BECKMAN COULTER
Life Sciences

Illumina Nextera XT DNA Library Preparation Kit Miniaturized on Biomek i5 Multichannel 384 & Echo 525 Liquid Handlers

Introduction

The Illumina Nextera XT DNA Library Preparation Kit (hereafter Nextera XT Prep Kit) can prepare up to 384 indexed paired-end libraries for sequencing on Illumina platforms. The kit uses only 1 ng of input DNA and fragments the DNA using an enzymatic reaction called Tagmentation. This reduces manual pipetting optimizations and hands-on time by providing master mixed reagents. The kit also accommodates various small genomes, amplicons, plasmids, etc. Previously this kit has been automated successfully on the Biomek i-Series liquid handlers, as well as miniaturized (20X) successfully and efficiently on Echo 525 liquid handlers.

This application overview flyer demonstrates how this Nextera XT Preparation Kit automated method increases throughput and miniaturizes reactions without sacrificing data metrics and sequencing quality on the integrated Biomek i5 Multichannel (MC) 384 and Echo 525 LH platforms. The Nextera XT DNA Prep Kit is miniaturized up to 20X and it can prepare up to 384 indexed paired-end libraries from DNA for sequencing on Illumina platforms. It uses as little as 50 pg of input sample for library prep. All miniaturized reactions are performed on an Echo 525 liquid handler, and source plate preparation, bead cleanups, thermocycling incubations, and shaking are handled by the Biomek i5 MC 384 with enhanced selective tip pipetting capability. Quantification setup is done by Biomek i5 MC 384 and Echo 525 liquid handlers for PicoGreen assay or Miseq, followed by direct normalization and pooling on the Echo 525 liquid handler. The Biomek i5's 360-degree gripper accesses the Echo 525 liquid handler without the need for additional integration.

Compared to manual pipetting, the Illumina Nextera XT DNA Library Prep Kit, automated on Biomek i5 MC384 & Echo 525 liquid handlers, provides:

- Lower reaction cost by reducing reaction volumes
- Reduced hands-on time and increased throughput through tip-less transfers for reaction setup
- Reduction in pipetting errors
- Reduced chances of cross contamination
- Standardized workflow for improved results

Spotlight

Biomek i5 MC 384 Liquid Handler

- 384 Multichannel head with 1-60 µL pipetting capability for efficient cleanups
- Enhanced Selective Tip Pipetting to transfer custom array of samples
- Independent 360˚ rotating gripper with offset fingers
- High deck capacity with 25 positions

- BioShakes, peltiers for incubations, 384 channel tip washing for sample processing and bead cleanups
- Quick installation with ready-to-implement methods
- Knowledgeable support
- Spacious, open platform design to integrate on-deck and off-deck elements (e.g. thermo cyclers)
- Guided Labware Setup (GLS) and DeckOptix Final Check to ensure accurate system setup and reagent calculations

Echo 525 Liquid Handler

- Designed for biochemical and genomic sample/reagent transfer
- 25 nL droplet enabling highly precise and accurate miniaturized assay
- Supports wide range of fluids (including viscous fluids)
- Rapid transfers at up to 6 µL/sec
- Reduced possibility for cross-contamination
- Accelerate normalization and pooling step with contact–free any well to any well transfers

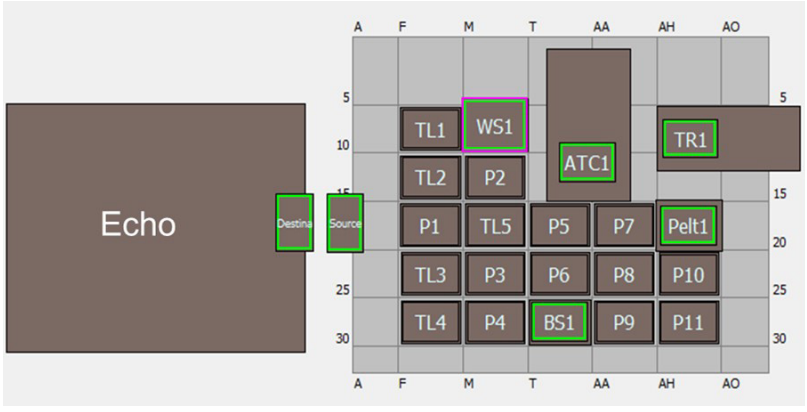


Figure 1. Biomek i5 MC 384 integrated with Echo 525 liquid handler. The deck layout shown above was used to demonstrate the NexteraXT Library Preparation automated method described in this app note.

Automated method

The automated, miniaturized Nextera XT Library Preparation method is constructed in a modular fashion and can be run start to finish with full walk-away capability. It also includes optional start and stop points based on Illumina's recommendations, thus providing users flexibility in scheduling their workflow and breaking the process into pre- and post-PCR steps. PCR reactions can be performed on-deck with an integrated thermocycler or with an off-deck thermocycler. A Bioshake is used to level the fluid meniscus for efficient Echo 525 liquid handler dispensing.

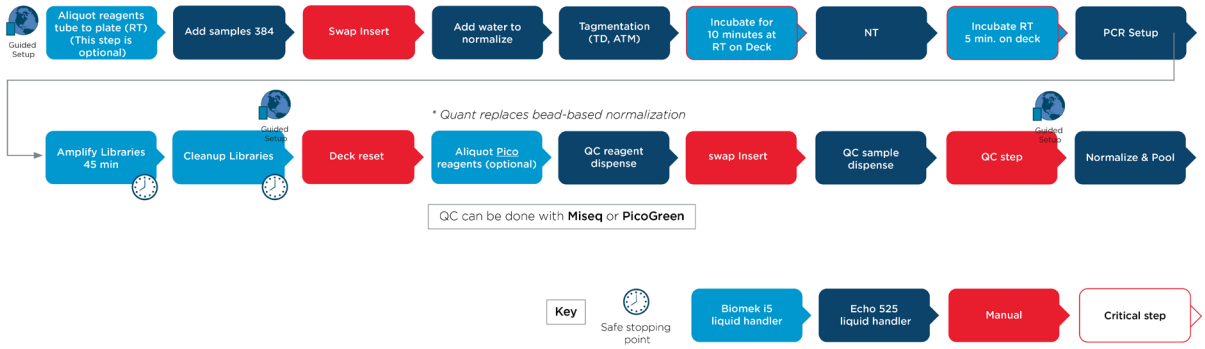


Figure 2. Illumina Nextera XT DNA Library Preparation automated workflow.

Miniaturized automated assays provide increased efficiency and higher throughput, reducing hands-on time. Table 1 details the estimated time to completion with 4 different input sample numbers.

Major Process Description	Turn-around time (in minutes)			
	48 Samples	96 Samples	192 Samples	384 Samples
Deck Setup Time	10	15	20	30
Tagmentation & Amplification & Cleanup				
Tag and Amp				
Echo Reagent Aliquoting	9	12	18	28
Tagmentation Run Time (10 min incubation)	28	38	38	48
Amplification (includes ATC)	40	46	46	52
Cleanup				
Reagent Aliquoting	9	16	29	56
Cleanup	24	24	24	24
Quantitation				
Deck Setup time	5	5	5	5
Quantitation by Pico /Miseq **	15/3	16/4	17/5	19/7
Normalization and pooling				
Deck Setup time	2	2	2	2
Direct Normalization and pooling	3	4	5	7
Total	**2 hr 25 min/ 2 hr 13 min**	**2 hr 58 min/ 2 hr 46 min**	**3 hr 33 min/ 3 hr 12 min**	**4 hr 36 min/ 4 hr 24 min**

* Timing estimates include thermocycling, incubations and Echo times. Timing does not include Plate reader and Miseq quant times.
* Timing do not include reagent thawing.

Table 1. Estimated run times for Illumina Nextera XT DNA Library Prep Kit miniaturized on the Biomek i5 MC & Echo 525 liquid handlers. Timing estimates include thermocycling, incubations and Echo 525 liquid handler times, but do not include sample preparation or reagent thawing. Echo 525 liquid handler times are estimates based on 1 sec/well.

The software provides several user-friendly features, such as:

1. Biomek Method Launcher (BML)

BML is a secure interface for method implementation that prevents compromising method integrity. It allows users to remotely monitor the progress of the run. It also features DeckOptix Final Check software to reduce deck setup errors, and thus prevent failed experiments due to missing or misplaced labware and incorrect tip or plate type. The manual control options provide the opportunity to interact with the instrument, if needed.

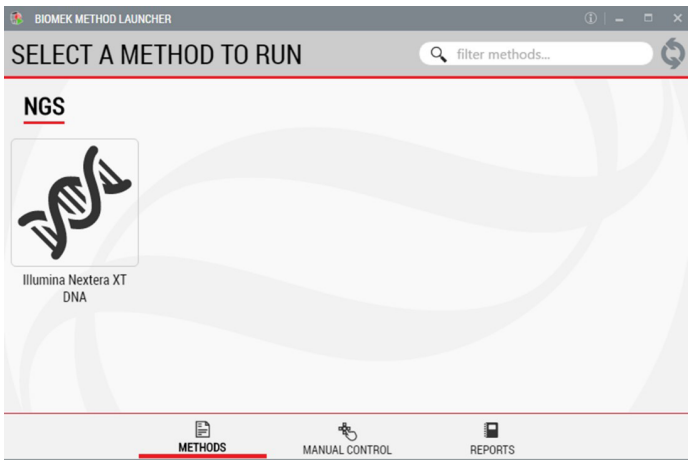


Figure 3. Biomek Method Launcher interface.

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.

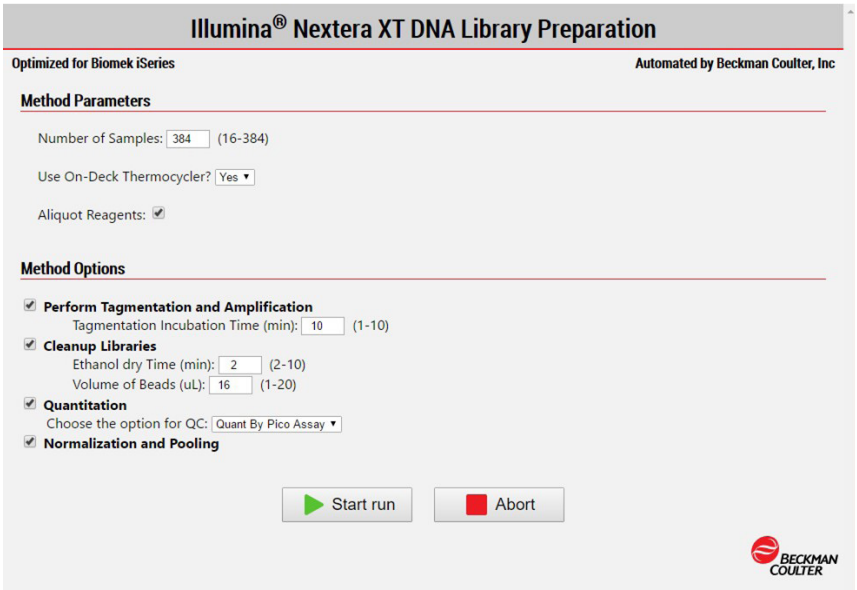
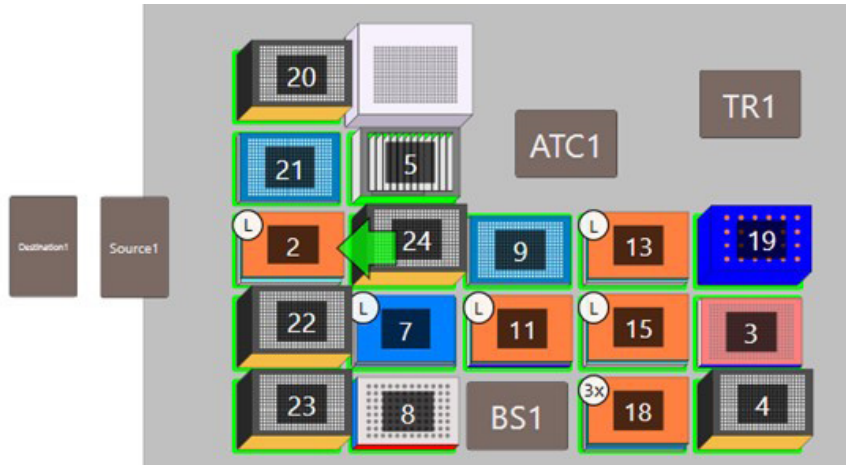


Figure 4. Biomek Method Options Selector enables users to select sample number and on- or off-deck Thermocycler option, choice for aliquoting reagents and all the processing options for the miniaturized workflow.

3. Guided Labware Setup (GLS)

GLS provides the user with specific graphical setup, as well as reagent volume calculation and step-by-step instructions to prepare master mixes. The specific setup of the deck changes to accommodate the specific method parameters chosen in the MOS.



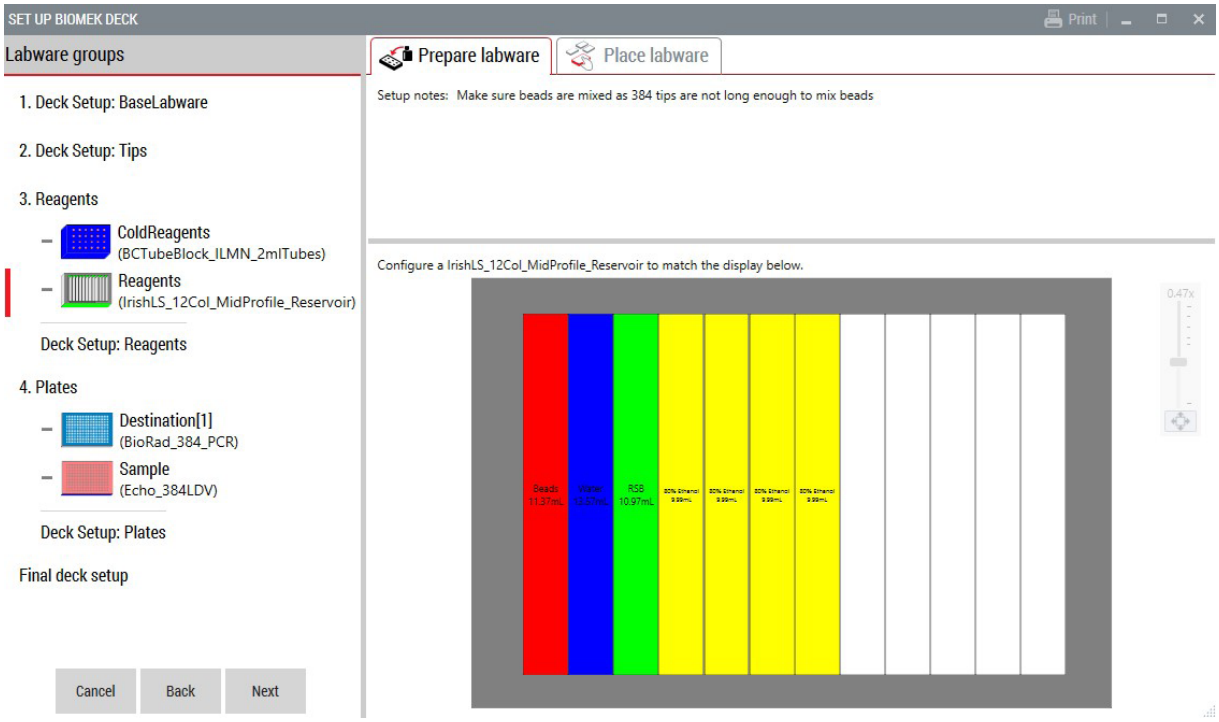


Figure 5. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup.

Experimental Design

To demonstrate capabilities to perform a 384-sample library preparation on this integrated system we prepared libraries from 384 samples of 3 different ATCC strains (*Bacillus Cereus* ATCC strains 14579 D-5, *Escherichia Coli* 700926 D-5, *Rhodobacter Sphaeroides* 17023 D-5 respectively). The 3 strains were staggered across the 384-plate, (Fig 6) and 50 pg of input DNA was used per well to generate libraries with the Nextera XT DNA kit miniaturized to ~20X.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	BC1	E coli 1	R Sp 1	BC17	E coli 17	R Sp 17	BC33	E coli33	R Sp33	BC49	E coli49	R Sp49	BC65	E coli65	R Sp65	BC81	E coli81	R Sp81	BC97	E coli97	R Sp97	BC113	E coli113	R Sp113
B	BC2	E coli 2	R Sp 2	BC18	E coli 18	R Sp 18	BC34	E coli34	R Sp34	BC50	E coli50	R Sp50	BC66	E coli66	R Sp66	BC82	E coli82	R Sp82	BC98	E coli98	R Sp98	BC114	E coli114	R Sp114
C	BC3	E coli 3	R Sp 3	BC19	E coli 19	R Sp 19	BC35	E coli35	R Sp35	BC51	E coli51	R Sp51	BC67	E coli67	R Sp67	BC83	E coli83	R Sp83	BC99	E coli99	R Sp99	BC115	E coli115	R Sp115
D	BC4	E coli 4	R Sp 4	BC20	E coli 20	R Sp 20	BC36	E coli36	R Sp36	BC52	E coli52	R Sp52	BC68	E coli68	R Sp68	BC84	E coli84	R Sp84	BC100	E coli100	R Sp100	BC116	E coli116	R Sp116
E	BC5	E coli 5	R Sp 5	BC21	E coli 21	R Sp 21	BC37	E coli37	R Sp37	BC53	E coli53	R Sp53	BC69	E coli69	R Sp69	BC85	E coli85	R Sp85	BC101	E coli101	R Sp101	BC117	E coli117	R Sp117
F	BC6	E coli 6	R Sp 6	BC22	E coli 22	R Sp 22	BC38	E coli38	R Sp38	BC54	E coli54	R Sp54	BC70	E coli70	R Sp70	BC86	E coli86	R Sp86	BC102	E coli102	R Sp102	BC118	E coli118	R Sp118
G	BC7	E coli 7	R Sp 7	BC23	E coli 23	R Sp 23	BC39	E coli39	R Sp39	BC55	E coli55	R Sp55	BC71	E coli71	R Sp71	BC87	E coli87	R Sp87	BC103	E coli103	R Sp103	BC119	E coli119	R Sp119
H	BC8	E coli 8	R Sp 8	BC24	E coli 24	R Sp 24	BC40	E coli40	R Sp40	BC56	E coli56	R Sp56	BC72	E coli72	R Sp72	BC88	E coli88	R Sp88	BC104	E coli104	R Sp104	BC120	E coli120	R Sp120
I	BC9	E coli 9	R Sp 9	BC25	E coli 25	R Sp 25	BC41	E coli41	R Sp41	BC57	E coli57	R Sp57	BC73	E coli73	R Sp73	BC89	E coli89	R Sp89	BC105	E coli105	R Sp105	BC121	E coli121	R Sp121
J	BC10	E coli 10	R Sp 10	BC26	E coli 26	R Sp 26	BC42	E coli42	R Sp42	BC58	E coli58	R Sp58	BC74	E coli74	R Sp74	BC90	E coli90	R Sp90	BC106	E coli106	R Sp106	BC122	E coli122	R Sp122
K	BC11	E coli 11	R Sp 11	BC27	E coli 27	R Sp 27	BC43	E coli43	R Sp43	BC59	E coli59	R Sp59	BC75	E coli75	R Sp75	BC91	E coli91	R Sp91	BC107	E coli107	R Sp107	BC123	E coli123	R Sp123
L	BC12	E coli 12	R Sp 12	BC28	E coli 28	R Sp 28	BC44	E coli44	R Sp44	BC60	E coli60	R Sp60	BC76	E coli76	R Sp76	BC92	E coli92	R Sp92	BC108	E coli108	R Sp108	BC124	E coli124	R Sp124
M	BC13	E coli 13	R Sp 13	BC29	E coli 29	R Sp 29	BC45	E coli45	R Sp45	BC61	E coli61	R Sp61	BC77	E coli77	R Sp77	BC93	E coli93	R Sp93	BC109	E coli109	R Sp109	BC125	E coli125	R Sp125
N	BC14	E coli 14	R Sp 14	BC30	E coli 30	R Sp 30	BC46	E coli46	R Sp46	BC62	E coli62	R Sp62	BC78	E coli78	R Sp78	BC94	E coli94	R Sp94	BC110	E coli110	R Sp110	BC126	E coli126	R Sp126
O	BC15	E coli 15	R Sp 15	BC31	E coli 31	R Sp 31	BC47	E coli47	R Sp47	BC63	E coli63	R Sp63	BC79	E coli79	R Sp79	BC95	E coli95	R Sp95	BC111	E coli111	R Sp111	BC127	E coli127	R Sp127
P	BC16	E coli 16	R Sp 16	BC32	E coli 32	R Sp 32	BC48	E coli48	R Sp48	BC64	E coli64	R Sp64	BC80	E coli80	R Sp80	BC96	E coli96	R Sp96	BC112	E coli112	R Sp112	BC128	E coli128	R Sp128

Figure 6. Schematic representation of the sample plate showing distribution of the 384 samples.

Individual sample cleanup was done for 384 samples in parallel, with final elution volume of 14 µL. The quality of the libraries was assessed using with Agilent TapeStation 2200 and High Sensitivity D5000 tape and samples were quantified with 1X hsDs DNA Qubit™. The libraries were quantified via Quant-IT Pico assay. Normalization and pooling were done by Echo 525 liquid handler. The transfer volume of each library was calculated such that the final amount of DNA was 750 pg. Pooled libraries were then quantified using 1X hsDs DNA Qubit™. The libraries were diluted to be at 4 nM or 0.6 ng/µL concentration, denatured and loaded at 10 pM on the MiSeq (Illumina) using the MiSeq Reagent Kit v2 300-cycle from Illumina. For the alignment analysis, reads from each sample were processed through the BWA alignment tool.

Results

15 library preps randomly selected across the plate were run on the Agilent TapeStation High Sensitivity D5000 tape. Traces indicate library prep had fragments between 500 – 1500 bps (Figure 7). For the Quant-IT Pico assay, standard curve generated had R^2 value of 0.999 (Figure 8A). Figure 8B is the chart that shows the concentration for each library in ng/µL. 3 libraries (F10, N7 and J24) were less than 0.2 ng/µL. The remaining 381 samples were then carried forward for sequencing. The %Q30 for the run was 92.93% (Figure 9A) and the % PF shows equal index representation across the samples at expected % (Figure 9B). Overall the Quality read scores per cycle depict that the quality scores are similar to conventional 2x150 genomic runs. The % alignment for *B. Cereus* (Figure 10 A and B) and *E. coli* (Figure 11 A and B) was 99%, and for *R. Sphaeroides* was 93.5% (Figure 12 A and B).

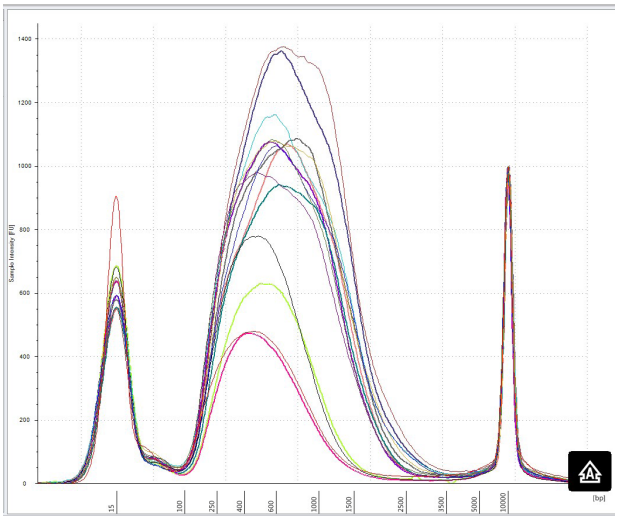


Figure 7. DNA strains analyzed on TapeStation 2200 with High Sensitivity DNA 5000 tape, yield was around 20 ng.

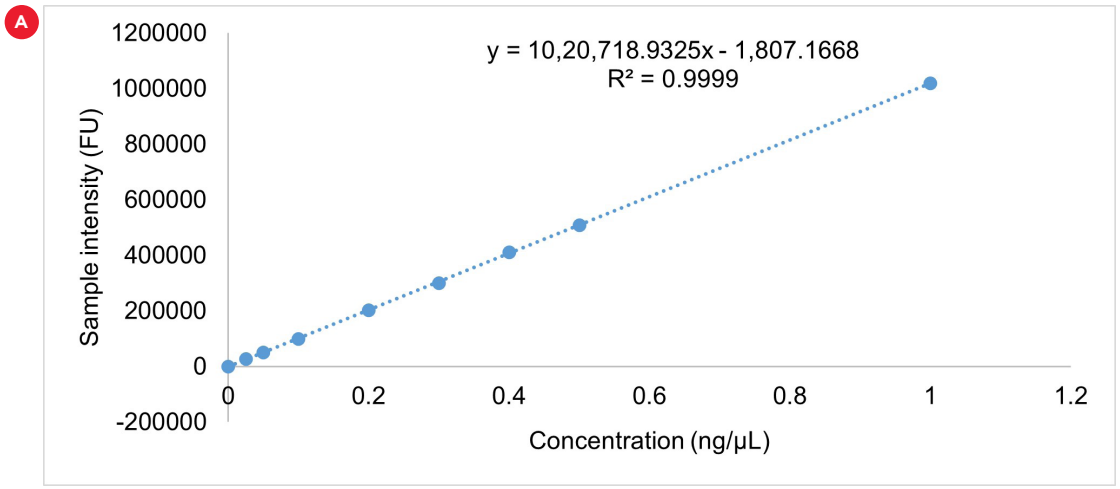


Figure 8 (A). Standard curve with equation displayed for the standards for the Quant-IT Pico Assay.

B

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	B Cer	Ecoli	R Sph	B Cer	Ecoli	R Sph	B Cer	Ecoli	R Sph	B Cer	Ecoli	R Sph	B Cer	Ecoli	R Sph	B Cer	Ecoli	R Sph	B Cer	Ecoli	R Sph	B Cer	Ecoli	R Sp
A	0.85	1.85	2.05	0.52	2.29	1.71	0.88	1.53	1.89	0.26	1.87	2.07	0.46	1.94	2.12	0.58	2.12	2.02	0.67	1.72	2.26	0.86	1.96	2.20
B	0.92	2.20	2.13	0.63	2.10	2.13	0.63	2.21	0.98	1.05	1.55	1.77	0.73	0.96	1.95	0.80	2.01	2.44	0.30	1.71	2.17	0.79	2.49	2.32
C	1.05	2.47	2.30	1.47	2.39	2.74	0.99	0.81	2.28	1.22	2.19	2.15	0.79	1.92	2.40	0.64	1.63	0.88	0.27	1.80	2.31	1.10	2.45	1.78
D	1.41	1.22	2.53	0.92	2.50	2.56	0.32	0.58	2.20	1.17	2.13	0.66	1.03	1.79	2.33	0.73	1.82	1.62	1.24	1.58	2.55	0.95	2.43	2.46
E	0.21	1.84	2.85	1.11	1.92	2.47	1.00	1.78	2.71	1.18	1.76	1.75	0.86	1.99	2.24	0.80	2.35	2.60	0.80	1.58	2.39	0.92	2.62	3.21
F	1.39	1.74	3.68	1.27	2.10	2.47	1.11	2.35	2.76	0.15	2.29	2.61	1.20	2.26	2.70	0.98	2.19	1.99	0.84	1.70	2.73	1.14	2.01	2.32
G	1.07	1.76	1.45	0.43	2.25	1.95	1.15	2.17	0.59	0.92	2.90	2.78	0.93	0.94	2.37	1.23	2.25	2.30	0.92	2.14	2.03	0.95	2.26	1.70
H	1.07	1.92	2.33	0.87	3.33	3.11	0.87	0.69	2.67	1.23	2.45	2.41	1.16	2.22	2.16	1.01	2.14	2.17	0.93	2.16	2.40	1.00	1.85	2.72
I	0.71	1.97	2.93	1.14	2.41	2.73	1.02	2.21	2.63	0.96	1.89	2.39	1.10	2.40	2.04	1.04	2.39	2.23	0.98	1.78	2.27	1.12	1.68	2.41
J	0.95	2.74	2.45	1.49	3.60	3.10	1.13	2.51	2.87	1.06	2.09	2.72	0.98	2.29	2.34	1.09	2.35	2.59	0.88	2.03	2.02	1.39	2.49	0.17
K	1.34	2.77	2.44	1.35	1.29	2.44	1.29	1.96	2.72	0.32	2.91	2.53	1.14	2.76	2.04	1.97	1.79	2.11	1.00	1.85	1.93	1.51	2.19	2.95
L	0.98	2.05	3.14	0.37	2.59	2.01	1.38	2.32	2.33	0.98	2.18	2.71	0.89	1.99	2.59	0.83	2.09	2.19	0.72	3.25	2.26	1.23	1.88	2.05
M	1.17	3.07	2.32	1.23	2.53	2.05	1.19	2.54	2.30	1.16	2.65	2.66	1.03	2.13	2.70	1.14	2.42	1.71	0.75	1.75	2.14	1.14	1.80	1.97
N	1.05	2.54	1.93	1.51	1.97	2.52	0.18	2.17	2.58	0.88	2.09	1.92	1.18	2.54	2.62	0.84	1.97	1.46	0.69	1.94	2.20	0.94	2.24	2.74
O	1.01	2.28	1.77	0.76	2.75	1.35	0.70	2.54	3.14	1.05	2.64	2.59	0.77	2.27	3.01	1.05	2.20	2.39	0.82	2.08	2.06	0.78	2.19	1.69
P	1.18	2.29	2.79	1.01	1.84	2.88	1.04	1.63	3.00	0.24	1.85	1.74	1.25	2.51	2.97	0.73	1.74	1.61	1.04	1.74	2.39	0.82	3.21	2.86

Figure 8 (B). Chart of the concentrations in ng/µL for each library.

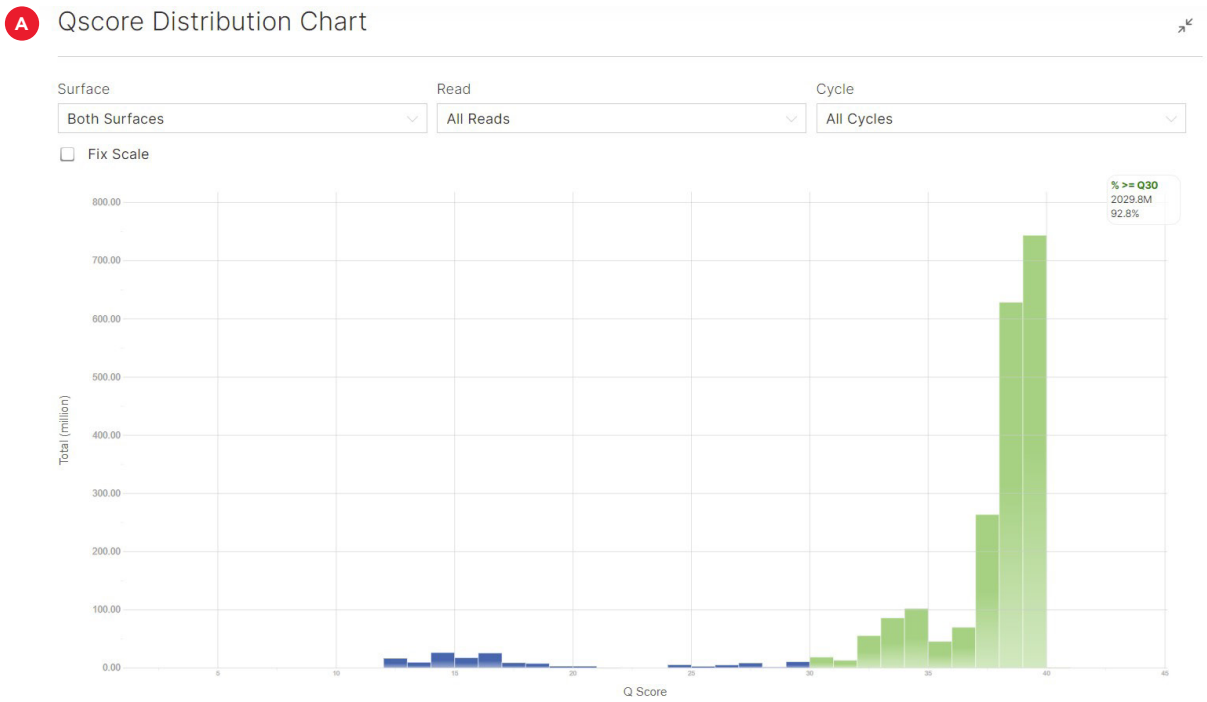


Figure 9 (A). Q Score distribution for the MiSeq Run for Nextera XT DNA.

B

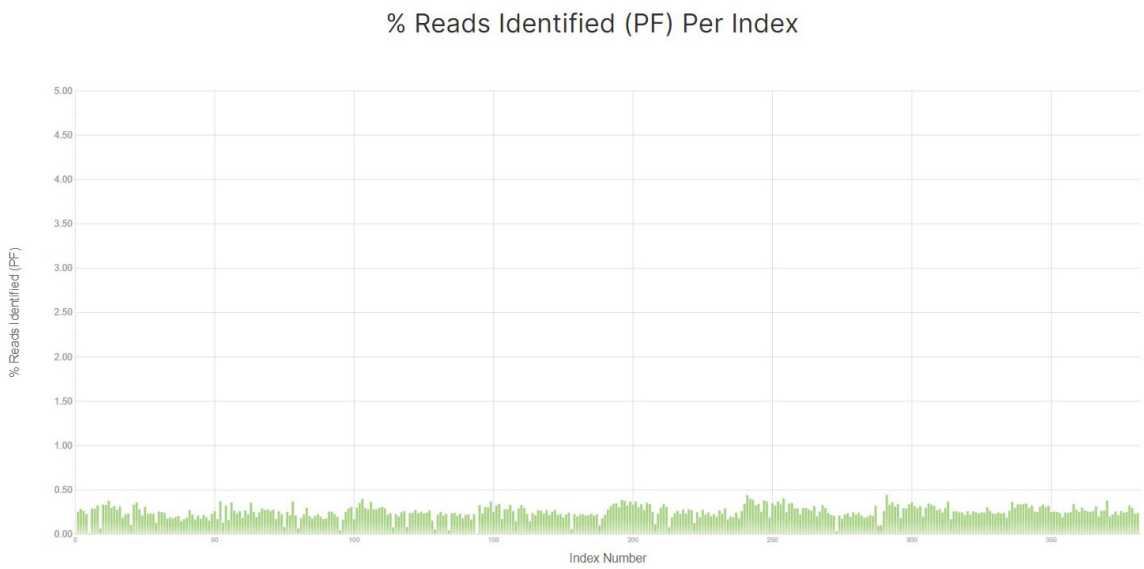
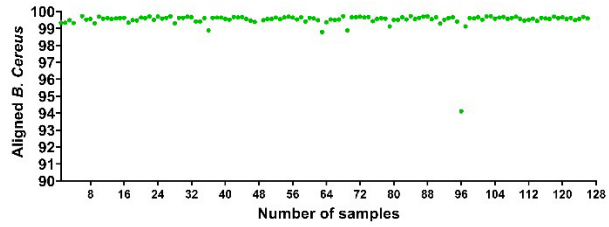


Figure 9 (B). % reads identified for each index for Nextera XT.

A



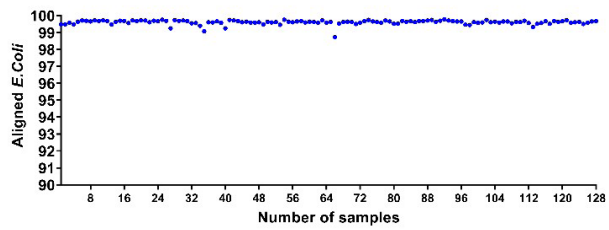
B

	Reads	Percentage
Total PF	46,548	100.00%
Paired	46,442	99.77%
Read 1	23,221	49.89%
Read 2	23,221	49.89%
Aligned	46,402	99.69%
Properly Paired	46,088	99.32%
Singletons	10	0.02%
Secondary Alignments	106	0.23%
Supplementary Alignments	0	0.00%
Duplicates	450	0.97%

Please note that "Paired", "Read 1", "Read 2" and "Aligned" percentages are calculated based on the "Total PF" value. All other percentages are calculated based on

Figure 10. (A) % aligned for 128 samples of *Bacillus Cereus*. **(B)** Alignment statistics for a representative sample of *Bacillus Cereus*.

A

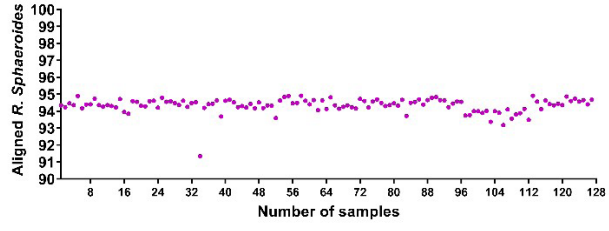


B

	Reads	Percentage
Total PF	51,065	100.00%
Paired	50,840	99.56%
Read 1	25,420	49.78%
Read 2	25,420	49.78%
Aligned	50,887	99.65%
Properly Paired	50,276	98.80%
Singletons	42	0.08%
Secondary Alignments	225	0.44%
Supplementary Alignments	0	0.00%
Duplicates	612	1.20%

Figure 11. (A) % aligned for 128 samples of *Escherichia Coli*. **(B)** Alignment statistics for a representative sample of *Escherichia Coli*.

A



B

	Reads	Percentage
Total PF	34,709	100.00%
Paired	34,432	99.20%
Read 1	17,216	49.60%
Read 2	17,216	49.60%
Aligned	32,590	93.89%
Properly Paired	31,808	97.60%
Singletons	359	1.10%
Secondary Alignments	277	0.85%
Supplementary Alignments	0	0.00%
Duplicates	307	0.94%

Figure 12. (A) % aligned for 128 samples of *Rhodobacter Sphaeroides*. **(B)** Alignment statistics for a representative sample of *Rhodobacter Sphaeroides*.

Summary

We have demonstrated that the automated workflow for the Nextera XT Library Prep kit from aliquoting reagents to final normalization pooling can be done in 4.5 hours for 384 samples on the Biomeki5-Echo 525 integrated system, including 35 minutes of deck set up time. This is the same amount of time taken on a Biomek i7 hybrid workstation for 96 samples without pooling. The integrated system provides the ability for on-deck incubations, sample clean ups and increase walk away time as compared to an Echo 525 system alone. Individual sample cleanups provide an advantage for more accurate quantification of individual library prep which helps in pooling by mass thus resulting in uniform index representation during sequencing run. Overall, combining the capabilities of Echo 525 liquid handler with Biomek i5 Workstation allows for 20X reduced reaction volume of Nextera XT reagents resulting in a 10X cost savings on reagents and a 2X cost savings on consumables as compared to Biomek i7 hybrid system while obtaining effective sequencing data.

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