**Asterand**

Partners in Human Tissue Research

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### RNA Quality and Yields from Frozen Tissues

# QC or Not QC?

- **Pro**
  - Don't waste good experiments on bad samples
  - Know what you have
  - Appropriate materials for each experiment

- **Cons**
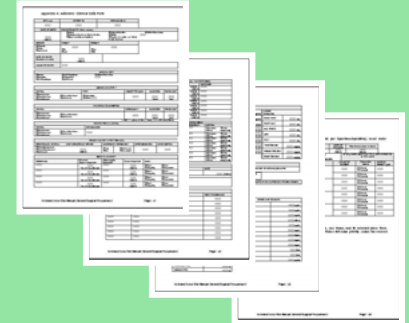  - Expensive
  - Time consuming

**(1)  Recovery**

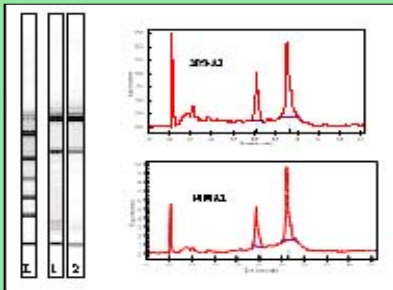**(2) Receive Materials**

**(3) Clinical Data Review**

**(8) Biorepository**

# Quality Control Processing

**(4) H&E Processing**

**(7) RNA Quality Testing**

**(6) Mirror Inference**

**(5) Path QA**

# RNA QC

- **Small sample (~50 mg) cut on dry ice**

- **TRIzol extraction**

- **Agilent Bioanalyzer 2100 analysis**

- **Manual Grading from 2001 until 2006**

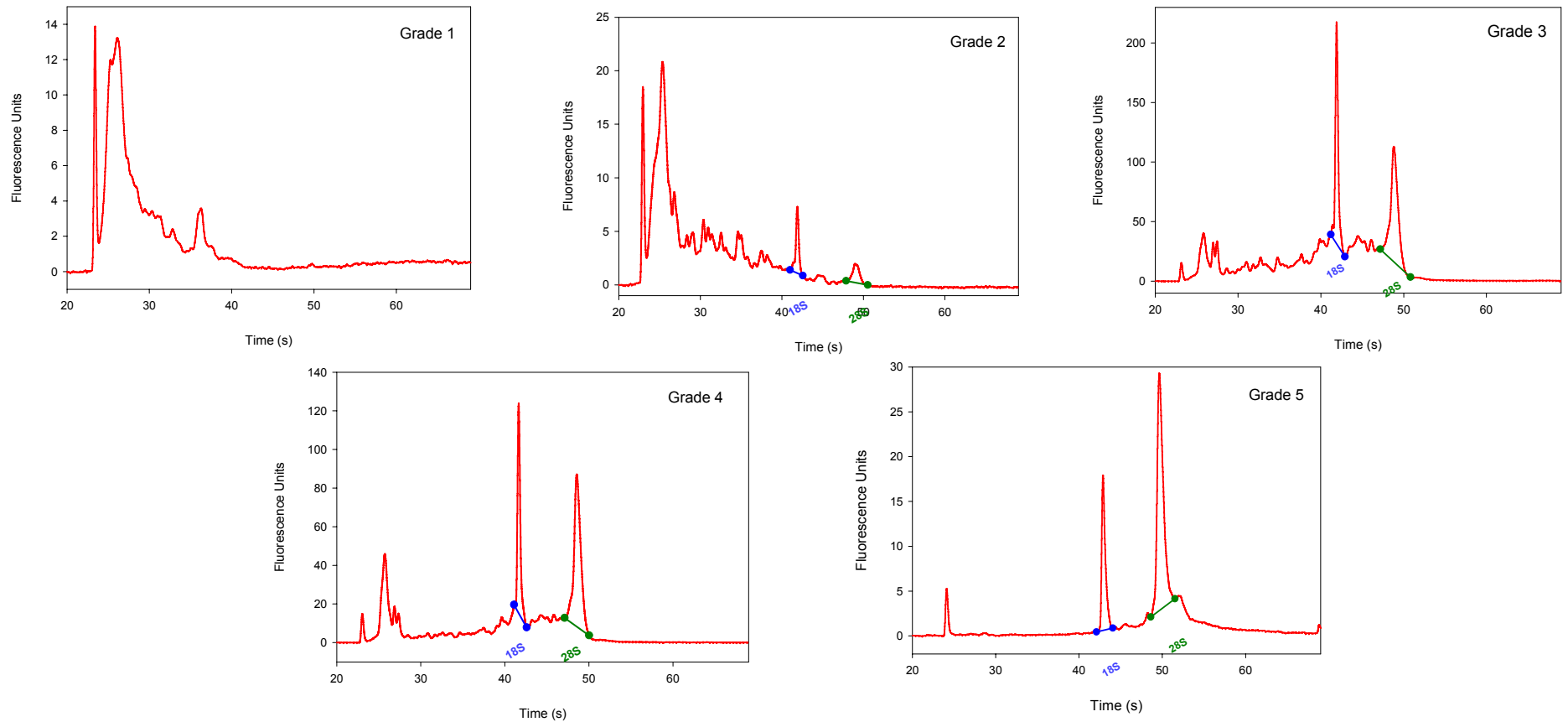- **Agilent RNA Integrity Number (RIN)**

# Asterand Grading System

One point each:

- Ratio of 28S to 18S peaks ≥1.3.

- Area under 28S and 18S peaks combined ≥30% of the total area.

- Widths of 18S and 28S peaks ≤4 seconds.

- No distinct peaks between 28S and 18S peaks or between 18S peak and lower marker peak.

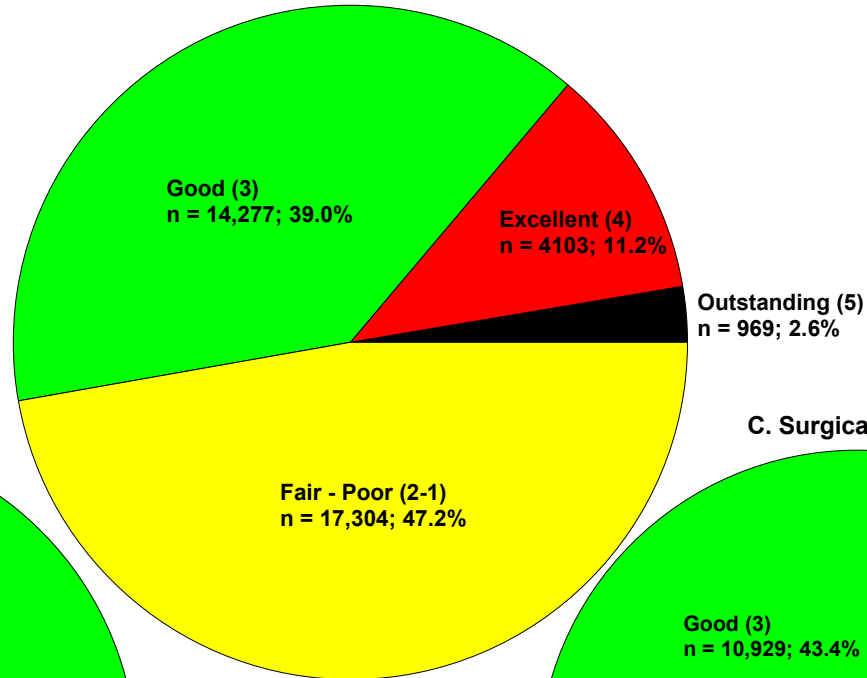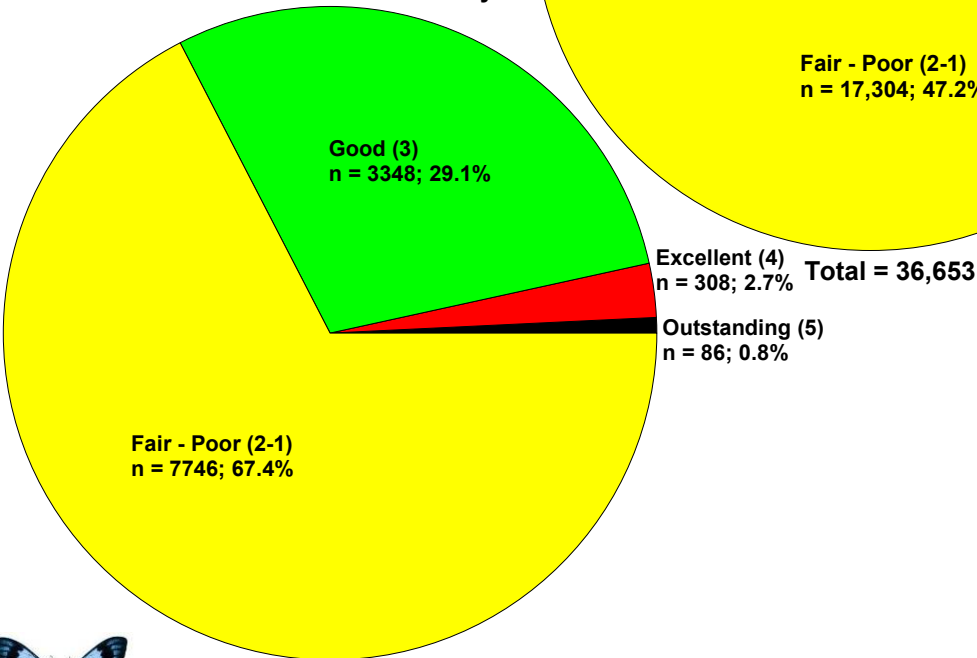- Area under degradation peaks <combined areas of 28S and 18S peaks.

# Asterand RNA Grading Categories

# Comparison of RNA Quality : Surgical and Postmortem Recoveries



**A. All Samples**

Good (3)
n = 14,277; 39.0%

Excellent (4)
n = 4103; 11.2%

Outstanding (5)
n = 969; 2.6%

Fair - Poor (2-1)
n = 17,304; 47.2%

Total = 36,653

**B. Postmortem Recovery**

Good (3)
n = 3348; 29.1%

Excellent (4)
n = 308; 2.7%

Outstanding (5)
n = 86; 0.8%

Fair - Poor (2-1)
n = 7746; 67.4%

Total = 11,488

**C. Surgical Samples**

Good (3)
n = 10,929; 43.4%

Excellent (4)
n = 3795; 15.1%

Outstanding (5)
n = 883; 3.5%

Fair - Poor (2,1,0)
n = 9558; 38.0%
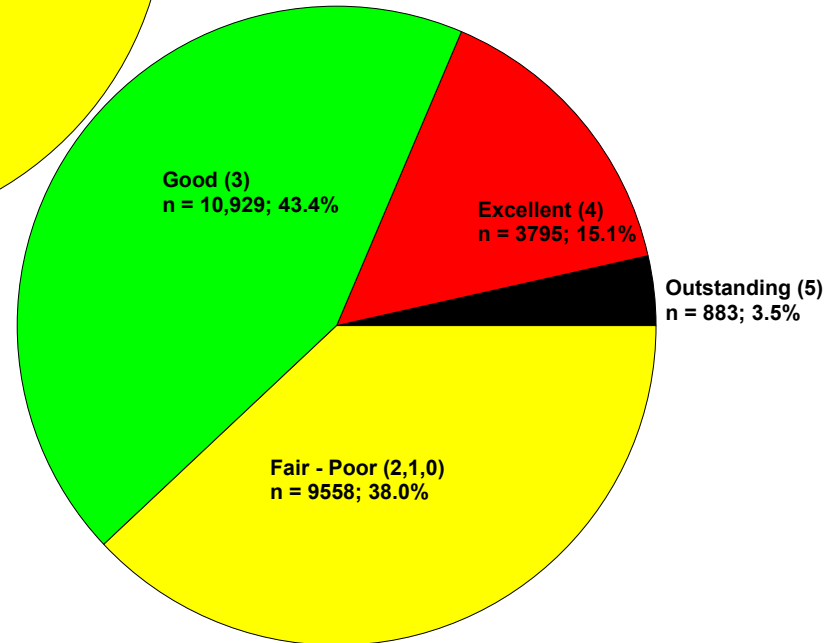
Total = 25,165

# Comparison RIN v Grading (n=2688)
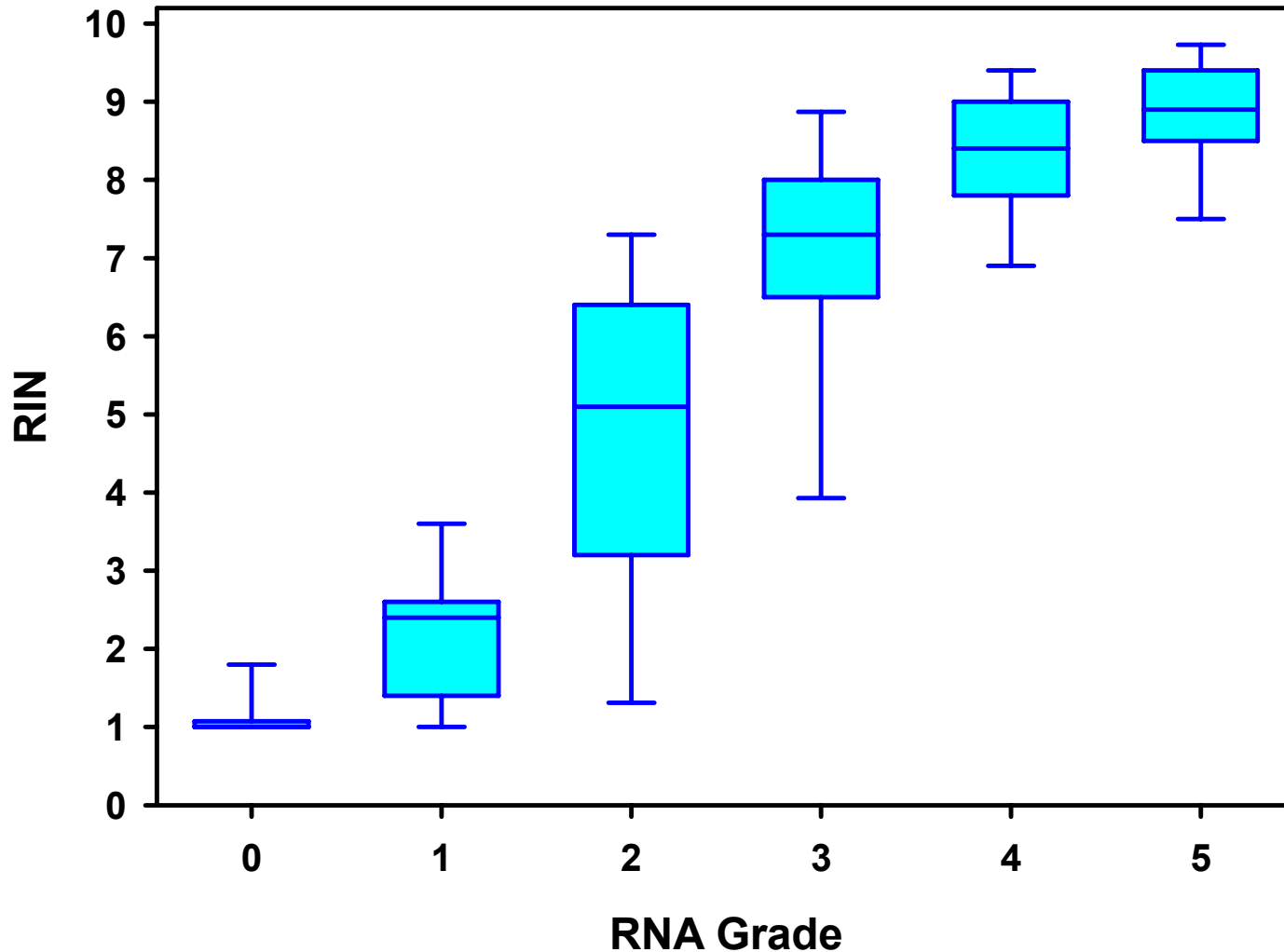
# RIN v Grade

| Grade | n | RIN | | | |
|---|---|---|---|---|---|
| | | Mean ± s.d. | Median | 25% | 75% |
| 0 | 84 | 1.3 ± 0.9 | 1.0 | 1.0 | 1.0 |
| 1 | 287 | 2.4 ± 1.4 | 2.4 | 1.4 | 2.6 |
| 2 | 760 | 4.8 ± 2.0 | 5.1 | 3.2 | 6.4 |
| 3 | 752 | 6.9 ± 2.0 | 7.3 | 6.5 | 8.0 |
| 4 | 609 | 8.0 ± 1.9 | 8.4 | 7.8 | 9.0 |
| 5 | 196 | 8.7 ± 1.2 | 8.9 | 8.5 | 9.4 |

# Human v Computer Grading: Non-linear with Emphasis on Highest Quality
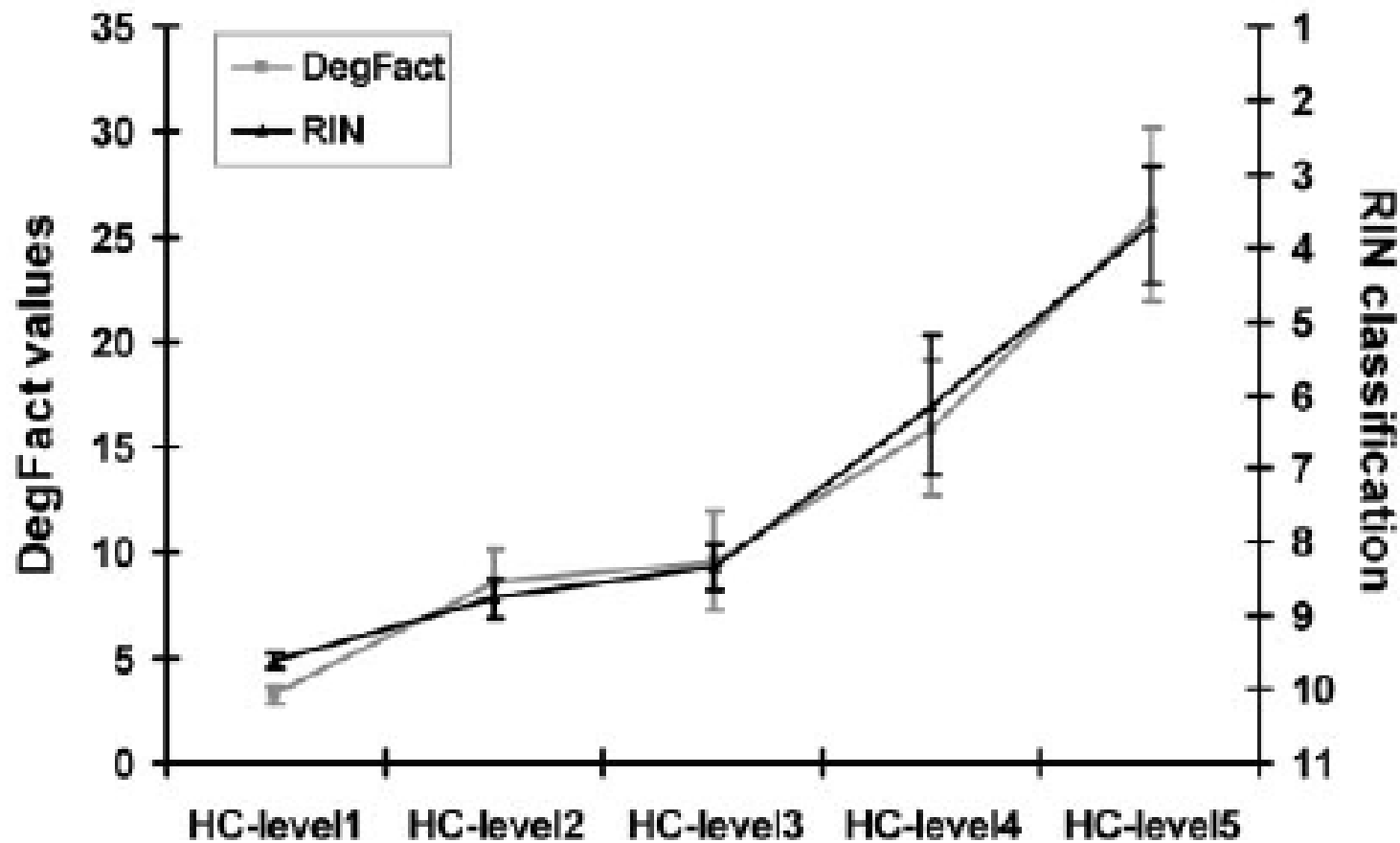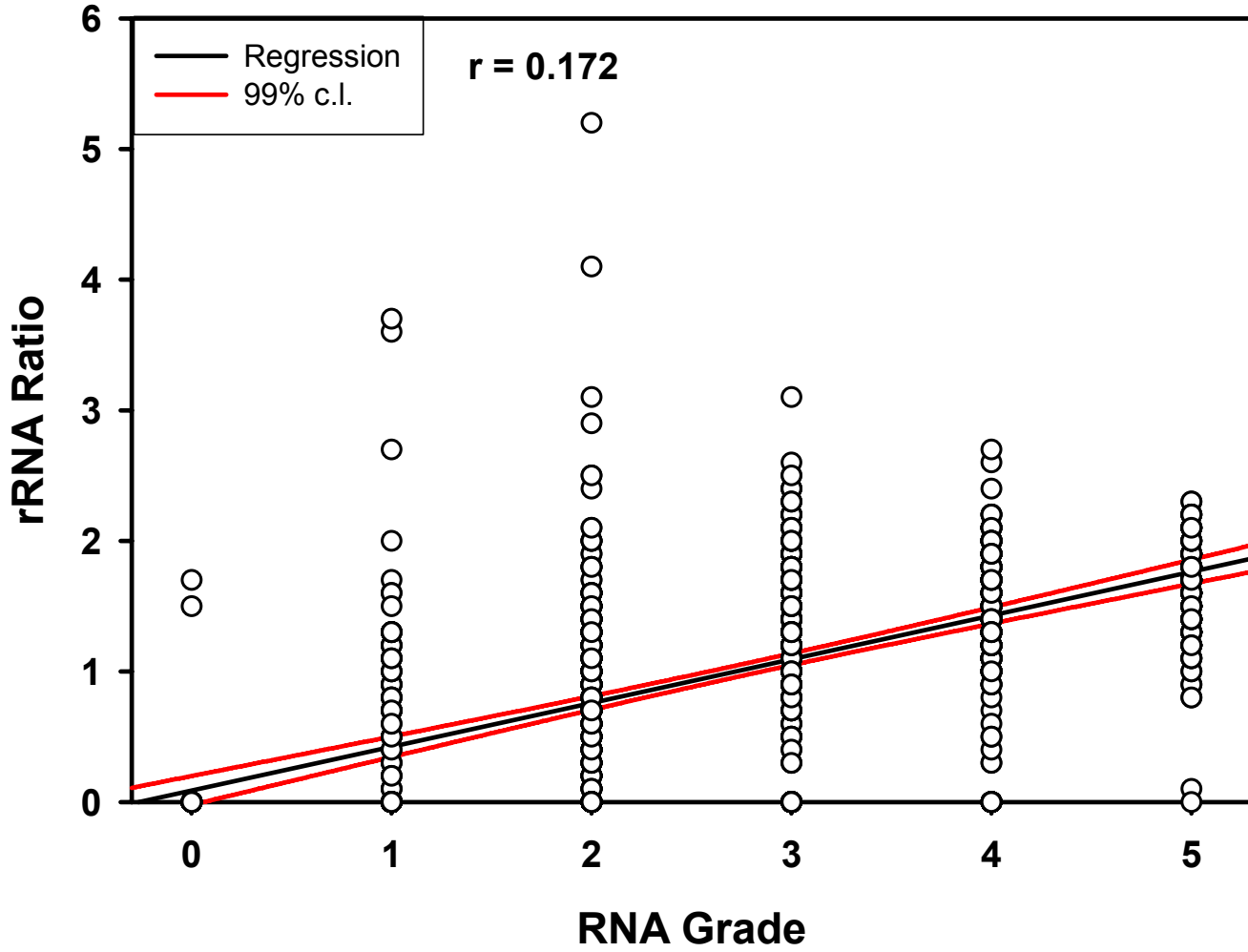


**Imbeaud et al. Nucleic Acids Res 33, e56, 2005**

# rRNA Ratio v RNA Grade

# RIN v rRNA ratio

# RIN v rRNA Area



$$R = 0.981$$

$$y = 1.663 + 7.556(1 - 0.933^x)$$
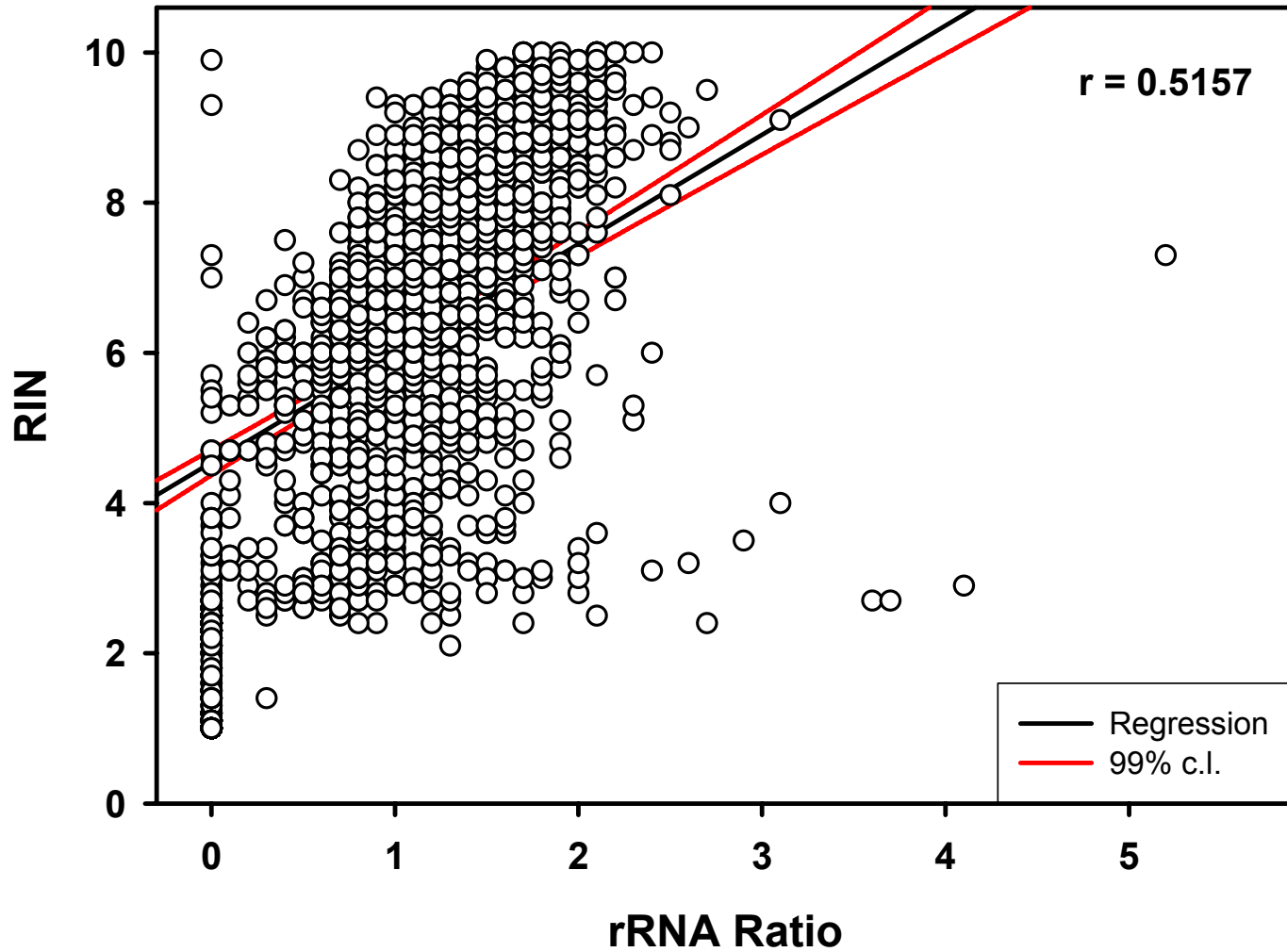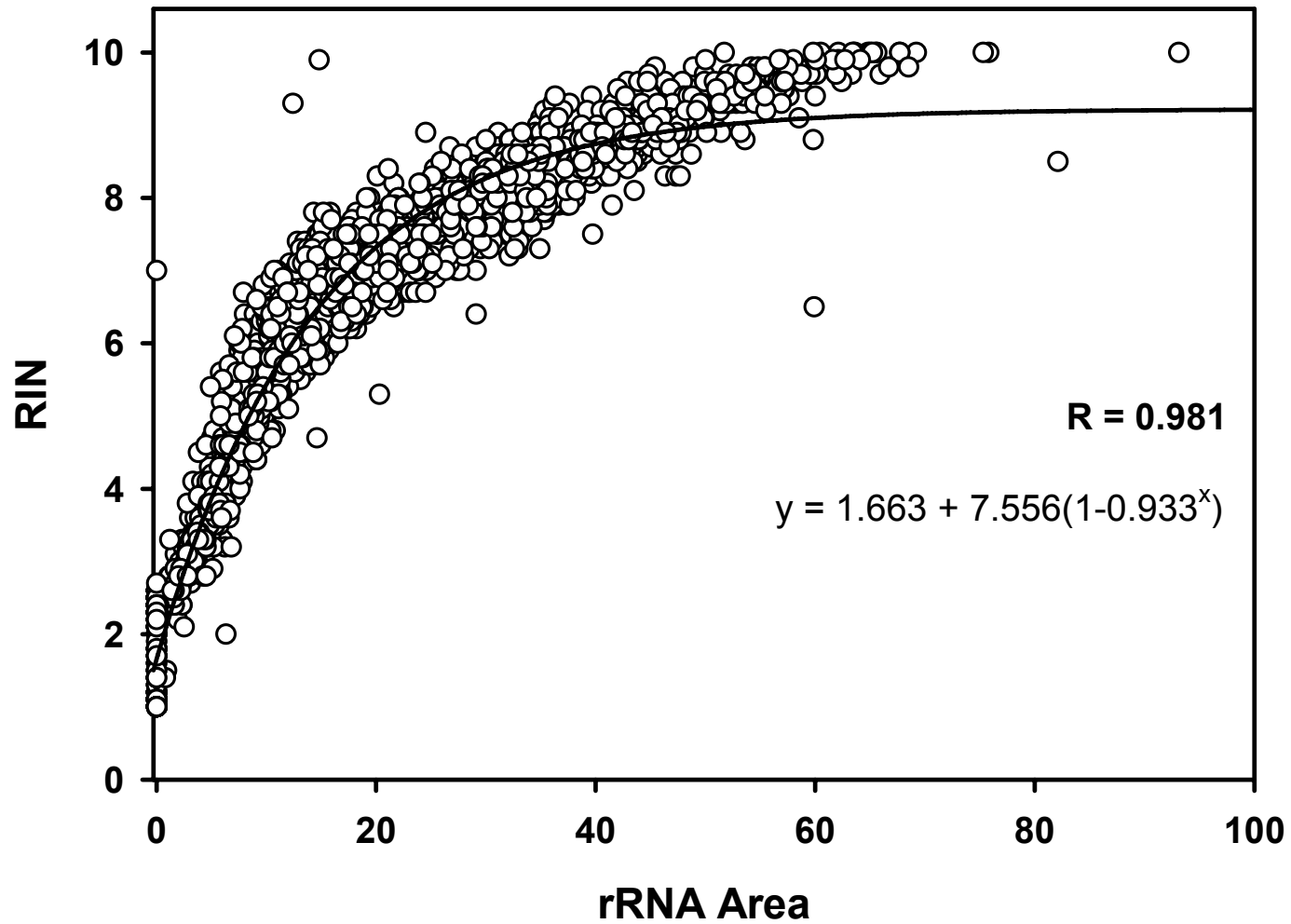
# Summary RNA Quality Measures

- **Good correlation between the RNA quality grades and RIN**

- **RNA grades 3-5 cover a small range of RIN.**

- **rRNA ratio correlates very poorly with other measures of RNA quality.**

- **RIN correlates well with the area under the rRNA peaks, but it is a non-linear.**

- **RIN classification:**
  - **High: RIN ≥7**
  - **Medium: 2.5 ≥ RIN <7**
  - **Low: RIN <2.5**

# RIN Categories

| RIN Category | Number | Mean RIN ± SD |
|---|---|---|
| High RIN ≥7 | 14922 | 8.4 ± 0.8 |
| Medium 2.5 ≥ RIN <7 | 9963 | 4.6 ± 1.6 |
| Low RIN <2.5 | 7375 | 1.0 ± 1.1 |
| Total | 32260 | 5.5 ± 3.2 |

# Heat Treatment (2 min. @ 70°C)

| Group | Treatment | number | Mean ± SEM | |
| --- | --- | --- | --- | --- |
| | | | RIN | rRNA ratio |
| Set 1 | none | 83 | 7.4 ± 0.2 | 1.6 ± 0.04 |
| | HT | | 7.4 ± 0.1 | 1.0 ± 0.03* |
| Set 2 | none | 76 | 4.2 ± 0.3 | 1.0 ± 0.1 |
| | HT | | 4.2 ± 0.3 | 0.8 ± 0.1* |

* $P<0.001$

**Heat Treatment significantly affects rRNA ratio but not RIN**

# RNA Yields (µg/mg) for each RIN category: surgical normal and cancer samples

| RIN Category | Tissue type | Samples | mean ± SEM | Median |
|---|---|---|---|---|
| High | Normal | 1831 | 0.94 ± 0.03 | 0.57 |
| | Tumor | 3033 | 1.72 ± 0.05 | 1.27* |
| Medium | Normal | 1345 | 0.67 ± 0.03 | 0.27 |
| | Tumor | 853 | 0.92 ± 0.06 | 0.40* |
| Low | Normal | 435 | 0.38 ± 0.06 | 0.06 |
| | Tumor | 272 | 0.73 ± 0.12 | 0.18* |
| Total | | 7769 | 1.16 ± 0.02 | |

# RNA Yields (µg/mg): surgical normal and cancer samples 11 major tissues

| Tissue | Normal | | | Tumor | | |
|---|---|---|---|---|---|---|
| | Number | Mean ± SEM | Median | Number | Mean ± SEM | Median |
| Breast | 565 | 0.13 ± 0.03 | 0.04 | 989 | 0.89 ± 0.04 | 0.51* |
| Kidney | 561 | 1.22 ± 0.06 | 0.97 | 915 | 1.21 ± 0.04 | 0.90 |
| Stomach | 308 | 1.70 ± 0.12 | 1.23 | 370 | 1.83 ± 0.09 | 1.33 |
| Uterus | 263 | 0.53 ± 0.04 | 0.34 | 329 | 1.16 ± 0.08 | 0.51* |
| Bladder | 92 | 0.54 ± 0.10 | 0.19 | 286 | 1.84 ± 0.12 | 1.32* |
| Colon | 261 | 1.04 ± 0.07 | 0.77 | 273 | 2.26 ± 0.12 | 1.82* |
| Ovary | 80 | 0.54 ± 0.05 | 0.44 | 220 | 1.94 ± 0.16 | 1.30* |
| Skin | 137 | 0.19 ± 0.04 | 0.07 | 155 | 1.78 ± 0.16 | 1.32* |
| Soft Tissues | 346 | 0.18 ± 0.02 | 0.04 | 140 | 1.29 ± 0.13 | 0.85* |
| Bronchus and Lung | 136 | 0.62 ± 0.08 | 0.44 | 136 | 1.46 ± 0.14 | 1.32* |
| Rectum | 133 | 0.96 ± 0.09 | 0.73 | 115 | 2.38 ± 0.26 | 1.79* |

# Conclusion Yield

- **Yield of RNA decreases with decreasing RIN of samples.**

- **In general, tumor tissues have significantly higher RNA yields than normal tissues.**

  - **By RNA Quality level**

  - **By tissue with some exceptions**

# Advantages of RIN

- **RIN values provide advantages over previous grading systems**
  - ❏ **Objective**
  - ❏ **Quantitative**
  - ❏ **Wide continuous scale**
  - ❏ **Labor saving**
  - ❏ **Likely to gain widespread use**