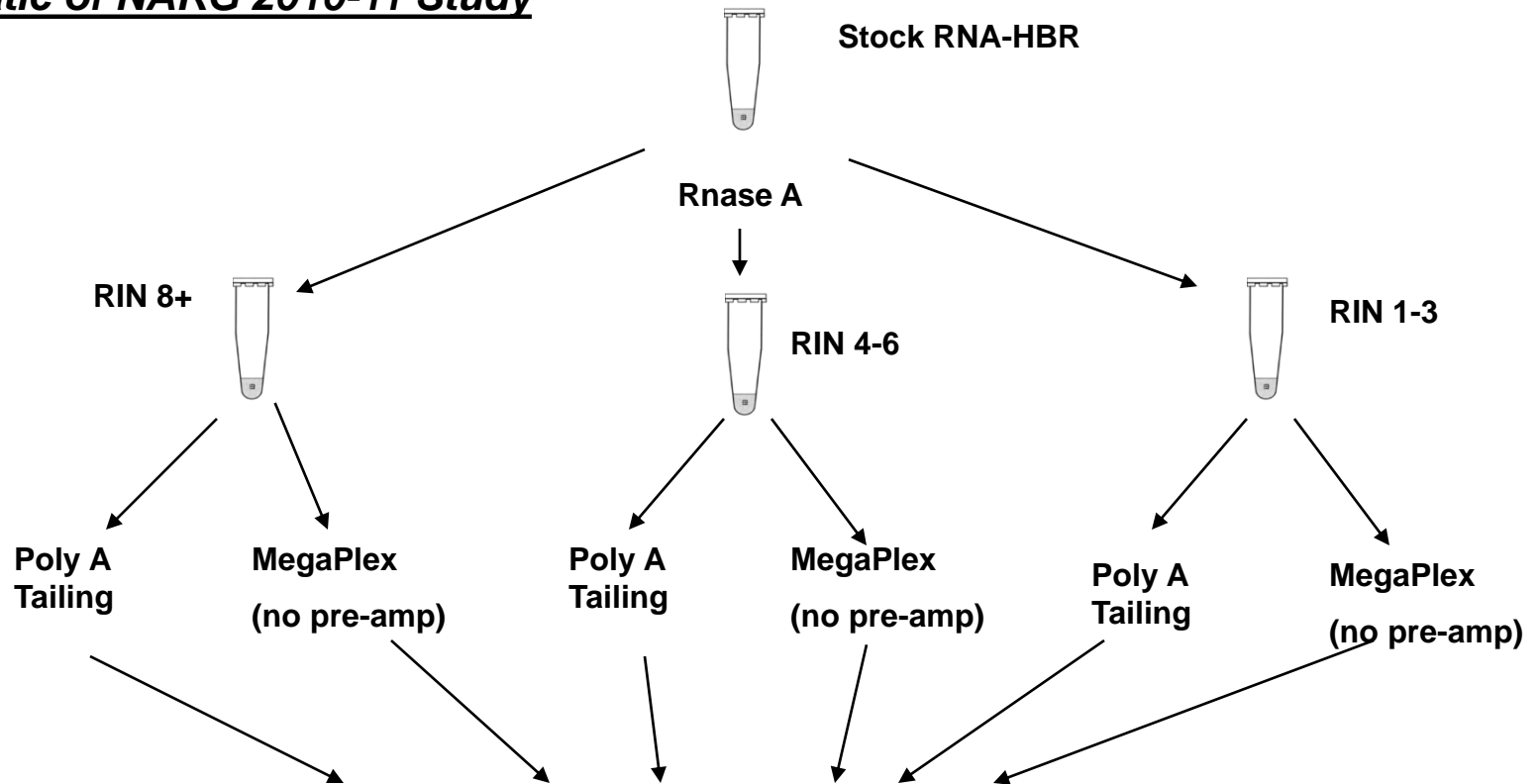


2010-11 Study

Determining miRNA expression Levels in Degraded RNA Samples Using Real-Time RT-QPCR and Microarray Technologies

Schematic of NARG 2010-11 Study



hsa-miR-122
hsa-miR-192
hsa-miR-194
hsa-miR-19a
hsa-miR-9

hsa-miR-149
hsa-miR-135a
hsa-miR-382
hsa-miR-370

Samples analyzed in duplicate RT reactions and in duplicate qPCR reactions

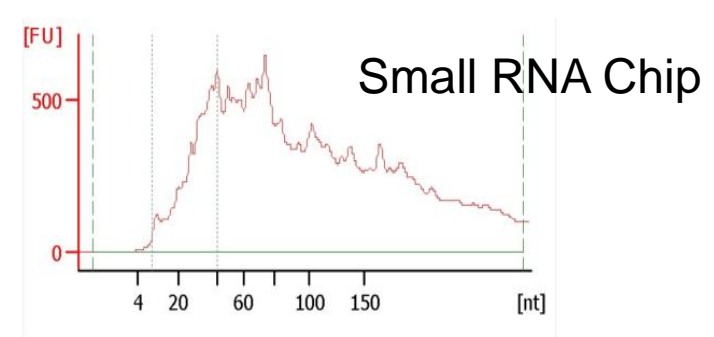
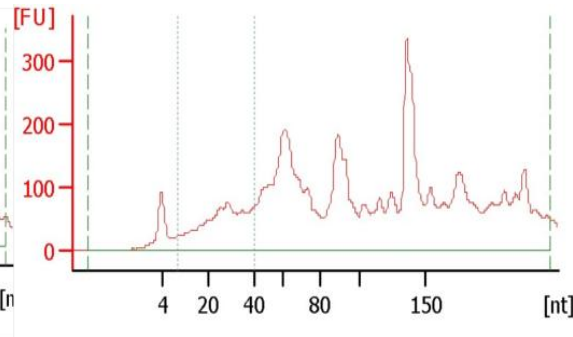
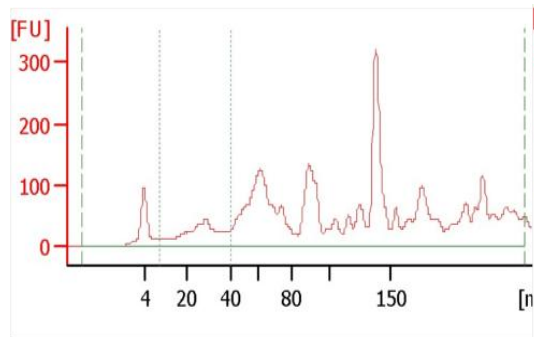
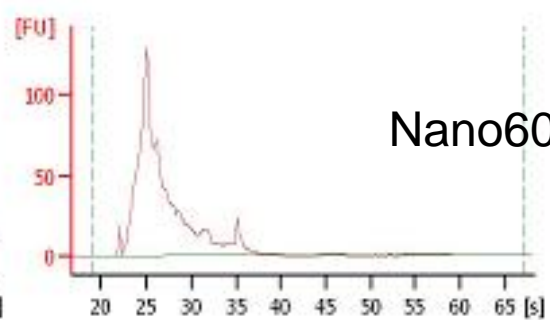
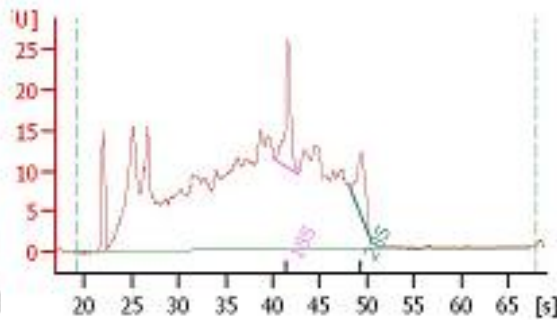
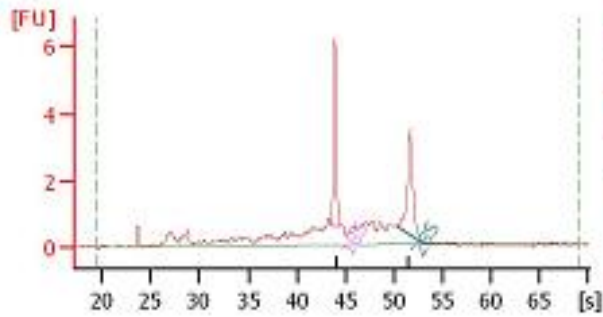
Mirror mirror on the wall, who is the prettiest of them all????

Sample R8:
high quality

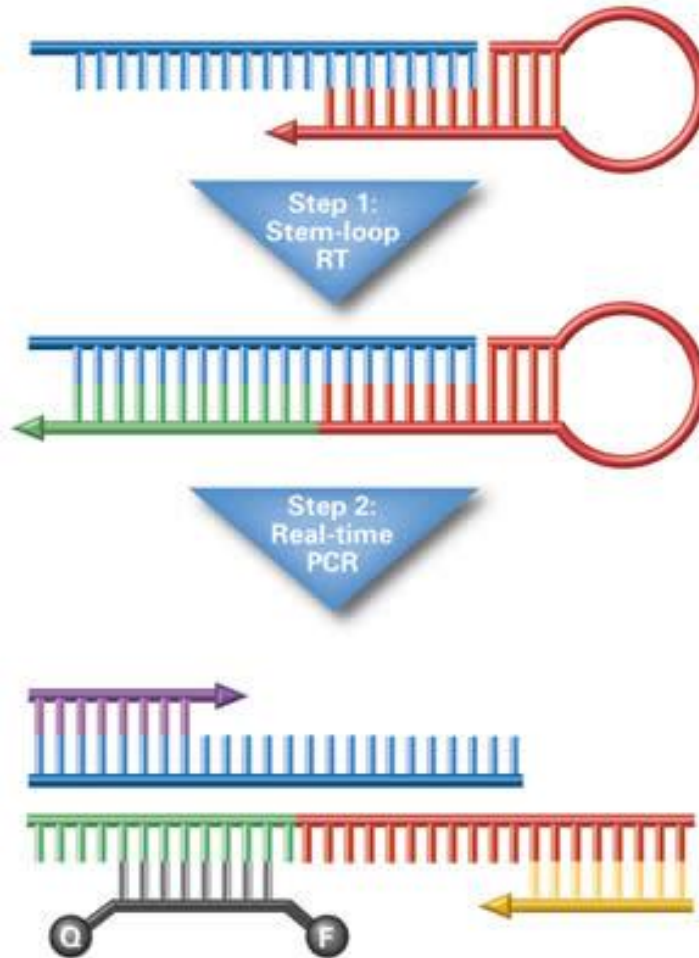
Sample R4:
partially degraded

Sample R2:
fully degraded

Nano6000 Chip



Megaplex miRNA Assay

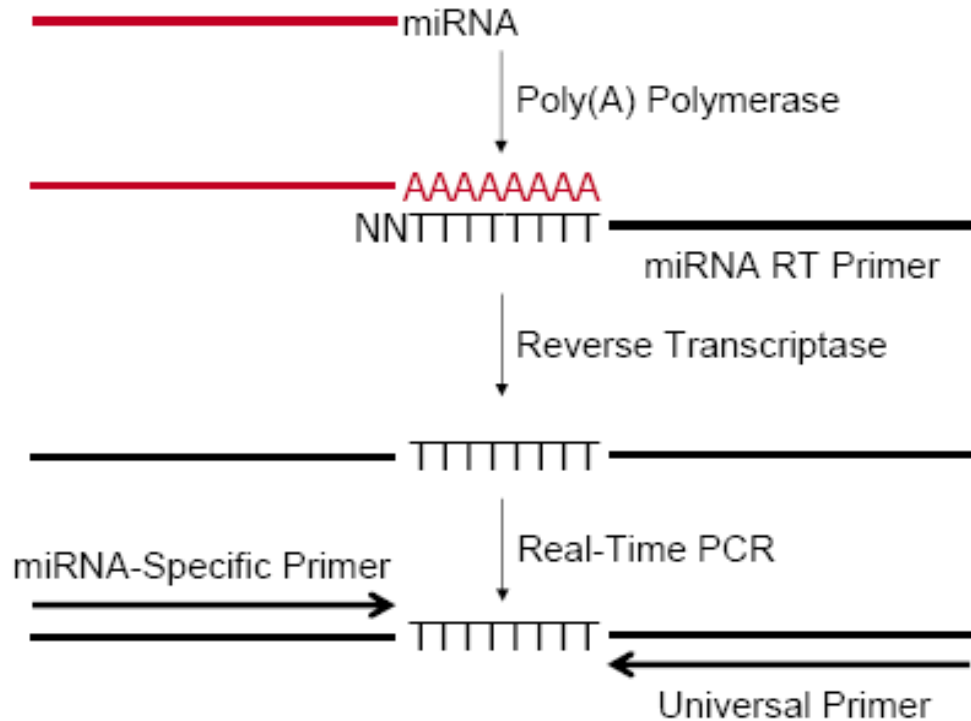


Pool of 384 specific miRNA stemloop primers

Taqman assays run with primers specific to each miRNA being assayed

384 well plate, 10ul rxn volume, and cycled using an ABI 7900HT under recommended conditions
96 well plate, 25ul rxn volume and cycled using Stratagene MX3000P or ABI 7500fast instrument specific recommended conditions.

PolyA Tailing Assay



SYBR Green assays run with primers specific to each miRNA being assayed

384 well plate, 10ul rxn volume, and cycled using an ABI 7900HT under recommended conditions
96 well plate, 25ul rxn volume and cycled using Stratagene MX3000P or ABI 7500fast instrument specific recommended conditions.

9 miRNAs were assayed using both methods

miRNA name	copy number *	mature miRNA sequence	ABI Assay ID	Qiagen Catalog Number	mirBASE 14.0 Accession number
hsa-miR-122a	1.3 x 10 ³	UGGAGUGUGACAAUGGUGUUUG	000445	MPH00020A	MIMAT0000421
hsa-miR-192	8.9 x 10 ³	CUGACCUAUGAAUUGACAGCC	000491	MPH00080A	MIMAT0000222
hsa-miR-194	1.3 x 10 ⁴	UGUAACAGCAACUCCAUGUGGA	000493	MPH00083A	MIMAT0000460
hsa-miR-370	7.6 x 10 ⁴	GCCUGCUGGGGUGGAACCUGGU	000558	MPH00185A	MIMAT0000722
hsa-miR-19a	1.6 x 10 ⁵	UGUGCAAUCUAUGCAAACUGA	000395	MPH01214A	MIMAT0000073
hsa-miR-382	1.8 x 10 ⁵	GAAGUUGUUCGUGGUGGAUUCG	000572	MPH00201A	MIMAT0000737
hsa-miR-135a	3.2 x 10 ⁵	UAUGGCUUUUUUAUCCUAUGUGA	000460	MPH00035A	MIMAT0000428
hsa-miR-149	1.2 x 10 ⁶	UCUGGCUCCGUGUCUUCACUCCC	000472	MPH00053A	MIMAT0000450
hsa-miR-9a	9.3 x 10 ⁷	UCUUUGGUUAUCUAGCUGUAUGA	000583	MPH00456A	MIMAT0000441

* source: miRNAmap 2.0

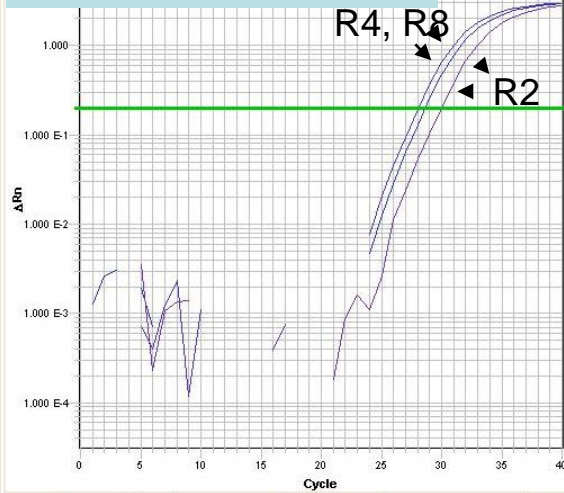
Other studies:

Ambion TechNotes, 2007, 14:2

Liang Y *et al.* BMC Genomics 2007 8:166

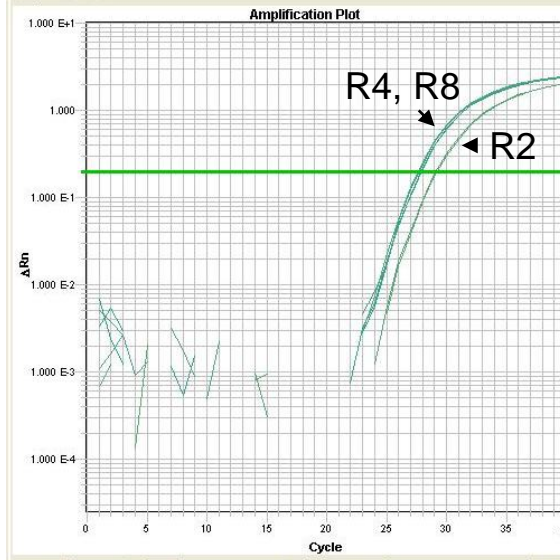
Low copy number miR-192

Stemloop-Taqman



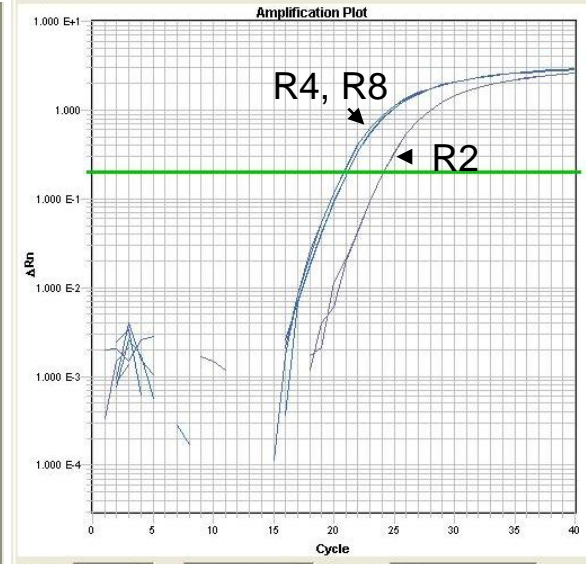
Detector: ABI_miR-2 Plot: ΔRn vs. Cycle Threshold: 0.20

Medium copy number miR-382



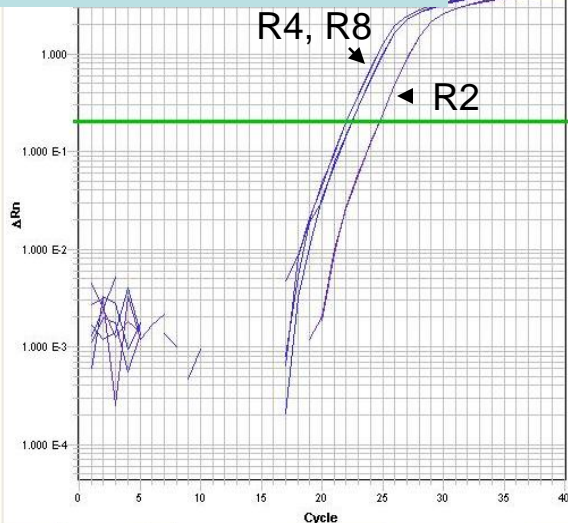
Detector: ABI_miR-8 Plot: ΔRn vs. Cycle Threshold: 0.20

High copy number miR-9

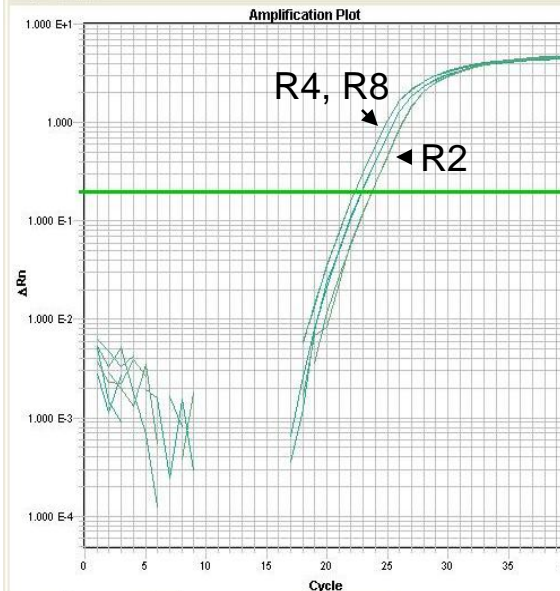


Detector: ABI_miR-5 Plot: ΔRn vs. Cycle Threshold: 0.20

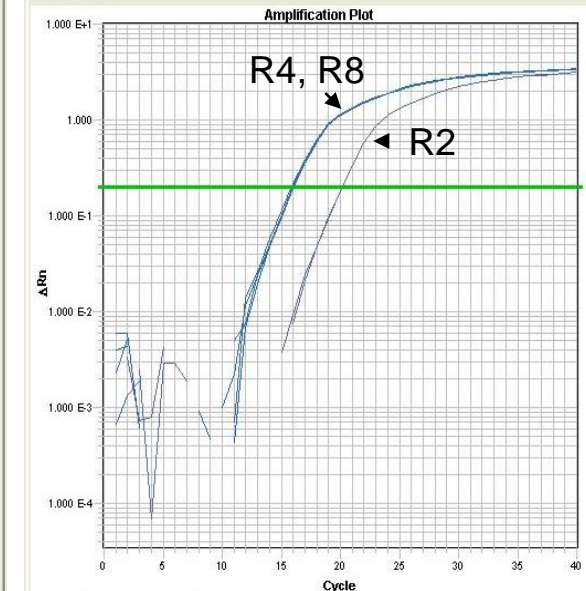
PolyA-SYBR green



Detector: miR-192 Plot: ΔRn vs. Cycle Threshold: 0.20



Detector: miR-382 Plot: ΔRn vs. Cycle Threshold: 0.20

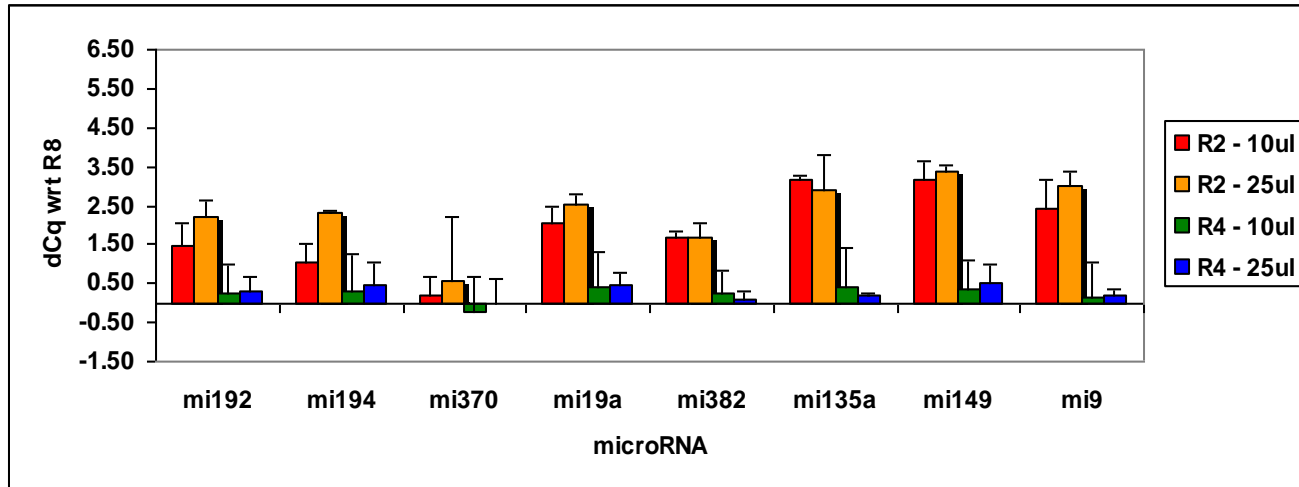


Detector: miR-9-SAB Plot: ΔRn vs. Cycle Threshold: 0.20

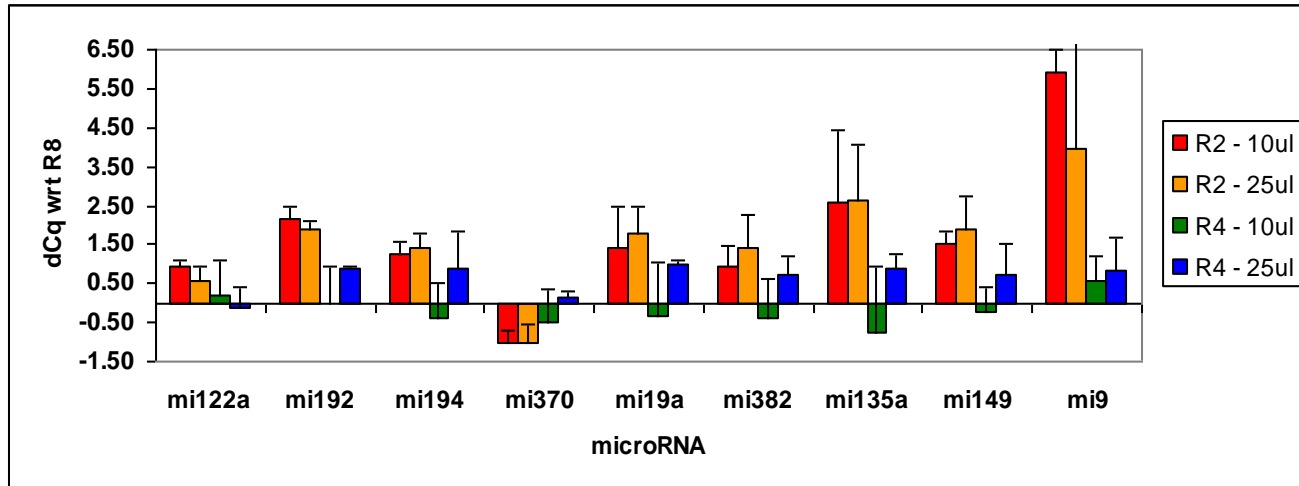
Stem-loop method - TaqMan

10ul rxn
384 well
ABI 7900HT

25ul rxn
96 well
ABI 7500fast
Stratagene
MX3000P



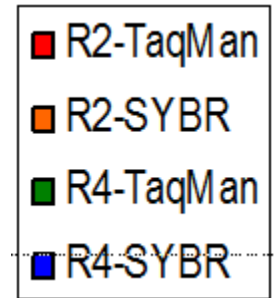
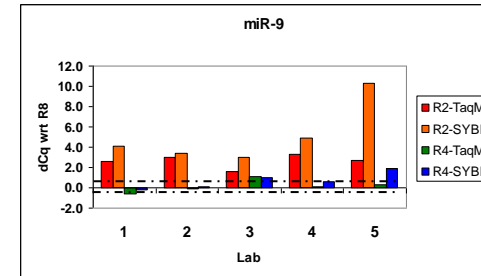
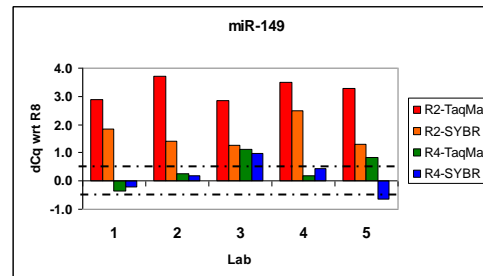
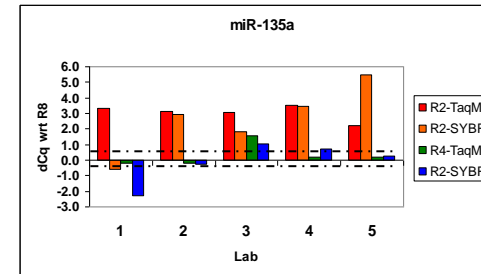
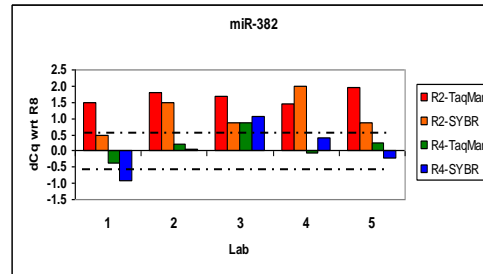
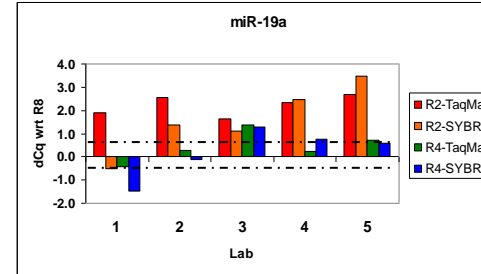
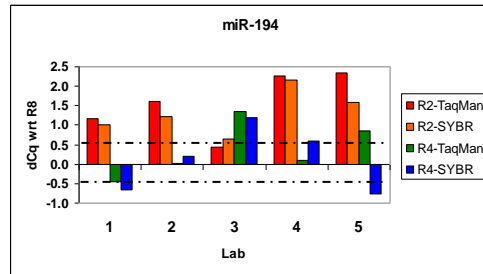
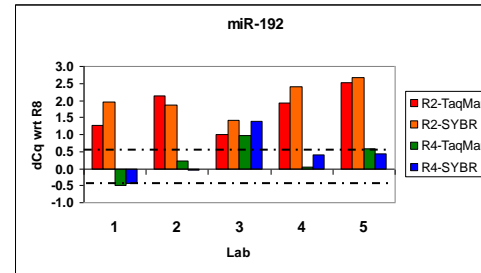
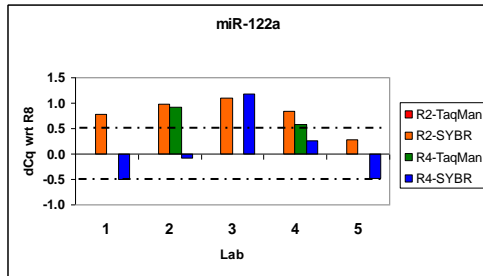
Poly-A method – SYBR Green

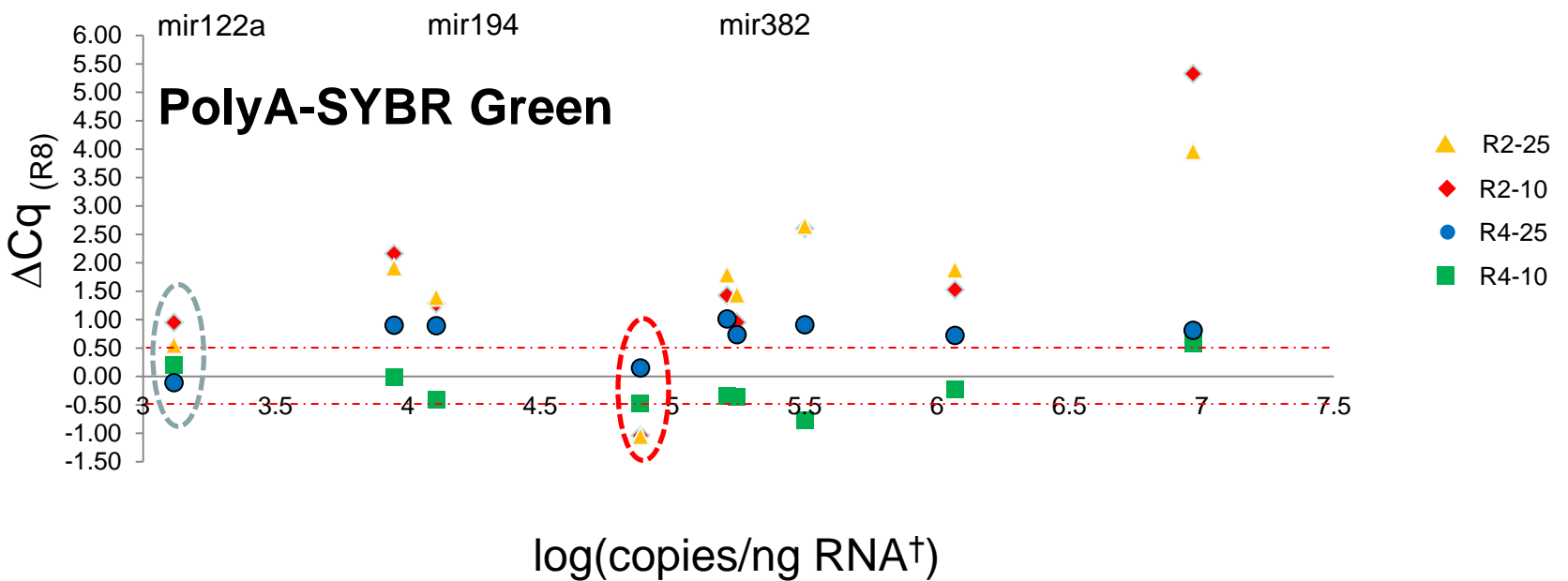
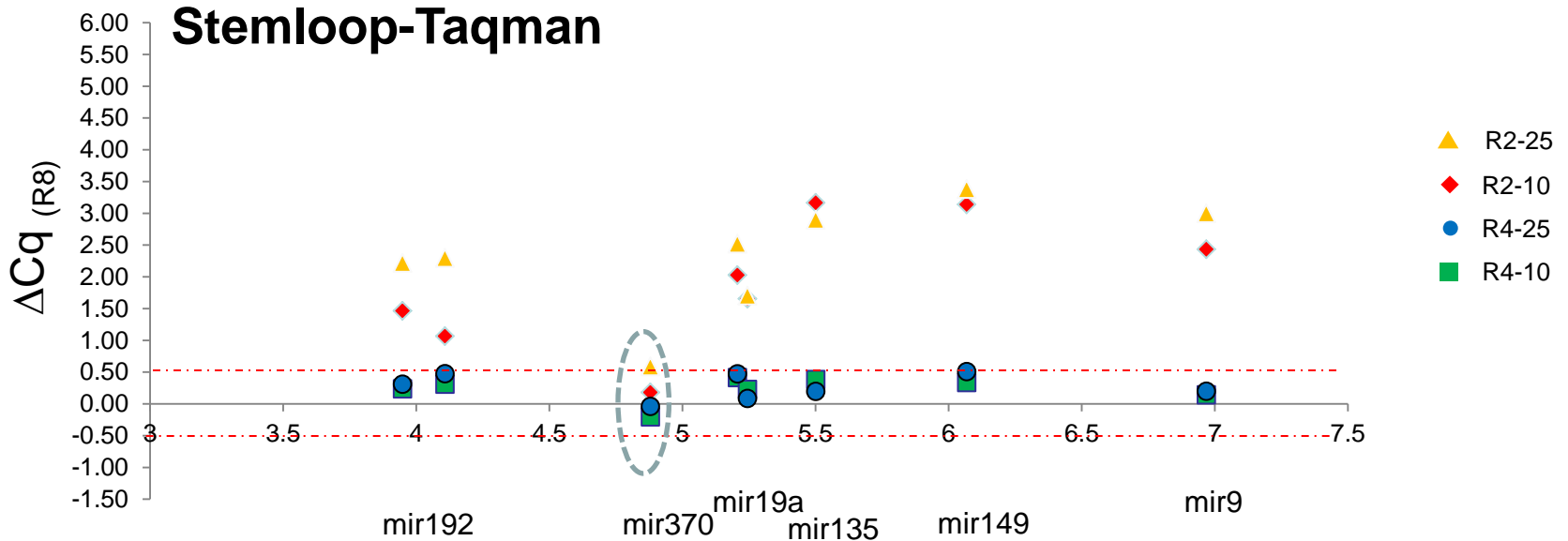


miRNA name	copy number *	mature miRNA sequence	%GC content
hsa-miR-122a	1.3×10^3	UGGAGUGUGACAAUGGUGUUUG	45
hsa-miR-192	8.9×10^3	CUGACCUAUGAAUUGACAGCC	48
hsa-miR-194	1.3×10^4	UGUAACAGCAACUCCAUGUGGA	45
hsa-miR-370	7.6×10^4	GCCUGCUGGGGUGGAA CCUGGU	68
hsa-miR-19a	1.6×10^5	UGUGCAAUCUAUGCAAACUGA	53
hsa-miR-382	1.8×10^5	GAAGUUGUUCGUGGUGGAUUCG	50
hsa-miR-135a	3.2×10^5	UAUGGCUUUUUUAUCCUAUGUGA	30
hsa-miR-149	1.2×10^6	UCUGGCUCCGUGUCUUCACUCCC	61
hsa-miR-9a	9.3×10^7	UCUUUGGUUAUCUAGCUGUAUGA	53

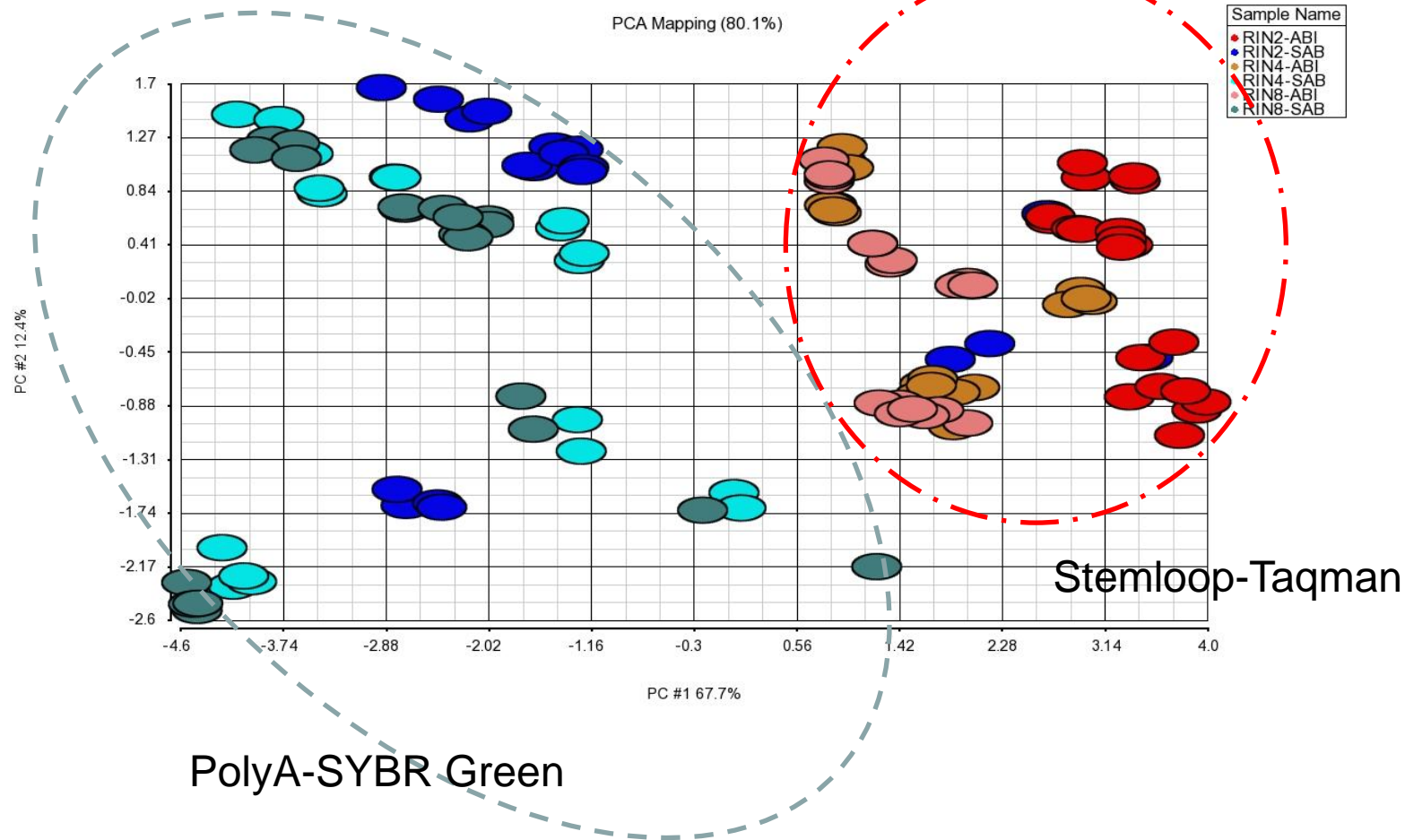


ΔCq of each miRNA per method for each participating laboratory

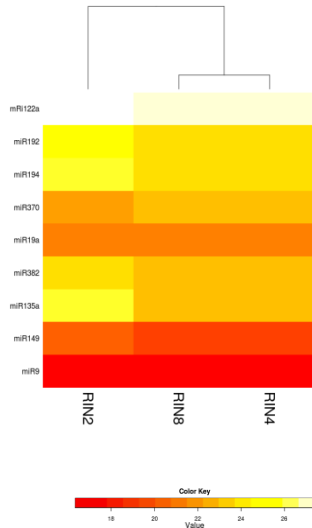
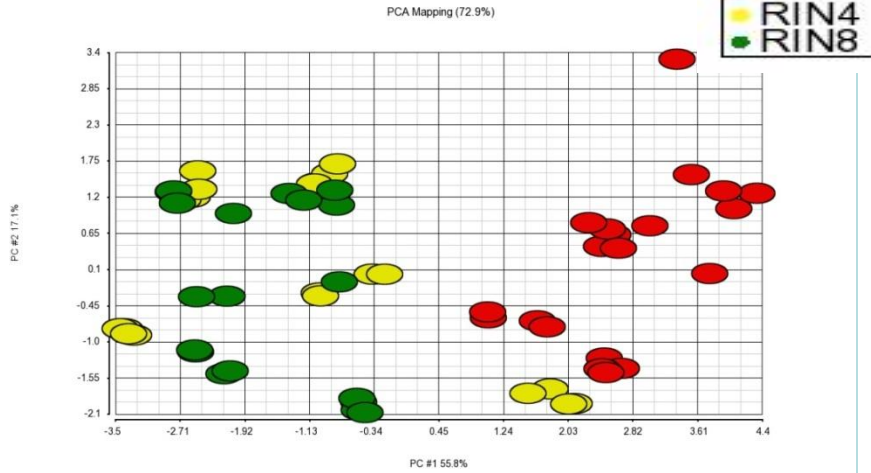




†source:miRNAmap2.0

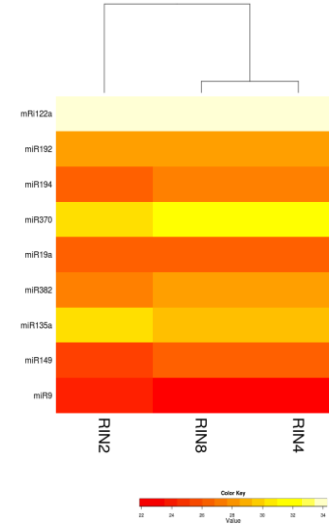
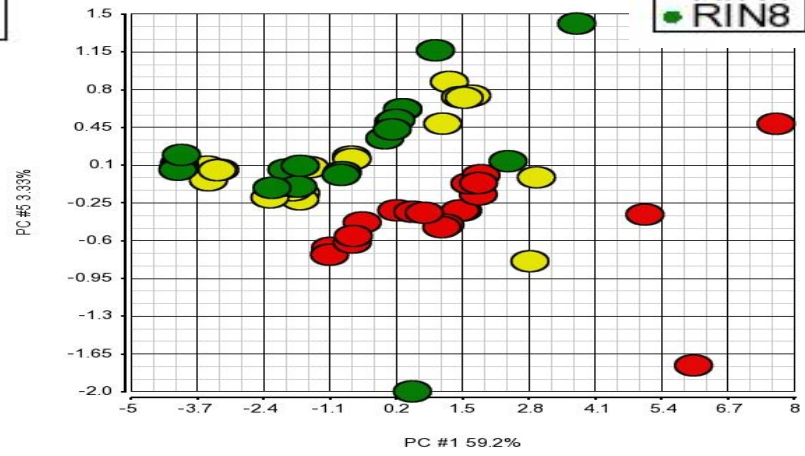


Stemloop-Taqman



PolyA-SYBR Green

PCA Mapping (62.5%)



Conclusions from miRNA qPCR assays

- There seems to be a fair amount of inter-lab variability in the qPCR Cq values for miRNA. We believe that this is probably at the level of the RT step as determined previously in the mRNA qPCR studies.
- Increased degradation is directly proportional to Cq regardless of method. There was not a significant difference between R4 and R8 indicating that some degradation is tolerated. The discrimination between R2 and R4/R8 was better using the Megaplex stem-loop method.
- hsa-mir-122 was only detected by 2 labs (and only with R8 and R4) using the MegaPlex method. This miRNA was however, detected in other studies (previous Ambion study) using the MegaPlex method with pre-amplification step. This miRNA was detected by all labs using the polyA tailing method suggesting a lower detection threshold
- The polyA tailing method tended to give more false positives for the hsa-mir-370 assay.
- There was little difference in Cq value between R8, R4 and R2 for the stem-loop method for hsa-mir-370.
- We recommend that total RNA samples below RIN5 be treated cautiously for miRNA profiling

Acknowledgements

➤ **ABRF Nucleic Acids Research Group**

Russ Carmical	UTMB
Sridar Chittur	Ualbany (Co-chair)
Jennifer Holbrok	Nemours
Vijay Nadella	Ohio Univ
Scott Tighe	UVM (Co-chair)



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Don Baldwin	UPenn
Sridar Chittur	UAlbany
Nalini Raghavachari	NIH
Scott Tighe	UVM

➤ **Bioinformatics**

Jeff Bond	UVM
Julie Dragon	UVM

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