BECKMAN COULTER Life Sciences

Biomek i-Series Automated AmpliSeq for Illumina® Library Prep Kit

Introduction

The AmpliSeq for Illumina Library Prep Kit is used to prepare dual indexed libraries for targeted sequencing on Illumina platforms. Sample inputs range from 2-200 ng DNA and 2-200 ng RNA and also compatible with FFPE samples thus allowing researchers to work with low input DNA and RNA.

In this flyer, we demonstrate the automated performance of AmpliSeq for Illumina Library Prep Kit on the Biomek i5 Span-8 Genomics Workstation for three of the workflows compiled in a single automated method.

- Ampliseq for Illumina Cancer Hotspot Panel v2 (for max 96 DNA samples with single DNA panel)
- Ampliseq for Illumina Comprehensive Panel v3 (for max 48 DNA and 48 RNA samples with 2 tube panel for each)
- Ampliseq for Illumina Focus Panel (for max 48 DNA and 48 RNA samples with single tube panel for each)

In comparison to the manual pipetting, the AmpliSeq for Illumina Library Prep Kit automated on Biomek platform provides:

- Reduced hands-on time and increased throughput
- Reduction in system setup and potential pipetting errors
- Standardized workflow for improved results
- Quick install with ready-to-implement methods
- Knowledgeable support

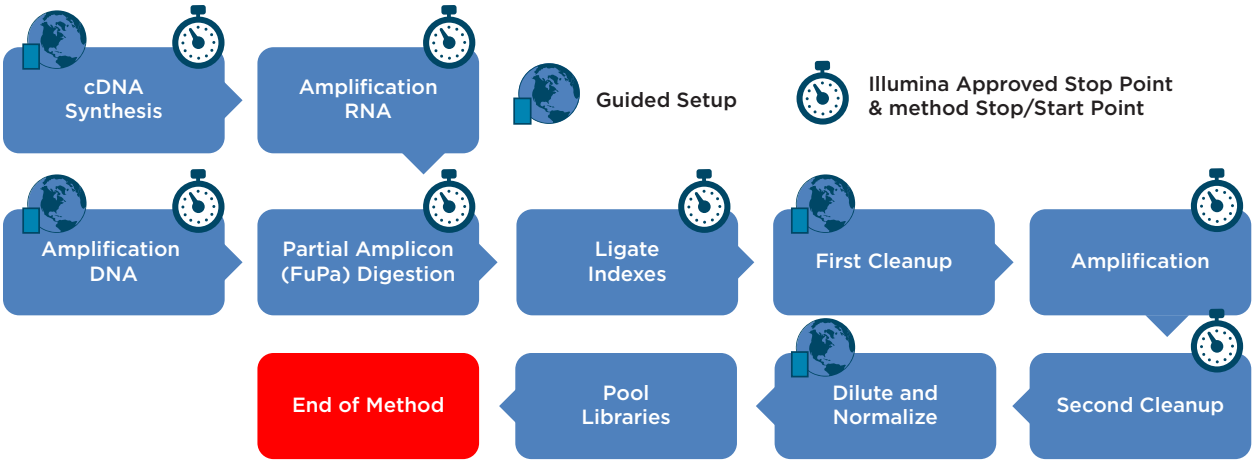
Spotlight

Biomek i5 Span-8 Genomics Workstation

System features deliver reliability and efficiency to increase user confidence and walk-away time

- Span-8 pod with 1-1000 uL pipetting capability
- Independent 360˚ rotating gripper with offset fingers
- High deck capacity with 25 positions
- Orbital Shakers, Peltiers, Span-8 Tip Wash for efficient and cost-effective control of sample processing
- Open platform design to integrate on-deck and off-deck elements (e.g. thermocyclers)
- Optional Enclosure

Figure 1. Biomek i5 Span-8 Genomics Workstation with optional Enclosure on a Biomek Mobile Workstation. Deck layout in the lower image.



- This workflow is for Comprehensive V3 Panel
- RNA and/or DNA workflow can be run independently
- The method is modularly written and each process can be run independent of each other.

Figure 2. AmpliSeq for Illumina Library Prep Kit automated workflow

Automated Method

The method can be run using the interactive Guided Labware Setup software. It provides logical start and stop points assigned based on Illumina's recommendations, enabling flexibility to the users for scheduling their workflow and allowing each laboratory to address their individual requirements for sample processing and throughput.

Application is designed to run up to 96 total samples. The instrument has 1 each Static Peltier and Orbital Shaker. Application can run DNA and RNA workflow individually or simultaneously, if applicable.

Major Process Description

Kit Type	Hot Spot V2 Panel			Focus Panel			V3 Panel		
Sample Number	**24**	**48**	**96**	**24**	**48**	**96**	**24**	**48**	**96**
Instrument Setup Time	10 min	15 min	20 min	10 min	15 min	20 min	10 min	15 min	20 min
cDNA Synthesis	NA	NA	NA	NA	8 min	15 min	NA	8 min	15 min
Amplification	9 min	18 min	31 min	14 min	20 min	34 min	21 min	37 min	1 hr 8min
Digest Amplicons	8 mins	14 min	27 min	7 min	14 min	27 min	7 min	14 min	27 min
Ligate indexes	12 mins	22 min	43 min	13 min	22 min	43 min	13 min	22 min	43 min
Instrument Setup Time	15 min	20 min	30 min	15 min	20 min	30 min	15 min	20 min	30 min
First Cleanup and Amplification	39 min	49 min	1 hr 7 min	42 min	49min	1 hr 7 min	42 min	49min	1 hr 7 min
Second Cleanup	55 min	1 hr 9 min	1hr 37min	58 min	1 hr 9 min	1hr 37min	58 min	1 hr 9 min	1hr 37min
Normalize and Pooling	13 min	15 min	17 min	13 min	15 min	17 min	13 min	15 min	17 min
Method Run Time	**2hr 58min**	**4hr 16min**	**6hr 39min**	**3hr 9min**	**4hr 26min**	**6hr 57min**	**3hr 26min**	**4hr 43min**	**7hr 31min**
Total Time	**8hr 58min**	**10hr 16 min**	**12hr 39 min**	**9hr 9min**	**10hr 26min**	**12hr 57min**	**9hr 26min**	**10hr 43min**	**13hr 31 min**

*Total timing estimates include Thermocycling but do not include reagent thawing
* Focus and V3 Panel can have a maximum of 48 DNA and 48 RNA samples.

Table 1. Estimated run times for AmpliSeq for Illumina Library Prep Kit on the Biomek i5 Span8 Genomics Workstation.

The software provides several user friendly features such as:

1. Biomek Method Launcher (BML)

BML is a secure interface for method implementation without affecting method integrity. It allows the users to remotely monitor the progress of the run. The manual control options provide the opportunity to interact with the instrument, if needed.

Figure 3. Biomek Method Launcher provides a straight-forward interface to launch the method

2. Method Options Selector (MOS)

MOS enables selection of plate processing and sample number options to maximize flexibility, adaptability and the ease of method execution.

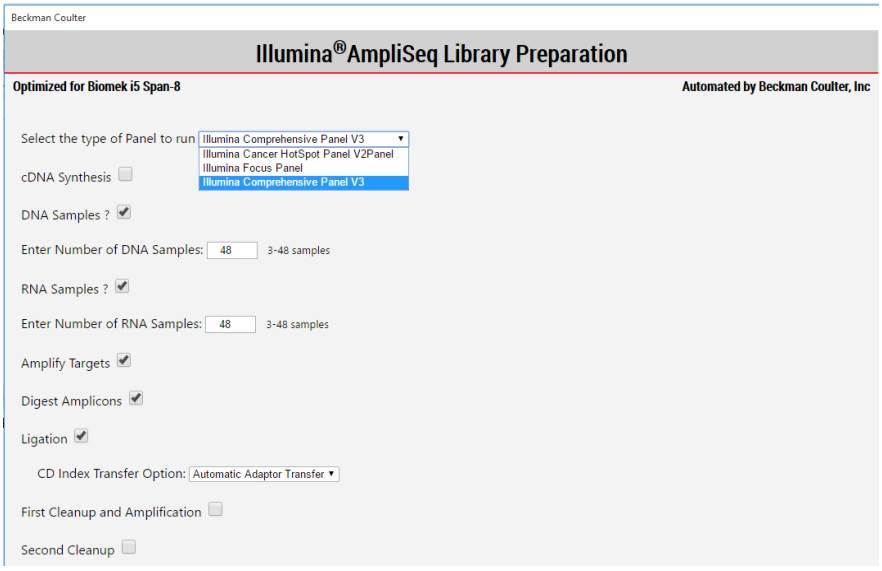


Figure 4. AmpliSeq for Illumina Method Options Selector enables one to select DNA and/or RNA to run, batch size and other processing options.

3. Guided Labware Setup (GLS)

GLS is generated based on options selected in the MOS, and provides the user specific graphical setup instructions with reagent volume calculation and step by step instructions to prepare reagents.

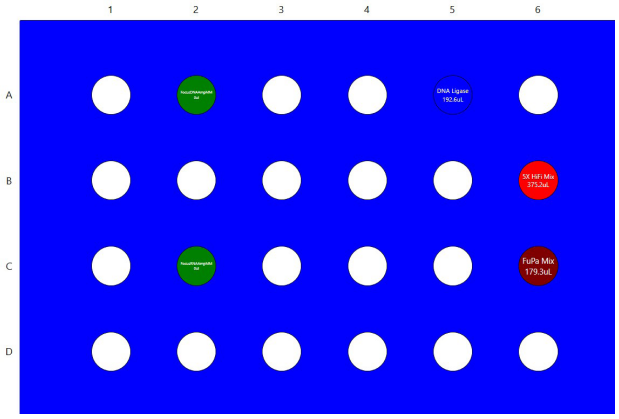


Figure 5A. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup

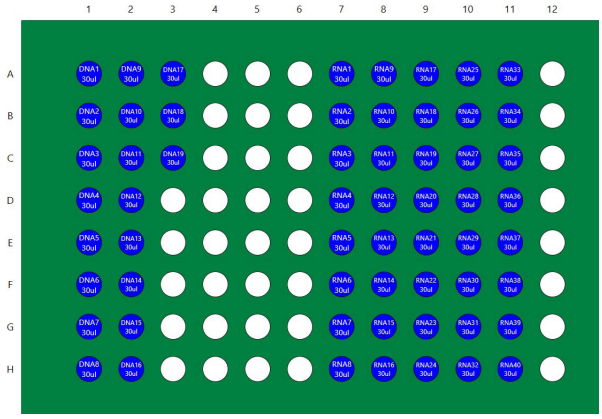


Figure 5B. Application allows DNA and RNA to be processed in parallel post amplification on a single plate.

Experimental Design

The AmpliSeq for Illumina Comprehensive Cancer Panel v3 was run with 10ng each of 8 DNA samples (4 replicates of Horizon™ HD 798 and 4 replicates of HD 803) and 10ng of 8 RNA samples (4 replicates of Agilent® Universal Human Reference RNA and 4 replicates of Seracare® Fusion RNA mix v3 Catalog No. 0710-0431) to generate libraries for sequencing on MiSeq. The quantity of the original sample was assessed with Qubit™ ds HS DNA Kit and Qubit™ HS RNA Kit. Libraries were normalized and pooled and After paired end 2x151 bp sequencing run, analysis was performed using the BaseSpace Sequence Hub.

Results

A robust and reliable automation solution for NGS library prep is essential to take full advantage of Illumina's sequencing technology. Automation saves up valuable time and money and helps the researchers to obtain an efficient process with more walk-away time.

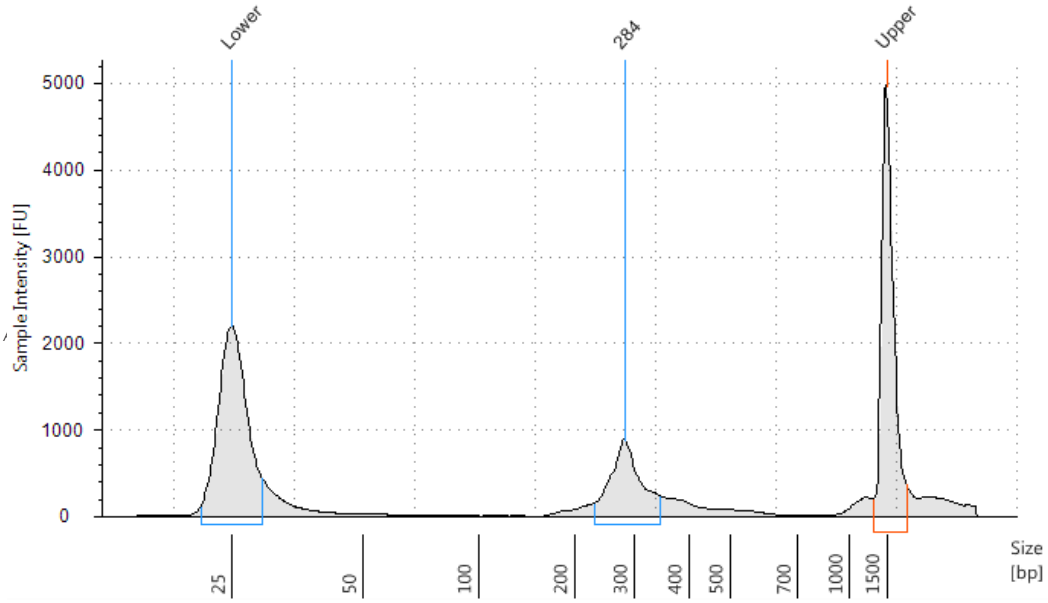


Figure 6. Seracare Fusion RNA Mix v3 (0710-0431) library distribution on D1000 tape run on Agilent TapeStation 2100.

Mean Autosome Rate	Percent Q30 Bases	Percent On-target Aligned Reads	Uniformity of Coverage
99.2%	92.3%	95.0%	93.4%

Figure 7. Average sequencing matrix for 8 DNA samples

Sample Index	Sample Id	Sample Group	Total Aligned Reads (R1/R2)	Percent Aligned Reads (R1/R2)	Overall Percent Aligned Reads
1	Seracare1	SeraCare-RNA	77,463 / 77,463	77.1% / 77.1%	77.1%
2	UHRRNA1	UHR-Control	53,922 / 53,922	71.4% / 71.4%	71.4%
3	Seracare3	SeraCare-RNA	77,841 / 77,841	78.3% / 78.3%	78.3%
4	UHRRNA2	UHR-Control	42,973 / 42,973	74.4% / 74.4%	74.4%
5	Seracare2	SeraCare-RNA	45,134 / 45,134	83.5% / 83.5%	83.5%
6	UHRRNA3	UHR-Control	54,737 / 54,737	72.8% / 72.8%	72.8%
7	Seracare4	SeraCare-RNA	71,616 / 71,616	84.7% / 84.7%	84.7%
8	UHRRNA4	UHR-Control	56,214 / 56,214	74.3% / 74.3%	74.3%

Figure 8. Aggregate Summary of RNA samples

Gene A	Gene B	Score	Fusion Call
TPM3	NTRK1	139.4	Present
FGFR3	TACC3	51.5	Present
KIF5B	RET	51.1	Present
FGFR3	BAIAP2L1	50.7	Present
SLC45A3	BRAF	48.9	Present
ETV6	NTRK3	48.4	Present
EML4	ALK	46.6	Present
EGFR	SEPT14	39.9	Present
PAX8	PPARG	26.0	Present
CD74	ROS1	22.7	Present
NCOA4	RET	21.9	Present
SLC34A2	ROS1	15.5	Present
LMNA	NTRK1	14.9	Present
TMPRSS2	ERG	8.3	Present

Figure 9. The fusion calls that are present in Seracare RNA are detected in this run as seen in table above.

Summary

We've demonstrated automation of AmpliSeq for Illumina Library Preparation Kit on the Biomek i5 Span-8 Genomics Workstation. The solution delivers libraries that yield quality sequencing results. It's flexible and scalable thus providing fast and efficient library construction

If preparing the maximum number of libraries per kit, more than one kit may be required to accommodate for higher dead volume requirements associated with automated platforms and any variation in overfill volumes by original reagent manufacturer.

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

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