Illumina DNA PCR-Free Prep App on Biomek NGeniuS System

App Template Version 1.0.0

BECKMAN COULTER
Life Sciences

2024-GBL-EN-104856-v1

This method has been demonstrated for use on the Biomek NGeniuS System but has not been validated by Beckman Coulter for use in the diagnosis of disease or other clinical conditions. All products are for Research Use Only. Not for use in Diagnostic Procedures.

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App Template Description

The Illumina* DNA PCR-Free Prep App Template enables the generation of libraries compatible with Illumina sequencing platforms. The App Template allows the user to produce between 4 to 24 libraries in a single batch run. The user may utilize genomic DNA (gDNA) as starting material. Blood and saliva inputs are not supported by the App Template. This app supports the Standard Input protocol with gDNA inputs in the range of 100-2000 ng. Inputs within the range of 25-99 ng are not supported by this Standard Input app. The user has the option to specify the concentration of the starting material to enable normalization as well as the bead drying time following ethanol washes. 80% ethanol wash volumes have been reduced from 180 µL to 100 µL. Automated pooling at the end of the protocol is not supported.

The App template was designed using the Illumina DNA-PCR Free Reference guide (Document # 1000000086922 v04). The App Template utilizes the Illumina DNA PCR-Free Library Prep (24 Samples) kit (Illumina Part Number 20041794) in conjunction with the IDT® for Illumina® DNA/RNA UD Indexes Sets A, B, C, or D (Illumina Part Numbers 20027213, 20027214, 20042666, or 20042667) or the Illumina DNA/RNA UD Indexes Sets A, B, C or D (Illumina Part Numbers 20091654, 20091656, 20091658, 20091660).

*Illumina is a trademark of Illumina, Inc. IDT is a trademark of Integrated DNA Technologies.

2023-GBL-EN-101890-v1

Scoping

Scoping

- Author
  - Illumina scientists with support from Beckman Coulter Life Sciences
- Kit
  - Illumina DNA PCR-Free Prep
    - Version 1000000086922 v04, compatible with v03*
- Supported
    - “Standard Input” protocol, DNA Input 100 - 2000 ng
    - Genomic DNA
- Excluded
    - “Low Input” protocol, DNA Input 25-99 ng
    - Blood protocol
    - Saliva protocol

*Differences between the IFU versions were communicated to Beckman Coulter at the time of Demonstration. v04 documentation not yet released by Illumina at time of NGeniuS App release.

Scoping

- Part numbers
  - 20041794 – 24 Samples
  - 20041795 – 96 Samples, *not supported*
  - 20027213 – IDT for Illumina DNA/RNA UD Indexes Set A
  - 20027214 – IDT for Illumina DNA/RNA UD Indexes Set B
  - 20042666 – IDT for Illumina DNA/RNA UD Indexes Set C
  - 20042667 – IDT for Illumina DNA/RNA UD Indexes Set D
  - 20091654 – Illumina DNA/RNA UD Indexes Set A
  - 20091656 – Illumina DNA/RNA UD Indexes Set B
  - 20091658 – Illumina DNA/RNA UD Indexes Set C
  - 20091660 – Illumina DNA/RNA UD Indexes Set D

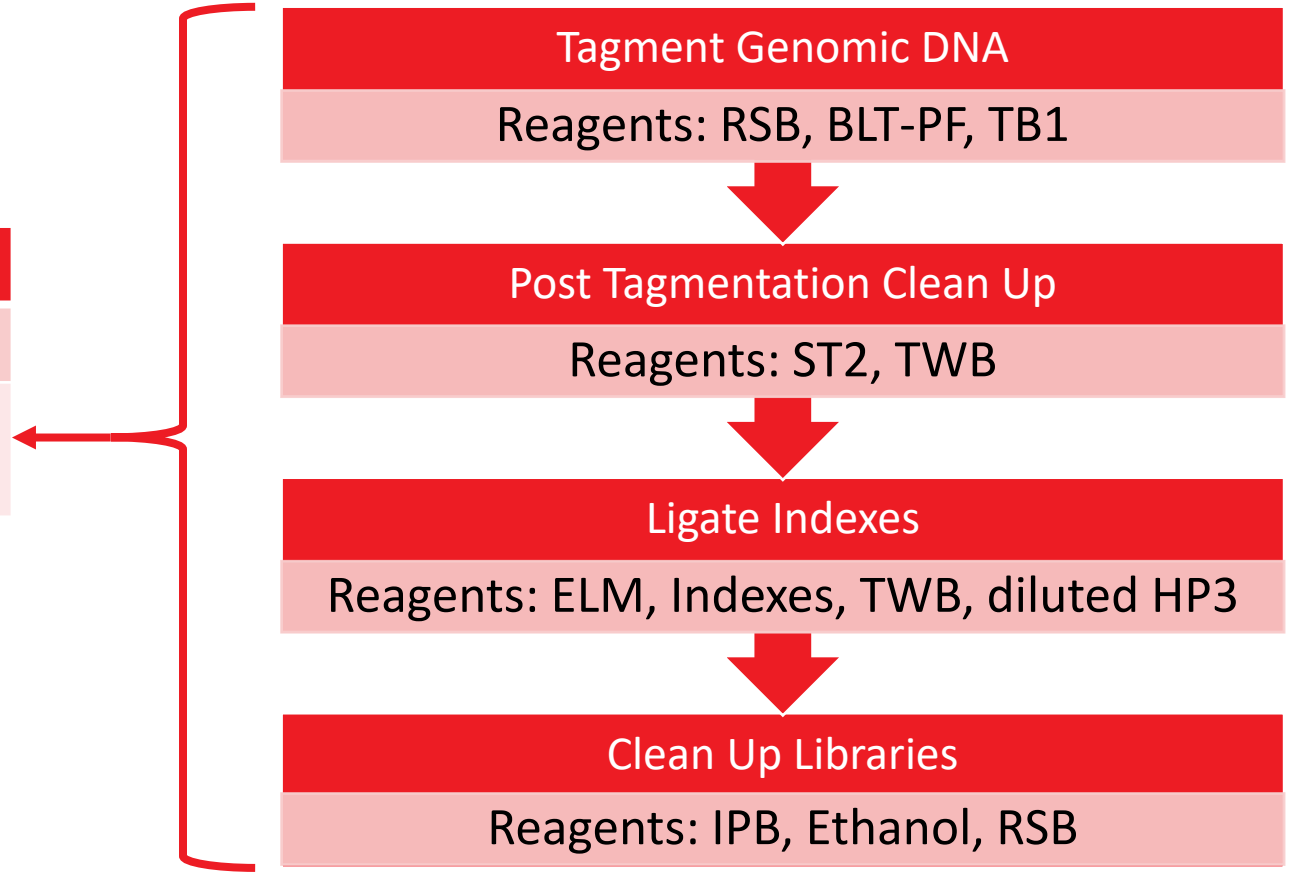
EOL March 30, 2025

Illumina Equivalency document PON2023-1440

App Details

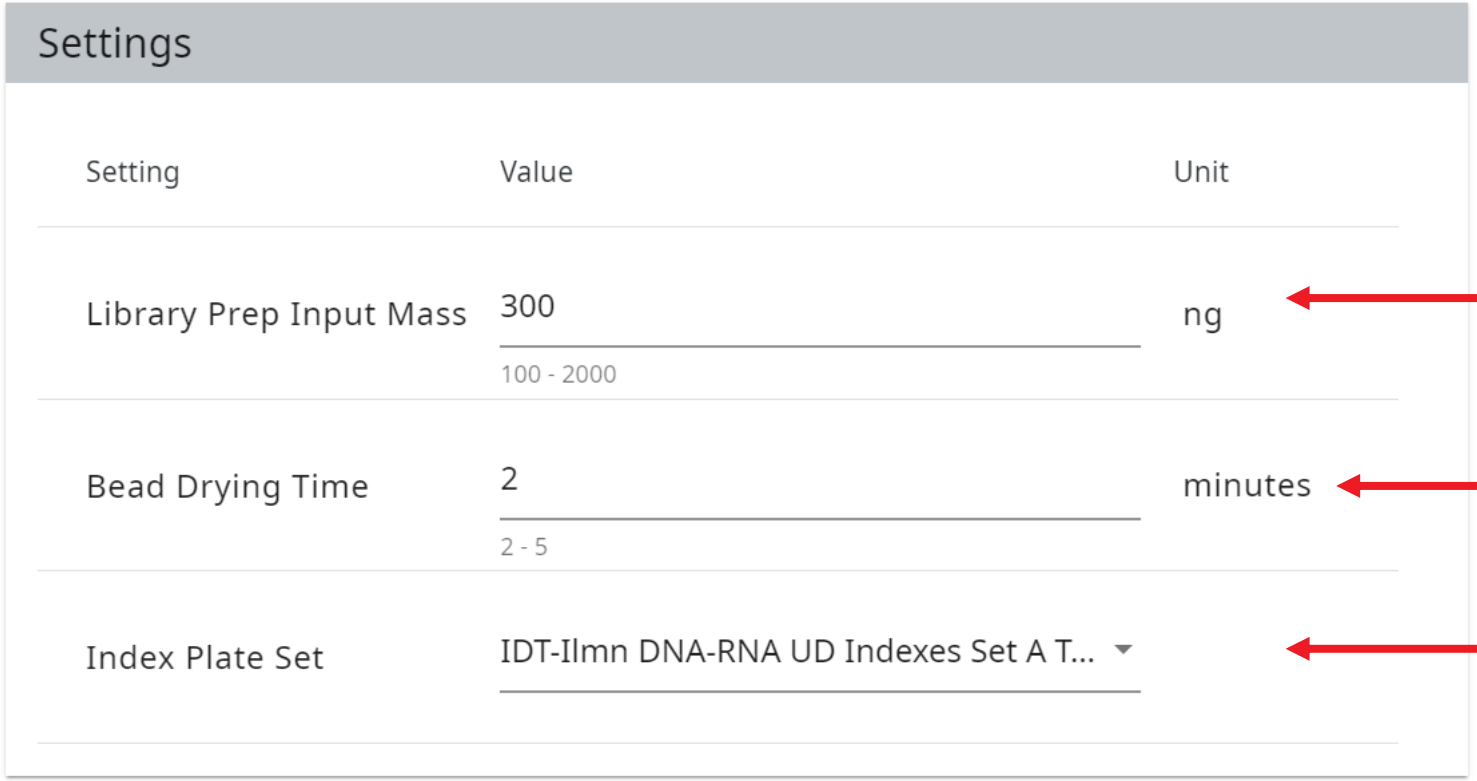
Sections Automated

App Sections
Normalize Samples
Tagment Genomic DNA, Post Tagmentation Clean-Up, Ligate Indexes, Clean Up Libraries



Pooling and sequencing done off-instrument

App Settings



Default input mass for Standard Input workflow

Adjust drying time according to laboratory conditions

IDT for Illumina or Illumina index plates supported. Naming lines up with BaseSpace naming conventions.

Kit Volumes

Reagent	Stated vial volume *	4 samples volume requested	8 samples volume requested	16 samples volume requested	24 samples volume requested
BLT-PF	440	103	166	292	418
TB1	290	72	114	198	282
ST2	1400	84	128	216	304
HP3**	400	229	418	796	1174
ELM	1600	229	418	796	1174
IPB***	10000	617.6	985.2	1640.4	2295.6
RSB****	20000	2619.7	2739.4	2978.8	3218.3
TWB*****	41000	3400	4300	6100	7900

* All values in µL, consumed volumes are less than requested due to source labware dead volume requirements
** HP3 comes in a vial and must be diluted 1:10 into a 2.0 mL Sarstedt vial
*** IPB comes in a 15 mL conical and must be reformatted into a 5 mL Sarstedt vial
**** RSB comes in a 50 mL conical and is stored in a Bulk Reservoir. Volume shown does not include reagent needed for normalization.
***** TWB comes in a 50 mL conical and is stored in a Bulk Reservoir

Batch Runs Per Kit

Batch size	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Batches per kit	5	4	3	3	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
Samples	20	20	18	21	16	18	20	22	12	13	14	15	16	17	18	19	20	21	22	23	24
Largest batch with leftover volume	-	-	-	-	6	4	-	-	11	10	9	8	7	6	5	4	-	-	-	-	-
Total samples from kit	20	20	18	21	22	22	20	22	23	23	23	23	23	23	23	23	20	21	22	23	24

- The ***Batch size*** can be run ***Batches per kit*** times, leaving enough reagent volume to do one additional batch with ***Largest batch with leftover volume*** samples.
- Run combinations calculated based on **published** reagent vial volumes.
- BLT-PF and TB1 are limiting reagents.

Estimated Time of Completion

Samples	4	8	16	24
Index Aliquot	00:02	00:02	00:04	00:06
Reagent Aliquot	00:22	00:29	00:35	00:39
Processing	02:29	02:45	04:27	06:10
Total ETC	02:54	03:17	05:07	06:56

Times (hours:minutes) calculated based on default 2 minute bead dry time, with all plate-based indices contiguous starting with well A1. Does not include times needed for manual interactions *(e.g.*, reagent thawing, placing labware into Biomek NGeniuS System, …). Illumina Reference Guide suggests 01:30 total manual preparation time for a single sample.

Consumables

		Batch Size (samples)			
Consumable	**Part number**	**4**	**8**	**16**	**24**
RVs	C62705	7	7	7	7
Bulk Reservoirs	C62707	2	2	2	2
Lids	C62706	5	5	5	5
Millitips (boxes)	C59585	38 (1)	76 (1)	138 (2)	200 (3)
Microtips (boxes)	C62712	128 (1)	244 (1)	472 (2)	704 (2)
5.0 mL Sarstedt vial	60.611	1	1	1	1
2.0 mL Sarstedt vial	72.664	1	1	1	1
Seal plate	C70665	1	1	1	1
Price Per Sample ($)*	-	39.09	19.55	14.74	12.03

* Costs assume using fresh tip boxes. Some clean tips will remain each run, reducing cost of subsequent runs. Costs do not include Sarstedt reformat vials or empty tip boxes for tip disposal.

Demonstration Data (App Template Version 1.0.0)

Experimental Design for Demonstration Run Conditions

Experiment	Sample number	Target Sample Mass (ng)	Bead Dry Time (min)	Instrument	Operator
1	9*	100	2	B	A
2	24**	300	2	A	B
3	4*	2000	2	A	A

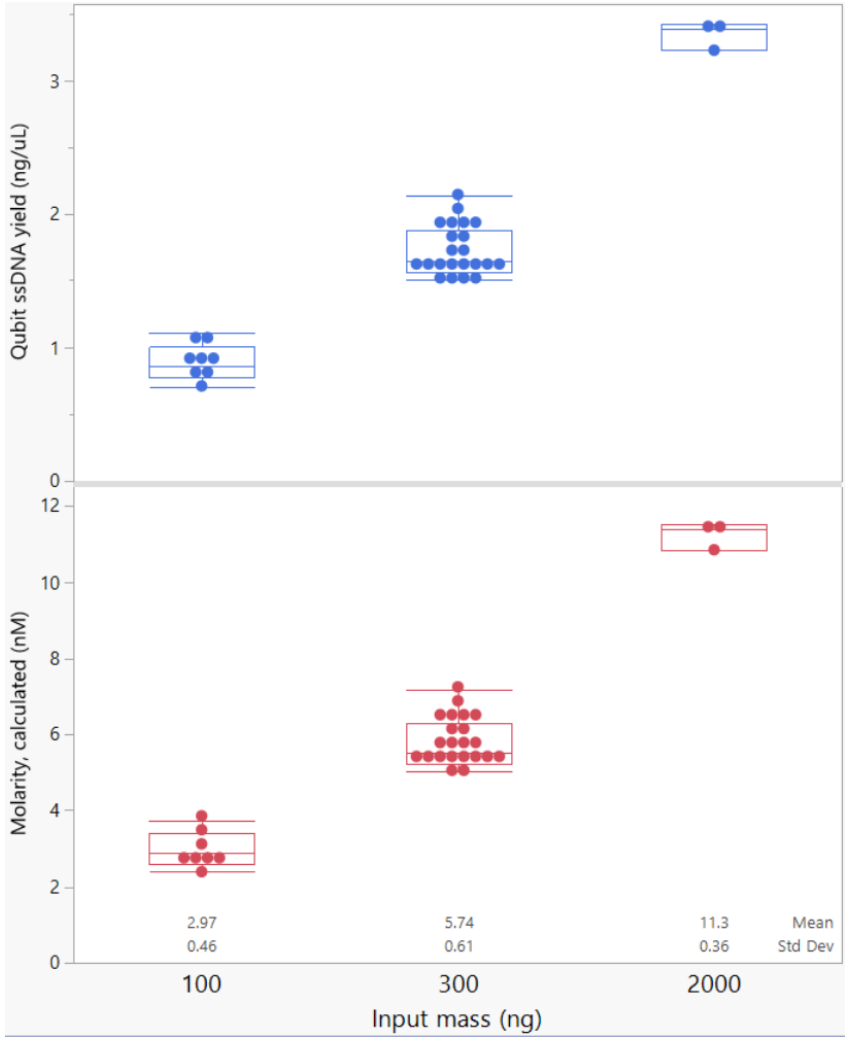
* One negative control (H_2O) per experiment

** Two negative controls (H_2O) per experiment

Library Construction Pass Criteria:

- More than 80% of samples giving a yield of 0.75 nM or greater as measured by qPCR or Qubit ssDNA quantification, using 450 bp as the average library size and 660 g/mol as the DNA mass (100-299 ng input).
- More than 80% of samples giving a yield of 2.8 nM or greater as measured by qPCR or Qubit ssDNA quantification, using 450 bp as the average library size and 660 g/mol as the DNA mass (300+ ng input).

Yield Summary



Experiment	Input mass (ng)	Sample count	Negative controls	Average yield (ng/µL)	Average yield, calculated (nM)
1	100	9	1	0.884	2.97
2	300	24	2	1.71	5.74
3	2000	4	1	3.35	11.3

All experiments used IDT for Illumina DNA/RNA UD Indexes Set A.
Yield, ng/µL, assessed by Qubit ssDNA Assay on Qubit 4 Fluorometer. All negative controls measured as too low / Out of range. All yields above minimum Library Construction metrics.
Molarity calculated as 3.36 X concentration, per Illumina IFU based on 450 bp library.

Sequencing

Pooling (off instrument)

Library Prep Input Mass	100 ng	300 ng	2000 ng
Strategy	By mass	By volume	By mass
Samples	8	22	3
Pool volume (µL)	160	320	180
Pool molarity (nM)	2	2	2

- By mass
  - Calculated volume required for each library needed to make 2 nM in desired pool volume. Pooled those volumes and diluted to desired pool volume using Resuspension Buffer (RSB).
- By volume
  - 9 µL of each library pooled. Concentration measured using Qubit ssDNA Assay Kit. Pool diluted with RSB to achieve 2 nM final pooled concentration.

Sequencing Details

- Pooled libraries sent to Illumina Solutions Center, Baltimore
- Pools denatured and diluted to 400 pM following NovaSeq 6000 Standard Protocol with 1% PhiX spike-in
- 100 and 2000 ng pooled inputs run on S2 flowcell
- 300 ng pooled input run on S4 flowcell
- 151 | 10 | 10 | 151 cycles
- Data mapped against hg38 reference human genome in DRAGEN Germline App v4.2.4

NovaSeq Data

Metric	100 ng input (n = 8)	300 ng input (n = 22)	2000 ng input (n = 3)
Flow cell	S2	S4	S2
Instrument Model	NovaSeq6000	NovaSeq6000Dx	NovaSeq6000
%Q30 AVG	89.03	89.19	86.78
Pass Filter (%)	72.07	77.00	66.50*
Lane QC Status	QcPassed	QcPassed	QcPassed
Flow Cell Status	QcPassed	QcPassed	QcPassed
Yield (Tbp)	1.32	3.75	1.22
Indexing QC Reads Identified, PF (%)	94.2869	92.1646	92.0427
Indexing QC CV (%)	21.28	21.83	19.00

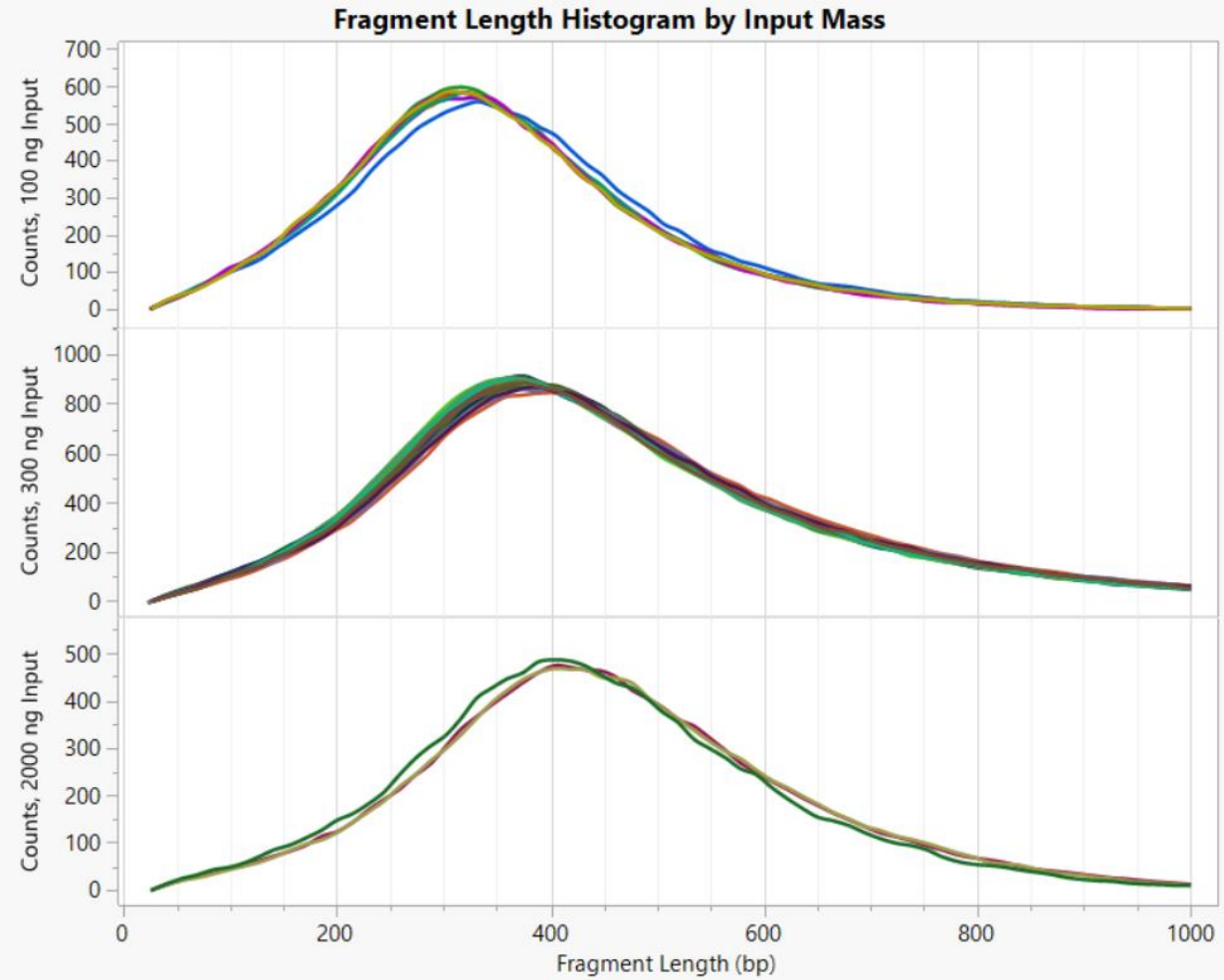
* %PF below 70 due to sample overloading, but per-sample sequencing yield was sufficient to meet required autosomal coverage for DRAGEN Germline analysis

DRAGEN Germline Analysis

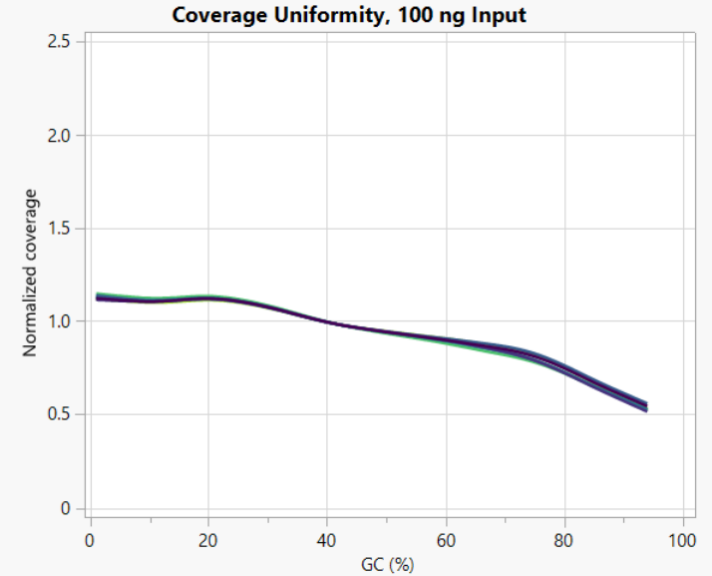
	Measured [StdDev]		
	100 ng Input Mass (mean, n = 8)	**300 ng Input Mass (mean, n = 22)**	**2000 ng Input Mass (mean, n = 3)**
Mean Insert Size (bp)	348 [5]	453 [7]	453 [9]
% Mapped reads (downsampled 30x)	97.17 [0.08]	97.12 [0.10]	96.88 [0.11]
% Duplicate Marked Reads	7.73 [0.37]	7.22 [0.44]	9.08 [1.08]
% Q30 bases read 1	90.86 [0.22]	92.28 [0.15]	88.64 [0.74]
% Q30 bases read 2	87.99 [0.74]	87.44 [0.56]	85.69 [1.03]
Normalized coverage at GC regions 20-39%	1.056 [0.005]	1.014 [0.006]	1.037 [0.006]
Normalized coverage at GC regions 60-79%	0.876 [0.011]	0.991 [0.018]	0.900 [0.000]
Average Autosomal Coverage (x)	41.84 [8.20]	42.99 [9.04]	100.00 [14.58]
Percent Autosome Callability	97.50 [0.10]	97.72 [0.12]	97.97 [0.07]
Percent Autosome Exome Callability	98.92 [0.05]	99.04 [0.05]	99.13 [0.02]

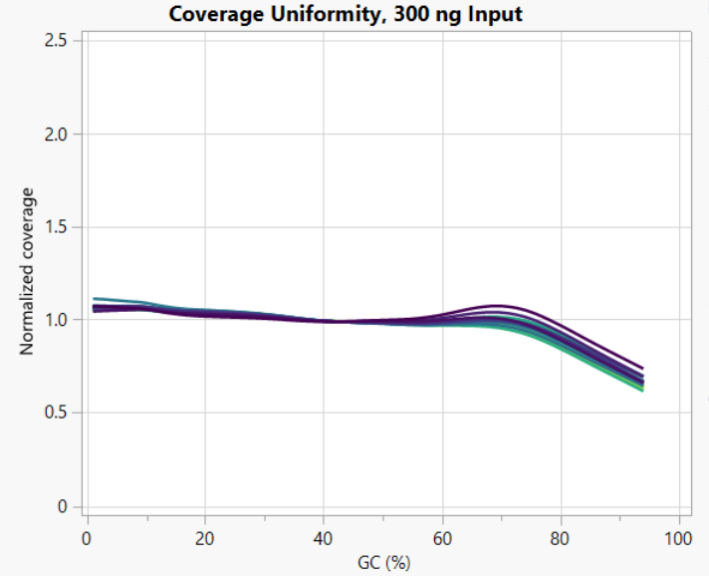
DRAGEN Germline Analysis: Fragment Length

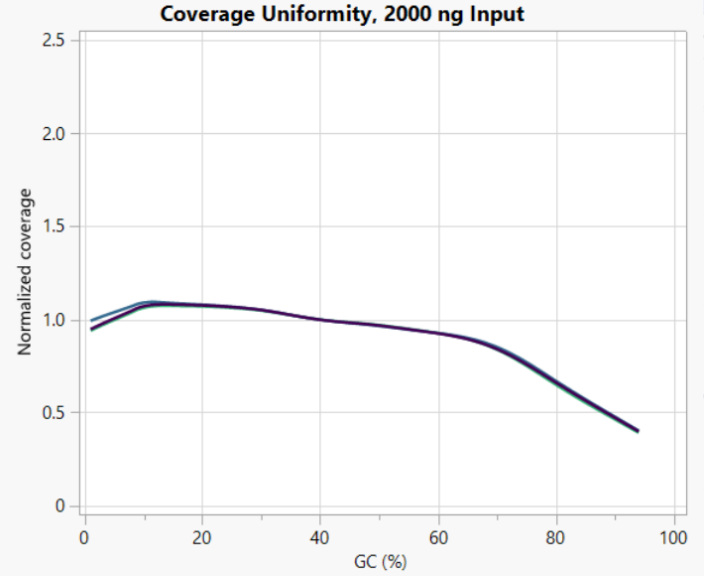
Bias toward smaller peak fragment size at lower input masses. Similar phenomenon observed by Illumina in internal 200 ng input mass data.



DRAGEN Germline Analysis: Coverage Uniformity







	100 ng Input (mean, n=8)	300 ng Input (mean, n=22)	2000 ng Input (mean, n=3)
Normalized coverage at GC regions 20-39% should fall in the range 0.97 ≤ x ≤ 1.06	1.056	1.014	1.037
Normalized coverage at GC regions 60-79% should fall in the range 0.82 ≤ x ≤ 1.13	0.876	0.991	0.900

Demonstration Summary

Demonstration Summary

- The Illumina DNA PCR-Free Prep App 1.0.0, written by Illumina, on the Biomek NGeniuS Next Generation Library Prep System prepares libraries at input masses between 100 and 2000 ng of genomic DNA
- Yield at all tested masses exceeded 2 nM final concentrations as required for Illumina NovaSeq sequencing
- NovaSeq 6000 sequencing data of prepared libraries passes internal Illumina metrics for:
  - % Mapped reads
  - % Duplicate marked reads
  - % Q30 bases reads
  - Normalized coverage at GC regions 20-39% and 60-79%
  - Average autosomal coverage
  - % Autosome callability
  - % Autosome exome callability

General automation considerations

- Please read and understand Biomek NGeniuS System IFU, C43212.
- Spin down index plate before use to make sure indices are at the bottom of wells
- Do not use unsupported index plates (see slide 6 for part numbers)
  - If the plate geometry is not the same, it could result in an instrument crash
- Make sure foil of each index well is widely opened to prevent tip-friction binding and lifting of Index Plate
  - Use a *new* P200 or P1000 to pierce and widen *each* well being used
- Do not use other sized kits as their reagents might come in an unexpected tube size
  - Chance for misread OCR token on vial
  - Chance for failed chemistry
  - Chance for damaged instrument
- Avoid bubbles in reagent tubes to ensure accurate liquid level sensing and aliquoting
  - BLT-PF, TB1, and TWB are bubbly
- The Work Aid requests more volume than what is consumed
  - Dead volume is needed in source tubes to ensure enough is available due to tolerance stack-ups
- Dead volume will be left behind in some storage wells
  - The nature of automation, tolerance stack-ups, and environment necessitates some overage
- Make sure bulk reagents wet the entire length of reservoir
  - Ensures accurate liquid volume sensing
- Start with sample volumes >= 2 μL.
  - Biomek NGeniuS System will calculate needed sample volume based on provided sample concentration
  - System *will* accept smaller input volumes, but more variation is observed