BECKMAN COULTER
Life Sciences

Illumina TruSight® Oncology 500 DNA/RNA Automation Kit App Template Setup Guide for Biomek NGeniuS System

Introduction

Purpose

This manual provides instructions for users running the Illumina TruSight® Oncology 500 DNA/RNA Automation Kit App Template on the Biomek NGeniuS Next Generation Library Prep System.

The Illumina TruSight® Oncology 500 assay is a comprehensive next-generation sequencing (NGS) assay targeting the full coding regions of 523 genes implicated in the pathogenesis of solid tumors. Using enrichment-based library preparation techniques for use with formalin-fixed, paraffin-embedded (FFPE) samples, the Illumina TruSight® Oncology 500 assay when running the DNA/RNA workflow can detect single nucleotide variants (SNVs), indels, amplifications, and multinucleotide variants (MNVs) in a single sequencing run. The Illumina TruSight® Oncology 500 assay also detects immunotherapy biomarkers for tumor mutational burden (TMB) and microsatellite instability (MSI) in DNA samples. Additionally, processing RNA samples adds the ability to detect known or novel gene fusion events, providing a comprehensive survey of the tumor genome in a single assay.

The Illumina TruSight® Oncology 500 DNA/RNA Automation Kit App Template allows for the creation of Illumina TruSight® Oncology 500 DNA and RNA libraries compatible with Illumina sequencing platforms. The user will first create a batch in the Biomek NGeniuS Portal Software. If running RNA samples as part of the batch, the user will then add pre-normalized RNA samples to the input reaction vessel (RV) and place the input reaction vessel on the Biomek NGeniuS system. The Biomek NGeniuS system will then perform Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA in a single run. If running DNA samples, the user will then shear the DNA samples using a Covaris® instrument as detailed in the Illumina TruSight® Oncology 500 Reference Guide. Covaris® sheared DNA samples can be loaded onto the library preparation reaction vessel and prepared into Illumina libraries through End Repair/A-tailing, Adapter Ligation, and Index PCR. Regions of interest are hybridized to probes, magnetically captured, cleaned, and eluted. Enriched libraries are then PCR-amplified. An optional fluorometric quantification step may be used to ensure there is sufficient library available before Bead-Based Normalization. After Normalization, the libraries are ready to pool for sequencing. The specific automated and manual steps of the workflow are detailed in Figure 1 below.

The Illumina TruSight® Oncology 500 DNA/RNA Automation Kit App Template allows for the creation of Illumina TruSight® Oncology 500 DNA and RNA libraries compatible with Illumina sequencing platforms. The App Template allows the user to produce between four and 24 libraries in a single batch with any combination of DNA and RNA samples required. If following the recommended schedule in the Illumina TruSight® Oncology 500 Reference Guide (Document# 1000000067621 v10), sample preparation will take approximately 3 workdays to complete. Users are to follow the Illumina TruSight® Oncology 500 Reference Guide for manual steps and this guide for automated steps.

The Illumina TruSight® Oncology 500 DNA/RNA Automation Kit App Template utilizes the TruSight® Oncology 500 DNA/RNA Automation Kit (16 indexes, 32 Samples) (Illumina Part Number 20045508).

Other Illumina TruSight® Oncology 500 kit part numbers are not supported. Supplementary HRD enrichment workflow is not supported by the App Template. 80% ethanol wash volumes have been reduced to 50 µL from 200 µL to reduce tip consumption. First and Second Hybridization times have been limited to the minimum time listed in the manual protocol (8 hours for First Hybridization and 1.5 hours for Second Hybridization) to reduce application run time and cannot be changed.

The user has the option of specifying if the batch contains high-quality or low-quality RNA samples and will adjust the RNA fragmentation program accordingly. Refer to the Illumina TruSight® Oncology 500 Reference Guide for details concerning RNA input mass and sample quality. DNA samples are pre-sheared on a Covaris® ® instrument prior to loading onto the Biomek NGeniuS Next Generation Library Prep system. Refer to the Illumina TruSight® Oncology 500 Reference Guide for details concerning DNA input mass, sample quality, and shearing parameters.

Beckman Coulter makes no warranties of any kind whatsoever express or implied, with respect to this App Template, including but not limited to warranties of fitness for a particular purpose or merchantability or that the App Template is non-infringing. All warranties are expressly disclaimed. Your use of the App Template 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.

Products and demonstrated applications are not intended or validated for use in diagnostic procedures.

References

- Illumina TruSight® Oncology 500 Reference Guide (Document# 1000000067621 v10)
- Biomek NGeniuS Next Generation Library Prep System Instructions for Use (Beckman Reference Number C432122AB or later)
- Illumina NextSeq 500 and NextSeq 550 Sequencing Systems Denature and Dilute Libraries Guide (Illumina Reference Number 15048776 v18 or later)
- Illumina NextSeq 500 and NextSeq 550 Sequencing Systems Safety and Compliance Guide (Illumina Reference Number 15046564 v03 or later)
- Beckman Coulter Life Sciences references can be found at **https://www.beckman.com/support**
- Illumina references can be found at **https://support.illumina.com/documentation.html**

Safety Notices

This guide does not replace the Biomek NGeniuS Next Generation Library Prep System Instructions for Use (Beckman Reference Number C432122AB or later versions). The user bears responsibility to review the Safety Notice published in Biomek NGeniuS Next Generation Library Prep System Instructions for Use prior to operating the Biomek NGeniuS Next Generation Library Prep System.

This guide does not replace Illumina TruSight® Oncology 500 Reference Guide (Document# 1000000067621 v10). The user bears responsibility to review this guide prior to using the Illumina TruSight® Oncology 500 DNA/RNA Automation Kit (16 indexes, 32 Samples) (Illumina Part Number 20045508).

Safety Data Sheets (SDS) for the Illumina TruSight® Oncology 500 DNA Automation Kit may be obtained at https://support.illumina.com/sds.html

Review the Illumina NextSeq 500 and NextSeq 550 Sequencing Systems Safety and Compliance Guide for safety information about the Illumina NextSeq 500/550 sequencer.

System and Workflow Overview

Method Description

The Illumina TruSight® Oncology 500 DNA/RNA Automation Kit App Template contains logical start/stop points as listed in the manual protocol to allow for more efficient workflow planning. The App Template utilizes the Biomek NGeniuS Next Generation Library Prep System's built-in thermal cycler unit for all sample workflow heating and cooling steps, including both hybridizations and polymerase chain reaction (PCR) reactions. To reduce the risk of cross-contamination, the App Template utilizes unique filtered tips for each pipetting operation while the pipetting pod employs defined fly-over paths so that used tips are not moved over a reaction vessel (RV) containing samples. The Biomek NGeniuS Next Generation Library Prep System's built-in cold storage positions are used to ensure that reagents which require chilled storage are kept cold until needed or retrieved (for up to 16 hours).

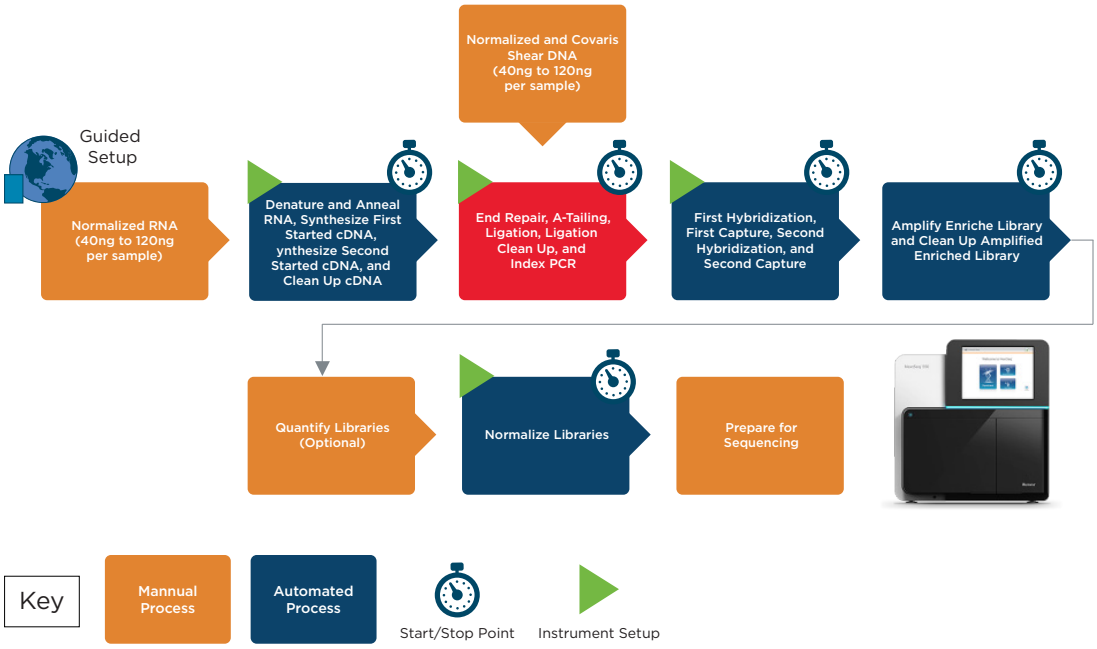


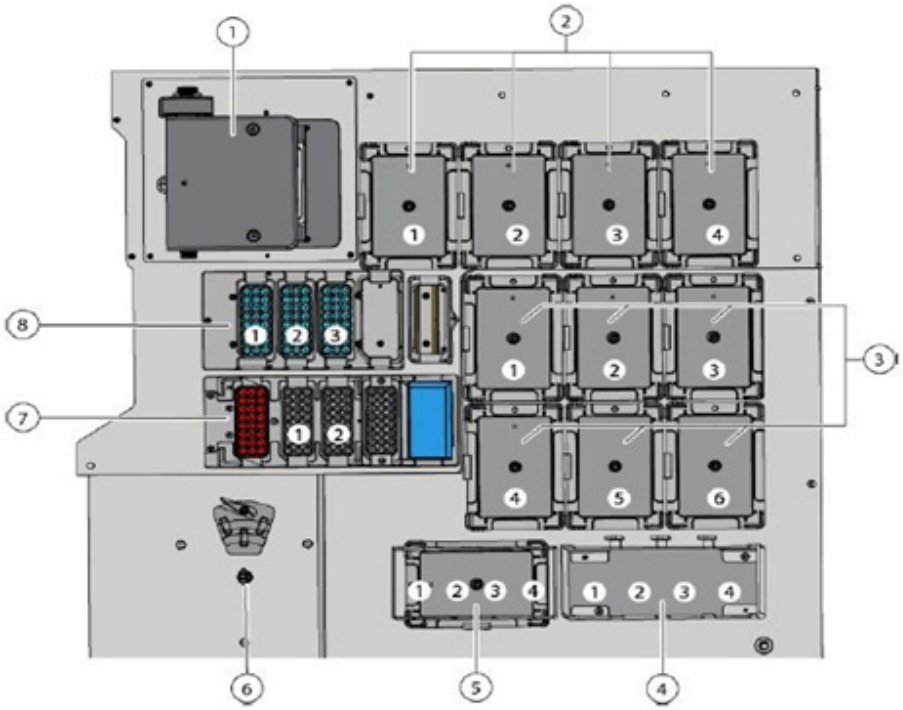
Figure 1. The Illumina TruSight® Oncology 500 DNA/RNA Automation App Template Workflow.

Hardware

The Illumina TruSight® Oncology 500 DNA/RNA Automation Kit App Template was developed on the Biomek NGeniuS Next Generation Library Prep System.

Deck and Deck Elements

The Biomek NGeniuS system is equipped with several deck elements, including a Thermal Cycler, Cold Reagent Storage, Magnet Station, Warm and Ambient Reagent Storage, Reaction Vessel Exchange, Liquid Waste Station, Reagent Input Carousels, Bulk Reservoir Station, Reaction Vessel Input and Waste Station, and finally storage locations for Biomek 70 µL tips and Biomek 1025 µL tips. All required hardware is included with each Biomek NGeniuS system, and the deck layout is the same for all Biomek NGeniuS systems.



1. *Thermal Cycler*
2. *Biomek 70 µL Conductive Filtered Tip Storage*
3. *Biomek 1025 µL Conductive Filtered Tip Storage*
4. *Reaction Vessel Input and Waste Station*
5. *Bulk Reservoir Station*
6. *Reagent Input Carousels* position
7. *Warm and Ambient Temperature Storage, Reaction Vessel Exchange, Liquid Waste*
8. *Cold Reagent Storage, Magnet Station*

Figure 2. Biomek NGeniuS Deck.

Pipettor

The Biomek NGeniuS system is equipped with an eight channel pipettor capable of loading either the Biomek 1025 µL tips or Biomek 70 µL tips. The Biomek NGeniuS system pipettor has two pipetting ranges, a low volume range from 1 µL to 10 µL and a high volume range from 10 µL to 1000 µL.

Status Indicator Light Bars

Multi-color LED light bars (Table 1) along all four sides at the top of the Biomek NGeniuS instrument provide a color-coded indication of the current run status, such as ready, running, and user interaction needed. The status is also displayed on the Head-Up Display (HUD) and on the Biomek NGeniuS Portal software.

Color	Instrument State	Operational Status
White	Power On, Starting, Updating	The instrument is starting up or a software update is underway. For software updates, see the HUD for additional information.
Amber Solid	Power On, Not Ready	The instrument is doing background processing in preparation for operation. It is safe to access the instrument.
Blue Solid	Power On, Ready	The instrument is in a ready state. It is safe to access the instrument.
Green Scrolling	Power On, Running	Instrument operations are in process.
Amber Light and Dark Alternating	Power On, User Interaction Needed	User interaction is needed to proceed. The Head-up display and batch card in the user portal display the cause.
Red Solid Flashing	Power On, Error	An error has occurred.
Rainbow Motion	Get Sample	The run is complete, the sample is ready.
No Lights	Power Off	Off

Table 1. Status Indicator Light Bar Status.

Instrument User Interface

The Biomek NGeniuS System Instrument User Interface consists of the HUD and the Software Navigation Dial on the front of the instrument. The Software Navigation Dial on the front of the instrument provides navigation of the information on the HUD (the screen in the rear of the instrument) by turning the dial left or right to highlight an item on the HUD and pressing the center button to select the highlighted item.

Figure 3. TInstrument User Interface showing the Software Navigation Dial (1) and the Head-Up Display (2).

Reagents and Consumables

The following reagents and consumables are required for running the Illumina TruSight® Oncology 500 DNA/RNA Automation Kit App Template on the Biomek NGeniuS Next Generation Library Prep System.

Part Number	Number of each used per run	Quantity of Part Number to Order	Vendor	Description
C59585	8	2	Beckman Coulter	Case, Tips, 1025 µL, Conductive, Filtered, 480 Tips
C62712	7	1	Beckman Coulter	Case, Tips, 70 µL, Conductive, Filtered, 3840 Tips
C62705	20	1	Beckman Coulter	Biomek Reaction Vessel, 24 well
C62707	4	1	Beckman Coulter	Biomek Bulk Reservoir, 25 mL/section
C62706	20	1	Beckman Coulter	Biomek Lid, 24 well
C70665	2	1	Beckman Coulter	Biomek Seal Plate, 24 well
C70672	1	1	Beckman Coulter	Case, Biomek 1025 µL Tip Box, Empty, 5 Racks
C70673	1	1	Beckman Coulter	Case, Biomek 70 µL Tip Box, Empty, 10 Racks
20045508	1	1	Illumina	TruSight® Oncology 500 DNA/RNA Automation Kit (16 indexes, 32 Samples)
NC9236303	3	1	Fisher Scientific	Sarstedt Inc 5 mL SCTUBE 15.3X92FCBSG/PK1000 (Sarstedt Part Number 62.611)
NC0308763	2	1	Fisher Scientific	Sarstedt Inc 1.5 mL SC MTUBE CAP PCR/PK1000 (Sarstedt Part Number 72.692)
BP2818500	1	1	Fisher Scientific	Ethanol, Absolute (200 Proof), Molecular Biology Grade, Fisher BioReagents™
10-977-023	1	1	Fisher Scientific	Invitrogen™ UltraPure™ DNase/RNase-Free Distilled Water

Table 2. Consumables purchases. Number of pieces used per run refers to the number of tip racks, reaction vessels, etc. that are needed for the maximum number of samples being processed in a batch. The table above shows the consumables required for a 12 DNA 12 RNA sample batch.

Approximate Time for Processing

Customer Portal Section	Operation	4 DNA / 4 RNA Batch Run Time (hr:min)	8 DNA / 8 RNA Batch Run Time (hr:min)	12 DNA / 12 RNA Batch Run Time (hr:min)
1	Reagent Aliquoting- Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA	0:13	0:16	0:19
1	Sample Processing- Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA	2:14	2:27	2:58
3	Reagent Aliquoting- End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR	0:38	0:55	0:48
3	Sample Processing- End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR	4:48	6:40	8:22
5 and 6	Reagent Aliquoting- First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section and the Amplify Enriched Libraries and Clean Up Amplified Enriched Library	1:06	1:21	1:37
5 and 6	Sample Processing- First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section and the Amplify Enriched Libraries and Clean Up Amplified Enriched Library	16:40	19:48	22:44
8	Reagent Aliquoting- Normalize Libraries	0:30	0:34	0:38
8	Sample Processing- Normalize Libraries	1:21	2:10	3:04
All	Total Instrument Run Time	27:30	36:11	42:10

Tip: If running RNA samples, the most efficient way to operate the App Template is to perform the first section (Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA) and then start the third section (End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR) and allow it to run overnight so that the fifth and sixth sections (First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section and the Amplify Enriched Libraries and Clean Up Amplified Enriched Library) can be started on the morning of Day 2. This in turn should allow the operator to complete the batch by the end of Day 3. If running only DNA samples the Biomek NGeniuS system should be able to complete the batch by the end of Day 2 by allowing overnight runs.

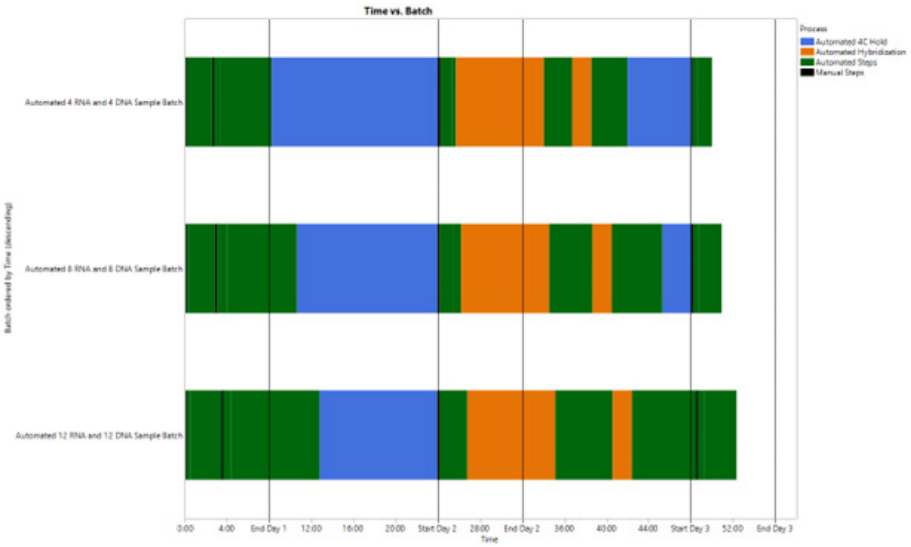


Figure 4. Sample batch run time estimates.

User Guide Workflow Instructions

The following instructions assume that the user has access to the tenant on the Biomek NGeniuS Portal Software and that the Illumina TruSight® Oncology 500 DNA/RNA Automation Kit App Template has been downloaded to that tenant. If either of these two things need to be done, consult the Biomek NGeniuS Next Generation Library Prep System Instructions for Use (Beckman Reference Number C432122AB or later version) chapters Getting Started with the Biomek NGeniuS Portal Software, System Administration, and Configuring Apps in the Biomek NGeniuS Portal Software.

For detailed instructions on how to operate the Biomek NGeniuS instrument, please refer to the Biomek NGeniuS Next Generation Library Prep System Instructions for Use (Beckman Reference Number C432122AB or later version) chapter, **Starting a Batch Run**.

Preparing the Batch

1. Navigate to the Biomek NGeniuS Portal Software using either Google Chrome or Microsoft Edge.
2. In the login screen, enter your email and password. Click "Sign In" to sign into the Biomek NGeniuS Portal Software.

Figure 5. Login Screen with entry for email address (1), password (2), Forgot Password Link (3), Sign In button (4), and Help Link (5) highlighted.

3. If you are working with more than one lab, click on the appropriate lab in the "What Lab are you working with?" screen.
4. The Biomek NGeniuS Portal Software will open on the Biomek NGeniuS Portal Software User Interface. Click the "Create" button to open the Configure Run Screen

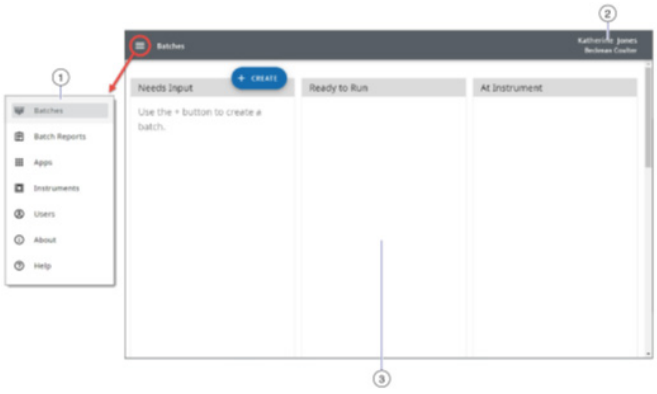


Figure 6. DBiomek NGeniuS Portal Software User Interface with the Main Menu (1), the Active User Information (2) and the Workspace (3) highlighted.

5. In the Configure Run Screen, enter a name for the batch and the number of samples included in the batch. The Illumina TruSight® Oncology 500 DNA/RNA Automation Kit App Template supports running any number of samples between 4 and 24, with any combination of DNA and RNA samples being possible.

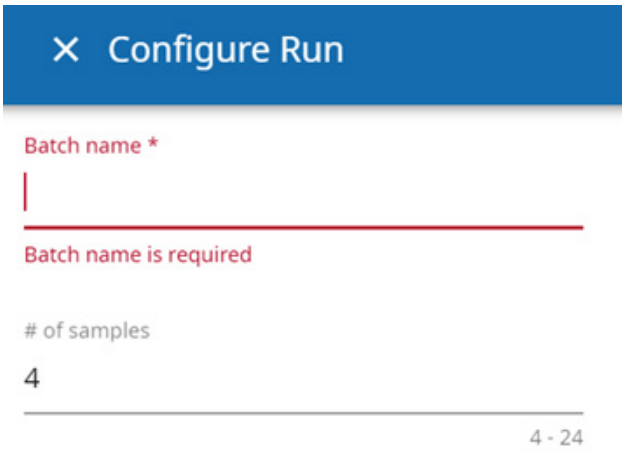


Figure 7. Configure Run field.

Tip: When running the Illumina TruSight® Oncology 500 DNA/RNA Automation Kit App Template consider the sample number listed in the Biomek NGeniuS Customer Portal (4-24) to be the number of wells that are being processed in a single batch. Some users refer to "samples" as both the DNA and RNA components isolated from a single source, which can cause confusion about the total number of samples that can be processed.

6. In the Settings Screen, select quality of the RNA being processed if running RNA samples. This will specify the RNA fragmentation parameters utilized in the "Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA" section. If using fragmented RNA or RNA derived from FFPE material, select the Low Quality RNA option. If using intact RNA or RNA derived from cell lines, select the High Quality RNA option. This parameter applies ONLY to samples designated as RNA in the Sample Data Sheet (described below) and will have no impact on samples designed as DNA. Refer to the Illumina TruSight® Oncology 500 Reference Guide for more information concerning sample quality.

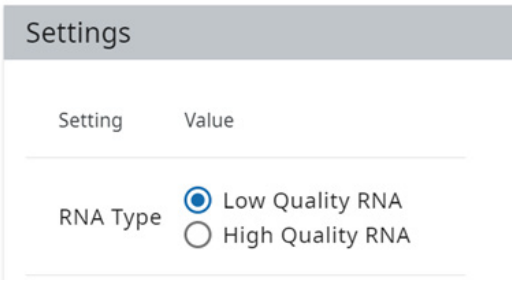


Figure 8. Settings field.

7. Proceed to the Sections field of the Configure Run Screen. The "Start at Section" drop down allows the user to choose where the batch starts. If the batch includes RNA samples, select the "Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA" section as the starting section.

If running a DNA Only batch, select the “End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR” section as the starting section. The blue slider to the left will allow the user to select how much of the App Template is being run.

If starting with RNA samples in the “Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA” section, the slider appears as a blue dot due to the presence of the off-system section “Add Sheared DNA to LPRV”, which forces the batch to wait while the user adds the sheared DNA samples to the reaction vessel containing the purified double-stranded cDNA.

If running a DNA Only batch, the slider appears as a blue dot due to the presence of the “Retrieve ALS Product from Cold Storage 3” off-system section and the batch cannot continue automatically until the off-system section has been completed.

If the batch is started at the “First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two” section, the slider will allow the user to select the “Amplify Enriched Libraries and Clean Up Amplified Enriched Library” section as well and additional user intervention will not be required until arriving at the “Quantify Libraries Off System” off-system section. Once completed, the batch can proceed to the Normalize Libraries section.

fragmented RNA or RNA derived from FFPE material, select the Low Quality RNA option. If using intact RNA or RNA derived from cell lines, select the High Quality RNA option. This parameter applies ONLY to samples designated as RNA in the Sample Data Sheet (described below) and will have no impact on samples designed as DNA. Refer to the Illumina TruSight® Oncology 500 Reference Guide for more information concerning sample quality.

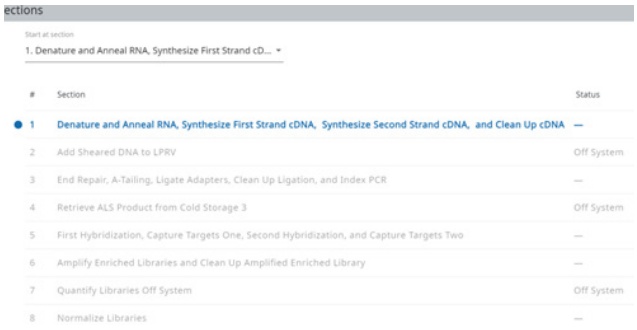


Figure 9. Sections field.

8. Proceed to the Sample Data field of the Configure Run Screen. Click the “Download Sample Data Template” csv file and fill in the Sample_ID (the name of the sample) and the Index (UP01 – UP16) associated with that sample. Click the Upload button to import the completed Sample Data Template into the Biomek NGeniuS Portal Software.

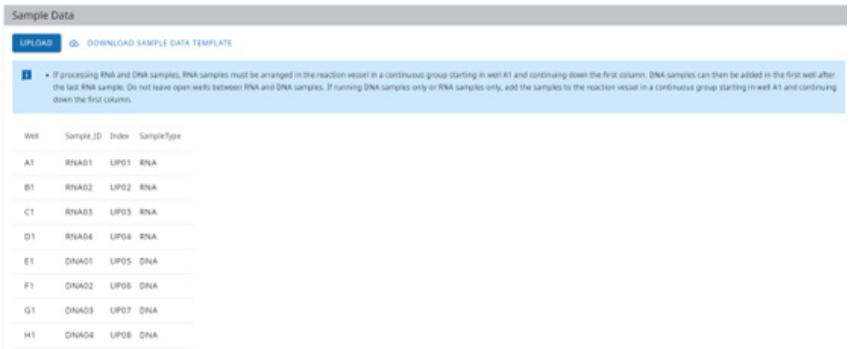


Figure 10. Sample Data field after Sample Data Template upload.

Tip: RNA samples are always loaded first onto the input reaction vessel when running mixed input DNA & RNA runs. Additionally, sheared DNA samples are added to the input reaction vessel starting in the next contiguous empty well. This sample layout is enforced to ensure the most efficient use of the Biomek NGeniuS system and cannot be altered. If a Sample Data Sheet contains a non-continuous sample layout the batch will not be allowed to proceed.

9. If the Batch settings pass the error checking built into the Biomek NGeniuS Portal Software (in this case ensuring there are no duplicate Sample_IDs or the Index names have been properly formatted "UP01" instead of "UP1"), the "Ready to Run" button will appear. Click the button to send the batch from the "Needs Input" column to the "Ready to Run" column in the Biomek NGeniuS Portal Software.

10. Click the "Download Work Aid" link on the Batch Card to download the Work Aid for this batch. The Work Aid contains information such as the volume of reagents needed for the batch, reagent preparation information, where to load the reagents on the carousel and/or bulk reservoir, and how to set up the input reaction vessel containing the sheared DNA samples for processing the batch. Even if following a lab-developed SOP, it is advisable to download the Work Aid at this point.

Warning: Once the batch has been retrieved on a Biomek NGeniuS instrument and setup has begun, the Work Aid can no longer be downloaded from the customer portal.

Prepare Reagents

Reagents will need to be prepared in accordance with the Illumina TruSight® Oncology 500 Reference Guide for the sections of the App Template being performed. The volume of reagents and preparation instructions are included in the Work Aid and are dependent on the number of samples being processed. For convenience, the volumes required for each batch size have been included in Appendix A of this guide. Using the Work Aid, prepare the reagents needed for the batch run.

Quantifying Nucleic Acids and Shearing DNA

The Illumina TruSight® Oncology 500 Reference Guide (Document# 1000000067621 v10) details all requirements for DNA sample quality and input mass. Additionally, the Illumina TruSight® Oncology 500 Reference Guide provides detailed instructions on how to fragment the DNA samples using various Covaris® platforms. This section does not replace the Illumina TruSight® Oncology 500 Reference Guide and users should consult the TruSight® Oncology 500 Reference Guide for this information. The information is presented in this guide for reference purposes only and has been modified for the Biomek NGeniuS system.

DNA and RNA Input Recommendations

The TruSight® Oncology 500 assay is optimized to prepare libraries from gDNA that are fragmented to 90–250 bp.

Use a minimum of 40 ng of DNA/RNA input with the TruSight® Oncology 500 Kit assay. Inputs lower than 40 ng can decrease library yield and quality. Quantify the input nucleic acids before beginning the protocol. To obtain sufficient nucleic acid material, isolate nucleic acid from a minimum of 2 mm3 of FFPE tissue. Use a fluorometric quantification method that uses DNA binding dyes such as AccuClear™ (DNA) or QuantiFluor® (RNA).

Assess Sample Quality

For optimal performance, assess DNA sample quality before using the TruSight® Oncology 500 assay. DNA samples can be assessed using the Illumina FFPE QC Kit (Illumina Part Number WG-321-1001). Use DNA samples that result in a delta Cq value ≤ 5. Samples with a delta Cq > 5 might result in decreased assay performance.

RNA samples can be assessed using Advanced Analytical Technologies Fragment Analyzer™ (Standard Sensitivity RNA Analysis Kit) or Agilent Technologies 2100 Bioanalyzer (Agilent RNA 6000 Nano Kit). Use RNA samples that result in a DV200 value of ≥ 20%. Using samples with a DV200 value < 20% might result in decreased assay performance.

DNA Shearing Recommendations

The TruSight® Oncology 500 assay is optimized using the Covaris® E220 evolution™, LE220, or ME220 Focused-ultrasonicator with the parameters provided in Fragment gDNA on page 18 of the Illumina TruSight® Oncology 500 Reference Guide. Fragment size distribution can vary due to differences in sample quality and the sonication instrument used for fragmentation.

Use the following guidelines for shearing.

- Avoid excessive bubbles or an air gap in the shearing tube as it can lead to incomplete shearing.
  - Load the gDNA into the Covaris® tube slowly to avoid creating bubbles.
  - Centrifuge the Covaris® tube to collect the sample at the bottom of the tube before shearing.
- If using the LE220 Covaris® instrument, fill unused Covaris® 8 microTUBE Strip wells with 52 µL water to provide substance for optimal machine performance.
- [Optional] Assess fragment size distribution of sheared samples using the Agilent DNA 1000 Kit with the Agilent Bioanalyzer 2100 (Agilent Part Numbers 5067–1504 and G2940CA).

Fragment gDNA

This process fragments gDNA to a 90–250 bp fragment size using the Covaris® E220 evolution, LE220, or ME220 Focused-ultrasonicator. Covaris® shearing generates dsDNA fragments with 3′ and 5′ overhangs.

Consumables

- TEB (Tris-EDTA Buffer)
- Covaris® 8 microTUBE Strip with foil seals
- Biomek NGeniuS Reaction Vessel (RV)

Preparation

1. Remove TEB from 2°C to 8°C storage. Bring to room temperature. Invert to mix.
2. Turn on and set up the Covaris® instrument according to manufacturer guidelines. The instrument requires ~1 hour to de-gas.
3. Thaw purified gDNA samples at room temperature.
4. Invert to mix.
5. Refer to DNA/RNA Input Recommendations to qualify and quantify samples.
6. Dilute a minimum of 40 ng of each purified DNA sample in TEB for a final volume of 12 µL.

Procedure

1. Add 12 µL of each diluted, purified DNA sample into a Covaris® 8 microTUBE Strip.
2. Add 40 µL TEB to each sample.
3. Pipette to mix.
4. Fill any unused Covaris® 8 microTUBE Strip wells with 52 µL water.
5. Seal the microTUBE Strip with the foil seal.
6. Centrifuge briefly.
7. If using the Covaris® E220 evolution, LE220, or ME220 model, fragment the gDNA using the following settings.

Setting	E220evolution	LE220	ME220
Peak Incident Power	175 watts	450 watts	50 watts
Duty Factor	10%	30%	30%
Cycles per Burst	200	200	1000
Treatment Time	280 seconds	250 seconds	10 seconds
Temperature	7°C	7°C	12°C
Intensifier	Yes	Not applicable	Not applicable
Other	Intensifier	Not applicable	Wave guide
Pulse Reports	Not applicable	Not applicable	20
Average Power	Not applicable	Not applicable	15 watts

Table 3. Covaris® shearing settings.

8. Centrifuge tube strip briefly to collect droplets.

9. Transfer 50 µL of each sheared gDNA sample to the corresponding wells of the Library Prep Reaction Vessel (LP RV).

Tip: Use a 20 µL pipette with fine tips when transferring sheared gDNA sample to the LP RV. Pipette 20 µL, an additional 20 µL, and then the remaining 10 µL.

SAFE STOPPING POINT

If you are stopping, transfer the sheared gDNA to a PCR plate, apply Microseal 'B' to the plate, and briefly centrifuge at 280 × g. Store at -25°C to -15°C for up to 7 days.

Preparing the Biomek NGeniuS System

1. Power up the Biomek NGeniuS instrument by pressing the power button.
2. The Biomek NGeniuS instrument will then prepare to run a Self Check. The user will need to do the following:
   - Ensure the deck is clear of labware.
   - Place an empty carousel on the deck.
   - Highlight and select Start using the Software Navigation Dial.
   - Follow the instructions on the Head-Up Display to complete the Self Check.

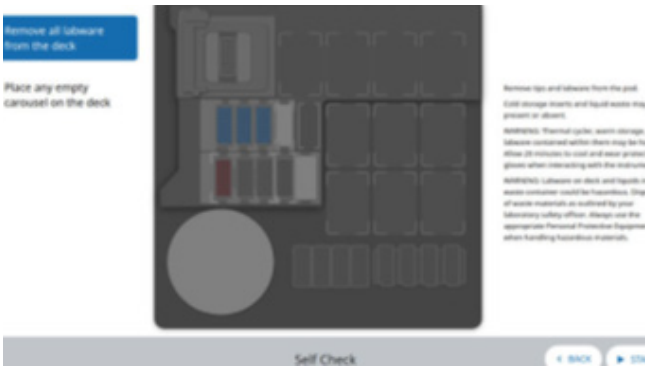


Figure 11. Self Check Opening Screen.

Figure 12. Self Check Succeeded Message.

3. Navigate to the Batches option in the Main Menu using the Software Navigation Dial.

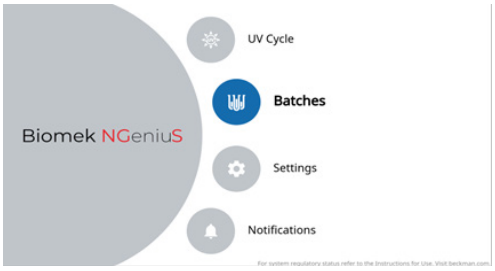


Figure 13. Main menu with Batches selected.

4. Select the batch run to be executed using the Software Navigation Dial.

Running the Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA Section (Section 1)

This section is only run if running a batch with RNA samples. The Biomek NGeniuS Setup Screen displayed on the Head-Up Display will guide the user through the setup process, highlighting positions on the Deck Diagram to be interacted with and actively checking the positions utilizing the Biomek NGeniuS DeckOptix® system to make sure that labware is placed correctly on the deck.

The following table details the reagents to be used in this section and details their preparation. Please consult the Work Aid for volumes required, which will vary based on the number of samples being processed. Preparation instructions have been reproduced from the Illumina TruSight® Oncology 500 Reference Guide. This section does not replace the Illumina TruSight® Oncology 500 Reference Guide and users should consult that for this information. The information is presented in this guide for reference purposes only and has been modified for the Biomek NGeniuS system.

Reagent	Kit Tube	Storage Conditions	Preparation Instructions	Reformat or Bulk Reagent?
EPH3	0.5 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
FSM	0.5 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
RVT	0.5 mL Sarstedt Tube	-25°C to -15°C	Keep on ice. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
SSM	2.0 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Invert 10 times to mix. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
RSB	15 mL Falcon Tube	2°C to 8°C or -25°C to -15°C	Bring to room temperature. If stored at -25°C to -15°C, thaw at room temperature and vortex before use.	Reformat tube. Add reagent to a 1.5ml conical Sarstedt tube as instructed by the Work Aid.
SPB	15 mL Falcon Tube	2°C to 8°C	Bring to room temperature for at least 30 minutes. Vortex for 1 minute before use.	Bulk reservoir. Add reagent to the bulk reservoir as instructed by the Work Aid.
80% EtOH	N/A	Room Temperature	Dilute 100% EtOH in nuclease-free water to a final concentration of 80%.	Bulk reservoir. Add reagent to the bulk reservoir as instructed by the Work Aid.

Table 4. Reagents required by the Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA Section.

Warning: 80% Ethanol should be freshly prepared prior to the run.

To run the Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA Section, follow the steps below:

1. Utilizing the Biomek NGeniuS Setup Screen and the Work Aid, place the labware and reagents on the Biomek NGeniuS System to prepare for aliquoting reagents into Reagent Storage.
2. Once all positions have a green check mark close the door. Use the Navigation Dial to highlight and select START.
3. The door will then lock and the reagent aliquoting process will begin. The instrument will first check the Liquid Waste container to ensure there is sufficient space for the liquid waste generated by the run. If the system detects that the liquid waste container is not empty, the aliquoting process is stopped and an error message appears instructing you to empty the liquid waste.
4. The instrument will then check the volumes of the reagents in the bulk reservoirs. If insufficient liquid is determined in one of the bulk reservoir reagents, the aliquoting process is stopped and an error message appears informing you of the problematic bulk reservoirs.
5. The instrument will then proceed to the carousel-based reagents. The carousel will be identified using the carousel barcode, after which the tube presence will be confirmed. The instrument will then confirm that the caps have been removed. If the carousel camera fails to detect a tube, the tube must be manually confirmed. After visually ensuring a tube with the proper reagent is loaded, highlight the tube(s) in question on the HUD and perform a long press on the Software Navigation Dial to manually confirm the tube(s).
6. The instrument will then proceed to confirm the identity of the reagents. If all the reagent tubes are successfully identified, aliquoting the reagents to reagent storage will begin automatically.

Note: In cases where the camera is unable to positively identify an item, or identifies a cap on a tube, the Head-Up Display will show a graphic of the carousel with the failed locations marked. To see more information about the failure, highlight each item's location. To replace the incorrectly placed tubes with correct ones or to remove caps, highlight and select Unlock. Open the door and replace the tube, then close the door. Highlight and select START. If the camera fails to identify a tube that you visually confirm as correct, highlight the position on the Head-Up Display, select and hold the location to manually confirm it.

Note: A manual confirmation can be removed by highlighting the position and selecting and holding the software navigation dial button again.

7. The system determines aliquot locations and senses liquid levels in the reagent tubes. Only the required amount of reagent volume will be transferred to the reagent storage module. The system displays an estimated time of completion for the aliquot process. If there is insufficient volume in a reagent tube, the tube is skipped and the aliquot process proceeds. The estimated time will be shortened if insufficient liquid is in the tube for transfer.

8. Once aliquoting has begun, prepare the Input Reaction Vessel (Input RV) by adding the RNA samples to it as directed by the Work Aid.

9. Once reagent aliquoting is complete, the door will unlock, and the user will be instructed to remove the carousel containing the reagents from Biomek NGeniuS instrument. Store the remaining reagents at their appropriate storage conditions. Place the Input Reaction Vessel containing the RNA samples on the instrument as directed by the Head-Up Display, close the door, and select "Start" to begin running the section.

10. Once the section is completed the ds cDNA samples will be stored in the thermal cycler at 4°C until the user highlights and selects the "RETRIEVE SAMPLES" button on the Instrument User Interface, at which point the user can retrieve the LP RV in the usual manner through the Teardown Procedure.

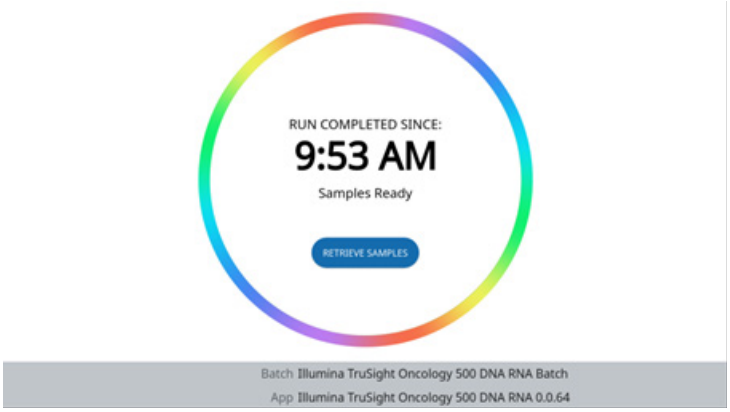


Figure 14. Run completed dialog.

11. At the end of the Teardown Procedure, the user will need to highlight and select the "LIQUID WASTE EMPTIED" button to confirm to the Biomek NGeniuS instrument that the liquid waste has been emptied. The user will receive a message that "Teardown is complete". The user will then highlight and select the "BACK TO HOME" button to return to the Main Menu.

Tip: Consult Appendix A of this guide to determine which reagents are needed for the next sections so that they can begin thawing while Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA Section is being run if proceeding on with the run the same day.

Add Sheared DNA to LPRV (Off System Section 2)

This off-system section allows the user to add sheared DNA to the LPRV for processing in the End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR Section if running a batch with both RNA and DNA samples.

Running the End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR Section (Section 3)

The Biomek NGeniuS Setup Screen displayed on the Head-Up Display will guide the user through the setup process, highlighting positions on the Deck Diagram to be interacted with and actively checking the positions utilizing the Biomek NGeniuS DeckOptix® system to make sure that labware is placed correctly on the deck.

The following table details the reagents to be used in this section and details their preparation. Please consult the Work Aid for volumes required, which will vary based on the number of samples being processed. Preparation instructions have been reproduced from the Illumina TruSight® Oncology 500 Reference Guide. This section does not replace the Illumina TruSight® Oncology 500 Reference Guide and users should consult that for this information. The information is presented in this guide for reference purposes only and has been modified for the Biomek NGeniuS system.

Reagent	Kit Tube	Storage Conditions	Preparation Instructions	Reformat or Bulk Reagent?
ERA1-A	0.5 mL Sarstedt Tube	-25°C to -15°C	Keep on ice. Centrifuge briefly, and then pipette to mix.	No. Kit tubes are loaded directly onto the Biomek NGeniuS System.
ERA1-B	0.5 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Centrifuge briefly, and then pipette to mix. If precipitates are present, warm the tube in your hands, and then pipette to mix until the crystals dissolve.	No. Kit tubes are loaded directly onto the Biomek NGeniuS System.
ALB1	2.0 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex ≥ 10 seconds to resuspend. Centrifuge briefly.	No. Kit tubes are loaded directly onto the Biomek NGeniuS System.
LIG3	0.5 mL Sarstedt Tube	-25°C to -15°C	Keep on ice. Centrifuge briefly, and then pipette to mix.	No. Kit tubes are loaded directly onto the Biomek NGeniuS System.
STL	2.0 mL Sarstedt Tube	-25°C to -15°C	Thaw and bring to room temperature. Vortex to resuspend. Centrifuge briefly	No. Kit tubes are loaded directly onto the Biomek NGeniuS System.
UMI1	0.5 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex ≥ 10 seconds to resuspend. Centrifuge briefly.	No. Kit tubes are loaded directly onto the Biomek NGeniuS System.
SUA1	0.5 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex ≥ 10 seconds to resuspend. Centrifuge briefly.	No. Kit tubes are loaded directly onto the Biomek NGeniuS System.
EPM	2.0 mL Sarstedt Tube	-25°C to -15°C	Thaw on ice. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
UPxx	0.5 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex to resuspend.Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
RSB	15 mL Falcon Tube	2°C to 8°C or -25°C to -15°C	Bring to room temperature.If stored at -25°C to -15°C, thaw at room temperature and vortex before use.	Reformat tube. Add reagent to a 1.5ml conical Sarstedt tube as instructed by the Work Aid.
SUA1	0.5 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex ≥ 10 seconds to resuspend. Centrifuge briefly.	No. Kit tubes are loaded directly onto the Biomek NGeniuS System.
SUA1	0.5 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex ≥ 10 seconds to resuspend. Centrifuge briefly.	No. Kit tubes are loaded directly onto the Biomek NGeniuS System.

Table 5. Reagents used by the End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR Section.

Note: UMI1 is required for DNA samples and SUA1 is required for RNA samples. If running a batch with no RNA samples present, SUA1 will not be listed in the Work Aid as it is not required.

Warning: If running a batch with both DNA and RNA samples, ensure that the sheared DNA samples have been added to the RV containing the purified double-stranded cDNA from the previous section as outlined in the Work Aid.

Warning: 80% Ethanol should be freshly prepared prior to the run.

To run the End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR Section, follow the steps below:

1. Utilizing the Biomek NGeniuS Setup Screen and the Work Aid, place the labware and reagents on the Biomek NGeniuS System to prepare for aliquoting reagents into Reagent Storage.
2. Once all positions have a green check mark close the door. Use the Navigation Dial to highlight and select START.
3. The door will then lock and the reagent aliquoting process will begin. The instrument will first check the Liquid Waste container to ensure there is sufficient space for the liquid waste generated by the run. If the system detects that the liquid waste container is not empty, the aliquoting process is stopped and an error message appears instructing you to empty the liquid waste.
4. The instrument will then check the volumes of the reagents in the bulk reservoirs. If insufficient liquid is determined in one of the bulk reservoir reagents, the aliquoting process is stopped and an error message appears informing you of the problematic bulk reservoirs.
5. The instrument will then proceed to the carousel-based reagents. The carousel will be identified using the carousel barcode, after which the tube presence will be confirmed. The instrument will then confirm that the caps have been removed. If the carousel camera fails to detect a tube, the tube must be manually confirmed. After visually ensuring a tube is loaded, highlight the tube(s) in question on the HUD and perform a long press on the Software Navigation Dial to manually confirm the tube(s).
6. The instrument will then proceed to confirm the identity of the reagents. If all the reagent tubes are successfully identified, aliquoting the reagents to reagent storage will begin automatically.

Note: In cases where the camera is unable to positively identify an item, or identifies a cap on a tube, the HUD will show a graphic of the carousel with the failed locations marked. To see more information about the failure, highlight each item's location. To replace the incorrectly placed tubes with correct ones or to remove caps, highlight and select Unlock. Open the door and replace the tube, then close the door. Highlight and select START. If the camera fails to identify a tube that you visually confirm as correct, highlight the position on the HUD, select and hold the location to manually confirm it.

Note: A manual confirmation can be removed by highlighting the position and selecting and holding the software navigation dial button again.

7. The system determines aliquot locations and senses liquid levels in the reagent tubes. Only the required amount of reagent volume will be transferred to the reagent storage module. The system displays an estimated time of completion for the aliquot process. If there is insufficient volume in a reagent tube, the tube is skipped and the aliquot process proceeds. The estimated time will be shortened if insufficient liquid is in the tube for transfer.

8. Once aliquoting has begun, prepare the Library Prep Reaction Vessel (LP RV) by adding the sheared DNA samples to it as directed by the Work Aid unless this has been done already.

9. Once reagent aliquoting is complete, the door will unlock, and the user will be instructed to remove the carousel containing the reagents from Biomek NGeniuS instrument. Store the remaining reagents at their appropriate storage conditions.

10. Add the LP RV containing the sheared DNA samples to the RV Exchange position as shown by the Biomek NGeniuS Setup Screen. Close the door. Highlight and select START to begin running the section.

11. Once the section is completed the libraries will be stored in the thermal cycler at 4°C until the user highlights and selects the "RETRIEVE SAMPLES" button on the Instrument User Interface, at which point the user can retrieve the HYB1 RV in the usual manner through the Teardown Procedure.

Retrieve ALS Product from Cold Storage 3 (Off System Section 4)

Following the completion of the End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR Section, the Biomek NGeniuS instrument will split the amplified library samples (ALS) product in preparation for running the First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section. 20 µL of ALS product will remain in the HYB1 RV and stored in the thermal cycler at 4°C until the user highlights and selects the "RETRIEVE SAMPLES" button on the Instrument User Interface, at which point the user can retrieve the HYB1 RV in the usual manner through the Teardown Procedure.

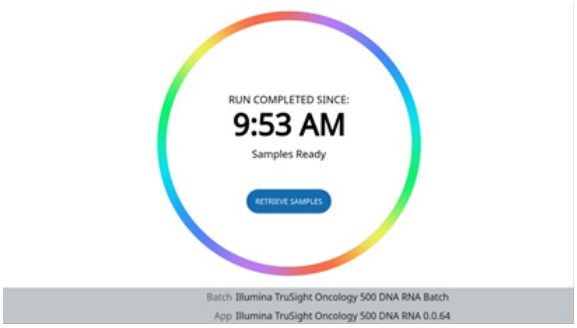


Figure 15. Run completed dialog.

The remaining 30 µL of ALS product that can be used as an archival stock is stored at 4°C in the Cold Storage 3 position (the right-most of the blue Cold Storage locations), as shown in the figure below.

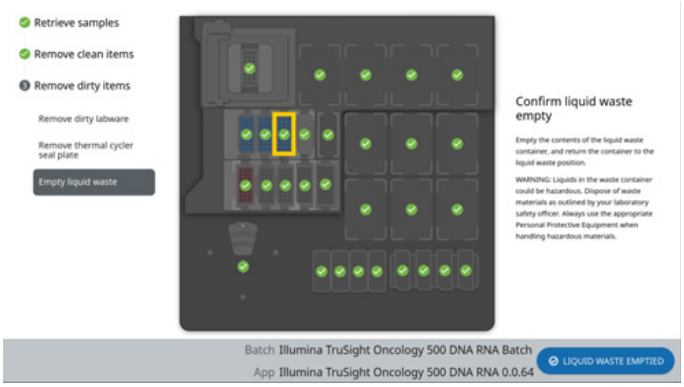


Figure 16. Teardown Procedure with Cold Storage 3 position highlighted on the Deck Diagram.

Instead of discarding the remaining ALS product, the user is free to save the product as an archival stock if desired. The archival stock should be removed from the Biomek NGeniuS instrument in 16 hours or less to prevent the accumulation of condensation. Beckman Coulter and Illumina make no claims about the stability of the archival stock under these storage conditions.

At the end of the Teardown Procedure, the user will need to highlight and select the "LIQUID WASTE EMPTIED" button to confirm to the Biomek NGeniuS instrument that the liquid waste has been emptied. The user will receive a message that Teardown is complete. The user will then highlight and select the "BACK TO HOME" button to return to the Main Menu.

Figure 17. Teardown complete dialog.

Note: If stopping at the end of the End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR Section, apply Microseal 'B' to the HYB1 RV (as well as the residual ALS stock from Cold Storage 3 if it is being retained), and briefly centrifuge at 280 × g. Store at -25°C to -15°C for up to 30 days.

Running the First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section and the Amplify Enriched Libraries and Clean Up Amplified Enriched Library Section (Sections 5 and 6)

The Biomek NGeniuS Setup Screen displayed on the Head-Up Display will guide the user through the setup process, highlighting positions on the Deck Diagram to be interacted with and actively checking the positions utilizing the Biomek NGeniuS DeckOptix® system to make sure that labware is placed correctly on the deck.

The following table details the reagents to be used in this section and details their preparation. Please consult the Work Aid for volumes required, which will vary based on the number of samples being processed. Preparation instructions have been reproduced from the Illumina TruSight® Oncology 500 Reference Guide. This section does not replace the Illumina TruSight® Oncology 500 Reference Guide and users should consult that for this information. The information is presented in this guide for reference purposes only and has been modified for the Biomek NGeniuS system.

Reagent	Kit Tube	Storage Conditions	Preparation Instructions	Reformat or Bulk Reagent?
OPD2	0.5 mL Sarstedt Tube with a yellow cap.	-25°C to -15°C	Thaw to room temperature. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
OPR1	0.5 mL Sarstedt Tube with a red cap.	-25°C to -15°C	Thaw to room temperature. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
TCA1	2.0 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
TCB1	2.0 mL Sarstedt Tube	2°C to 8°C	Thaw to room temperature. Centrifuge briefly, then pipette to mix. Inspect for precipitates. If present, warm the tube in your hands, then pipette to mix until the crystals dissolve.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
EE2	2.0 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
EEW	Bottle	-25°C to -15°C	Thaw to room temperature. Vortex for 1 minute to resuspend.	Bulk reservoir. Add reagent to the bulk reservoir as instructed by the Work Aid.
ET2	0.5 mL Sarstedt Tube	2°C to 8°C	Bring to room temperature. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
HP3	0.5 mL Sarstedt Tube	2°C to 8°C	Bring to room temperature. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
SMB	15 mL Falcon Tube	2°C to 8°C	Bring to room temperature for 30 minutes. If the bead pellet is present, pipette up and down to release the pellet, and then vortex to resuspend.	**Reformat Tubes**. Add reagent to the 5 mL Sarstedt tubes as instructed by the Work Aid for **SMB1** and **SMB2.**
EPM	2.0 mL Sarstedt Tube	-25°C to -15°C	Thaw on ice. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
PPC3	0.5 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex to resuspend Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
RSB	15 mL Falcon Tube	2°C to 8°C or -25°C to -15°C	Bring to room temperature. If stored at -25°C to -15°C, thaw at room temperature and vortex before use.	**Bulk reservoir**. Add reagent to the bulk reservoir as instructed by the Work Aid.
SPB	15 mL Falcon Tube	2°C to 8°C	Bring to room temperature for at least 30 minutes. Vortex for 1 minute before use.	**Reformat Tube**. Add reagent to the 5 mL Sarstedt tube as instructed by the Work Aid.
80% EtOH	N/A	Room Temperature	Dilute 100% Ethanol in nuclease-free water to a final concentration of 80%.	Bulk reservoir. Add reagent to the bulk reservoir as instructed by the Work Aid.

Table 6. Reagents used in the First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section and the Amplify Enriched Libraries and Clean Up Amplified Enriched Library Section.

Note: OPD2 is required for DNA samples and OPR1 is required for RNA samples. If running a batch with no RNA samples present, OPR1 will not be listed in the Work Aid as it is not required.

Warning: 80% Ethanol should be freshly prepared prior to the run.

To run the First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section and the Amplify Enriched Libraries and Clean Up Amplified Enriched Library Section, follow the steps below:

1. Return to the Biomek NGeniuS Portal Software and log in. The batch card for the run will now be found in the Needs Input column of the Workspace.
2. Select the batch card and click the Ready to Run button. This will move the batch card to the Ready to Run column of the Workspace and generate the Work Aid.
3. Return to the Biomek NGeniuS instrument. Navigate to the Batches menu. Highlight and select the batch to continue the run.
4. Utilizing the Biomek NGeniuS Setup Screen and the Work Aid, place the labware and reagents on the Biomek NGeniuS System to prepare for aliquoting reagents into Reagent Storage.
5. Once all positions have a green check mark, close the door, highlight and select START.
6. The door will then lock and the reagent aliquoting process will begin. The instrument will first check the Liquid Waste container to ensure there is sufficient space for the liquid waste generated by the run. If the system detects that the liquid waste container is not empty, the aliquoting process is stopped and an error message appears instructing you to empty the liquid waste.
7. The instrument will then check the volumes of the reagents in the bulk reservoirs. If insufficient liquid is determined in one of the bulk reservoir reagents, the aliquoting process is stopped and an error message appears informing you of the problematic bulk reservoirs.
8. The instrument will then proceed to the carousel-based reagents. The carousel will be identified, after which the tube presence will be confirmed. The instrument will then confirm that the caps have been removed. If the carousel camera fails to detect a tube, the tube must be manually confirmed. After visually ensuring a tube is loaded, highlight the tube(s) in question on the HUD and perform a long press on the Software Navigation Dial to manually confirm the tube(s).
9. The instrument will then proceed to confirm the identity of the reagents. If all the reagent tubes are successfully identified, aliquoting the reagents to reagent storage will begin automatically.

Note: In cases where the camera is unable to positively identify an item, or identifies a cap on a tube, the HUD will show a graphic of the carousel with the failed locations marked. To see more information about the failure, highlight each item's location. To replace the incorrectly placed tubes with correct ones or to remove caps, highlight and select Unlock. Open the door and replace the tube, then close the door. Highlight and select START. If the camera fails to identify a tube that you visually confirm as correct, highlight the position on the HUD, select and hold the location to manually confirm it.

Note: A manual confirmation can be removed by highlighting the position and selecting and holding the software navigation dial button again.

10. The system determines aliquot locations and senses liquid levels in the reagent tubes. Only the required amount of reagent volume will be transferred to the reagent storage module. The system displays an estimated time of completion for the aliquot process. If there is insufficient volume in a reagent tube, the tube is skipped and the aliquot process proceeds. The estimated time will be shortened if insufficient liquid is in the tube for transfer.

11. Once reagent aliquoting is complete, the door will unlock, and the user will be prompted to remove the carousel containing the reagents from Biomek NGeniuS instrument. Store the remaining reagents in their appropriate storage conditions.

12. Add the HYB1 RV containing the ALS product used for the First Hybridization to the RV Exchange position as shown by the Biomek NGeniuS Setup Screen. Close the door. Highlight and select START to begin running the section.

13. When the sections have completed, the enriched libraries will be located in the Purified Library Reaction Vessel (PL RV) and stored in the thermal cycler at 4°C until the user highlights and selects the "RETRIEVE SAMPLES" button on the Instrument User Interface, at which point the user can retrieve the PL RV in the usual manner through the Teardown Procedure.

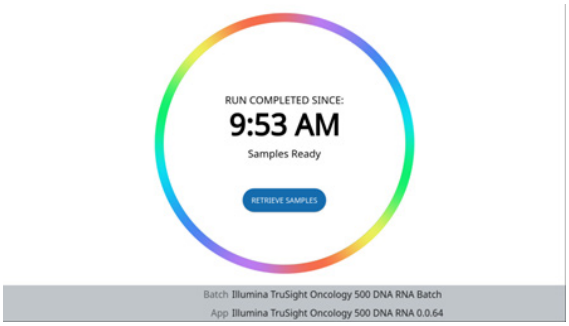


Figure 18. Run completed dialog.

At the end of the Teardown Procedure, the user will need to highlight and select the "LIQUID WASTE EMPTIED" button to confirm to the Biomek NGeniuS instrument that the liquid waste has been emptied. The user will receive a message that Teardown is complete. The user will then highlight and select the "BACK TO HOME" button to return to the Main Menu.

Quantify Libraries (Off System Section)

The Illumina TruSight® Oncology 500 Reference Guide (Document# 1000000067621 v10) details how to perform the optional Quantify Libraries procedure. This section does not replace the Illumina TruSight® Oncology 500 Reference Guide and users should consult that for this information. The information is presented in this guide for reference purposes only.

Accurately quantify to make sure that there is sufficient library available for clustering on the flow cell. Use a fluorometric quantification method to assess the quantity of enriched libraries before library normalization. Efficient bead-based library normalization requires ≥ 3 ng/µL of each library. The AccuClear Ultra High Sensitivity dsDNA Quantitation Kit (Biotium Catalog Number 31029) has been demonstrated to be effective for quantifying libraries in this protocol.

[AccuClear] Recommended Guidelines

1. Combine 6 µL DNA standard with 44 µL RSB to dilute DNA standard to 3 ng/µL.
2. Use RSB as blank.
3. Run the diluted AccuClear DNA standard and blanks in triplicate.
4. Run libraries in single replicates.
5. Determine the average relative fluorescence unit (RFU) for DNA standard and blank.
6. Calculate the Normalized Standard RFU using the following formula:

Average Standard RFU – Average Blank RFU = Normalized Standard RFU

7. Calculate the Normalized RFU for each library using the following formula:

Library RFU – Average Blank RFU = Normalized RFU for each library

Assess Quantity

Assess the resulting Normalized RFU for each library against the following criteria.

Fluorescence Measurement	Recommendation
≤ Average Blank RFU	Repeat library preparation and enrichment if purified DNA sample meets quantity and quality specifications.
> Average Blank RFU (and) < Normalized Standard RFU	Proceed to Normalize Libraries. Using libraries with RFU below the Normalized Standard RFU might not yield adequate sequencing results to confidently call variants that can be present in the sample.
≥ Normalized Standard RFU	Proceed to Normalize Libraries.

Table 7. Quantity Assessment Table

Running the Normalize Libraries Section

The Biomek NGeniuS Setup Screen displayed on the Head-up Display will guide the user through the setup process, highlighting positions on the Deck Diagram to be interacted with and actively checking the positions utilizing the Biomek NGeniuS DeckOptix® system to make sure that labware is placed correctly on the deck.

The following table details the reagents to be used in this section and details their preparation. Please consult the Work Aid for volumes required, which will vary based on the number of samples being processed. Preparation instructions have been reproduced from the Illumina TruSight® Oncology 500 Reference Guide. This section does not replace the Illumina TruSight® Oncology 500 Reference Guide and users should consult that for this information. The information is presented in this guide for reference purposes only and has been modified for the Biomek NGeniuS system.

Reagent	Kit Tube	Storage Conditions	Preparation Instructions	Reformat or Bulk Reagent?
EE2	2.0 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System
HP3	0.5 mL Sarstedt Tube	2°C to 8°C	Bring to room temperature. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
LNB1	5.0 mL Sarstedt Tube	2°C to 8°C	Bring to room temperature for at least 30 minutes. Pipette LNB1 pellet up and down to resuspend.	No. Kit vials are loaded directly onto the Biomek NGeniuS System
LNA1	5.0 mL Sarstedt Tube	-25°C to -15°C	Thaw to room temperature. Vortex to resuspend. Centrifuge briefly.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.
LNS1	5.0 mL Sarstedt Tube	2°C to 8°C	Bring to room temperature. Vortex to resuspend. Centrifuge briefly.	No. Kit vials loaded directly onto the Biomek NGeniuS System.
LNW1	5.0 mL Sarstedt Tube	2°C to 8°C	Bring to room temperature. Vortex to resuspend.	No. Kit vials are loaded directly onto the Biomek NGeniuS System.

Table 8. Reagents used in the Normalize Libraries Section.

To run the Normalize Libraries Section, follow the steps below:

1. Return to the Biomek NGeniuS Portal Software and log in. The batch card for the run will now be found in the Needs Input column of the Workspace.
2. Select the batch card and click the Ready to Run button. This will move the batch card to the Ready to Run column of the Workspace and generate the Work Aid.
3. Return to the Biomek NGeniuS instrument. Navigate to the Batches menu. Highlight and select the batch to continue the run.
4. Utilizing the Biomek NGeniuS Setup Screen and the Work Aid, place the labware and reagents on the Biomek NGeniuS System to prepare for aliquoting reagents into Reagent Storage.
5. Once all positions have a green check mark, close the door, highlight and select START.
6. The door will then lock and the reagent aliquoting process will begin. The instrument will first check the Liquid Waste container to ensure there is sufficient space for the liquid waste generated by the run. If the system detects that the liquid waste container is not empty, the aliquoting process is stopped and an error message appears instructing you to empty the liquid waste.
7. The instrument will then check the volumes of the reagents in the bulk reservoirs. If insufficient liquid is determined in one of the bulk reservoir reagents, the aliquoting process is stopped and an error message appears informing you of the problematic bulk reservoirs.

8. The instrument will then proceed to the carousel-based reagents. The carousel will be identified, after which the tube presence will be confirmed. The instrument will then confirm that the caps have been removed. If the carousel camera fails to detect a tube, the tube must be manually confirmed. After visually ensuring a tube is loaded, highlight the tube(s) in question on the HUD and perform a long press on the Software Navigation Dial to manually confirm the tube(s).

9. The instrument will then proceed to confirm the identity of the reagents. If all of the reagent tubes are successfully identified, aliquoting the reagents to reagent storage will begin automatically.

Note: In cases where the camera is unable to positively identify an item, or identifies a cap on a tube, the HUD will show a graphic of the carousel with the failed locations marked. To see more information about the failure, highlight each item's location. To replace the incorrectly placed tubes with correct ones or to remove caps, highlight and select Unlock. Open the door and replace the tube, then close the door. Highlight and select START. If the camera fails to identify a tube that you visually confirm as correct, highlight the position on the HUD, select and hold the location to manually confirm it.

Note: A manual confirmation can be removed by highlighting the position and selecting and holding the software navigation dial button again.

10. The system determines aliquot locations and senses liquid levels in the reagent tubes. Only the required amount of reagent volume will be transferred to the reagent storage module. The system displays an estimated time of completion for the aliquot process. If there is insufficient volume in a reagent tube, the tube is skipped and the aliquot process proceeds. The estimated time will be shortened if insufficient liquid is in the tube for transfer.

11. Once reagent aliquoting is complete, the door will unlock, and the user will be instructed to remove the carousel containing the reagents from Biomek NGeniuS instrument. Store the remaining reagents at their appropriate storage conditions.

12. Add the PL RV containing the ALS product used for the First Hybridization to the RV Exchange position as shown by the Biomek NGeniuS Setup Screen. Close the door. Highlight and select START to begin running the section.

13. Once the section is completed the libraries will be stored in the thermal cycler at 4°C until the user highlights and selects the "RETRIEVE SAMPLES" button on the Instrument User Interface, at which point the user can retrieve the NL RV in the usual manner through the Teardown Procedure.

Note: Only 20 µL of libraries in the PL RV are utilized for the Normalize Libraries section. The remaining volume (approximately 8-10 µL depending on if the Quantify Libraries procedure is performed), will be transferred to a Reaction Vessel at the Cold Storage 3 position and can be retained by the user at this section. Consult the "Retrieve ALS Product from Cold Storage 3 (Off System Section)" part of this guide if more information on the location of the Cold Storage 3 position is required.

When the section has completed, the normalized libraries will be located in the Neutralized Library Reaction Vessel (NL RV) and stored in the thermal cycler at 4°C until the user highlights and selects the "RETRIEVE SAMPLES" button on the Instrument User Interface, at which point the user can retrieve the NL RV in the usual manner through the Teardown Procedure.

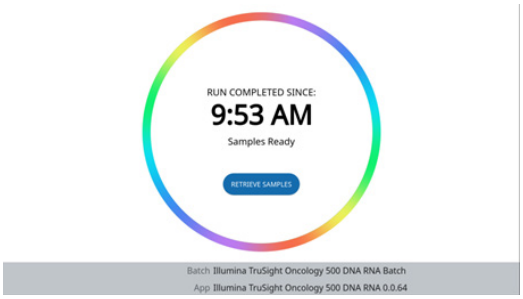


Figure 19. Run completed dialog.

At the end of the Teardown Procedure, the user will need to highlight and select the "LIQUID WASTE EMPTIED" button to confirm to the Biomek NGeniuS instrument that the liquid waste has been emptied. The user will receive a message that Teardown is complete. The user will then highlight and select the "BACK TO HOME" button to return to the Main Menu.

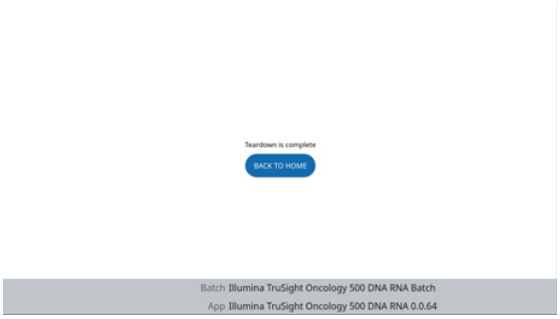


Figure 20. Teardown complete dialog.

The batch run is now complete. The batch report will be generated and made available on the Biomek NGeniuS Portal Software. To access the batch report, perform the following steps:

1. Navigate to the Biomek NGeniuS Portal Software using either Google Chrome or Microsoft Edge.
2. In the login screen, enter your email and password. Click "Sign In" to sign into the Biomek NGeniuS Portal Software.

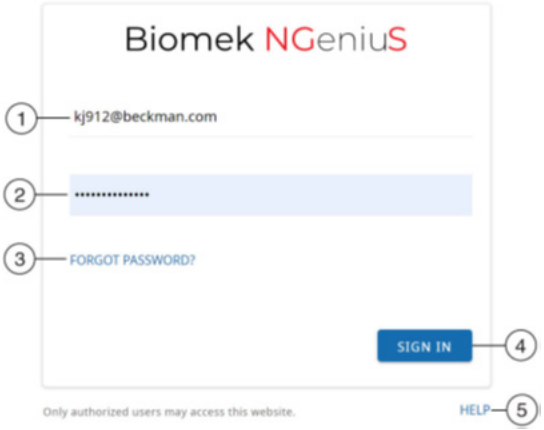


Figure 21. Login Screen with entry for email address (1), password (2), Forgot Password Link (3), Sign In button (4), and Help Link (5) highlighted.

3. If you are working with more than one lab, click on the appropriate lab in the "What Lab are you working with?" screen.

4. The Biomek NGeniuS Portal Software will open on the Biomek NGeniuS Portal Software User Interface. Click the Main Menu and select "Batch Reports".

5. Batches are listed in the order of the time and date it was created in Biomek NGeniuS Portal Software, with the most recent batch appearing first. Find the desired batch by its meta data.

6. Click the download button (with the cloud icon) in the Report column that corresponds to the desired batch. Depending on your Internet connection speed, the PDF rendering time will vary.

7. Open the report and/or save it to the location of your choice. Batch reports are automatically named as follows: <BatchName>-BatchReport.pdf.

Preparing the Libraries for Sequencing (Off System)

Consult Illumina NextSeq 500 and NextSeq 550 Sequencing Systems Denature and Dilute Libraries Guide (Illumina Reference Number 15048776 v18 or later versions) for details on how to pool, dilute, and denature the libraries for sequencing.

Troubleshooting

If an error occurs with the Biomek NGeniuS system, please consult the Biomek NGeniuS Next Generation Library Prep System Instructions for Use (Beckman Reference Number C432122AB or later versions) chapter, **Troubleshooting & Handling Errors.** Depending on the nature of the error, it may be necessary to contact Beckman Coulter Life Sciences. Please have the instrument's instance number (which should be on a sticker placed on the instrument by the field service engineer on installation) or the instrument's serial number (located on the back of the instrument) ready to facilitate Beckman Coulter Service.

Appendix A

This appendix is designed to provide the user with information to help facilitate running the Illumina TruSight® Oncology 500 DNA Automation Kit App Template on the Biomek NGeniuS Next Generation Library Prep System.

Reagents Needed Based on Batch Run Size

The following table represents the volume of reagents needed for each section based on the batch size being processed. Please note that the volume here assumes that the First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section and the Amplify Enriched Libraries and Clean Up Amplified Enriched Library Section are being run sequentially. If the user chooses to stop between these sections some reagent volumes will not be accurate.

Labware	Kit Vial	Kit Vial	Kit Vial	Kit Vial	1.5ml conical Sarstedt Reformat tube	Bulk Reservoir	Bulk Reservoir
RNA Samples	**EPH3 Volume (µL)**	**FSM Volume (µL)**	**RVT Volume (µL)**	**SSM Volume (µL)**	**cDNA_RSB Volume (µL)**	**cDNA 80%_ EtOH Volume (µL)**	**cDNA SPB Volume (µL)**
1	22.9	36.2	23.6	48.3	83.6	3100.0	1085.0
2	35.9	62.4	37.2	82.5	147.3	3200.0	1170.0
3	48.8	71.8	38.2	108.8	210.9	3300.0	1255.0
4	65.7	81.3	39.3	135.0	274.6	3400.0	1340.0
5	74.6	90.7	40.3	161.3	338.2	3500.0	1425.0
6	65.7	100.2	41.4	187.5	401.8	3600.0	1510.0
7	92.5	109.6	42.4	213.8	465.5	3700.0	1595.0
8	101.4	119.0	43.4	240.0	545.1	3800.0	1680.0
9	110.3	128.5	44.5	266.3	600.8	3900.0	1765.0
10	119.3	138.0	45.6	292.5	656.4	4000.0	1850.0
11	128.2	147.4	46.6	318.8	712.0	4100.0	1935.0
12	137.1	156.9	47.7	345.0	767.7	4200.0	2020.0
13	146.0	166.3	48.7	371.3	823.3	4300.0	2105.0
14	155.0	175.8	49.8	397.5	879.0	4400.0	2190.0
15	163.9	185.2	50.8	423.8	934.6	4500.0	2275.0
16	172.8	194.7	51.9	450.0	990.2	4600.0	2360.0
17	181.7	204.1	52.9	476.3	1045.9	4700.0	2445.0
18	190.7	213.6	53.9	502.5	1101.5	4800.0	2530.0
19	199.6	223.0	55.0	528.8	1157.2	4900.0	2615.0
20	208.5	232.5	56.1	555.0	1212.8	5000.0	2700.0
21	217.4	241.9	57.1	581.3	1268.4	5100.0	2785.0
22	226.4	251.4	58.2	607.5	1324.1	5200.0	2870.0
23	235.3	260.8	59.2	633.8	1379.7	5300.0	2955.0
24	244.2	270.3	60.3	660.0	1435.4	5400.0	3040.0

Table 9. Volumes used in the Denature and Anneal RNA, Synthesize First Strand cDNA, Synthesize Second Strand cDNA, and Clean Up cDNA Section.

Labware	Kit Vial	Kit Vial	Kit Vial	Kit Vial	Kit Vial	Kit Vial	Kit Vial	Kit Vial	Kit Vial	1.5ml conical Sarstedt Reformat tube	Bulk Reservoir	Bulk Reservoir
Batch Size	ERA1-A Volume (µL)	ALB1 Volume (µL)	LIG3 Volume (µL)	UMI1 Volume (µL)	SUA1 Volume (µL)	EPM Volume (µL)	UP01-UP16 (Volume (µL Per Sample)	ERA1-B Volume (µL)	STL Volume (µL)	RSB Volume (µL)	80%	cDNA SPB Volume (µL)
4	41.1	292.0	51.0	67.0	67.0	114.0	12.1	77.8	61.0	169.7	3400.0	1420.0
5	44.7	355.0	56.3	77.5	77.5	135.0	12.1	86.8	66.3	207.1	3500.0	1525.0
6	48.2	418.0	61.5	88.0	88.0	156.0	12.1	95.9	71.5	244.6	3600.0	1630.0
7	51.8	481.0	66.8	98.5	98.5	177.0	12.1	104.9	76.8	282.0	3700.0	1735.0
8	55.3	544.0	72.0	109.0	109.0	198.0	12.1	114.0	82.0	335.4	3800.0	1840.0
9	58.8	607.0	77.3	119.5	119.5	219.0	12.1	123.1	87.3	364.8	3900.0	1945.0
10	62.4	670.0	82.5	130.0	130.0	240.0	12.1	132.1	92.5	394.3	4000.0	2050.0
11	65.9	733.0	87.8	140.5	140.5	261.0	12.1	141.2	97.8	423.7	4100.0	2155.0
12	69.5	796.0	93.0	151.0	151.0	282.0	12.1	150.2	103.0	453.1	4200.0	2260.0
13	73.0	859.0	98.3	161.5	161.5	303.0	12.1	159.3	108.3	482.5	4300.0	2365.0
14	76.6	922.0	103.5	172.0	172.0	324.0	12.1	168.3	113.5	512.0	4400.0	2470.0
15	80.1	985.0	108.8	182.5	182.5	345.0	12.1	177.4	118.8	541.4	4500.0	2575.0
16	83.6	1048.0	114.0	193.0	193.0	366.0	12.1	186.4	124.0	570.8	4600.0	2680.0
17	87.2	1111.0	119.3	203.5	203.5	387.0	12.1	195.5	129.3	600.2	4700.0	2785.0
18	90.7	1174.0	124.5	214.0	214.0	408.0	12.1	204.6	134.5	629.7	4800.0	2890.0
19	94.3	1237.0	129.8	224.5	224.5	429.0	12.1	213.6	139.8	659.1	4900.0	2995.0
20	97.8	1300.0	135.0	235.0	235.0	450.0	12.1	222.7	145.0	688.5	5000.0	3100.0
21	101.4	1363.0	140.3	245.5	245.5	471.0	12.1	231.7	150.3	717.9	5100.0	3205.0
22	104.9	1426.0	145.5	256.0	256.0	492.0	12.1	240.8	155.5	747.4	5200.0	3310.0
23	108.5	1489.0	150.8	266.5	266.5	513.0	12.1	249.8	160.8	776.8	5300.0	3415.0
24	112.0	1552.0	156.0	277.0	277.0	534.0	12.1	258.9	166.0	806.2	5400.0	3520.0

Table 10. Reagent Volumes for the End Repair, A-Tailing, Ligate Adapters, Clean Up Ligation, and Index PCR Section.

Note: Reagent volumes requested for RNA or DNA specific reagents will correlate to the number of each type of sample in the batch rather than the total batch size. Smallest batch size possible for this section is 4 samples.

Labware	Kit Vial	Kit Vial	Kit Vial	Kit Vial	Kit Vial	Kit Vial	Kit Vial	Kit Vial	Kit Vial
Batch Size	**EPM Volume (µL)**	**OPD2 Volume (µL)**	**OPR1 Volume (µL)**	**TCA1 Volume (µL)**	**HP3 Volume (µL)**	**ET2 Volume (µL)**	**PPC3 Volume (µL)**	**TCB1 Volume (µL)**	**EE2 Volume (µL)**
4	114.0	57.0	57.0	134.0	27.9	92.0	41.0	186.0	96.1
5	135.0	67.5	67.5	155.0	35.7	102.5	46.3	217.5	172.2
6	156.0	78.0	78.0	176.0	43.6	113.0	51.5	249.0	248.2
7	177.0	88.5	88.5	197.0	95.4	123.5	56.8	280.5	348.3
8	198.0	99.0	99.0	218.0	97.7	134.0	62.0	312.0	389.9
9	219.0	109.5	109.5	239.0	99.9	144.5	67.3	343.5	431.6
10	240.0	120.0	120.0	260.0	102.1	155.0	72.5	375.0	473.2
11	261.0	130.5	130.5	281.0	104.3	165.5	77.8	406.5	514.8
12	282.0	141.0	141.0	302.0	106.6	176.0	83.0	438.0	556.4
13	303.0	151.5	151.5	323.0	108.8	186.5	88.3	469.5	598.0
14	324.0	162.0	162.0	344.0	111.0	197.0	93.5	501.0	639.7
15	345.0	172.5	172.5	365.0	113.2	207.5	98.8	532.5	681.3
16	366.0	183.0	183.0	386.0	115.5	218.0	104.0	564.0	722.9
17	387.0	193.5	193.5	407.0	117.7	228.5	109.3	595.5	764.5
18	408.0	204.0	204.0	428.0	119.9	239.0	114.5	627.0	806.2
19	429.0	214.5	214.5	449.0	122.1	249.5	119.8	658.5	847.8
20	450.0	225.0	225.0	470.0	124.4	260.0	125.0	690.0	889.4
21	471.0	235.5	235.5	491.0	126.6	270.5	130.3	721.5	931.0
22	492.0	246.0	246.0	512.0	128.8	281.0	135.5	753.0	972.6
23	513.0	256.5	256.5	533.0	131.0	291.5	140.8	784.5	1014.3
24	534.0	267.0	267.0	554.0	133.3	302.0	146.0	816.0	1055.9

Table 11. Reagent Volumes for the First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section and the Amplify Enriched Libraries and Clean Up Amplified Enriched Library Section Part 1

Note: Reagent volumes requested for RNA or DNA specific reagents will correlate to the number of each type of sample in the batch rather than the total batch size. Smallest batch size possible for these sections is 4 samples.

Labware	5 mL Reformat Tube	5 mL Reformat Tube	5 mL Reformat Tube	Bulk Reservoir	Bulk Reservoir	Bulk Reservoir
Batch Size	**EPCR_SPB Volume (µL)**	**SMB1 Volume (µL)**	**SMB2 Volume (µL)**	**EEW Volume (µL)**	**RSB Volume (µL)**	**80% EtOH Volume (µL)**
4	723.0	896	896	4900.0	2474.4	3400.0
5	841.3	1057.5	1057.5	5500.0	2718.0	3500.0
6	959.5	1219	1219	6100.0	2961.6	3600.0
7	1077.8	1380.5	1380.5	6700.0	3205.2	3700.0
8	1292.0	1630	1630	7300.0	3448.8	3800.0
9	1402.3	1787.5	1787.5	7900.0	3692.4	3900.0
10	1512.5	1945	1945	8500.0	3936.0	4000.0
11	1622.8	2102.5	2102.5	9100.0	4179.6	4100.0
12	1733.0	2260	2260	9700.0	4423.2	4200.0
13	1843.3	2417.5	2417.5	10300.0	4666.8	4300.0
14	1953.5	2575	2575	10900.0	4910.4	4400.0
15	2063.8	2732.5	2732.5	11500.0	5154.0	4500.0
16	2174.0	2890	2890	12100.0	5397.6	4600.0
17	2284.3	3047.5	3047.5	12700.0	5641.2	4700.0
18	2394.5	3205	3205	13300.0	5884.8	4800.0
19	2504.8	3362.5	3362.5	13900.0	6128.4	4900.0
20	2615.0	3520	3520	14500.0	6372.0	5000.0
21	2725.3	3677.5	3677.5	15100.0	6615.6	5100.0
22	2835.5	3835	3835	15700.0	6859.2	5200.0
23	2945.8	3992.5	3992.5	16300.0	7102.8	5300.0
24	3056.0	4150	4150	16900.0	7346.4	5400.0

Table 12. Reagent Volumes for the First Hybridization, Capture Targets One, Second Hybridization, and Capture Targets Two Section and the Amplify Enriched Libraries and Clean Up Amplified Enriched Library Section Part 2

Note: Reagent volumes requested for RNA or DNA specific reagents will correlate to the number of each type of sample in the batch rather than the total batch size. Smallest batch size possible for these sections is 4 samples.

Labware	Kit Vial	Kit Vial	Kit Vial	Kit Vial	Kit Vial	Kit Vial
Batch Size	LNB1 Volume (µL)	LNA1 Volume (µL)	LNS1 Volume (µL)	HP3 Volume (µL)	EE2 Volume (µL)	LNW1 Volume (µL)
4	314.8	480.6	396.0	90.0	251.1	660.0
5	323.2	526.8	427.5	91.7	283.6	762.5
6	331.6	573.0	459.0	93.4	316.2	865.0
7	340.0	619.2	490.5	95.1	348.7	967.5
8	348.4	665.4	522.0	96.7	381.2	1166.0
9	356.8	711.6	553.5	98.4	413.7	1260.5
10	365.2	757.8	585.0	100.1	446.3	1355.0
11	373.6	804.0	616.5	101.8	478.8	1449.5
12	382.0	850.2	648.0	103.5	511.3	1544.0
13	390.4	896.4	679.5	105.2	543.9	1638.5
14	398.8	942.6	711.0	106.9	576.4	1733.0
15	407.2	988.8	742.5	108.6	608.9	1827.5
16	415.6	1035.0	774.0	110.3	641.4	1922.0
17	424.0	1081.2	805.5	112.0	674.0	2016.5
18	432.4	1127.4	837.0	113.7	706.5	2111.0
19	440.8	1173.6	868.5	115.4	739.0	2205.5
20	449.2	1219.8	900.0	117.1	771.6	2300.0
21	457.6	1266.0	931.5	118.8	804.1	2394.5
22	466.0	1312.2	963.0	120.5	836.6	2489.0
23	474.4	1358.4	994.5	122.2	869.1	2583.5
24	482.8	1404.6	1026.0	123.9	901.7	2678.0

Table 13. Reagent Volumes for the Normalize Libraries Section

Note: Reagent Smallest batch size possible for these sections is 4 samples.

2024-GBL-EN-105480-v1

BECKMAN COULTER Life Sciences

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