MIT BioMicro Center: Sample Quantification

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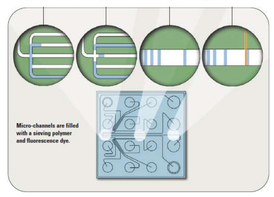
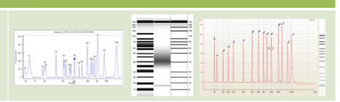
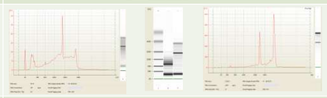
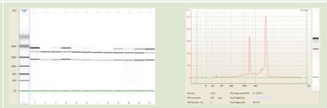
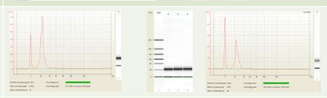
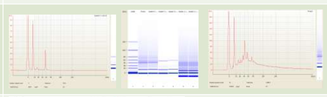
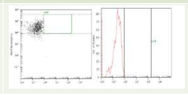
MICROFLUIDICS



Agilent 2100 Bioanalyzer and Caliper LabChip GX

MICROFLUIDIC ASSAYS		
High Sensitivity DNA -Bioanalyzer -LabChip GX	•DNA samples 50-7000bp in size •5-500 pg/µL concentration	
Nano RNA -Bioanalyzer -LabChip GX	• Total & mRNA, 25-500 ng/µL • Gives a RIN or RQS as a standard measure of RNA degradation	
Pico RNA -Bioanalyzer	• 50 pg/µL total RNA • 250 pg/µL mRNA • Gives a RIN or RQS as a standard measure of RNA degradation	
Small RNA -Bioanalyzer	• small RNA 6 to 150 nt in size • 50 to 2000 pg/µL concentration	
High Sensitivity Protein -Bioanalyzer	•Cleaner than Silver • Uses 4µl Protein • Quantify to 100pg • Detect to 20pg	
Cell Fluorescence -Bioanalyzer	• two-color flow cytometry • on-chip cell staining • results in 25 minutes	 •Sample data from Agilent

RT-PCR

Thing 1 **Thing 2** **Thing 3**

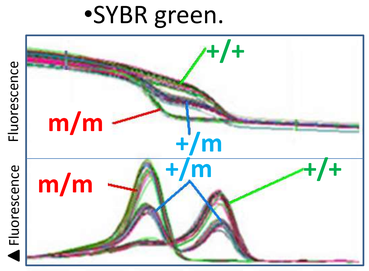
Many thanks to the **Guarente** lab for providing Thing 3.

General Information

•The MIT Biomicro Center now offers three Roche Lightcycler 480s, a fully-integrated, high throughput real-time PCR platform for highly accurate qualitative and quantitative detection of nucleic acids.
•96 well and 384 well plates
•Rapid cycle times
•Can detect **>4 fluors**

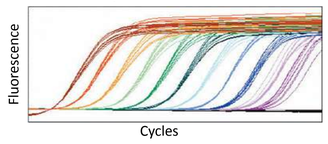
Applications:

Melting Curve Genotyping
•SYBR green.

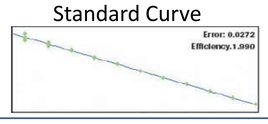


Absolute Quantification
Illumina QC

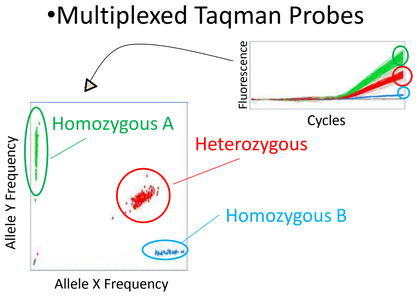
Amplification Curve



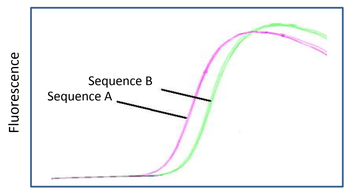
Standard Curve



Endpoint Genotyping
•Multiplexed Taqman Probes

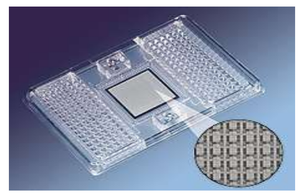


Relative Quantification
•Compare expression levels of 2 target sequences in a single sample.

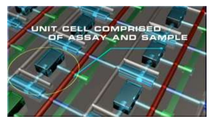


FLUIDIGM BIOMARK

Massively parallel real-time PCR machine



Integrated Fluid Circuit (IFC)

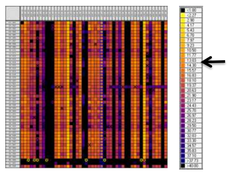


• Dense network of channels able to regulate flow of solution down to picoliter scale
• Nanoflex valves open and close by application of air pressure
• Reaction volumes fixed by chamber volume, consistent.

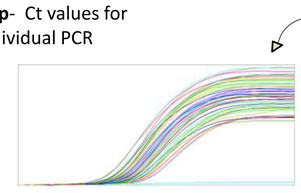
Digital PCR

• 9,216 qPCR data points
• **Requires pre-amplification**
• 1/200th of typical reagents:

qPCR	384 well	96.96 Dynamic Array
Master Mix	46,000µL	240µl
Primer-probe	9,200µL	480µl
Plates	24	1
Pipette steps	18,432	192



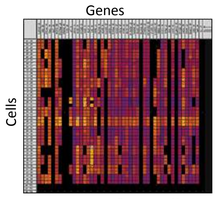
Heatmap- Ct values for each individual PCR reaction



Amplification curve for selected samples

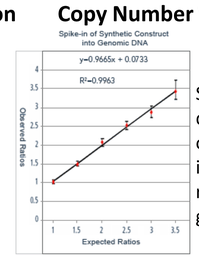
Other Applications

Single Cell Gene Expression



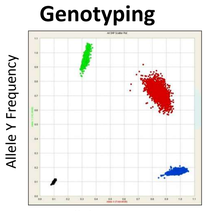
Heatmap of 48 cells tested against 46 genes

Copy Number Variation



Simultaneous quantification of gene of interest and reference gene.

Genotyping



9,216 data points from a single 96.96 dynamic array

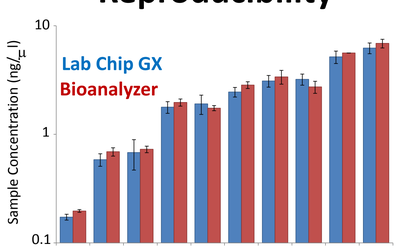
CALIPER v. BIOANALYZER: DNA

The **Caliper Labchip GX** and **Agilent Bioanalyzer** both provide a platform for analyzing DNA samples at high sensitivity. We preformed several validation assays to compare the performance of the two platforms on samples prepared for Illumina sequencing.

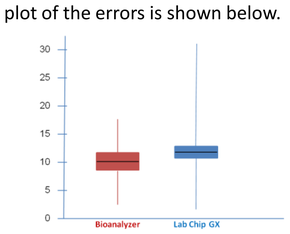
Manufacturers' Specifications

	BIOANALYZER	LABCHIP
Size range	50-7000bp	50-5000bp
Linear Range	5-500 pg/µl	10-500 pg/µl
Accuracy	+/- 20%	+/- 30%
Batch Size	11 samples	96 samples

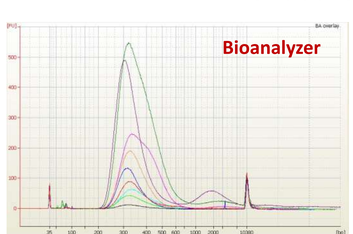
Reproducibility

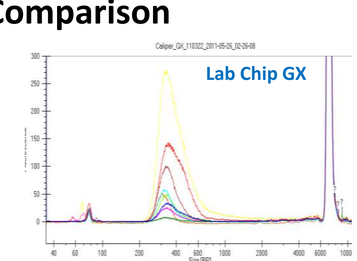


10 DNA samples run six times on the Lab Chip and twice on the BioAnalyzer. Error bars indicate the standard deviation, a box plot of the errors is shown below.

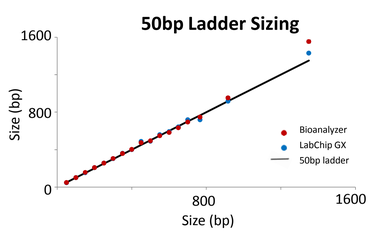


Cross Comparison

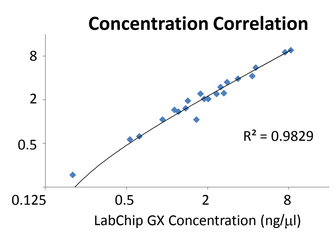




50bp Ladder Sizing



Concentration Correlation

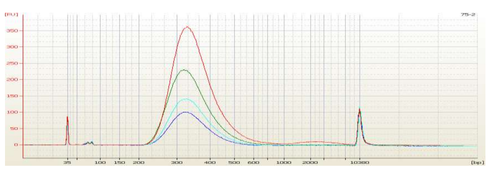


NEB 50bp ladder and 21 samples were compared on both the Bioanalyzer and the LabChip. Results indicate the two instruments have very similar behaviors as expected.

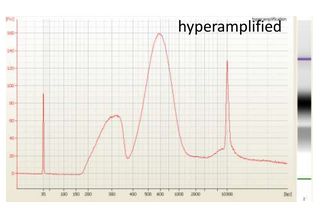
ILLUMINA LIBRARY QUALITY CONTROL

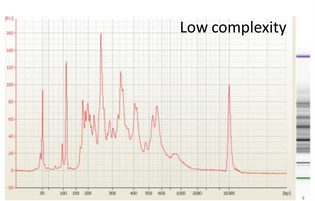
Evaluation of libraries prior to clustering to ensure consistent sequencing results.

Bioanalyzer



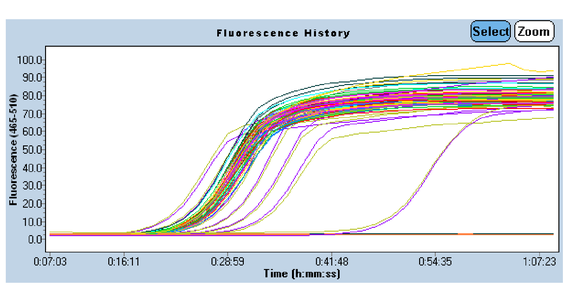
The Bioanalyzer or Labchip GX confirms there is sample, and provides the average size, approximate concentration and molarity. It also can identify samples with problems such as hyperamplification, "primer-dimer" or low complexity





RT PCR

Provides accurate concentration measurement for clustering. Absolute quantification based on protocols developed at the Broad Institute.



The Ct of library samples is compared to that of a standard curve

Multiplexing

Data obtained from RT PCR is used to pool samples of varying concentrations for equal or biased representation in multiplexed lanes.