#7555

Detection of miRNAs Associated with Alzheimer's Disease by Electrochemiluminescence

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❶ Purpose

MicroRNA (miRNA) detection has become a powerful tool to understand disease pathology, which has led to an increasing interest in the use of miRNAs as molecular biomarkers. However, miRNA quantification is almost exclusively conducted through miRNA sequencing or singleplex measurements that require amplification. We sought to develop a simple-to-use, multiplexed assay panel for measuring miRNAs associated with Alzheimer's disease from brain tissue across multiple species.

❷ Methods

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.
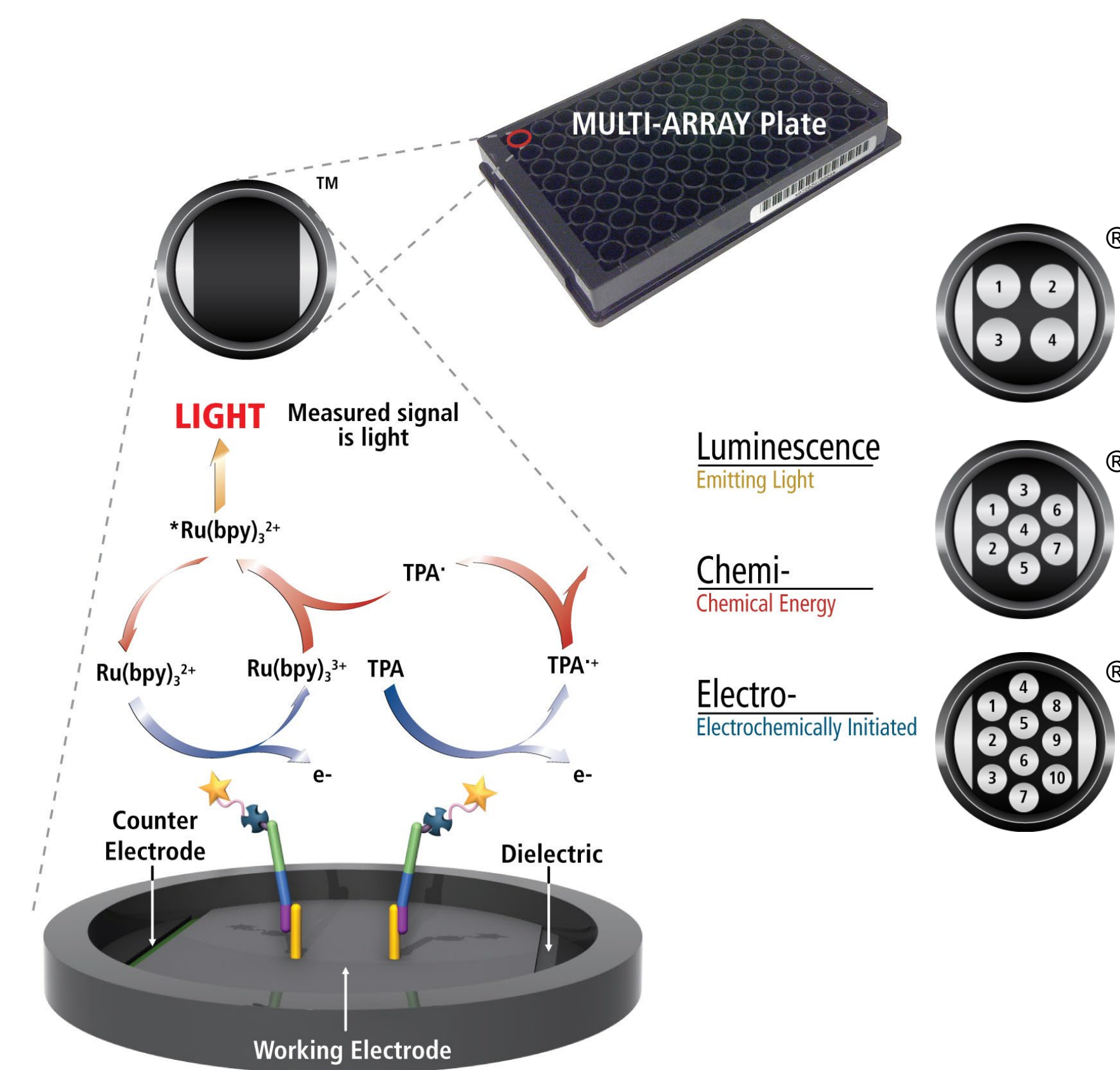
Electrochemiluminescence Technology
- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

Basis of the N-PLEX platform

N-PLEX® plates contain 10 unique capture oligonucleotides that are bound to their corresponding spots on the electrode surface. Detection of a nucleic acid sequence is accomplished by hybridization of one or more probes with sequence complementary to these capture oligos and the nucleic acid of interest, followed by detection via electrochemiluminescence (i.e. biotin/streptavidin SULFO-TAG interactions). Blocking, hybridization, and detection are completed using proprietary MSD® buffers and diluents.

miRNA detection via two-probe approach

The two-probe detection assay used probes that were each complementary to the nucleotides of one-half of the miRNAs. The upstream probes contain spot-specific sequences at the 5' ends that allow for hybridization to the N-PLEX plates. The use of spot-specific sequences enables detection of up to 10 distinct miRNAs per well. The downstream probes contain biotin on the 3' ends for detection. The probes were hybridized to the miRNA and then to spot-specific capture oligos on the N-PLEX plates. SULFO-TAG labeled streptavidin was then used to detect the captured miRNAs.

Preparation of tissue lysate samples

Brain tissues were harvested from cynomolgus monkey (N = 1), mouse (N = 4), and rat (N = 2), and homogenized in lysis buffer. Lysates were collected and heat treated at 55°C for 30 minutes. No extraction step or amplification was necessary.
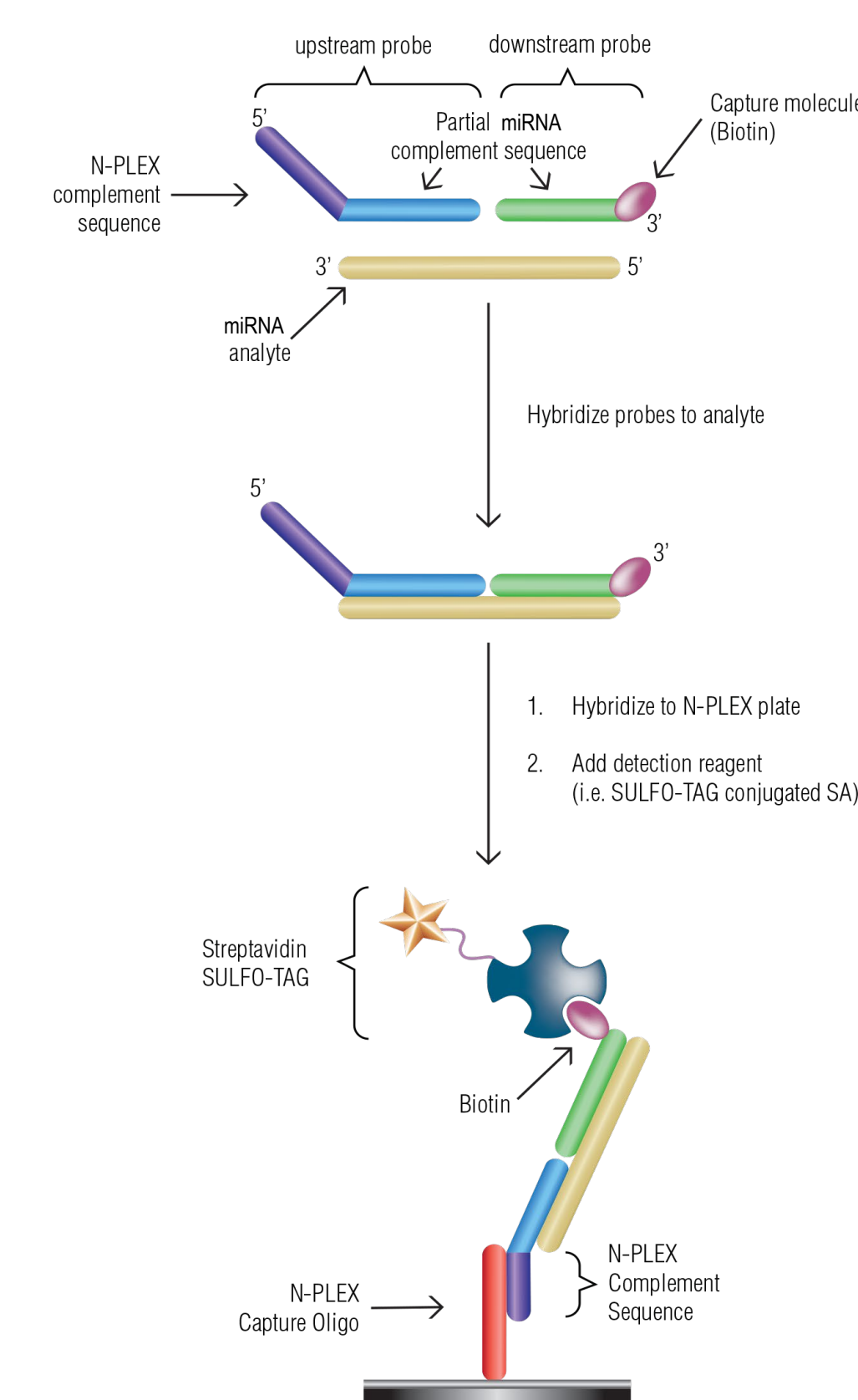
❸ Results

Target miRNAs in Panel

miRNA	LLOD (pM)	LLOD (pg/mL)	Hill Slope	miRNA Sequence	% Conserved Bases Human	Mouse	Rat	NHP
miR-342-3p	0.09	0.7	1.0	UCUCACACAGAAAUCGCACCCGU	100%	100%	100%	100%
miR-125b-5p	0.07	0.5	1.0	UCCCUGAGACCCUAACUUGUGA	100%	100%	100%	100%
miR-9-5p	0.08	0.6	1.0	UCUUUGGUUAUCUAGCUGUAUGA	100%	100%	100%	100%
miR-26b-5p	0.19	1.3	1.1	UUCAAGUAAUUCAGGAUAGGU	100%	100%	100%	100%
miR-146a-5p	0.10	0.7	1.0	UGAGAACUGAAUUCCAUGGGUU	100%	100%	100%	100%
miR-93-5p	0.04	0.3	1.0	CAAAGUGCUGUUCGUGCAGGUAG	100%	100%	100%	96%
miR-34a-5p	0.08	0.5	1.0	UGGCAGUGUCUUAGCUGGUUGU	100%	100%	100%	100%
miR-107-3p	0.05	0.4	1.0	AGCAGCAUUGUACAGGGCUAUCA	100%	100%	100%	100%
miR-132-3p	0.10	0.7	1.0	UAACAGUCUACAGCCAUGGUCG	100%	100%	100%	100%

Figure 1. Probes were designed against several putative Alzheimer's miRNA biomarkers and were run in a multiplex probe system. The selected miRNAs displayed high sequence similarity across species, with 96%–100% concordance among human, mouse, rat, and NHP. Calibration curves were run using synthetic miRNA templates, and the lower limit of detection (LLOD) was determined. The optimized assays were able to identify target miRNAs with a LLOD of lower than 1.27 pg/mL.
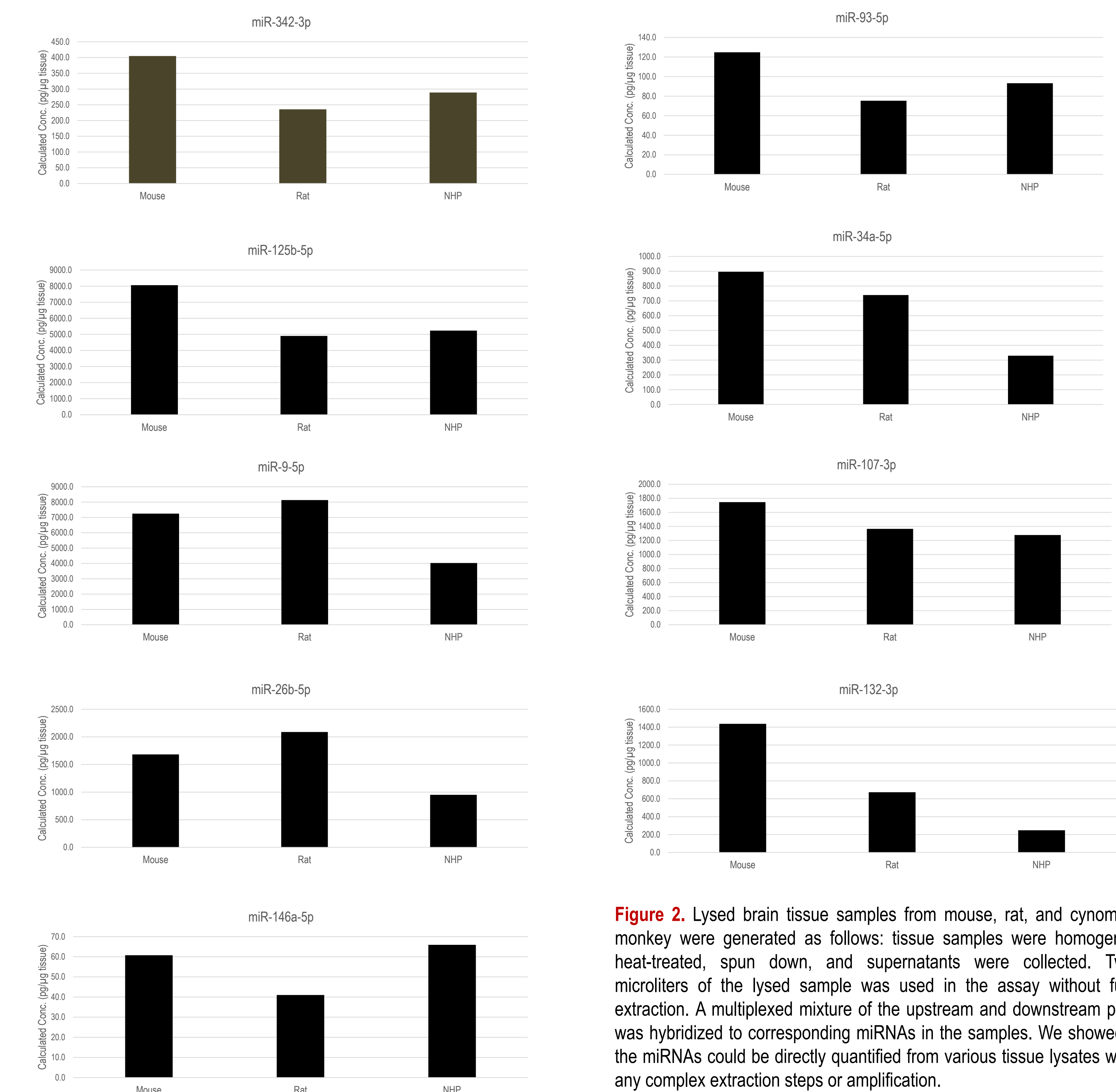
Sample Testing

Figure 2. Lysed brain tissue samples from mouse, rat, and cynomolgus monkey were generated as follows: tissue samples were homogenized, heat-treated, spun down, and supernatants were collected. Twenty microliters of the lysed sample was used in the assay without further extraction. A multiplexed mixture of the upstream and downstream probes was hybridized to corresponding miRNAs in the samples. We showed that the miRNAs could be directly quantified from various tissue lysates without any complex extraction steps or amplification.

Specificity

Target Sequence ECL Signal	Similar Sequence ECL Signal	% ECL Signal	# of Conserved Bases
miR-93-5p	miR-20b-5p	3%	20/23 (87%)
384103	13223		
miR-107-3p	miR-103a-3p	71%	22/23 (96%)
275918	197166		
miR-26b-5p	miR-26a-5p	3%	19/22 (87%)
74497	2231		

Figure 3. miRNAs that have similar sequences to the target miRNAs of interest were identified using BLAST. Each of the three similar miRNAs were produced synthetically and tested in the N-PLEX assays to evaluate specificity. ECL signals from the similar miRNAs were ≈ 3% of the target signal except for miR-103a-3p, which had an especially high percentage of conserved bases to miR-107-3p and displayed 71% of target signal (all miRNAs were tested at 125 pM).

Reproducibility

A.

High Quantitative Control

Target	Conc. (pg/mL)
miR-342-3p	738
miR-125b-5p	699
miR-9-5p	735
miR-26b-5p	1360
miR-146a-5p	1430
miR-93-5p	501
miR-34a-5p	714
miR-132-3p	744
miR-107-3p	1420

Mid Quantitative Control

Target	Conc. (pg/mL)
miR-342-3p	148
miR-125b-5p	140
miR-9-5p	147
miR-26b-5p	271
miR-146a-5p	285
miR-93-5p	100
miR-34a-5p	143
miR-132-3p	149
miR-107-3p	284

Low Quantitative Control

Target	Conc. (pg/mL)
miR-342-3p	30
miR-125b-5p	28
miR-9-5p	29
miR-26b-5p	54
miR-146a-5p	57
miR-93-5p	20
miR-34a-5p	29
miR-132-3p	30
miR-107-3p	57

B.

ULOQ

Target	Conc. (pg/mL)
miR-342-3p	2580
miR-125b-5p	2450
miR-9-5p	2570
miR-26b-5p	4750
miR-146a-5p	4990
miR-93-5p	1760
miR-34a-5p	2500
miR-132-3p	2600
miR-107-3p	4970

LLOQ

Target	Conc. (pg/mL)
miR-342-3p	6.6
miR-125b-5p	6.3
miR-9-5p	6.6
miR-26b-5p	12
miR-146a-5p	13
miR-93-5p	4.5
miR-34a-5p	6.4
miR-132-3p	6.7
miR-107-3p	13

Mouse Brain Lysate Sample

Target	Average Calc. Conc. (pg/mL)
miR-342-3p	92
miR-125b-5p	1810
miR-9-5p	1360
miR-26b-5p	209
miR-146a-5p	12
miR-93-5p	25
miR-34a-5p	70
miR-132-3p	445
miR-107-3p	385

Rat Brain Lysate Sample

Target	Average Calc. Conc. (pg/mL)
miR-342-3p	49
miR-125b-5p	1320
miR-9-5p	1510
miR-26b-5p	407
miR-146a-5p	8.7
miR-93-5p	18
miR-34a-5p	257
miR-132-3p	141
miR-107-3p	289

Legend:
- miR-342-3p
- miR-125b-5p
- miR-9-5p
- miR-26b-5p
- miR-146a-5p
- miR-93-5p
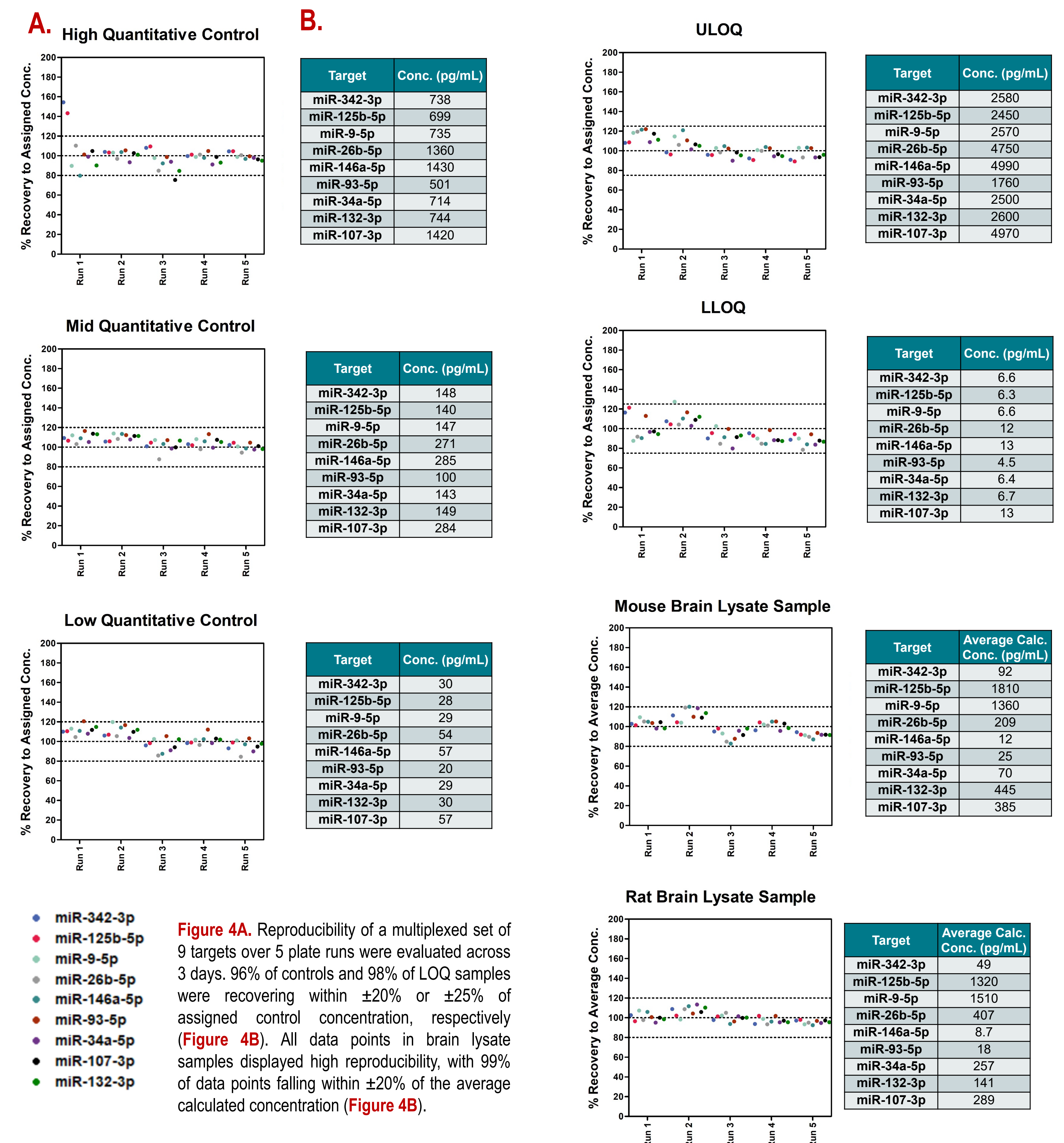- miR-34a-5p
- miR-107-3p
- miR-132-3p

Figure 4A. Reproducibility of a multiplexed set of 9 targets over 5 plate runs were evaluated across 3 days. 96% of controls and 98% of LOQ samples were recovering within ±20% or ±25% of assigned control concentration, respectively (**Figure 4B**). All data points in brain lysate samples displayed high reproducibility, with 99% of data points falling within ±20% of the average calculated concentration (**Figure 4B**).

❹ Conclusion

A multiplexed miRNA assay has been demonstrated that uses a simple, rapid workflow (<4 hours to result). It does not require RNA extraction and does not use a polymerase-based detection system. In a single 96-well plate run, the assay can detect nine miRNAs in 40 tissue lysate samples, providing a method that is both quantitative for multiple miRNAs and that has the potential for high-throughput screening.

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