External RNA Quality Control by RT-PCR

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Quality Control of RNA Transcript

- *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 a 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***