SHORT PROTOCOL MAQ Control

Preparation of Master Reaction Mix

1. Remove the PCR mix from the -20 °C freezer and allow complete thawing on ice.
2. Vortex thoroughly and centrifuge the tubes at 12,000 x g for 10 s before use.
3. Mix together **3 µl** PCR mix with **2 µl** optimized primer mix (for optimization, see user guide You MAQ) and **0.075 µl** Taq DNA polymerase.
4. Vortex briefly and centrifuge the tubes at 12,000 x g for 10 s.

PCR Reaction Setup

5. For each sample, combine **20-50 ng** genomic DNA with **5 µl** of the master reaction mix.
6. Adjust with distilled water to final volume of **15 µl**.
7. Vortex briefly and centrifuge the tubes at 12,000 x g for 10 s.

PCR Cycling Profile

98 °C – 10 min
95 °C – 45 s
60 °C – 45 s } x23
68 °C – 2 min
72 °C – 10 min

Note: Keep the ramp rate of the machine below 2.5 °C/s.

Fragment Analysis

8. Prepare the size standard mix by combining **10 µl** of HiDi-Formamide (not provided) with **0.3 µl** GS500-liz size standard (not provided).
9. For each reaction, dispense **10 µl** of size standard mix per well into a 96-well plate.
10. Add **2 µl** of the MAQ PCR product to a well containing the size standard mix.
11. Denature samples at 95 °C for 3 min and put on ice immediately.
12. Centrifuge the plate at 1,000 x g for 10 s.
13. Load onto the fragment analyzer.
14. Analyse data with MAQ-S analysis software (freely downloadable from www.agilent.com)

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