Manual MPS Library Quantitation with PowerSeq® Quant MS

1 Purpose

1.1 To determine the DNA concentrations of the purified libraries, in order to establish the appropriate dilution schemes for normalization.

2 Preparation

2.1 Refer to the PowerSeq® Quant setup sheet and/or the LIMS test batch for your quantitation run.

2.2 Retrieve the following reagents and allow to equilibrate to room temperature.

PowerSeq® Quant MS 2x qPCR Master Mix
PowerSeq® Quant MS 10x Primer Mix
PowerSeq® Quant MS DNA Standard (200 pM)
PowerSeq® Quant MS 1x Library Dilution buffer

2.3 Log all reagent lot numbers in LIMS as appropriate.

2.4 Vortex the reagents thoroughly. It is suggested to vortex three times for 10 seconds each time. Centrifuge briefly.

2.5 Retrieve a reagent reservoir.

2.6 Retrieve the purified library plate to be quantified and allow to equilibrate to room temperature.

2.7 Vortex the library plate on a plate mixer for 1 minute at 1000 rpm and centrifuge the plate at 1000 rpm for 1 minute.

2.8 Prepare standards:

2.8.1 Label five (5) -1.5 mL micro centrifuge tubes with the standard concentrations (20 pM, 2 pM, 0.2 pM, and 0.02 pM, NTC).

2.8.2 Pipette 45 µL of PowerSeq® Quant MS 1x Dilution Buffer into each labelled DNA standard tube.

- Pipette 5 µL of the 200 pM DNA standard into the 20 pM tube. Vortex for 10 seconds and centrifuge briefly.

- Pipette 5 µL of the 20 pM standard into the 2 pM tube. Vortex for 10 seconds and centrifuge briefly.
- Pipette 5 µL of the 2 pM standard into the 0.2 pM tube. Vortex for 10 seconds and centrifuge briefly.
- Pipette 5 µL of the 0.2 pM standard into the 0.02 pM tube. Vortex and centrifuge briefly.

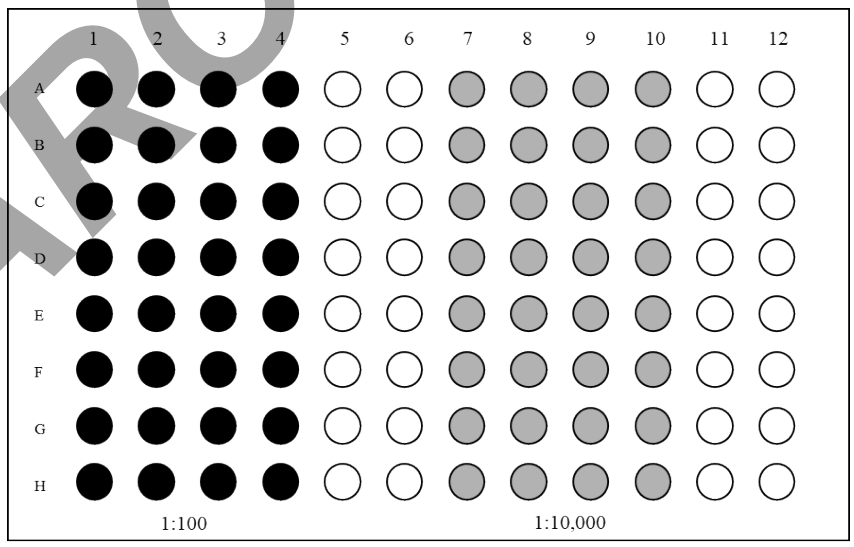
2.8.3 Standards may be stored in a refrigerator and used for up to two (2) weeks. If storing, record your initials and the date.

2.9 Create 1:10,000 dilutions of libraries:

2.9.1 The following instructions are for the creation of 1:10,000 dilutions when a complete set of libraries is being quantified (I.e., the initial quantification of the purified or normalized libraries).

2.9.2 If quantifying re-normalized libraries, the dilution described in steps – below may be performed in 1.5 mL microcentrifuge tubes using a single channel pipet.

2.9.3 Label a new 96-well plate.
Columns 1-4 will be used to create 1:100 dilutions of columns 2-5 from the purified library plate.
Columns 7-10 will be used to create the final 1:10,000 dilutions from the columns 1-4.



2.9.4 Pour approximately 15 mL of 1x Dilution Buffer into the reagent reservoir.

2.9.5 Remove the seal from the library plate.

2.9.6 Using a multi-channel pipette, add 2 µL of the purified library plate, into the appropriate wells of the plate.

2.9.7 Add 198 µL of PowerSeq® Quant MS 1x Dilution Buffer into each well.

2.9.8 Seal the plate, vortex on plate mixer at 1000 rpm for 1 minute and centrifuge at 1000 rpm for 1 minute. This creates a 1:100 dilution.

2.9.9 Transfer 2 µL of the 1:100 libraries into the appropriate 1:10,000 column.

2.9.10 Add 198 µL of PowerSeq® Quant MS 1x Dilution Buffer into each well.

2.9.11 Seal the plate, vortex on a plate mixer at 1000 rpm for 1 minute and centrifuge at 1000 rpm for 1 minute. This creates the final, 1:10,000 dilution.

2.9.12 Reseal the purified libraries plate using a BioRad Microseal B film and return to -20°C freezer.

Note: Unpooled, purified libraries may be stored for up to six months at -20° C.

2.10 If a dilution factor other than 1:10,000 is necessary, follow the instructions below.

2.10.1 <u>1:1000 dilution</u>:

2.10.1.1 Transfer 2 µL of the 1:100 libraries into a new column. You may use a new plate.

2.10.1.2 Add 18 µL of PowerSeq® Quant MS 1x Dilution Buffer into each well to complete 1:1000 dilution.

2.10.1.3 Seal the plate, vortex on a plate mixer at 1000 rpm for 1 minute and centrifuge at 1000 rpm for 1 minute.

2.10.2 <u>1:100,000 dilution</u>:

2.10.2.1 Transfer 2 µL of the 1:10,000 libraries into a new column. You may use a new plate.

2.10.2.2 Add 18 µL of PowerSeq® Quant MS 1x Dilution Buffer into each well to complete 1:100,000 dilution.

2.10.2.3 Seal the plate, vortex on a plate mixer at 1000 rpm for 1 minute and centrifuge at 1000 rpm for 1 minute.

2.11 Prepare reaction mix:

2.11.1 Referring to the PowerSeq® Quant setup sheet for your run, create the master mix by adding the appropriate volumes of each of the following reagents. Vortex for 10 seconds to mix.

Reagent	Per Reaction
2x qPCR Master Mix	10.0 µL
10x Primer mix	2.0 µL
Water	4.0 µL

3 Procedure

3.1 Obtain a new Applied Biosystems® MicroAmp® Optical 96-Well Reaction Plate.

3.2 Add 16.0 µL of reaction mix to each well in use according to the plate layout in the PowerSeq® Quant setup sheet for your run.

3.3 Add 4 µL of the Quant MS standards, NTC, and the 1:10,000 dilution of the libraries according to the plate layout in the PowerSeq® Quant setup sheet for your run.

3.4 Seal the dilution plate with optical adhesive film and store at 4°C until the quantitation run is complete. If rework must be performed these dilutions may be used.

3.5 Seal the quantitation plate with optical adhesive film, use a straight edge or tube opener to secure the seal.

3.6 Centrifuge for 1 minute at 3000 rpm.

3.7 Ensure that there are no bubbles in the reaction wells before placing the plate on the 7500.

4 Software Operations

4.1 Turn on the Applied BioSystems® 7500 Real-Time PCR System. Allow time for instrument to warm up.

4.2 Press the tray door to open and place the plate on the instrument. Be sure the plate is correctly aligned when position A12 is in the top right corner of the tray.

4.3 Close the tray door by pushing the depressed imprint on the right side of the tray. Do not push from the center.

4.4 Double click icon HID Real-Time PCR Analysis Software v1.2.

4.5 Click the *Custom Assay* icon.

4.6 Click the down arrow for *New Experiment.*

4.7 Click From Template.

4.8 Open the "PowerQuant" template.

4.9 Inside the Experiment Menu on the left side of the screen, click *Setup » Experiment Properties*.

4.10 Enter run name into the field labeled Experiment Name.

4.11 Click Setup » Plate Setup » Assign Targets and Samples.

4.12 To import samples, click *File » Import*. Locate file in the LIMS file share folder. Click *Start Import.*

4.13 Plate set-up imported successfully » click *OK.*

4.14 Check the top header and ensure the following:

- **Experiment Name:** Current Run Name
- **Type:** Standard Curve
- **Reagents:** SYBR® Green Reagents

7500 **PowerQuant MS**	The PowerQuant MS file is as follows: Soak at 95°C for 2 minutes <u>30 cycles:</u> Denature at 95°C for 15 seconds Anneal at 60°C for 15 seconds Extend at 72°C for 45 seconds – fluorescent collection

4.15 Click *Start Run*. Run time is ~1:15 hour.

4.16 Once run is complete, discard the plate and turn the instrument off.

5 Exporting Results

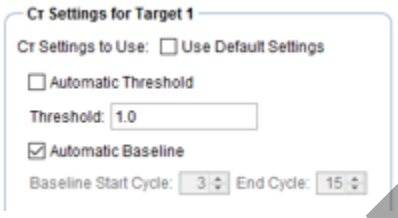
5.1 Open HID Real-Time PCR Analysis Software v1.2 on the desktop, if needed.

5.2 If the assay that needs analysis is not currently open, click *File* » *Open*. Navigate to desired file, select the file, and click *Open*.

5.3 In the *Experiment Menu* located on the left side of the screen, click *Analysis*.

5.4 In the *Analysis* tab on the top right side of the screen, click *Analysis Settings* » *CT Settings*.

5.5 Verify the settings below and click *Cancel.*



5.6 Click Analyze.

5.7 After analysis, results can be exported. Click *View Plate Layout » Highlight All Wells*.

5.8 Located on the top toolbar, click *Export.*

5.8.1 Select data to export » *Results.*

5.8.2 Select one file or separate files » *One File.*

5.8.3 Ensure the correct file name.

5.8.4 In the Custom Export tab check the data is exporting columns (A1, B1, etc.)

5.8.5 Click Start Export.

5.9 With all wells still highlighted, click *Print Report* located on the top toolbar. Select *All Report Types.*

5.10 Click *Print* and chose to save as a *.PDF*. Ensure the correct run name is listed. Add *reports* to the end of the file name.

5.11 Save file in appropriate LIMS folder and click *Save*.

5.12 Transfer the raw data .EDS files from the instrument PC to the Forensic Biology network drive. These files should be saved in the respective instrument folders that are in the "Quant Trio" folder.

6 Data Analysis

6.1 Use the standard curve to determine the concentration in sample wells, average sample replicates and account for the 1:10,000 dilution factor.

6.2 If using LIMS, import the data into the LIMS test batch.

6.3 Use the reports generated to interpret the results for each assay.

6.3.1 Using the standard curve reports, ensure the following parameters are met for the slope, Y-intercept and R^2 value. All three parameters must fall within the quality criteria for the quantitation to pass.

6.3.1.1 Standard slope must be between –3.2 and –3.8

6.3.1.2 R^2 values must be ≥ 0.990

6.3.1.3 The Y-Intercept value must be between 12.5 and 18.5

6.3.2 If using LIMS, record the QCBatch parameters located at the top of the screen. Make sure to release and save all data stored in the QCBatch Parameters tab.

6.3.3 Evaluate the NTC. The library quant must quant at 0 pM for the quantitation batch to pass.

6.3.4 Extraction negative controls and amplification negative controls are not evaluated for pass/fail during this library quantification procedure.

6.3.5 Sample interpretations

6.3.5.1 Samples whose dilutions are reported outside the range of 0.02pM to 20pM, should be scheduled for requant at a dilution whose quantitation value would be expected to fall within that range.

6.3.5.2 The difference between replicate quantitations of the same sample must fall within 60% of each other. Replicates whose quantitation values fall outside of this range should be requanted.

6.3.6 The pushed concentration for each sample will be the average of the quantitation values of both of its two replicate quantitations.

6.3.7 The NTC will only be quantified once per run. If using LIMS, remove the replicate quantitation row from the data entry screen.

6.3.8 Record the results of your analysis.