

A⁴: Amorfix Aggregated A β Assay

**for Quantitative, Highly Sensitive
Measurement of A β Aggregates for
Alzheimer's Disease (AD) Research**

Technology Overview

A4: Amorfix Aggregated A β Assay

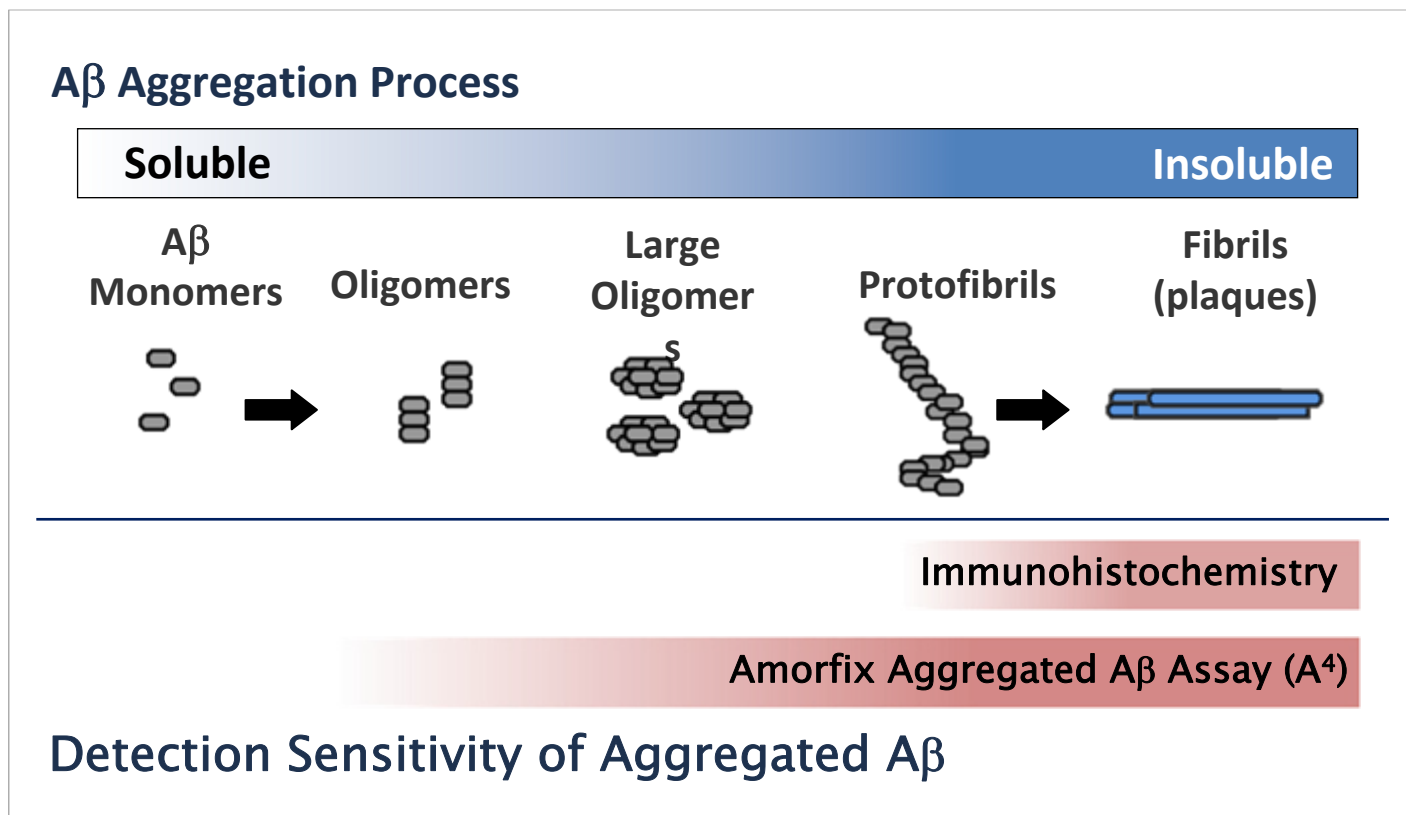
1. A **quantitative** and **sensitive biomarker** assay that detects **aggregated A β** in brain, plasma, and CSF from animal models used for Alzheimer's Disease research.
2. The A⁴ can detect A β aggregates early in disease progression, **before plaque formation** in the brain.
3. Early detection of aggregates will **reduce time and cost** for preclinical trials, allowing for faster screening of lead candidates.

A β Plaque Detection Using Immunohistochemistry (IHC)



- A β plaques are traditionally detected using IHC
- Using standard IHC techniques, it is difficult to detect A β plaques in APP/PS1 mice prior to 6 months of age

Alzheimer's Disease Progression: A β Aggregation and Detection

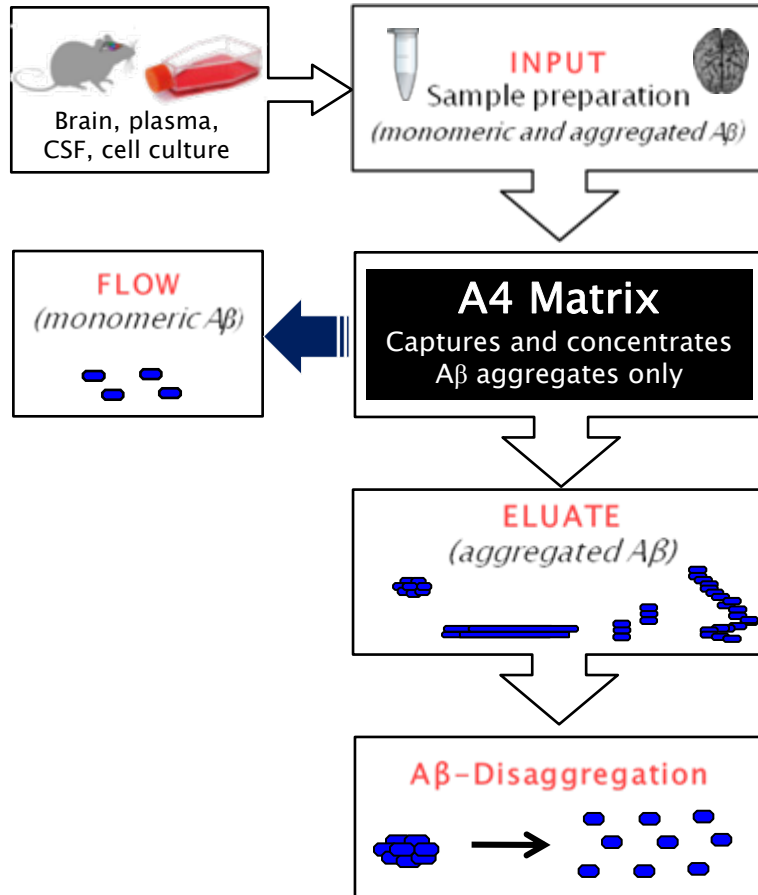


- Formation of insoluble plaques is the final step in A β aggregation
- A⁴ can detect A β aggregation before plaques are visible using IHC

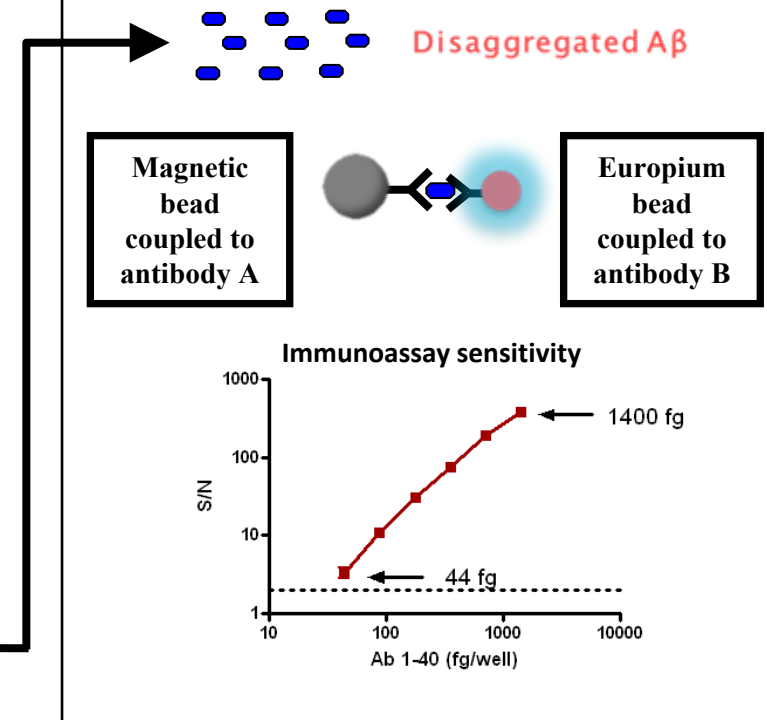
A⁴ Overview

Part 1: A β Aggregate Isolation

Part 2: A β Quantification



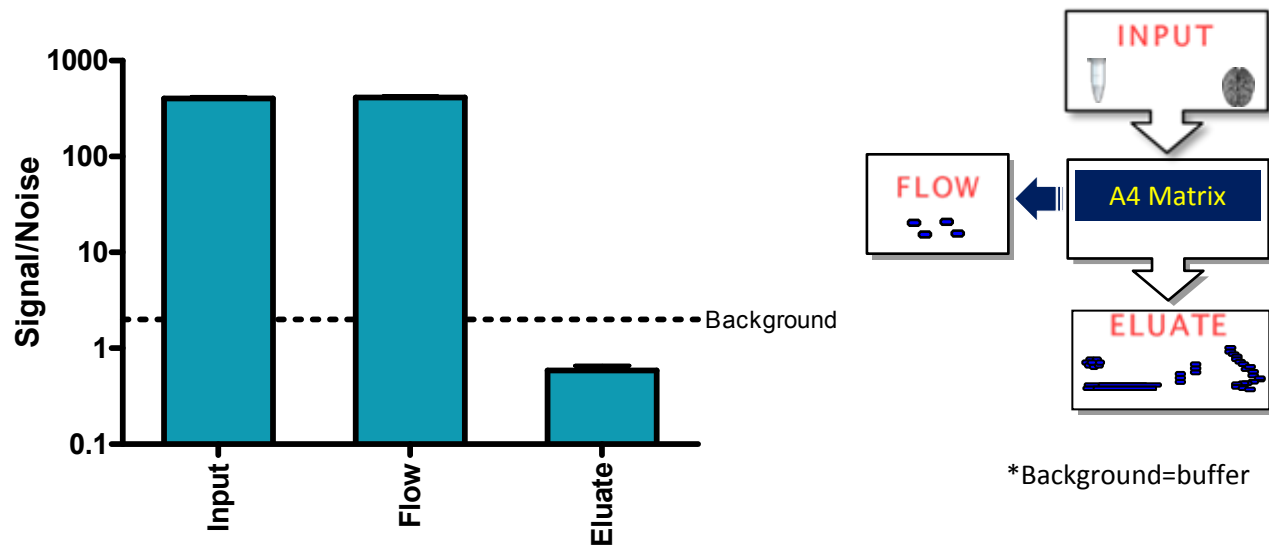
A β Ultra-sensitive Immunoassay Quantifies A β in its monomeric form



Enrichment of Aggregated $A\beta$ by the A^4 Matrix

A4 Matrix Enriches for A β Aggregates Only

Analysis of buffer spiked with monomeric A β 1-40 using A β ultrasensitive immunoassay

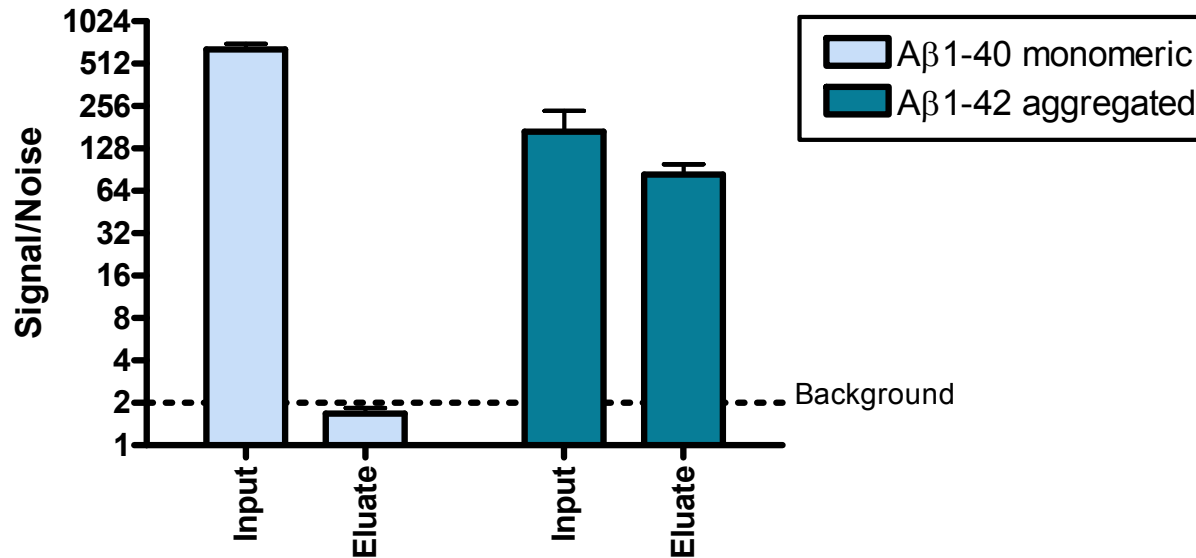


- Signal from flow-through is equivalent to that of input.
- Eluate does not contain monomeric A β 1-40

- **A⁴ Matrix** does not capture monomeric form of A β

A4 Matrix Enriches for A β Aggregates Only

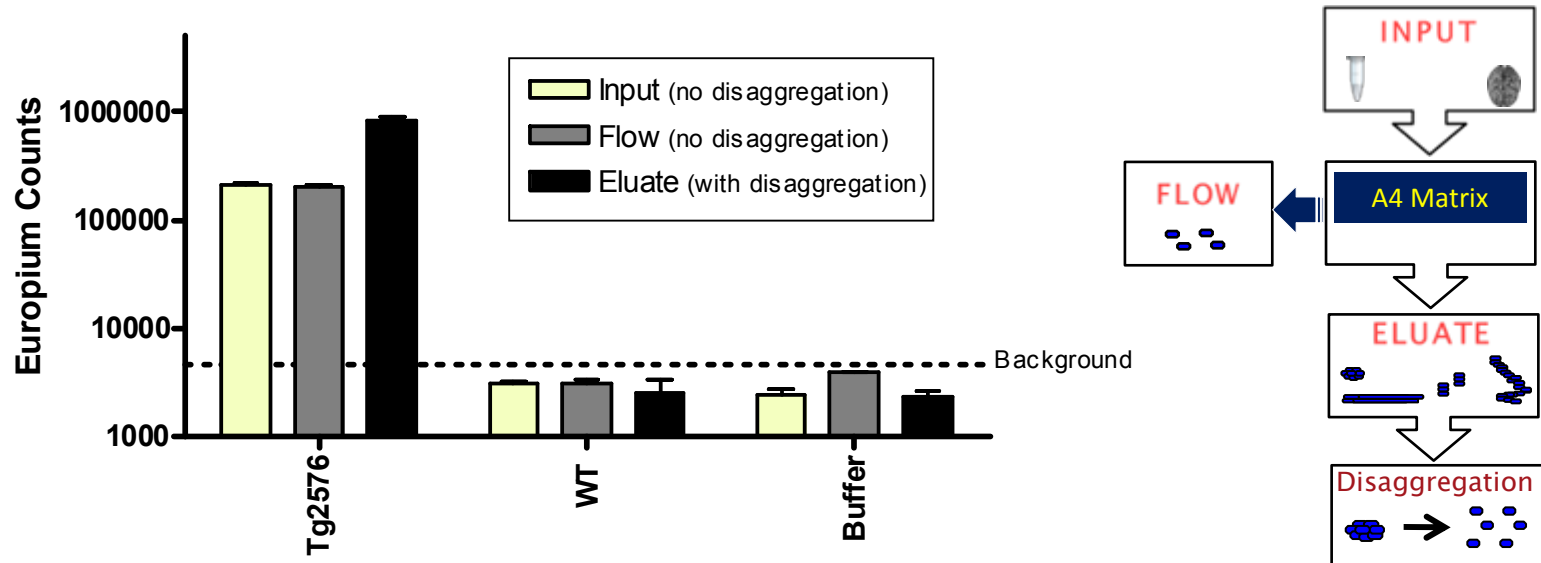
Analysis of tissue culture medium (10% FBS) spiked with monomeric A β 1-40 or aggregated A β 1-42



- No Signal from monomeric A β in eluates
- A⁴ Matrix captures aggregated A β

A4 Matrix Enriches for A β Aggregates Only

Analysis of A⁴ fractions from Tg2576 mice using A β ultrasensitive immunoassay



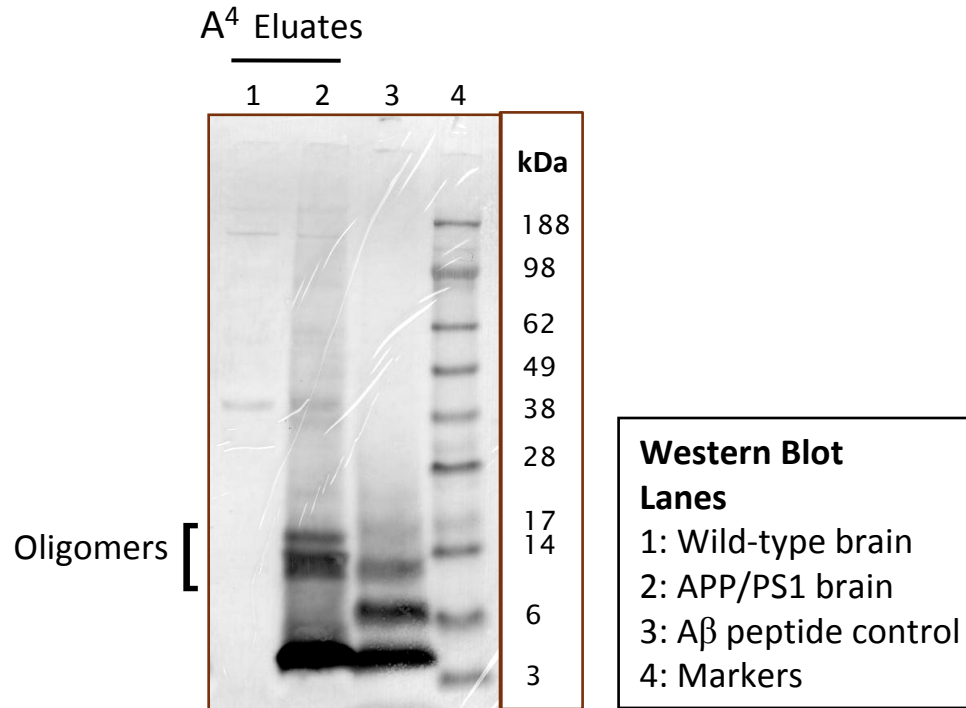
→ Signal from monomeric A β in flow-through is equivalent to that from input
 → Signal from eluted sample is due to A⁴ matrix enrichment of aggregated A β followed by disaggregation step

*Background=buffer

- A⁴ Matrix does not capture monomeric A β from Tg2576 brains

Small Oligomers are Captured by A⁴ Matrix

SDS insoluble A β oligomers are enriched by A⁴ Matrix in APP/PS1 mice



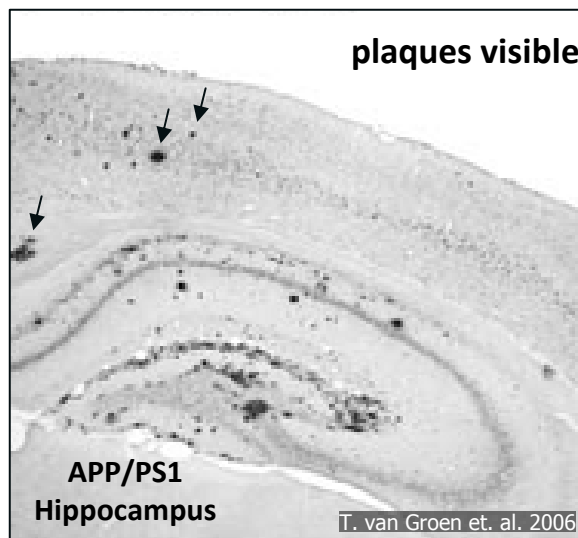
- Western blotting shows that A⁴ Matrix enriches for A β aggregates present in AD brain

Detection of A β Aggregates in Brain Homogenate

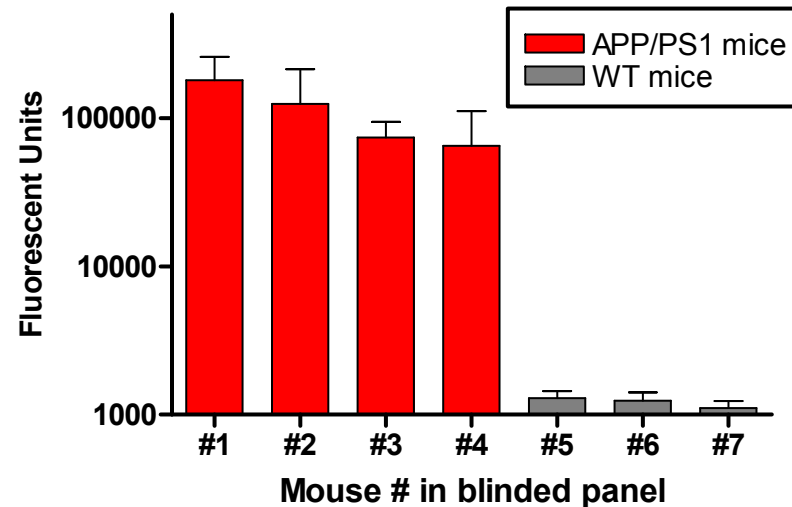
Detection of A β Aggregates in Brain

Detection of A β aggregates in brain of AD mice (APP/PS1) at 14 months

Using IHC



Using A⁴

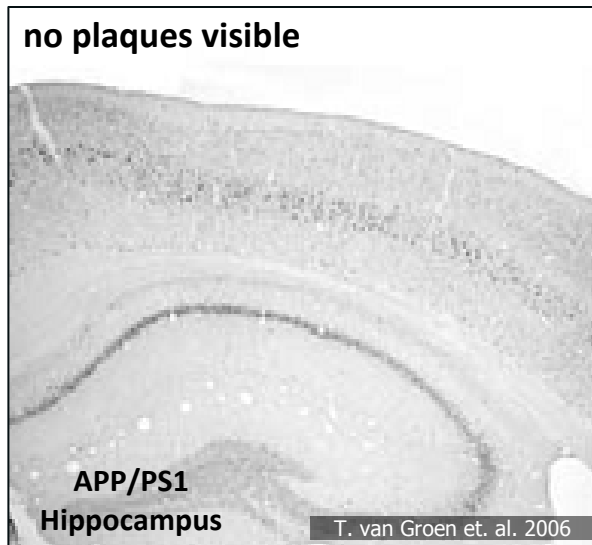


- A⁴ provides a quantitative measure of A β aggregation

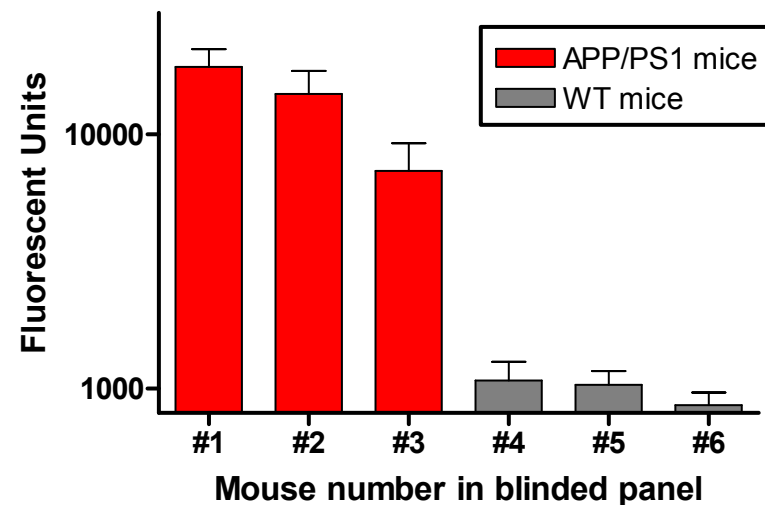
Detection of A β Aggregates in Brain

Detection of A β aggregates in brain of AD mice (APP/PS1)

6 months of age
Using IHC

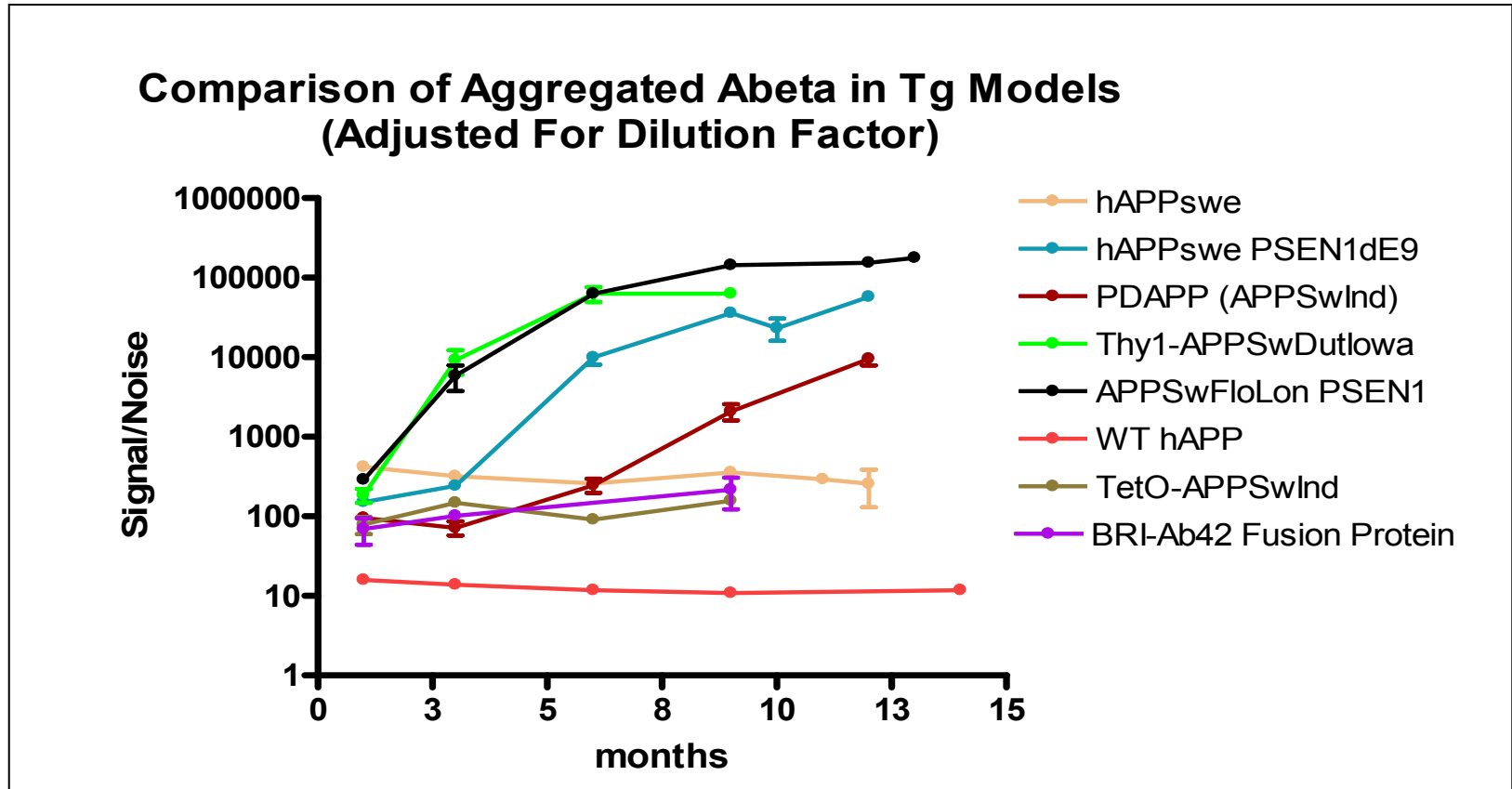


3 months of age
Using A⁴



- A⁴ is sensitive enough to detect A β aggregates several months before IHC can detect plaques (3 months vs 6 months)

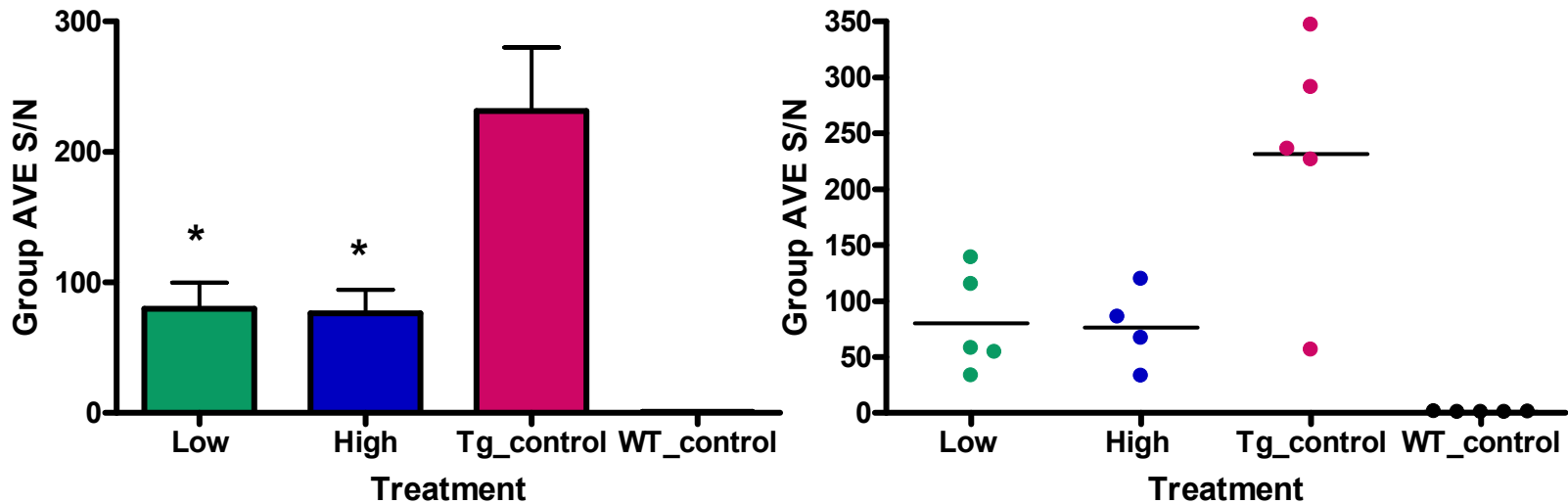
Comparison of Aggregated A β Levels in Brains from Transgenic AD Models



- The A⁴ can be used to monitor the progression and severity of A β aggregation in various models of AD

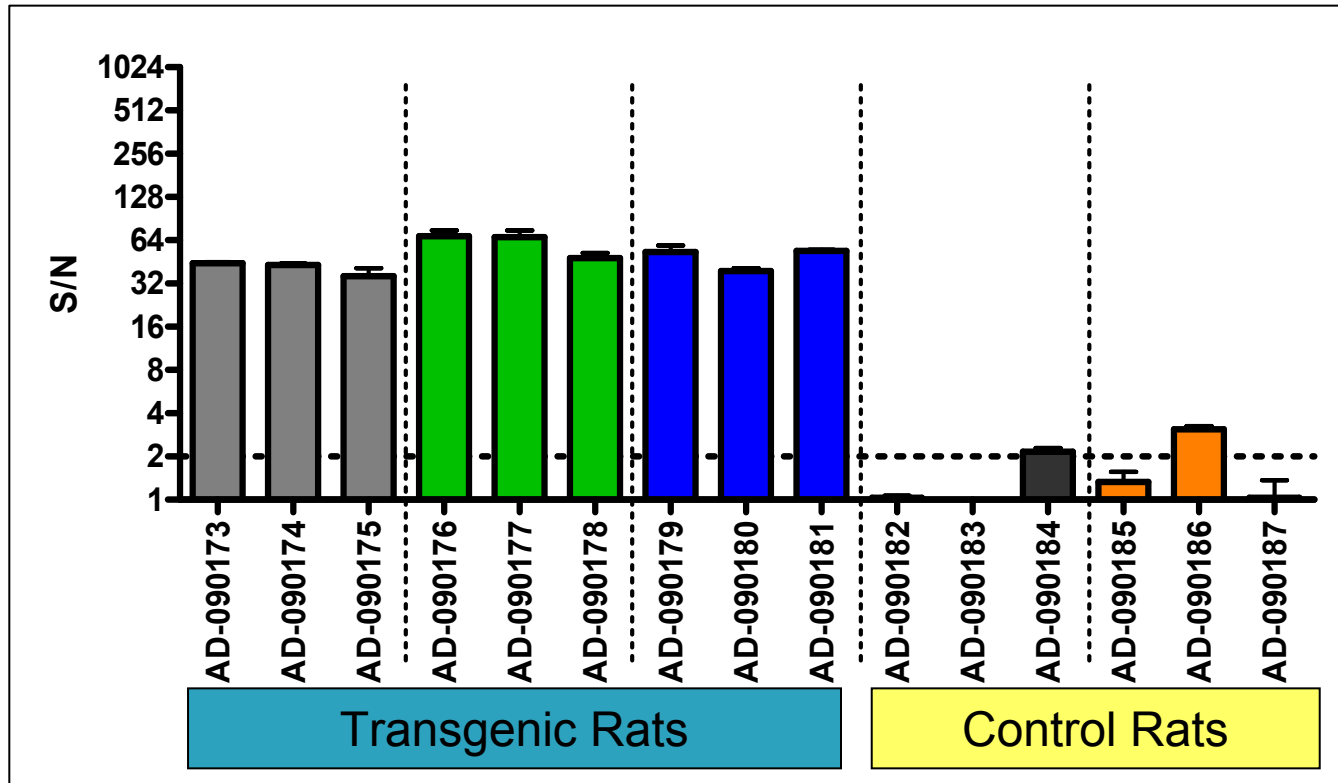
Results from Blinded Study

**A β aggregates decrease in the brains of Tg2576 mice
Following treatment with γ -secretase modulator**



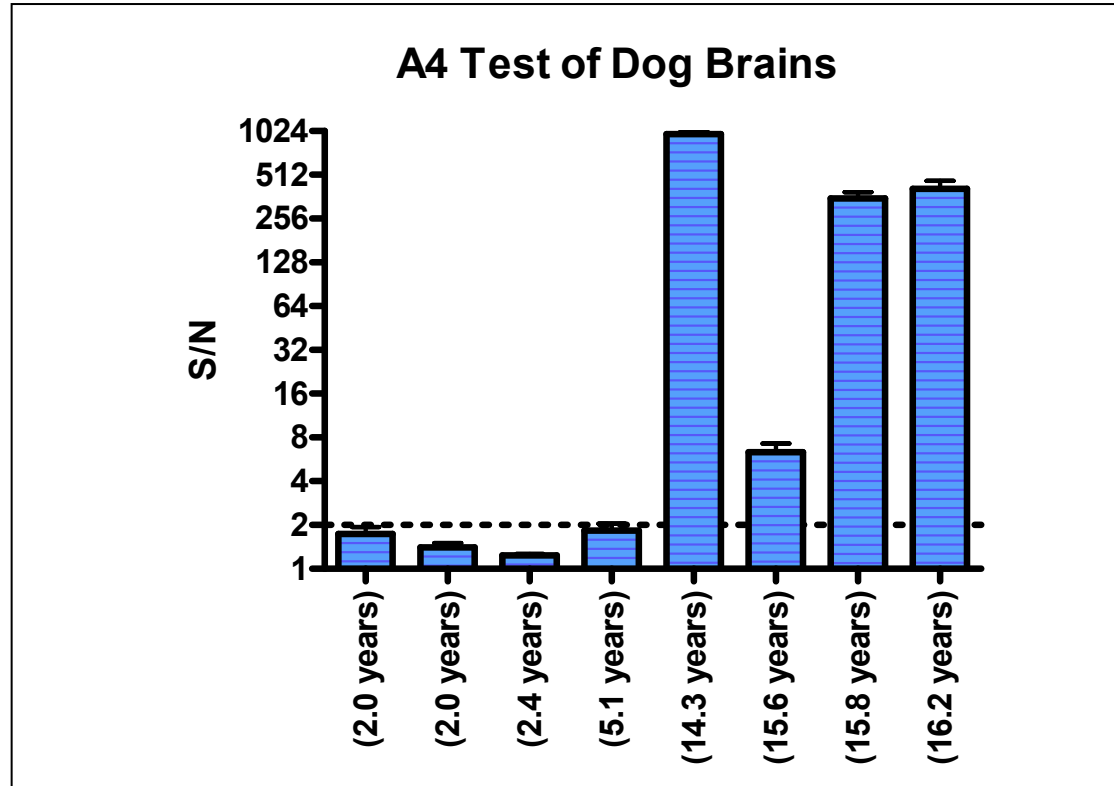
- The A⁴ can be used to monitor efficacy of candidate therapeutics in preclinical trials

A4 Analysis of Brains from hAPPswe Rat Transgenic Model



- The A⁴ can detect the presence of human A β in a variety of transgenic animal models

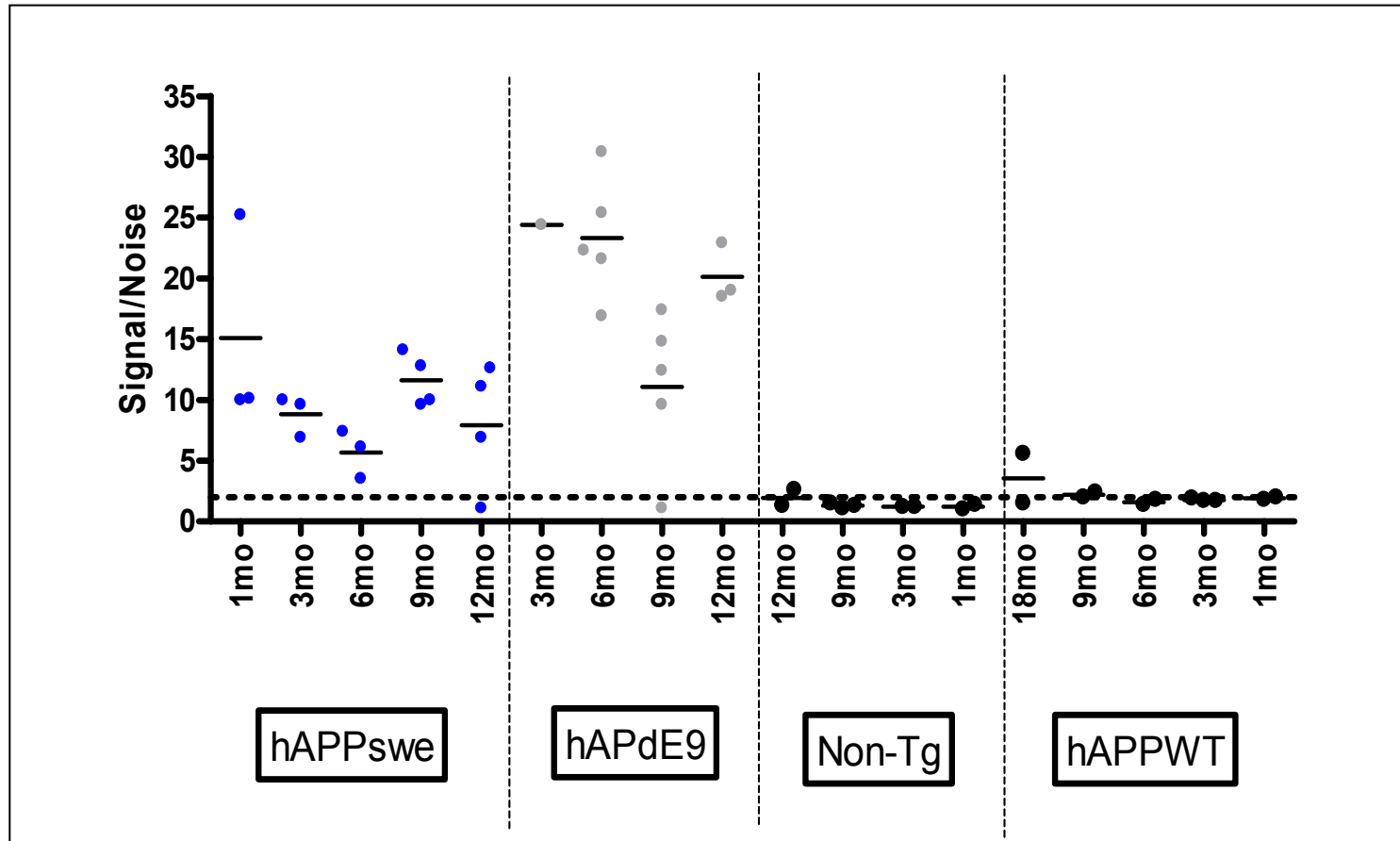
Detection of Aggregated A β in Canine Model of Aging



↑
This animal scored very high
on cognition tests

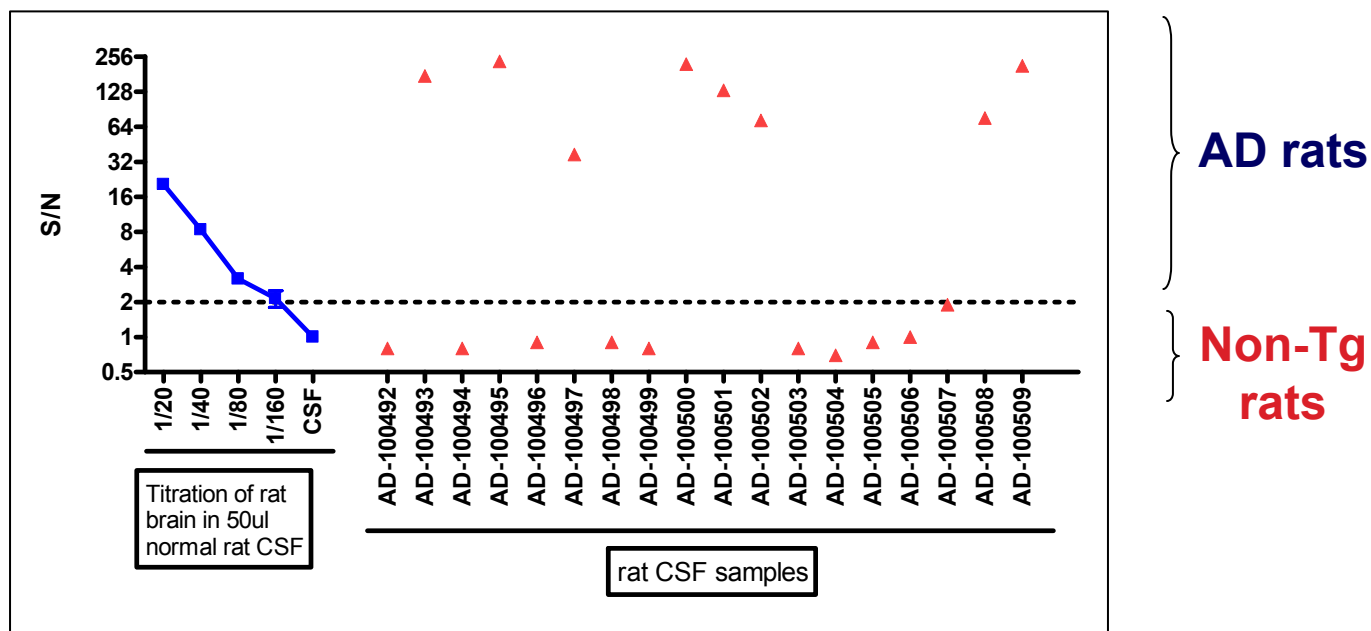
Detection of Aggregated A β in Plasma and CSF

A4 Detection of $A\beta$ Aggregates in AD Mouse Plasma



- This data provides proof-of-concept evidence of aggregated $A\beta$ in plasma

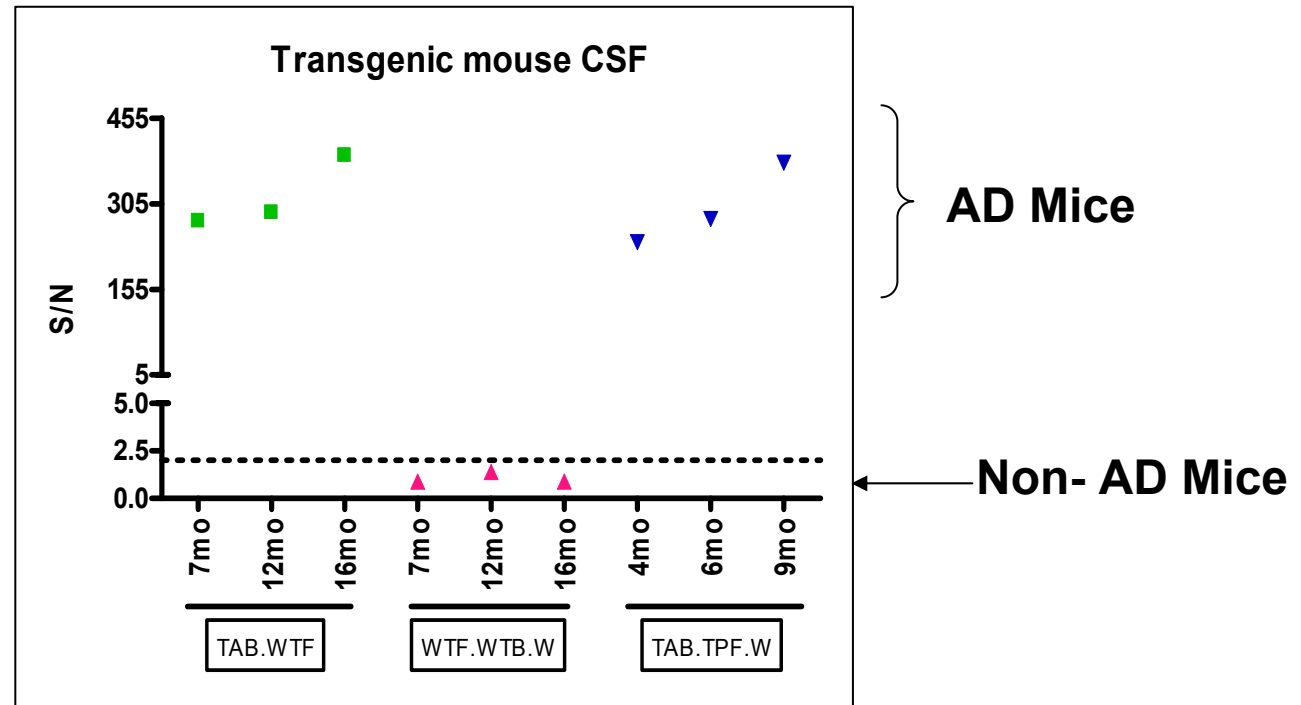
A4 Analysis of CSF from hAPPswe Transgenic Rat Model



CSF tested as single replicate for each sample.

- Confirmation for the use of A⁴ in detection of aggregated A β in CSF

Detection of A β Aggregates in CSF



- 20 ml pooled CSF from each age
- Tested as single replicates
- Increased signal from aggregates with age in AD mice
- Confirmation for the use of A⁴ in detection of aggregated A β in CSF

Summary

A4 Summary

- Provides quantitative and sensitive measurements of A β aggregates
- A⁴ has been validated for analysis of aggregates in brain using 11 different animal models
 - 9 transgenic mouse models
 - 1 transgenic rat model
 - Canine model (non-transgenic beagles)
- Proof-of-Concept data for presence of aggregated A β in CSF and plasma from transgenic animal models.
 - 3 transgenic mouse models
 - 1 transgenic rat model
- Potential for dramatically changing pre-clinical trials using longitudinal sampling of animals.

For More Information

- For more information, or for a quote for the A⁴ diagnostic service, please contact:

Email: alzheimers@amorfix.com