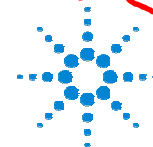
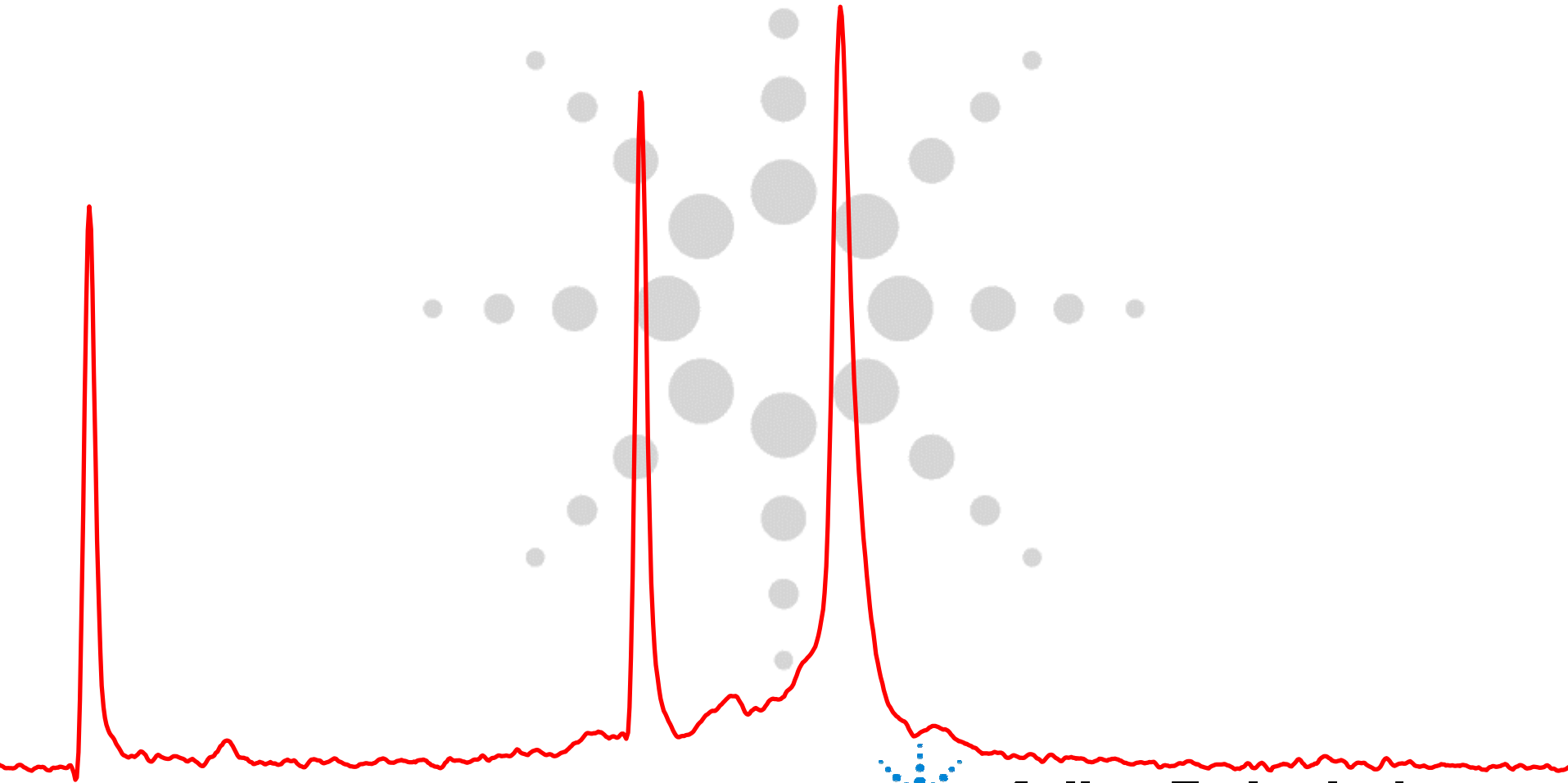


RNA Integrity Number (RIN)

- A standardized approach for RNA integrity assessment



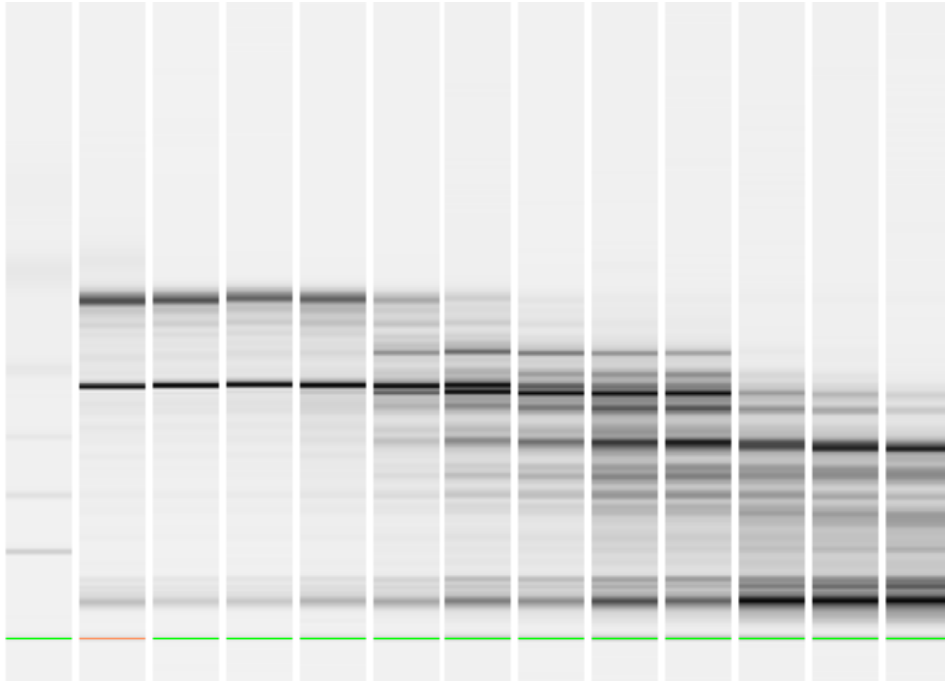
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Overview

- **Problem Description**
- **Approach**
- **Results**



Problem Description

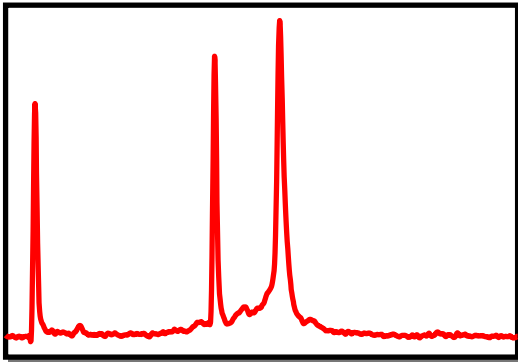


- The ratio of ribosomal bands is not sufficient to describe RNA integrity!
- RNA degradation is a gradual process.
- Results have to be interpreted by visual inspection.
- Overlay of electropherograms only works well for samples with the same concentration.
- Instrument dependency in signal height



Introducing the RNA Integrity Number (RIN)

- The RIN is a software algorithm that allows classification of RNA integrity
- It extracts a number of characteristic features from the bioanalyzer electropherogram
- The RIN returns a value that is characteristic for the integrity of a specific sample.

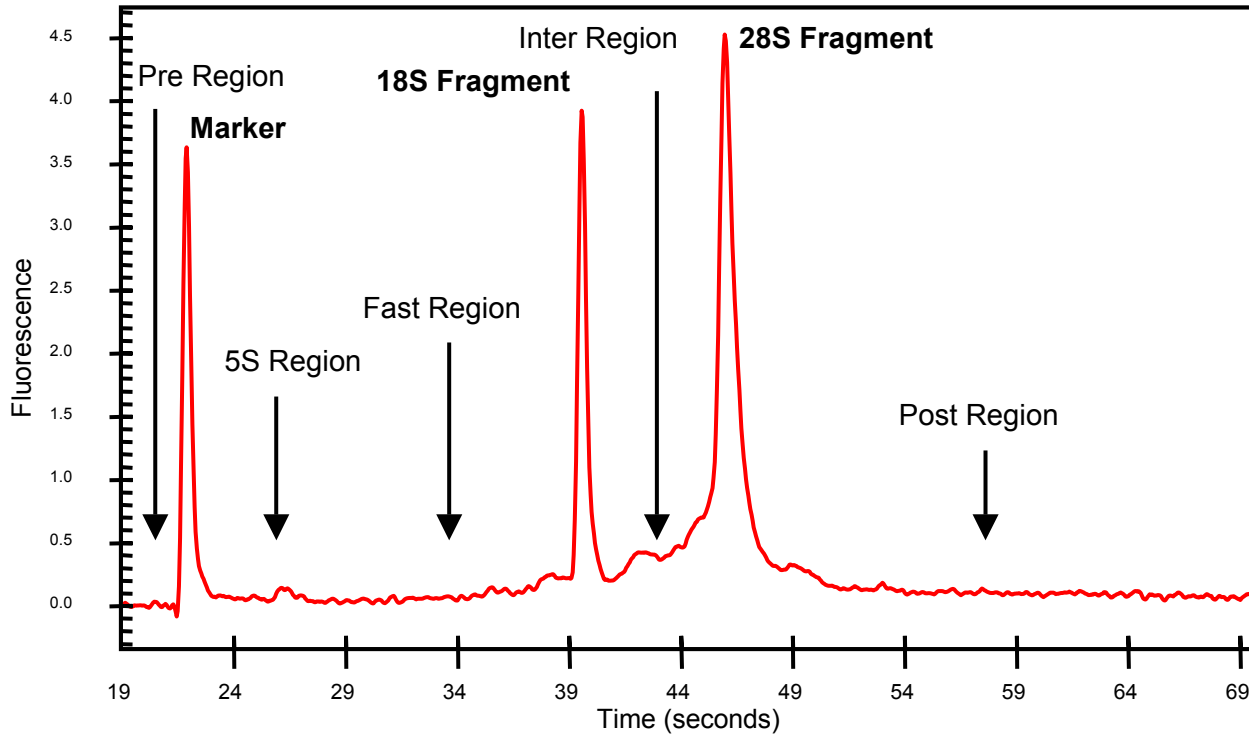


RNA Area:	26.3	
RNA Concentration:	75	ng/ μ l
rRNA Ratio [28s / 18s]:	1.9	
RNA Integrity Number (RIN):	9.6	



Feature Generation

Approach: Training of regression models for peak positions and Intensities based on peak labels



Features

- Peak height/position
- Areas/Area ratios
- S/N ratio
- Max, Min values
- Waviness of the curve
- ...

Selection of the best feature combination

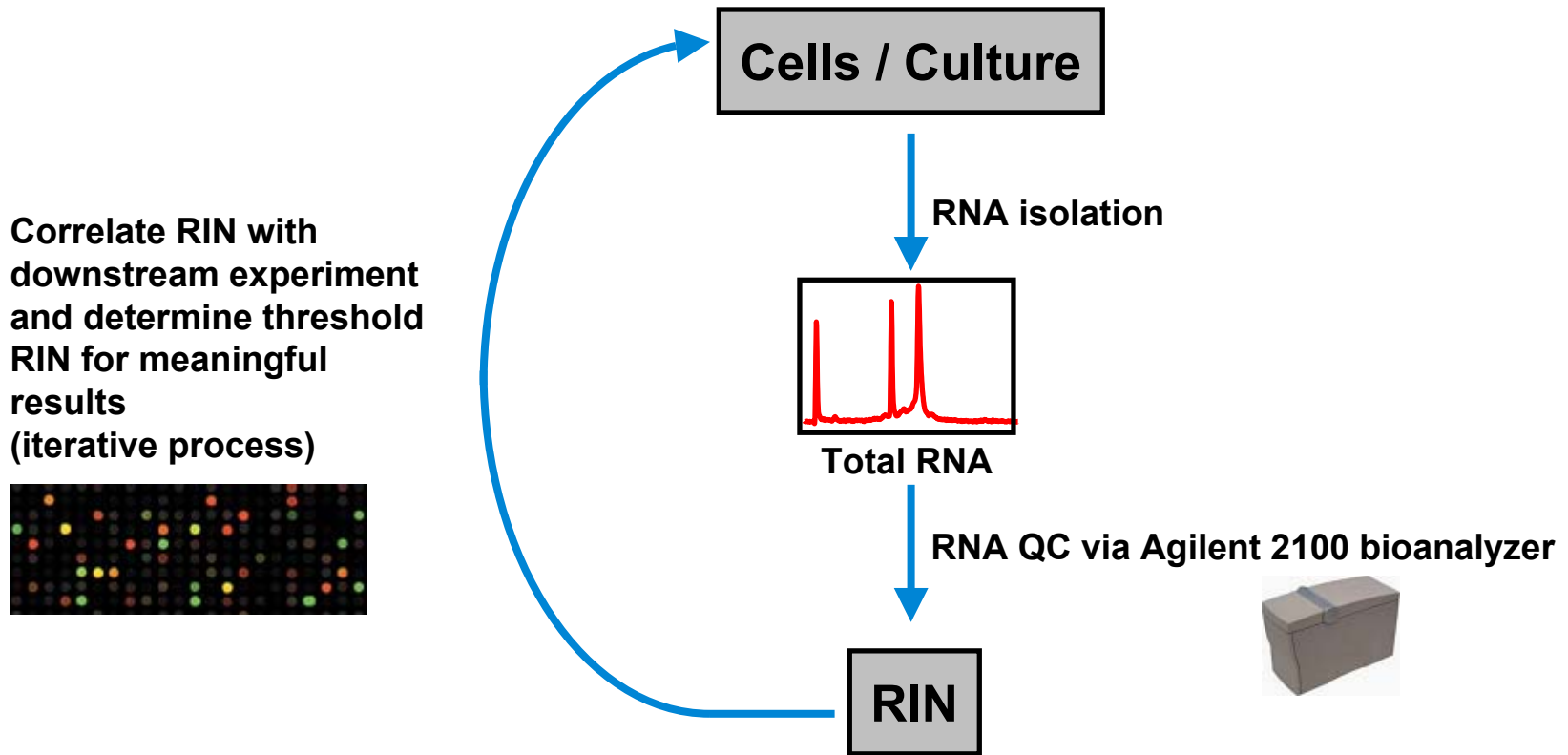


RIN - Limitations

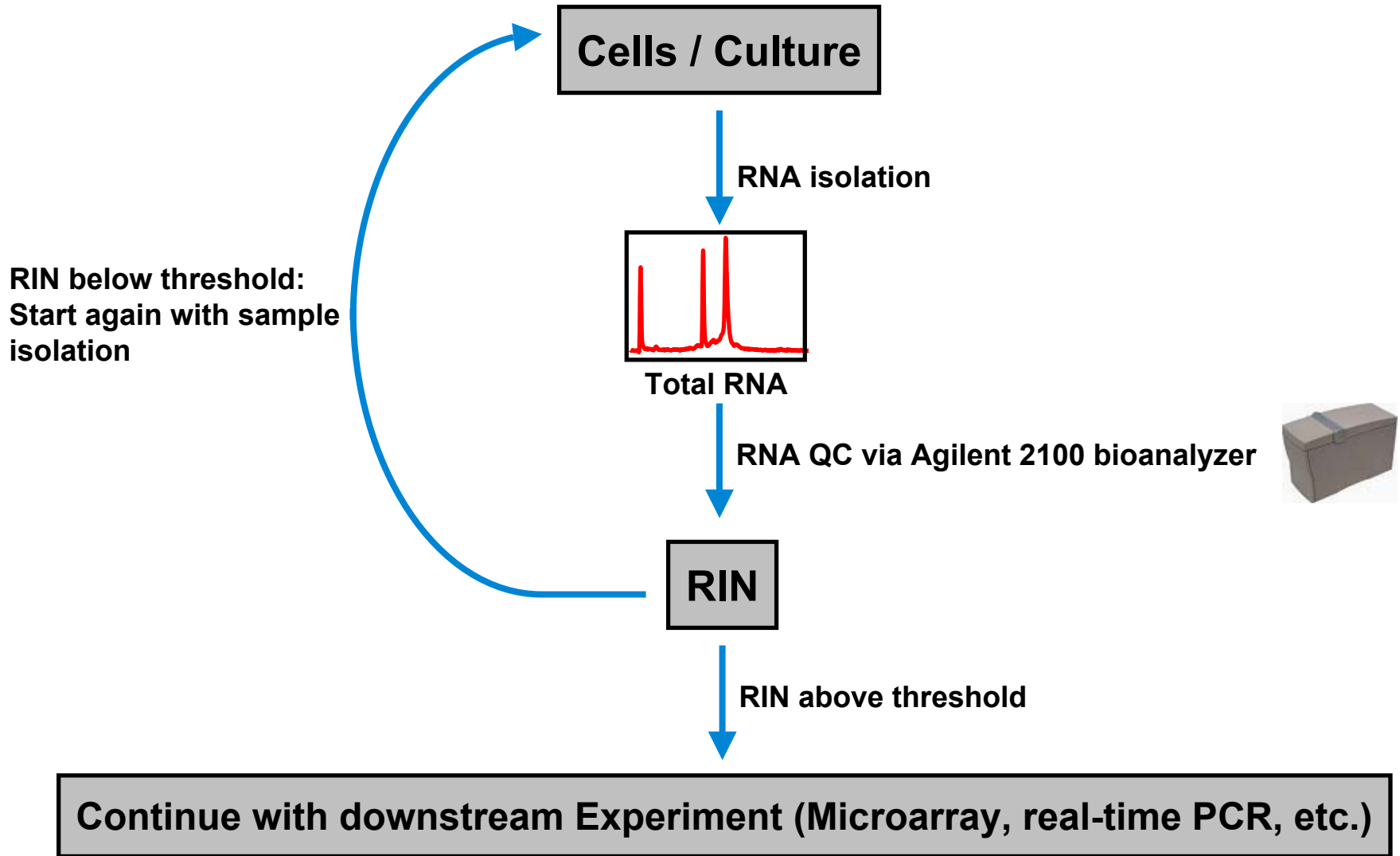
- ***What the RIN can do:***
 - Obtain an assessment of RNA integrity.
 - Directly compare RNA samples
 - Ensure repeatability of experiments
- ***What it CANNOT do:***
 - Predict the outcome of an experiment if no prior validation was done



RIN Validation for Gene Expression Experiments



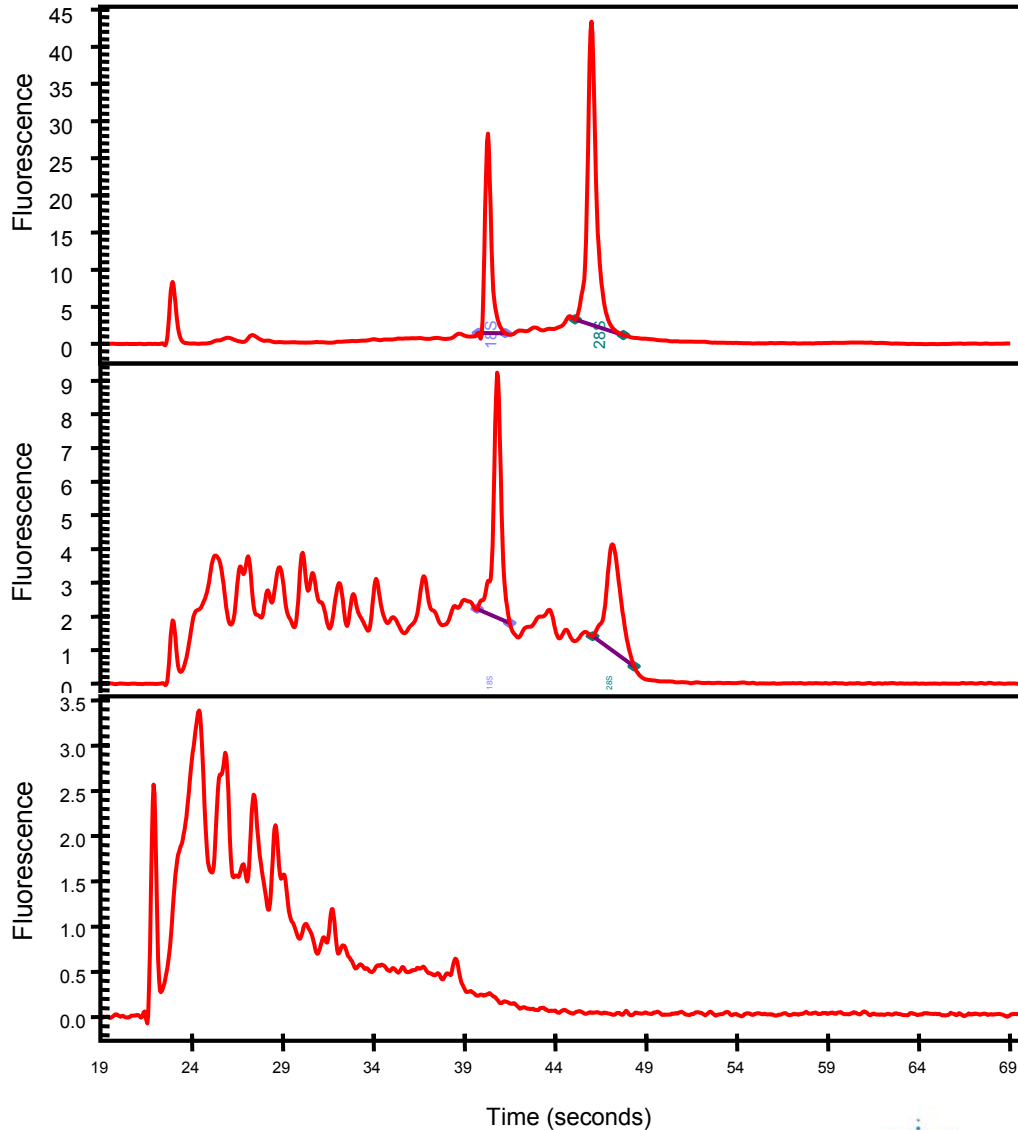
RNA QC in Routine Gene Expression Workflow



Results



RIN Application – Assessment of RNA Integrity



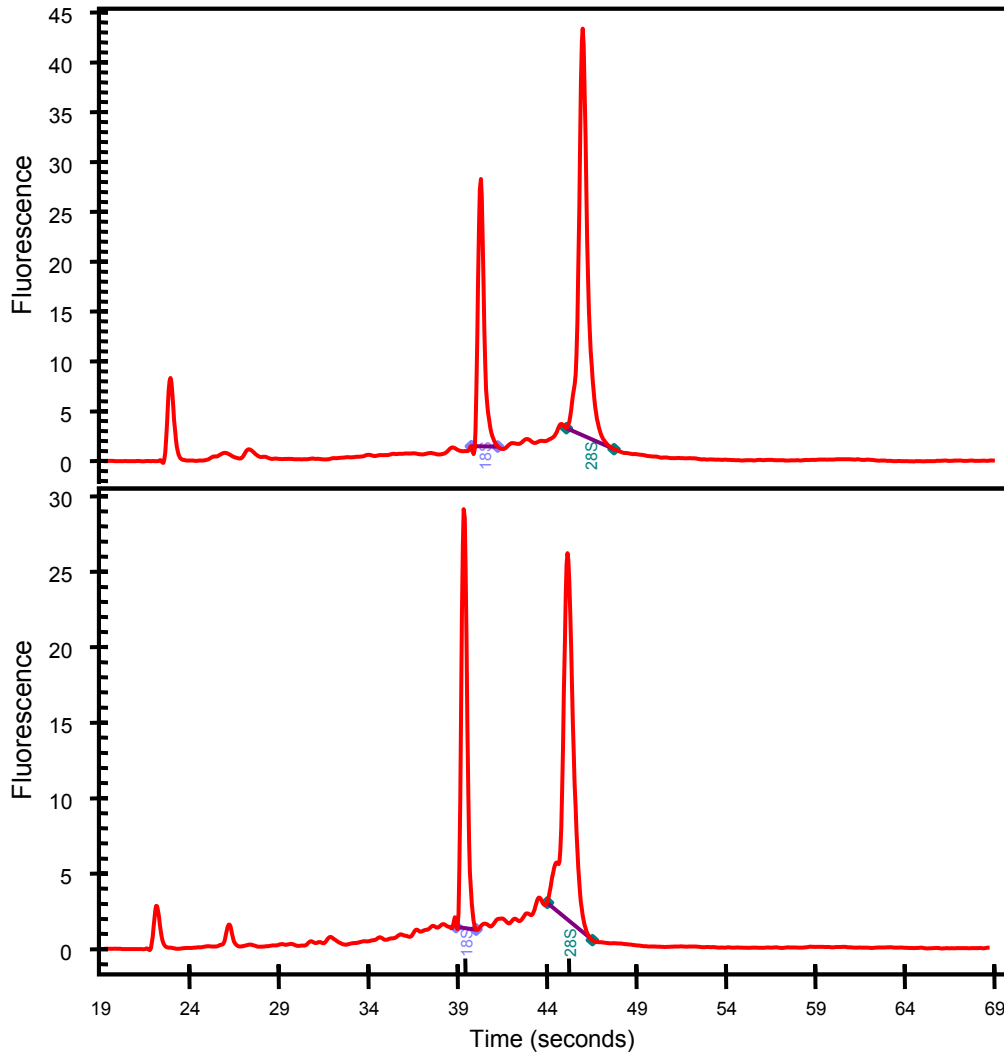
Intact RNA: RIN 10

Partially degraded RNA: RIN 5

Strongly Degraded RNA: RIN 3



RIN Application – Assessment of RNA Integrity



Intact RNA: RIN 10

ribosomal ratio: 1.9

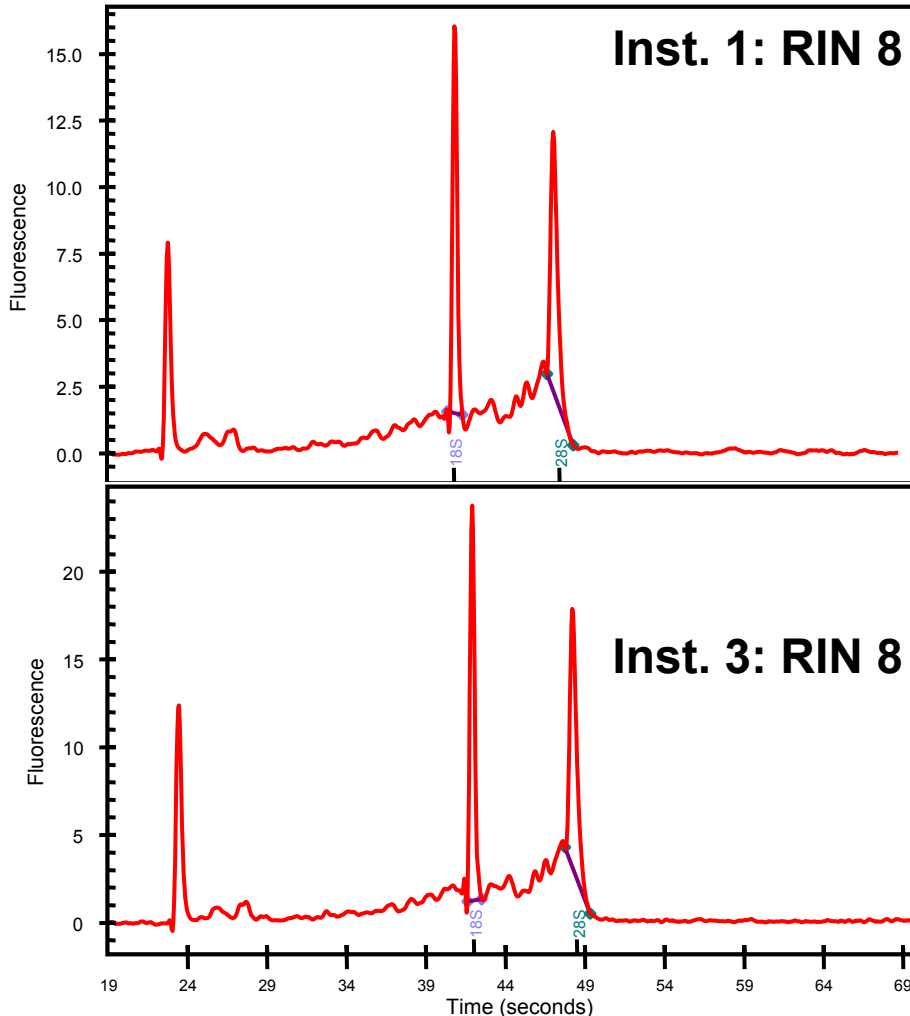
Intact RNA: RIN 10

ribosomal ratio: 1,4



RIN Application – Directly Compare Samples

same sample on different instruments



When testing an identical RNA sample on various instruments, identical RINs are obtained – within narrow limits

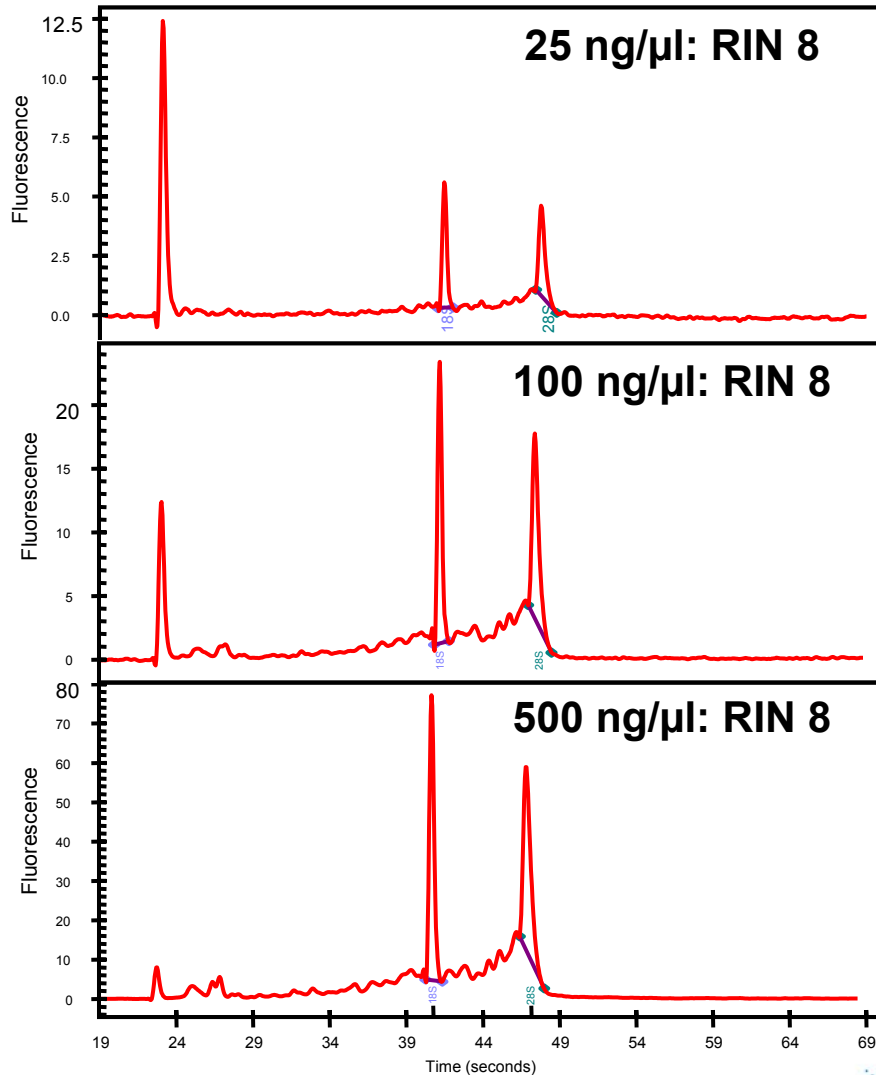
36 samples 3 instruments
CV RIN: 1.4 %
CV ribosomal ratio: 5.1* %

* Improved with new software



RIN Application – Directly Compare Samples

same sample in different dilutions



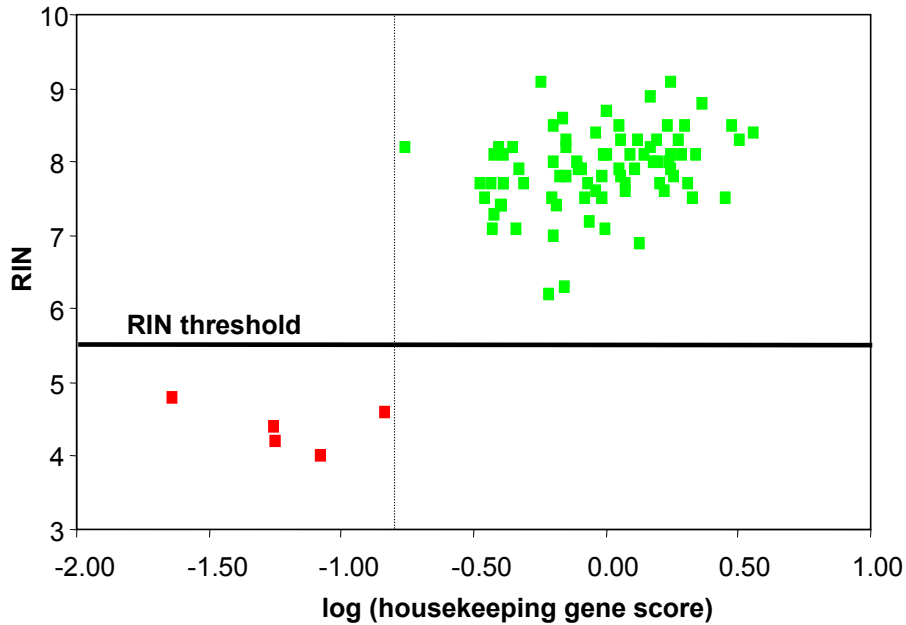
When testing an identical RNA sample in various dilutions, identical RINs are obtained – within narrow limits

108 samples 3 dilutions
CV RIN: 3 %
CV ribosomal ratio: 22 %

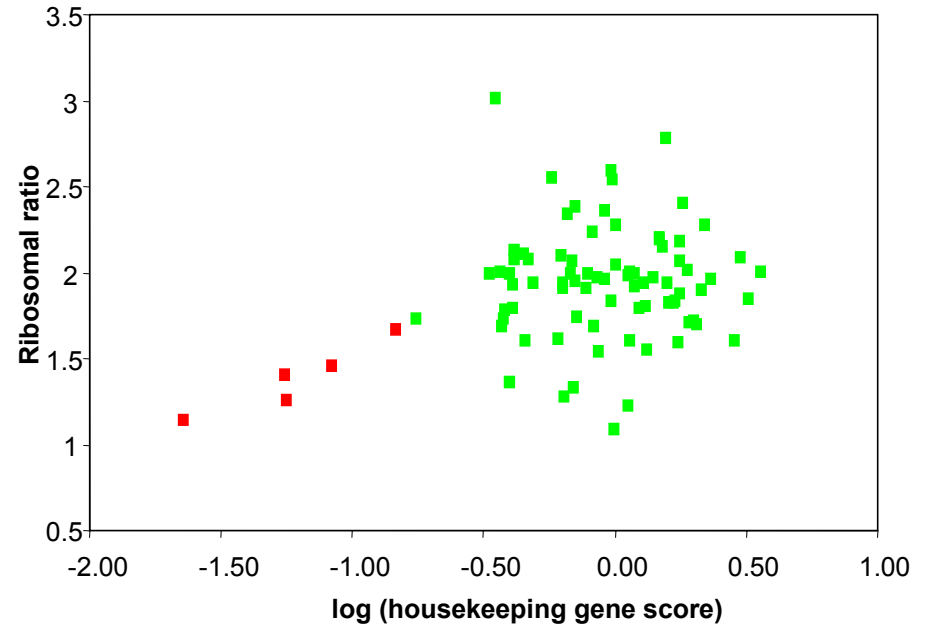


Determination of Threshold for Real-Time PCR

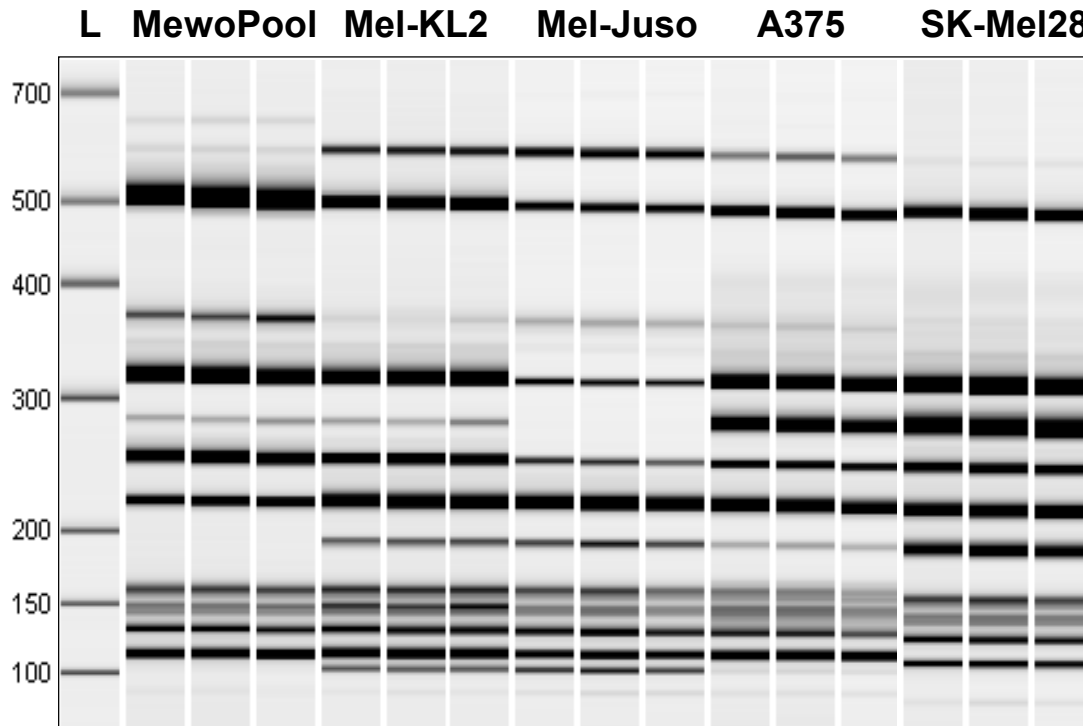
RIN vs. log housekeeping gene score



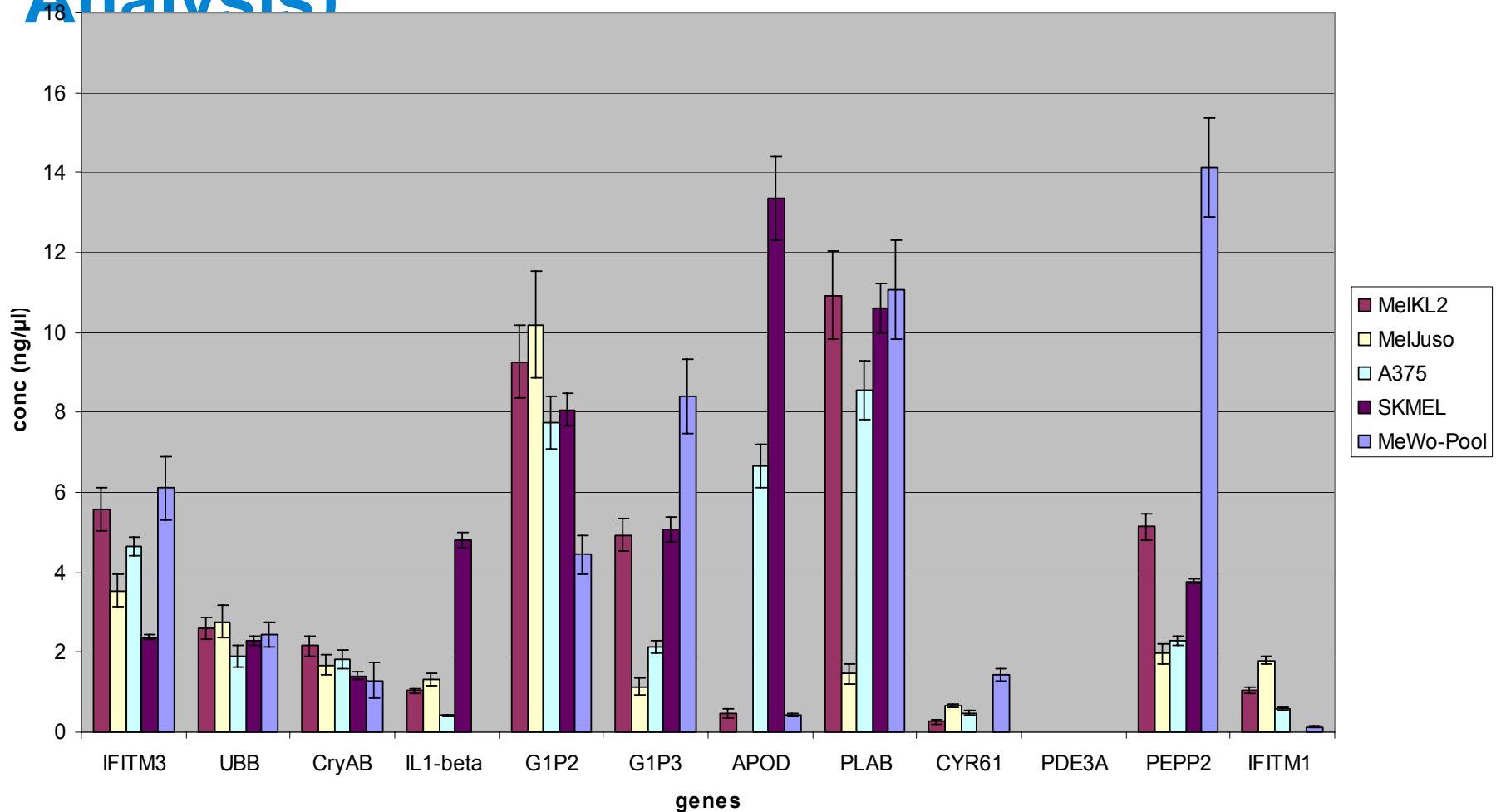
Ribosomal ratio vs. log housekeeping gene score



Comparative Expression Analysis of 5 Melanoma Cell Lines (12-plex PCR)



Comparative Expression Analysis of 5 Melanoma Cell Lines – Semi-quantitative Analysis)



Conclusion

- **A software algorithm was presented that allows standardization of RNA integrity assessment**
- **Initial results indicate that the software algorithm is independent of instrumentation and can ensure repeatability of results (e.g. RT-PCR or microarray)**
- **Further validation of the algorithm is necessary**



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- **Software**

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*Johns Hopkins Medical Institute

+ German Cancer institute (DKFZ)

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- **Ruediger Salowsky**
- **Marcus Gassmann**
- **Christine Miller***
- **Rainer Wittig+**

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