Pillar Biosciences oncoReveal™ Nexus 21 Gene Panel on the Biomek NGeniuS System

App Template Version 1.0.0

BECKMAN COULTER
Life Sciences

2024-GBL-EN-107053-v1

Beckman Coulter makes no warranties of any kind whatsoever express or implied, with respect to this protocol, including but not limited to warranties of fitness for a particular purpose or merchantability or that the protocol is non-infringing. All warranties are expressly disclaimed. Your use of the method is solely at your own risk, without recourse to Beckman Coulter. This App Template has been demonstrated for use on the Biomek NGeniuS system for the chemistry kit version and the release date shown at the time when the App is selected and created but has not been validated by Beckman Coulter for use in the diagnosis of disease or other clinical conditions.

Biomek NGeniuS Next Generation Library Preparation System is not labeled for IVD use and is not intended or validated for use in the diagnosis of disease or other conditions.

©2024 Beckman Coulter, Inc. All rights reserved. Beckman Coulter, the stylized logo, and the Beckman Coulter product and service marks mentioned herein, including Biomek and Biomek NGeniuS, are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries. All other trademarks are the property of their respective owners.

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

App Template Description

The App Template for Pillar Biosciences* oncoReveal™ Nexus 21 gene panel prepares sample DNAs for sequencing by amplifying target regions containing mutational hot spots using SLIMamp® (stem-loop inhibition mediated amplification) technology. Per PCR reaction, the oncoReveal™ Nexus 21 gene panel App Template supports a DNA input mass of 20-60 ng for both standard genomic DNA and FFPE DNA. Libraries can be sequenced on any low- to mid- throughput sequencers such as Illumina MiSeq™ or NextSeq™ Systems.

The App Template allows the user to produce between 4 and 24 libraries in a single continuous batch run. Optionally, users may select from multiple starting and stopping points. Additional stop points with timely user interaction are present to support pre/post PCR environments. The App Template was designed using the Pillar oncoReveal™ Nexus 21 gene kit (HDA-HS-1013-24). Ethanol wash volumes have been reduced to 60µL from 150µL to reduce tip consumption and sample processing time. Recommended indexing PCR cycle count has been changed to 5 to facilitate more balanced amplicon coverage.

In addition to the consumables listed in the oncoReveal™ Nexus 21 gene assay User Guide (UM-0081), the following consumables are required for a full run:

- 2.0mL Sarstedt Tubes – Skirted Base (Sarstedt P/N: 72.664)
- 5.0mL Sarstedt Tubes – False Bottom with Flat Base (Sarstedt P/N: 60.611.310)
- Axygen® 96-well Polypropylene PCR Microplate, Full Skirt, Clear, Nonsterile (Corning P/N: PCR-96-FS-C)

For research use only. Not for use in diagnostic procedures.

* Pillar®, SLIMamp® and oncoReveal™ are trademarks of Pillar Biosciences, Inc. Current as of 03.12.2024

2024-GBL-EN-106758-v1

Scoping

Scoping

- Author
  - Pillar scientists with support from Beckman Coulter Life Sciences
- Panel
  - oncoReveal™ Nexus 21 Gene Panel
    - Version 1.0 (User Guide UM-0081)
- Supported DNA Input
    - Standard genomic or FFPE DNA: 20-60 ng in 4.75 µL

Scoping

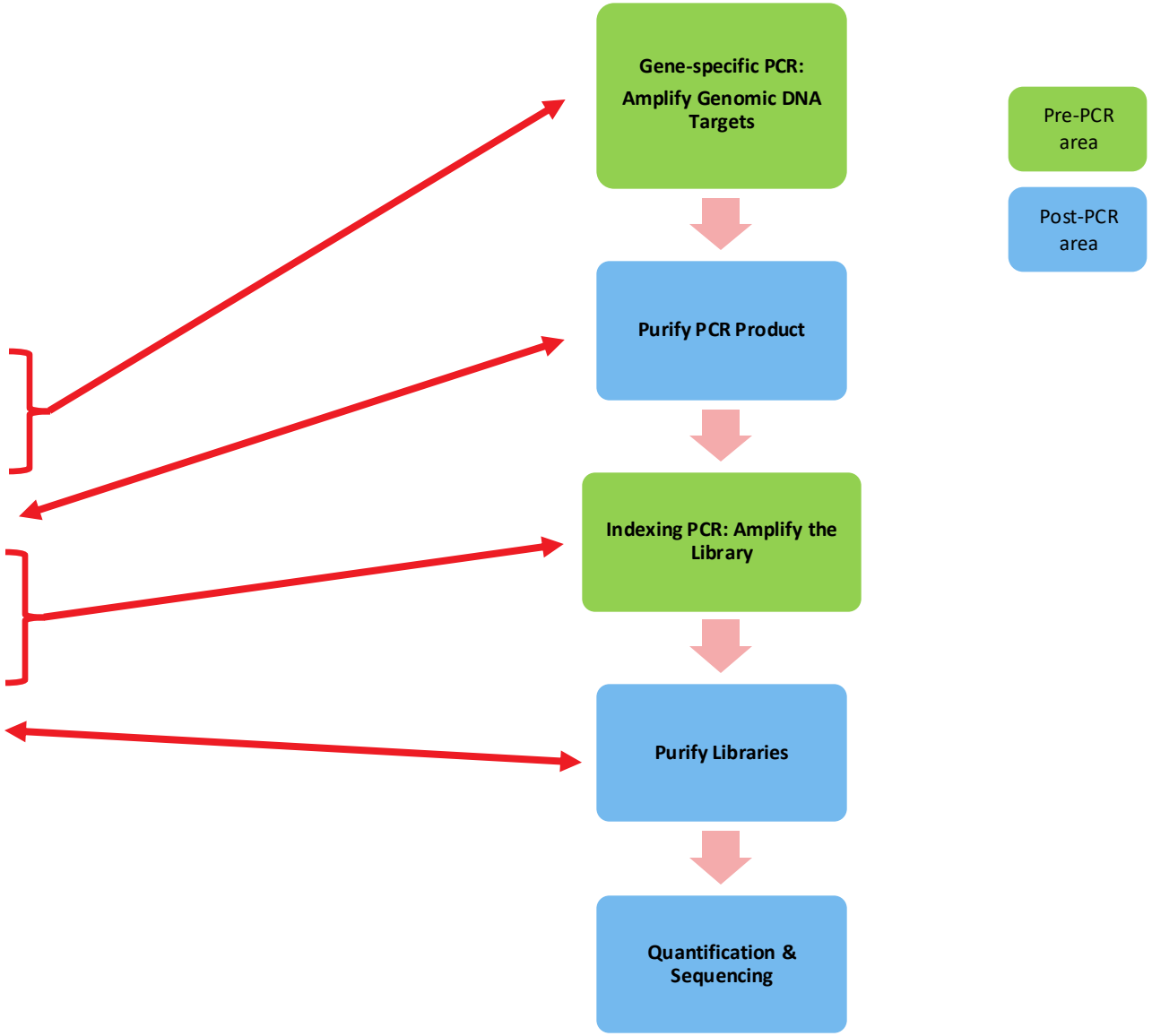
- Panel kit
  - oncoReveal™ Nexus 21 Gene Panel kit (HDA-HS-1013-24)

- Indexing kit
  - Pillar Custom Indexing Primers Kit A, 32 Combinations, 96 reactions (IDX-PI-1001-96)

App Details

Sections Automated

App Sections
Gene-Specific PCR Sample Prep
Gene-Specific PCR Amplification
Gene-Specific PCR Product Purification
Indexing PCR Sample Prep
Indexing PCR Amplification
Indexing PCR Product Purification



Section Details

App Sections
Gene-Specific PCR Sample Prep
Gene-Specific PCR Amplification
Gene-Specific PCR Product Purification
Indexing PCR Sample Prep
Indexing PCR Amplification
Indexing PCR Product Purification

PCR steps are each split into two sections.
* Sample Prep adds the reaction mixes to the DNA
* Amplification does the thermal cycling

This allows for the Sample Prep and Amplification to be prepared in pre-PCR / post-PCR settings if desired (and if equipment allows), per the User Guide, although it is not necessary in automation.

If starting at Gene-Specific PCR Amplification, the Gene-Specific PCR Master Mix, Nexus 21 Gene Oligo Pool, and GC Rescue G must have already been added to the samples.

If starting at Indexing PCR Amplification, the Indexing PCR Master Mix and indices must have already been added to the samples.

If stopping after Gene-Specific PCR Sample Prep or Indexing PCR Sample Prep, samples should be retrieved and stored in a timely manner.

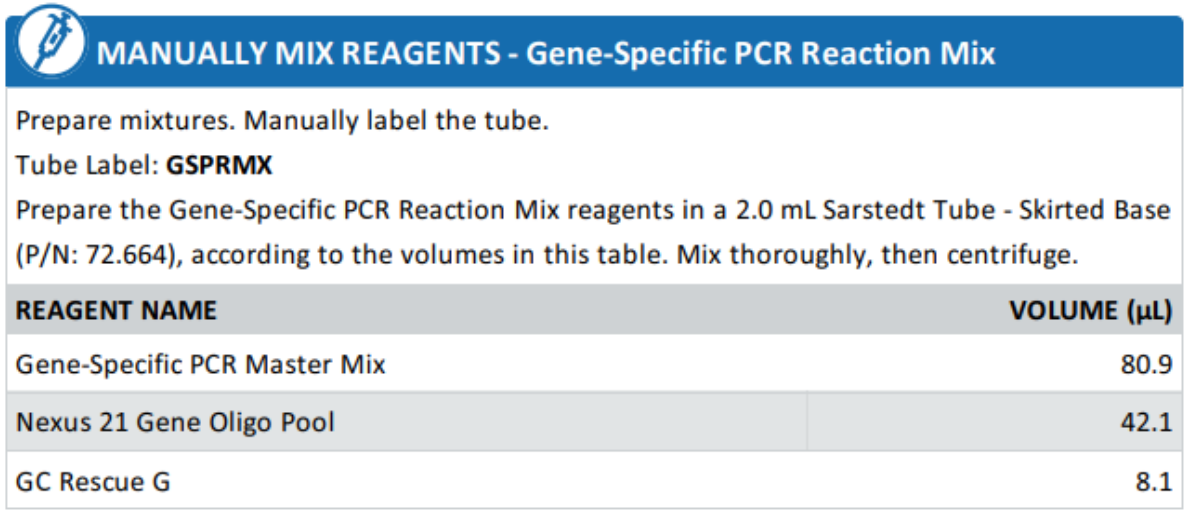
Section Details

App Sections
Gene-Specific PCR Sample Prep
Gene-Specific PCR Amplification
Gene-Specific PCR Product Purification
Indexing PCR Sample Prep
Indexing PCR Amplification
Indexing PCR Product Purification

The App allows for processing both gDNA and FFPE in a single batch run.

- The Gene-Specific PCR *Reaction* Mix is provided to the system as a user-created manual mix.

In the Work Aid's Reagent Preparation and Manually Mix Reagents sections, the above is mentioned. The Work Aid provides reagent volumes appropriate for the batch size.



MANUALLY MIX REAGENTS - Gene-Specific PCR Reaction Mix

Prepare mixtures. Manually label the tube.

Tube Label: **GSPRMX**

Prepare the Gene-Specific PCR Reaction Mix reagents in a 2.0 mL Sarstedt Tube - Skirted Base (P/N: 72.664), according to the volumes in this table. Mix thoroughly, then centrifuge.

REAGENT NAME	VOLUME (µL)
Gene-Specific PCR Master Mix	80.9
Nexus 21 Gene Oligo Pool	42.1
GC Rescue G	8.1

Section Details

App Sections
Gene-Specific PCR Sample Prep
Gene-Specific PCR Amplification
Gene-Specific PCR Product Purification
Indexing PCR Sample Prep
Indexing PCR Amplification
Indexing PCR Product Purification

The App, as designed by Pillar, does not make use of the Micronic-compatible carousel for introducing indices onto the Biomek NGeniuS system.

- 5 µL of each forward and reverse primer are manually added to an Axygen 96-well PCR Microplate (P/N: PCR-96-FS-C)
- Biomek NGeniuS system will aliquot out of the microplate into a cold storage RV.

In the Work Aid's Reagent Preparation section, the above is mentioned.

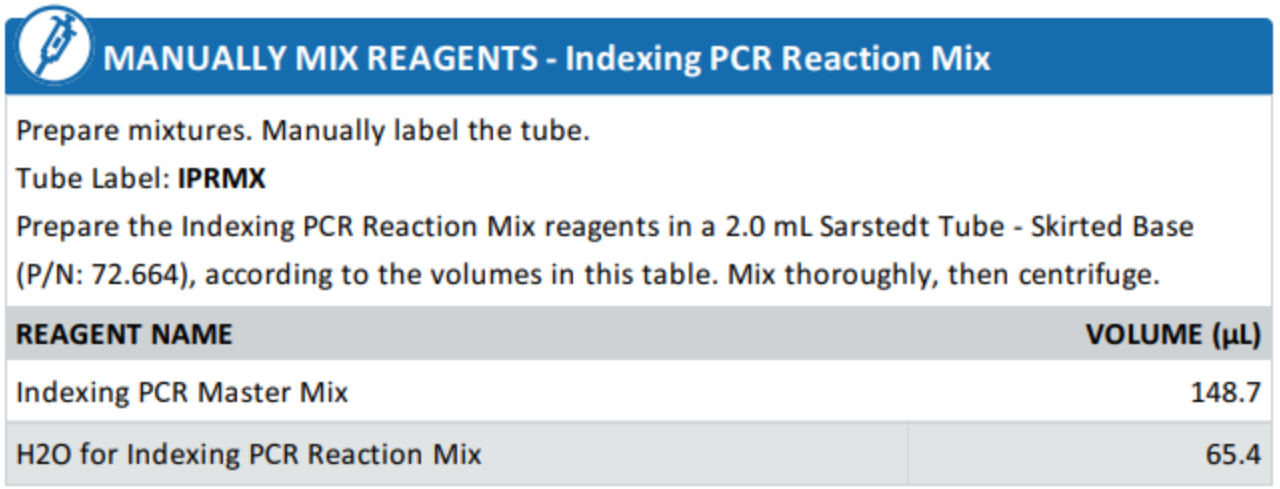
Indices IndexPlate: default	Add 5 µL of the assigned forward and reverse indexing primers to wells of an Axygen 96-well Polypropylene PCR Microplate, Full Skirt, Clear, Nonsterile (P/N: PCR-96-FS-C). It is recommended to match sample and index well positions. Centrifuge before use to ensure there are no bubbles. Wells: A1, B1, C1, D1	10.0

Section Details

App Sections
Gene-Specific PCR Sample Prep
Gene-Specific PCR Amplification
Gene-Specific PCR Product Purification
Indexing PCR Sample Prep
Indexing PCR Amplification
Indexing PCR Product Purification

The Indexing PCR *Reaction* Mix is provided to the system as a user-created manual mix (a dilution of the stock Indexing PCR Master Mix per the User Guide).

In the Work Aid's Reagent Preparation section, the above is mentioned. The Work Aid provides reagent volumes appropriate for the batch size.



MANUALLY MIX REAGENTS - Indexing PCR Reaction Mix

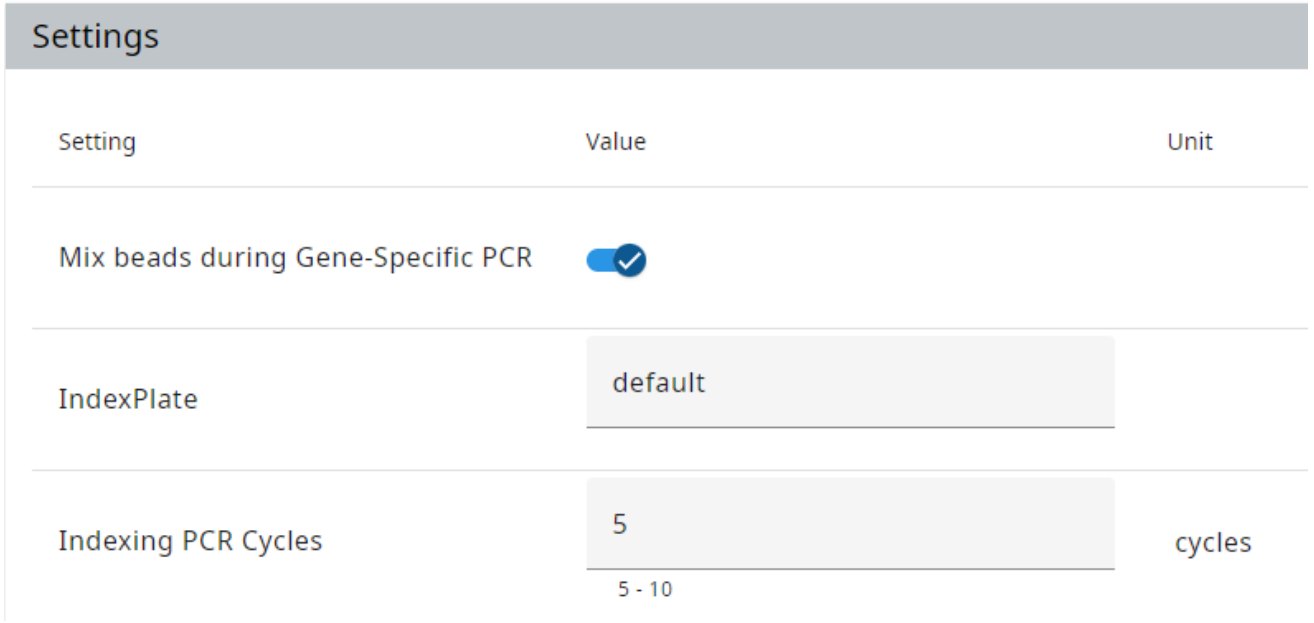
Prepare mixtures. Manually label the tube.

Tube Label: **IPRMX**

Prepare the Indexing PCR Reaction Mix reagents in a 2.0 mL Sarstedt Tube - Skirted Base (P/N: 72.664), according to the volumes in this table. Mix thoroughly, then centrifuge.

REAGENT NAME	VOLUME (µL)
Indexing PCR Master Mix	148.7
H2O for Indexing PCR Reaction Mix	65.4

App Settings



Setting	Description
Mix beads during Gene-Specific PCR	When on, mixes AMPure beads during the Gene-Specific PCR section to prevent them from settling. If not selected, mixing will only occur directly before Gene-Specific Product Purification.
IndexPlate	Allows the operator to enter in a name for the index plate being used in the batch.
Indexing PCR Cycles	Allows the operator to set the number of indexing PCR cycles performed within a range of 5-10. To facilitate balanced amplicon coverage, 5 Indexing PCR cycles are recommended when performing indexing PCR on the oncoReveal™ Nexus 21 Gene application. Additional Indexing PCR cycles can be performed if library yields are low.

Requested Reagent Volumes

Reagent	HDA-HS-1013-24 kit volumes[1]	4 samples volume requested	8 samples volume requested	16 samples volume requested	24 samples volume requested
Gene-Specific PCR Master Mix [2]	470	80.9	130.9	230.9	330.9
Nexus 21 Gene Oligo Pool [2]	250	42.1	68.1	120.1	172.1
GC Rescue G [2]	50	8.1	13.1	23.1	33.1
Indexing PCR Master Mix [3]	910	148.7	248.7	448.7	648.7
AMPure XP	User supplied	720	1240	2120	3000
70% EtOH	User supplied	4960	5920	7840	9760

[1] All values in µL, consumed volumes are less than requested due to source labware dead volume requirements
[2] Reagents are combined as a manual mixture to create Gene-Specific PCR Reaction Mix
[3] Reagent is combined with H_2O as a manual mixture to create Indexing PCR Reaction Mix

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	5	4	3	3	3	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1
Samples	20	25	24	21	24	27	20	22	24	26	28	30	32	17	18	19	20	21	22	23	24
Largest batch with leftover volume	0	0	0	6	0	0	10	8	6	4	0	0	0	15	14	13	12	11	10	9	8
Total estimated samples from kit	20	25	24	27	24	27	30	30	30	30	28	30	32	32	32	32	32	32	32	32	32

- 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 reagent vial volumes provided by Pillar.
- 5 µL of each index (PI50*X* and PI7*XX*) is required per sample. The index primer kits have enough reagent per-index to support 96 samples-worth of Biomek NGeniuS system batch runs.
- Numbers indicate maximum potential uses on optimally configured Biomek NGeniuS System

Estimated Time of Completion

Samples	4	8	16	24
Index Aliquot	00:01	00:01	00:02	00:04
Reagent Aliquot	00:12	00:15	00:18	00:20
Processing	04:18	04:25	05:03	05:43
Total ETC	04:32	04:41	05:24	06:07

Times (hours:minutes) calculated based on 5 indexing PCR cycles, bead mixing, and with all plate-based indices contiguous starting with well A1. Does not include times needed for manual interactions *(e.g.*, reagent thawing, manual pipetting, placing labware into Biomek NGeniuS System, …).

Consumables

		Batch Size (samples)			
Consumable	Part number	4	8	16	24
RVs	C62705	7	7	7	7
Bulk Reservoirs	C62707	1	1	1	1
Lids	C62706	4	4	4	4
Millitips (boxes)	C59585	6 (1)	11 (1)	12 (1)	12 (1)
Microtips (boxes)	C62712	116 (1)	228 (1)	388 (2)	644 (2)
Seal plate	C70665	1	1	1	1
5.0 mL Sarstedt® vial	60.611*	1	1	1	1
2.0 mL Sarstedt® vial	72.664*	2	2	2	2
Axygen® 96-well PCR Microplate	PCR-96-FS-C*	1	1	1	1
Price Per Sample, fresh ($)**	-	37.84	18.92	11.14	7.42
Price Per Sample, per tip ($)***	-	20.80	11.72	6.59	5.14

* 3rd party part numbers, not included in cost calculations.
** Costs assume a single batch run using fresh tip boxes. Some clean tips will remain each run, reducing per-sample cost of subsequent runs.
*** Costs on a per tip basis, only considering tips consumed, since batch runs do not consume entire tip boxes.

Demonstration Data

Experimental Design for Demonstration Run Conditions

Experiment	Sample Throughput	Target Sample Mass (ng)	gDNA (sample count)	fcDNA (sample count)	Myeloid (sample count)	Negative control (count)	PCR Cycles	Operator
1	4	20	1	1	1	1	5	Pillar Biosciences
2	15	40	6	6	1	2	5	Pillar Biosciences
3	24	60	10	10	1	3	5	Beckman Coulter Life Sciences

gDNA: Coriell NA12878

fcDNA: Horizon Mimix™ Quantitative Multiplex fcDNA (moderate) Reference Standard, HD799

Myeloid: Horizon Mimix™ Myeloid DNA Reference Standard, HD829

5 Indexing PCR cycles was used since that was found to provide balanced amplicon coverage.

The ng/µL concentration of the samples was determined using the Qubit dsDNA HS Assay Kit.

The nM concentration was determined using the following formula:

LibraryConcentration[nM] = LibraryConcentration[ng/µL] x 5.

Library Construction Pass Criteria:

- Sample library yields >= 8 nM
- Non-Template control < 2.5 nM

Demo run 1, 4 samples, low input mass

Target Library Prep Input Mass (ng):	20
PCR Amplification Cycles:	5
Version:	1.0.0

All sample yields >= 8 nM
All control yields < 2.5 nM

Sample	Stock for run start Concentration (ng/ul)	Library input (ng)	Library yield (ng/ul)	Library yield (nM)	Pass/Fail
gDNA Sample 1	4.25	20.4	17.4	87	Pass
fcDNA Sample 1	4.29	20.58	8.9	44.5	Pass
Negative Control	N/A	0	0.0508	0.254	Pass
Myeloid Sample 1	4.19	20.125	17.4	87	Pass

Demo run 2, 15 samples, mid input mass

Target Library Prep Input Mass (ng):	40
PCR Amplification Cycles:	5
Version:	1.0.0

All sample yields >= 8 nM
All control yields < 2.5 nM

Sample	Stock for run start Concentration (ng/ul)	Library input (ng)	Library yield (ng/ul)	Library yield (nM)	Pass/Fail
gDNA Sample 1	8.5	40.8	20.3	101.5	Pass
fcDNA Sample 1	8.49	40.752	11	55	Pass
gDNA Sample 2	8.5	40.8	18.4	92	Pass
fcDNA Sample 2	8.49	40.752	11.2	56	Pass
gDNA Sample 3	8.5	40.8	19.2	96	Pass
Negative Control	N/A	N/A	0.0515	0.2575	Pass
fcDNA Sample 3	8.49	40.752	10.4	52	Pass
gDNA Sample 4	8.5	40.8	18.4	92	Pass
fcDNA Sample 4	8.49	40.752	10.7	53.5	Pass
fcDNA Sample 5	8.49	40.752	11	55	Pass
Negative Control	N/A	N/A	0.0405	0.2025	Pass
Myeloid Sample 1	8.505	40.824	17.6	88	Pass
gDNA Sample 5	8.5	40.8	15.7	78.5	Pass
fcDNA Sample 6	8.49	40.752	9.75	48.75	Pass
gDNA Sample 6	8.5	40.8	16.6	83	Pass

Demo run 3, 24 samples, high input mass

Sample	Stock for run start Concentration (ng/ul)	Library input (ng)	Library yield (ng/ul)	Library yield (nM)	Pass/Fail
gDNA Sample 1	12	57.6	17	85	Pass
fcDNA Sample 1	10.9	52.32	10.8	54	Pass
gDNA Sample 2	12	57.6	15.8	79	Pass
fcDNA Sample 2	10.9	52.32	11.4	57	Pass
gDNA Sample 3	12	57.6	16.1	80.5	Pass
NTC-1	N/A	N/A	0.08	0.4	Pass
gDNA Sample 4	12	57.6	14.5	72.5	Pass
fcDNA Sample 3	10.9	52.32	10.5	52.5	Pass
fcDNA Sample 4	10.9	52.32	11.1	55.5	Pass
gDNA Sample 5	12	57.6	13.4	67	Pass
fcDNA Sample 5	10.9	52.32	9.44	47.2	Pass
gDNA Sample 6	12	57.6	15.7	78.5	Pass
fcDNA Sample 6	10.9	52.32	10.3	51.5	Pass
gDNA Sample 7	12	57.6	16	80	Pass
fcDNA Sample 7	10.9	52.32	11	55	Pass
NTC-2	N/A	N/A	0.101	0.505	Pass
gDNA Sample 8	12	57.6	16.5	82.5	Pass
fcDNA Sample 8	10.9	52.32	10.6	53	Pass
gDNA Sample 9	12	57.6	14	70	Pass
fcDNA Sample 9	10.9	52.32	9.05	45.25	Pass
gDNA Sample 10	12	57.6	14.6	73	Pass
fcDNA Sample 10	10.9	52.32	10.6	53	Pass
NTC-3	N/A	N/A	0.062	0.31	Pass
Myeloid Sample 1	12.2	58.56	15.1	75.5	Pass

Target Library Prep Input Mass (ng):	60
PCR Amplification Cycles:	5
Version:	1.0.0

All sample yields >= 8 nM

All control yields < 2.5 nM

Sequencing and Variant Analysis

Sequencing Setup

Experiment	Sequencer
1	MiSeq
2	NextSeq 500
3	MiSeq

Sequencing conducted at Pillar
Experiment libraries were pooled and then loaded on an Illumina Sequencer:

- Runs 1 & 3 were sequenced concurrently on a MiSeq at 12.5 pM.
- Run 2 was sequenced on a NextSeq 500 at 1.8 pM.

Analysis App: Pillar PiVAT RUO, version 2023.1.1

Sequencing QC Results – Samples by Run

- Coverage mean for all samples are ≥ 1500x.

  Pass ✓

- Overall Q=30 for all samples are ≥ 80%.

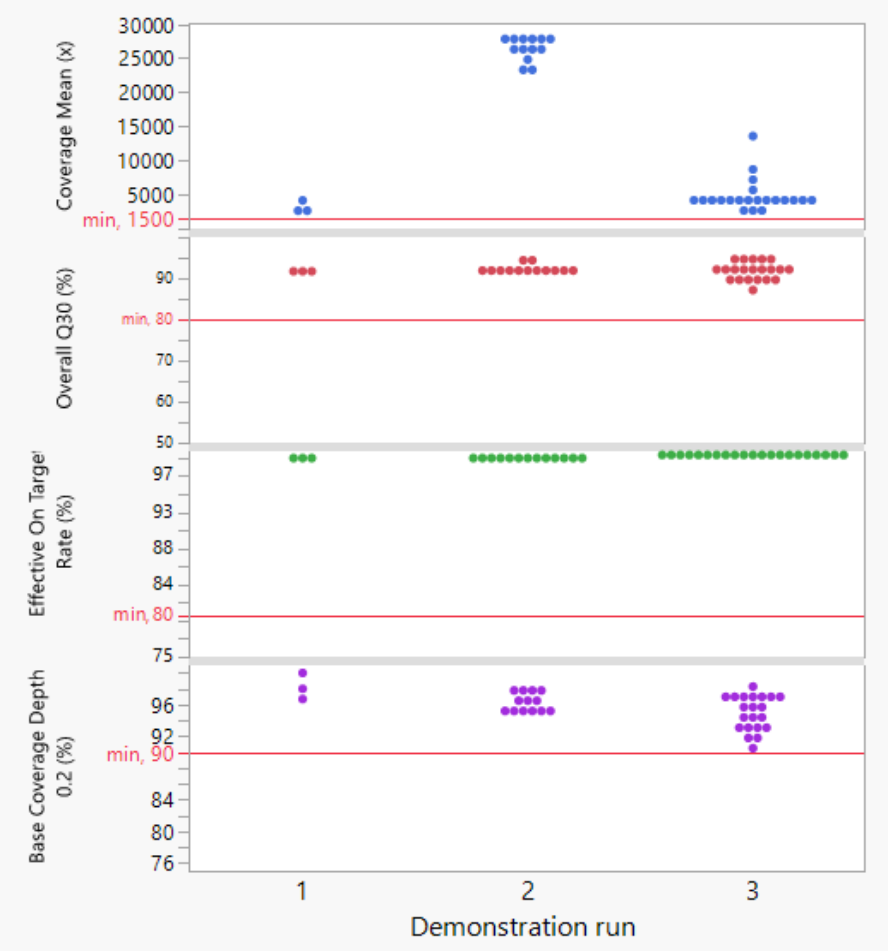
  Pass ✓

- Effective On Target Rate for all samples are ≥ 80%.

  Pass ✓

- Base_Coverage_Depth_>_(Nx)_Relative_to_Mean _Coverage 0.2 for all samples is ≥ 90%.

  Pass ✓



Note: Run 2 sequenced on NextSeq 500 giving higher coverage

Sequencing QC Results – Samples by Type

- Coverage mean for all samples are ≥ 1500x.

  Pass ✓

- Overall Q=30 for all samples are ≥ 80%.

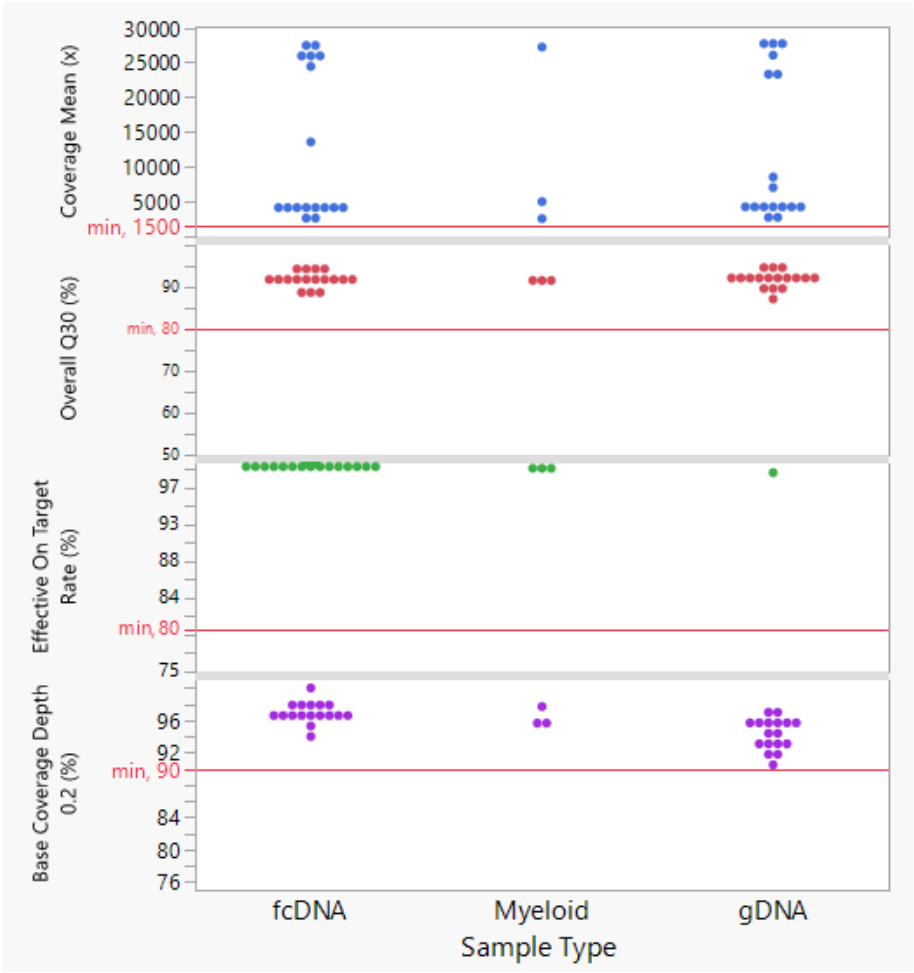
  Pass ✓

- Effective On Target Rate for all samples are ≥ 80%.

  Pass ✓

- Base_Coverage_Depth_>_(Nx)_Relative_to_Mean _Coverage 0.2 for all samples is ≥ 90%.

  Pass ✓



Note: Coverage Mean spread due to use of multiple sequencers

Sequencing QC Results – Details

Numbers reported as: average (low)	1 (n=3)	2 (n=13)	3 (n=21)
Sequencer	MiSeq	NextSeq 500	MiSeq
Coverage mean for all samples are ≥ 1500x.	2790 (2569)	26110 (23293)	4026 (2498)
Overall Q=30 for all samples are ≥ 80%	91.7 (91.5)	92.0 (91.4)	90.7 (87.2)
Effective On Target Rate for all samples are ≥ 80%	99.2 (99.0)	99.2 (98.8)	99.6 (99.23)
Base_Coverage_Depth_>_(Nx)_Relative_to_Mean_Coverage 0.2 for all samples is ≥ 90%	98.1 (96.7)	96.2 (94.8)	94.3 (90.5)
	Pass	**Pass**	**Pass**

Sequencing QC Results – NTC Variant Calls

Run	Sample	Variant Calls	Pass Calls Metric (0)
1	C1	0	Pass
2	F1	0	Pass
2	C2	0	Pass
3	F1	0	Pass
3	H2	0	Pass
3	G3	0	Pass

Expected Variants for Horizon FFPE (fcDNA) Libraries

Chromosome	Variant	Approximate Allele Frequency (%)	Average Variant Read Frequency (%), (StdDev)	Variant Detection Rate (%)	Detection Pass Metric (%)	Pass Detection Metric (95%)
7	BRAF V600E	10.5	13.05 (0.58)	100	95	Pass
7	EGFR G719S	24.5	24.88 (0.48)	100	95	Pass
7	EGFR L858R	3	3.43 (0.25)	100	95	Pass
4	KIT D816V	10	9.83 (0.50)	100	95	Pass
12	KRAS G12D	6	6.22 (0.49)	100	95	Pass
12	KRAS G13D	15	15.46 (0.66)	100	95	Pass
1	NRAS Q61K	12.5	11.64 (0.40)	100	95	Pass
7*	EGFR DeltaE746 - A750	2	2.12 (0.10)	41.2	N/A	N/A*
7*	EGFR T790M	1	1.28 (0.14)	82.4	N/A	N/A*

Variants at or above an expected frequency of 3% are specified. Across all replicates, at least 95% of variants should be called.

*The expected allele frequency is below the 3% cutoff for this assay. As such, a 100% detection rate is not expected, and variants are excluded from pass criteria.

Expected Variants for Horizon Myeloid Libraries

Chromosome	Variant	Approximate Allele Frequency (%)	Average Variant Read Frequency (%), (StdDev)	Variant Detection Rate (%)	Detection Pass Metric (%)	Pass Detection Metric (95%)
13	FLT D835Y	5	4.60 (0.16)	100	95	Pass
2	IDH1 R132C	5	4.61 (0.19)	100	95	Pass
15	IDH2 R172K	5	4.84 (0.16)	100	95	Pass
9	JAK2 F537-K539>L	5	5.30 (0.15)	100	95	Pass
9	JAK2 V617F	5	4.34 (0.29)	100	95	Pass
12	KRAS G13D	40	39.59 (0.31)	100	95	Pass
5	NPM1 W288Cfs*12	5	4.50 (0.47)	100	95	Pass
1	NRAS Q61L	10	10.89 (0.74)	100	95	Pass
17	TP53 S241F	5	5.28 (0.24)	100	95	Pass

Variants at or above an expected frequency of 3% are specified. Across all replicates, at least 95% of variants should be called.

Variants Expected for NA12878 gDNA Libraries

Chromosome	Gene	Location	Approximate Allele Frequency (%)	Average Variant Read Frequency (%), gDNA (StdDev)	Variant Detection Rate (%)	Detection Pass Metric (%)	Pass
4	PDGFRA	chr4:55141055-55141055	99.5	99.53 (0.19)	100	100	Pass
4	PDGFRA	chr4:55152040-55152040	47.5	49.47 (0.57)	100	100	Pass
7	EGFR	chr7:55249063-55249063	49.5	50.31 (1.01)	100	100	Pass
17	TP53	chr17:7579472-7579472	48.3	49.43 (1.02)	100	100	Pass

Coriell NA12878 is a high-quality genomic DNA (gDNA) sample extracted from a well-characterized genome in a bottle cell line. All variants specified should be called.

Variants Not Expected for NA12878 gDNA Libraries

Chromosome	Variant	Approximate Allele Frequency (%)	Average Variant Read Frequency (%), gDNA (StdDev)	Variant *Not* Detected Rate (%)	Detection Pass Metric (%)	Pass Detection Metric (0%)
7	BRAF V600E	0	0 (0)	100	90	Pass
7	EGFR G719S	0	0 (0)	100	90	Pass
7	EGFR L858R	0	0 (0)	100	90	Pass
4	KIT D816V	0	0 (0)	100	90	Pass
12	KRAS G12D	0	0 (0)	100	90	Pass
12	KRAS G13D	0	0 (0)	100	90	Pass
1	NRAS Q61K	0	0 (0)	100	90	Pass
7	EGFR DeltaE746 - A750	0	0 (0)	100	90	Pass
7	EGFR T790M	0	0 (0)	100	90	Pass
13	FLT D835Y	0	0 (0)	100	90	Pass
2	IDH1 R132C	0	0 (0)	100	90	Pass
15	IDH2 R172K	0	0 (0)	100	90	Pass
9	JAK2 F537-K539>L	0	0 (0)	100	90	Pass
9	JAK2 V617F	0	0 (0)	100	90	Pass
5	NPM1 W288Cfs*12	0	0 (0)	100	90	Pass
1	NRAS Q61L	0	0 (0)	100	90	Pass
17	TP53 S241F	0	0 (0)	100	90	Pass

No variants specified above should be called in NA12878 libraries. 90% of NA12878 libraries must pass.

Unexpected High Impact Variants for gDNA Libraries

No high impact variants (PiVAT Max_Impact = HIGH), should be called. 90% of NA12878 libraries must pass. Unexpected variants not specified in previous slide that are not high impact are acceptable.

Run	Sample	Count Max_Impact = HIGH	Metric
1	A1	0	Pass
2	A1	0	Pass
2	C1	0	Pass
2	E1	0	Pass
2	H1	0	Pass
2	E2	0	Pass
2	G2	0	Pass
3	A1	0	Pass
3	C1	0	Pass
3	E1	0	Pass
3	G1	0	Pass
3	B2	0	Pass
3	D2	0	Pass
3	F2	0	Pass
3	A3	0	Pass
3	C3	0	Pass
3	E3	0	Pass

Demonstration Summary

Demonstration Metrics Summary

Metric	Run 1	Run 2	Run 3
All sample yields >= 8 nM	Pass	Pass	Pass
All negative controls < 2.5 nM	Pass	Pass	Pass
No NTC Variant Calls	Pass	Pass	Pass
Coverage mean for all samples are ≥ 1500x	Pass	Pass	Pass
Overall Q=30 for all samples are ≥ 80%	Pass	Pass	Pass
Effective On Target Rate for all samples are ≥ 80%	Pass	Pass	Pass
Base_Coverage_Depth_>_(Nx)_Relative_to_Mean_Coverage 0.2 for all samples is ≥ 90%	Pass	Pass	Pass
Horizon FFPE and Myeloid samples should have the specified variants called	Pass	Pass	Pass
No variants specified for FFPE samples should be called in NA12878 libraries	Pass	Pass	Pass
NA12878 gDNA samples should have the specified variants called	Pass	Pass	Pass
No unexpected high impact variants should be called in NA12878 gDNA samples	Pass	Pass	Pass

Demonstration Summary

- The oncoReveal™ Nexus 21 Gene App, written by Pillar, on the Biomek NGeniuS Next Generation Library Prep System prepares libraries at input masses between 20 and 60 ng of genomic DNA and FFPE DNA
- Yield at all tested input masses exceeded 8 nM final concentration
- gDNA and FFPE samples can be processed simultaneously
- Sequencing data of prepared libraries passes all Pillar metrics for:
  - Mean coverage and depth
  - Overall %Q30
  - Effective on target rate
  - Variant Detection rate for genes of interest

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
  - Only Axygen® PCR-96-FS-C is supported for this App
  - If the plate geometry is not the same, it could result in an instrument crash
- Make sure foil, if present, 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
- Avoid bubbles in reagent tubes to ensure accurate liquid level sensing and aliquoting
- 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
- Prepare samples while Biomek NGeniuS System is aliquoting reagents
  - Avoids sample evaporation while Biomek NGeniuS System is preparing run

oncoReveal™ Nexus 21 Gene App specific considerations

- Read and understand the oncoReveal™ Nexus 21 Gene user guide, UM-0081
- Master mixes are manually prepared to reduce dead volumes and maximize kit usage
- It's recommended to set Mix beads during Gene-Specific PCR setting to ON for application runs, as it reduces sample processing time and bead settling prior to Gene-Specific PCR Product Purification.
- To facilitate balanced amplicon coverage, 5 Indexing PCR cycles are recommended for the oncoReveal™ Nexus 21 Gene App. Users have the option to increase the Indexing PCR cycle count, but results may differ from those presented in this document.
- Index plate is manually prepared to reduce dead volumes and maximize kit usage
- An excess (~4mL) of H_2O and EtOH is called for in the Work Aid to reduce sample processing time

App Template Revision Notes

- 1.0.0 Demonstration data obtained.

This document contains proprietary information owned by Beckman Coulter, Inc.

Any disclosure, use, copying or distribution of this information without the express written consent of Beckman Coulter, Inc. is strictly prohibited.

©2024 Beckman Coulter, Inc. All rights reserved. Beckman Coulter, the stylized logo, and the Beckman Coulter product and service marks mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries. All other trademarks are the property of their respective owners.