Quantitative Real-time RT-PCR Protocol

Materials required:

- Eppendorf tube
- 200 µl PCR tubes
- automatic pipettor
- pipette tips (10µl; 20µl; 100µl)
- metal cooler
- SYBR Green and Rox Master Mix (in the kit) (MM)
- Polg2 and Ercc2 primer working solutions (QIAGEN, IDT primers, prepared)
- cDNA from reverse transcription
- RNase and DNase free water

Preparation of reagents:

Measure the appropriate amounts of reagents in an Eppendorf tube and mix well. Centrifuge briefly and keep ice-cold.

- ✓ **MM-primer solution1 for one sample:**
  - SYBR Green Master mix: 12.5 µl
  - Polg2 primer working solution: 1 µl

  13.5 µl/tube
- ✓ **MM-primer solution2 for one sample:**
  - SYBR Green Master mix: 12.5 µl
  - Ercc2 primer working solution: 1 µl

  13.5 µl/tube
- ✓ **cDNA solution for one sample:**
  - Gli265 0Gy / 3Gy cDNA: 1 µl
  - RNase and DNase free water: 10.5 µl

  11.5 µl/tube

Total volume: 25 µl/sample

- Be sure to prepare duplicates and cDNA negative controls. Disperse the solutions into 0.2 ml tubes.
- Place the tubes into the 36 rotor. Place the rotor into the thermal cycler.
- Start the RotoGene program.
- New run: template: Templates/mRNS QIAGEN

PCR program:

1. 10 min. 95 °C
2. a.) 15 s 95 °C
   b.) 30-40 s 55-60 °C } 40x
   c.) 30 s 72 °C

Melt: Polg2: 79 °C 1peak
Ercc2: 80 °C 1peak

95 °C 1 min.
65 °C 2 min.
65-95 °C; 2 °C/ min

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