Beckman Coulter
NGS Sample Prep Solutions

Alisa Jackson
Marketing Manager, Genomic Solutions
acjackson@beckman.com
Beckman Coulter’s Products for Genomic Sample Prep

- **DNA Isolation**
- **RNA Isolation**
- **PCR Purification & Clean-up**
- **NGS Library Construction**
- **Size Selection**

**Agencourt**
- DNAdvance
- Genfind v2
- CosMCPrep
- RNAdvance Cell v2
- RNAdvance Blood
- RNAdvance Tissue
- FormaPure - FFPE
- AMPure XP
- CleanSEQ
- RNAClean XP

**SPRIworks**
- Fragment Library Systems
- HT
- SPRIselect for size selection
- Demonstrated LC for popular kits
Beckman Coulter’s Sample Prep Solutions

NGS Library Construction and Size Selection

AMPure XP for DNA Purification
SPRIworks Library Construction Systems
SPRIselect for Size Selection
Demonstrated methods for popular NGS kits
NGS Sample Preparation Challenges

• Library construction is laborious and time consuming
• Errors and inconsistent results
• Increased throughput due to multiplexing
• Limited human resources in laboratory
• Multiple vendors needed to complete workflow process
• Size selection not integrated and low throughput
Workflow Comparison

**Manual**
- Up to 8 libraries per day
  - DNA Extraction
  - Shear DNA
  - End Repair
  - A-Tailing
  - Ligation
  - Size Selection
  - PCR Set-up
  - PCR
  - PCR Clean-up
  - qPCR
  - Normalization
  - Pooling
  - Sequencing

**SPRiworks**
- Up to 30 libraries per day
  - DNA Extraction
  - Shear DNA
  - End Repair
  - A-Tailing
  - Ligation
  - Size Selection
  - PCR Set-up
  - PCR
  - PCR Clean-up
  - qPCR
  - Normalization
  - Pooling
  - Sequencing

**SPRiworks HT**
- Up to 96 libraries per day
  - DNA Extraction
  - Shear DNA
  - End Repair
  - A-Tailing
  - Ligation
  - Size Selection
  - PCR Set-up
  - PCR
  - PCR Clean-up
  - qPCR
  - Normalization
  - Pooling
  - Sequencing

Manual: 1-8 fully sized libraries per day per person

Automated (optional)
Covaris E-series can be integrated on Biomek FX®

Automated
1-96 fully sized libraries per day

Automated (optional)
T-robot on Biomek FX®

Automated with method suite

Automated with method suite
Quality NGS Sequencing *Starts with* Better Sample Prep!

**Improve your results!**
- Automate size selection with SPRIworks chemistry
- Standardize your process and increase reproducibility

**Get your results faster!**
- Increase throughput and reduce processing time
- Multiplex for greater efficiency
- Track your samples with confidence

*Empowering real discoveries.*
Automated Library Construction with **SPRI-based Size Selection**

- Beckman Chemistry *and* automation combined for maximum efficiency
  - Low- to high-throughput options
  - Consistent results
  - Validated solutions

**SPRIworks Fragment Library System**

**SPRIworks HT* Library Prep System**
Medium to High Throughput NGS Sample Prep

Increasing throughput and flexibility
SPRIworks HT Solution

Proven SPRIworks Chemistry + Proven Biomek Automation

= High Throughput Library Construction with built-in SPRI size selection and Clean-up

Empowering real discoveries.
SPRIworks HT Solution
High Throughput Library Construction with built-in SPRI size selection and Clean-up

- 48 samples per kit
- Kit contains enzymes, buffers, AMPure XP, Sizing Solution, and PCR master mix for library construction
- Flexibility in choice of library adapters

- Pre-defined Biomek Configuration
- Suite of Methods
- Library Construction – 96 S /6hrs
- Per well Size Selection (4 options)
- PCR setup
- AMPure XP PCR clean-up
- qPCR setup
- Normalization
- Pooling

Biomek FX® Dual Multi 96 and Span 8
- 4X3 ALP
- Orbital Shaker
- 96 Channel Wash Station
- Optional on deck Thermocycler
## System Capabilities

<table>
<thead>
<tr>
<th></th>
<th>SPRIworks HT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Throughput</strong></td>
<td>• With size selection: 96 samples in ~6 hrs</td>
</tr>
<tr>
<td></td>
<td>• Without size selection: 96 samples in ~4 hrs</td>
</tr>
<tr>
<td><strong>DNA input amount</strong></td>
<td>1 ug*</td>
</tr>
<tr>
<td><strong>Size Selection Options</strong></td>
<td>• No size selection</td>
</tr>
<tr>
<td></td>
<td>• 150-350 bp (insert size)</td>
</tr>
<tr>
<td></td>
<td>• 250-450 bp (insert size)</td>
</tr>
<tr>
<td></td>
<td>• 350-700 bp (insert size)</td>
</tr>
<tr>
<td><strong>Supported input sample types</strong></td>
<td>Sheared DNA: gDNA, cDNA, amplicons</td>
</tr>
</tbody>
</table>

*Validation amount
Method Suite with Simple Setup Steps
Simple Setup Steps

Set runtime options
Select from 1-96 samples
Select Start Step
Select size ranges *per well*
Select whether to perform PCR setup
Simple Setup Steps

Reagent Calculator

System calculates required reagents and volume based on steps chosen and number samples to run.
Simple Setup Steps

Setup Deck & Load Reagents

Place tips, plates and reagents on Deck. **Click OK and walk away!**

96 libraries are prepped with size selection and ready for PCR in ~ 6 hours.

Entire hands on processing takes ~30 minutes.
Reproducible Data

Experimental Design

1. 1ug in 50 ul (20ng/ul) of shear used per sample
2. 8 samples for each of the 4 size selections
   - No SS, Small & Medium = TruSeq shear
   - Large = 500bp Covaris shear
3. 20ul of Library PCR’d for 10 cycles
4. Purification of amplified samples using AMPure XP
5. Eluted in 25ul
6. Quantification using 1ul on Agilent High Sensitivity chip
NGS HT Validation

Shear Data for DNA Sample Input
20ng/ul

<table>
<thead>
<tr>
<th>TruSeq (Enrichment)</th>
<th>Covaris 500pb (PN 400056)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duty Cycles</td>
<td>10% 5%</td>
</tr>
<tr>
<td>Intensity</td>
<td>5.0 3</td>
</tr>
<tr>
<td>Cycles / burst</td>
<td>200 200</td>
</tr>
<tr>
<td>Duration (sec)</td>
<td>120 90</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>5.5-6 6-8</td>
</tr>
</tbody>
</table>

![Graph showing shear data for different DNA samples.](image)
Reproducibility of System

- 150 – 350 bp (insert) size selection (Small Size Selection)
- Trace reflects addition of ~ 100 bp adapter
- > 85% of sample in defined region
Reproducibility of System

- 250 – 450 bp (insert) size selection
- Trace reflects addition of ~ 100 bp adapter
- > 85% of sample in defined region
Reproducibility of System

- 350 – 700 bp (insert) size selection
- Trace reflects addition of ~ 100 bp adapter
- > 85% of sample in defined region
Comparative Data

- **Illumina TruSeq DNA v2 and Sage Pippen Prep**
- **SPRIworks HT**
  - 250 – 450 bp (insert) size selection
  - 10 cycles of PCR used for SPRIworks HT
  - 8 cycles of PCR used for Illumina/Sage
Summary on SPRIworks HT

- 96 libraries created in under 6 hours including size selection
- User friendly method suite
- High reproducibility
- Equivalent to Illumina TruSeq DNA kit
### Biomek validated and Demonstrated Methods for Popular NGS Kits and Applications

- Flexibility to automate protocols for multiple applications and platforms
- Standardized process, decreased errors
- Quick implementation
- Top notch support for optimization to address individual needs.
- Simple User Interface
- Increased throughput
- Includes methods of SPRIselect size selection

Contact local Sales Representative for Updated methods list

*Illumina has reviewed and qualified sequencing results for relevant samples.*

---

<table>
<thead>
<tr>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPRIworks HT – Illumina platforms</td>
</tr>
<tr>
<td>TruSeq DNA v2</td>
</tr>
<tr>
<td>Truseq RNA v2</td>
</tr>
<tr>
<td>TruSeq Stranded mRNA – Illumina- Qualified*</td>
</tr>
<tr>
<td>Nimblegen SeqCap EZ</td>
</tr>
<tr>
<td>Agilent SureSelect XT</td>
</tr>
<tr>
<td>NEB Library Construction</td>
</tr>
<tr>
<td>Nextera ScriptSeq Complete Gold Low Input</td>
</tr>
<tr>
<td>Roche GS Flex Rapid</td>
</tr>
<tr>
<td>Roche REMe</td>
</tr>
<tr>
<td>Roche 454 Emulsion PCR SETUP</td>
</tr>
<tr>
<td>KAPA Biosystems Library Quantification Kit – Illumina</td>
</tr>
<tr>
<td>qPCR setup 96/ 384</td>
</tr>
<tr>
<td>SPRIselect Size Selection</td>
</tr>
<tr>
<td>Quantitation /Normalization</td>
</tr>
<tr>
<td>Sample Pooling for Multiplexing</td>
</tr>
</tbody>
</table>
RNASeq workflow
Example of RNASeq sample prep Workflow automated with multiple BEC products

Options for Beckman provided solutions

Blue text = Beckman products
Black text= Other vendor chemistry automated on the Biomek Platform

RNA Extraction FFPE, Tissue
PolyA/cDNA Synthesis
Library Construction w/Size Selection
qPCR/Norm and Pooling
Cluster Generation and RNA Sequencing

FormaPure RNAdvance Tissue RNAdvance Cell V2 RNAdvance Blood RNAClean

TruSeq RNA Other

SPRIworks HT AMPure XP SPRIselect TruSeq RNA kits NEB kit

SPRIworks HT Method Suite qPCR setup 96 or 384

C-bot Illumina Sequencer
Learn more about automating RNASeq sample prep from poster and Webinar from Michaela Bowden Ph.D. of Dana Farber Cancer Institute.

Available in the Beckman Coulter exhibit under NGS Sample Prep

Automated TruSeq RNA Sample Preparation from FFPE tissue specimens utilizing the Biomek FX Liquid Handler

Overview
The poster describes a TruSeq RNA library preparation for FFPE samples using the Biomek automation platform, capable of constructing 96 libraries in 7 hr. The Biomek platform provides a solution for high throughput library construction, producing sensitive and reproducible sequencing data, which facilitates biomarker discovery in archival FFPE tissues.

Figure 1: Representative RNA QC plots with associated solution bands on the left for Tumor / Benign pair. A

RNA concentration was determined by Ribocount Assay. Concentrations

Single-End Sequencing Performance
Sequencing was performed on the Illumina HiSeq platform. 50bp single reads were mapped using TopHat and transcript abundance in FPKM units (fragments per kilobase of mRNA per 10^6 reads) calculated using Cufflinks. Multiplexed sequencing was performed in such that each lane contained 3 samples. RNASeq library construction / sequencing were successful in 12/12 samples with aligned reads ranging from 21% - 72% relative to the total # reads.

A wide ranging panel of endogenous controls [1] were found to be good concordance between the manual and sequenced libraries, with a Pearson coefficient r calculated. The insets are plots of the average Log values of all samples for each library preparation method, highlighting the stability of these endogenous controls in sample types, irrespective of preparatory method or morphology.

Empowering real discoveries.
NGS in Cancer Research

Example of Target/Exome Capture Workflow automated with BEC products

DNA/RNA Extraction:
- Agencourt FormaPure
- Agencourt RNAAdvance Tissue
- Agencourt RNAAdvance Blood
- Agencourt GenFind V2
- Agencourt DNAdvance

If RNA Poly A/cDNA synthesis:
- TruSeq RNA

Library Construction W/Size Selection:
- SPRiworks HT
- AMPure XP
- SPRIselect
- TruSeq RNA
- TruSeq DNA
- Agilent SureSelect NEB

Pre and post Hybridization:
- Agilent SureSelect
- Nimblegen SeqEZ

Cluster Generation and RNA Sequence:
- C-bot
- Illumina Sequencer

Blue text = Beckman products
Black text= Other vendor chemistry automated on the Biomek Platform

Empowering real discoveries.
Agencourt AMPure XP

DNA Purification
Process using SPRI* magnetic beads

1. PCR reaction
2. Binding of PCR amplicons to magnetic beads
3. Separation of PCR amplicons bound to magnetic beads from contaminants
4. Washing of PCR amplicons with Ethanol
5. Elution of PCR amplicons from the magnetic particles
6. Transfer away from the beads into a new plate

*Learn more about SPRI magnetic bead technology in our booth under Nucleic Acid Extraction and Purification.
Features

• High recovery of Double stranded and single stranded DNA templates
• Efficient removal of unincorporated dNTPs, primers, primer dimers, salts and other contaminants
• No PCR degradation after storage at 4° C for seven days
• Automated 96, 384 well formats (Biomek® 3000, NX, and FX)

• Down Stream Applications:
  • PCR
  • Genotyping, SNP detection
  • Fragment analysis
  • Sequencing (Sanger and Next generation)
  • Cloning
  • Primer walking
## Fast Processing Time

<table>
<thead>
<tr>
<th>Agencourt AMPure XP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bind Inc</td>
<td>5min</td>
</tr>
<tr>
<td>Bind settle</td>
<td>2 min</td>
</tr>
<tr>
<td>EtOH settle</td>
<td>30 sec</td>
</tr>
<tr>
<td>Air Dry</td>
<td>0 min</td>
</tr>
<tr>
<td>Elute Inc</td>
<td>2 min</td>
</tr>
<tr>
<td>Elute Settle</td>
<td>1 min</td>
</tr>
</tbody>
</table>

**Total**  
**10.5 min**
High Recovery

![Bar graph showing concentration (ng/µL) against template size (bp) for different methods: Agencourt AMPure XP, QIAquick, and QuickStep. The x-axis represents template size in bp, ranging from 200bp to 10kb. The y-axis represents concentration in ng/µL, ranging from 0 to 30 ng/µL. The graph indicates higher concentration for Agencourt AMPure XP compared to QIAquick and QuickStep across all template sizes.](image-url)
Pure PCR Products: No Dimers, No Salts

No Primer Dimers

No Salt Carry Over

* Trade marks are property of their respective owners
Stable PCR Products

PCR Products Post PCR Clean up on 1.2% Agarose

Day 0

Day 7 at 4°C

> 50% degradation
Faster PCR Clean up

<table>
<thead>
<tr>
<th>Method</th>
<th>Time to Process 96 Samples (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agencourt AMPure XP</td>
<td>100</td>
</tr>
<tr>
<td>QIAGenorm QuickStep</td>
<td>60</td>
</tr>
<tr>
<td>QuickStep</td>
<td>40</td>
</tr>
<tr>
<td>ExoSAP-IT</td>
<td>20</td>
</tr>
</tbody>
</table>

**Empowering real discoveries.**
SPRIselect
DNA Size Selection
What is Size Selection?

• Sheared DNA contains an assortment of DNA lengths
• Size Selection is a process that targets a specific range of Nucleic Acid fragment sizes while removing undesired fragments
• Required step for most Next Generation Sequencing platforms and their corresponding applications
Challenges size selection

<table>
<thead>
<tr>
<th>Challenges</th>
<th>SPRIselect answers with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual gel based process is long, tedious, low throughput and prone to variability.</td>
<td>Proven SPRI magnetic bead technology simplifies process and provides consistent results</td>
</tr>
<tr>
<td>Other automated solutions are low throughput and lack processing scalability</td>
<td>High throughput processing in microtiter plates or tubes enabled manual or automated prep. Biomek template methods available.</td>
</tr>
<tr>
<td>Some processes require additional devices and costly consumables</td>
<td>All that’s needed are the beads and a magnet. Low cost and efficient.</td>
</tr>
<tr>
<td>Production of waste and potential exposure to harmful toxins</td>
<td>All that’s needed are the beads and a magnet. Low cost and efficient.</td>
</tr>
</tbody>
</table>
SPRIselect for DNA Sizing

- SPRI-based* chemistry for DNA size selection.
- Similar to Sizing Solution in SPRIworks HT.
- Manufactured to a tight specification to provide lot to lot consistency.
- 3 Sizes, 5, 60 and 450 ml
- Validated to target fragments between 150 bp to 800 bp in length.
- Guideline to aide customers in optimizing to their desired size range.
  - Customers register and download from the website.
- Biomek template methods available for BFXP, BNXPS8 and B4K.

*Learn more about SPRI magnetic bead technology in our booth under Nucleic Acid Extraction and Purification.
How Does SPRIselect Work?

Same benefits and function of SPRI - Solid Phase Reversible Immobilization
SPRIselect Size Selection Options

**Left Side Size Selection**
- Excludes fragments below target cutoff

**Right Side Size Selection**
- Excludes fragments above target cutoff

**Double Size Selection**
- Excludes fragments above and below target cutoffs
- Captures a targeted region of specific fragment sizes
Increasing the ratio of SPRIselct volume to sample volume will increase the efficiency of binding smaller fragments.
Right Side Size Selection

Increasing the ratio of SPRIselct volume to sample volume will decrease the efficiency of binding larger fragments.
Double Size Selection

The Left Side Size Selection ratio always needs to be greater than the Right Side Size Selection ratio.

Peak at about 330bp ideal for Ion Torrent 200 bp read application.
Case Study: Achieving a median target of 350 bp size selection with ±200 bp.

- Human DNA sample sheared in water and 1x TE shear and ran each on 4 Agilent chips and they traces ran as seen in graph.
- Evaluation of the 50-2000 bp smear region showed concentrations of 5.3 and 5.9 ng/ul respectively.
- Concentrations confirmed at 10 ng/ul on Nanodrop.
Case Study: Achieving a median target of 350 bp size selection with ±200 bp.

Processed 1 ug (100 ul) through a One-Step Double Size Selection* using the ratios in the table below.

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th></th>
<th></th>
<th></th>
<th>1x TE</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>A</td>
<td>0.80-0.70</td>
<td>0.80-0.70</td>
<td>0.80-0.70</td>
<td>0.80-0.70</td>
<td>0.80-0.70</td>
<td>0.80-0.70</td>
<td>0.80-0.70</td>
<td>0.80-0.70</td>
</tr>
<tr>
<td>B</td>
<td>0.85-0.70</td>
<td>0.85-0.70</td>
<td>0.85-0.70</td>
<td>0.85-0.70</td>
<td>0.85-0.70</td>
<td>0.85-0.70</td>
<td>0.85-0.70</td>
<td>0.85-0.70</td>
</tr>
<tr>
<td>C</td>
<td>0.86-0.68</td>
<td>0.86-0.68</td>
<td>0.86-0.68</td>
<td>0.86-0.68</td>
<td>0.86-0.68</td>
<td>0.86-0.68</td>
<td>0.86-0.68</td>
<td>0.86-0.68</td>
</tr>
<tr>
<td>D</td>
<td>0.83-0.68</td>
<td>0.83-0.68</td>
<td>0.83-0.68</td>
<td>0.83-0.68</td>
<td>0.83-0.68</td>
<td>0.83-0.68</td>
<td>0.83-0.68</td>
<td>0.83-0.68</td>
</tr>
</tbody>
</table>

*Procedure available
Double Size Selection of 0.83 - 0.68 yielded the target of 350bp average range

4 replicates 10ng/ul hDNA in water

3 replicates 10ng/ul hDNA in TE
Biomek Template Method for SPRIsselect

- Optional
- Currently available for BFXP and BNXPS8 And B4K
- No active ALPs required
- Flexible
- Excel spreadsheet to define ratios
- Quick Start Guide
- FAS support
Extended!
A Suite of Products to Improve Efficiency of NGS Sample Prep

NA extraction & purification

Library Construction and Size Selection (low to high throughput)

Downstream platforms and applications

Agencourt SPRI Reagent Kits for Extraction and Purification

- SPRIworks Fragment Library Systems, chemistry and automation (low to high throughput)
- SPRIselect for size selection
- Demonstrated methods for popular reagent kits
Questions:

Email questions to acjackson@beckman.com

Or call us at 1-800-742-2345
Disclaimers:

Beckman Coulter, the stylized logo, Biomek, SPRI, and SPRIsel ect are trademarks of Beckman Coulter, Inc. and are registered with the USPTO.

All trademarks are the property of their respective owners.
Thank you!