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

- P301S/E4
 - Control (C57Bl/6)
 - n = 8 M/F group (8 wk)
- AAV9hAPOE2-mirAPOE4
 - AAV9hAPOE2
 - AAV9mCherry-mirAPOE4
 - AAVnull
 - PBS

10¹¹ gc, intracisternal



P301S/E4 mice express mutant human tau and human APOE4

Evaluate

- Behavior / Cognitive Function
- Brain APOE2 & APOE4 expression
- Total and phospho-tau
- Neurofibrillary tangles
- Microglial activation
- Pathology of hippocampus / cortex

FIG. 8