



Plasma-SeqSensei™

IVD Software

Instructions for Use

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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 PSS 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.
- TM and ® are not explicitly indicated in this IFU.

2.4 Licenses

2.4.1 End-user license

The user of the Plasma-SeqSenseiTM 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 ► 9 Appendix A, page 49/53.

2.4.2 COSMIC license (Qiagen)

Utilisation of the COSMIC Dynamic Software Tool Large Enterprise in the PSS 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.*

3.2.1 Plasma-SeqSensei™ IVD Software maintenance

Sysmex Inostics will notify you if a new release of the PSS IVD Software is available for download. Always use the most recent PSS IVD Software version available.

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: <i>This is a single test type, other assays cannot be pooled at the same time on the sequencing device.</i>

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 connection.

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 NextSeq500 and Illumina NextSeq550 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) 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 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 $\leq 125\%$.
- Download the program at:
www.sysmex-inostics.com/products/plasma-seqsensei-kits/.
- 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 www.sysmex-inostics.com/products/plasma-seqsensei-kits/ 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.

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




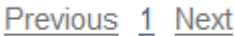
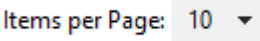


1. Close the program by either clicking on the X () on the upper right corner of the software window or on the X in the grey circle () on the lower left corner of the software window.
2. A window will appear to verify if closing of the program is desired.
3. Click on [Yes] for quitting or [No] for resuming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

5.2 Overview of icons and functions

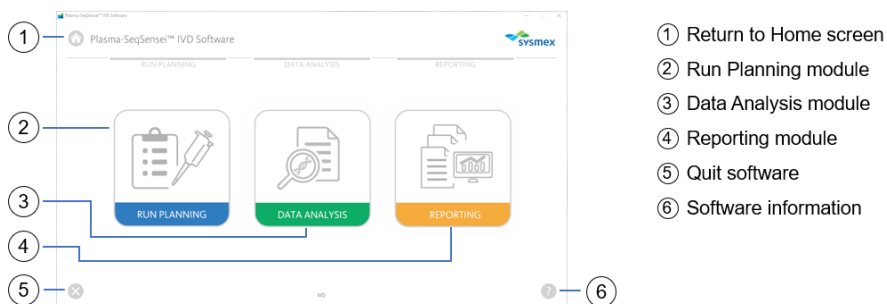
Icon	Function
	Return to Home Screen
	Software information
	Quit software
	Back to Overview in Reporting Module
	Module selection tabs are always visible to change between modules.

5 Initial steps and program window layout

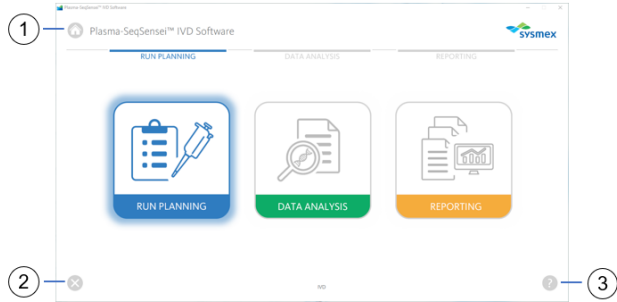
Icon	Function
	Run Planning module
	Data Analysis module
	Reporting module
	Deletes all input on the current page in Run Planning module.
	Go to previous page in Run Planning module.
	Go to next page in Run Planning module.
	Delete sample in Run Planning module.
	Export the specific file in Run Planning module.
	Navigate to select the desired folder/files in Data Analysis module.

Icon	Function
	Initiate the data analysis.
	Reload page to include newest data 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.
	Stop and cancel the analysis process in Data Analysis module.
	Delete data of a specific run in Reporting module

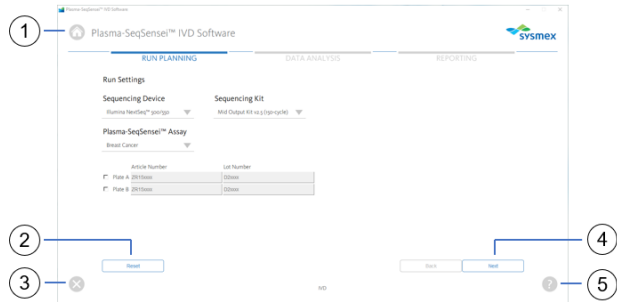
5.3 Overview of the user interface



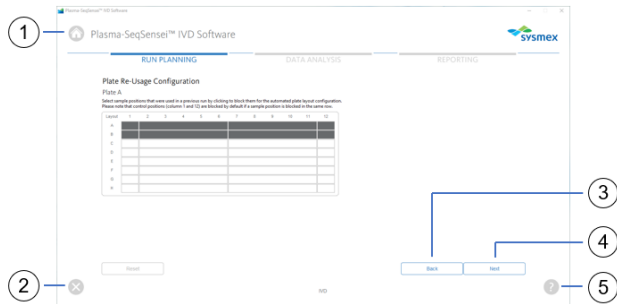
5.3.1 Run Planning module



- ① Return to Home screen
- ② Quit software
- ③ Software information

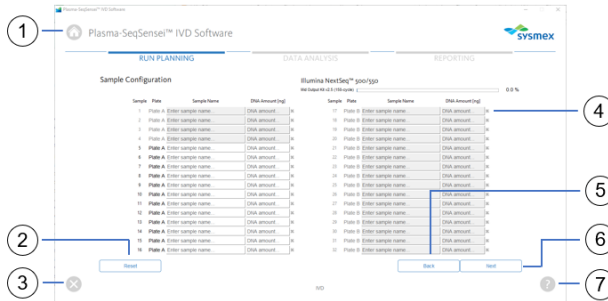


- ① Return to Home screen
- ② Reset input
- ③ Quit software
- ④ Next page
- ⑤ Software information

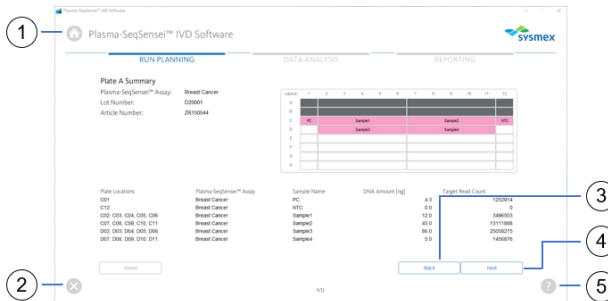


- ① Return to Home screen
- ② Quit software
- ③ Previous page
- ④ Next page
- ⑤ Software information

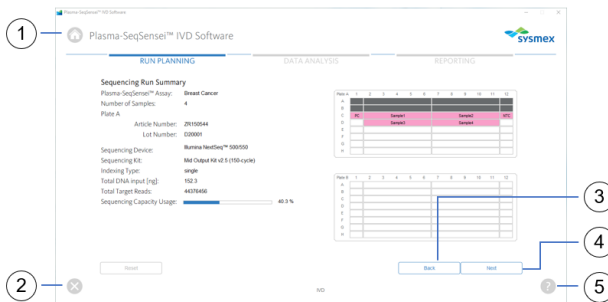
5 Initial steps and program window layout



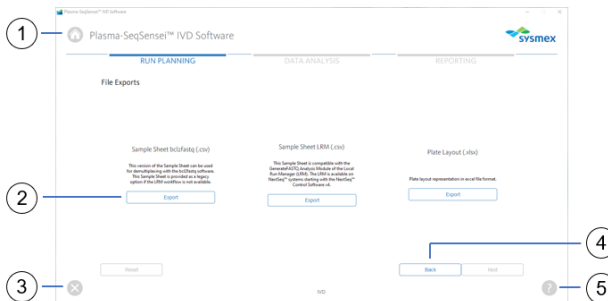
- ① Return to Home screen
- ② Reset input
- ③ Quit software
- ④ Delete sample
- ⑤ Previous page
- ⑥ Next page
- ⑦ Software information



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



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

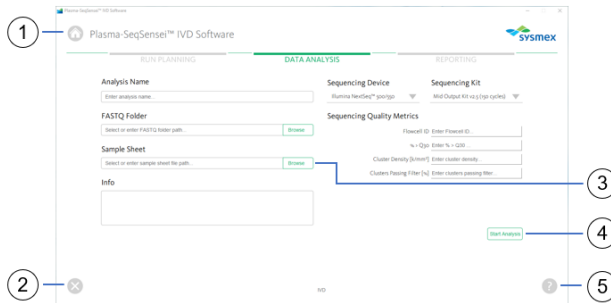


- ① Return to Home screen
- ② Export files
- ③ Quit software
- ④ Previous page
- ⑤ Software information

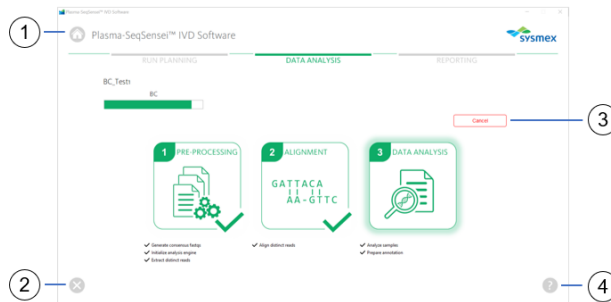
5.3.2 Data Analysis module



- ① Return to Home screen
- ② Quit software
- ③ Software information

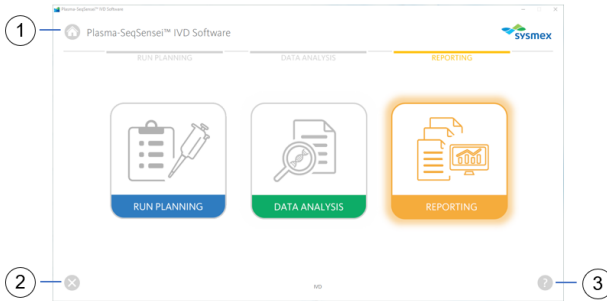


- ① Return to Home screen
- ② Quit software
- ③ Browse files / folders
- ④ Start analysis
- ⑤ Software information

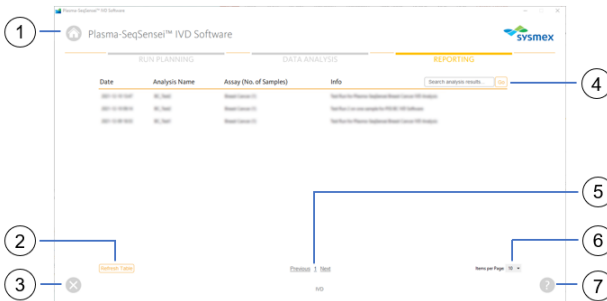


- ① Return to Home screen
- ② Quit software
- ③ Cancel analysis
- ④ Software information

5.3.3 Reporting module



- ① Return to Home screen
- ② Quit software
- ③ Software information



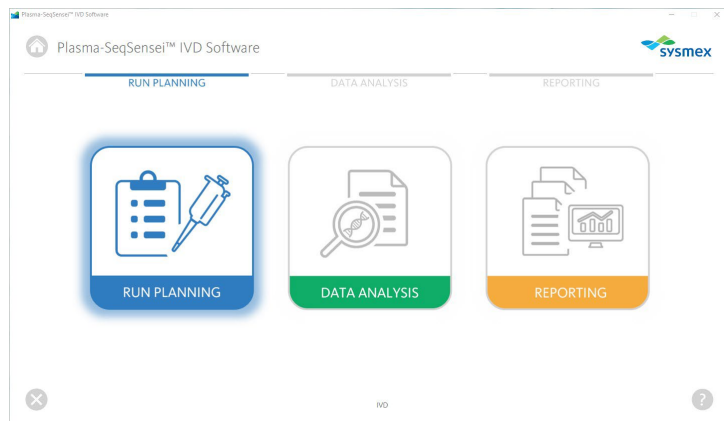
- ① Return to Home screen
- ② Refresh / reload table
- ③ Quit software
- ④ Search analysis results input field
- ⑤ Previous / next page
- ⑥ Adjust number of visible items per page
- ⑦ Software information



- ① Return to Home screen
- ② Export all pdf reports
- ③ Return to Reporting module overview
- ④ Quit software
- ⑤ Delete results
- ⑥ 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 8/53).

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).

Plasma-SeqSensei™ IVD Software

sysmex

RUN PLANNING DATA ANALYSIS REPORTING

Run Settings

Sequencing Device
Illumina NextSeq™ 500/550

Sequencing Kit
Mid Output Kit v2.5 (150-cycle)

Plasma-SeqSensei™ Assay
Breast Cancer

	Article Number	Lot Number
<input type="checkbox"/> Plate A	ZR15xxxx	D2xxxx
<input type="checkbox"/> Plate B	ZR15xxxx	D2xxxx

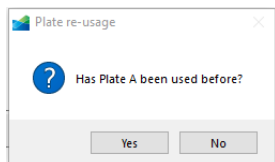
Reset Back Next

1. Click on the Run Planning module (blue).
2. Select run settings
 - a. Select a sequencing device.
 - b. Select the sequencing kit to be used.
 - c. Select the Plasma-SeqSensei™ Assay 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 PSS IVD kits and the PSS 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 PSS 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.

Plate Re-Usage Configuration

Plate A

Select sample positions that were used in a previous run by clicking to block them for the automated plate layout configuration. Please note that control positions (column 1 and 12) are blocked by default if a sample position is blocked in the same row.

Layout	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

- 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.

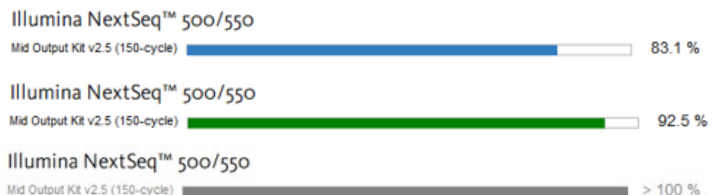
Sample Configuration

Illumina NextSeq™ 500/550

Max sequenced reads per lane: 80.3 %

Sample	Plate	Sample Name	DNA Amount [ng]	Sample	Plate	Sample Name	DNA Amount [ng]
1	Plate A	Enter sample name	DNA amount	17	Plate D	Enter sample name	DNA amount
2	Plate A	Enter sample name	DNA amount	18	Plate D	Enter sample name	DNA amount
3	Plate A	Enter sample name	DNA amount	19	Plate D	Enter sample name	DNA amount
4	Plate A	Enter sample name	DNA amount	20	Plate D	Enter sample name	DNA amount
5	Plate A	Sample1	12	21	Plate D	Enter sample name	DNA amount
6	Plate A	Sample2	4.3	22	Plate D	Enter sample name	DNA amount
7	Plate A	Sample3	85	23	Plate D	Enter sample name	DNA amount
8	Plate A	Sample4	81	24	Plate D	Enter sample name	DNA amount
9	Plate A	Sample5	27.7	25	Plate D	Enter sample name	DNA amount
10	Plate A	Sample6	43	26	Plate D	Enter sample name	DNA amount
11	Plate A	Sample7	89	27	Plate D	Enter sample name	DNA amount
12	Plate A	Enter sample name	DNA amount	28	Plate D	Enter sample name	DNA amount
13	Plate A	Enter sample name	DNA amount	29	Plate D	Enter sample name	DNA amount
14	Plate A	Enter sample name	DNA amount	30	Plate D	Enter sample name	DNA amount
15	Plate A	Enter sample name	DNA amount	31	Plate D	Enter sample name	DNA amount
16	Plate A	Enter sample name	DNA amount	32	Plate D	Enter sample name	DNA amount

- 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.

Plate A Summary
 Plasma-SeqSensei™ Assay: Breast Cancer
 Lot Number: D20001
 Article Number: ZR150544

Layout	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C	PC		Sample1					Sample2				NTC
D			Sample1					Sample4				
E												
F												
G												
H												

Plate Locations	Plasma-SeqSensei™ Assay	Sample Name	DNA Amount [ng]	Target Read Count
D01	Breast Cancer	PC	4.0	1252914
C12	Breast Cancer	NTC	8.0	0
C02, C03, C04, C05, C06	Breast Cancer	Sample1	12.0	3490503
C07, C08, C09, C10, C11	Breast Cancer	Sample2	40.0	13111886
D02, D03, D04, D05, D06	Breast Cancer	Sample3	86.0	25056275
D07, D08, D09, D10, D11	Breast Cancer	Sample4	9.0	1456876

Reset Back Next

- 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™ IVD Software

Sequencing Run Summary

Plasma-SeqSensei™ Assay: Breast Cancer
 Number of Samples: 4
 Plate A
 Article Number: ZR150544
 Lot Number: D20001
 Sequencing Device: Illumina NextSeq™ 500/550
 Sequencing Kit: Mid Output Kit v2.5 (150-cycle)
 Indexing Type: single
 Total DNA input (ng): 152.3
 Total Target Reads: 44376456
 Sequencing Capacity Usage: 40.3 %

Plate A

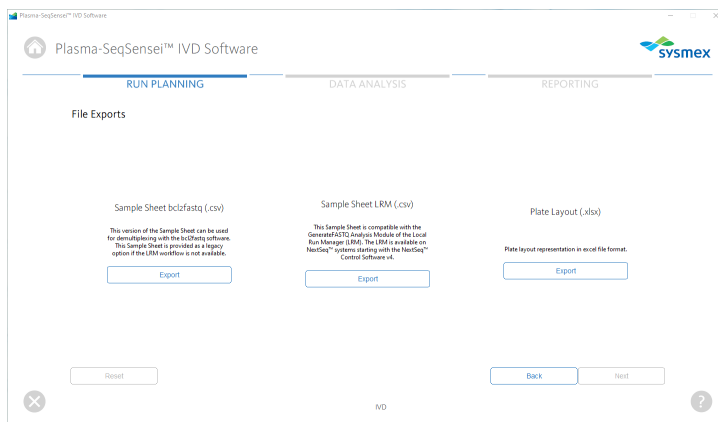
Plate A	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D	PC											NTC
E		Sample						Sample				
F		Sample						Sample				
G												
H												

Plate B

Plate B	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Buttons: Reset, Back, Next

- 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.



- 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].
- You can export the plate layout (.xlsx) for documentation purposes.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1													
2													
3													
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9													
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21													
22													
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25													
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36													
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100													

- 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	!EMFileVersion,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,CTCAGACTAG,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,ACTGTCAGAG,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):

	A	B	C	D	E	F	G	H	I	J	K	L
1	[Header],,,,,,											
2	!EMFileVersion,4,,,,,											
3	Experiment Name,BCIVDLRM2,,,,,											
4	Date,2022-05-09 12:16:33.881398,,,,,											
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	Adapter,AGATCGGAAGAGCACACGTCTGAACTCCAGTCA,,,,,											
17												
18	[Data],,,,,,											
19	Sample_ID,Sample_Name,Sample_Plate,Sample_Well,I7_Index_ID,index,I5_Index_ID,index2,Sample_Project,Description											
20	BC_IVD1_NTcplateapldx0_0_A12_a,BC_IVD1_NTcplateapldx0_0_A12_a,a,A12,A12,ACTAGATCGT,a,TTGTATCTGG,BCIVDLRM2,											
21	BC_IVD1_NTcplateapldx1_0_A12_a,BC_IVD1_NTcplateapldx1_0_A12_a,a,A12,A12,ACTAGATCGT,a,CAACCAAGCA,BCIVDLRM2,											
22	BC_IVD1_NTcplateapldx2_0_A12_a,BC_IVD1_NTcplateapldx2_0_A12_a,a,A12,A12,ACTAGATCGT,a,GGACGGTGTT,BCIVDLRM2,											
23	BC_IVD1_NTcplateapldx3_0_A12_a,BC_IVD1_NTcplateapldx3_0_A12_a,a,A12,A12,ACTAGATCGT,a,CCACGGGTAA,BCIVDLRM2,											
24	BC_IVD1_NTcplateapldx0_0_A12_b,BC_IVD1_NTcplateapldx0_0_A12_b,b,A12,A12,ACTAGATCGT,b,CTCCACTGAA,BCIVDLRM2,											
25	BC_IVD1_NTcplateapldx1_0_A12_b,BC_IVD1_NTcplateapldx1_0_A12_b,b,A12,A12,ACTAGATCGT,b,GTCCGGTACT,BCIVDLRM2,											
26	BC_IVD1_NTcplateapldx2_0_A12_b,BC_IVD1_NTcplateapldx2_0_A12_b,b,A12,A12,ACTAGATCGT,b,CTACTACTTG,BCIVDLRM2,											
27	BC_IVD1_NTcplateapldx3_0_A12_b,BC_IVD1_NTcplateapldx3_0_A12_b,b,A12,A12,ACTAGATCGT,b,ACTTGGGATA,BCIVDLRM2,											
28	BC_IVD1_PCplateapldx0_43_A01_a,BC_IVD1_PCplateapldx0_43_A01_a,a,A01,A01,CTACAGCAGT,a,TTGTATCTGG,BCIVDLRM2,											
29	BC_IVD1_PCplateapldx1_43_A01_a,BC_IVD1_PCplateapldx1_43_A01_a,a,A01,A01,CTACAGCAGT,a,CAACCAAGCA,BCIVDLRM2,											
30	BC_IVD1_PCplateapldx2_43_A01_a,BC_IVD1_PCplateapldx2_43_A01_a,a,A01,A01,CTACAGCAGT,a,GGACGGTGTT,BCIVDLRM2,											
31	BC_IVD1_PCplateapldx3_43_A01_a,BC_IVD1_PCplateapldx3_43_A01_a,a,A01,A01,CTACAGCAGT,a,CAACGGTAA,BCIVDLRM2,											
32	BC_IVD1_PCplateapldx0_43_A01_b,BC_IVD1_PCplateapldx0_43_A01_b,b,A01,A01,CTACAGCAGT,b,CTCCACTGAA,BCIVDLRM2,											
33	BC_IVD1_PCplateapldx1_43_A01_b,BC_IVD1_PCplateapldx1_43_A01_b,b,A01,A01,CTACAGCAGT,b,GTCCGGTACT,BCIVDLRM2,											
34	BC_IVD1_PCplateapldx2_43_A01_b,BC_IVD1_PCplateapldx2_43_A01_b,b,A01,A01,CTACAGCAGT,b,CTACTACTTG,BCIVDLRM2,											
35	BC_IVD1_PCplateapldx3_43_A01_b,BC_IVD1_PCplateapldx3_43_A01_b,b,A01,A01,CTACAGCAGT,b,ACTTGGGATA,BCIVDLRM2,											
36	BC_IVD1_Sample1pldx0_120_A02_a,BC_IVD1_Sample1pldx0_120_A02_a,a,A02,A02,ACACTGATG,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):

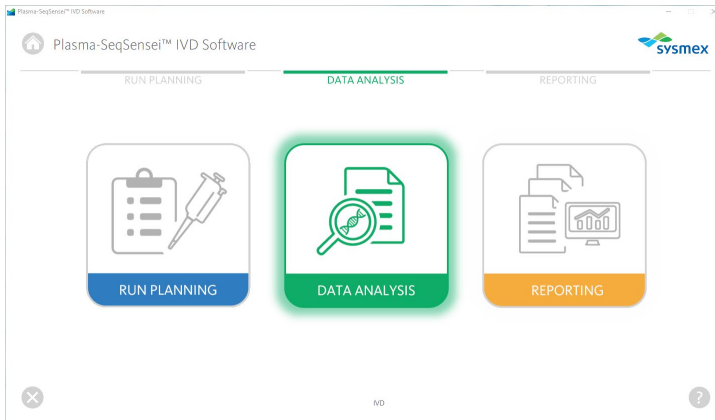
#	A	B	C	D	E	F	G	H	I	J
1	[Header],,,,,,									
2	1EMFileVersion,4,,,,,									
3	Experiment Name,BCIVDbcl2fastq,,,,,									
4	Date,2022-05-09 12:07:50.975042,,,,,									
5	Workflow,GenerateFASTQ,,,,,									
6	Application,FASTQ Only,,,,,									
7	Assay,TruSeq LT,,,,,									
8	safeseq_sw,1.1.7,,,,,									
9	Chemistry,Default,,,,,									
10	BC_IVD1,D20000,None,,,,,									
11										
12	[Reads],,,,,,									
13	148,,,,,									
14										
15	[Settings],,,,,,									
16	FilterPCRDuplicates,0,,,,,									
17	ReverseComplement,0,,,,,									
18	VariantFilterQualityCutoff,30,,,,,									
19	outputgenomevcf,FALSE,,,,,									
20	Adapter,AGATCGGAAGAGCACAGTCGTGAATCCAGTCA,,,,,									
21										
22	[Data],,,,,,									
23	Sample_ID,Sample_Name,Sample_Plate,Sample_Well,I7_Index_ID,index,Sample_Project,Description									
24	BC_IVD1_NTcplatea_0_C12_a,BC_IVD1_NTcplatea_0_C12_a,a,C12,C12,TCGTCTACTAC,BCIVDbcl2fastq,									
25	BC_IVD1_PCplatea_43_C01_a,BC_IVD1_PCplatea_43_C01_a,a,C01,C01,CATGTGATAC,BCIVDbcl2fastq,									
26	BC_IVD1_Sample1_120_C02_a,BC_IVD1_Sample1_120_C02_a,a,C02,C02,GTACAGATAG,BCIVDbcl2fastq,									
27	BC_IVD1_Sample1_120_C03_a,BC_IVD1_Sample1_120_C03_a,a,C03,C03,ATAGATCGCG,BCIVDbcl2fastq,									
28	BC_IVD1_Sample1_120_C04_a,BC_IVD1_Sample1_120_C04_a,a,C04,C04,TCGTGACACAG,BCIVDbcl2fastq,									
29	BC_IVD1_Sample1_120_C05_a,BC_IVD1_Sample1_120_C05_a,a,C05,C05,ATCAGAGAGAG,BCIVDbcl2fastq,									
30	BC_IVD1_Sample1_120_C06_a,BC_IVD1_Sample1_120_C06_a,a,C06,C06,TACTGCGAGAG,BCIVDbcl2fastq,									
31	BC_IVD1_Sample2_450_C07_a,BC_IVD1_Sample2_450_C07_a,a,C07,C07,AGTGTCTCTG,BCIVDbcl2fastq,									
32	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	1EMFileVersion,4,,,,,											
3	Experiment Name,BCIVDbcl2fastq,,,,,											
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,AGATCGGAAGAGCACAGTCGTGAATCCAGTCA,,,,,											
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_b,BC_IVD1_NTcplatea_0_A12_b,b,A12,A12,ACTAGATCGT,b,TGCTGTGGTG,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_b,BC_IVD1_NTcplatea_0_A12_b,b,A12,A12,ACTAGATCGT,b,TTACGTTGGAG,BCIVDbcl2fastq2,											
28	BC_IVD1_NTcplateb_0_A12_b,BC_IVD1_NTcplateb_0_A12_b,b,A12,A12,ACTAGATCGT,b,TTACGTTGGAG,BCIVDbcl2fastq2,											
29	BC_IVD1_NTcplateb_0_A12_b,BC_IVD1_NTcplateb_0_A12_b,b,A12,A12,ACTAGATCGT,b,AGTACCGGAG,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_b,BC_IVD1_PCplatea_43_A01_b,b,A01,A01,CTACAGCAGT,b,TGCTGTGGTG,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_b,BC_IVD1_PCplatea_43_A01_b,b,A01,A01,CTACAGCAGT,b,TTACGTTGGAG,BCIVDbcl2fastq2,											
36	BC_IVD1_PCplateb_43_A01_b,BC_IVD1_PCplateb_43_A01_b,b,A01,A01,CTACAGCAGT,b,TTACGTTGGAG,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 BaseSpace™ all .fastq.gz files need to be copied to a single folder location.

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 8/53).

6 Plasma-SeqSensei™ IVD Software modules

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/mm²
- Q30 score: ≥ 80 %
- Clusters Passing Filter (PF): ≥ 80 %

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

The screenshot displays the Plasma-SeqSensei™ IVD Software interface. At the top, there are three tabs: RUN PLANNING, DATA ANALYSIS (which is highlighted in green), and REPORTING. The DATA ANALYSIS tab contains several input fields and buttons. On the left, there is a section for 'Analysis Name' with a text input field and a 'Browse' button. Below this is a 'FASTQ Folder' section with a text input field and a 'Browse' button. Further down is a 'Sample Sheet' section with a text input field and a 'Browse' button. At the bottom left is an 'Info' section with a large empty text area. On the right side, there are two dropdown menus for 'Sequencing Device' (set to 'Illumina NextSeq™ 500/550') and 'Sequencing Kit' (set to 'Mid Output kit v2.5 (50 cycles)'). Below these are 'Sequencing Quality Metrics' with four input fields: 'Flowcell ID', '% > Q30', 'Cluster Density [k/mm²]', and 'Clusters Passing Filter [%]'. Each of these fields has a 'Browse' button next to it. At the bottom right of the form is a green 'Start Analysis' button. The bottom of the window shows a status bar with a close button (X), a question mark icon, and the text 'IVD'.

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/mm²]
 - d. Clusters Passing Filter [%]

When navigating to the next page 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.*

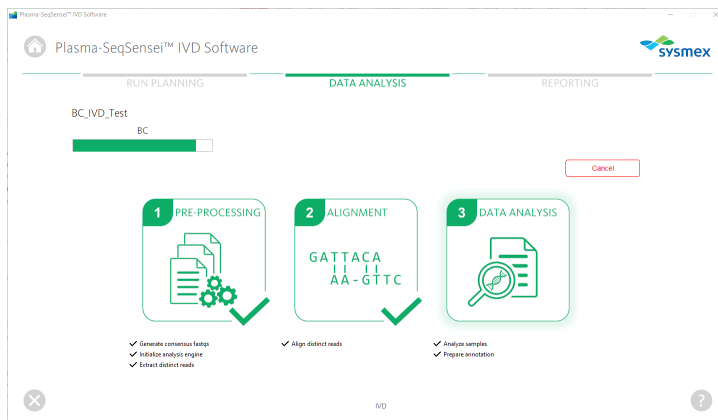
7. Select the sample sheet that was created in the Run Planning module (.csv) by clicking on the [Browse] button and navigating to the file to be selected.
8. Fill in information regarding the experiment, sequencing run or analysis (optional).
9. Click [Start Analysis].

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

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 46/53.

10. 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.
11. 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.

6.3 Reporting module

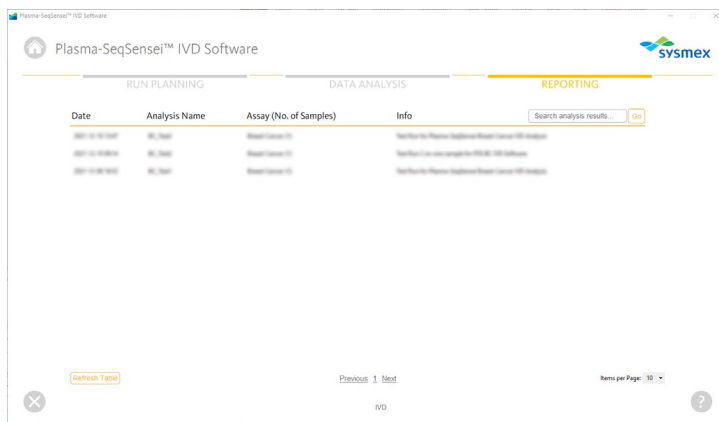


The Reporting module (orange) is used to save and manage all analysis results performed using the Plasma-SeqSensei™ IVD Software. It allows the user to:

- 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.

6 Plasma-SeqSensei™ IVD Software modules



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](#)

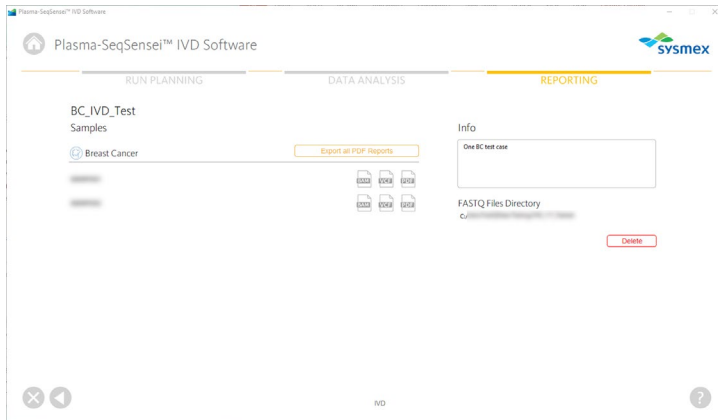
- b. The number of items per page can be changed on the bottom right-hand corner of the page.

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.

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

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

Export all PDF Reports

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.

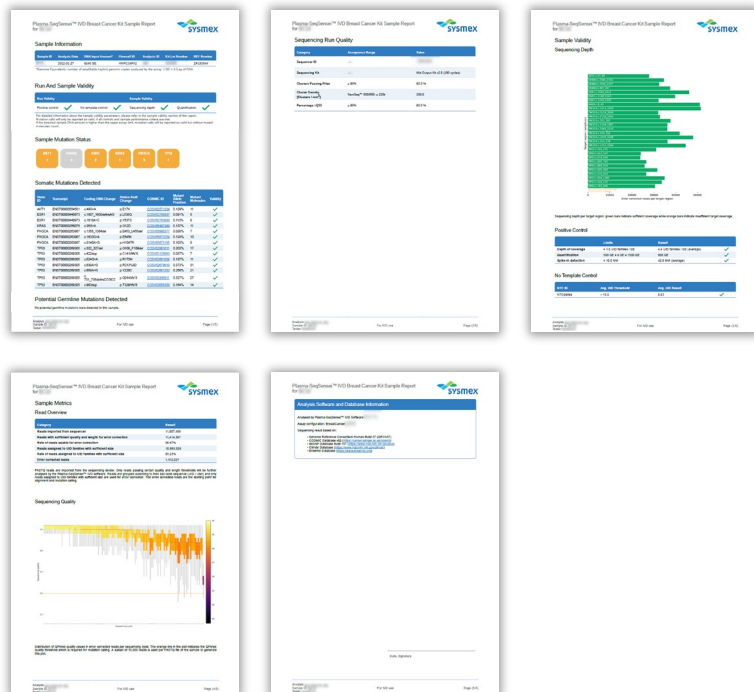
Delete

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, only for valid samples) 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 of these 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.

The reports generated by the PSS IVD Software contain multiple sections:

- Sample information

- Run and Sample validity
 - Sequencing depth
 - Positive control
 - No template control
- Sample mutation status
- Mutations detected
 - Somatic mutations
 - Invalid 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
	2022-01-20	8346 GE	HMFCJAFX2			

*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
	2022-02-03	Not quantifiable	HNLGFAFX2			

*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.

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.
- PSS IVD Kit Lot number used
- The REF number specifies the article number of the PSS kit used.

Run And Sample Validity

Run Validity		Sample Validity	
Positive control:	✓	No template control:	✓
		Sequencing depth:	✓
		Quantification:	✗

For detailed information about the sample validity parameters, please refer to the sample validity section of this report.
Mutation calls will only be reported as valid, if all controls and sample performance criteria are met.
If the detected sample DNA amount is higher than the upper assay limit, mutation calls will be reported as valid but without mutant molecules count.

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.

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 below 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.

If the DNA input amount is above the allowed input range, the sample quantification will be depicted as invalid (✗) and only MAF values will be reported without the respective mutant molecule (MM) counts.

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

Sample Mutation Status



The **Sample Mutation Status** gives an overview of how many mutations were detected per gene analysed by the PSS 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:

Somatic Mutations Detected

Gene ID	Transcript	Coding DNA Change	Amino Acid Change	COSMIC ID	Mutant Allele Fraction	Mutant Molecules	Validity
AKT1	ENST00000554581	c.49G>A	p.E17K	COSV62571334	0.129%	11	✓
ESR1	ENST00000440973	c.1607_1608delinsAG	p.L536Q	COSV52785937	0.091%	8	✓
ESR1	ENST00000440973	c.1610A>C	p.Y537S	COSV52783938	0.113%	9	✓
KRAS	ENST00000256078	c.35G>A	p.G12D	COSV55497369	0.137%	11	✓
PIK3CA	ENST00000263967	c.1356_1364del	p.E453_L455del	COSV55900377	0.088%	7	✓
PIK3CA	ENST00000263967	c.1633G>A	p.E545K	COSV55873239	0.124%	10	✓
PIK3CA	ENST00000263967	c.3140A>G	p.H1047R	COSV55873195	0.103%	9	✓
TP53	ENST00000269305	c.322_327del	p.G108_F109del	COSV52901611	0.203%	17	✓
TP53	ENST00000269305	c.422dup	p.C141Wfs*8	COSV53125883	0.087%	7	✓
TP53	ENST00000269305	c.524G>A	p.R175H	COSV52661038	0.137%	11	✓
TP53	ENST00000269305	c.639A>G	p.R213K3D	COSV52679610	0.373%	31	✓
TP53	ENST00000269305	c.659A>G	p.Y220C	COSV52661282	0.256%	21	✓
TP53	ENST00000269305	c.731_736delinsCCGCC	p.G244Afs*3	COSV53345811	0.327%	27	✓
TP53	ENST00000269305	c.983dup	p.T329Hfs*8	COSV53056388	0.164%	14	✓

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 PSS IVD Software in the covered gene regions of the PSS IVD Kit used, when Run and Sample Validity criteria were fulfilled (with the possible exception of Quantification).

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
- COSMIC ID, if available (COSV number) 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).

The MM value will not be displayed if the DNA input is above the input range of the assay.

- Validity of the mutation

Example A:

Invalid Somatic Mutations Detected

Reason	Number of Invalid Mutations
Failure of Positive Control (PC)	14

Presence of invalid mutation calls detected, please refer to the VCF output for additional information and consider re-running the sample.

Example B:

Invalid Somatic Mutations Detected

Reason	Number of Invalid Mutations
Failure of No Template Control (NTC)	14

Presence of invalid mutation calls detected, please refer to the VCF output for additional information and consider re-running the sample.

Example C:

Invalid Somatic Mutations Detected

Reason	Number of Invalid Mutations
Sequencing depth of amplicon below threshold	20

Presence of invalid mutation calls, please refer to the VCF output for additional information and consider re-running the sample.

Example D:

Invalid Somatic Mutations Detected

Reason	Number of Invalid Mutations
Lower limit for DNA input not reached	17

Presence of invalid mutation calls detected, please refer to the VCF output for additional information and consider re-running the sample.

In the **Invalid Somatic Mutations Detected** section of the report the number of invalid mutations is shown that were detected by the PSS IVD Software in the covered gene regions of the PSS IVD Kit used. The reason for invalidity is also given.

Invalid mutations can be viewed in the .vcf file of the respective sample.

Example A:

Potential Germline Mutations Detected

Gene ID	Transcript	Coding DNA Change	Amino Acid Change	dbSNP	MAF	Mutant Molecules	Validity
PIK3CA	ENST00000263967	c.363C>T	p.I121=	rs115746478	99.0%	4950	✓
TP53	ENST00000269305	c.329G>T	p.R110L	rs11540654	51.0%	2550	✓

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 between 40 % and 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
- dbSNP 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). The MM value will not be displayed if the DNA input is above the input range of the assay.
- Validity of the possible germline mutation

Sequencing Run Quality

Category	Acceptance Range	Value
Sequencer ID	- / -	
Sequencing Kit	- / -	Mid Output Kit v2.5 (150 cycles)
Clusters Passing Filter	≥ 80%	80.0 %
Cluster Density [Clusters / mm ²]	NextSeq™ 500/550: ≤ 220k	200.0
Percentage >Q30	≥ 80%	80.0 %

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

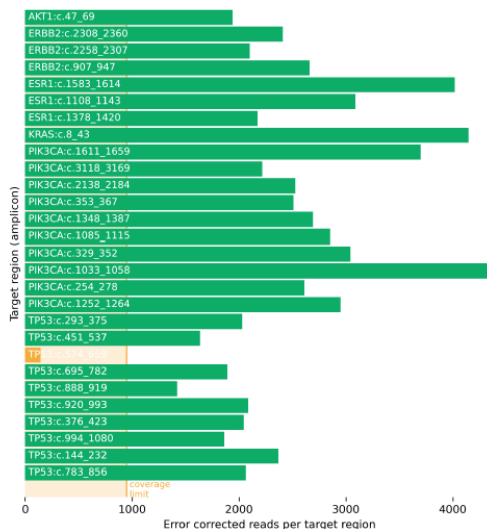
The following categories are shown:

- Sequencer ID
- Sequencing kit used
- Clusters passing filter
- Cluster density in clusters/mm²
- Percentage > Q30

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

Sample Validity

Sequencing Depth



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 PSS IVD Kit is depicted. Green bars represent amplicons with sufficient target coverage, orange bars show amplicons that have insufficient target coverage. 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”.

Positive Control

	Limits	Result	
Depth of coverage	≥ 1.0 UID families / GE	0.1 UID families / GE (average)	✗
Quantification	500 GE ≤ x GE ≤ 1500 GE	773 GE	✓
Spike-In detection	≥ 10.0 MM	58.1 MM (average)	✓

If depth of coverage failed, not all amplicons were detected above or equal to the lower coverage limit. For simplicity, only the avg. detection result of all amplicons is shown in this report.

The **Positive Control** table shows the limits to be reached by the positive control of the assay for valid PSS 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 mutations.
- 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.

Sample Metrics

Read Overview

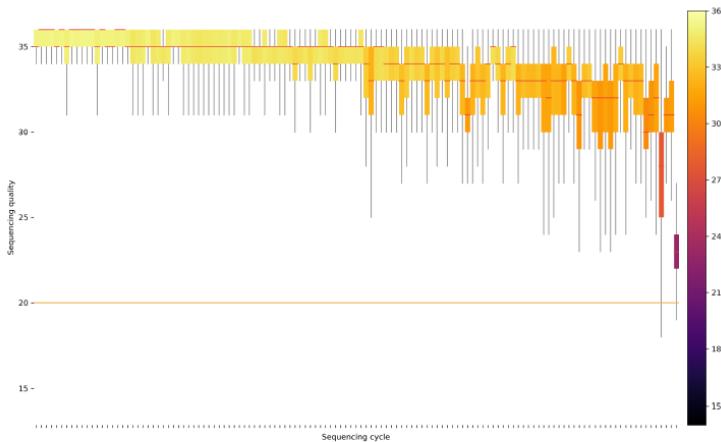
Category	Result
Reads imported from sequencer	2,759,170
Reads with sufficient quality and length for error correction	1,831,255
Rate of reads usable for error correction	66.37%
Reads assigned to UID families with sufficient size	1,779,693
Rate of reads assigned to UID families with sufficient size	97.18%
Error corrected reads	101,635

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.

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.

Analysis Software and Database Information

Analysed by Plasma-SeqSensei™ IVD Software

Assay configuration: BreastCancer_IVD1

Sequencing result based on:

- Genome Reference Consortium Human Build 37 (GRCh37)
- COSMIC Database V92 (<https://cancer.sanger.ac.uk/cosmic>)
- dbSNP Database Build 151 (<https://www.ncbi.nlm.nih.gov/snp>)
- ClinVar Database (<https://www.ncbi.nlm.nih.gov/clinvar>)
- Ensembl Database (<https://www.ensembl.org>)

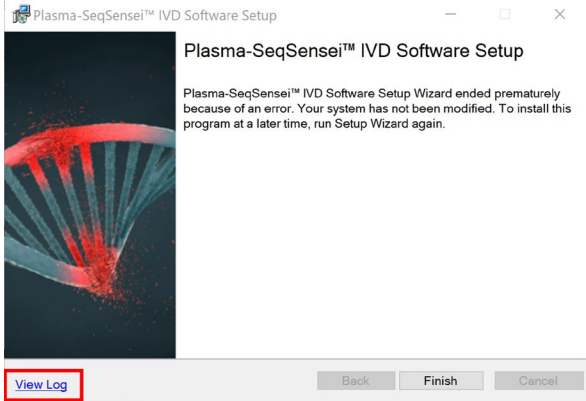
The last section of the report depicts the **Analysis Software and Database Information** used for the analysis of this sample.

Date, Signature

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™ PSS Software or contact your local authorised Sysmex representative for further information.

Problem	Solution
The software installation does not finish successfully.	<p>Please make sure you have followed exactly the instructions given in section 5.1.1 of this manual for the installation of the software. 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):</p> 
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 license key is not accepted during installation of the software.	Check if 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.
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: <ol style="list-style-type: none"> 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 Plasma-SeqSensei™ 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™?	<p>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 PSS IVD Software will concatenate those files automatically for further analysis.</p> <p>When using the PSS Extension IVD kit and two plates are being sequenced on the same sequencing run, each well will result in 16 files.</p>
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.
I cannot start the analysis because 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.	During demultiplexing, FASTQ files are generated. Depending on your demultiplexing pipeline, either 5 FASTQ files per sample, 5 wells per sample or one file per well is/are generated. This usually is the outcome if you use bcl2fastq for demultiplexing. Alternatively, 20 FASTQ files per sample are generated – 5 wells per sample, 4 files per lane, 1 file per lane. This is the case if you use the “GenerateFASTQ” module of the Local Run Manager on your NextSeq™ device.

	<p>When two PSS plates are run at the same time, 80 FASTQ files per sample are generated - 5 wells per sample, 4 files per lane, 4 files per plate index.</p> <p>In summary, there should be 5, 20, or 80 FASTQ files present for a single sample in the FASTQ input folder. Positive and No Template controls however only receive 1, 4 or 16 FASTQ files.</p>
My analysis takes forever.	<p>The analysis usually takes less than 6 hours. If longer analysis times are noticed multiple issues could have occurred:</p> <ul style="list-style-type: none"> • 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 <p>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.</p>
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.
Some amplicons are invalid, what does this mean for my report?	<p>If more than 10 % of all target regions do not reach the target sample coverage, the entire sample analysis is invalid. If less than 10 % of all target regions do not reach the target sample coverage, only those amplicons are invalid, while the rest of the regions are valid.</p> <p>All detected mutations (valid and invalid ones) can be found in the .vcf files.</p>

9 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.



May 2022

PSSSWIFU.R1

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