DNA Casework Unit
Procedures for Post-Amplification Quantification of Mitochondrial DNA

1 Scope

These procedures describe the process for quantification of amplified mitochondrial deoxyribonucleic acid (mtDNA). Following post-amplification purification, amplicons are quantified with Agilent 2100 Bioanalyzer and DNA1000 or 7500 Series II LabChip kit.

2 Equipment/Materials/Reagents

Equipment/Materials

- General laboratory supplies (e.g., pipettes, centrifuge)
- 2100 Bioanalyzer, Agilent Technologies
    - 2100 Expert software (Rev. B.02.03 or above).
- Bioanalyzer Chip priming station, Agilent Technologies
- Vortex mixer
- DNA LabChip®, Agilent Technologies

Reagents

- DNA1000 or 7500 Kit, Agilent Technologies.
    - DNA1000 or 7500 Dye concentrate, if needed
    - DNA1000 or 7500 DNA gel matrix, if needed
    - DNA1000 or 7500 gel-dye mix
    - DNA1000 or 7500 markers
    - DNA1000 or 7500 ladder
- Water, Reagent Grade or equivalent

3 Standards and Controls

All corresponding reagent blanks (RB) and amplification controls (Negative Control [NC] and Positive control [i.e., HL60]) to a sample must be quantified.

For evaluation of the quantification data, refer to the last section of this procedure.

4 Procedures

Refer to DNA Procedures Introduction (DNA QA 600) and follow applicable general precautions and cleaning instructions.

4.1 DNA 1000 Series II Kit

The DNA 1000 kit is typically used for samples amplified with HV1A, HV1B, HV2A, HV2B, HV1, HV2 or mini-primer sets.

4.1.1 DNA 1000 Gel-Dye Mix Preparation

4.1.1.1	Allow dye concentrate and DNA gel matrix to equilibrate to room temperature for approximately 30 minutes. Add 25 µL of dye concentrate into DNA 1000 gel matrix vial. Cap and vortex well.	

4.1.1.2	Transfer gel-dye mix to spin filter. Spin at approximately 2240 X g for 15 minutes. Discard spin filter. Label tube (include date of preparation).	

Use gel-dye mix within 4 weeks of preparation. Protect from light and store at 4ºC.

4.1.2 DNA 1000 Chip Loading

4.1.2.1	Add 9 µL gel-dye mix to the well marked "G" (Circled G). Place chip into priming station (syringe clip adjusted to lowest notch) with syringe set at 1 mL. Close priming station and press plunger until held by syringe clip. Wait 60 seconds, then release clip and pull back syringe plunger to 1 mL. Open priming station and remove chip.	

4.1.2.2	Add 9 µL gel-dye mix to 2 wells marked "G". Add 1 µL of DNA 1000 ladder (yellow tube) to ladder well. Add 5 µL of DNA 1000 markers (green tube) to ladder well. Add 5 µL of DNA 1000 markers (green tube) to each sample well to be used. Add 6 µL of DNA 1000 markers (green tube) to each unused sample well. Add 1 µL control/sample to appropriate wells.	

4.1.2.3	Place chip into vortex mixer and vortex for 60 seconds at 2400 rpm (speed may be lowered).	

The chip should be run within 5 minutes of preparation to avoid failure due to evaporation.

4.2 DNA 7500 Series II Lab Kit

The DNA 7500 kit is typically used for samples amplified with WCR primers.

4.2.1 DNA 7500 Gel-Dye Mix Preparation

4.2.1.1	Allow dye concentrate and DNA gel matrix to equilibrate to room temperature for approximately 30 minutes. Add 25 µL of dye concentrate into DNA 7500 gel matrix vial. Cap and vortex well.	

4.2.1.2	Transfer gel-dye mix to spin filter. Spin at approximately 1500 X g for 10 minutes. Discard spin filter. Label tube (include date of preparation).	

Use gel-dye mix within 4 weeks of preparation. Protect from light and store at 4ºC.

4.2.2 DNA 7500 Chip Loading

4.2.2.1	Add 9 µL gel-dye mix to well marked "G". (Circled G) Place chip into priming station (syringe clip adjusted to highest notch) with syringe set at 1 mL. Close priming station and press plunger until held by syringe clip. Wait 30 seconds, then release clip and pull back syringe plunger to 1 mL. Open priming station and remove chip.	

4.2.2.2	Add 9 µL gel-dye mix to 2 wells marked "G". Add 1 µL of DNA 7500 ladder (yellow tube) to ladder well. Add 5 µL of DNA 7500 markers (green tube) to ladder well. Add 5 µL of DNA 7500 markers (green tube) to each sample well to be used. Add 6 µL of DNA 7500 markers (green tube) to each unused sample well. Add 1 µL control/sample to appropriate wells.	

4.2.2.3	Place chip into vortex mixer and vortex for 60 seconds at 2400 rpm (speed may be lowered).	

The chip should be run within 5 minutes of preparation to avoid failure due to evaporation.

4.3 Quantification Using Agilent 2100 Bioanalyzer

4.3.1	Turn on Bioanalyzer and computer. Start software by double-clicking 2100 Expert icon. Click on Instrument context and select Bioanalyzer. Insert chip into Bioanalyzer and close lid.	

Screen should now display a chip in upper left. If a chip is not displayed, remove chip from instrument and inspect wells for appropriate volume.

4.3.3	Select DNA 1000 II or DNA 7500 II from Assay Selection button at upper right of Instrument screen. Enter Laboratory number (and sample name, if possible) in file prefix box in Destination section. Enter number of wells to be run. Click Start button at upper right (under Assay Selection button). Fill out Sample Information table by clicking on "Data File: name" or by clicking on Data and Assay icon in Contexts panel.	

4.3.4	When run is complete, place electrode cleaner (containing 350 µL water) in Bioanalyzer and close lid for 10 seconds. Open the lid, remove electrode cleaner, and let dry 10 seconds. Close lid.	

4.4 Evaluation of Results

4.4.1 The software calculates sample concentration. Multiple peak concentrations should be added together when appropriate. If a single peak concentration exceeds 50 ng/µL, a dilution will be prepared and quantified.

4.4.2 If a peak of interest is not integrated, it can be manually integrated by decreasing analysis thresholds. In most instances decreasing Height Threshold is sufficient for integration. If this is not successful, decrease Height Threshold and adjust one or more thresholds as detailed below.

Click on expansion tab on right side of sample electropherogram panel and select LOCAL tab.
Change value of following threshold(s) as appropriate and press ENTER

- Height Threshold (default value = 20) can be decreased to between 19 – 0.02
- Slope Threshold (default value = 0.5) can be decreased to between 0.45 – 0.05
- Area Threshold (default value = 0.1) can be decreased to between 0.09 – 0.02
- Width Threshold (default value = 0.5) can be decreased to between 0.45 – 0.1

Save file and reprint sample of interest if necessary.

Do NOT manually integrate the peak of interest by changing the start and stop points for integration on the electropherogram of the peak of interest.

4.4.3 Samples may proceed to cycle sequencing of a region when:

- The sample and positive control concentrations are ≥ 1 ng/µL.
  AND
- The NC and RB concentrations are < 10% of the corresponding sample concentration.

Samples that do not meet these criteria will not be taken on to cycle-sequencing. Sample and positive control concentrations may be rounded up from 0.96 ng/µL.

5 Sampling

Not applicable.

6 Calculations

Not applicable.

7 Measurement Uncertainty

Not applicable.

8 Limitations

8.1 If a single peak concentration exceeds 50 ng/µL, a dilution will be prepared and quantified.

8.2 Minimum sample and positive control concentration and maximum RB and NC concentrations are empirically determined to provide a level at which the cycle-sequence data is expected to not be interpretable.

9 Safety

9.1 Refer to the "Hazardous Waste Disposal" section of the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in these procedures.

9.2 Procedural Specific Chemical Hazards:

- Agilent kit components contain dimethyl sulfoxide (DMSO). This dye binds to nucleic acids and is treated as a potential mutagen.

10 References

FBI Laboratory Safety Manual

DNA Procedures Manual

Agilent 2100 Bioanalyzer 2100 Expert User's Guide. Available online at www.agilent.com.

Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Guide. Available online at www.agilent.com

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0	02/05/16	Reformatted from Mitochondrial DNA Analysis Laboratory Procedures.

Approval

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