# Pillar Biosciences oncoReveal™ Solid Tumor v2 Gene Panel on Biomek NGeniuS System

## App Template Version 1.0.0-1.0.2

BECKMAN COULTER
Life Sciences

2024-GBL-EN-106698-v1

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# App Template Description

The App Template for Pillar Biosciences* oncoReveal™ Solid Tumor v2 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™ Solid Tumor v2 panel App Template supports a DNA input mass of 20-60 ng for standard genomic DNA, 20-80 ng for good quality FFPE DNA, and a minimum 40 ng for severely degraded FFPE DNA. Mixed sample type inputs require the addition of Uracil-DNA glycosylase (UDG) for all samples. Libraries can be sequenced on any low- to mid- throughput sequencers such as Illumina MiSeq or NextSeq System.

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™ Solid Tumor v2 kit (HDA-ST-1002-24). Ethanol wash volumes have been reduced to 60µL from 150µL to reduce tip consumption and sample processing time.

In addition to the consumables listed in the oncoReveal™ Solid Tumor v2 assay User Guide (UM-0065), 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.



2024-GBL-EN-105198-v4

# Scoping

# Scoping

- Author
  - Pillar scientists with support from Beckman Coulter Life Sciences
- Panel
  - oncoReveal™ Solid Tumor v2
    - Version 3.0 (User Guide UM-0065)
- Supported DNA Input
    - Genomic DNA: 20-60 ng in 4.25 µL
    - FFPE DNA: 20-80 ng good quality, minimum 40 ng severely degraded, in 4.25 µL

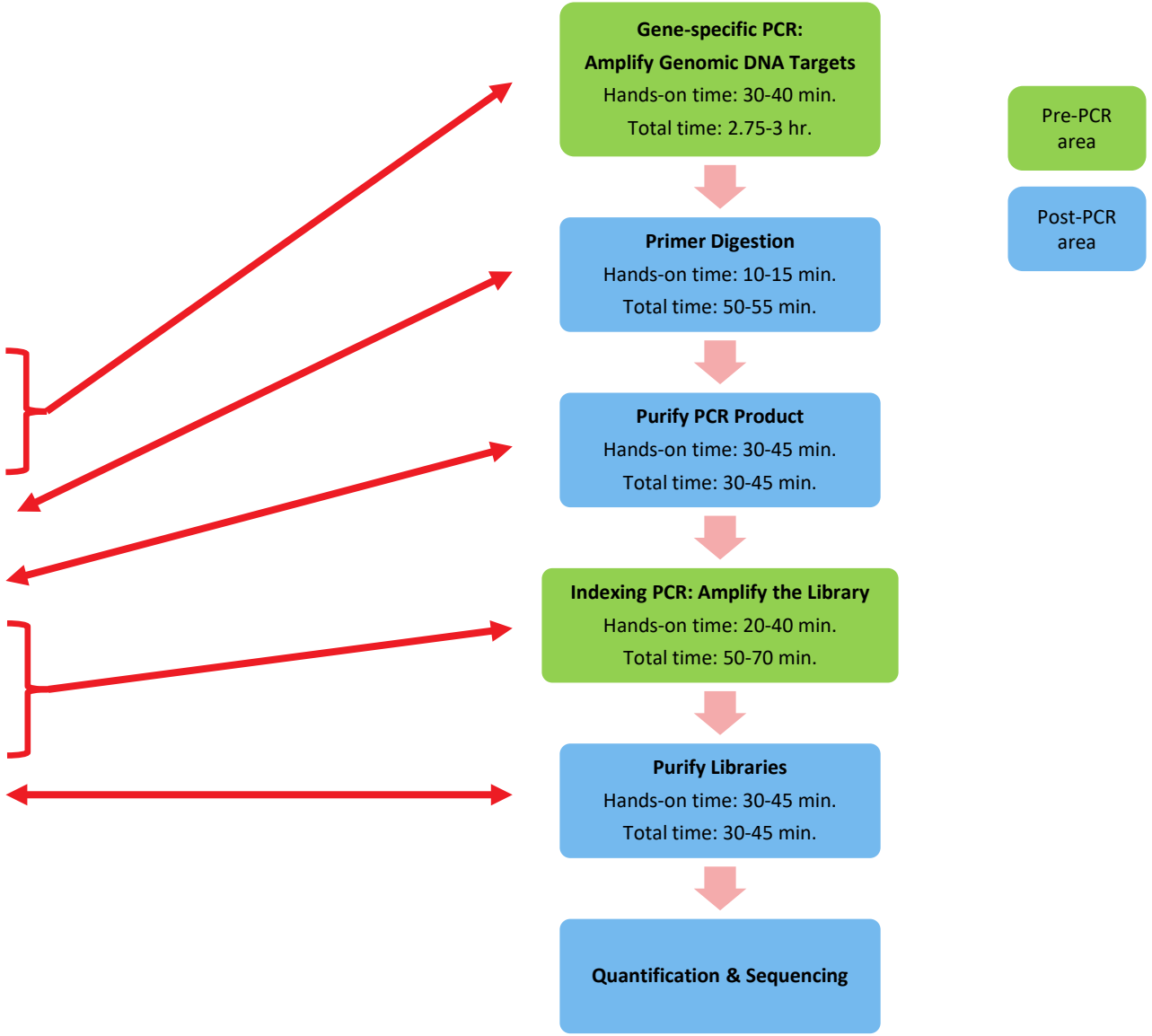
# Scoping

- Panel kit
  - oncoReveal™ Solid Tumor v2 Panel kit (HDA-ST-1002-24)
- 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 Exo Digestion |
| Gene-Specific PCR Product Purification |
| Indexing PCR Sample Prep |
| Indexing PCR Amplification |
| Indexing PCR Product Purification |




Workflow adapted from ONCO/Reveal™ Solid Tumor Panel Library Preparation User Guide, Figure 2

Times listed are from User Guide

# Section Details

| App Sections |
| --- |
| Gene-Specific PCR Sample Prep |
| Gene-Specific PCR Amplification |
| Gene-Specific PCR Exo Digestion |
| 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, Solid Tumor v2 Oligo Pool, UDG, 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.

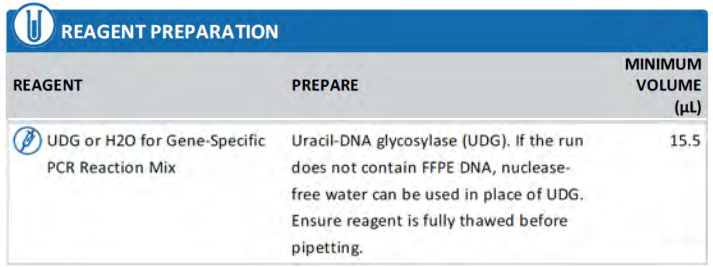
# Section Details

| App Sections |
| --- |
| Gene-Specific PCR Sample Prep |
| Gene-Specific PCR Amplification |
| Gene-Specific PCR Exo Digestion |
| 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.

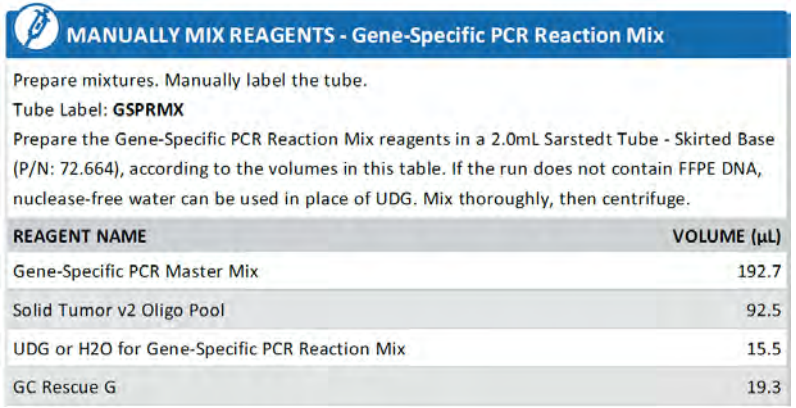
- If *any* sample is FFPE, then Uracil-DNA Glycosylase (UDG) must be included. UDG will not negatively impact performance of gDNA samples. UDG is user-supplied.
- If only gDNA is being processed, the UDG is optional, and can be replaced with $H_2O$.
- The Gene-Specific PCR *Reaction* Mix is provided 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.



**REAGENT PREPARATION**

| REAGENT | PREPARE | MINIMUM VOLUME (µL) |
| --- | --- | --- |
| UDG or H2O for Gene-Specific PCR Reaction Mix | Uracil-DNA glycosylase (UDG). If the run does not contain FFPE DNA, nuclease-free water can be used in place of UDG. Ensure reagent is fully thawed before pipetting. | 15.5 |



**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 2.0mL Sarstedt Tube - Skirted Base (P/N: 72.664), according to the volumes in this table. If the run does not contain FFPE DNA, nuclease-free water can be used in place of UDG. Mix thoroughly, then centrifuge.

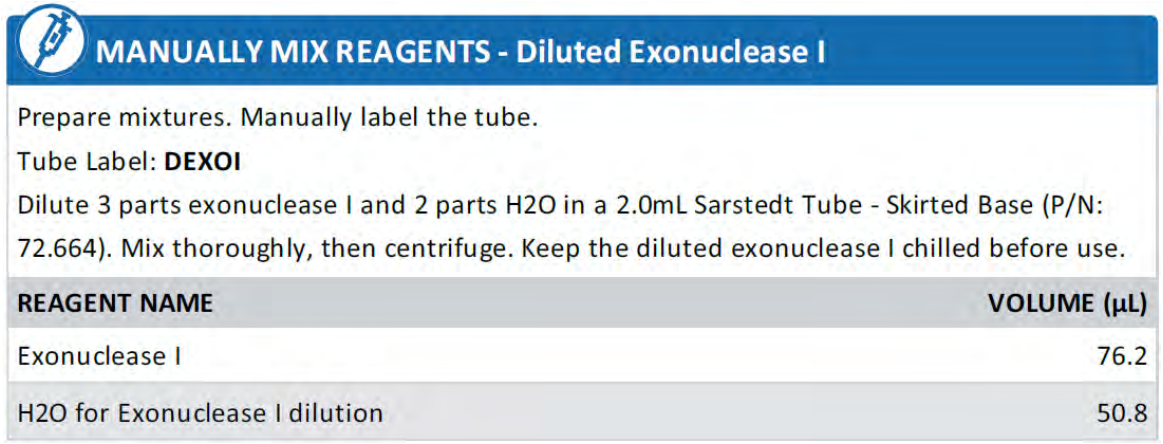
| REAGENT NAME | VOLUME (µL) |
| --- | --- |
| Gene-Specific PCR Master Mix | 192.7 |
| Solid Tumor v2 Oligo Pool | 92.5 |
| UDG or H2O for Gene-Specific PCR Reaction Mix | 15.5 |
| GC Rescue G | 19.3 |

# Section Details

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

The Diluted Exonuclease I is provided to the system as a user-created manual mix (a dilution of the stock Exonuclease I per the User Guide).

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 - Diluted Exonuclease I**

Prepare mixtures. Manually label the tube.

Tube Label: **DEXOI**

Dilute 3 parts exonuclease I and 2 parts H2O in a 2.0mL Sarstedt Tube - Skirted Base (P/N: 72.664). Mix thoroughly, then centrifuge. Keep the diluted exonuclease I chilled before use.

| REAGENT NAME | VOLUME (µL) |
| --- | --- |
| Exonuclease I | 76.2 |
| H2O for Exonuclease I dilution | 50.8 |

# Section Details

| App Sections |
| --- |
| Gene-Specific PCR Sample Prep |
| Gene-Specific PCR Amplification |
| Gene-Specific PCR Exo Digestion |
| 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.

**REAGENT PREPARATION**

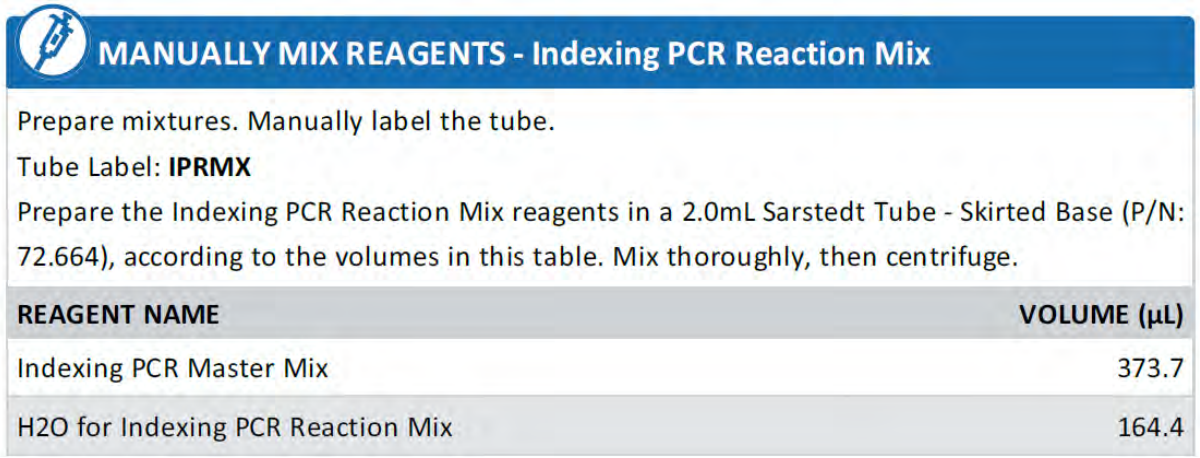
| REAGENT | PREPARE | MINIMUM VOLUME (µL) |
| --- | --- | --- |
| Indices<br>IndexPlate: default | Add 5 µL of the assigned forward and reverse indexing primers to wells of an Axygen 96-well Polypropylene PCR Microplate, Full Skirt, Clear, Nonsterile (P/N: PCR-96-FS-C). It is recommended to match sample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

# Section Details

| App Sections |
| --- |
| Gene-Specific PCR Sample Prep |
| Gene-Specific PCR Amplification |
| Gene-Specific PCR Exo Digestion |
| 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**

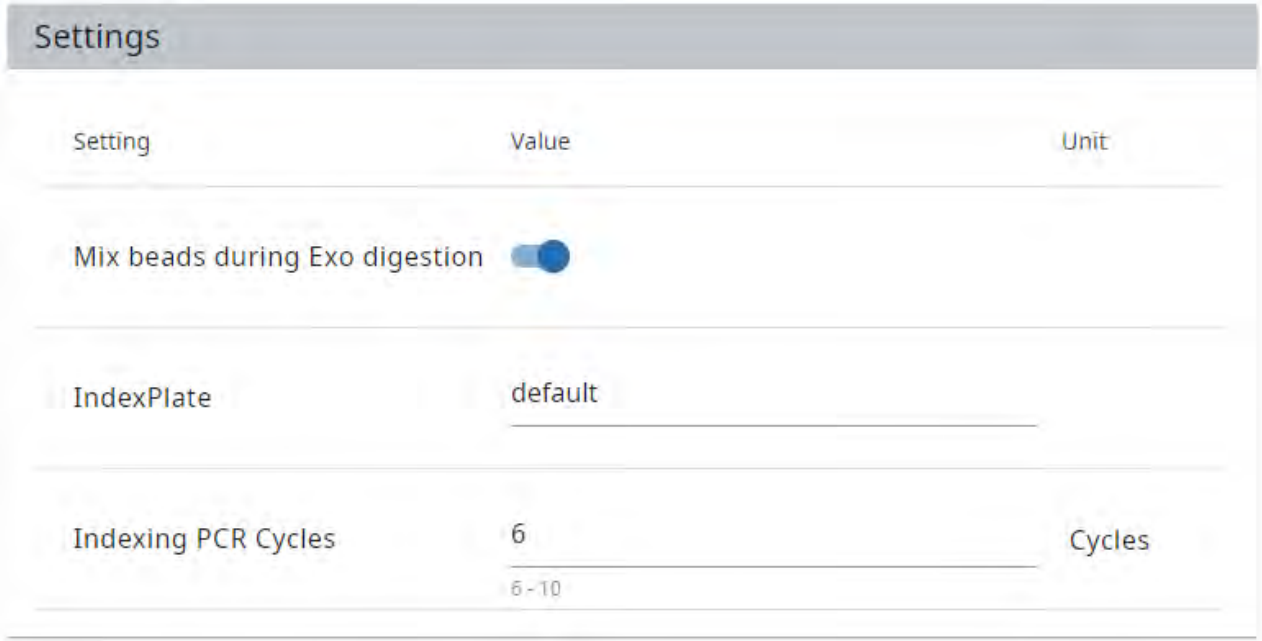
Prepare mixtures. Manually label the tube.

Tube Label: **IPRMX**

Prepare the Indexing PCR Reaction Mix reagents in a 2.0mL 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 | 373.7 |
| H2O for Indexing PCR Reaction Mix | 164.4 |

# App Settings




| Setting | Description |
|---|---|
| **Mix beads during Exo digestion** | When on, mixes AMPure beads during the Exonuclease Digestion 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 6-10. |

# Requested Reagent Volumes

| Reagent | HDA-ST-1002-24 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 | 80.2 | 130.2 | 230.2 | 330.2 |
| Solid Tumor v2 Oligo Pool [2] | 230 | 38.5 | 62.5 | 110.5 | 158.5 |
| GC Rescue G [2] | 50 | 8.1 | 13.1 | 23.1 | 33.1 |
| UDG [2,3] | User supplied | 6.6 | 10.5 | 18.5 | 26.5 |
| Exonuclease I [4] | 190 | 49.2 | 61.2 | 85.2 | 109.2 |
| Indexing PCR Master Mix [5] | 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 with user-supplied UDG or $H_20$ to create Gene-Specific PCR Reaction Mix
[3] UDG volumes are listed as if at least one FFPE sample was being processed, otherwise UDG is replaced by $H_2O$
[4] Reagent is combined with $H_2O$ as a manual mixture to create Diluted Exonuclease I
[5] 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 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Samples | 20 | 25 | 24 | 21 | 24 | 27 | 30 | 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 | 0 | 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.

# Estimated Time of Completion

| Samples | 4 | 8 | 16 | 24 |
|---|---|---|---|---|
| Index Aliquot | 00:01 | 00:01 | 00:02 | 00:04 |
| Reagent Aliquot | 00:15 | 00:18 | 00:21 | 00:23 |
| Processing | 05:12 | 05:21 | 06:08 | 06:57 |
| Total ETC | 05:29 | 05:41 | 06:32 | 07:25 |

Times (hours:minutes) calculated based on 6 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 | 121 (1) | 237 (1) | 453 (2) | 669 (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* | 3 | 3 | 3 | 3 |
| Axygen® 96-well PCR Microplate | PCR-96-FS-C* | 1 | 1 | 1 | 1 |
| Price Per Sample ($)** | - | 37.84 | 18.92 | 11.14 | 7.42 |

Costs assume a single batch run using fresh tip boxes. Some clean tips will remain each run, reducing cost of subsequent runs.
* 3rd party part numbers.
** Costs do not include Sarstedt reformat vials, Axygen plate, or empty tip boxes for tip disposal.

# Demonstration Data

# Experimental Design for Demonstration Run Conditions

| Experiment | Sample Throughput | Target Sample Mass (ng) | PCR Cycles | Site |
|---|---|---|---|---|
| 1 | 4* | 60 ng gDNA, 80 ng FFPE (Moderate), 80 ng FFPE (Severe) | 6 | Pillar Biosciences |
| 2 | 13* | 40 ng gDNA, 50 ng FFPE (Moderate), 50 ng FFPE (Severe) | 6 | Beckman Coulter Life Sciences |
| 3 | 24** | 20 ng gDNA, 20 ng FFPE (Moderate), 40 ng FFPE (Severe) | 6 | Pillar Biosciences |

* One negative control (NTC) per experiment
** Three negative controls (NTC) per experiment

gDNA: Coriell NA12878.
FFPE: Horizon Discovery Quantitative Multiplex Reference Standard fcDNA Moderate and Severe

The nM concentration was determined using the following formula:
LibraryConcentration[nM] = LibraryConcentration[ng/µL] x 5.
The ng/µL concentration of the samples was determined using a 1X Qubit dsDNA HS Assay Kit.

Library Construction Pass Criteria:
- Sample library yields >3.5 nM
- Non-Template control <2.5 nM

# Demo run 1, 4 samples, high input mass

| | |
|---|---|
| Target Library Prep Input Mass, gDNA (ng): | 60 |
| Target Library Prep Input Mass, FFPE Moderate (ng): | 80 |
| Target Library Prep Input Mass, FFPE Severe (ng): | 80 |
| PCR Amplification Cycles: | 6 |
| Version: | 1.0.0 |

| Sample | Stock conc (ng/μL) | Index Pair | Library Yield (nM) |
|---|---|---|---|
| gDNA Sample 1 | 13.95 | Pi712 / Pi501 | 73.5 |
| FFPE Moderate Sample 1 | 18.6 | Pi712 / Pi502 | 80.5 |
| Negative Control | N/A | Pi712 / Pi503 | 0.278 |
| FFPE Severe Sample 1 | 18.6 | Pi712 / Pi504 | 66.5 |

All sample yields > 3.5 nM
All control yields < 2.5 nM

# Demo run 2, 13 samples, mid input mass

| | |
|---|---|
| Target Library Prep Input Mass, gDNA (ng): | 40 |
| Target Library Prep Input Mass, FFPE Moderate (ng): | 50 |
| Target Library Prep Input Mass, FFPE Severe (ng): | 50 |
| PCR Amplification Cycles: | 6 |
| Version: | 1.0.1 |

| Sample | Stock conc (ng/μL) | Index Pair | Library Yield (nM) |
|---|---|---|---|
| gDNA Sample 1 | 9.3 | Pi702 / Pi514 | 135 |
| FFPE Moderate Sample 1 | 11.6 | Pi702 / Pi515 | 121.25 |
| FFPE Severe Sample 1 | 11.6 | Pi702 / Pi516 | 92.25 |
| gDNA Sample 2 | 9.3 | Pi703 / Pi509 | 141 |
| FFPE Moderate Sample 2 | 11.6 | Pi703 / Pi510 | 109.5 |
| FFPE Severe Sample 2 | 11.6 | Pi703 / Pi511 | 107.25 |
| gDNA Sample 3 | 9.3 | Pi703 / Pi512 | 132.5 |
| FFPE Moderate Sample 3 | 11.6 | Pi703 / Pi513 | 130 |
| FFPE Severe Sample 3 | 11.6 | Pi703 / Pi514 | 52 |
| Negative Control | 0 | Pi703 / Pi515 | 0.3075 |
| gDNA Sample 4 | 9.3 | Pi703 / Pi516 | 102.75 |
| FFPE Moderate Sample 4 | 11.6 | Pi704 / Pi509 | 80.75 |
| FFPE Severe Sample 4 | 11.6 | Pi704 / Pi510 | 68.5 |

All sample yields > 3.5 nM
All control yields < 2.5 nM

| Sample | Stock conc (ng/µL) | Index Pair | Library Yield (nM) |
|---|---|---|---|
| gDNA Sample 1 | 4.65 | Pi709 / Pi501 | 150.5 |
| FFPE Moderate Sample 1 | 4.65 | Pi709 / Pi502 | 81.5 |
| FFPE Severe Sample 1 | 9.3 | Pi709 / Pi503 | 119.5 |
| Negative Control | 0 | Pi709 / Pi504 | 1.21 |
| gDNA Sample 2 | 4.65 | Pi709 / Pi505 | 144.5 |
| FFPE Moderate Sample 2 | 4.65 | Pi709 / Pi506 | 74.5 |
| FFPE Severe Sample 2 | 9.3 | Pi709 / Pi507 | 110 |
| gDNA Sample 3 | 4.65 | Pi709 / Pi508 | 154 |
| FFPE Moderate Sample 3 | 4.65 | Pi710 / Pi501 | 59.5 |
| FFPE Severe Sample 3 | 9.3 | Pi710 / Pi502 | 102 |
| gDNA Sample 4 | 4.65 | Pi710 / Pi503 | 135 |
| FFPE Moderate Sample 4 | 4.65 | Pi710 / Pi504 | 55.5 |
| FFPE Severe Sample 4 | 9.3 | Pi710 / Pi505 | 88 |
| Negative Control | 0 | Pi710 / Pi506 | 1.09 |
| gDNA Sample 5 | 4.65 | Pi710 / Pi507 | 139 |
| FFPE Moderate Sample 5 | 4.65 | Pi710 / Pi508 | 56.5 |
| FFPE Severe Sample 5 | 9.3 | Pi711 / Pi501 | 96 |
| Negative Control | 0 | Pi711 / Pi502 | 1.16 |
| gDNA Sample 6 | 4.65 | Pi711 / Pi503 | 115.5 |
| FFPE Moderate Sample 6 | 4.65 | Pi711 / Pi504 | 51 |
| FFPE Severe Sample 6 | 9.3 | Pi711 / Pi505 | 79 |
| gDNA Sample 7 | 4.65 | Pi711 / Pi506 | 129 |
| FFPE Moderate Sample 7 | 4.65 | Pi711 / Pi507 | 68.5 |
| FFPE Severe Sample 7 | 9.3 | Pi711 / Pi508 | 94 |

# Demo run 3, 24 samples, low input mass

| | |
|---|---|
| Target Library Prep Input Mass, gDNA (ng): | 20 |
| Target Library Prep Input Mass, FFPE Moderate (ng): | 20 |
| Target Library Prep Input Mass, FFPE Severe (ng): | 40 |
| PCR Amplification Cycles: | 6 |
| Version: | 1.0.0 |

All sample yields > 3.5 nM
All control yields < 2.5 nM

# Sequencing and Variant Analysis

# Sequencing Setup

| Experiment | Sequencer |
|---|---|
| 1 | NextSeq500 |
| 2 | MiSeq |
| 3 | NextSeq500 |

All experiment runs pooled to 3.85 nM and then loaded on flow cell at 1.8pM.
Runs 1 & 3 were sequenced concurrently.
Run 2 was sequenced individually.

Analysis App: Pillar PiVAT RUO, version 2023.1.1

# Sequencing QC Results – NTC Variant Calls

| Run | Sample | Variant Calls | Pass Calls Metric (0) |
|---|---|---|---|
| 1 | 01C_20240805_Demo1_NTC | 0 | Pass |
| 2 | 240903-STV2-Demo2-B2-NTC | 0 | Pass |
| 3 | 01D_20240807_Demo3_NTC | 0 | Pass |
| 3 | 02F_20240807_Demo3_NTC | 0 | Pass |
| 3 | 03B_20240807_Demo3_NTC | 0 | Pass |

# Sequencing QC Results – Samples by Run

- Coverage mean for all samples are ≥ 1500x.

  Pass ✓

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

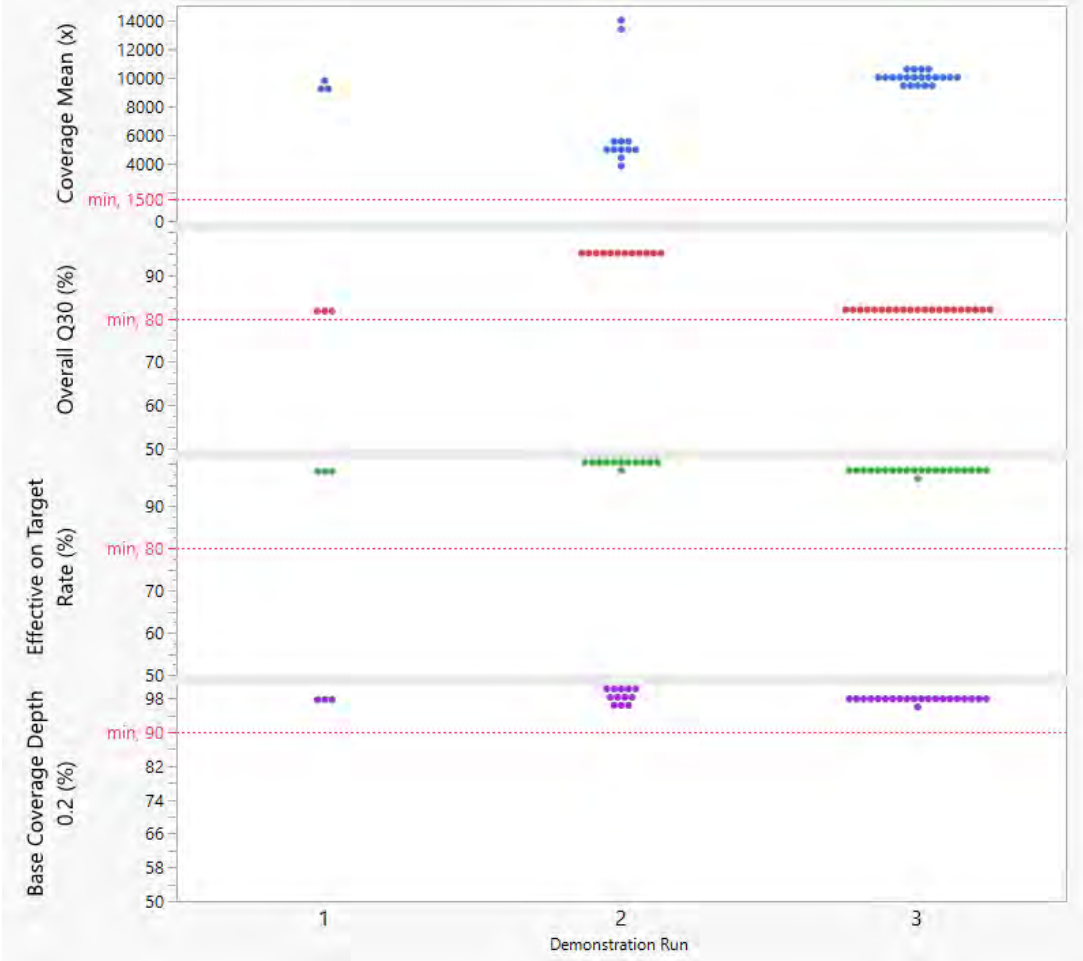
  Pass ✓

- 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 ✓



# Sequencing QC Results – Samples by Type

- Coverage mean for all samples are ≥ 1500x.

  Pass ✓

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

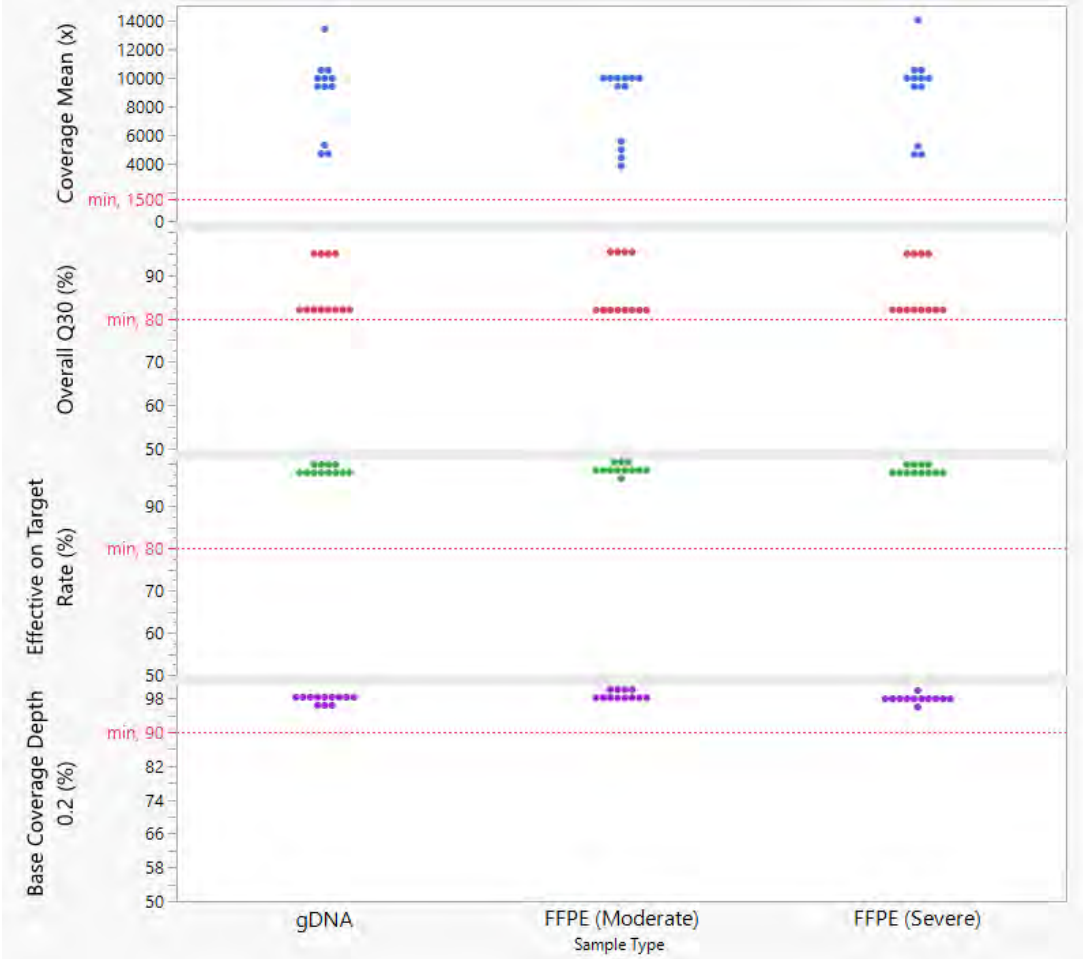
  Pass ✓

- 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 ✓



# Expected Variants for Horizon Libraries (FFPE Samples)

| Chromosome | Variant | Approximate Allele Frequency (%) | Average Variant Read Frequency (%), FFPE Moderate (StdDev) | Average Variant Read Frequency (%), FFPE Severe (StdDev) | Variant Detection Rate (%) | Detection Pass Metric (%) | Pass Detection Metric (95%) |
|---|---|---|---|---|---|---|---|
| 7 | BRAF V600E | 10.5 | 11.92 (0.63) | 12.49 (0.60) | 100 | 95 | Pass |
| 7 | EGFR G719S | 24.5 | 22.88 (0.88) | 23.20 (0.95) | 100 | 95 | Pass |
| 7 | EGFR L858R | 3 | 3.36 (0.43) | 4.01 (0.39) | 100 | 95 | Pass |
| 4 | KIT D816V | 10 | 8.20 (0.46) | 9.92 (0.52) | 100 | 95 | Pass |
| 12 | KRAS G12D | 6 | 6.29 (0.65) | 6.24 (0.53) | 100 | 95 | Pass |
| 12 | KRAS G13D | 15 | 15.17 (0.99) | 15.90 (1.24) | 100 | 95 | Pass |
| 1 | NRAS Q61K | 12.5 | 13.00 (1.02) | 13.18 (1.11) | 100 | 95 | Pass |
| 3 | PIK3CA E545K | 9 | 7.50 (0.56) | 8.22 (0.75) | 100 | 95 | Pass |
| 3 | PIK3CA H1047R | 17.5 | 17.96 (1.13) | 18.72 (0.68) | 100 | 95 | Pass |
| 7* | EGFR DeltaE746 - A750 | 2 | 2.10 (0.04) | 2.34 (0.30) | 50 | N/A | N/A* |
| 7* | EGFR T790M | 1 | 0 (0) | 0 (0) | 0 | 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.

# Variants Not Expected for 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 |
| 3 | PIK3CA E545K | 0 | 0 (0) | 100 | 90 | Pass |
| 3 | PIK3CA H1047R | 0 | 0 (0) | 100 | 90 | Pass |
| 7* | EGFR DeltaE746 - A750 | 0 | 0 (0) | 100 | N/A | N/A* |
| 7* | EGFR T790M | 0 | 0 (0) | 100 | N/A | N/A* |

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

*Variants are excluded from pass criteria.

# Variants Expected for gDNA Libraries

| Chromosome | Gene | Location | Approximate Allele Frequency (%) | Average Variant Read Frequency (%), gDNA (StdDev) | Variant Detection Rate (%) | Detection Pass Metric (%) | Pass |
|---|---|---|---|---|---|---|---|
| 7 | EGFR | chr7:55249063-55249063 | 49.2 | 49.64 (0.70) | 100 | 100 | PASS |
| 4 | FGFR3 | chr4:1807894-1807894 | 99.5 | 98.71 (0.15) | 100 | 100 | PASS |
| 19 | KEAP1 | chr19:10600442-10600442 | 50 | 49.97 (0.72) | 100 | 100 | PASS |
| 4 | PDGFRA | chr4:55141055-55141055 | 99.9 | 99.43 (0.14) | 100 | 100 | PASS |
| 4 | PDGFRA | chr4:55152040-55152040 | 50 | 47.48 (1.19) | 100 | 100 | PASS |
| 12 | POLE | chr12:133236000-133236000 | 99.7 | 99.04 (0.26) | 100 | 100 | PASS |
| 12 | POLE | chr12:133257837-133257837 | 48.5 | 49.80 (0.89) | 100 | 100 | PASS |
| 10 | RET | chr10:43613843-43613843 | 99.7 | 99.30 (0.10) | 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.

# Unexpected High Impact Variants for gDNA Libraries

| Run | Sample | Count Max_Impact = HIGH | Metric |
|---|---|---|---|
| 1 | 01A_20240805_Demo1_TV2_60ng | 0 | Pass |
| 2 | 240903_STV2_Demo2_A1_TV2_40ng | 0 | Pass |
| 2 | 240903_STV2_Demo2_D1_TV2_40ng | 0 | Pass |
| 2 | 240903_STV2_Demo2_G1_TV2_40ng | 0 | Pass |
| 2 | 240903_STV2_Demo2_C2_TV2_40ng | 0 | Pass |
| 3 | 01A_20240807_Demo3_TV2_20ng | 0 | Pass |
| 3 | 01E_20240807_Demo3_TV2_20ng | 0 | Pass |
| 3 | 01H_20240807_Demo3_TV2_20ng | 0 | Pass |
| 3 | 02C_20240807_Demo3_TV2_20ng | 0 | Pass |
| 3 | 02G_20240807_Demo3_TV2_20ng | 0 | Pass |
| 3 | 03C_20240807_Demo3_TV2_20ng | 0 | Pass |
| 3 | 03F_20240807_Demo3_TV2_20ng | 0 | Pass |

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.

# Demonstration Summary

# Demonstration Metrics Summary

| Metric | Run 1 | Run 2 | Run 3 |
|---|---|---|---|
| All sample yields > 3.5 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 Moderate and Severe FFPE 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™ Solid Tumor v2 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, 20 – 80 ng of moderately degraded FFPE DNA, and a minimum of 40 ng severely degraded FFPE DNA
- Yield at all tested input masses exceeded 3.5 nM final concentration
- gDNA and FFPE samples can be processed simultaneously
- NextSeq™ 550 sequencing data of prepared libraries passes all Pillar metrics for:
  - Mean coverage
  - 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™ Solid Tumor v2 App specific considerations

- Read and understand the oncoReveal™ Solid Tumor v2 user guide, UM-0065
- Master mixes are manually prepared to reduce dead volumes and maximize kit usage
- It's recommended to set Mix beads during Exo digestion setting to ON for application runs, as it reduces sample processing time and bead settling prior to Gene-Specific PCR Product Purification.
- Prepared Diluted Exonuclease I reagent is highly viscous. Operators should thoroughly centrifuge the prepared reagent tube until no liquid remains on vial walls.
- 6 Indexing PCR cycles is recommended for the oncoReveal™ Solid Tumor v2 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 speed sample processing

# App Template Revision Notes

- 1.0.0 Demonstration data obtained.
- 1.0.1 App Template Description updated.  Chemistry unaffected.
- 1.0.2 App Template Description updated.  Chemistry unaffected.