External RNA Quality Control by RT-PCR

Walter Koch and Xiaoning Wu, Roche Molecular System
Garry Miyada, Affymetrix
Patrick Gilles, Invitrogen life technologies
Paul Wolber, Agilent Technologies
Reinhold Mueller, Stratagene
Daya Ranamukha-arachchi, FDA/CDRH

ERCC Workshop, Dec. 2, 2003
Quality Control of RNA Transcripts

- **Integrity (full length)**
- **Purity** (e.g., DNA contamination)
- **Specificity**
- **Absolute or relative concentrations in their pure forms**
- **Absolute or relative conc. in RNA pools or spikes**
Conventional QC

- **Absolute conc.**, e.g. absorbance at 260 nm
- **Electrophoresis**, e.g. Agilent BioAnalyzer, expected sizes and purity
- **RiboGreen assays**, relative conc.

QC by Kinetic RT-PCR

- **Specificity**
- **Integrity and purity**
- **Relative conc. in their pure forms**
- **Relative conc. in RNA pools or spiked preps**
Kinetic RT-PCR

- Single tube and SYBR Green I –based
  - Simple and low costs
  - Expandable

- Two sets of transcript-specific PCR primers covering both 3’ and 5’ ends of each RNA transcript
  - Highly specific and sensitive
  - Approximately 200 bp amplicon allowing easy conversion to other probe-based assays

- Common reagents and instruments from major manufacturers, such as ABI, Invitrogen, etc.
  - Easy to obtain
  - Maintain consistency
Basic QC Assessment of RNA Controls

- *Gel electrophoresis, e.g. Agilent BioAnalyzer (integrity, sizes and purity)*
- *Absorbance at 260/280 nm (purity and absolute conc.)*
- *RT-PCR using both 3’ and 5’ primer sets (integrity) with and without RT step (purity)*
- *Encapsulated RNA controls*
Determination of Relative Conc. for Pure RNA Transcripts

• Choose one RNA transcript as a standard

• Use an “end-point” limiting dilution assay to determine its relative conc.

• Determine relative concentrations of other RNA transcripts using the first as a standard
QC Assay Validation for RNA controls

- Specificity
- Sensitivity and efficiency
- Linearity
- Quantitation ranges
- Precision and reproducibility
- Detection and quantitation limits
RNA Control Pooling and Spiking

- Pools and pooling
- Numbers of RNA transcripts in each pool
- Total RNA preps for spiking
- Numbers of RNA transcripts and their “copy numbers” in each total RNA prep
QC Assessment for Pooled and Spiked RNA Controls

- Specificity
- Sensitivity and efficiency
- Linearity
- Quantitation ranges
- Precision and reproducibility
- Detection and quantitation limits
Issues for Discussion

• **Statistical analysis**
• **Resources and timelines**
  • QC for 1st batch (lot)
  • Ongoing QC for subsequent lots
  • Determination of RNA stability