Scaling Up NGS
Sample Preparation:
Automation and Lab Preparation

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AGILENT AUTOMATED NGS SAMPLE PREPARATION

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Benefits of Automation

• Scale Up Sample Throughput
  • Increase throughput up to 10 times

• Reduced Hands on time

• Increased reproducibility
  • Reduce variation in library prep yield
Automation Increases Reproducibility

• Increased reproducibility

• CV for automated preparation is lower than CV for manual preparation
## Automation Increases Laboratory Throughput

<table>
<thead>
<tr>
<th></th>
<th>MANUAL METHOD</th>
<th>AUTOMATED METHOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hands-On Time</td>
<td>Maximum Number of Samples Processed/Day</td>
</tr>
<tr>
<td>Library Prep</td>
<td>375</td>
<td>20</td>
</tr>
<tr>
<td>Pre-Capture PCR</td>
<td>90</td>
<td>20</td>
</tr>
<tr>
<td>Hybridization Prep.</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>Capture</td>
<td>210</td>
<td>20</td>
</tr>
<tr>
<td>Post-Capture PCR</td>
<td>90</td>
<td>20</td>
</tr>
<tr>
<td><strong>Total Minutes</strong></td>
<td>825</td>
<td>20 - 40 per week</td>
</tr>
<tr>
<td><strong>Total Hours</strong></td>
<td>13.75</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Assumes individual tube preps with an experienced user staggering sample processing

<sup>2</sup>Library prep master mixes done once
Agilent NGS Automation Instruments
Agilent NGS Bravo Option A

• Capable of automating almost any NGS reagents
• Higher throughput and greater reproducibility than manual preparation
• Deck features:
  • Temperature control
  • Shaking
  • Magnetic bead station
Agilent NGS Option B

- Additional automation and labware storage offer greater walkaway time
- Some NGS protocols include >55 pipetting steps
- BenchCel stores pipette tips to reduce number of manual interventions
Workstation Options for Increasing Walkaway Time

- Preconfigured workstations are designed for increasing walkaway time
- Bravo liquid handler is capable of automating common library preparation and sequence capture methods
Agilent TapeStation 2200 Simplifies Sample QC

- DNA sample QC is required at 3 steps in targeted enrichment workflow
- TapeStation processes 96 samples in 1.5 hours
- Significant time savings over BioAnalyzer
Agilent PlateLoc Thermal Microplate Sealer

- Ideal for sealing plates for heated overnight incubations
- Prevents evaporation of precious samples
- Works as a walk-up instrument so it can be used to seal other plates in lab for PCR or other applications
Example VWorks Form for NGS Protocols

- VWorks software forms available to streamline processing
- Diagrams show location of accessories, labware, and reagents for Bravo deck and Minihub
Agilent NGS Workstation is an Open Platform

- Automation protocols are available for both Illumina and SOLiD sequencers
- Optimized protocols and user manual available for SureSelectXT and HaloPlex for Illumina
- Vworks automation files available for:
  - Illumina TruSeq RNA and DNA
  - NimbleGen SeqCapEZ
  - KAPA
Automated Agilent NGS Protocols

- Optimized Vworks protocols are available for many Agilent NGS reagents
- Step-by-step user manuals
- Genomics and automation technical support
  - SureSelect\textsuperscript{XT}
  - HaloPlex
3rd Party NGS Reagents

- VWorks protocol available for fast scale-up
- VWorks protocols are open so users can modify protocols as necessary
  - Illumina TruSeq DNA and RNA
  - Roche NimbleGen SeqCap EZ
  - KAPA Library Prep Kit for Illumina
CONSIDERATIONS FOR AUTOMATING NGS SAMPLE PREPARATION

Steve Lappin
Application Scientist
Lab Space Requirements

• Space Requirements for instruments and computer
  • Option A: 48” w, 34” h, 20”d
  • Option B: 105” w, 50” h, 20”d

• PCR instrument and plate sealer should be in close proximity to the robot

• Clean-dry 100 psi compressed air is required for BenchCel and PlateLoc

• House air or compressor options
Option B Workstation Dimensions

- 1435.2
- 56.50  867mm Rack
- 1257.4
- 49.50  660mm Rack
- 1028.8
- 40.50  457mm Rack
- 841.2
- 33.12  Top of Bravo

Dimensions:
- 503.4  19.82
- 216.5  8.53
- 1864.3  73.40
Lab Environmental Considerations

• Instruments should not be placed directly under air vents (heating/cooling/dust)

• Third party vendors can provide containment solutions

• Ambient temp should be ~22-27°C
  • If outside of normal range, additional temperature controlled steps can be introduced as needed

• Hybridization solutions are especially sensitive to cold
Multiple Systems Drive Throughput and Consistency

- Multiple systems enable users to increase throughput and build redundancy
- Multiple System Examples from current customers
  - Separate Pre/Post PCR Workstations
    - Pre-PCR library prep: NGS Option B
    - Post-PCR: NGS Option A
  - Labs in different locations: 1 lab director running similar projects in Singapore and USA
  - Massive throughput: >40 Bravos at the Broad institute
  - Ultra High throughput with maximum walkaway time: 6 NGS Option B workstations at 1 Sequencing Center
Labware Considerations

• Check protocols and order labware early, some protocols require labware from 4 or more vendors

• PCR plates determined by your thermalcycler
  – Full/half skirted with base

• Pipette Tips
  – Protocols with many pipetting steps require many pipette tips
    • Halo: 25 tips/sample
    • SureSelect: 55 tips/sample
  – Tips should be sterile, DNA/RNA free, we suggest filtered
Labware used:

- Agilent Filtered 250µL tips (180µL capacity)
- Thermo/Nunc 1.3mL DeepWell Plate
- Thermo Reservoir
- E&K Scientific 2.2mL plate
- ABI MicroAmp Plate with Black Carrier
- Eppendorf Twin.tec plate
Overview of NGS Sample Preparation Workflow

Library Prep highlighted in green

Target Enrichment highlighted in blue
Automation of Next-Gen Sequencing Using SureSelect

SureSelect XT Library Prep

- Shear Genomic DNA
- Repair Ends
- 3’-dA Addition
- Adapter Ligation
- PCR Enrichment
- Prepped Library

SureSelect Target Enrichment

- Automated Purification
- Automated Purification
- Automated Purification
- Automated Purification
- Automated Purification

- SureSelect Oligo Capture Library

- Library Hybridization
- Automated Bead Capture
- PCR
- QA

Sequence

Agilent Technologies
### Processing 192 Samples: An Example Workweek

<table>
<thead>
<tr>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purify 45 min</td>
<td>Prepare Hybs 1.5 Hours</td>
<td>Capture/Wash 3.5 hours</td>
<td>Prepare Hybs 1.5 hours</td>
<td>Capture/Wash 3.5 hours</td>
</tr>
<tr>
<td>Quantify** 1.5 – 4 hours</td>
<td>Purify 45 min</td>
<td>Purify 45 min</td>
<td>Quantify** 1.5 – 4 hrs</td>
<td>Purify 45 min</td>
</tr>
<tr>
<td>Library Prep 4 hrs</td>
<td>Quantify** 1.5 – 4 hrs</td>
<td>PCR* 45 min</td>
<td>PCR 45 min’</td>
<td>PCR 45 min</td>
</tr>
<tr>
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<td>Purify 45 min</td>
<td>Purify 45 min</td>
<td>Purify 45 min</td>
</tr>
<tr>
<td>Purify 45 min</td>
<td>PCR* 30 min</td>
<td>Quantify** 1.5 – 4 hrs</td>
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<td>Quantify** 1.5 – 4 hrs</td>
</tr>
<tr>
<td>Quantify** 1.5 – 4 hours</td>
<td>Purify 45 min</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Procedures take place off Bravo deck

**Quantification is off deck. Time for 96 samples: BioAnalyzer=4 hours, TapeStation=1.5 hours
Quality Control Scale Up

- With increased sample processing capacity, it is necessary to plan for scale up of sample QC
- Three QC steps are required in targeted resequencing protocols
- QC process for 96 samples can take 4 hours with BioAnalyzer
- TapeStation processes 96 samples in 90 minutes
- TapeStation offers time savings of 7.5 hours per plate of samples
Minimize Reagent Waste

- Automation protocols require more dead volume than the same process done manually
- Many kits do not have enough dead volume to provide the advertised number of samples when automating
  - This adds to the cost per sample
- Even with “Automation Friendly” reagent kits, dead volumes can be an issue
  - Goal is to maximize reagent yield and minimize “dead volume” of residual reagents left in bottom of tubes
- For Agilent automation kits, to achieve the advertised number of samples, batch in minimum (or multiples) of 24 samples for highest reagent yield
VWorks Automation Software

- Tiered access for power user and end users
- Forms functionality to create GUI. Easily managing daily runs with less training needed
- Open system to completely control all aspects of automation protocols
- Modifications can often be made easily
Normalization and Pooling

• Before sequencing and hybridization, normalization and/or pooling is performed on NGS libraries
  • These processes can be error-prone and tedious
• Time savings is offered along with precision pipetting, utilizing data generated from your analysis device output
  • TapeStation, Pico, or BioA
• Protocols are included with NGS methods, but authoring modified protocols for specific applications is facilitated with a hit-picking wizard
Normalization and Pooling

Hit Picking Wizard to automate the setup and data handling of these processes
Setting up PCR plates with Bravo

Developing protocols such as PCR setup for other applications is very manageable for lab personnel using drag-and-drop tasks.

Protocol examples are available from the Field Applications team.
Setting up PCR plates with Bravo

Overview of VWorks Software User Interface

1 Available Tasks area

2 Protocol area

3 Task Parameters area
Citations for Agilent NGS Automation

  - 1200 samples/week

  - ChIP Seq sample preparation

In Summary:

Benefits to automating NGS sample preparation:
Increase throughput, increase reproducibility

Why Automate with Agilent?

• Comprehensive Solution
  • Automation and reagents developed and supported by one company
• Automation Expertise
  • Applications team focused on instrumentation
• Flexibility
  • Automation can be easily set up to perform a variety of tasks
  • Potential to build scalable high throughput systems
• Satisfied Customers
  • Over 100 Agilent NGS Systems in use worldwide
  • Key sequencing centers (Broad, BGI, Sanger) use Agilent Bravo
  • Three peer-reviewed publications