Mitochondrial DNA Deletion by Real-time PCR Protocol

Reagents and Materials:

- **Template:** 500 ng total DNA
- **Primer set:**
  - Genomic housekeeping gene: GAPDH
  - Mitochondrial housekeeping gene: TotMT
  - WT mitochondrial specific: WT
  - Common deletion specific: DEL
- **Master MIX (MM):** SYBR Green Master mix
- **Prepare Master Mix with the appropriate primer:**
  Measure the appropriate amounts of primer with MM in an Eppendorf tube and mix well. Centrifuge briefly and keep ice-cold.

Work flow:

- **Primer solution:**
  - **MM-primer solution1:**
    SYBR Green Master mix: 12.5 µl
    pimer GAPDH: 3 µl
    } 15.5 µl/tube
  - **MM-primer solution2:**
    SYBR Green Master mix: 12.5 µl
    pimer TotMT: 3 µl
    } 15.5 µl/tube
  - **MM-primer solution3:**
    SYBR Green Master mix: 12.5 µl
    pimer WT: 3 µl
    } 15.5 µl/tube
  - **MM-primer solution4:**
    SYBR Green Master mix: 12.5 µl
    pimer DEL: 3 µl
    } 15.5 µl/tube
- **Template solution:**
  DNA: x µl (=500 ng DNA)
  DNase free water: 9.55 µl –x µl
  } 9.5 µl/tube

Mix the MM primer solution (1 or 2 or 3 or 4) with Template solution

Total volume: 25 µl

- Aliquot the solutions into the 0.2 ml tubes.
- Place the tubes into the 36 rotor. Place the rotor into the thermal cycler.
- Start the RotoGene program.
- New run: mt DEL

PCR program: 10 min. 95 °C

15 s 95 °C
30 s 60 °C
30 s 72 °C
} 40x

Melt:

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

Save. File name: