Case Study:

Quantification (PK evaluation) of small anti-sense oligonucleotides (ASOs) in animal tissues and studying the effect of ASO modifications on tissue accumulation and activity

Obective:

To standardize quantification methods for small ASOs in various animal tissues

Study design:

- Tissues received from the ASO dosed animals were lysed for evaluation
- Hybridization based qPCR and ELISA methods were evaluated for ASO quantification
- Gene expression analysis using RT-qPCR to assess ASO activity

Challenge:

Several methods are available to quantify ASOs (Ex. LC-MS, qRT-PCR or chromatography-based). However, these methods require analyte extraction, enzymatic modifications or lysate dilution. Such sample processing steps lead to analyte loss, requirement of large volumes of sample or reduction in analyte concentration. Most tissues do not accumulate ASOs in high quantities barring a few, such as kidney or liver. Thus, a method with better sensitivity is required for PK studies in tissues with low levels of ASO accumulation.

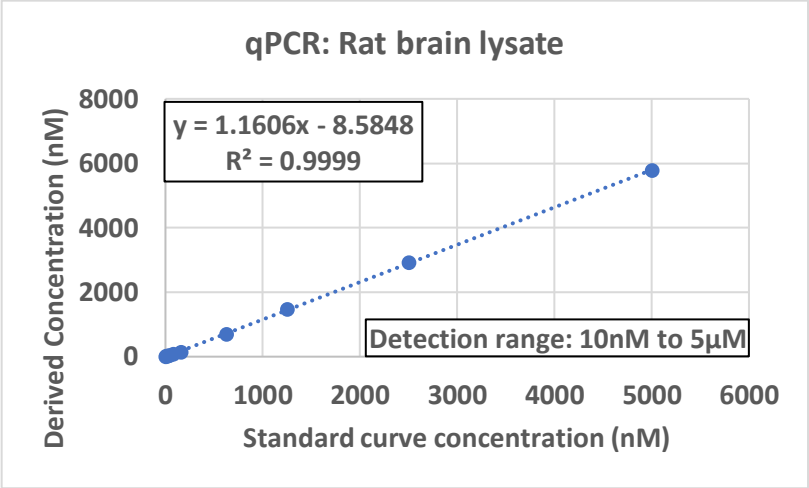
Aurigene Solution:

- Standardized tissue lysis with minimum volume of lysis buffer, effectively concentrating the ASO
- Standardized ASO quantification directly from the lysate without extensive processing

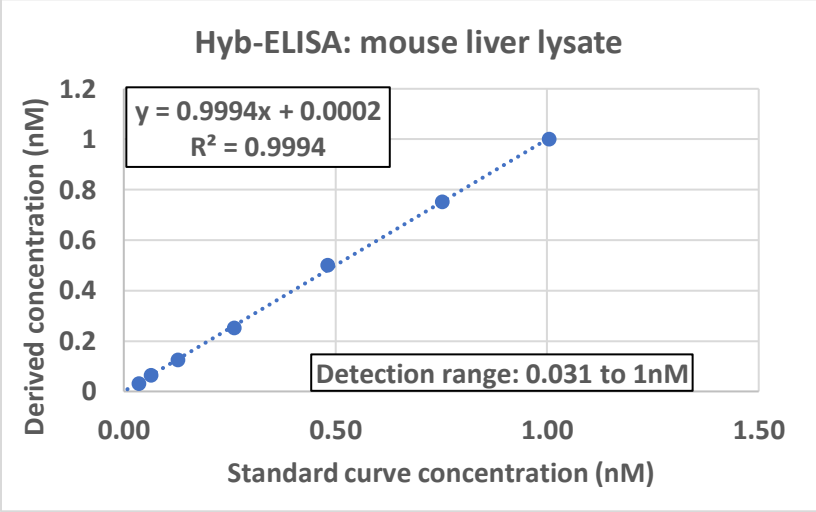
Outcomes:

- Quantified small ASOs with two methods: a) qRT-PCR (Figure A) and b) Hybridization ELISA (Figure B) in liver, brain, kidney, plasma and CSF
- Quantified several modified (such as LNA, GalNAc, PS, MOE) and unmodified ASO variants
- Hybridization ELISA-based quantification is highly sensitive with an LLOQ of 0.031 nM (Figure B), while qRT-PCR has LLOQ of 10 nM (Figure A)
- GalNAc conjugated ASO not only preferentially accumulated in the liver but also showed better activity as compared to non-GalNac ASO (Figure C)

A) qPCR method:



B) Hyb-ELISA method:



C) ASO activity: