Plasma-SeqSensei™

IVD Software

Instructions for Use

April 2024

Document version: PSSSWIFU.R4
Software version: 1.3.1 onwards

en

Table of Contents

1 Intended purpose 2
2 Introduction 3
2.1 Product concept 3
2.2 Runtime environment specifications 3
2.3 Trademarks 4
2.4 Licenses 4
2.4.1 End-user license 4
2.4.2 COSMIC license (Qiagen) 4
2.4.3 GNU 4
2.5 Personal data protection 4
3 Warnings and precautions 5
3.1 Operators 5
3.2 Plasma-SeqSensei™ IVD Software product assurance 5
3.2.1 Plasma-SeqSensei™ IVD Software maintenance 6
3.2.2 Microsoft operating system 7
3.2.3 System limitations 7
3.2.4 Liability limitations 7
3.3 Computer viruses 8
3.4 Operational environment 8
4 Specifications for sequencing device 9
4.1 Data acquisition 9
5 Initial steps and program window layout 11
5.1 Initial steps 11
5.1.1 Download and installation of the software 11
5.1.2 Acquiring a license key 11
5.1.3 Starting the program 12
5.1.4 Closing the program 12
5.2 Overview of icons and functions 12
5.3 Overview of the user interface 15
5.3.1 Run Planning module 15
5.3.2 Data Analysis module 17
5.3.3 Reporting module 18
6 Plasma-SeqSensei™ IVD Software modules 20
6.1 Run Planning module 20
6.2 Data Analysis module 29
6.3 Reporting module 33
7 Reports 37
8 Troubleshooting 48
9 Glossary and terminologies 52
10 Revision history 53
11 Appendix A 54

1 Intended purpose

The Plasma-SeqSensei™ IVD Software is intended to analyse Sysmex Inostics' Plasma-SeqSensei™ *Assay-Specific* IVD Kit sequencing results (Next-Generation Sequencing (NGS) data) for validity control and to detect and report mutations within the assays target regions.

The software can detect single-nucleotide variant substitutions (SNVs), insertion- and deletion-alterations, as well as deletion-insertion mutations (delins) as specified per the respective Plasma-SeqSensei™ *Assay-Specific* IVD assay.

The software is to be used with a specific Plasma-SeqSensei™ *Assay-Specific* IVD Kit to support the clinician in determining potential benefit of therapy to cancer patients. The information generated by the software should never be the sole determinant for making medical decisions. It should be complemented with other clinical findings and patient history.

The software is to be used by trained personnel in a professional laboratory environment.

Important: *The software can only be used with and according to Sysmex Inostics' Plasma-SeqSensei™* Assay-Specific *IVD Kit IFU and not with other types of products or laboratory developed tests.*

2 Introduction

These instructions for use (IFU) describe the use of the Plasma-SeqSensei™ IVD Software for the analysis of Plasma-SeqSensei™ *Assay-Specific* IVD Kits from Sysmex Inostics.

Please read this manual carefully before operating the software. Keep this manual in a safe and accessible place for future reference.

While we have taken numerous precautions to ensure the quality in the content of this manual, please contact the Service department of your authorised local Sysmex representative if you find any errors or omissions.

Modification, translation, reverse engineering, decompiling and disassembly of this manual and the software is prohibited. The creation of derivative works based on this manual, or the software is prohibited. Copying this manual or the software for purposes other than backup based on the license agreement is prohibited.

For further information, please contact your authorised local Sysmex representative.

2.1 Product concept

The Plasma-SeqSensei™ IVD Software allows the user to plan and analyse Plasma-SeqSensei™ *Assay-Specific* IVD Kit sequencing runs, and to generate reports for the analysed samples.

2.2 Runtime environment specifications

Operating Systems:	Windows® 10 (64 Bit)
CPU:	Recent CPU (Intel® Core™ i5/7 or AMD Ryzen™)
RAM:	16 GB
Storage:	10 GB free disk space
Screen resolution:	≥ 1440 x 810 pixel

2.3 Trademarks

- Company and product names in this IFU are the registered trademarks or trademarks of their respective owners.
- The fact that a trademark is not explicitly indicated in this IFU does not authorise its use.
- ™ and ® are not explicitly indicated in this IFU.

2.4 Licenses

2.4.1 End-user license

The user of the Plasma-SeqSensei™ IVD Software needs to accept the license agreement with Sysmex Inostics GmbH prior to its installation. For the full text of the *General Terms and Conditions for Software Licenses of Sysmex Inostics GmbH* refer to ► *11 Appendix A*, page 54/57.

2.4.2 COSMIC license (Qiagen)

Utilisation of the COSMIC Dynamic Software Tool Large Enterprise in the Plasma-SeqSensei™ IVD Software is covered by a license agreement with Qiagen K. K.

2.4.3 GNU

The GNU general public license policy (www.gnu.org/licenses) applies to some parts of this software. Please contact your nearest branch or sales office, if you would like to get the source code or detailed information about the software parts to which GNU general public license policy is applied. On the part of the software which is out of scope of the GNU general public license, accessing the source code, doing reverse engineering, reverse compiling, or attempting to disassemble the software is not allowed.

2.5 Personal data protection

Insofar as personal data is processed, the user shall comply with the statutory provisions on data protection.

3 Warnings and precautions

The information generated using this product should never be the sole determinant for making medical decisions. It should be complemented with other clinical findings and patient history.

Important: *The software can only be used with and according to Sysmex Inostics' Plasma-SeqSensei™* Assay-Specific *IVD Kit IFU and not with other types of products or laboratory developed tests.*

Any use outside of the specified intended purpose is considered as off-label use.

Sysmex rejects any liability for damages or losses, which result from off-label use.

3.1 Operators

The Plasma-SeqSensei™ IVD Software must only be used by trained personnel in a professional laboratory environment.

In the event of malfunction, consult the IFU. For further assistance, please contact your authorised local Sysmex representative.

3.2 Plasma-SeqSensei™ IVD Software product assurance

To ensure optimal performance of the Plasma-SeqSensei™ IVD Software, regular maintenance of the Plasma-SeqSensei™ IVD Software and the Microsoft Windows® operating system is required. The following chapters explain the required tasks.

Note: *For the installation and update process of the Plasma-SeqSensei™ IVD Software, local admin rights are required on the device.*

Important: *An active internet connection is mandatory to receive update notifications within the software.*

3.2.1 Plasma-SeqSensei™ IVD Software maintenance

The updater service of the Plasma-SeqSensei™ IVD Software checks upon start of the software if an active internet connection and a connection to the update server is available. If this connection cannot be made a button with a red cross will be visible at the bottom right side of the application screen. Clicking the button requests to verify your internet connection and firewall settings to ensure the proper execution of the software update service.

When the updater service is connecting properly, the Plasma-SeqSensei™ IVD Software will notify you, if a new update to the Plasma-SeqSensei™ IVD Software is available for download. If a new version of the Plasma-SeqSensei™ IVD Software is to be installed, you can either start the process upon start of the software or use the [update available] button on the application screen at your preferred time. You will require admin rights for this step.

In the case of an update to existing Plasma-SeqSensei™ IVD assays, only the [update available] button on the application screen will appear to start the update process. Always use the most recent Plasma-SeqSensei™ IVD Software and assay version available, if possible. After installing the newest software version, you will not be able to restore a previous version.

When new Plasma-SeqSensei™ IVD assays will be available, the software will notify you by displaying a [new assay available] button on the application screen.

A release note with details about the new version of the Plasma-SeqSensei™ IVD Software will be available for download at https://sysmex-inostics.com/products/kit-specs/ or using the button for the user manual at the bottom of the application screen.

3.2.2 Microsoft operating system

Installation, updates, and security of the Microsoft operating system are under the responsibility of the user. It is recommended that the update service is activated regularly. Please be aware that an automatically triggered restart of the operating system of Windows® updates after the installation might interrupt a running Plasma-SeqSensei™ data analysis. It is recommended to disable automatic reboots or to configure the “system active hours” in the Windows® update setting accordingly.

3.2.3 System limitations

Input file types	.fastq.gz
Sequencing devices to be used	Illumina NextSeq500/550
Minimum number of samples to be tested per run	2
Maximum number of samples to be tested per run	16 (standard kit) or 32 (with extension kit)
Maximum number of plates to be tested per run	1 (standard kit) or 2 (with extension kit)
Number of controls to be run per plate per run	2 (Positive Control [PC] and No Template Control [NTC])
Number of assays to be pooled per sequencing run	None **Note:** *This is a single test type, other assays cannot be pooled at the same time on the sequencing device.*

3.2.4 Liability limitations

Sysmex bears no liability for any failures of the Plasma-SeqSensei™ IVD Software arising from:

- not following the above-described maintenance procedures,
- using the system beyond the system limitations.

3.3 Computer viruses

It has been verified that the product you can download from www.sysmex-inostics.com is free of computer viruses.

3.4 Operational environment

For optimal performance, the Plasma-SeqSensei™ IVD Software should be installed on the same computer where the sequencing data is stored. If connected via a network, analysis times may increase, depending on upload/download speed of the network connection, or the analysis can freeze completely.

The network is expected to be managed under the full responsibility of the operator's organisation, providing effective network security that guarantees the security of its assets. Recommended security features include, but are not limited to, the authorisation of network access, restricted internet access, implementation of hardware/software technologies preventing virus/malware intrusion.

4 Specifications for sequencing device

The Plasma-SeqSensei™ Software is developed for the analysis of raw sequencing data (supplied as .fastq.gz files) obtained from the use of different Illumina sequencing devices. Only Illumina NextSeq™500 and Illumina NextSeq™550 devices are to be used in conjunction with the Plasma-SeqSensei™ IVD Software.

The following control software has been used during the development of the Plasma-SeqSensei™ IVD Kits. When using a later version of the control software, verify its functionality before starting the sequencing run. Additionally, verify the functionality of the sample sheet generated by the Plasma-SeqSensei™ IVD Software in combination with the later version of Illumina's control software.

Software Name	Manufacturer/Vendor
NextSeq™ Control Software v4.0.1.41	Illumina, Inc.
NextSeq™ Local Run Manager Software v2.4.0	Illumina, Inc.

4.1 Data acquisition

Depending on the setup of downstream processing, different paths to data acquisition can be followed. These paths all build on the sample sheet setup carried out in the run planning step for the sequencing run.

The NextSeq™ sequencing device can be run using two different paths.

1. The Local Run Manager (LRM) of the NextSeq™ device is the recommended path. The LRM can be used to generate FASTQ files directly using the sequencer, when selecting the "GenerateFASTQ Module" in the settings. Here the software performs demultiplexing, and adapter trimming using the provided sample sheet and adapter settings. The outputted FASTQ files (.fastq.gz) need to be made available to the PC running the Plasma-SeqSensei™ IVD Software.

   Important: *When using the LRM, the 'Adapter' with its sequence (found in the sample sheet, see example sample sheet in ► chapter* 6.1 Run Planning module*, step 7.c page 26/57) needs to be added to the 'Advanced Module Settings' for correct adapter trimming to take place.*

2. During the manual setup the NextSeq™ device writes the sequencing information to a run folder in the binary base call format (.bcl files) and does not perform demultiplexing or adapter trimming. Demultiplexing and adapter trimming is performed manually by the customer after sequencing using the bcl2fastq software provided by Illumina together with a bcl2fastq compatible sample sheet which can be generated during run planning. The resulting FASTQ files (.fastq.gz) need to be made available to the analysis machine on which the Plasma-SeqSensei™ IVD Software is running.

5 Initial steps and program window layout

5.1 Initial steps

- Scale of computer screen needs to be set to 100 % (if screen resolution is 1440 x 810 pixel). A warning message will appear if your screen resolution or scaling is out of the accepted range. The software will automatically direct you to the display settings if required.
- Download the program at: https://sysmex-inostics.com/products/kit-specs/.
- Acquire license key from Sysmex Inostics GmbH prior to installation of the program.
- Admin rights on the computer device need to be available.

5.1.1 Download and installation of the software

The Plasma-SeqSensei™ IVD Software can be downloaded from https://sysmex-inostics.com/products/kit-specs/ as a compressed .zip file.

Download this file to the Windows® 10 download folder, right-click on the file and then select “Extract All…”. In the following window, click on “Extract”. The folder with the extracted files will open automatically. To start the installation process, double-click on the “Plasma-SeqSensei™ IVD Software” file (do not extract the assay zip files which are also located in this folder). Follow the installation instructions as seen on the screen. Accept license agreement and enter the license key when prompted.

During the installation process admin rights are necessary for complete installation of the software.

The software needs to be installed locally on the hard drive of the device. Do not execute the .msi file (Windows® installer file) on a network drive.

5.1.2 Acquiring a license key

Upon purchase of the Plasma-SeqSensei™ IVD Kits one license key per customer will be provided by Sysmex Inostics GmbH.

5.1.3 Starting the program

Double-click on the Plasma-SeqSensei™ IVD icon on the desktop: 

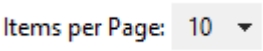
5.1.4 Closing the program

1. Close the program by either clicking on the X () on the upper right corner of the software window or on the X in the grey circle () on the lower left corner of the software window.
2. A window will appear to verify if closing of the program is desired.
3. Click on [Yes] for quitting or [No] for resuming the session in the Plasma-SeqSensei™ IVD Software.

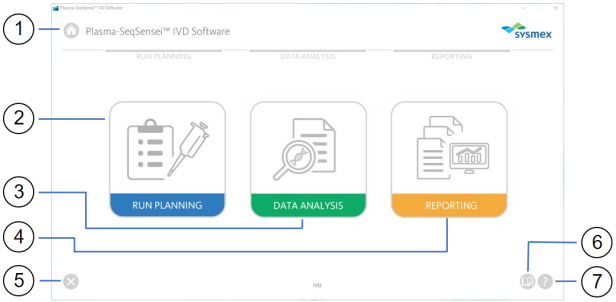
5.2 Overview of icons and functions

Icon	Function
	Return to Home Screen
	Quit software
	An active connection to updater server is unavailable
	Link to technical information and IFU for Plasma-SeqSensei™ IVD Kit and Software
	Software/Open Source Software information
	Plasma-SeqSensei™ IVD Software update available
	New Plasma-SeqSensei™ IVD assay available

Icon	Function
	Module selection tabs are always visible to change between modules.
	Run Planning module
	Data Analysis module
	Reporting module
	Deletes all input on the current page in Run Planning module.
	Go to previous page in Run Planning module.
	Go to next page in Run Planning module.
	Delete sample in Run Planning module.
	Export the specific file in Run Planning module.
	Navigate to select the desired folder/files in Data Analysis module.
	Initiate the data analysis.

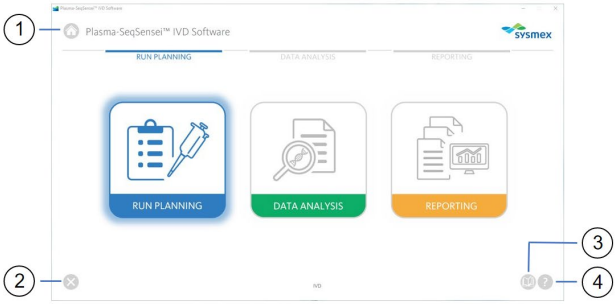
Icon	Function
	Reload page to include newest data in Reporting module.
	Back to Overview in Reporting module
	Search for analysis results in Reporting module.
	Skip to different pages for analysis results in Reporting module.
	Change number of visible items per page (5 to 50) in Reporting module.
	Export all pdf reports from selected run.
	Download bam file from selected sample.
	Download vcf file from selected sample.
	Download pdf file/report from selected sample.
	Stop and cancel the analysis process in Data Analysis module.
	Delete data of a specific run in Reporting module

5.3 Overview of the user interface

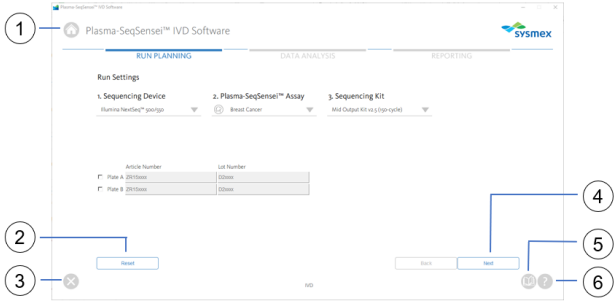


① Return to Home screen
② Run Planning module
③ Data Analysis module
④ Reporting module
⑤ Quit software
⑥ User manual
⑦ Software information

5.3.1 Run Planning module

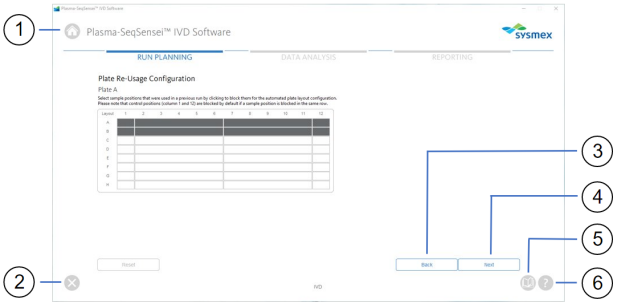


① Return to Home screen
② Quit software
③ User manual
④ Software information

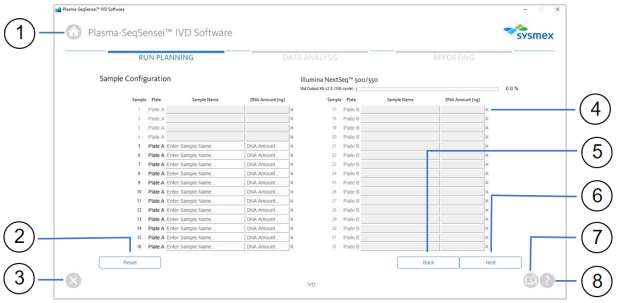


① Return to Home screen
② Reset input
③ Quit software
④ Next page
⑤ User manual
⑥ Software information

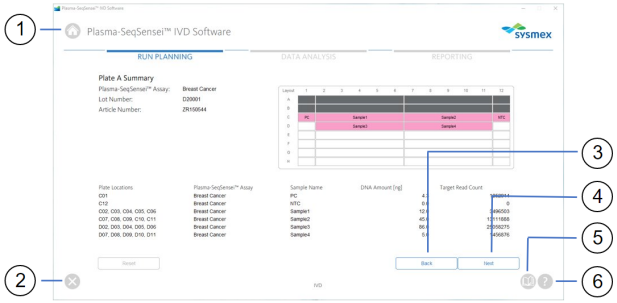
5 Initial steps and program window layout



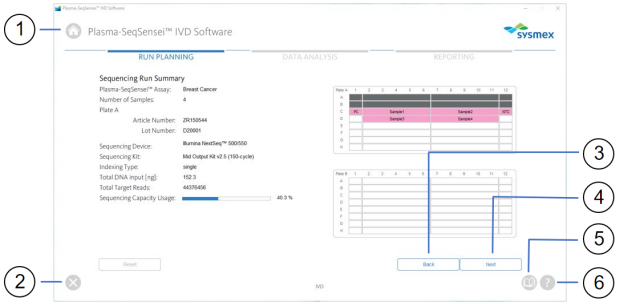
① Return to Home screen
② Quit software
③ Previous page
④ Next page
⑤ User manual
⑥ Software information



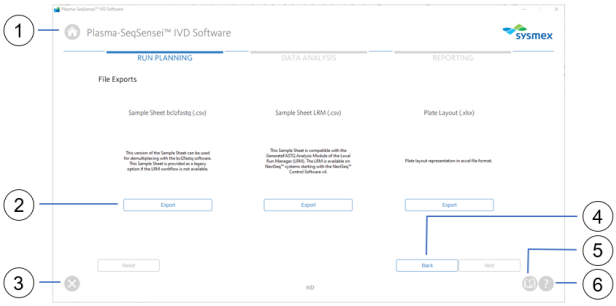
① Return to Home screen
② Reset input
③ Quit software
④ Delete sample
⑤ Previous page
⑥ Next page
⑦ User manual
⑧ Software information



① Return to Home screen
② Quit software
③ Previous page
④ Next page
⑤ User manual
⑥ Software information

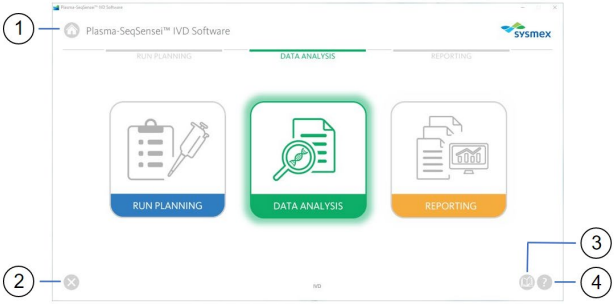


① Return to Home screen
② Quit software
③ Previous page
④ Next page
⑤ User manual
⑥ Software information

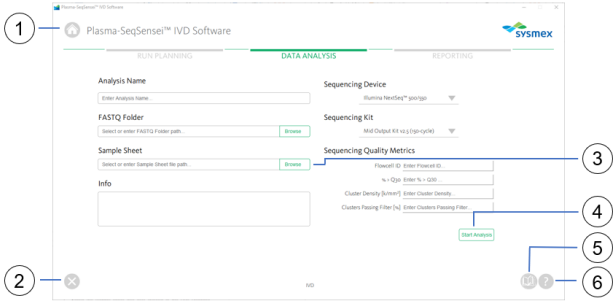


① Return to Home screen
② Export files
③ Quit software
④ Previous page
⑤ User manual
⑥ Software information

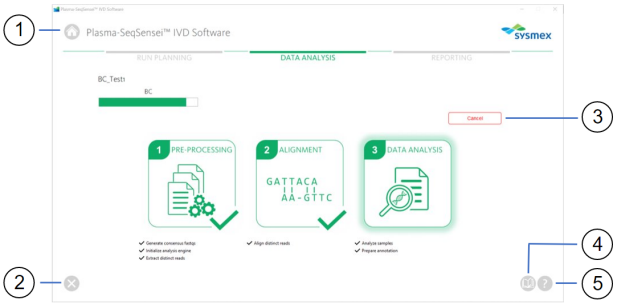
5.3.2 Data Analysis module



① Return to Home screen
② Quit software
③ User manual
④ Software information

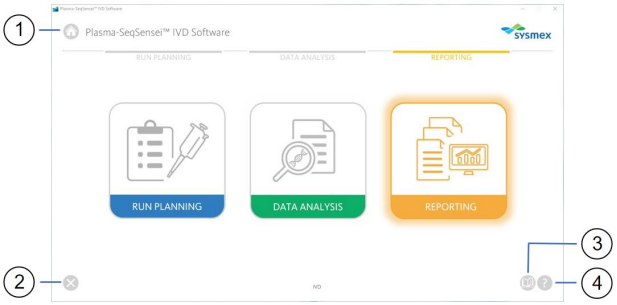


① Return to Home screen
② Quit software
③ Browse files / folders
④ Start analysis
⑤ User manual
⑥ Software information

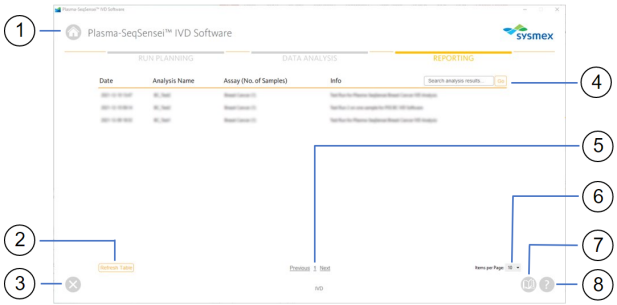


① Return to Home screen
② Quit software
③ Cancel analysis
④ User manual
⑤ Software information

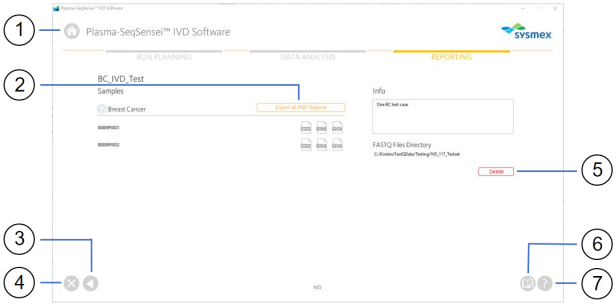
5.3.3 Reporting module



① Return to Home screen
② Quit software
③ User manual
④ Software information



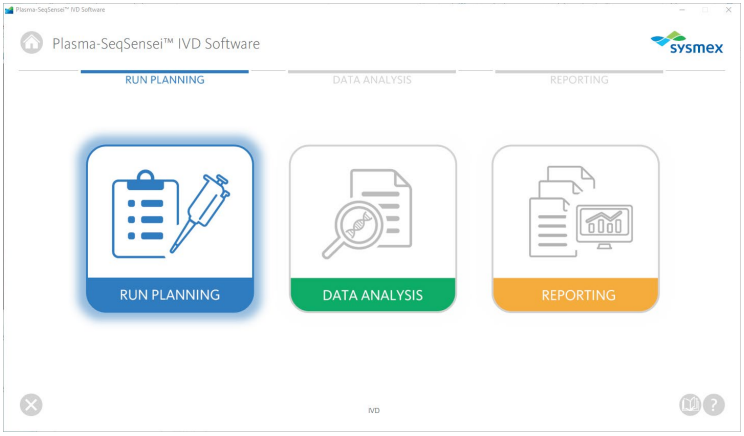
① Return to Home screen
② Refresh / reload table
③ Quit software
④ Search analysis results input field
⑤ Previous / next page
⑥ Adjust number of visible items per page
⑦ User manual
⑧ Software information



1. Return to Home screen
2. Export all pdf reports
3. Return to Reporting module overview
4. Quit software
5. Delete results
6. User manual
7. Software information

6 Plasma-SeqSensei™ IVD Software modules

6.1 Run Planning module



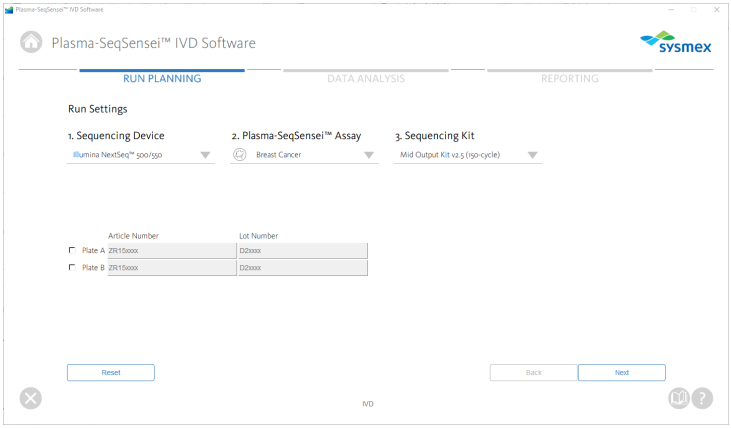
The Run Planning module (blue) is used to plan the sequencing run including:

- Assay type
- Sequencing device
- Sequencing kit usage
- Plate type (A and/or B)
- Sample number
- Sample name
- Sample concentration
- Sample location on plate
- Sample sheet generation
- Plate layout

Use the sample sheet to enable downstream demultiplexing and adapter trimming of the data in different possible set-ups. The demultiplexing and adapter trimming itself is not part of the analysis software provided here (see ► chapter *4.1 Data acquisition*, page 9/57).

You must perform the run planning after Qubit™ quantification of cfDNA samples and before starting the UID-PCR.

Note: *Qubit measurement of the samples represents a rough approximation of input DNA content to determine the sample load. The final (and possibly differing) quantification of the samples will be performed during the sequencing of the library using the internal quantifier (Quantispike).*



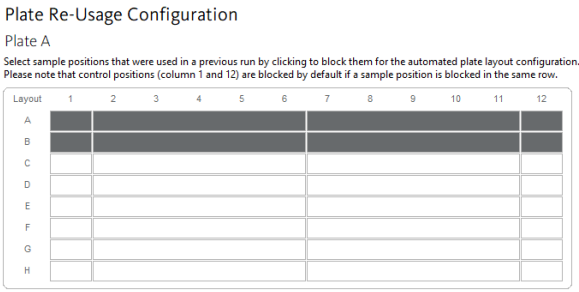
1. Click on the Run Planning module (blue).
2. Select run settings.
    a. Select a sequencing device.
    b. Select the Plasma-SeqSensei™ Assay to be used.
    c. Select the sequencing kit to be used.
    d. Select plate to be used (A and/or B) and fill in the article number (format ZR15xxxx) and Lot Number (format D2xxxx) from the Plasma-SeqSensei™ *Assay-Specific* Kit to be used.

        Note: *If more than 16 samples are processed in one run (maximum of up to 32 samples), two Plasma-SeqSensei™ IVD Kits and the Plasma-SeqSensei™ Extension IVD Kit with plate B are needed.*

        Important: *Do not use the same plate type twice for the same run!*
    e. If a mistake was made, you can delete all input by clicking on the [Reset] button on the lower left-hand side of the page.
    f. A window will appear asking to confirm resetting the page.

3. Click on [Next]. A window will appear asking if the marked plate has been used before.

   a. Select [Yes] or [No].
   b. If [Yes] was selected, a new screen will appear, where you can mark plate positions of previous runs, to prevent repeated usage of empty wells of the Plasma-SeqSensei™ Index Primer plate. These wells will not be selectable in further planning steps of this run.
   Note: *Wells in column 1 (positive control) and column 12 (no template control) will be selected automatically.*



   c. Click [Next] for the next page or go [Back] one page to change input.

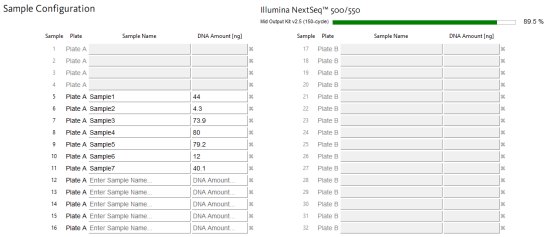
4. In the Sample Configuration table fill in the name and concentration of the samples.

   Note: *Sample names and concentrations can be easily inserted by using copy/paste from two columns of an excel sheet.*

   a. Fill in unique sample names (minimum of 2 samples) without using special characters; only alpha-numeric characters are allowed. A check on the conformance is carried out by the software.

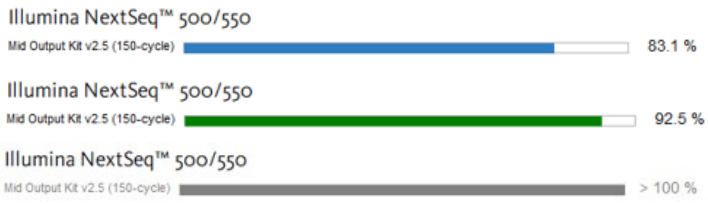
Note: *Samples are automatically sorted for the topmost empty well location of the individual plate by the software when navigating to the next page.*

b. Enter sample concentration in ng/116 µl eluate per sample (including “point” as decimal separator, e.g. 8.5 ng). Sample input must be within the assay-specific input ranges. A check on the conformance is carried out by the software when navigating to the next page.



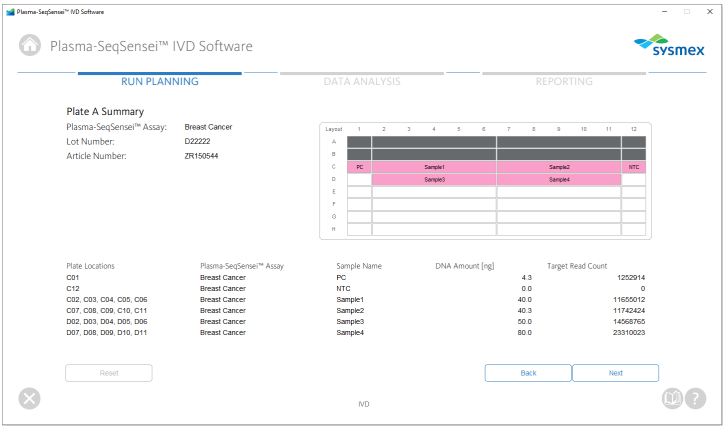
c. You can delete sample names including concentrations by clicking on the “X” at the end of the line or reset all input by clicking on the [Reset] button on the lower left-hand corner of the window.
   i. A window will open verifying the need for deleting the selected sample or entire page.
   ii. After selecting [OK] or [Yes], you can add a new sample name and concentration.

d. On the top right-hand corner, the read capacity of the selected sequencing kit is depicted (blue- and green-coloured bars are within accepted ranges, a grey bar shows overloading of selected sequencing kit).



e. Click [Next] for the next page or go [Back] one page to change the input.

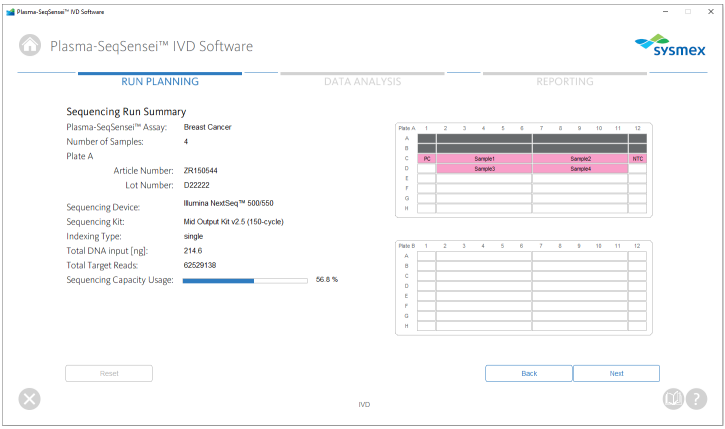
5. Revise the input by carefully checking the plate-specific Summary.



   a. Plasma-SeqSensei™ assay, lot number and article number are shown.
   b. Wells that are to be used for the run are highlighted with sample names as well as positive control (PC) and no template control (NTC) are included.
   c. Previously used wells are marked in dark grey.
   d. Plate location, Plasma-SeqSensei™ assay, sample name, sample concentration in ng and target read counts are stated in a list in the bottom half of the screen.
   e. Click [Next] for the next page or go [Back] one page to change input.

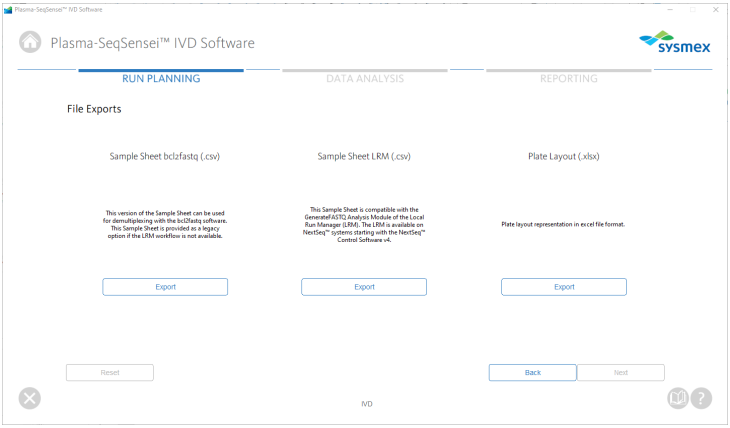
6. Revise the input by checking the Sequencing Run Summary that specifies the following parameters:

   - Plasma-SeqSensei™ assay
   - Number of samples
   - Plate used, with article and lot number
   - Sequencing device
   - Sequencing kit
   - Indexing type (automatically filled)
   - Total DNA input (in ng)
   - Total target reads (automatically filled)
   - Sequencing capacity usage (automatically filled)
   - Layout of both possible plates

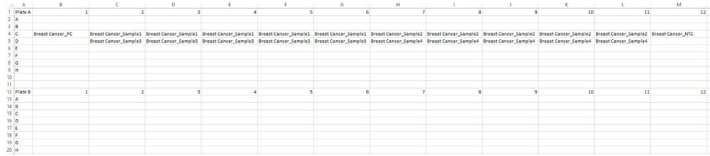


a. Click [Next] for the next page or go [Back] one page to change input.

7. On the file exports page three files will be displayed. They can be exported as .csv files (sample sheet) or .xls file (plate layout) as needed.



a. To export a specific sample sheet or the plate layout, click on the [Export] button, select the location on your computer or network and click [Save].
b. You can export the plate layout (.xlsx) for documentation purposes.



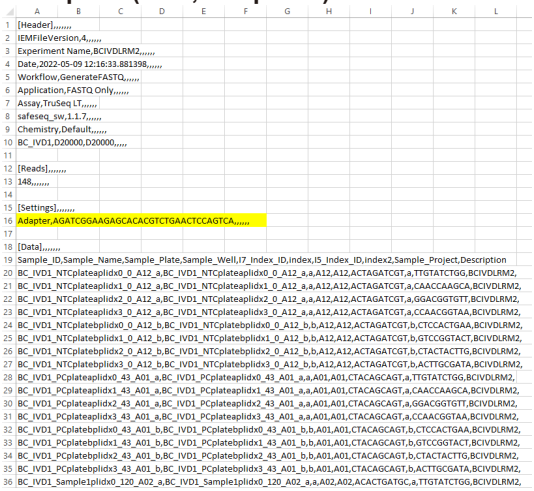
c. Sample sheet LRM (.csv) in the middle of the screen is applied when starting the sequencing run using the local run manager (LRM) from Illumina, Inc. When uploading the LRM sample sheet in the LRM software of the sequencing device, the GenerateFastQ module needs to be selected.

Important: *In the 'Advanced Module Settings' the 'Adapter' with its sequence (as highlighted in yellow in the example sample sheet below) must be included for correct adapter trimming to take place.*

Example A (LRM, one plate):

	A	B	C	D	E	F	G	H	I	J
1	[Header],,,,,,,									
2	IEMFileVersion,4,,,,,,									
3	Experiment Name,BCIVDLRM,,,,,,									
4	Date,2022-05-09 12:08:02.863719,,,,,,									
5	Workflow,GenerateFASTQ,,,,,,									
6	Application,FASTQ Only,,,,,,									
7	Assay,TruSeq LT,,,,,,									
8	safeseq_sw,1.1.7,,,,,,									
9	Chemistry,Default,,,,,,									
10	BC_IVD1,D20000,None,,,,									
11										
12	[Reads],,,,,,,									
13	148,,,,,,,									
14										
15	[Settings],,,,,,,									
16	Adapter,AGATCGGAAGAGCACACGTCTGAACTCCAGTCA,,,,,,									
17										
18	[Data],,,,,,,									
19	Sample_ID,Sample_Name,Sample_Plate,Sample_Well,I7_Index_ID,index,Sample_Project,Description									
20	BC_IVD1_NTCplatea_0_C12_a,BC_IVD1_NTCplatea_0_C12_a,a,C12,C12,TCGCTACTAC,BCIVDLRM,									
21	BC_IVD1_PCplatea_43_C01_a,BC_IVD1_PCplatea_43_C01_a,a,C01,C01,CATGTGATAC,BCIVDLRM,									
22	BC_IVD1_Sample1_120_C02_a,BC_IVD1_Sample1_120_C02_a,a,C02,C02,GTCAGACTAG,BCIVDLRM,									
23	BC_IVD1_Sample1_120_C03_a,BC_IVD1_Sample1_120_C03_a,a,C03,C03,ATAGATCGCG,BCIVDLRM,									
24	BC_IVD1_Sample1_120_C04_a,BC_IVD1_Sample1_120_C04_a,a,C04,C04,TCGTACACAG,BCIVDLRM,									
25	BC_IVD1_Sample1_120_C05_a,BC_IVD1_Sample1_120_C05_a,a,C05,C05,ATCGAGAGAG,BCIVDLRM,									
26	BC_IVD1_Sample1_120_C06_a,BC_IVD1_Sample1_120_C06_a,a,C06,C06,TACTGCAGAG,BCIVDLRM,									
27	BC_IVD1_Sample2_450_C07_a,BC_IVD1_Sample2_450_C07_a,a,C07,C07,AGTGACTCTG,BCIVDLRM,									
28	BC_IVD1_Sample2_450_C08_a,BC_IVD1_Sample2_450_C08_a,a,C08,C08,CACAGTCTCA,BCIVDLRM,									
29	BC_IVD1_Sample2_450_C09_a,BC_IVD1_Sample2_450_C09_a,a,C09,C09,TATGACTCGC,BCIVDLRM,									

Example B (LRM, two plates):



```
[Header],,,,,,,
IEMFileVersion,4,,,,,,
Experiment Name,BCIVDLRM2,,,,,,
Date,2022-05-09 12:16:33.881398,,,,,,
Workflow,GenerateFASTQ,,,,,,
Application,FASTQ Only,,,,,,
Assay,TruSeq LT,,,,,,
safeseq_sw,1.1.7,,,,,,
Chemistry,Default,,,,,,
BC_IVD1,D20000,D20000,,,,,

[Reads],,,,,,,
148,,,,,,,

[Settings],,,,,,,
Adapter,AGATCGGAAGAGCACACGTCTGAACTCCAGTCA,,,,,,

[Data],,,,,,,
Sample_ID,Sample_Name,Sample_Plate,Sample_Well,I7_Index_ID,index,I5_Index_ID,index2,Sample_Project,Description
BC_IVD1_NTCplateaplidx0_0_A12_a,BC_IVD1_NTCplateaplidx0_0_A12_a,a,A12,A12,ACTAGATCGT,a,TTGTATCTGG,BCIVDLRM2,
BC_IVD1_NTCplateaplidx1_0_A12_a,BC_IVD1_NTCplateaplidx1_0_A12_a,a,A12,A12,ACTAGATCGT,a,CAACCAAGCA,BCIVDLRM2,
BC_IVD1_NTCplateaplidx2_0_A12_a,BC_IVD1_NTCplateaplidx2_0_A12_a,a,A12,A12,ACTAGATCGT,a,GGACGGTGTT,BCIVDLRM2,
BC_IVD1_NTCplateaplidx3_0_A12_a,BC_IVD1_NTCplateaplidx3_0_A12_a,a,A12,A12,ACTAGATCGT,a,CCAACGGTAA,BCIVDLRM2,
BC_IVD1_NTCplatebplidx0_0_A12_b,BC_IVD1_NTCplatebplidx0_0_A12_b,b,A12,A12,ACTAGATCGT,b,CTCCACTGAA,BCIVDLRM2,
BC_IVD1_NTCplatebplidx1_0_A12_b,BC_IVD1_NTCplatebplidx1_0_A12_b,b,A12,A12,ACTAGATCGT,b,GTCCGGTACT,BCIVDLRM2,
BC_IVD1_NTCplatebplidx2_0_A12_b,BC_IVD1_NTCplatebplidx2_0_A12_b,b,A12,A12,ACTAGATCGT,b,CTACTACTTG,BCIVDLRM2,
BC_IVD1_NTCplatebplidx3_0_A12_b,BC_IVD1_NTCplatebplidx3_0_A12_b,b,A12,A12,ACTAGATCGT,b,ACTTGCGATA,BCIVDLRM2,
BC_IVD1_PCplateaplidx0_43_A01_a,BC_IVD1_PCplateaplidx0_43_A01_a,a,A01,A01,CTACAGCAGT,a,TTGTATCTGG,BCIVDLRM2,
BC_IVD1_PCplateaplidx1_43_A01_a,BC_IVD1_PCplateaplidx1_43_A01_a,a,A01,A01,CTACAGCAGT,a,CAACCAAGCA,BCIVDLRM2,
BC_IVD1_PCplateaplidx2_43_A01_a,BC_IVD1_PCplateaplidx2_43_A01_a,a,A01,A01,CTACAGCAGT,a,GGACGGTGTT,BCIVDLRM2,
BC_IVD1_PCplateaplidx3_43_A01_a,BC_IVD1_PCplateaplidx3_43_A01_a,a,A01,A01,CTACAGCAGT,a,CCAACGGTAA,BCIVDLRM2,
BC_IVD1_PCplatebplidx0_43_A01_b,BC_IVD1_PCplatebplidx0_43_A01_b,b,A01,A01,CTACAGCAGT,b,CTCCACTGAA,BCIVDLRM2,
BC_IVD1_PCplatebplidx1_43_A01_b,BC_IVD1_PCplatebplidx1_43_A01_b,b,A01,A01,CTACAGCAGT,b,GTCCGGTACT,BCIVDLRM2,
BC_IVD1_PCplatebplidx2_43_A01_b,BC_IVD1_PCplatebplidx2_43_A01_b,b,A01,A01,CTACAGCAGT,b,CTACTACTTG,BCIVDLRM2,
BC_IVD1_PCplatebplidx3_43_A01_b,BC_IVD1_PCplatebplidx3_43_A01_b,b,A01,A01,CTACAGCAGT,b,ACTTGCGATA,BCIVDLRM2,
BC_IVD1_Sample1plidx0_120_A02_a,BC_IVD1_Sample1plidx0_120_A02_a,a,A02,A02,ACACTGATGC,a,TTGTATCTGG,BCIVDLRM2,
```

d. Sample sheet bcl2fastq (.csv) on the left side of the screen is used when Illumina bcl2fastq software is applied for demultiplexing, adapter trimming and FASTQ file generation.

Example A (bcl2fastq, one plate):

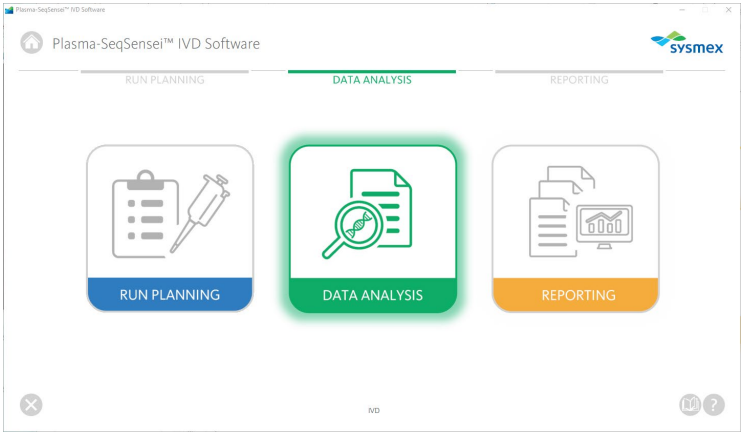
```
[Header],,,,,,,
IEMFileVersion,4,,,,,
Experiment Name,BCIVDbcl2fastq,,,,,,
Date,2022-05-09 12:07:50.975042,,,,,,
Workflow,GenerateFASTQ,,,,,,
Application,FASTQ Only,,,,,,
Assay,TruSeq LT,,,,,,
safeseq_sw,1.1.7,,,,,,
Chemistry,Default,,,,,,
BC_IVD1,D20000,None,,,,,

[Reads],,,,,,,
148,,,,,,,

[Settings],,,,,,,
FilterPCRDuplicates,0,,,,,,
ReverseComplement,0,,,,,,
VariantFilterQualityCutoff,30,,,,,,
outputgenomevcf,FALSE,,,,,,
Adapter,AGATCGGAAGAGCACACGTCTGAACTCCAGTCA,,,,,,

[Data],,,,,,,
Sample_ID,Sample_Name,Sample_Plate,Sample_Well,I7_Index_ID,index,Sample_Project,Description
BC_IVD1_NTCplatea_0_C12_a,BC_IVD1_NTCplatea_0_C12_a,a,C12,C12,TCGCTACTAC,BCIVDbcl2fastq,
BC_IVD1_PCplatea_43_C01_a,BC_IVD1_PCplatea_43_C01_a,a,C01,C01,CATGTGATAC,BCIVDbcl2fastq,
BC_IVD1_Sample1_120_C02_a,BC_IVD1_Sample1_120_C02_a,a,C02,C02,GTCAGACTAG,BCIVDbcl2fastq,
BC_IVD1_Sample1_120_C03_a,BC_IVD1_Sample1_120_C03_a,a,C03,C03,ATAGATCGCG,BCIVDbcl2fastq,
BC_IVD1_Sample1_120_C04_a,BC_IVD1_Sample1_120_C04_a,a,C04,C04,TCGTACACAG,BCIVDbcl2fastq,
BC_IVD1_Sample1_120_C05_a,BC_IVD1_Sample1_120_C05_a,a,C05,C05,ATCGAGAGAG,BCIVDbcl2fastq,
BC_IVD1_Sample1_120_C06_a,BC_IVD1_Sample1_120_C06_a,a,C06,C06,TACTGCAGAG,BCIVDbcl2fastq,
BC_IVD1_Sample2_450_C07_a,BC_IVD1_Sample2_450_C07_a,a,C07,C07,AGTGACTCTG,BCIVDbcl2fastq,
BC_IVD1_Sample2_450_C08_a,BC_IVD1_Sample2_450_C08_a,a,C08,C08,CACAGTCTCA,BCIVDbcl2fastq,
```

Example B (bcl2fastq, two plates):

	A	B	C	D	E	F	G	H	I	J	K	L
1	[Header],,,,,,,,											
2	IEMFileVersion,4,,,,,,											
3	Experiment Name,BCIVDbcl2fastq2,,,,,,											
4	Date,2022-05-09 12:16:25.131648,,,,,,											
5	Workflow,GenerateFASTQ,,,,,,											
6	Application,FASTQ Only,,,,,,											
7	Assay,TruSeq LT,,,,,,											
8	safeseq_sw,1.1.7,,,,,,											
9	Chemistry,Default,,,,,,											
10	BC_IVD1,D20000,D20000,,,,,											
11												
12	[Reads],,,,,,,,											
13	148,,,,,,,											
14												
15	[Settings],,,,,,,,											
16	FilterPCRDuplicates,0,,,,,,											
17	ReverseComplement,0,,,,,,											
18	VariantFilterQualityCutoff,30,,,,,,											
19	outputgenomevcf,FALSE,,,,,,											
20	Adapter,AGATCGGAAGAGCACACGTCTGAACTCCAGTCA,,,,,,											
21												
22	[Data],,,,,,,,											
23	Sample_ID,Sample_Name,Sample_Plate,Sample_Well,I7_Index_ID,index,I5_Index_ID,index2,Sample_Project,Description											
24	BC_IVD1_NTCplatea_0_A12_a,BC_IVD1_NTCplatea_0_A12_a,a,A12,A12,ACTAGATCGT,a,CCAGATACAA,BCIVDbcl2fastq2,											
25	BC_IVD1_NTCplatea_0_A12_a,BC_IVD1_NTCplatea_0_A12_a,a,A12,A12,ACTAGATCGT,a,TGCTTGGTTG,BCIVDbcl2fastq2,											
26	BC_IVD1_NTCplatea_0_A12_a,BC_IVD1_NTCplatea_0_A12_a,a,A12,A12,ACTAGATCGT,a,AACACCGTCC,BCIVDbcl2fastq2,											
27	BC_IVD1_NTCplatea_0_A12_a,BC_IVD1_NTCplatea_0_A12_a,a,A12,A12,ACTAGATCGT,a,TTACCGTTGG,BCIVDbcl2fastq2,											
28	BC_IVD1_NTCplateb_0_A12_b,BC_IVD1_NTCplateb_0_A12_b,b,A12,A12,ACTAGATCGT,b,TTCAGTGGAG,BCIVDbcl2fastq2,											
29	BC_IVD1_NTCplateb_0_A12_b,BC_IVD1_NTCplateb_0_A12_b,b,A12,A12,ACTAGATCGT,b,AGTACCGGAC,BCIVDbcl2fastq2,											
30	BC_IVD1_NTCplateb_0_A12_b,BC_IVD1_NTCplateb_0_A12_b,b,A12,A12,ACTAGATCGT,b,CAAGTAGTAG,BCIVDbcl2fastq2,											
31	BC_IVD1_NTCplateb_0_A12_b,BC_IVD1_NTCplateb_0_A12_b,b,A12,A12,ACTAGATCGT,b,TATCGCAAGT,BCIVDbcl2fastq2,											
32	BC_IVD1_PCplatea_43_A01_a,BC_IVD1_PCplatea_43_A01_a,a,A01,A01,CTACAGCAGT,a,CCAGATACAA,BCIVDbcl2fastq2,											
33	BC_IVD1_PCplatea_43_A01_a,BC_IVD1_PCplatea_43_A01_a,a,A01,A01,CTACAGCAGT,a,TGCTTGGTTG,BCIVDbcl2fastq2,											
34	BC_IVD1_PCplatea_43_A01_a,BC_IVD1_PCplatea_43_A01_a,a,A01,A01,CTACAGCAGT,a,AACACCGTCC,BCIVDbcl2fastq2,											
35	BC_IVD1_PCplatea_43_A01_a,BC_IVD1_PCplatea_43_A01_a,a,A01,A01,CTACAGCAGT,a,TTACCGTTGG,BCIVDbcl2fastq2,											
36	BC_IVD1_PCplateb_43_A01_b,BC_IVD1_PCplateb_43_A01_b,b,A01,A01,CTACAGCAGT,b,TTCAGTGGAG,BCIVDbcl2fastq2,											

e. If changes of the input need to be done, go back multiple pages by clicking on [Back] on the bottom right-hand corner of the window.

6.2 Data Analysis module



The Data Analysis module (green) is used to start the sequencing analysis employing:

- Compressed FASTQ files (.fastq.gz)
- Sample sheet of the run prepared in Run Planning module

The FASTQ files should be saved locally on the same drive as the Plasma-SeqSensei™ IVD Software in one single folder per sequencing run. No subfolders are allowed. If transferring data from Illumina's BaseSpace™ all .fastq.gz files need to be copied to a single folder location.

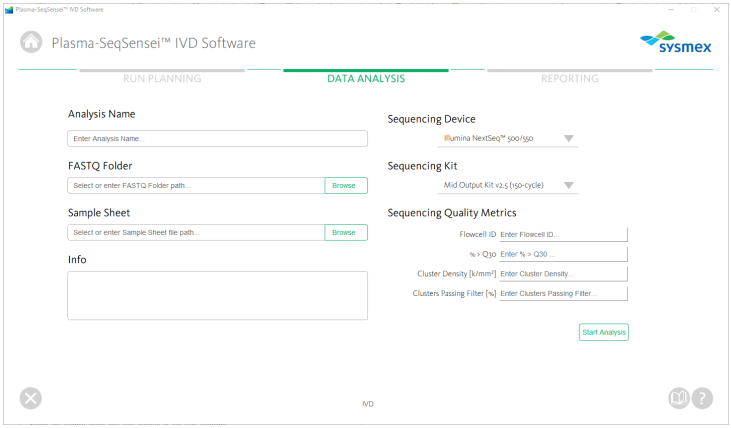
Note: *.fastq.gz files that are named 'Undetermined` must not be present in the folder to be analysed by the software.*

The analysis will be carried out after the sequencing run and subsequent demultiplexing, adapter trimming and FASTQ file generation was performed. The demultiplexing and adapter trimming itself is not part of the analysis software provided here and need to be performed prior to data analysis (see ► chapter *4.1 Data acquisition*, page 9/57).

Before starting data analysis using the Plasma-SeqSensei™ IVD Software, check the run validity parameters in Illumina's instrument software:

- Cluster density:
  NextSeq™: Average 0 to 220 K/mm^2
- Q30 score: ≥ 80 %
- Clusters Passing Filter (PF): ≥ 80 %

If run validity parameters are not reached, the run is invalid.



1. Click on the Data Analysis module (green).
2. Type in a name for the analysis.
3. Select the sequencing device used.
4. Select the sequencing kit.
5. Fill in the sequencing quality metrics/run validity criteria as shown on the sequencing device:
   a. Flowcell ID
   b. % > Q30
   c. Cluster Density [k/mm^2]
   d. Clusters Passing Filter [%]

   When starting the analysis an error message appears if Flowcell ID is not complete or does not match ID included in the files to be analysed, or if run validity criteria are out of acceptable ranges.

6. Select the folder that contains the FASTQ files from the sequencing run to be analysed (.fastq.gz) by clicking on the [Browse] button and navigating to the folder to be selected.

   Note: *The .fastq.gz files will not be visible when selecting the folder.*

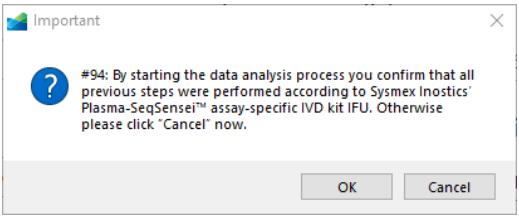
7. Select the sample sheet that was created in the Run Planning module (.csv) for this sequencing run by clicking on the [Browse] button and navigating to the file to be selected.

8. Fill in information regarding the experiment, sequencing run or analysis (optional).

9. Click [Start Analysis].

   If files are missing, sample sheet and file names do not match, or the wrong sample sheet was selected an error message will be displayed by the software.

10. A window will appear asking to confirm adherence to the IVD workflow according to the Plasma-SeqSensei™ *Assay-Specific* IVD Kit Instructions for Use.



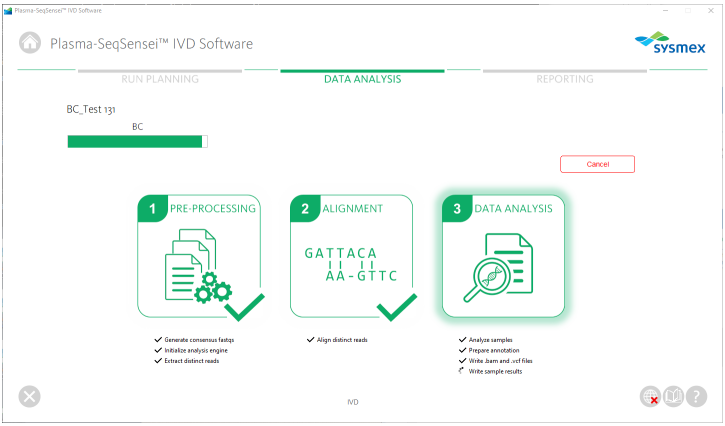
   a. Select [Ok] if you followed the Instructions for Use to start the IVD-certified analysis of your sequencing results.

   b. Select [Cancel] if you did not adhere to the Instructions for Use for the Plasma-SeqSensei™ Assay-Specific IVD Kit, as these sequencing results are not eligible for IVD-certified analysis.

Note: *It is recommended to close all other applications during the analysis and that the standby functionality of your Windows® computer is switched off.*

Depending on available memory analysis can take up to 6 hours. If analysis takes longer refer to ► chapter *8 Troubleshooting*, page 48/57.

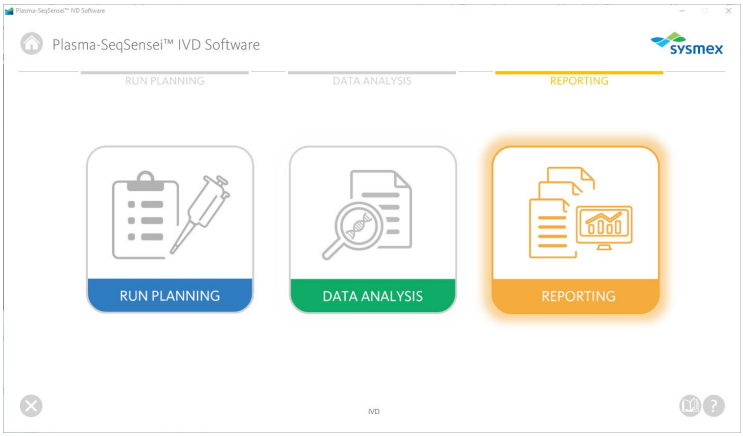
11. A new window appears showing the process and progress of data analysis.



    a. You can stop the analysis by clicking on the red [Cancel] button on the right-hand side of the window. The analysis will have to be started anew after cancellation; it cannot be paused.

12. After the analysis of the data is finished, the software will automatically switch to the Reporting module and open the page with the results of the sequencing run analysis.

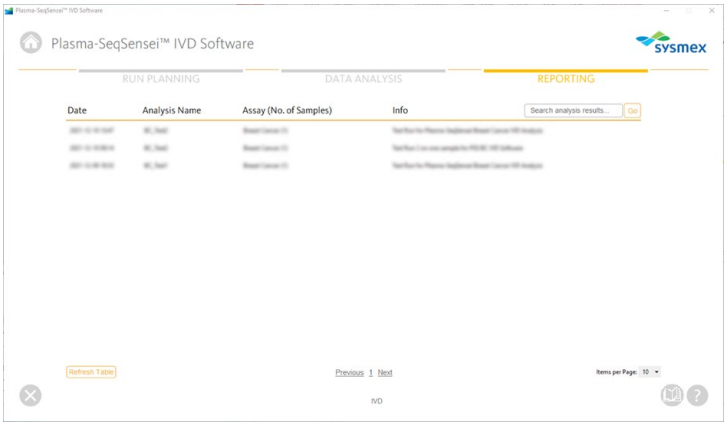
6.3 Reporting module



The Reporting module (orange) is used to save and manage all analysis results performed using the Plasma-SeqSensei™ IVD Software. It allows the user to:

- see all runs analysed on the device
- see the files directory of FASTQ files for each run
- download reports (.pdf), .vcf and .bam files from each run
- delete runs/data

The reporting module will automatically start after an analysis is finished. All previous analyses on the device can be accessed at any time to download or delete data. Data can be downloaded in .pdf format (reports), .vcf format or .bam format.



1. Click on the Reporting module (orange).
2. Here, an overview of all analyses performed with the Plasma-SeqSensei™ IVD Software on the device is shown.
    a. To skip to a specific page in this overview, click on the page number on the bottom of the screen or [Previous]/[Next].

    Previous 1 Next

    b. The number of items per page can be changed on the bottom right-hand corner of the page.

    Items per Page: 10

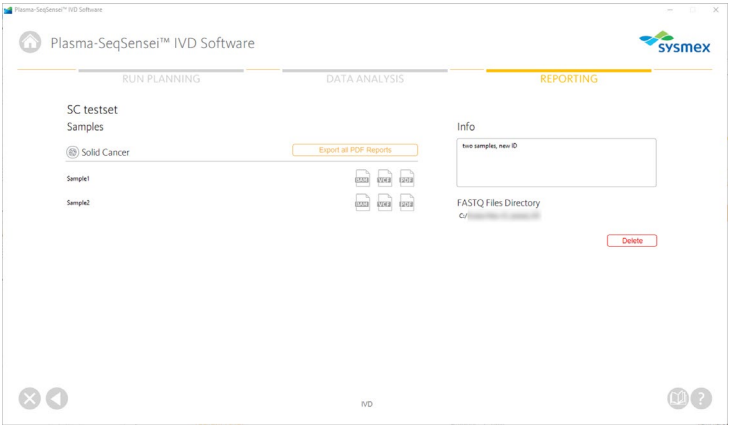
    c. To search for specific analysis results, type in the name of the run in the search field on the top right-hand corner of the window and click on the [Go] button.

    Search for Analysis Name or Info ... Go

    d. If you are waiting for new data, click on the button [Refresh Table] on the lower left-hand corner of the window to reload the results table and see new analysis results.

    Refresh Table

3. Select an analysis result of interest by clicking on it.
4. In the new window the analysis results are shown including:



   a. Name of the run
   b. Assay used for the run
   c. Name of all samples in that run
   d. Information of the run (if included in the Data Analysis module)
   e. Location of the FASTQ file directory used for data analysis
   f. Icons to export .pdf reports, .vcf files and .bam files individually per sample

   g. Button to export all .pdf reports of selected run as a .zip file

5. When exporting files, click on the icon or button and select the name and location on your device/server for export.

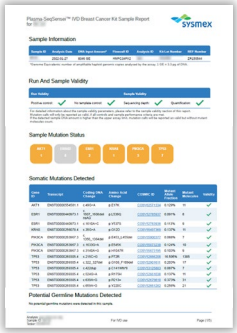
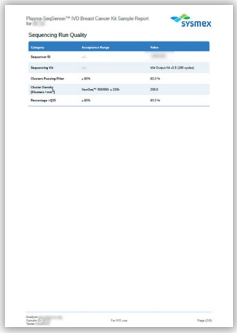
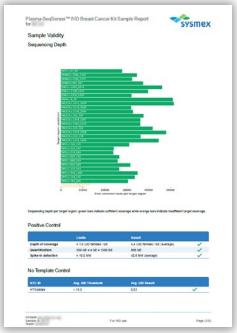
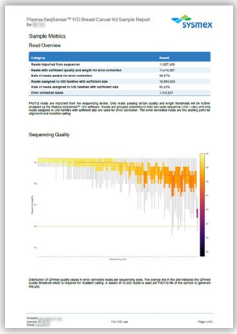
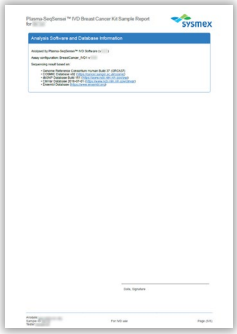
6. To delete all analysis results of this specific run, click on the red [Delete] button on the lower right-hand corner of the screen.

A window verifies if final deletion of all data is desired.

7. To navigate back to the Reporting module overview, click on the white triangle in the grey circle on the lower left-hand corner of the software window.

7 Reports

Reports are available in .pdf format.

In addition, .vcf (variant call format) and .bam (binary alignment map) files can be downloaded. .vcf files contain, among other information, all mutations in mutant allele fraction (MAF) and mutant molecules (MM) as shown in the report in a standardised format. .bam files contain the alignment information of the generated UID consensus reads against the assay amplicons. Both files can be used for a detailed inspection of the analysis results using third-party software (e.g. the Integrative Genomics Viewer (IGV, https://software.broadinstitute.org/software/igv/)). If using IGV, please select “Human hg19” as the reference genome.

7 Reports

The reports generated by the Plasma-SeqSensei™ IVD Software contain multiple sections:

- Sample information
- Run and Sample validity
  - Sequencing depth
  - Positive control
  - No template control
- Sample mutation status
  - Invalid amplicons
- Mutations detected
  - Somatic mutations
  - Potential germline mutations
- Sequencing run quality
- Sample metrics
  - Read overview
  - Sequencing quality
- Analysis software and database information

Example A:

Sample Information

Sample ID	Analysis Date	DNA Input Amount*	Flowcell ID	Analysis ID	Kit Lot Number	REF Number
SampleB	2024-03-15	5290 GE	HHSIG0FX2	BC_Test 131	D20001	ZR150544

*Genome Equivalents: number of amplifiable haploid genomic copies analysed by the assay. 1 GE = 3.3 pg of DNA.

Example B:

Sample Information

Sample ID	Analysis Date	DNA Input Amount*	Flowcell ID	Analysis ID	Kit Lot Number	REF Number
SampleA	2024-03-15	Not quantifiable	HHSIG0FX2	BC_Test 131	D20001	ZR150544

*Genome Equivalents: number of amplifiable haploid genomic copies analysed by the assay. 1 GE = 3.3 pg of DNA.

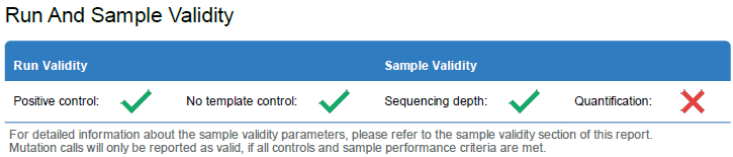
The DNA input is listed as not quantifiable if the detected DNA amount for this sample is outside the valid input range. Alternatively, this error message is also displayed if the quantification of the positive control fails regardless of the sample DNA input or if the coverage of the sequences used for quantification is insufficient.

The **Sample Information** contains a summary of the specific sample analysis including:

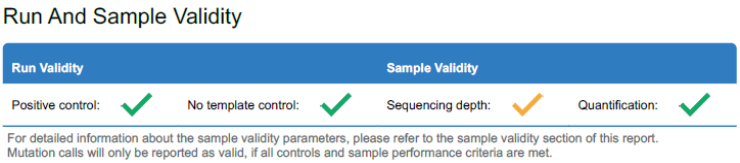
- Sample ID
- Analysis Date

- DNA Input Amount in genome equivalents (GE) calculated using the internal quantifier (Quantispike); if DNA Input Amount is outside of valid input ranges or the quantification of the positive control fails, it will be marked as 'Not quantifiable'.
- Flowcell ID
- Analysis ID given by the user for the analysis of this set of samples.
- Plasma-SeqSensei™ IVD Kit Lot number used
- The REF number specifies the article number of the Plasma-SeqSensei™ kit used.

Example A:



Example B:



The **Run and Sample Validity** table on the first page of the report shows if the analysed sample fulfils the validity criteria. Green check marks (✓) show valid results, red crosses (✗) show invalid results and an orange check mark (✓) for sequencing depth informs the user of at least one invalid amplicon due to low sequencing coverage. More information about this invalid amplicon is found in the Sample Mutation Status section and the Sequencing Depth section of the report.

Quantification of the samples are initially performed using Qubit measurement, which represents a rough approximation of input DNA content for correct sample load. Quantification in the report refers to the DNA content of the sample as determined by the internal quantifier (Quantispike).

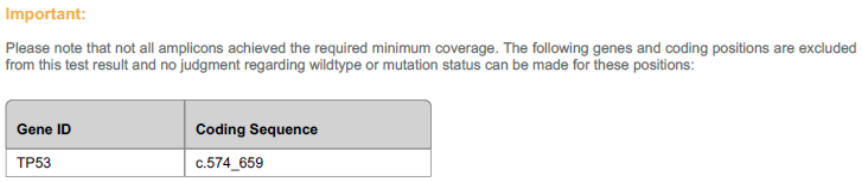
If the quantification value is outside of the allowed input range (visible in Sample Information/DNA Input Amount), the sample will be invalid, and no results will be shown on the report.

The analysis of the sample is also invalid if positive control, no template control, or Sequencing metrics are outside allowed criteria.

Example:

The **Sample Mutation Status** gives an overview of how many mutations were detected per gene analysed by the Plasma-SeqSensei™ IVD Kit. If a mutation was found, the box is depicted in orange with the number of mutations found in the gene underneath the gene name. If no mutation was found in a gene, the box with the gene name is depicted in grey with a zero underneath.

Example:



A note (Important) will be included if one or more amplicons did not achieve sufficient sequencing depth and are excluded from reporting (see Run and Sample Validity and Sequencing Depth section of this report). The coding positions affected within the specified gene are depicted in a table. Possible mutations located within these regions are not shown and the mutation status of these gene positions cannot be judged.

Example A:

Somatic Mutations Detected

Gene ID	Transcript	Coding DNA Change	Amino Acid Change	COSMIC ID	ClinVar ID	Mutant Allele Fraction	Mutant Molecules
AKT1	ENST00000554581.1	c.49G>A	p.E17K	COSV62571334	13983	0.129%	11
TP53	ENST00000269305.4	c.422dup	p.C141Wfs*8	COSV53125883	844977	0.087%	7
TP53	ENST00000269305.4	c.524G>A	p.R175H	COSV52661038	12374	0.137%	11
TP53	ENST00000269305.4	c.639A>G	p.R213=	COSV52679610	43591	0.373%	31
TP53	ENST00000269305.4	c.659A>G	[1]p. Y220C	COSV52661282	127819	0.256%	21

[1] Partially covered amino acid triplet detected. The amino acid annotation in this report is made based on the assumption that the bases, which are not covered by this assay correspond to the reference sequence.

Example B:

Somatic Mutations Detected

No valid somatic mutations were detected in this sample.

In the **Somatic Mutations Detected** section of the report all mutations are shown that were detected by the Plasma-SeqSensei™ IVD Software in the covered gene regions of the Plasma-SeqSensei™ IVD Kit used, when Run and Sample Validity criteria were fulfilled and the sequencing depth was reached for the amplicon.

In the table the following information is shown:

- Gene ID
- Transcript number of the gene used during analysis
- Coding DNA change detected
- Amino acid change resulting from coding DNA change
  - if mutation is found within the intronic region of the gene, the amino acid change will be shown as “p.?”
  - if the base pair change is detected in an amino acid-encoding nucleotide triplet, that is only partially covered by the assay, the amino acid change is marked with a [1]. Here, the amino acid annotation is made based on the assumption that the bases, which are not covered by the assay correspond to the reference sequence.
- COSMIC ID, if available (COSV number) in the database version used (refer to last page of report)
- ClinVar ID, if available in the database version used (refer to last page of report)
- Mutant allele fraction

- Mutant molecules (MM) per mutation detected in the sample utilising the DNA content calculated with the internal quantifier (Quantispike).

Example A:

Potential Germline Mutations Detected

Gene ID	Transcript	Coding DNA Change	Amino Acid Change	dbSNP ID	ClinVar ID	Mutant Allele Fraction	Mutant Molecules
TP53	ENST00000269305.4	c.215C>G	p.P72R	rs1042522	12351	44.211%	2339

Potential germline mutations were detected in this sample.
This classification is based on a mutant allele fraction above 40% and below or equal 60% (heterozygous) or above 90% (homozygous) for the listed mutations.

Example B:

Potential Germline Mutations Detected

No potential germline mutations were detected in this sample.

Potential germline mutations (SNPs) will be listed in an additional table if found. Mutations are listed as potential germline mutations when they are present at a MAF > 40 % to ≤ 60 % (heterozygous) or ≥ 90 % (homozygous). Entry in the dbSNP database is optional. To validate the depicted mutation as a real germline mutation, additional testing of genomic DNA would need to be performed.

In the table the following information is shown:

- Gene ID
- Transcript number of the gene used during analysis
- Coding DNA change detected
- Amino acid change resulting from coding DNA change
  - if the base pair change is detected in an amino acid-encoding nucleotide triplet, that is only partially covered by the assay, the amino acid change is marked with a [1)]. Here, the amino acid annotation is made based on the assumption that the bases, which are not covered by the assay correspond to the reference sequence.
- dbSNP ID, if available in the database version used (refer to last page of report)
- ClinVar ID, if available in the database version used (refer to last page of report)
- Mutant allele fraction

- Mutant molecules (MM) per mutation detected in the sample utilising the DNA content calculated with the internal quantifier (Quantispike).

Example:

Sequencing Run Quality

Category	Acceptance Range	Value
Sequencer ID	- / -	[redacted]
Sequencing Kit	- / -	Mid Output Kit v2.5 (150-cycle)
Clusters Passing Filter	≥ 80%	91.8 %
Cluster Density [Clusters / mm^2]	NextSeq™ 500/550: ≤ 220k	207.0
Percentage >Q30	≥ 80%	92.3 %

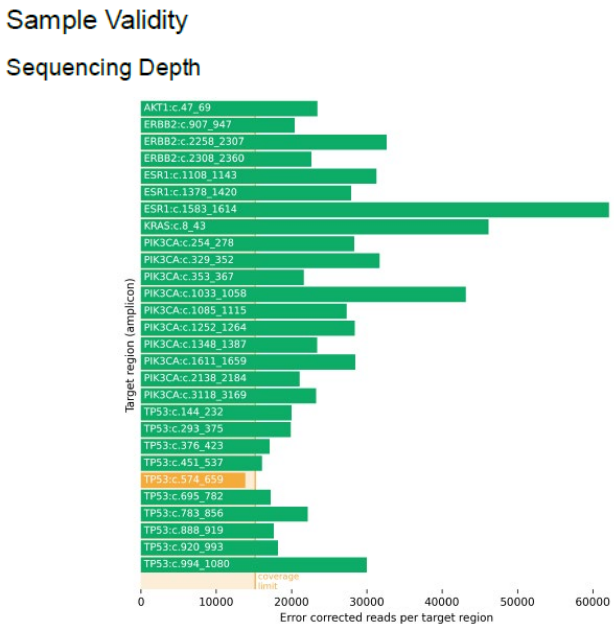
The **Sequencing Run Quality** table includes the sequencing quality metrics/run validity criteria as filled in by the user into the Plasma-SeqSensei™ IVD Software in the Analysis module. Acceptance ranges (if applicable) of these criteria are shown in the table.

The following categories are shown:

- Sequencer ID
- Sequencing kit used
- Clusters passing filter
- Cluster density in clusters/mm^2
- Percentage > Q30

If any parameter is out of range, the sequencing run is invalid and needs to be repeated.

Example:



Sequencing depth per target region: green bars indicate sufficient coverage while orange bars indicate insufficient target coverage.

The second part of the **Sample Validity** section includes further details to determine the reason for a possible failure of the sample analysis.

In the **Sequencing Depth** section, the sequencing coverage of all target regions/amplicons analysed in the Plasma-SeqSensei™ IVD Kit is depicted. Green bars represent amplicons with sufficient target coverage, orange bars show amplicons that have insufficient target coverage. The orange marked amplicons are invalid and no judgement regarding the mutational status of this target region can be made. The coverage limit is visualised at the bottom of the graph in light orange. The unit used for the calculation of coverage is in “error corrected reads per target region”.

Example:

Positive Control

	Limits	Result	
Depth of coverage	≥ 1.0 UID families / GE	0.8 UID families / GE (minimum)	✗
Quantification	500 GE ≤ x GE ≤ 1500 GE	808 GE	✓
Spike-in detection	≥ 10.0 MM	42.8 MM (average)	✓

The **Positive Control** table shows the limits to be reached by the positive control of the assay for valid Plasma-SeqSensei™ runs. Green check marks (✓) show valid results, red crosses (✗) show invalid results. Here, the following values for the control and the sample need to be within valid ranges:

- Depth of sequencing coverage must be at least 1 UID family per GE for the positive control for all included amplicons.
- Quantification (in GE) of the positive control needs to be within shown acceptance ranges.
- A detection limit of at least 10 MM per mutation included in the positive control must be reached.

Example A:

No Template Control

NTC ID	Avg. UID Threshold	Avg. UID Result	
NTCplatea	< 15.0	0.03	✓

Example B:

No Template Control

NTC ID	Avg. UID Threshold	Avg. UID Result	
NTCplatea	< 15.0	3577.34	✗

The **No Template Control** section shows if a possible contamination of the no template control has occurred. Green check marks (✓) show valid results, red crosses (✗) show invalid results. If the average UID result is above 15, the samples could be contaminated, and the run is therefore invalid.

Example:

Read Overview

Category	Result
Reads imported from sequencer	18,611,038
Reads with sufficient quality and length for error correction	17,612,879
Rate of reads usable for error correction	94.64%
Reads assigned to UID families with sufficient size	17,136,078
Rate of reads assigned to UID families with sufficient size	97.29%
Error corrected reads	812,656

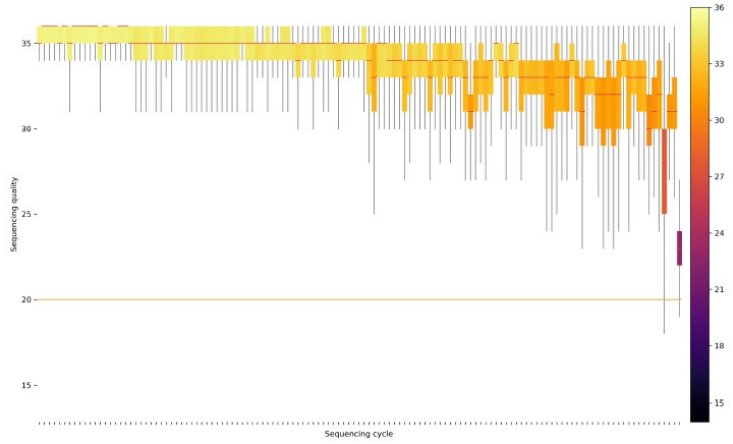
FASTQ reads are imported from the sequencing device. Only reads passing certain quality and length thresholds will be further analysed by the Plasma-SeqSensei™ IVD software. Reads are grouped according to their bar-code sequence (UID / UMI) and only reads assigned to UID families with sufficient size are used for error correction. The error corrected reads are the starting point for alignment and mutation calling.

You can find more information about the sequencing run and analysis in the **Sample Metrics** section.

The **Read Overview** gives information about the numbers and percentages of different types of reads during the analysis of the data.

Example:

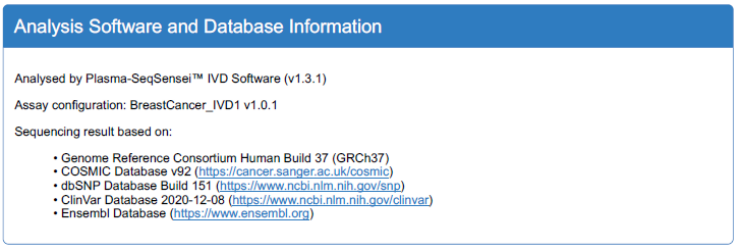
Sequencing Quality



Distribution of QPhred quality values in error corrected reads per sequencing cycle. The orange line in the plot indicates the QPhred quality threshold which is required for mutation calling. A subset of 10,000 reads is used per FASTQ file of the sample to generate this plot.

The **Sequencing Quality** section shows a plot with an average of all QPhred quality values of a subset of reads of the specific sample according to the sequencing cycle number. QPhred scores above 20 (orange line) are acceptable.

Example:

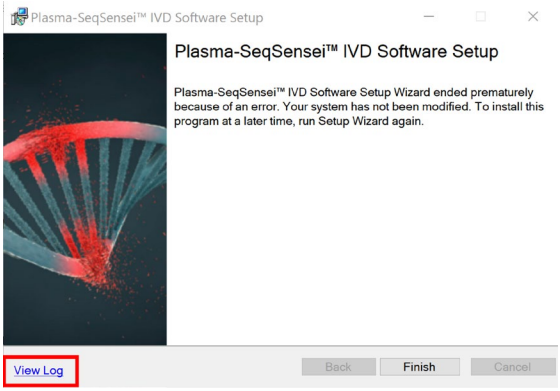


The last section of the report depicts the **Analysis Software and Database Information** used for the analysis of this sample.

A **signature field** at the end of the report is included.

8 Troubleshooting

Please refer to the following table if problems occur during the usage of the Plasma-SeqSensei™ Software or contact your local authorised Sysmex representative for further information.

Problem	Solution
The software installation does not finish successfully.	Please make sure you have followed exactly the instructions given in section 5.1.1 of this manual for the installation of the software. This includes especially the location of the downloaded folder (locally) and the unpacking of the .zip file prior to installation. Only unpack the first folder, do not unpack the subfolders. If performing the installation a second time, please make sure to delete the folder C:\Users\Public\SysmexInostics\ivd, if it already exists. If you still cannot install the software successfully, please contact Sysmex Customer Support and provide a copy of the installation log file which can be accessed here (red box): 
I cannot see the entire software window and cannot close it.	The settings of the computer screen need to be set to 125 % or lower to be able to show the entire software window.
The ‘start analysis` button is missing.	Please set your screen scale to 125% or lower and restart the Plasma-SeqSensei™ Software.
The license key is not accepted during installation of the software.	Verify that there are no additional spaces added to the license key or the customer name and check that you downloaded the correct software (RUO or IVD) or contact the Customer Support of Sysmex.

Run Planning module	
Article number or lot number is not accepted.	Make sure to use the entire number including the capital letters ZR + six numbers for the article number or D + five numbers for the lot number. Only the article number of the Plasma-SeqSensei™ *Assay-Specific* IVD Kit is to be filled in. Do not include the article number of the Extension IVD Kit.
No assays are selectable.	Please perform a second installation after deleting the folder: C:\Users\Public\SysmexInostics\ivd. Make sure that only the downloaded folder is unpacked, located in the download folder and that all other subfolders are still zipped. Do not unpack any subfolders.
A sample is missing in the Plate Summary page.	Make sure you entered the correct number of samples with sample names and DNA concentration in the Run Planning module.
Sample name is not accepted.	The sample name should be: 1. unique 2. no longer than 16 characters 3. consisting of only alpha-numeric characters.
DNA amount is not accepted.	DNA amount must be within IVD Kit-specific input range and a dot (.) is to be used as decimal separator.
The sample sheet is not accepted by the NextSeq™ sequencing device	If an older version (< v2.4.0) of the Local Run Manager is being used on the sequencing device, the current sample sheet might not be compatible. Try using the second version of the sample sheet available from the File Export page of the Run Planning module or contact the Customer Support of Sysmex.
Data Analysis module	
Where can I find the sequencing quality metrics?	The metrics will be displayed at the end of each run on the sequencing device. They can also be found when loading the sequencing run into the Illumina Sequencing Analysis Viewer (SAV) software.
I cannot select the FASTQ files when browsing from the IVD Software.	The FASTQ files will not be displayed in the selection window. It is the folder in which these files are located that needs to be selected.
Why are there 4 .fastq.gz files per well visible when I use the LRM on the NextSeq™?	When using the Local Run Manager (LRM) during the sequencing step on your NextSeq™ device, one file per lane of each well is created resulting in 4 files total. The Plasma-SeqSensei™ IVD Software will concatenate those files automatically for further analysis. When using the Plasma-SeqSensei™ Extension IVD Kit and two plates are being sequenced on the same sequencing run, each well will result in 16 files.

8 Troubleshooting

I cannot find the sample sheet for my run.	Try to use the Search option of your computer device or prepare a new sample sheet by using the Run Planning module again. Make sure to fill in the exact same names, concentrations, and layout of the plate.
The Flowcell ID is not accepted.	Use the entire Flowcell ID, as shown on the Flowcell, the Sequencing Device or the Sequencing Analysis Viewer.
I cannot start the analysis because files are missing.	Please check if you have the correct number of FASTQ files available in your analysis folder. During demultiplexing, FASTQ files are generated. Depending on your demultiplexing pipeline, the number of FASTQ files per sample can vary. If you use bcl2fastq for demultiplexing you usually have 5 FASTQ files per sample (one file per well). Alternatively, 20 FASTQ files per sample are generated (5 wells per sample, 4 lanes per well). This is the case if you use the “GenerateFASTQ” module of the Local Run Manager on your NextSeq™ device. When two Plasma-SeqSensei™ plates are run at the same time applying the LRM, 80 FASTQ files per sample are generated (5 wells per sample, 4 lanes per well, 4 plate indices). In summary, there should be 5, 20, or 80 FASTQ files present for a single sample in the FASTQ input folder. Additionally, Positive (PC) and No Template controls (NTC) receive 1, 4 or 16 FASTQ files, which are also located in the analysis folder.
I cannot start the analysis because additional files were found.	Please make sure that the FASTQ file called “Undetermined.fastq.gz” is not included in the analysis folder.
My analysis takes forever.	The analysis usually takes less than 6 hours. If longer analysis times are noticed multiple issues could have occurred: • Not enough memory available on the device. • Very large FASTQ files due to the use of an inappropriate sequencing kit, e.g. low number of samples/DNA input on a High Output Kit. • Plasma-SeqSensei™ IVD Software is not located on the same device as the data. When files are located on a network drive, access can be limited/slow, if the network drive is very busy with other processes. • Low quality of the reads In case this happens, either stop the run, remedy the problem (e.g. location of software) and start over again or wait for the analysis to finish. If the analysis still does not finish, please contact the technical support.

Reporting module	
I cannot export my reports when clicking on the icons or button.	That indicates an issue with the analysis, possibly it was terminated prematurely. The analysis will have to be repeated. Make sure that your runtime environment meets all requirements stated in ► chapter *2.2 Runtime environment specifications*, page 3/57.
Some amplicons are invalid, what does this mean for my report?	If more than 10 % of all target regions do not reach the target sequencing depth, the entire sample analysis is invalid. If less than 10 % of all target regions do not reach the target sequencing depth, only those amplicons are invalid, while the rest of the regions are valid.

9 Glossary and terminologies

Term	Definition
cfDNA	Cell-free DNA
ClinVar	Clinical Variation
COSMIC	Catalogue of somatic mutations in cancer
CPU	Central processing unit
ctDNA	Circulating tumour DNA
DNA	Desoxyribonucleic acid
GB	Gigabyte
GE	Genome equivalent
ID	Identifier
IFU	Instructions for use
IVD	In-vitro diagnostic
LRM	Local run manager
MAF	Mutant allele fraction
MM	Mutant molecules
NGS	Next-generation sequencing
NTC	No template control
PC	Positive control
PCR	Polymerase chain reaction
PF	Passing filter
RUO	Research use only
SNP	Single nucleotide polymorphism
SNV	Single-nucleotide variant substitution
UID	Unique identifier

10 Revision history

Document version	Date	Change description	Section
R4	April 2024	Addition and information about updater service and new button in software window	3.2.1 5.2
		Information about new step in Analysis Module	6.2, step 10
		Additional information in report for section • Run and Sample Validity • Sample Mutation Status • Somatic Mutations Detected • Potential Germline Mutations	7
		Deletion of Invalid Mutations Detected section in report	7
		Deletion of mutation information for samples with DNA input above allowed ranges	7
		Update of Troubleshooting table	8
R3	December 2023	Addition of note about necessity of active internet connection for automatic update information and process	3.2
		Update of troubleshooting table	8
		Update of troubleshooting table	8
		Update of website address for download	5.1
		Addition of icons	5.2
		Update of user interface	5.3
		Order during first steps of Run Planning switched	6.1, step 2
		Update of screenshots and order of appearance for Valid Somatic Mutations, Potential Germline Mutations and Invalid Mutations in report	7
		Update of Troubleshooting table	8
		Addition of Glossary and terminology table	9
		Addition of Revision history table	10
R1	July 2022	Initial release	N/A

11 Appendix A

General Terms and Conditions

for Software Licenses of Sysmex Inostics GmbH

1. Subject matter

Sysmex Inostics GmbH, Falkenried 88, 20251 Hamburg, Germany (hereinafter referred to as "SIG") grants the customer a non-exclusive, non-transferable, temporary license to use the following software:

- Plasma-SeqSensei™ IVD Software for analysis of NGS data ("SIG Software")

Title, ownership rights and intellectual property rights in the SIG Software shall not pass to the customer. The license is granted in connection with the purchase of Plasma-SeqSensei™ IVD Kits for the duration of the use of the Plasma-SeqSensei™ IVD Kits.

2. Delivery

The SIG Software will be delivered as part of the delivery of the Plasma-SeqSensei™ IVD Kits. The SIG Software will be provided in its current version.

3. Licensed products from third party suppliers

If third party software products are also provided with the SIG Software that are not open-source software, these may only be used in conjunction with the SIG Software. SIG will draw the customer's attention to any special licensing conditions in an appropriate manner.

4. Prohibition of copying

The SIG software as well as the documentation must not be copied by the customer in whole or in part, with the exception of the production of a machine-readable copy of the SIG software for backup or archiving purposes. Any copy made by the customer for these purposes must be clearly and legibly marked with a complete reference to confidentiality, title, ownership rights and the intellectual property rights of SIG.

5. Prohibition of modification

The customer is neither allowed to make any changes to the SIG software himself nor to allow any third party to make any changes.

6. Prohibition of transfer

The transfer of rights and obligations arising from the license agreement to third parties, even after termination of the agreement, is not permitted. It is not permitted to pass on the license key.

7. Unauthorized use

The customer undertakes to ensure that his employees or other persons subject to his instructions who have access to the SIG software comply with all duties of protection and care arising from this agreement. Furthermore, the customer undertakes to ensure that no one gains access to the SIG Software for the purpose of deriving the source codes. If the customer becomes aware that the SIG Software is being used by one of the aforementioned persons in contravention of the existing obligations to protect and exercise due care, he will immediately do everything in his power to prevent such use in contravention of the contract and notify SIG in writing of the use in contravention of the contract.

8. Claim for damages

SIG is entitled to the industrial property rights and copyrights to the SIG Software. The customer can be held liable by SIG for any infringement of such property rights for which he is responsible.

9. Warranty

9.1 For the quality of the SIG Software, only the description of the SIG Software provided by the licensor prior to the conclusion of the contract or agreed in a separate document (e.g. in the documentation) shall be binding. Within the scope of the maintenance obligation, the licensor is not obliged to adapt the software to changed conditions of use and technical and functional developments, such as changes in the IT environment.

9.2 The licensor does not provide any warranty for errors in the software,

- which have been caused by application errors on the part of the customer and which could have been avoided if the documentation had been consulted carefully; this shall also apply in the event of non-existent or insufficient backup measures which would have prevented data loss;
- due to virus attack or other external influences for which the licensor is not responsible, such as fire, accidents, power failure, etc.;
- which are based on the fact that the SIG Software was used in an operating environment other than that approved by the licensor, or which are due to faults in the hardware, the operating system or the software of other manufacturers;
- which are based on the fact that the software has been modified by the customer or third parties without authorisation.

9.3 The customer is obliged to notify the licensor of defects in the SIG Software immediately after their discovery. In the case of material defects, this shall be done by describing the time of occurrence of the defects and the more detailed circumstances. If the licensor carries out a fault analysis at the customer's request and it turns out that there is no defect which the licensor is obliged to rectify, the licensor may invoice the customer for the expenditure incurred on the basis of the licensor's hourly rates valid at the time.

Defects in the software shall be remedied by the licensor within a reasonable period of time (subsequent performance). This shall be done, at the licensor's discretion, by eliminating the defect by means of an update/patch/bugfix/upgrade or by delivering defect-free software or by demonstrating a workaround, the latter to the extent that this is reasonable for the customer, taking into account the effects of the defect and the circumstances of the demonstrated workaround.

10. Liability

10.1 The licensor shall be liable in accordance with the statutory provisions for damages for bodily injury and personal injury, for damages based on the Product Liability Act, for damages caused by fraudulent conduct or intent on the part of the licensor, and for damages caused by gross negligence on the part of the legal representatives or executive employees of the licensor.

10.2 Notwithstanding any liability for damages according to section 10.1, the licensor shall be liable for damages limited to the amount of the foreseeable damage typical for the contract at the time of the conclusion of the contract for damages resulting from a simple negligent breach of essential contractual obligations as well as for damages caused by vicarious agents of the licensor. Material obligations are obligations the fulfilment of which is essential for the proper performance of the contract and compliance with which the licensee may regularly rely on. The contract-typical, foreseeable damage arising from breaches of duty by the licensor shall correspond to the amount of the remuneration paid by the customer in the contract year of the damaging event, up to a maximum of EUR 50,000. If the maximum liability amount is not reached in one contract year, the maximum liability amount for the next contract year shall not be increased.

10.3 Any further liability on the part of the licensor is excluded, subject to any expressly deviating provisions in these General Terms and Conditions. In particular, the licensor shall not be liable for initial defects unless the conditions of Clauses 10.1 or 10.2 are met. The licensor shall not be liable for damages incurred by the Licensee due to failure to back up data.

10.4 Contributory negligence on the part of the customer shall be taken into account.

10.5 The above limitations of liability shall also apply to the personal liability of the licensor's employees, representatives and/or bodies. They also apply to the liability of the licensor with regard to the reimbursement of futile expenses or indemnification obligations.

11. Rights of third parties

If claims are asserted against the customer by third parties due to alleged infringement of a patent, copyright, or other industrial property right to which the third party is entitled in respect of the SIG Software, SIG will indemnify the customer against claims by third parties, provided that the customer informs SIG immediately in writing of the alleged infringement of industrial property rights and supports SIG in the conduct of any legal action.

In the event of such a claim against the customer by a third party, SIG is entitled, at its discretion, either to procure for the customer a corresponding license from the third party, to modify the SIG software or to supply the customer with equivalent other software.

SIG shall not be liable for infringements of property rights resulting from the customer modifying the licensed software or modifying it according to his own requirements, or from the SIG Software being used or sold in conjunction with other software, hardware or consumables not supplied by SIG. This subject matter liability is the entire liability of SIG for infringement of any patent, trademark, copyright, or other intangible property right.

12. Software updates

Updates to the SIG Software will be provided to the customer free of charge.

13. Payment

The license fee is discharged with the purchase of the Plasma-SeqSensei™ IVD Kits. No additional fee will be charged.

14. Contract period

The granted use of the SIG Software shall be valid for the agreed contract period (see clause 1).

The contract may be terminated in writing by either party without notice for good cause. Good cause exists, in particular, if the customer infringes the licensor's rights of use by using the software beyond what is permitted under these General Terms and Conditions and does not remedy the infringement within a reasonable period of time following a warning by the licensor. The licensor reserves the right to assert further claims for damages.

15. Data protection

Insofar as personal data is processed, the licensor shall comply with the statutory provisions on data protection. Details shall be set forth in a Data Processing Agreement to be concluded separately.

16. General provisions

These General Terms and Conditions shall be governed by the laws of Germany. Exclusive place of jurisdiction for all disputes arising from this contract shall be Hamburg.

Should one or more of the provisions of this contract be or become invalid, this shall not affect the validity of the rest of the contract.

No verbal agreements have been made. Amendments and supplements to this contract must be made in writing.

Sysmex Inostics GmbH
Falkenried 88
20251 Hamburg, Germany
www.sysmex-inostics.com

April 2024
PSSSWIFU.R4