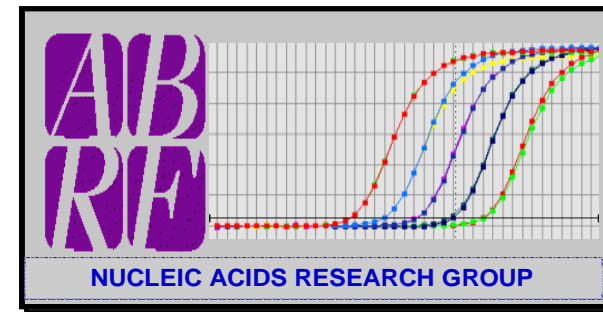




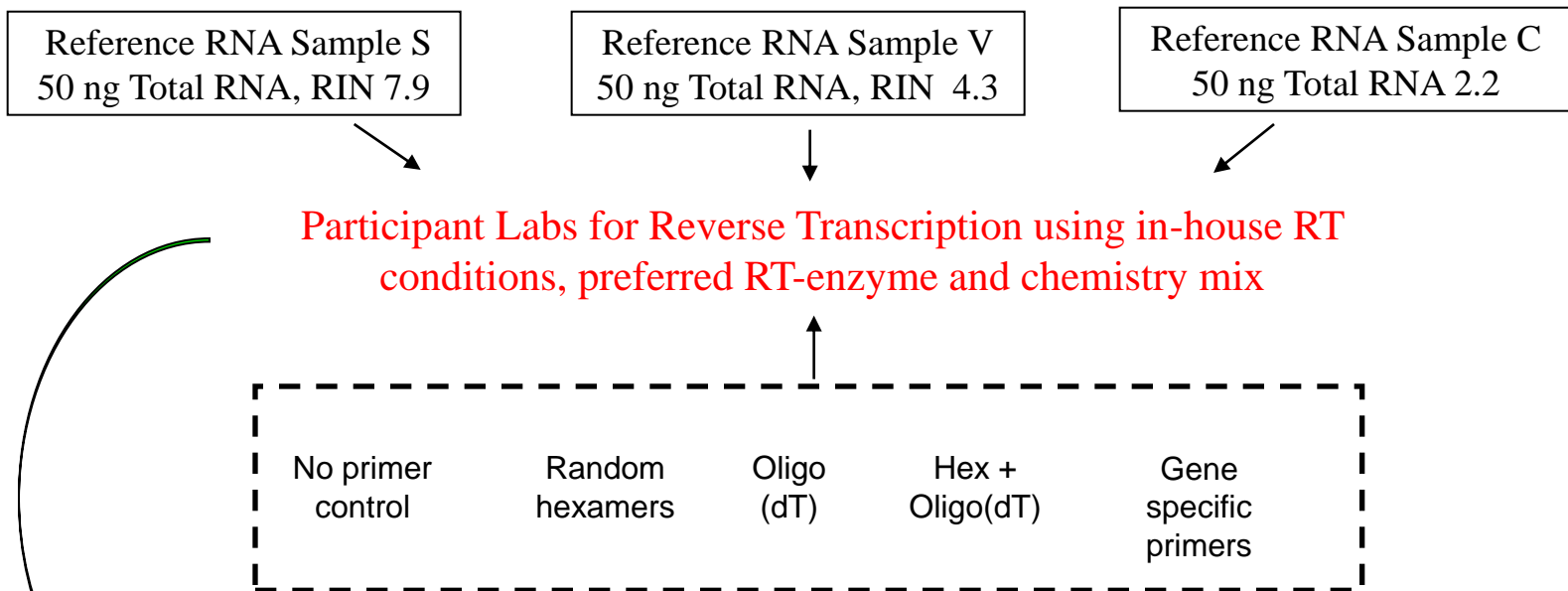
# NARG



- 2009-10 Study  
Priming strategies for cDNA synthesis using Real-Time qPCR (effect of RNA degradation)
- Results from samples with most degraded RNA

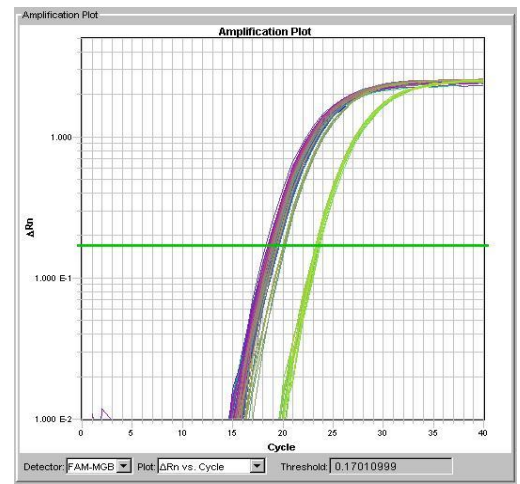
# 2009-10 Study Design

## A benchmarking opportunity, with a twist!



All cDNAs were shipped back to NARG for qPCR processing.

- 5 ng RNA equivalents cDNA were amplified using the ABI 2x master mix using 500 nM primers and 250 nM probe in a 5ul reaction volume.
- Cycling was performed on 384-well plates using the standard conditions recommended for the AB7900HT platform.



# Benchmarking Study

Participants from 20 labs

6 different types of RT enzymes

- 10 labs used AB's High Capacity RT at 37°C (7) or 42°C (3)
- 4 labs used Invitrogen's SuperScriptIII at 50°C
- 2 labs used AB's reverse Transcriptase at 37°C
- 2 labs used Roche's Transcriptor RT at 50°C or 55°C
- 1 lab used Clontech's SmartScribe at 42°C
- 1 lab used BioRad's iScript at 42°C

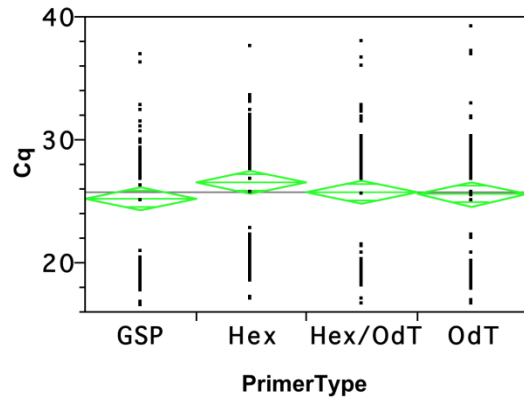
One-way analysis of variance (ANOVA) to determine:

- Effect of enzyme type and RT temperature on Cq
- Effect of RT priming strategy and qPCR assay location
- Effect of Lab on Cq
- Effect of overall RNA quality on RT-qPCR

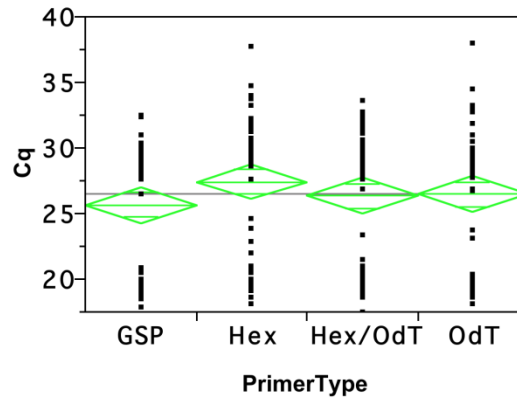
# Effect of RNA Quality on Cq By Primer Type And By Assay Location

## RNA (Quality)

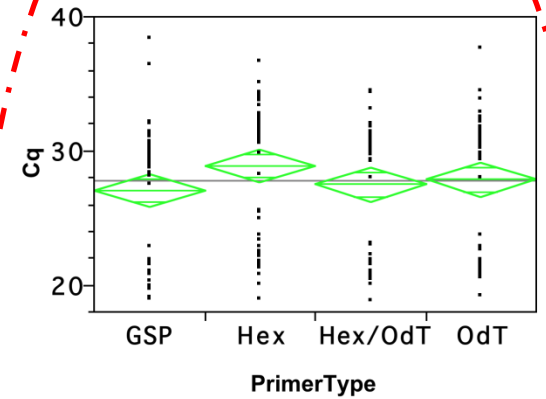
**S (RIN=8.1)**



**V (RIN=4.0)**



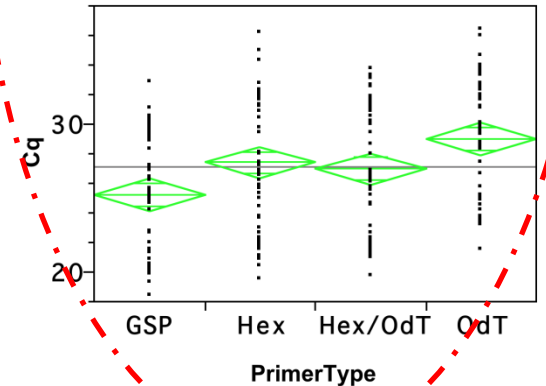
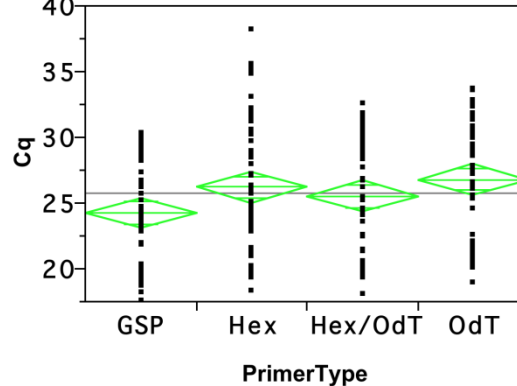
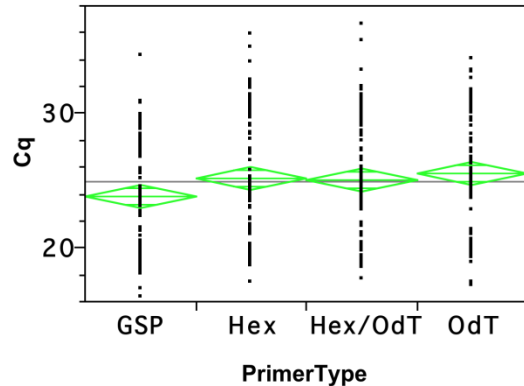
**C (RIN=2.2)**



Assay Location

3'

5'



# **What is the Best Priming Strategy to Use to Generate cDNA for Use in qPCR?**

**If the cDNA will be used for only 1 gene assay, the appropriate gene-specific primer may be a better choice**

**The use of randomer-oligo(dT) combinations in the RT reaction appear to give universally lower  $C_q$  values and higher  $\Delta C_q$  differences regardless of the assay location**

**RNA quality will significantly impact your qPCR results, however trends may be teased out even using a sub-optimal template in the RT (as sample V with RIN 4.3)**