PREPARATION OF NGS LIBRARIES FROM LIMITED SAMPLES IN A HIGH-THROUGHPUT, COST-EFFECTIVE MANNER

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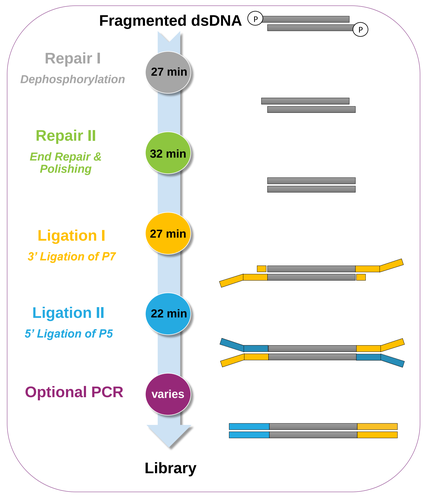
Abstract

Library preparations that maximize complexity and uniform representation of the genome make the most efficient use of next-generation sequencing (NGS) reads. Highly efficient library preparation is crucial for comprehensive analysis of DNA samples of limited quality or quantity. End repair of both the 3' and 5' DNA termini utilized in the Accel-NGS® 2S Plus and PCR-free DNA Library Kits allows greater efficiency of adapter ligation, requires no adapter titration for lower input quantities, and delivers a more complex library requiring less sequencing; thus reducing the overall sequencing cost for a given sample. Automation of Swift's high-quality library preparation kit permits a higher throughput prep and increased cost savings as compared to manual processing. Here we present use of the Accel-NGS 2S kits on the Beckman Coulter Biomek FX^P Laboratory Automation Workstation to produce high quality libraries from inputs as low as 100 pg and libraries from kidney FFPE with inputs of 50 ng and 10 ng. *E.coli* genomic DNA was titrated from 100 ng down to 100 pg while Coriell NA12878 gDNA was used for inputs of 500 ng, 250 ng, 100 ng, 10 ng, and 1 ng. Libraries were also prepared manually for comparison to automated preparation. Reproducibility and data quality was determined by analysis of data generated from sequencing on an Illumina® MiSeq®. The Accel-NGS 2S automated protocol produces libraries of quality comparable to that of manual preparation. Optimized Accel-NGS 2S kit configuration combined with the flexible workflow options and advanced pipetting performance of the Biomek FX^P Liquid Handler minimize consumable use and sample loss and increase lab efficiencies with a walk-away solution capable of generating up to 96 PCR-free libraries in 4.5 hours. The technology provides the opportunity to use one kit and one automated program to generate libraries from multiple sample types, regardless of their quality and quantity.

Accel-NGS 2S – dsDNA Library Preparation



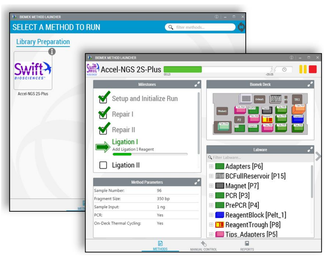
- ✓ Simple with-bead protocol
- ✓ Broad input range: 10 pg – 1 µg
- ✓ Sequential repair steps enable use of damaged DNA
- ✓ 5' and 3' repair enables more efficient adapter attachment
- ✓ Low dimer formation eliminates the need for adapter titration
- ✓ Compatible with cfDNA and FFPE samples
- ✓ Increased library complexity
- ✓ Balanced coverage of AT-/GC-rich regions
- ✓ Single and dual indexing available, and included in reaction price

Figure 1. Accel-NGS 2S Plus and PCR-free DNA Library kits. The Accel-NGS 2S family of kits was used to generate libraries. Accel-NGS 2S PCR-Free was used to generate libraries from 100ng or more and Accel-NGS 2S Plus was used for all inputs less than 100ng. The kit has four steps when used PCR-free: Repair I which dephosphorylates the 5' ends of the DNA, **Repair II** which performs end repair and polishing, **Ligation I** which adds the P7 adapter to the 3' terminus, and **Ligation II** which adds the P5 adapter to the 5' terminus.

Accel-NGS 2S Automation on Biomek FX^P

Biomek FX^P Hybrid Automated Laboratory Workstation

Biomek Method Launcher Software



- **96 PCR-Free libraries in ~ 4.5 hours**
- **Increased efficiency and flexibility through use of Span-8 and Multi-channel pods**
- **Single method executes either PCR-free or PCR protocol with optional on-deck thermal cycling.**

- **Guided labware and reagent setup saves time and reduces setup errors.**
- **Information tiles provide real-time run updates**
- **Remote run monitoring from any web-enabled device**

Automated vs. Manual Accel-NGS 2S: Control gDNA

FRAGMENT INSERT SIZE	NA12878 INPUT QUANTITY (ng)	LIBRARY YIELD (nM) AUTOMATED PREP	STD DEV AUTOMATED PREP	LIBRARY YIELD (nM) MANUAL PREP
350 bp	100	6.4	1.4	4.0
	10	26.1	10.6	27.0
200 bp	500	25.1	6.7	N/A
	250	16.9	3.3	15.2
	100	7.1	1.0	7.0
	10	30.6	8.3	N/A
	1	21.4	6.5	N/A

FRAGMENT INSERT SIZE	E.coli INPUT QUANTITY (ng)	LIBRARY YIELD (nM) AUTOMATED PREP	STD DEV AUTOMATED PREP	LIBRARY YIELD (nM) MANUAL PREP
200 bp	100	7.2	2.5	9.2
	10	11.8	2.7	25.4
	1	32.7	6.6	28.9
	0.1	29.7	8.6	25.6

	A	B	C	D	E	F
1	NTC		NTC		NTC	
2		NTC		NTC		NTC
3	NTC		NTC		NTC	
4		NTC		NTC		NTC
5	NTC		NTC		NTC	
6		NTC		NTC		NTC
7	NTC		NTC		NTC	
8		NTC		NTC		NTC

Tables 1, 2 and 3: Automated library prep yields from control gDNA. Yields are comparable to manually prepared libraries for NA12878 (Table 1) and E.coli (Table 2). NTCs were arrayed in checkerboard fashion across six columns on a plate, and had an average yield of 0.2 nM (Table 3). N=8 for each DNA source, insert size, and input quantity. 10 ng libraries were amplified with 6 cycles of PCR, 1 ng libraries with 9 cycles of PCR, and 100 pg libraries with 12 cycles of PCR.

Automated Accel-NGS 2S Sequencing Data: FFPE

FRAGMENT INSERT SIZE	DNA SOURCE	LIBRARY YIELD (nM)	MEDIAN INSERT SIZE (bp)	PCT ALIGNED	EST. LIBRARY COMPLEXITY (M)	PCT DUPLICATION
350 bp	Fresh Frozen	13.5	354	98.9%	1,821	0.1%
	6hr Fixation	3.0	285	95.6%	892	0.2%
	24hr Fixation	4.4	295	96.8%	647	0.3%
	48hr Fixation	3.0	260	93.9%	249	0.2%
200 bp	Fresh Frozen	27.3	273	98.6%	947	0.1%
	6hr Fixation	10.1	238	96.6%	705	0.1%
	24hr Fixation	4.0	239	85.9%	471	0.2%
	48hr Fixation	4.0	220	95.7%	385	0.2%

Table 4: High quality sequencing data from automated library preparations with FFPE. As fixation time increases, DNA quality decreases; leading to the challenge of obtaining a quality library to sequence. With the Accel-NGS 2S Plus kit, sequencable libraries are obtained from DNA heavily damaged in the fixation process. N=3 for each insert size and fixation time. Six cycles of PCR were performed for 10 ng. Libraries were sequenced on the Illumina MiSeq using a V2 standard flow cell. Accel-NGS 2S Plus and PCR-free kits readily produce high quality libraries from damaged DNA and low input quantities. Data were aligned to reference genomes using BWA (Li and Durbin, 2009) followed by collection of various performance metrics using Picard-tools (Broad Institute).

Sequencing Data of Control gDNA Automated Prep

FRAGMENT INSERT SIZE	DNA SOURCE	INPUT QUANTITY (ng)	MEDIAN INSERT SIZE (bp)	PCT ALIGNED	READS (K)	EST. LIBRARY COMPLEXITY (M)	PCT DUPLICATION
350 bp	NA12878	100	330	99.5%	800	1,786	N/A
		10	318	97.9%	500	1,071	0.1%
200 bp	NA12878	500	258	99.5%	4,928	4,364	N/A
		250	257	99.4%	3,318	3,318	N/A
		100	265	99.4%	950	1,981	N/A
		10	257	96.8%	630	977	0.1%
		1	249	99.0%	550	640	0.1%
	E.coli	100	260	99.4%	660	935	0.1%
		10	272	99.7%	600	786	0.1%
		1	243	98.9%	630	505	0.1%
		0.1	251	91.6%	300	94	0.3%

Table 5: NGS analysis of automated library prep with high quality DNA. A minimum of three libraries per DNA source, insert size, and input prepared via automation were selected for sequencing runs on the Illumina MiSeq using a V2 standard flow cell. Accel-NGS 2S Plus and PCR-free kits readily produce high quality libraries from high quality DNA with both high and low input quantities. Regardless of input quantity, adapter titration is not required. Data were aligned to reference genomes using BWA (Li and Durbin, 2009) followed by collection of various performance metrics using Picard-tools (Broad Institute).

Conclusions

- The Accel-NGS 2S Plus and PCR-free library kits were automated on the Beckman Coulter Biomek FX^P Laboratory Automation Workstation which offers an easy-to-use interface to set up and run the protocol.
- High quality Accel-NGS 2S Plus and PCR-free libraries prepared in an automated manner are comparable to that of manual preparation, enabling the use of one kit and one automated program for multiple sample types, including heavily damaged FFPE. Single and dual indexing is available, and adapter titration is not required.
- The automated Biomek FX^P method provides flexible workflow options, incorporation of on-deck incubations and thermal cycling, minimal user interventions and optimized pipetting which minimize consumable use and sample loss and increase lab efficiencies with a walk-away solution capable of generating up to 96 PCR-free libraries in approximately 4.5 hours.

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