vMiX™ Multiplex: A novel platform for enhanced and versatile miRNA-based gene silencing in neurodegenerative disorders

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AVIADOBIO

vMiX™

P0169

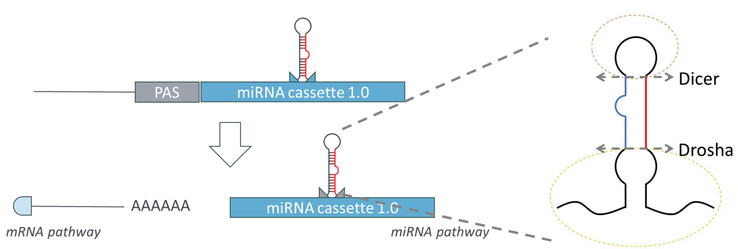
OBJECTIVE

To design an improved miRNA expression platform with enhanced versatility and expression.

INTRODUCTION

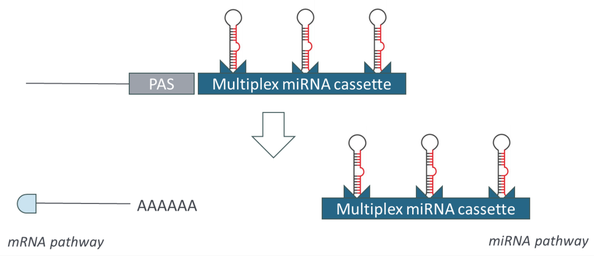
- Neurodegenerative diseases can be driven by protein aggregate accumulation (e.g., ALS, FTD, AD, PD, HD).
- AAV-delivered microRNAs offer a promising therapeutic approach by reducing protein expression. However, achieving consistent miRNA expression and processing across guide sequences remains challenging.
- We previously developed vMiX™ (here, vMiX™ 1.0), an AAV-based RNAi platform enabling efficient processing across diverse guide sequences and gene targets[1] (**Figure 1**).
- The design of vMiX 1.0 allowed early decoupling of miRNA and mRNA pathways to avoid competition between pathways (**Figure 1**).
- To enhance versatility and expression, we created vMiX™ Multiplex, able to express up to three miRNAs.

Figure 1: vMiX™ 1.0: First-generation AAV-based RNA interference platform[1]



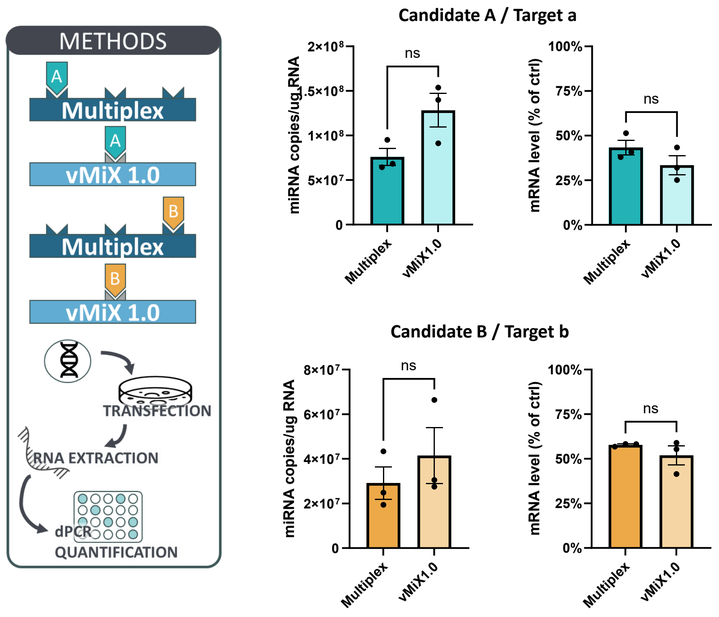
From vMiX 1.0 to Multiplex

Figure 2: vMiX™ Multiplex: Novel AAV-based RNA interference platform



- To build upon the vMiX™ 1.0 platform, we replaced the miRNA cassette sequence of vMiX™ 1.0 with a new Multiplex cassette enabling simultaneous expression of three hairpins (**Figure 2**).
- As with vMiX™ 1.0, the design allowed for decoupling of miRNA and mRNA pathways.

Figure 3: miRNA expression and target knockdown for a single hairpin in vMiX™ Multiplex is comparable to vMiX™ 1.0

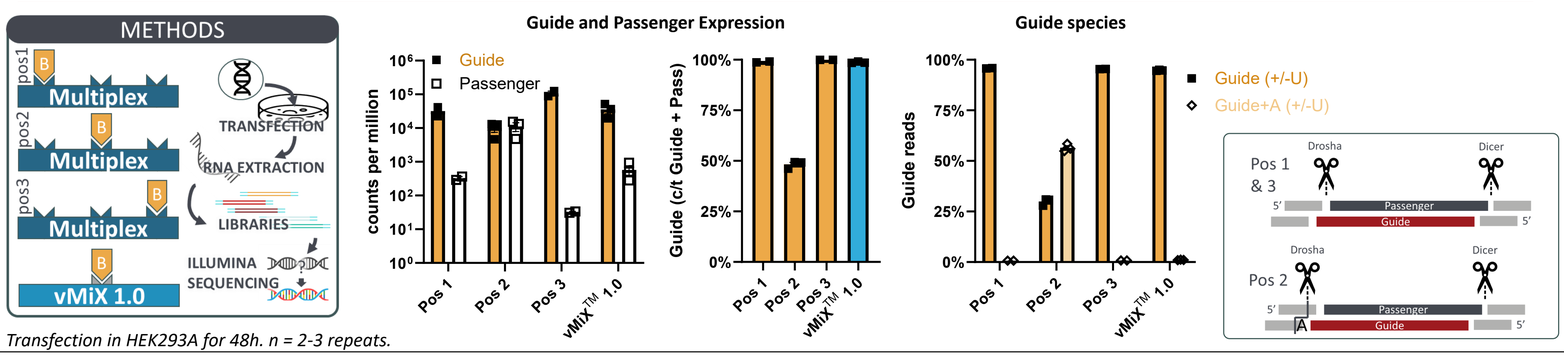


Transfection in HEK293A, n = 3 repeats with each data point as the average of 2 technical replicates. Analysed with Wilcoxon test.

- To test the vMiX™ Multiplex against vMiX™ 1.0, we cloned 2 miRNAs targeting 2 genes into both the vMiX™ 1.0 and the vMiX™ Multiplex.
- We transfected these plasmids into HEK293A cells for 48h before their RNA was extracted and analysed by RT-dPCR.
- Both plasmids expressed similar levels of desired miRNAs and knocked down target genes to similar degrees (**Figure 3**).

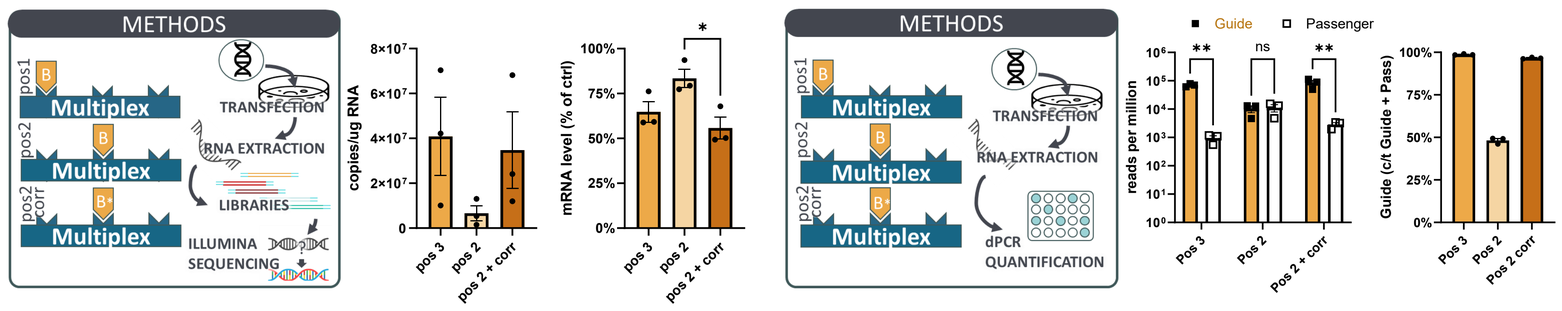
miRNA processing in vMiX™ Multiplex

Figure 4: miRNA processing in positions 1 and 3 reproduces vMiX™ 1.0 performance while hairpins in position 2 show altered Drosha cleavage



Transfection in HEK293A for 48h. n = 2-3 repeats.

Figure 5: Altered Drosha cleavage in position 2 can be restored with passenger sequence optimisation

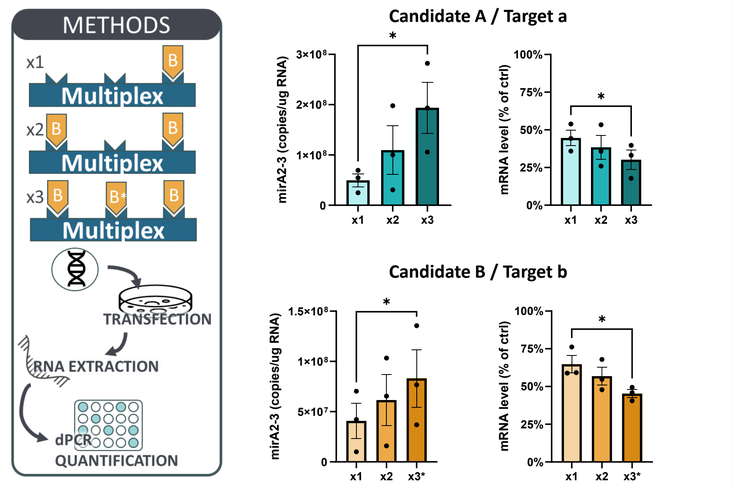


*Transfection in HEK293A for 48h. n = 3 repeats with each data point as the average (left) or n = 3 (right). Analysed with Friedman test (left) and 2-way mixed ANOVA (right). * p<0.05, ** p<0.01*

- To compare processing precision of the vMiX™ Multiplex against vMiX™ 1.0, we cloned one candidate in all three positions of the Multiplex cassette and transfected these plasmids into HEK293A cells for 48h before analysing small RNA expression using NGS small RNA sequencing (Illumina sequencing).
- Hairpins in positions 1 and 3 showed similar processing to vMiX™ 1.0 based on Guide species and Guide and Passenger expression (**Figure 4**).
- Position 2 showed a one-nucleotide Drosha cleavage shift that could affect Guide selection to RISC (**Figure 4**). Accounting for this shift with a minor change to the Passenger sequence ('corr') restored the expected Guide expression and Guide-to-Passenger ratio (**Figure 5**).

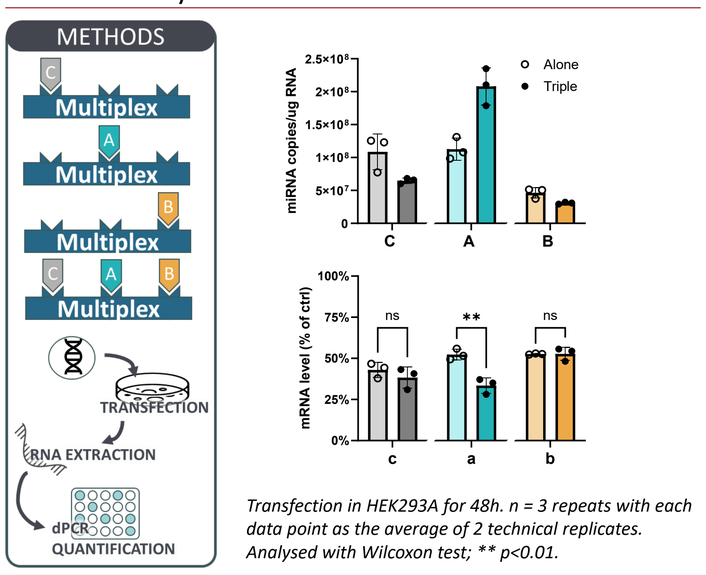
Enhanced miRNA expression and multi-target capability in vMiX™ Multiplex

Figure 6: vMiX™ Multiplex shows increases in miRNA expression and knockdown of target gene.



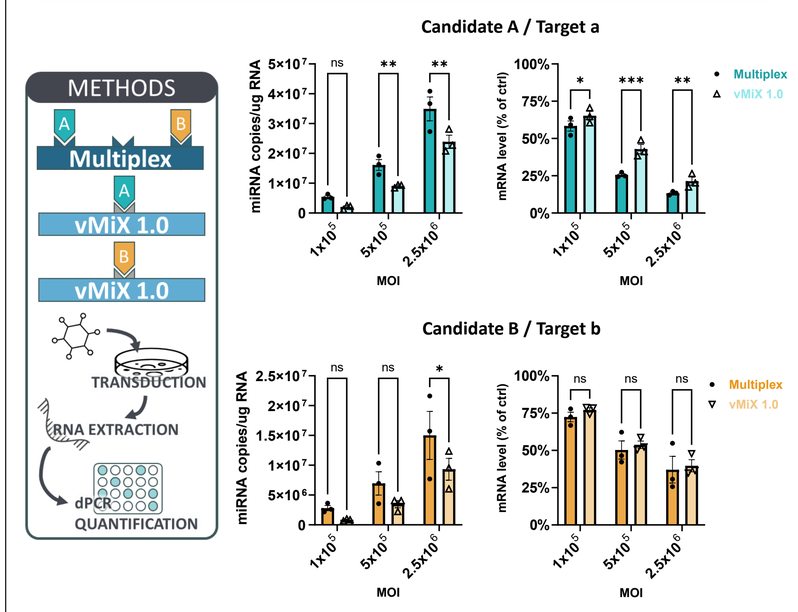
*Transfection in HEK293A for 48h. n = 3 repeats with each data point as the average of 2 technical replicates. Analysed with Friedman test; * p<0.05.*

Figure 7: vMiX™ Multiplex knocks down three targets simultaneously



*Transfection in HEK293A for 48h. n = 3 repeats with each data point as the average of 2 technical replicates. Analysed with Wilcoxon test; ** p<0.01.*

Figure 8: vMiX™ Multiplex packaged in AAV knocks down multiple targets

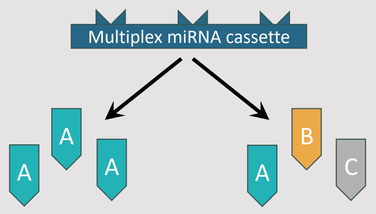


*Transduction in HeLa for 72h. n = 3 repeats with each data point as the average of 2 technical replicates. Analysed with 2-way repeated measures ANOVA. * p<0.05 ** p<0.01 ***p<0.001, **** p<0.0001*

- To test vMiX Multiplex expression and versatility, we cloned multiple copies of identical hairpins or distinct hairpins and transfected them into HEK293A cells for 48h.
- vMiX Multiplex showed that increased hairpin copies enhanced miRNA expression and knockdown (**Figure 6**), and that three distinct hairpins could simultaneously express three different miRNAs and knock down three target genes (**Figure 7**).
- To assess AAV-packaged performance, we packaged vMiX Multiplex with two hairpins and vMiX 1.0 with the same hairpins into AAV9s, then transduced HeLa cells for 72h before RNA extraction and analysis.
- The vMiX Multiplex AAV demonstrated successful transduction into cells and knocked down both target genes in a dose dependent manner (**Figure 8**).
- For further insights into vMiX™ Multiplex AAV packaging and transduction, please visit posters **P0177** and **P0178**.

CONCLUSIONS

- We assessed the novel vMiX™ Multiplex platform for expression efficiency and target knockdown using transfection and transduction.
- vMiX™ Multiplex platform permits the simultaneous expression of three identical or distinct hairpins without compromising expression and knockdown performance.
- vMiX™ Multiplex (vMiX™ 2.0) successfully builds upon the existing vMiX™ 1.0 to offer improved efficiency and versatility to miRNA-based therapeutic approaches.



REFERENCES: [1]Joubert R, et al. Poster presentation at ESGCT Annual Congress 2024.

ABBREVIATIONS: **AAV9:** adeno-associated virus serotype 9; **AD:** alzheimer's disease, **ALS:** amyotrophic lateral sclerosis; **FTD:** frontotemporal dementia; **HD:** huntington's disease; **miRNA:** micro RNA; PAS: polyadenylation sequence; **PAS**: polyadenylation signal; **RISC**: RNA-induced silencing complex.

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