Next Generation Sequencing: Improved Library Quantification with Agilent Technologies

Accurate quantification of in process Next Generation sequencing samples is critical for reliable sequence data. The inclusion of a new Agilent QPCR method and LabChip technology in typical NextGen workflows results in increased data quality with a more efficient use of reagents and starting DNA.
Agilent Genomics Workflow

Sample source
- Gene Expression, CGH, miRNA
- Gene Expression Hybridization Kits
- miRNA Labeling Reagent and Hybridization Kit
- DNA Microarray Hybridization Chamber
- Hybridization Chamber Gasket Slides
- SureHyb DNA Microarray Hybridization Kit

Sample preparation and isolation
- Absolutely RNA® Purification Kits
- miRACLE™ miRNA Kits
- RNA Isolation Kit/RNA Lysis Buffer
- RNase Block
- Total RNA Isolation Mini
- Plant RNA Isolation Mini
- 2100 Bioanalyzer
- RNA 6000 Series II

Sample amplification and labeling
- AffinityScript™ RT
- Fairplay™ III Labeling Kit
- T7 RNA Polymerase
- Salmon Sperm DNA
- Genomic DNA Labeling Kit PLUS
- Low RNA Input Linear Amplification Kit
- Low RNA Input Linear Amplification Kit, In solution
- Low RNA Input Linear Amplification Kit, On Array
- miRNA Labeling Reagent and Hybridization Kit

Microarray processing
- Analysis Software
  - GeneSpring

Microarray scanning
- Microarray Scanner
  - Feature Extraction Software

Microarray data analysis
- Validation

Kit
- Gene Expression Wash Buffer Kit
- Stabilization and Drying Solution
Outline

• Next Generation Sequencing (NGS) – workflow and technology
• Describe present challenges to NGS data generation
• Present improvements to workflow
  • QPCR quantification of library on Solid/Illumina
  • Capillary electrophoresis/LabChip characterization
  • Oligo Capture/Selection QPCR characterization
  • Real time monitoring of library amplification using Mx QPCR
• References:
  • Meyer, M. et al, Nucleic Acid Research 2007, From micrograms to picograms: QPCR reduces the material demands of high-throughput sequencing
  • Quail, MA. et al, Nature Methods 2008, A large genome center’s improvements to the Illumina sequencing system
  • Illumina QPCR Library Quantification Protocol 11322363_A
  • Hodges, E. et al, Nature Protocols 2009, Hybrid selection of discrete genomic intervals on custom-designed microarrays for massively parallel sequencing
Overview of Next Generation Sequencing

• What is next-gen sequencing (NGS)?
  • Ability to determine DNA base composition via millions of parallel sequence reads performed simultaneously
    • reducing cost and time for research purposes and discovery
    • Goal is to produce $1000 genome
  • A typical NGS platform generates data equivalent of ~200 hundred capillary sequencers in 24 hours

• Main NGS platforms
  • Illumina Genome Analyzer – bridge amplification synthesis on glass
  • Roche/454 GS FLX – emulsion PCR…pyrosequencing
  • ABI SOLiD – adaptor ligation emulsion PCR
  • All NGS platforms rely upon PCR amplification of target DNA library and accurate titration of amplified library into sequencing reaction
Next Generation Sequencing Platforms

- Each uses different sequencing chemistry
- 454 leader in read-length (250bp, std)
- Illumina leader in throughput (1.4GB/day)
- SOLiD advantage of read accuracy check
- Illumina over 400 publications and consortium endorsements
- Estimated cost per Mb: Illumina & SOLiD at $5.25, 454 at $75

### Chemistries

<table>
<thead>
<tr>
<th>Platform</th>
<th>Chemistry</th>
<th>454 GS FLX*</th>
<th>AB SOLiD</th>
<th>Illumina GAII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemistry</td>
<td>Pyrosequencing</td>
<td>Ligation based</td>
<td>Reversible terminators</td>
<td></td>
</tr>
<tr>
<td>Run Time</td>
<td>7 hours</td>
<td>3-6.5 days</td>
<td>3 days</td>
<td></td>
</tr>
<tr>
<td>Read Lengths (bp)</td>
<td>250</td>
<td>25, 50</td>
<td>35, 50</td>
<td></td>
</tr>
<tr>
<td>Ave. Reads per Run</td>
<td>400K</td>
<td>150x10^6</td>
<td>85x10^6</td>
<td></td>
</tr>
<tr>
<td>Data per run</td>
<td>100MB</td>
<td>up to 7GB</td>
<td>up to 4.3GB</td>
<td></td>
</tr>
<tr>
<td>Throughput</td>
<td>100MB</td>
<td>1.1GB/day</td>
<td>1.4GB/day</td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Platform</th>
<th>Titanium</th>
<th>Mate-Paired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run Time</td>
<td>10 hours</td>
<td>6.5 days</td>
</tr>
<tr>
<td>Read Lengths (bp)</td>
<td>400+</td>
<td>2x25, 2x35</td>
</tr>
<tr>
<td>Ave. Reads per Run</td>
<td>1x10^6</td>
<td>250x10^6</td>
</tr>
<tr>
<td>Data per run</td>
<td>400MB</td>
<td>90x10^6 pairs</td>
</tr>
<tr>
<td>Throughput</td>
<td>400MB</td>
<td>900MB/day</td>
</tr>
</tbody>
</table>

*Metrics apply to both Fragment and Mate-Paired runs.
NGS DNA Sample Prep Workflow

• Each platform unique sample prep, but the general steps are as follows:
  • Isolate gDNA and fragment…nebulization or sonication
  • Library Preparation…attach primers, single strand (unique for each platform)
  • Library QC & Quantification…size range, concentration molec./µL
  • Dilution and loading of library for amplification and sequencing
• All require accurate quantification to ensure high-quality reads and efficient generation of data
  • Too much DNA – mixed signals, un-resolvable data, lower number of single reads
  • Too little DNA – lower density sequenced increases, reduced sequencing coverage/read depth, empty runs, increased cost/run, wastes time
NGS DNA Sample Prep Workflow

• NGS provider suggest library quantification by Ribogreen or capillary electrophoresis/LabChip quantification
  • Limits of sensitivity of methods require starting with µgs DNA for ngs DNA library
  • Methods may overestimate library concentration since they measure total DNA
  • Rare or limited DNA samples are not appropriate given present mass limitations

• Present work-around is sequencing titration runs of library dilution, libraries run at dilution in NGS platform
  • Extensive time to get accurate DNA library loading information
  • Large cost associated with titration runs
  • Titration runs not always successful

• Recent improvements describe a more efficient manner of library titration using QPCR platform from Agilent
  • QPCR presents a unique departure in library characterization since it quantifies only those DNA in the library that will yield sequence information
From micrograms to picograms: quantitative PCR reduces the material demands of high-throughput sequencing

Matthias Meyer*, Adrian W. Briggs, Tomislav Maricic, Barbara Höber, Barbara Höffner, Johannes Krause, Antje Weihmann, Svante Pääbo and Michael Hofreiter

Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, D-04103 Leipzig, Germany

Received September 7, 2007; Revised October 15, 2007; Accepted November 23, 2007
Roche 454 Ancient DNA Library Titration Runs: The Problem

Typical result of 454 sequencing run on libraries with low DNA concentration from Neandertal (NT) ancient DNA

- Highly variable results
- Only one sample (NT3) generated the minimal sequence count required for good data
- Ran 16 samples and only 1 useable result
- In this example the cost per sample is ~$8000 instead of $500
- Problem attributed to under/overload of DNA

Table 1. Typical result of a sequencing run performed from 454 libraries with low concentrations derived from Neandertal ancient DNA extracts

<table>
<thead>
<tr>
<th>Sample</th>
<th>454 sequencing library dilution</th>
<th>Enriched beads</th>
<th>Filter passed sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT1</td>
<td>1:2, 1:150, 1:450</td>
<td>11 700, 8640, 13 860</td>
<td>2448, 1180, 314</td>
</tr>
<tr>
<td>NT2</td>
<td>1:2, 1:150, 1:450</td>
<td>2430, 1440, 1980</td>
<td>267, 52, 0</td>
</tr>
<tr>
<td>NT3</td>
<td>1:2, 1:150, 1:450</td>
<td>344 700, 324 900, 106 740</td>
<td>0, 1247, 6106</td>
</tr>
<tr>
<td>NT4</td>
<td>1:2, 1:150, 1:450</td>
<td>263 340, 29 520, 71 10</td>
<td>3805, 3312, 358</td>
</tr>
<tr>
<td>NT5</td>
<td>1:2, 1:150, 1:450</td>
<td>1980, 1440, 1440</td>
<td>36, 0, 0</td>
</tr>
<tr>
<td>NT6</td>
<td>1:2, 1:150, 1:450</td>
<td>3420, 2520, 2520</td>
<td>45, 4, 4</td>
</tr>
</tbody>
</table>
Emulsion PCR-Roche 454 (and SOLiD)

- Library (fragments with adaptors) are PCR amplified within a water:oil emulsification (emPCR) in the presence of beads
- One primer is attached to the surface of a bead
- DNA:Bead enrichment
- Fed into Roche 454 NGS platform picotiter plate, one bead per well
- emPCR relies on each micro reactor containing single bead and single template

From-Meyer, et al, NAR 2007 (Max Planck Leipzig, Germany)
Quantification of 454 Library by SYBR qPCR on the Mx3005P – qPCR Assay

• The goal is to determine DNA library:bead ratio for optimal sequencing results

• Created a Library standard- PCR off emPCR primers (adapters), TOPO cloned, amplify minprep, ran on gel and selected 450 bp amplicon for standard → OD260 quantification to molec./µL

• 10-fold dilution of standard, 1µL Library DNA-Agilent Brilliant SYBR core kit + AmpliTaq Gold, 200nM emPCR Primers ran Mx3005P QPCR

• Checked size distribution of product on gel → calculated molecules/µL using standard curve quant and size correction
Quantification of 454 Library by SYBR qPCR on the Mx3005P – qPCR Assay Performance

- Slopes of QPCR amp plots similar → complex template mix not affecting PCR efficiency across standard and samples
- Template:Bead ratios of 1-2 used in emPCR
- Able to compare sequencing results, template:bead ratio, and qPCR quantification to determine optimal DNA required-14-37 pg DNA
  - Using known starting concentrations calculated efficiency of library construction-0.4-1.7% yield library
  - pgs well lower than published requirements of DNA
  - Implies that improvements can be made to the existing protocols when true starting DNA template concentration is known
Quantification of 454 Library by SYBR qPCR on the Mx3005P – Sequencing results

Table 2. 454 library quantification and sequencing results obtained from fifteen samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial material (ng)</th>
<th>Mean fragment size (bp)</th>
<th>Concentration (molec./µl)</th>
<th>Recovery (%)</th>
<th>Copies per bead in emPCR</th>
<th>Enriched beads</th>
<th>Mixed sequences (%)</th>
<th>Filter passed sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neandertal 1</td>
<td>n/a</td>
<td>200</td>
<td>$2.00 \times 10^6$</td>
<td>n/a</td>
<td>2.17</td>
<td>144 275</td>
<td>8.7</td>
<td>15 541</td>
</tr>
<tr>
<td>Neandertal 2</td>
<td>n/a</td>
<td>200</td>
<td>$1.75 \times 10^7$</td>
<td>n/a</td>
<td>2.25</td>
<td>98 310</td>
<td>9.5</td>
<td>16 972</td>
</tr>
<tr>
<td>Neandertal 3</td>
<td>n/a</td>
<td>200</td>
<td>$1.88 \times 10^7$</td>
<td>n/a</td>
<td>2.08</td>
<td>109 330</td>
<td>8.2</td>
<td>16 447</td>
</tr>
<tr>
<td>Neandertal 4</td>
<td>n/a</td>
<td>200</td>
<td>$1.82 \times 10^7$</td>
<td>n/a</td>
<td>2.17</td>
<td>78 010</td>
<td>9.2</td>
<td>16 773</td>
</tr>
<tr>
<td>Neandertal 5</td>
<td>n/a</td>
<td>200</td>
<td>$5.48 \times 10^6$</td>
<td>n/a</td>
<td>2.19</td>
<td>101 790</td>
<td>9.4</td>
<td>17 323</td>
</tr>
<tr>
<td>Neandertal 6</td>
<td>n/a</td>
<td>200</td>
<td>$7.40 \times 10^7$</td>
<td>n/a</td>
<td>2.16</td>
<td>58 580</td>
<td>11.3</td>
<td>13 590</td>
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<tr>
<td>Neandertal 7</td>
<td>n/a</td>
<td>200</td>
<td>$9.74 \times 10^7$</td>
<td>n/a</td>
<td>2.14</td>
<td>55 680</td>
<td>10.8</td>
<td>19 639</td>
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<tr>
<td>Neandertal 8</td>
<td>n/a</td>
<td>200</td>
<td>$4.88 \times 10^7$</td>
<td>n/a</td>
<td>2.20</td>
<td>62 640</td>
<td>9.5</td>
<td>14 062</td>
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<tr>
<td>Neandertal 9</td>
<td>n/a</td>
<td>200</td>
<td>$3.99 \times 10^7$</td>
<td>n/a</td>
<td>2.15</td>
<td>62 640</td>
<td>11.5</td>
<td>15 582</td>
</tr>
<tr>
<td>Neandertal 10</td>
<td>n/a</td>
<td>200</td>
<td>$1.45 \times 10^8$</td>
<td>n/a</td>
<td>1.59</td>
<td>26 100</td>
<td>7.8</td>
<td>17 030</td>
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<tr>
<td>Neandertal 11</td>
<td>n/a</td>
<td>200</td>
<td>$7.85 \times 10^6$</td>
<td>n/a</td>
<td>2.33</td>
<td>54 230</td>
<td>8.9</td>
<td>14 592</td>
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<tr>
<td>Neandertal 12</td>
<td>n/a</td>
<td>200</td>
<td>$4.31 \times 10^7$</td>
<td>n/a</td>
<td>2.25</td>
<td>60 320</td>
<td>9.2</td>
<td>16 544</td>
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<tr>
<td>SAGE ditags</td>
<td>32</td>
<td>250</td>
<td>$9.83 \times 10^7$</td>
<td>0.5</td>
<td>1.64</td>
<td>76 270</td>
<td>3.4</td>
<td>21 429</td>
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<tr>
<td>Amplicons</td>
<td>85</td>
<td>320</td>
<td>$1.72 \times 10^8$</td>
<td>0.4</td>
<td>1.35</td>
<td>42 000</td>
<td>11.2</td>
<td>20 716</td>
</tr>
<tr>
<td>Bonobo</td>
<td>520</td>
<td>450</td>
<td>$1.50 \times 10^9$</td>
<td>1.7</td>
<td>2.33</td>
<td>87 800</td>
<td>20.3</td>
<td>12 232</td>
</tr>
</tbody>
</table>

- All NT ancient DNA samples amplified to adequate number of filtered sequences from successful reactions (Filter passed sequences)
- Low % of overloaded beads (%Mixed Sequences)
Quantification of 454 Library by SYBR qPCR on the Mx3005P

- Correlated this data with 454 results – all libraries yielded over 12,000 sequences
- qPCR results helped optimize emPCR and provide minimum sample requirement to generate good sequence result
- Provides ability to omit library titrations – improve economics of 454 sequencing...particularly small-scale projects
- Method could be adapted to Illumina and ABI...need adapter sequences
- Able to quantify ng & pg amounts material, million fold improvement
- $1000s of savings with the elimination of the titration run
Extreme Linearity and Sensitivity of Library Detection by Mx3005P QPCR System

Protocol
- Brilliant II SYBR Green Master Mix
- 10 min activation, 30 sec 95°C
- 60 sec 60°C for 40 cycles
- 400 nM each primer
- Standard is linearized plasmid template with 287 bp amplicon

• The plasmid standard 10-fold dilution in blue, LOD based on standard down to 1 aM (<100 molecules/µL)
• Library 10-fold dilutions in red.
A large genome center’s improvements to the Illumina sequencing system


*NATURE METHODS | VOL.5 NO.12 | DECEMBER 2008 | 1005*

• Improvements made to the standard Illumina protocols increase data quality and decrease cost, time and DNA requirements

• Changes in PCR amplification, library characterization and quantification are prominent improvements
Sanger Improvements to Illumina Sequencing

- Currently run 28 Illumina GA = 64 gigabases a week
- Improvements to Illumina protocols to make library preparation more reliable, reduce bias (PCR), tighten size distribution → reliably generate high yields sequencing data
- Use Agilent BioAnalyzer 2100 to check quality and estimate quantity
- Incorporate a QPCR method for final quantification
Bridge PCR-Illumina Genome Analyzer

- DNA fragments are flanked with adaptors.
- A flat surface coated with two types of primers, corresponding to the adaptors.
- Amplification proceeds in cycles, with one end of each bridge tethered to the surface.
- Cycling creates “clusters” that are fed into sequencing reactions
  - 40-44,000 clusters (20-25,000 filtered clusters) per tile optimal for typical library size
- Suboptimal cluster concentrations result in sequencing overlap or too few sequences
- Process relies on accurate library quantification prior to cluster generation
DNA QC in Next-Gen Sequencing – Illumina DNA Library Prep Workflow

Simplified Illumina GAII Workflow

Illumina Sequencing

Starting Material: Genomic DNA

Fragment DNA

Ligate Adapters

Purify Ligation Products

Amplify & Quantify Library

Sequence Library

Agilent 2100 Bioanalyzer

Check Size Distribution

QPCR Titration

Quantify, Size and QC DNA libraries
Quantification, Sizing and QC of NGS Samples

High Quality DNA library

Identification and quantification of primer dimers and PCR artifacts

Sizing

Quantification

Primer dimers

PCR artifact

DNA library
Agilent BioAnalyzer Microfluidic Capillary Electrophoresis is Routine for Characterization of DNA Libraries

- Optimization of PCR conditions and characterization with Agilent DNA assay
  - More homogeneous library amplification, more product
  - Fewer non specific products
  - Evident in electropherogram
Improving the next-gen sequencing Sample Prep Workflow

The library was enriched with Agilent’s SureSelect Target Enrichment platform

Post capturing - Pre-PCR
Gel purification, before PCR
No dilution

Non specific peak after PCR amplification

Post-capturing - Post-PCR
Amplified and purified prepped library
100 fold dilution
Sanger Improvements to Illumina Sequencing – Accurate Prediction of Cluster Density

- Sequenced cluster throughput as function total clusters detected
- Want highest number of filtered clusters for good data
- Want unbiased method based upon fragment size
- Want to achieve highest number of filtered clusters with the least amplification or smaller starting DNA
- Optimal to detect 40-44,000 total clusters
Sanger Improvements to Illumina Sequencing – QPCR Quantification, Accurate Prediction of Cluster Density

- BioAnalyzer quantification provides accurate and sensitive detection of ALL DNA species
- QPCR quantification detects only adapter ligated, sequenceable DNA
- Developed primers/TaqMan probe to Illumina paired-end adapter
- Construct standard from well characterized library (known size range*, cluster number, loading concentration*, sequencing result)
  - Standard run at 100, 10, and 1 pM, unknown run at ~10 pM *
- QPCR detection with TAMRA/FAM probe directed at adapters, 300nM primers (2.1, 2.2), Platinum Taq
- More accurately and successfully quantified DNA down to pM range → lower DNA concentrations can be used than previously stated in protocol
  * BA2100 measurements used
Sanger Improvements to Illumina Sequencing – Accurate Prediction of Cluster Density

- Sequenced cluster throughput improved after introduction of QPCR method (650)
- Stable cluster generation of 35-40,000
- Decreased across run variance (>800 runs)
qPCR Quantification Protocol Guide

FOR RESEARCH USE ONLY

Catalog # SY-930-1010
Part # 11322363 Rev. A
September 2009
Updated Illumina GA Library Quantification Method Recommends Agilent Mx3005P QPCR

Standard run in triplicate (1:2 dilutions) 100pM-1.5pM, dilute samples to ~10nM in EB

Ensure quality of run with standard efficiency (90-110%) Interpolate library samples against standard

http://www.illumina.com/support/documentation.ilmn
Updated Illumina GA Library Quantification Method Recommends Agilent Mx3005P - GC Content

- Optimization for GC content improves sequencing coverage and library complexity
- SYBR green PCR/Dissociation curves of 10nM library estimate Melting Temperature (Tm)
- GC enriched library will have a higher Tm for a given fragment size
- Additional method improvement that is achieved with the Mx3005p

http://www.illumina.com/support/documentation.ilmn
Protocol

Hybrid selection of discrete genomic intervals on custom-designed microarrays for massively parallel sequencing

Emily Hodges1,2, Michelle Rooks1,2, Zhenyu Xuan1, Arindam Bhattacharjee3, D Benjamin Gordon3, Leonardo Brizuela3, W Richard McCombie1 & Gregory J Hannon1,2

1Watson School of Biological Sciences and 2Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA. 3Agilent Technologies Inc., Santa Clara, California, USA. Correspondence should be addressed to G.J.H. (hannon@cshl.edu).

• Targeted sequencing following DNA capture of 1-2 μgs of DNA on Agilent Target Enrichment Array
• Protocol uses SYBR QPCR assay of 20ng/μL template pre and post
• Compare pre-hybridized to post-hybridized amplified template to validate capture or depletion
Agilent On-Array or In-solution Target Enrichment Formats

SureSelect Target Enrichment System*
Developed in collaboration with the Broad Institute
Dr. Chad Nusbaum et al.

SureSelect DNA Capture Array
Developed in collaboration with Cold Spring Harbor
Dr. Greg Hannon et al.

- 1-3 µg gDNA
- 1-5 µg gDNA (with WGA)
- 20 µg gDNA (unamplified)

*Flagship Method
## Agilent On-Array or In-solution Target Enrichment Formats

<table>
<thead>
<tr>
<th></th>
<th>SureSelect™ Target Enrichment System</th>
<th>SureSelect™ DNA Capture Array</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Throughput</strong></td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Study Sizes</strong></td>
<td>10-1,000s samples</td>
<td>1-10 samples (iterative designs)</td>
</tr>
<tr>
<td><strong>gDNA Input</strong></td>
<td>3 µg</td>
<td>3 µg</td>
</tr>
<tr>
<td><strong>Amplified library</strong></td>
<td>500 ng</td>
<td>20 µg</td>
</tr>
<tr>
<td><strong>Automation compatible</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Capture of Target DNA</strong></td>
<td>3.3⁺ Mb (2x tiling) (Custom 120-mer baits)</td>
<td>1Mb (20x tiling) (Custom 60-mer baits)</td>
</tr>
</tbody>
</table>
• QPCR for exons of 2 different genes

NTC (Water Blank) is negative, no detection of exon, no Ct

Pre-hyb samples are positive for selected exons, Ct=30

Post-hyb samples are positive for selected exons, Ct=20

ΔCt (pre-post)=~10, or 1024 fold enrichment of selected exons, in line with NGS enrichment metrics

Similar Ct for pre, post and ΔCt across genes support similar exon representation in amplified library before and after enrichment, and thus no bias…
Minimized or Amplification Free Sequencing

- Key aspect is upgrading the NGS protocol
  - eliminate or reduce the amount of PCR required, reduced artifact bias and time
  - Consolidate steps where possible
- Agilent Mx3005p QPCR system provides real time amplification curve management, with pause feature that allows start and stop of cycling
  - PCR of adapter ligated library step can be consolidated with quantification step by QPCR
  - Amplification of library proceeds in the presence of SYBR green
  - User stops cycling as library samples begin to amplify
  - Restart cycling for lower concentration samples
  - All samples would get a Ct to be interpolated against standard curve as in previous protocols
Minimized or Amplification Free Sequencing

Agilent Mx3005p can “pause”, provides minimal required amplification for each library expansion and simultaneous quantification by running only required number of cycles.

- Time Remaining: 02:05:11
  - Temperature: 94.5
  - Segment: 2
  - Cycle: 4 of 4
  - Plateau #: 1
  - Turn lamp off at end of run
  - E-mail experiment at end of run...

- Time Remaining: 02:19:17
  - Temperature: 94.5
  - Segment: 1
  - Cycle: 1 of 1
  - Plateau #: 1
  - Turn lamp off at end of run
  - E-mail experiment at end of run...

Library sample 1 done
Standard Curve Quant
Report of library concentration
Mx3005P QPCR system and Brilliant II QPCR reagents for NGS Library Quantification
Stratagene Mx3000P and Mx3005P QPCR System

Thermal system with +/- 0.25°C uniformity
- Highly reproducible results

Excitation: tungsten halogen lamp
- Wide range of excitation and dye choice

Scanning fiber optics
- High sensitivity and reduced noise

Detection: photomultiplier tube (PMT)
- Superior sensitivity of detection and dynamic range

4-color or 5-color optics filter wheel
- Multiplexing advantage and dye flexibility

View data in real-time with ability to pause run
- Monitor quantification with ability to pause run at given Ct value/quantity – useful for library amplification
Conclusions

• Next generation sequencing new technology has room for protocol optimization/improvement

• Desire to use smaller amounts of starting DNA, faster, less expensive
  • PCR introduces sequence bias, takes time

• QPCR assay advantage of quantifying libraries over total DNA methods
  • small amounts DNA accessible
  • accurate and reproducible assessment of concentration
  • uses adapters for sequencing, ensures quantification of “sequenceable” library
Thank You, Any Questions?

• References:
  Meyer, M. et al, Nucleic Acid Research 2007, From micrograms to picograms: QPCR reduces the material demands of high-throughput sequencing
  Quail, MA. et al, Nature Methods 2008, A large genome center’s improvements to the Illumina sequencing system
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