BECKMAN COULTER
Life Sciences

Biomek Automated Genomic Sample Prep Accelerates Research

Application: Biomek i-Series[1] Automated Sample Quality Control and Normalization Methods

- Agilent TapeStation 2200 Setup
- KAPA Illumina qPCR Quantification[2] and Normalization
- Quant-IT Picogreen ds DNA Assay and Normalization

Introduction

Sample quality is of utmost importance to genomic researchers to better ensure good results when running laborious and expensive processes such as Next Generation Sequencing. Here we demonstrate robust Biomek automated sample prep for three commonly used quality control and quantification protocols. These methods can be incorporated into genomic workflows such as NGS, qPCR and Microarray.

The QC Suite is automated on all Biomek workstations for
- Standardized workflow for improved results
- Reduce costly errors
- Reduced hands-on-time and increased throughput
- Ready-to-implement methods via knowledgeable support teams

Spotlight: Biomek i5 Span-8 liquid handling platform.

System features deliver reliability and efficiency to increase user confidence and walk-away time
- 1-1000uL pipetting capability
- Independent 360˚ rotating gripper with offset fingers
- 25 positions
- Orbital Shakers and Peltiers for controlling sample processing
- Optional Enclosure

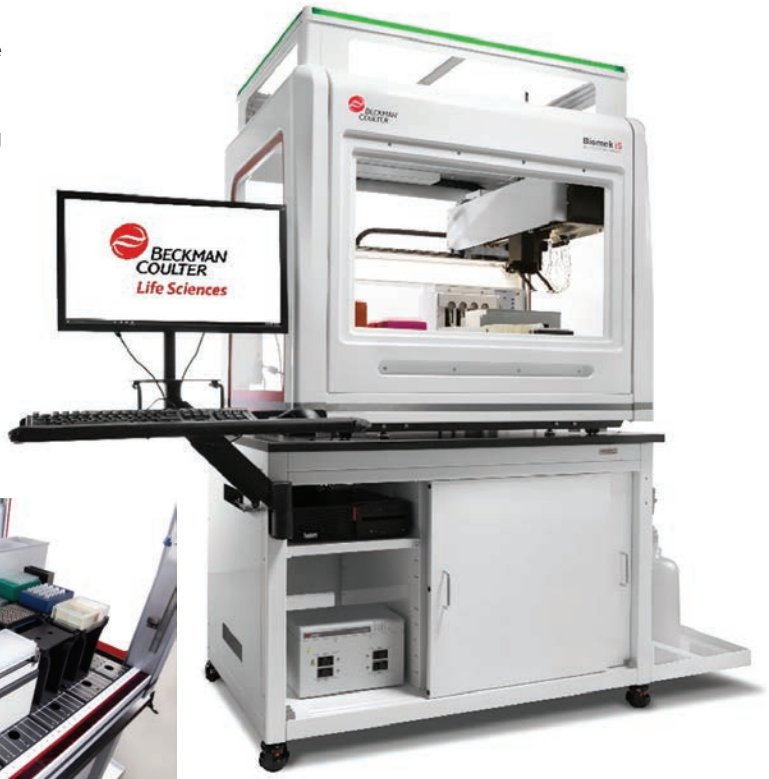


Figure 1. Biomek i5 Span-8 Genomics Workstation with optional enclosure on a Biomek Cart. High Capacity deck layout for increased walk away time.

Demonstrated Method Interface (DMI):

Three simple modules that provide the user full instructions to better ensure error-free method setup and provides users maximum flexibility for scheduling their day.

1. Biomek Method launcher (BML) — secure interface for selecting methods without affecting method integrity

Figure 2. Biomek Method Launcher provided an easy interface to start the method

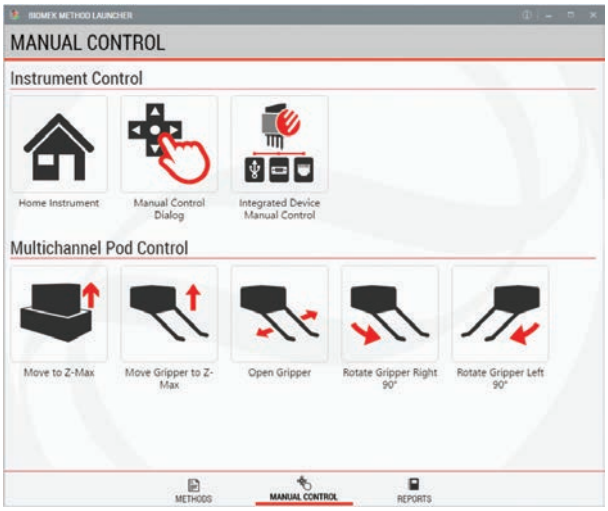


Figure 3. Manual Control can be run through the launcher interface

2. Method Options Selector (MOS) — Select run-time options and maximize flexibility in daily scheduling and method execution

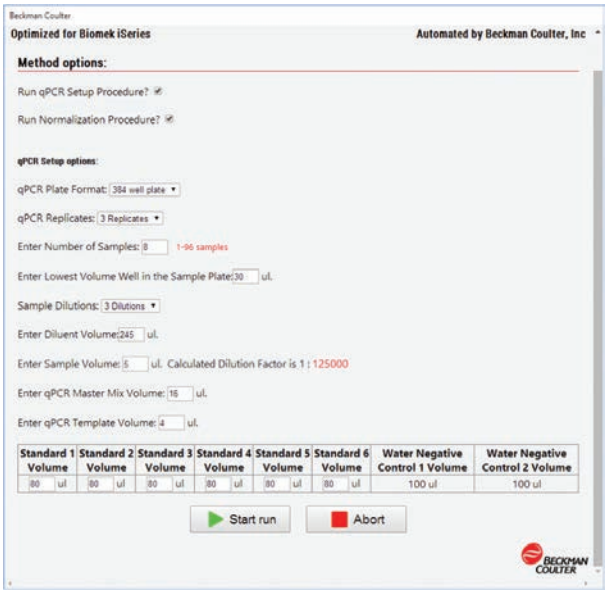


Figure 4. Example of Method Options Selector (MOS) for the KAPA qPCR Method. User can select what section of the method to be run, number of samples, plate type, replicates and details about the standards.

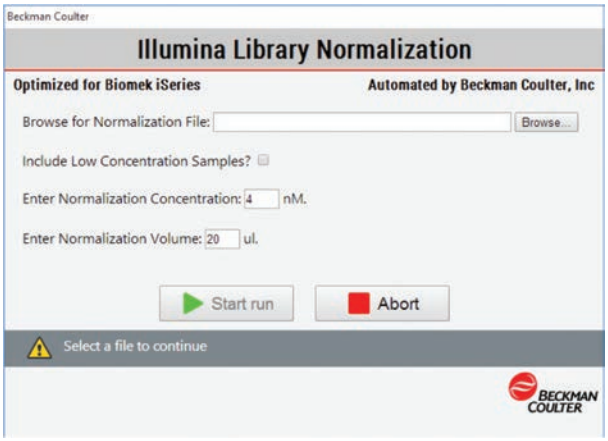


Figure 5. MOS for Normalization section of the method. User provides the Normalization file (with source concentration) and the desired concentration. The transfer volume for normalizing the samples is calculated within the method using scripting.

3. Guided Labware Setup (GLS) — Generated based on options selected in the MOS, and provides the user specific text and graphical setup instructions with reagent calculation

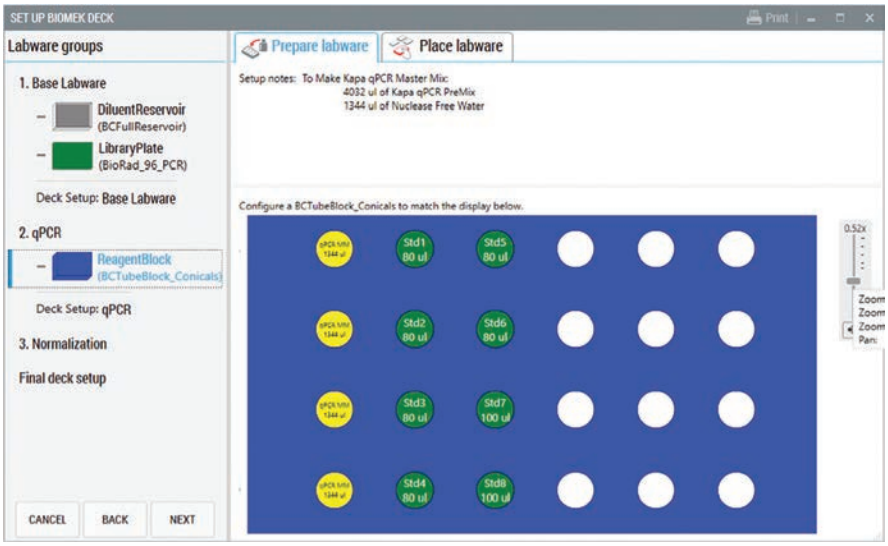


Figure 6. Guided Labware Setup with calculated required reagent volumes and setup notes for error free deck setup.

Biomek automated QC and Normalization methods, fast and efficient way to better ensure sample quality.

Major Process Description	12 samples	24 samples	48 samples	96 samples
Agilent TapeStation	-	-	-	10 mins
KAPA qPCR and Norm : 96 well (3 replicates, 3 dilutions)	24 mins	49 min	-	-
: 384 well (3 replicates, 3 dilutions)	25 mins	33 mins	50 mins	1 hr 26 mins
Quant-IT and Norm	28 mins	30 mins	36 mins	49 mins
Hands On Time	10 mins	10 mins	15 mins	15 mins
**Timing does not include thawing of reagents				

Table 1. Timings and throughput for the methods on i5 Span-8 Workstation

Experimental design[3]:

The following methods have been demonstrated on i-5 Span-8 Workstation:

- Agilent Tapestation 2200 Setup *(Genomic DNA Assay, D1000 DNA Assay, D1000 DNA High Sensitivity Assay, D5000 DNA Assay, D5000 DNA High Sensitivity Assay, RNA Assay and RNA High Sensitivity Assay)*
- Quant-IT Picogreen ds DNA Assay and Normalization
- KAPA Illumina Library Quantification Kit and Normalization

The samples analyzed were the final clean libraries generated from Coriell DNA using Illumina Nextera Rapid Capture Enrichment sample preparation kit. Agilent High Sensitivity D5000 DNA kit was used to generate data on Tapestation 2200. Data for Quant-IT Picogreen ds DNA Assay kit was generated using Paradigm plate reader (Florescence Ex 485nm and Em 530nm) and samples from KAPA Illumina Library Quantification kit were read on Applied Biosystem's 7900Ht real time PCR system.

TapeStation Results:

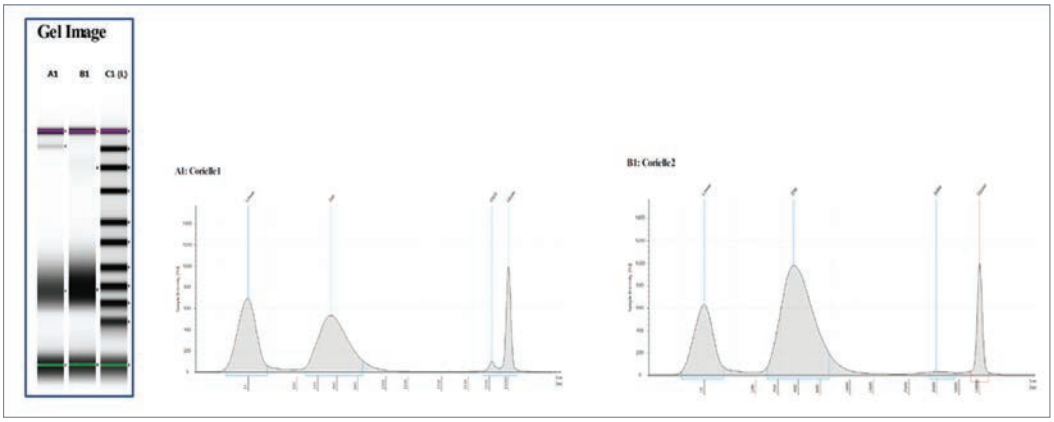
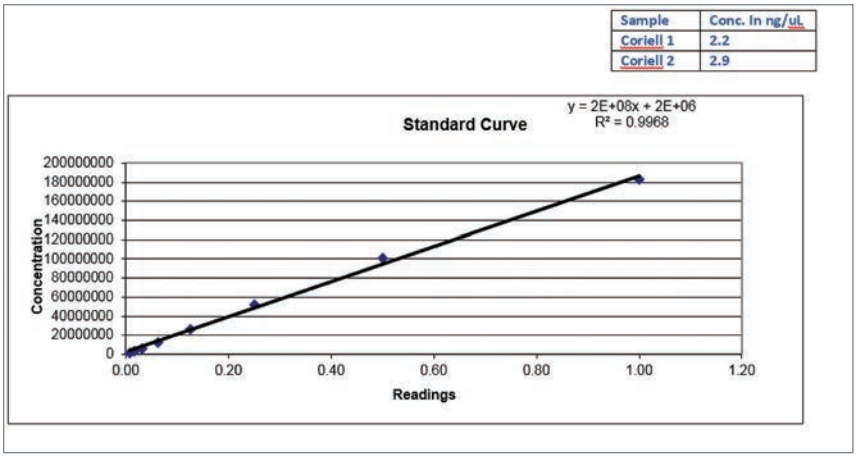


Figure 7. Coriell samples shown on gel and electropherogram after being analyzed on the Agilent TapeStation HS D5000 kit show a peak ~350bp as expected by Illumina's protocol.

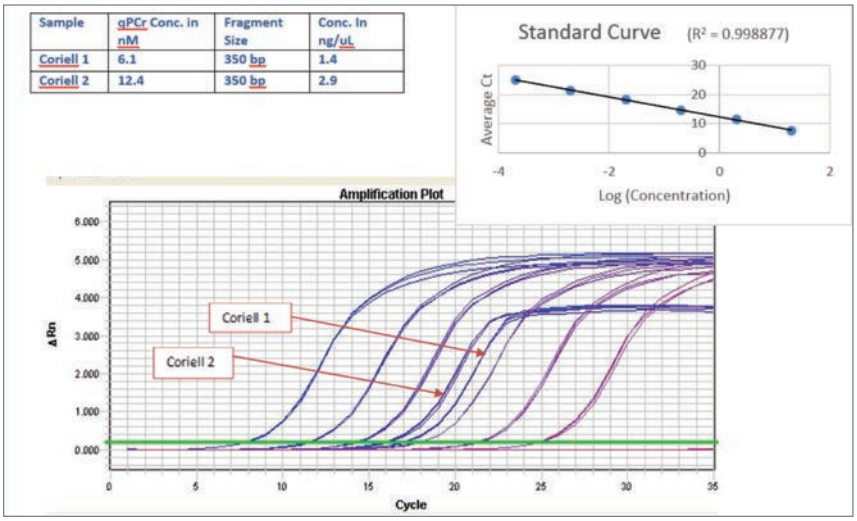
Quant-IT Assay Results:



Sample	Conc. In ng/uL
Coriell 1	2.2
Coriell 2	2.9

Figure 8. Standard curve for the Quant-IT assay and calculated concentrations of Coriell samples.

qPCR Results:



Sample	qPCr Conc. in nM	Fragment Size	Conc. In ng/uL
Coriell 1	6.1	350 bp	1.4
Coriell 2	12.4	350 bp	2.9

Figure 9. Amplification plot and standard curve for KAPA Library Quantification Kit and concentrations of Coriell DNA calculated from the curve

Conclusion

We have demonstrated that clean libraries from Coriell DNA shows comparable concentrations between two different quantification methods and the Tapestation data shows the quality of the library is acceptable for downstream applications.

1. Product in development
2. All trademarks are properties of their respective owners
3. Data obtained during development

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