Qlucore Diagnostics BCP-ALL

Instructions for Use

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1. Revision history

Date	Revision No.	Change
2023-10-04	Version 1.0	First version
2024-01-19	Version 2.0	Major update. Added performance data, analytical performance, mathematical approach, residual risks and other required information.
	Version 3.0	Added warning in section 19.3.3 Corrected heading for intended use Correction of performance data
2024-08-16	Version 4.0	Clarification of Qlucore Diagnostics in-vitro medical device software product classification ID number of notified body added to CE-symbol in table 2.
2025-01-31	Version 5.0	Updates to the License handling, Quality metrics and System requirements paragraphs. Added paragraph with information about only one open device. Clarified the RNA-seq classification result paragraph in the report. Added/removed some warning & error messages.
2025-02-24	Version 6.0	Update the warning and error tables, removed the signature section, adjusted header and footer.

2. Terminology

Table 1 lists definitions of terms, abbreviations, and expressions that are used in this document and in the Qlucore Diagnostics interface:

Table 1. Terminology.

Term	Explanation
Principal Component Analysis, PCA	PCA is a method for reducing high-dimensional data into lower dimensions. This is done by transforming the large set of variables into linear and orthogonal vectors, that preserves most of the variation in the dataset.
Case	The processing of data files from a sample in Qlucore Diagnostics (from files selected to results available)
Training data	The dataset used for training the machine learning model used in Qlucore Diagnostics.

Term	Explanation
QCSLS file	The Qlucore Diagnostics optional fields file format
QSD file	The Qlucore Diagnostics sample data file format
QDM file	The Qlucore Diagnostics Model file format

3. Writing conventions

Numbers presented in the user interface of Qlucore Diagnostics, on screen, and in reports use a point as a decimal separator. Large and small numbers are written using the *scientific e notation*. Thus, for instance:

6,100 is written as **6.1e3** or **6.1×10^{3}**,

5,400,000 is written as **5.4e6** or **5.4×10^{6}**,

0.06 is written as **6e-2** or **6×10^{-2}**.

4. Symbols used

Table 2 lists the symbols used in this document and in the Qlucore Diagnostics software.

Table 2. Symbols used in Qlucore Diagnostics.

Symbol	Title
CE 2797	CE marked product, followed by the ID number of the notified body.
	Indicates the medical device manufacturer. Can be combined with Date of manufacture.
	Indicates the date when the medical device was manufactured.
 https://qlucore.com/qd/eifu	Indicates the need for the user to consult the Instructions for use.
	Indicates the need for the user to consult the instruction for use for important cautionary information such as warnings and precautions that cannot be presented on the medical device itself.
UDI	Indicates a carrier that contains Unique Device Identifier information.
REF + catalogue number	Indicates the manufacturer's catalogue number so that the medical device can be identified.
IVD	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.

5. About Qlucore Diagnostics

5.1. Introduction

Qlucore Diagnostics BCP-ALL software is an in-vitro diagnostics medical device with the intended use of clinical precision cancer diagnostics based on RNA-seq data. It is designated for installation on a computer at hospitals and clinical labs for analysis, interpretation and display of results based on data from next-generation sequencing (NGS) of samples from bone marrow or peripheral blood.

Qlucore Diagnostics BCP-ALL software integrates an AI-powered cancer-specific machine-learning model, offering user-friendly reports and 3D visualizations. Moreover, it features a disease-agnostic platform and a specialized model, adapted to classifying and managing pediatric patients.

Installed in clinical labs, the software provides an easily navigable solution for diagnostics, outlined in this document. These instructions ensure comprehensive guidance, covering safety details, precautions, potential risks, and data security measures, facilitating effective utilization for precision cancer diagnostics in clinical settings.

Qlucore Diagnostics BCP-ALL consists of two components: a disease-agnostic platform and a dedicated disease-specific model. This Instructions for Use document is for the BCP-ALL model running on the Qlucore Diagnostics platform.

The Qlucore Diagnostics BCP-ALL in-vitro diagnostics medical device is intended to be installed on a computer in a clinical lab and used to aid in the initial classification and management of pediatric patients, based on genetic data from next-generation sequencing (NGS) of bone marrow or peripheral blood samples.

With a disease-specific (BCP-ALL) machine learning-based model to enable classification and a feature supporting clinical decisions using, among other things, 3D visualizations, Qlucore Diagnostics BCP-ALL aids the user in classifying samples into subtypes, using molecular information.

Qlucore Diagnostics BCP-ALL allows the user to import RNA-sequencing data that has been aligned and processed by gene fusion detection algorithms. Following import into the software, two key features are available:

1) gene fusion identification and
2) machine-learning-based classification of the analyzed sample into known subtypes of BCP-ALL, based on gene expression levels.

The gene fusions are automatically checked from a quality perspective and presented in different tiers, based on their relevance for BCP-ALL.

The gene expression analysis of BCP-ALL subtypes for the patient sample is performed using a BCP-ALL-dedicated classifier built by Qlucore. These two key features can support each other in subtype classification.

A clinical report, summarizing the result of the analysis, is automatically generated. The user can then add a conclusion text to the report if needed and export the report as a PDF file. In addition to the results, the report includes a 3D plot for illustration, showing the patient sample in relation to the training data.

The software can be run locally installed on a Windows or Mac computer. Users can choose between a graphical user interface and command-line mode.

5.2. Analytical Performance

The analytical performance has been evaluated using a dataset of 26 BCP-ALL samples and 20 T-ALL samples from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) ALL cohort. Each BAM file from the dataset was down-sampled by consecutively decreasing the proportion of the original reads obtained in the original sample. Furthermore, the BAM files were mixed with an increasing fraction of reads from a normal blood sample for analysis of the normal cell score.

The analytical sensitivity was assessed by the examination of the limits of detection. Hard limits and minimum or maximum cut-off values were determined for the following quality parameters in down-sampled BAM files:

- **Aligned read pairs**: The number of read pairs for paired-end sequencing in the sample that was mapped to the reference genome.
- **Read pairs mapping to features**: number of aligned read pairs that are unambiguously assigned to a feature (i.e., a gene in the reference genome).
- **Fraction of read pairs mapping to features (%)**: proportion of aligned read pairs that are unambiguously assigned to a feature (i.e., a gene in the reference genome).
- **Normal cell score**: indicator of the relative tumor cell content in a sample.
- **Local outlier factor**: indicator of the probability of a sample of being an outlier based on the comparison of local densities with its neighbors.

For the platform to accept a BAM file, its quality parameters must fall within specified hard limits and meet required cut-off values. The valid ranges, derived from these criteria, are summarized in the tables below.

Table 3. Analytical performance for Qlucore Diagnostics BCP-ALL subtype classification.

Parameter	Cut-off	Valid range
• Aligned read pairs (million)	≥10	[10, 100]
• Read pairs mapping to features (million)	none	[6, 100]
• Fraction of read pairs mapping to features (%)	≥60	[60, 100]
• Normal cell score	<2.5	[-3, 2.5)
• Local outlier factor	<1.3	[0, 1.3)

Table 4. Analytical Performance for Qlucore Diagnostics BCP-ALL gene fusion.

Parameter	Cut-off	Valid range
• Aligned read pairs (million)	≥10	[10, 100]

5.3. Clinical Performance

The clinical performance was examined on a representative sample of the intended population for the product. Genetic subtype classification and gene fusion detection were performed by comparing the outcome of Qlucore Diagnostics BCP-ALL to the known status of the sample. The results of the clinical data were analyzed to produce sensitivity, specificity, percent positive agreement (PPA), and percent negative agreement (PNA).

Table 5. Clinical Performance for Qlucore Diagnostics BCP-ALL subtype classification, overall parameters.

Overall Parameter	Result
Overall Sensitivity	91.5%, [95% CI (86.2% - 96.8%)]
Overall Specificity	98.3%, [95% CI (97.2% - 99.4%)]
Overall PPA	93.4%, [95% CI (90.3% - 96.4%)]
Overall PNA	98.9%, [95% CI (98.4% - 99.4%)]

Table 6. Clinical Performance for Qlucore Diagnostics BCP-ALL subtype classification, individual parameters.

Genetic Subtype	Individual Parameter	Result
BCR::*ABL1* or *BCR*::*ABL1*-like	Sensitivity (*)	80.0%, [95% CI (55.2% - 105%)]
	Specificity	-
	PPA	87.5%, [95% CI (76.0% - 99.0%)]
	PNA	99.1%, [95% CI (97.9% - 100%)]
ETV6::*RUNX1* or *ETV6*::*RUNX1*-like	Sensitivity (**)	100%
	Specificity	-
	PPA	100%
	PNA	99.5%, [95% CI (98.6% - 100%)]
DUX4-rearranged	Sensitivity	-
	Specificity	-
	PPA	100%
	PNA	100%
High hyperdiploidy	Sensitivity	88.5%, [95% CI (76.2% - 101%)]

Genetic Subtype	Individual Parameter	Result
	Specificity	100%
	PPA	83.7%, [95% CI (72.7%, 94.8%)]
	PNA	100%
KMT2A(MLL)-rearranged	Sensitivity	85.7%, [95% CI (59.8% - 112%)]
	Specificity	99%, [95% CI (97.1% - 101%)]
	PPA	80.0%, [95% CI (55.2%, 105%)]
	PNA	99.6%, [95% CI (98.8%, 100%)]
TCF3::*PBX1*	Sensitivity	100%
	Specificity	100%
	PPA	93.3%, [95% CI (80.7%, 106%)]
	PNA	100%

(*) Determined with samples *BCR*::*ABL1* or *BCR*::*ABL1-like* (*ABL*-class) for which reference methods were available as pediatric standard of care. (**) Determined with samples *ETV6*::*RUNX1* for which reference methods were available as pediatric standard of care.

Table 7. Clinical Performance for Qlucore Diagnostics BCP-ALL gene fusion detection.

Gene fusion	Parameter	Result
BCR::*ABL1*	PPA	100%
	PNA	100%
ETV6::*RUNX1*	PPA	100%
	PNA	100%
KMT2A(MLL)-rearranged	PPA	100%
	PNA	100%
TCF3::*PBX1*	PPA	100%
	PNA	100%

Table 8. Positive likelihood ratio and negative likelihood ratio

Likelihood ratio	Result
Positive	53.8
Negative	0.09

5.4. Mathematical approach

5.4.1. The classifier

One key element of the device is the capability to predict the specific patient's BCP-ALL subtype using gene expression levels (a measurement of how active different genes are in the specific patient sample). This is achieved by a machine learning based predictor, a classifier. Based on BCP-ALL characteristics, the classification problem decomposes into 6 independent binary classification problems and the classifier consists of six binary classifiers, one for each subtype, except BCP-ALL Other. Each binary classifier is a boosted trees classifier trained using the JrBoost software package [https://github.com/jrade]. The output from a boosted trees classifier is the probability that the tested sample is positive (i.e. is classified as belonging to the subtype). The threshold to distinguish between negative and positive samples is 0.5.

A machine learning predictor is a function that predicts the output variables, based on input data. The predictor is trained for a defined problem using training data. The training data for Qlucore Diagnostics BCP-ALL consists of a consecutive series of patients from southern Sweden. Of these, RNA or material suitable for RNA extraction from bone marrow (n=171) or peripheral blood (n=24) taken at diagnosis was available.

5.4.2. Diagnostic sensitivity, specificity, percent agreement, and likelihood ratio

Results were tabulated in a 2x2 contingency table according to Table 9 when the reference method for Qlucore Diagnostics BCP-ALL was the diagnostic accuracy criteria.

Table 9. Contingency table according to diagnostic accuracy criteria.

		True status		
		Positive	Negative	Total
Predicted outcome	Positive	# true positive (TP)	# false positive (FP)	TP+ FP
	Negative	# false negative (FN)	# true negative (TN)	FN + TN
	Total	TP + FN	FP + TN	N

Estimated diagnostic sensitivity and specificity were calculated with the following formulas:

$$Estimated\ sensitivity = 100 \times [TP/(TP + FN)]$$

$$Estimated\ specificity = 100 \times [TN/(TN + FP)]$$

Alternatively, when the reference method was not the diagnostic accuracy criteria, the results were tabulated according to Table 10 and expressed as percent positive agreement (PPA) and percent negative agreement (PNA).

Table 10. Contingency table according to non-diagnostic accuracy criteria comparator.

		True status		
		Positive	Negative	Total
Predicted outcome	Positive	a	b	a + b
	Negative	c	d	c + d
	Total	a + c	b + d	n

Overall percent agreement (OPA), percent positive agreement (PPA) and percent negative agreement (PNA) were calculated with the following formulas:

$$Overall\ percent\ agreement\ (OPA)\ =\ 100\ \times (a+d)/n$$

$$Percent\ positive\ agreement\ (PPA) = 100\ \times a/(a+c)$$

$$Percent\ negative\ agreement\ (PNA) =\ 100\ \times d/(b+d)$$

Results for the clinical performance parameters are expressed as proportions (%) and 95% confidence intervals (CI) calculated as:

$$95\ \%\ CI\ for\ sensitivity = sensitivity\ \pm 1.96\ \times \sqrt{sensitivity\ \times (1-sensitivity)/(TP+FN)}$$

$$95\ \%\ CI\ for\ specificity = specificity \pm 1.96\ \times \sqrt{specificity \times (1-specificity)/(TN+FP)}$$

Positive likelihood ratio and negative likelihood ratio were estimated with the following formulas:

$$Positive\ likelihood\ ratio\ [LR(+)] =\ sensitivity\ /\ (1\ -\ specificity)$$

$$Negative\ likelihood\ ratio\ [LR(-)] = (1-\ sensitivity)\ /\ specificity$$

6. Intended purpose

6.1. Qlucore Diagnostics Platform

The Qlucore Diagnostics Platform is a software specifically intended to be used together with one or several Qlucore Diagnostics Models, by providing it a hosting environment, execute it, and report analysis results.

The Qlucore Diagnostics Platform is intended to be used by trained healthcare professionals in a clinical laboratory setting.

6.1.1. Intended user

Qlucore Diagnostics BCP-ALL is intended to be used by trained healthcare professionals in a clinical laboratory setting. Especially roles such as:

- geneticist, laboratory scientist or bioinformatician for the pre-processing pipeline,
- geneticist or laboratory scientist for running a case
- bioinformatician, geneticist or laboratory scientist for command-line usage and

- geneticist/laboratory scientist/pathologist/Medical Doctor specialist within genetics and or oncology for interpreting the report.

6.1.2. Intended population

N/A: Qlucore Diagnostics platform has no medical purpose.

6.1.3. Contraindications

N/A: Qlucore Diagnostics platform has no medical purpose.

6.2. Qlucore Diagnostics BCP-ALL

The Qlucore Diagnostics BCP-ALL is software intended for qualitative determination of the presence of clinically relevant genetic markers in samples from bone marrow or peripheral blood during the genetic work-up of pediatric B-cell precursor Acute Lymphoblastic Leukemia (BCP-ALL).

The software supports an analysis of RNA-Seq data using gene expression-based classification and gene fusion identification. The classification of the sample is achieved by a machine learning-based classifier, which yields a probability score for the following defined genetic subtypes:

- *BCR::ABL1* or *BCR::ABL1-like*
- *DUX4*-rearranged
- *ETV6::RUNX1* or *ETV6::RUNX1-like*
- High hyperdiploidy
- *KMT2A(MLL)*-rearranged
- *TCF3::PBX1*

Gene fusion identification is performed by fusion callers and identified gene fusions are exported to a report along with fusion breakpoints.

The results obtained from the Qlucore Diagnostics BCP-ALL can be used to aid in the initial classification and management of pediatric patients from 1 up to 18 years of age, with suspected or diagnosed BCP-ALL. The results of the analyses are not intended for minimal residual disease (MRD) monitoring. Standard laboratory protocols for processing patient blood or bone marrow samples, library preparation from purified mRNA and whole-transcriptome RNA-sequencing should be followed.

The operation of the Qlucore Diagnostics BCP-ALL, as well as the interpretation of the analysis results is to be carried out by trained healthcare professionals in a clinical laboratory setting and used in conjunction with other clinical and laboratory findings. Test results are not to be interpreted as a negative result based on the absence of a fusion gene or absence of a specific subtype of BCP-ALL based on the gene expression probability score.

The Qlucore Diagnostics BCP-ALL is intended to be used together with the Qlucore Diagnostics Platform software.

6.2.1. Definitions

Suspected BCP-ALL: Clinical specialist suspects acute leukemia (pediatric oncologist).

Trained healthcare professionals: within relevant subjects of the diseases.

6.2.2. Intended User

Qlucore Diagnostics BCP-ALL is intended to be used by trained healthcare professionals in a clinical laboratory setting. Especially roles such as:

- geneticist, laboratory scientist or bioinformatician for the bioinformatic pipeline,
- geneticist or laboratory scientist for running a case,
- bioinformatician, geneticist or laboratory scientist for command-line usage and
- pathologist/Medical Doctor specialist within genetics and or oncology for interpreting the report.

6.2.3. Intended population

Children from 1 up to 18 years old with suspected or diagnosed BCP-ALL.

6.2.4. Contraindications

- Patient with ongoing infection
- Patient on cytotoxic drugs
- Purpose of analysis to follow up after cancer treatment

7. Warnings/precautions/contra-indications and limitations

Carefully study the meaning of safety alerts and symbols. Read all the safety information and instructions in this manual before you use this device / this system.

If you are not suitably trained or fail to follow the instructions provided in this manual, it can lead to system damage, degradation, or inaccurate results.

7.1. Safety alerts

Make sure that you always read, understand, and follow all safety alerts that may appear in this manual.

Report any serious incident that occurs about the device / system to the manufacturer and the competent authority in the European Union (EU) Member State where the user is located.

7.2. Warning messages

The following table lists the warning messages that may be displayed in the Qlucore Diagnostics interface. The signs “%1”, “%2”, “%3”, and “%n” are wildcards, that can take on and display different values depending on the context in which the message is displayed.

Table 11. Warning messages.

Warning	Description	Action
Result parameter outside of valid range.	A quality metric is outside the valid range set by the model.	Contact Qlucore Support.
Failed to delete a folder, please restart your computer.	A folder deletion was not successful.	If a restart does not solve this issue, contact Qlucore Support.

Warning	Description	Action
Failed to create a folder, please restart your computer.	A folder creation was not successful.	If a restart does not solve this issue, contact Qlucore Support.
Failed to remove INCOMPLETE files:	A temporary file could not be deleted.	Check that the file is not open in another application. If problem persists, restart the computer.
BAM file '%1' is malformed. %2 reads end outside of their scaffold. These reads cannot be used.	An error has occurred in the aligner tool or in the preprocessing pipeline, causing inconsistent reads.	Check settings, version and parameters of the preprocessing pipeline tools and re-run the alignment of the BAM file. If the problem persists, contact Qlucore Support.
Gene Fusion analysis failed.	The gene fusion analysis failed, and no gene fusion result will be available in the report.	Contact Qlucore Support
Failed to parse gene fusion file: %1	The parsing of a gene fusion file failed.	Contact Qlucore Support
Classification failed.	The classification failed, and no classification result will be available in the report.	Contact Qlucore Support
PCA plot generation failed	The PCA plot could not be generated.	If the problem persists over multiple different patient cases, contact Qlucore Support.
The reference genome '%1' specified in the supplied BAM file does not conform to the specification in the model	The reference genome file name used for alignment of the BAM file diverges from what is stated in section 18.6.2.	Ensure that the correct reference genome is used for BAM alignment.
The aligner command line option '%1' specified in the supplied BAM file does not conform to the specification in the model.	A parameter to the BAM aligner diverges from what is stated in section 18.6.2.	Ensure that the parameters to the aligner are compliant with the list of parameters in section 18.6.2.
DUX4 might not be detected without a FusionCatcher file.	DUX4 gene fusions may go undetected unless FusionCatcher is used.	Use FusionCatcher to maximize the likelihood that all DUX4 gene fusions are detected.

7.3. Residual risks

The device is intended to be used for mRNA-based BCP-ALL analysis. If the amount of RNA in the patient sample is too low, false negative and/or false positive results may occur even when all quality metrics for a case are within the approved limits.

The device may report errors during a case-run that stop the case execution and prevent erroneous results. If an error occurs, no report is produced for this case-run and the case needs to be restarted.

The device is intended to be used for mRNA-based BCP-ALL analysis. The device creates a report based on input files. If the report is created based on incorrect input files or data, the result report cannot be trusted for medical purposes.

The device is intended to be used for BCP-ALL gene fusion analysis. Significant fusions may be missed because callers do not always label them correctly and may therefore not be recognized by the device as tier 1. Instead, those fusions may be listed in the tier 2 or tier 3 tables.

The device is intended to be used for BCP-ALL gene fusion analysis. False negative results occur for so called “3-way fusions” in the Mitelman database as these are ignored by the device and the fusion callers.

8. Training requirements

N/A.

9. Workflow

The workflow from patient sample to the output from Qlucore Diagnostics begins with a laboratory workflow and a bioinformatic pipeline to create the files required for the subsequent analysis in Qlucore Diagnostics. The overall workflow is outlined in Figure 1 below:

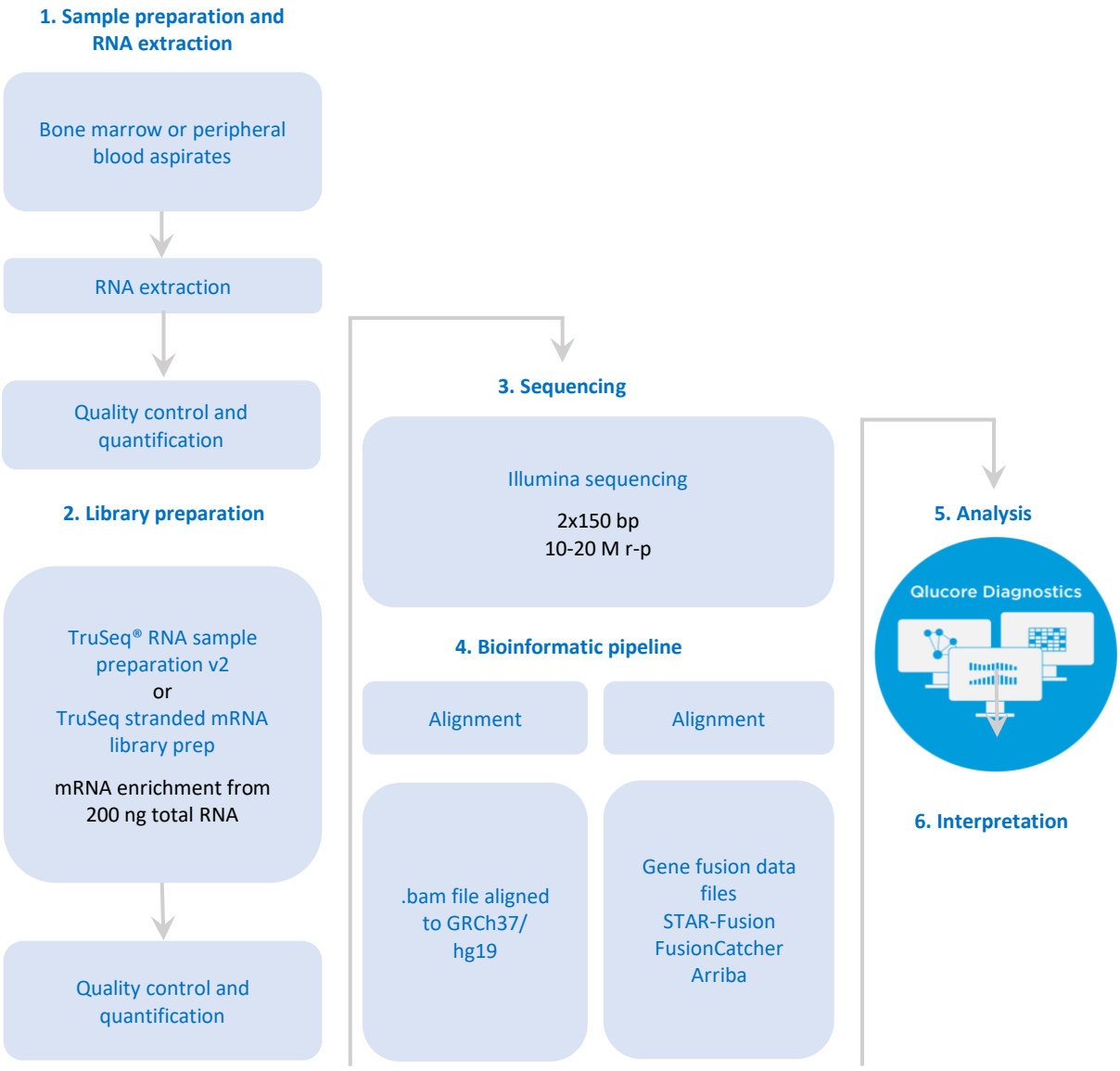


Figure 1. Qlucore Diagnostics workflow.

10. Prerequisites

For Qlucore Diagnostics to perform correctly, it is required that sample preparation, library preparation, sequencing and alignment is carried out according to the instructions outlined in section **18. Processing a case**.

11. Required materials and instruments

11.1. Required materials and reagents

- Human bone marrow or a blood sample collected in a cell culture flask (bone marrow) or EDTA-tube (blood).
- RNeasy Mini Kit (or a similar kit generating high-quality total RNA), including supplied reagents according to manufacturer's instructions (Qiagen, DE)

- TruSeq RNA Library Prep Kit v2, TruSeq Stranded mRNA Library Preparation Kit or Illumina Stranded mRNA Prep kit (or similar kit generating high-quality mRNA libraries compatible with an Illumina sequencing system) including supplied reagents according to manufacturer's instructions (Illumina, US)
- Sequencing reagents for your Illumina sequencing platform (Illumina, US)

11.2. Required instruments and equipment

- Lab equipment for RNA extraction according to manufacturer's instructions.
- Lab equipment for mRNA library preparation according to manufacturer's instructions.
- An Illumina sequencing platform (Illumina, US)

11.2.1. Pipeline software downloads

All pipeline software must be run on Linux or Mac computers using a command-line terminal. It is also possible to run them on virtual machines using Docker or Singularity containers. Follow the instructions for each operating system for installation.

The following sections list the sources for downloading the required software:

STAR

STAR v2.7.8a is the recommended version to use with STAR-Fusion and is compatible with STAR-Fusion v1.10.0 and above. STAR-Fusion v1.10.0 and above is also compatible with the latest CTAT Resource Library StarFv1.10 (i.e. the reference genome) for GRCh37 (hg19) at the time of writing.

STAR is included in the STAR-Fusion Singularity/Docker images.

Otherwise available from GitHub (https://github.com/alexdobin/STAR), direct download link: https://github.com/alexdobin/STAR/archive/refs/tags/2.7.8a.tar.gz. Binaries for Linux/macOS are in the **bin/** folder.

STAR-Fusion

STAR-Fusion v1.10.0 and above compatible with STAR v2.7.8a and CTAT Resource Library StarFv1.10.

Singularity image: https://data.broadinstitute.org/Trinity/CTAT_SINGULARITY/STAR-Fusion/

Docker image: https://hub.docker.com/r/trinityctat/starfusion/

Otherwise available from GitHub (https://github.com/STAR-Fusion/STAR-Fusion/wiki), direct download link: https://github.com/STAR-Fusion/STAR-Fusion/releases/download/STAR-Fusion-v1.10.0/STAR-Fusion-v1.10.0.FULL.tar.gz.

CTAT Resource Library

The GRCh37 reference genome from CTAT version StarFv1.10 is available here:

https://data.broadinstitute.org/Trinity/CTAT_RESOURCE_LIB/__genome_libs_StarFv1.10/GRCh37_gencode_v19_CTAT_lib_Mar012021.plug-n-play.tar.gz

FusionCatcher

FusionCatcher v1.33 is the latest release (release date 22 Jan 2021).

Available from GitHub following instructions found here: https://github.com/ndaniel/fusioncatcher

Arriba

Arriba v2.4.0 is the latest release (release date 8 Feb 2023).

Installation using Docker: https://arriba.readthedocs.io/en/latest/quickstart/#installation-using-docker

Installation using Singularity: https://arriba.readthedocs.io/en/latest/quickstart/#installation-using-singularity

Otherwise, available from GitHub:
https://github.com/suhrig/arriba/releases/download/v2.4.0/arriba_v2.4.0.tar.gz

Arriba should be run with reference genome GRCh37 and gene annotation Gencode19. Use the script download_references.sh provided by Arriba for this and follow the instructions in **18.6.4. Running Arriba**.

12. Safety considerations

The following safety related hazards have been identified:

- Delayed result: Errors in calculations or algorithms will give a wrong answer. (Delay is still within the stipulated time according to standard of care)
- No result
- False positive result
- False negative result
- Underfitting: Performance related hazard from AAMI CR34971:2022
- Unknown aspects or quality of third-party software
- Overfitting: Performance related hazard from AAMI CR34971:2022
- Security: Cyber security, data security, IT-security
- Selection bias: Performance related hazard from AAMI CR34971:2022
- Unauthorized patient data access
- Erroneous output
- Privacy failures: Performance related hazard from AAMI CR34971:2022
- Overtrust: Related hazard from AAMI CR34971:2022: User may perceive risk as lower and will trust the machine learning too much. Previous experiences can result in the user believing the application will work in all situations.

13. Security considerations

13.1. Windows PC/Mac Security

The software should be installed on a standard Windows PC or Mac, with minimum requirements as stated in section **15. System requirements**.

Virus protection: Virus protection software should be used. Any antivirus software supported by the host operating system may be used. Qlucore Diagnostics has no known limitations regarding virus protection.

Users and authentication: The Windows PC/Mac should be protected using a defined login procedure. Any automatic lock/log off features should be used to protect sensitive information.

The Windows PC/Mac may have multiple user profiles. If required, ensure that data used by each user may not be altered, corrupted, or shared by another user that is not intended to have that access.

Firewall: The software may trigger a local Windows/Mac firewall protection warning, especially when running the first case run.

Firewall settings depend on whether the computer is allowed to be directly connected to public Internet or not. Click **Allow access** if you have selected to activate the license via an Internet connection, see **17.4.1. License Activation**, or **Cancel** if you have selected to manually activate a license, via license file import, see **17.4.2. License file import**.

A residual risk exists that unauthorized users may gain access to the host computer and access and alter input files, output files, or settings, including the log file.
This risk may only be further reduced by the responsible healthcare organization (laboratory, hospital, or similar).

A residual risk exists that unauthorized users may gain access to the host computer and inject unauthorized python code.
This risk may only be further reduced by the responsible healthcare organization (laboratory, hospital, or similar), by preventing unauthorized access to the machine.

*If a model file is broken/corrupted, please re-download the relevant model file and re-install as described in **17. Running Qlucore Diagnostics for the first time**.*

Action

13.2. IT Network Security

Firewall: If the computer on which the software is installed is connected to an IT network, then a network firewall should be used, to protect from viruses and malware.

External devices: Do not connect the computer to external Bluetooth or Wi-Fi devices, or allow auto-execution of USB content, to prevent unauthorized access to Windows/Mac. To protect sensitive information, avoid using remote services on the computer.

Connection of the host computer to an IT network that includes other devices may introduce previously unidentified risks. Responsible personnel from the appropriate technical department need to identify all parts of the (IT) system, analyze, evaluate, and control any risks. Note that any subsequent changes to the IT network could introduce new risks or require additional analysis.

A residual risk exists that the local area network may be attacked if viruses and/or malware enter the host computer. This risk may only be further reduced by the responsible healthcare organization (laboratory, hospital, or similar).

14. Incident reporting

In case of a serious incident, defined as an incident that directly or indirectly led, might have led or might lead to any of the following: the death of a patient, user or other person, the temporary or permanent serious

deterioration of a patient's, user's or other person's state of health, or a serious public health threat, contact Qlucore and the relevant competent authority.

15. System requirements

15.1. Network/Firewall Configuration

The computer that the QD Platform is installed on must have the network port 52512 available for localhost usage.

15.2. Windows system requirements

- Windows 11 (64 bit)
- 8 GB of DDR4 RAM 2400MHz memory
- Graphics card supporting Open GL 3.3
- 50 GB free SSD hard disk space
- Intel 10th generation Comet Lake Core i5 10500 or better

15.3. MacOS system requirements

- MacOS 15.0, using an M1 Apple Silicon processor
- 8 GB of RAM
- 50 GB of free SSD hard disk space or better

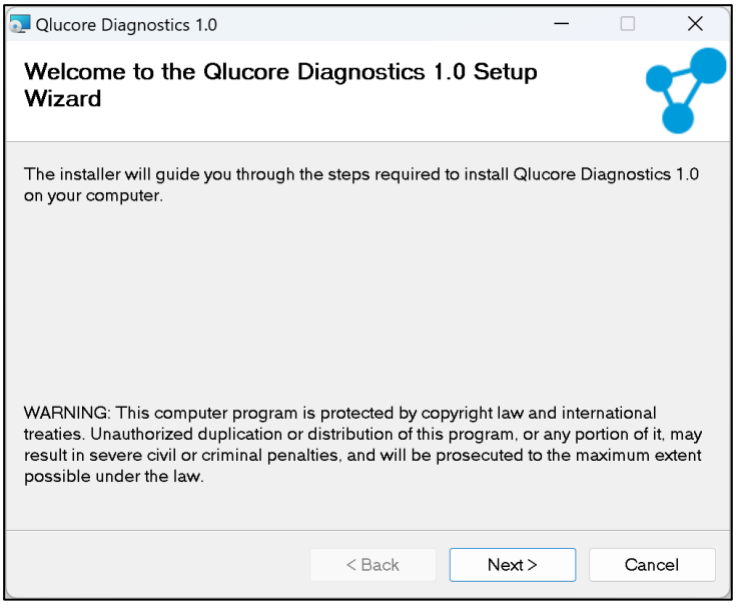
The platform will not run on Apple computers based on the x86 architecture.

16. Installation

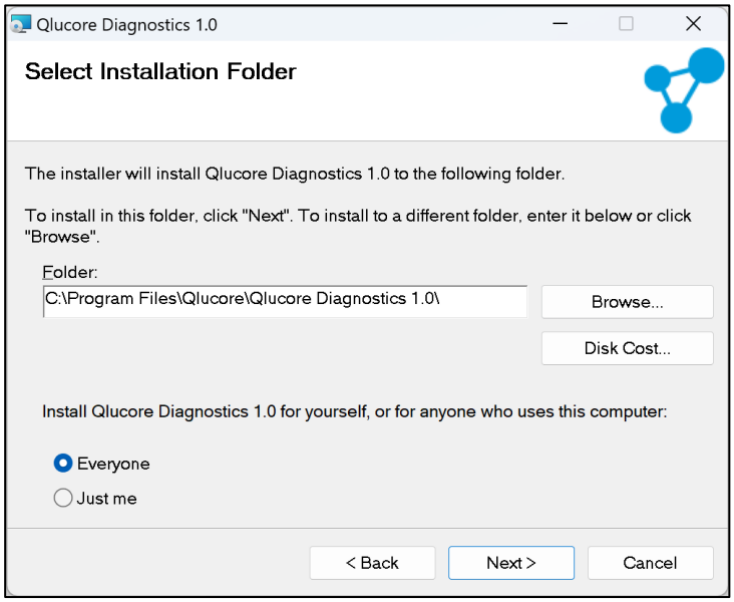
The Qlucore Diagnostics software can be obtained from the Download section of Qlucore.com where a login is required.

16.1. Installing Qlucore Diagnostics on a Windows system

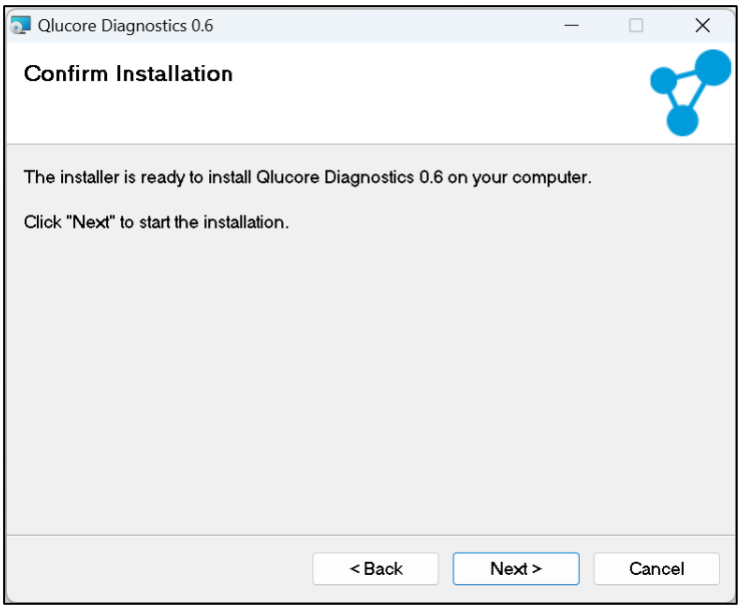
1. Download the installation file, named qlucore_diagnostics_xxx_setup.msi.
2. Double click the installation file icon to display the installation dialog.



3. Click **Next** to continue the installation. If you want to go back to a previous step and change something, click the **Back** button. If you want to interrupt the installation, click **Cancel**.
4. In the **Installation folder** dialog, you can select where to install the software if you do not want to use the default folder.



5. Select **Everyone** to make the installation available to all users of the computer, or **Just me** to restrict use to a single user.
6. Click **Next** to continue the installation. The **Confirm Installation** dialog will be displayed:

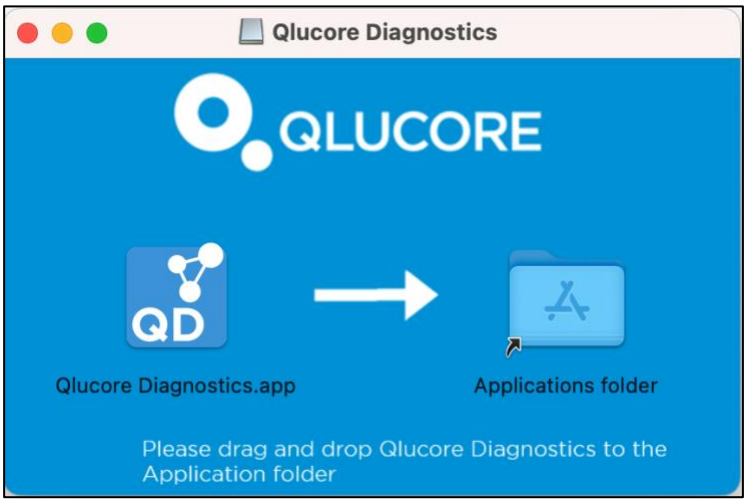


7. Click **Next** to start the installation. A progress bar will indicate the progress of the installation process.
8. When the installation is complete, close the Installation Wizard.

Once Qlucore Diagnostics is installed, you can launch it from the **Start** menu or by double clicking the desktop icon.

16.2. Installing Qlucore Diagnostics on a macOS system

1. Download the installation file, named qlucore_diagnostics_xxx_setup.dmg
2. Double-click the downloaded .dmg file. A new Finder window opens, where the user is asked to drag the Qlucore Diagnostics.app icon into the Applications folder.

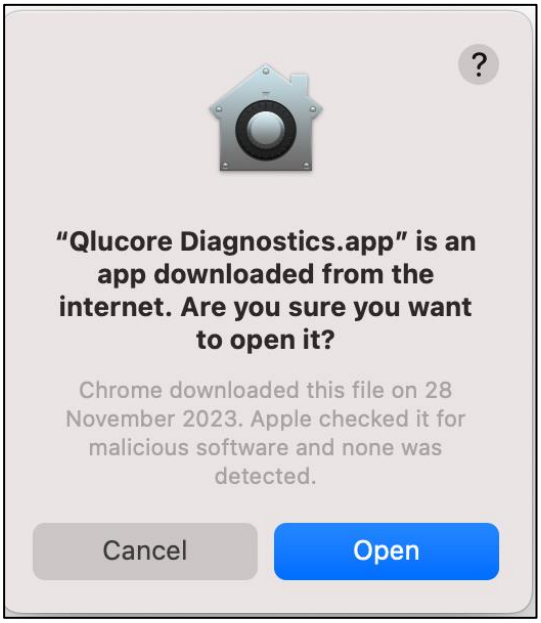


3. Drag the Qlucore Diagnostics.app icon to the Applications folder. This unpacks and installs the application.

4. Start Qlucore Diagnostics from the Applications folder, as with any other Mac program.

5. The first time Qlucore Diagnostics starts, macOS might show a dialog for confirming the installation of Rosetta. This is expected and needed for running Qlucore Diagnostics, and it will require system administrator credentials. When Rosetta has been installed, Qlucore Diagnostics can be started.

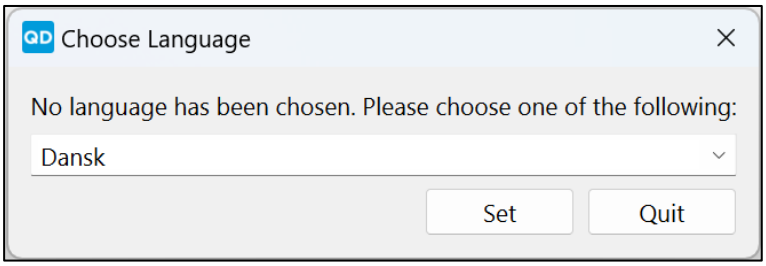
6. At first start, the user is also reminded by macOS that anything downloaded from the Internet is potential malware. Clicking 'Open' will start the program.



17. Running Qlucore Diagnostics for the first time

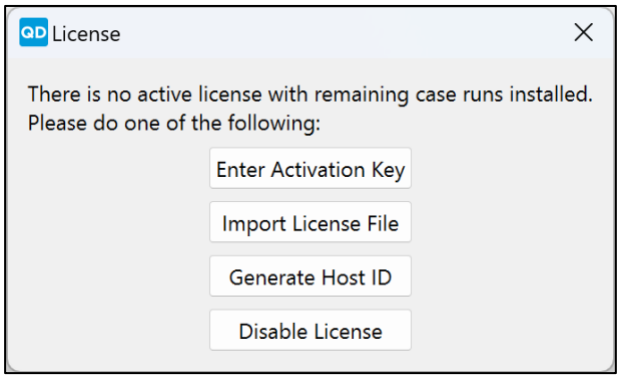
To start Qlucore Diagnostics, double click the icon on the desktop or select it from the **Start** menu (Windows) or click the application icon on the **Dock** (macOS).

1. Select the desired language in the dialog that is displayed:



2. Click **Set**.

Next, the **License** dialog will be displayed:



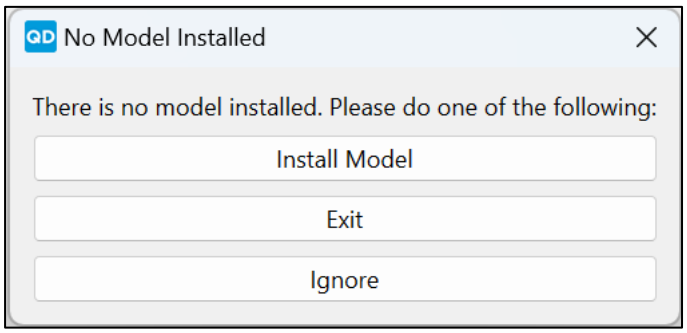
1. Click **Enter Activation Key** to activate a Qlucore Diagnostics license.
2. In the dialog displayed, enter the activation key obtained from Qlucore.
3. Click **OK**.

When the license has been validated and activated, the main Qlucore Diagnostics window will be displayed.

17.1. Installing a model

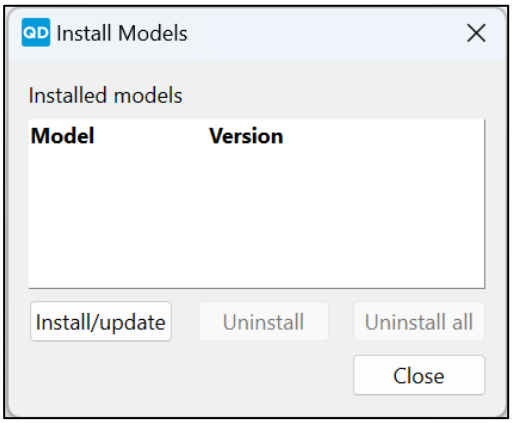
To enable analysis, the relevant model(s) must first be installed.

The first time that the software is started, and no model has yet been installed, a dialog will be displayed, prompting the installation of a model:

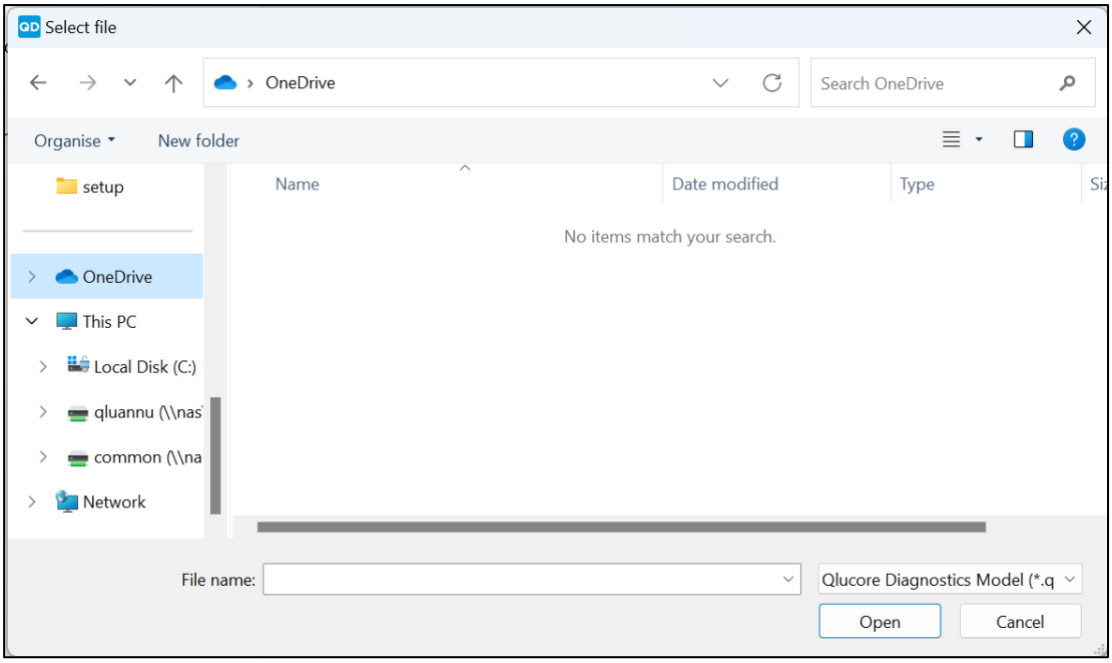


Clicking **Exit** will close the application. The **Ignore** button closes the **Install Model** dialog.

1. To install one or more models, click the **Install Model** button. The models dialog will be displayed:



2. Click the **Install/update** button. This will display a file selection window:



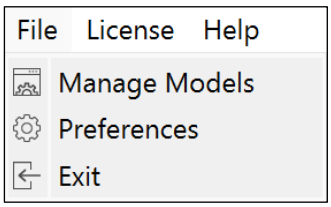
3. Locate and select the relevant model file.
4. Click **Open** to add the file to the **Installed models** list.

When a model has been installed, Qlucore Diagnostics is ready for performing analyses according to **18. Processing a case**.

17.2. An overview of the workspace

The Qlucore Diagnostics workspace has three menus: **File**, **License** and **Help**.

17.2.1. The File menu

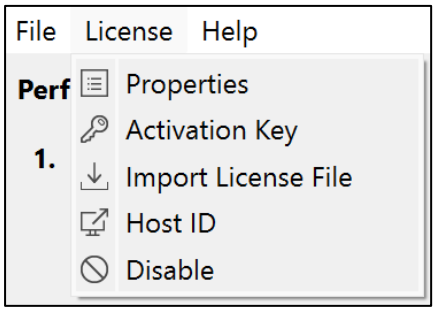


Manage Models displays the Model management dialog, where you can install or remove Models, according to the instructions in **17.1. Installing a model** above.

Preferences is where Qlucore Diagnostics can be customized, for instance by selecting the interface language and adding a logotype to use for the reports.

Exit closes the application.

17.2.2. The License menu



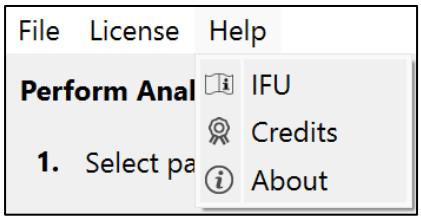
Properties displays details about the current license—its expiration date and the number of remaining cases.

Activation Key is used for adding licenses required for running Qlucore Diagnostics.

Host ID is used for providing Qlucore support with the host IDs required if there are license activation issues.

Disable is used when there is a need to disable any installed licenses.

17.2.3. The Help menu



IFU opens the **Instructions for Use** PDF file.

Credits displays information about third party manufacturers.

About displays information about the software and current version of it.

17.3. Setting the preferences

Use the **Preferences** dialog for customization.

You can customize reports by adding a logotype:

1. Click the path selector button [···] next to the **Custom logotype** field.
2. Select a logotype file to add.

The image file format of the logotype must be PNG.

Use the **Custom Patient Labels** option to use customized patient labels:

1. Click the path selector button [···] next to the **Custom Patient Labels** field.
2. Select the relevant QCSLS file, as defined in **18.7.3. Loading the analysis files**.

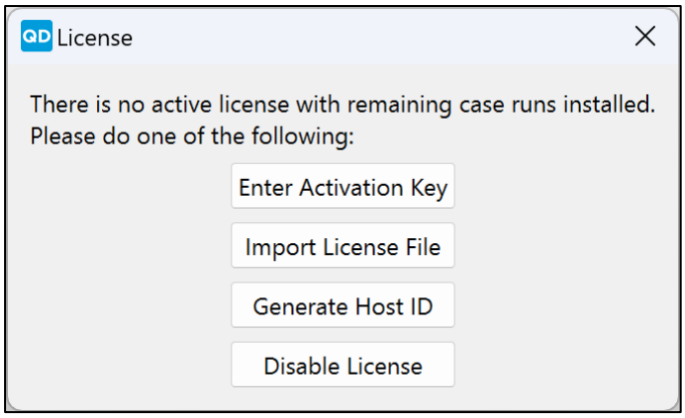
Use the **Language** dropdown list to select the display and report language. Note that file dialogs might still be displayed in the language you have chosen for your operating system regardless of the language you select here.

Click **OK** to save the preferences settings and exit the **Preferences** dialog.

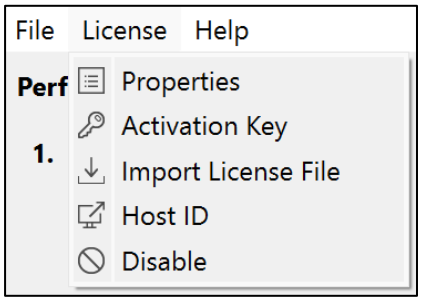
17.4. Managing licenses

Qlucore Diagnostics licenses can be managed using the menu that appears on startup, when no licenses are found, or through the License menu in the menu bar.

The following options are available on startup:

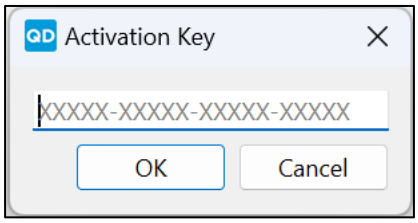


And on the License menu:



17.4.1. License Activation

When clicking **Activation Key**, a License Key Activation dialog will be displayed:



1. Enter the activation key.
2. Click **OK**.

The software will then attempt to activate the license on a server via the Internet. If the activation fails, contact Qlucore Support, via email or phone. Refer to section 28 Manufacturer information

for contact details.

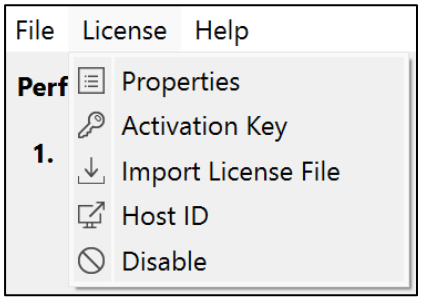
For security reasons, as described in section **13. Security considerations**, the computer may or may not be directly connected to public Internet and to a local network. To accommodate this, Qlucore Diagnostics supports two types of license activation:

1. Automatic license activation: Qlucore Diagnostics is activated with a license key, as described above. In this case, the client computer will connect to a license server via the Internet at the time of license activation (but not during normal operation of the application).
2. Manual license activation: Qlucore Diagnostics is activated with a license file with no need for an Internet or local network connection, as described in **17.4.2 License file import for manual license activation**. The license file is provided by Qlucore Sales & Support (e.g. via email) and does not require the computer running Qlucore Diagnostics to have Internet access at any time.

17.4.2. License file import for manual license activation

If license activation is not possible using an activation key, possibly because the computer is disconnected from Internet access, Qlucore support can provide the license file which can be imported via a file explorer.

To import a license file and manually activate the license, select **License File** Import from the License menu:



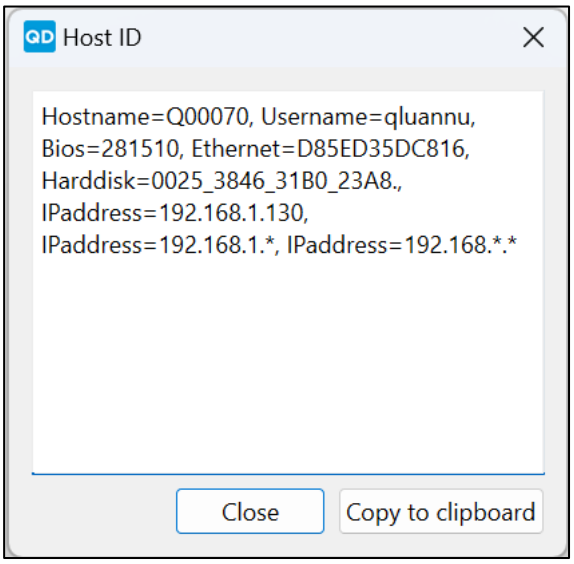
This brings up a file explorer window.

1. Locate and select the license file obtained from Qlucore customer support.
2. Click **OK** to activate the license.

17.4.3. Host IDs

In case of license issues, Qlucore support might need to know a user computer's Host ID that the user can obtain via the License menu:

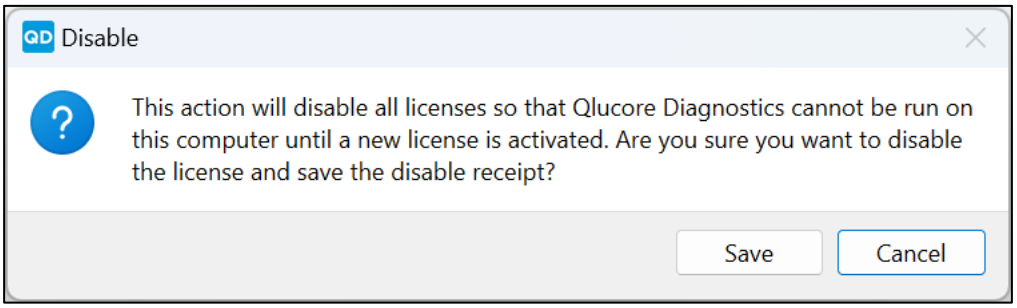
1. On the License menu, click the **Host ID** button. The **Generate Host ID** dialog will be displayed, showing a list of all Host IDs. This list can be copied to the clipboard.



17.4.4. Disabling Licenses

The **Disable** option on the License menu uninstalls all Qlucore Diagnostics licenses. Do this only if Qlucore customer support recommends it.

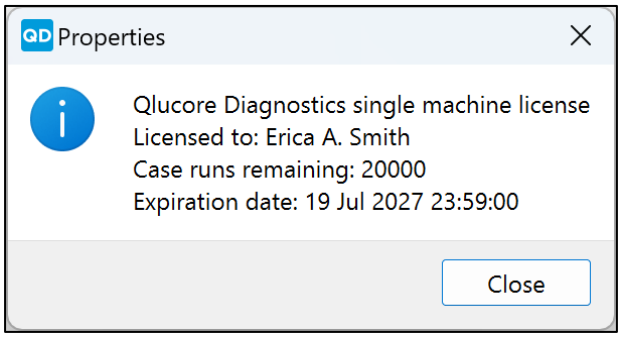
1. Click **Disable** to display the **Disable** dialog:



2. Click **Save** to confirm the deactivation of all Qlucore Diagnostics licenses. All licenses will be deactivated, and the platform cannot be used until a new license has been activated.
3. When prompted, select a location to which the **Disable Receipt** text file will be saved. Provided with this file, Qlucore customer support will be able to help solve Qlucore Diagnostics license issues.

17.4.5. License Properties

Select the **Properties** option in the License menu to view Qlucore Diagnostics license information:



18. Processing a case

The following files are required for processing a case:

- Model file
- Patient file
- Aligned BAM file
- Gene fusion files

The model file to be used is the one supplied with Qlucore Diagnostics, which corresponds to the analysis to be carried out. The management of models is described in detail in section **22. Managing Qlucore Diagnostics models**.

The patient file, BAM file and gene fusion files are created in the library preparation and bioinformatics pipeline steps, described in the following sections.

18.1. A short workflow summary

The workflow, as outlined in Figure 1 above, includes the following steps:

Sample preparation and RNA extraction

High quality RNA extracted from blood or bone marrow human samples.

Library preparation

Library preparation utilizing oligo-dT selection for mRNA purification from e.g., 100—1000 ng total RNA.

Sequencing

Paired (e.g., 2x150 bp) Illumina sequencing generating >20 M read pairs (r-p) per sample.

Bioinformatic pipeline

Align RNA sequencing reads to references genome GRCh37 (hg19) using STAR v2.7.8a and run fusion callers STAR-Fusion, FusionCatcher and/or Arriba.

Analysis

Qlucore Diagnostics.

Interpretation

Evaluating the report exported from Qlucore Diagnostics.

18.2. Sample preparation and RNA extraction

Diagnostic bone marrow (BM) or peripheral blood (PB) samples are collected from patients with suspected diagnosis of B-cell Acute Lymphoblastic Leukemia (BCP-ALL). Samples are collected in tissue culture flasks (BM) or in EDTA-tubes (PB), kept on ice, and proceeded to RNA extraction on the same day. If extraction on the same day as sample collection is not possible, samples need to be stabilized and stored in a way that does not disrupt the RNA integrity. RNA extraction is recommended to be performed with the RNeasy Mini Kit (Qiagen, DE) (or similar kit generating high-quality total RNA), according to manufacturer's instructions. DNAse treatment is optional but recommended. Contaminating DNA will be omitted at mRNA enrichment in the library preparation, however it can affect accurate quantification of input total RNA prior to library preparation. For additional instructions and recommendations, follow the manufacturer's instructions. RNA is sensitive for degradation and care should be taken prior, during, and after extraction. Final RNA samples are kept at -80° C. Repeated freeze and thaw cycles should be avoided.

18.2.1. Quality control and quantification

Quantification of total RNA and RNA integrity should be carried out according to manufacturer's instructions. A total amount of 100-1000 ng total RNA with an RNA Integrity Number (RIN) of ≥ 8, or an RNA Quality Number (RQN) ≥ 8 is recommended for downstream library preparation. For additional information and recommendations regarding quality control and quantification consult the RNeasy Mini Handbook (Qiagen, DE) (or corresponding total RNA extraction handbook) and the TruSeq RNA Library Prep Kit v2, TruSeq Stranded mRNA Reference Guide or Illumina Stranded mRNA Reference Guide (or corresponding mRNA library preparation handbook).

18.3. Library preparation

Sequencing ready libraries are prepared according to the TruSeq RNA Library Prep Kit v2, TruSeq Stranded mRNA Reference Guide or Illumina Stranded mRNA Reference Guide (Illumina, US) (or corresponding Reference Guide). Messenger RNA (mRNA) is enriched from total RNA, and cDNA libraries prepared. Modifications in the fragmentation step can be applied to achieve longer mRNA fragments which aid downstream gene fusion analysis. Consult the corresponding reference guide for such modifications. Protocol summary

- Poly-A containing mRNA is purified using oligo-dT attached magnetic beads or polyA capture and fragmented.
- Fragments are copied into first strand cDNA using reverse transcriptase and random primers.
- Second strand cDNA synthesis is performed using DNA Polymerase I and RnaseH. Strand specificity, if applied, is achieved by replacing the dTTP with dUTP in the Second Strand Marking mix.
- The 3' ends are adenylated and indexed adapters are ligated.
- The product is purified and amplified using PCR to create the final cDNA library.

18.4. Quality control, normalization and pooling

For quality control, normalization and pooling of the final libraries, consult the corresponding Library preparation Reference Guide. Quantification and normalization can also be carried out as specified in the Illumina Stranded mRNA Prep reference guide (Illumina, CA). Make sure to apply a protocol that fits your corresponding Illumina System.

18.5. Sequencing

Prior to loading the samples on your Illumina sequencing platform follow the Denature and Dilute guide for your Illumina system. Paired, 2x150 bp sequencing reads, with >20 M read pairs (r-p) per sample are recommended for downstream analysis using Qlucore Diagnostics.

18.6. Bioinformatic pipeline

Aligned BAM files are required as input data to Qlucore Diagnostics and the classifier model. The BAM files are assembled from raw unfiltered sequencing reads in FASTQ format, using the GRCh37 (hg19) reference genome. The CTAT resource library listed in **11.2.1. Pipeline software** is required for this step. The bioinformatics tools are run from the command-line using a terminal. Follow the instructions for each tool for how to install them. STAR is required for the alignment of BAM files, using the commands below.

18.6.1. Running STAR

Run STAR with the default commands recommended for compatibility with STAR-Fusion, with one critical addition; **--outFilterMultimapNmax 200**, which is required to properly handle genes in repetitive regions.

This is the complete set of commands:

```
STAR \
   --genomeDir path/to/GRCh37_gencode_v19_CTAT_lib_Mar012021.plug-n-play/ctat_genome_lib_build_dir/ref_genome.fa.star.idx \
   --readFilesIn R1.fastq.gz R2.fastq.gz \
   --runThreadN 40 \
   --outReadsUnmapped None \
   --twopassMode Basic \
   --readFilesCommand zcat \
   --outSAMstrandField intronMotif \
   --outSAMunmapped Within \
   --chimSegmentMin 12 \
   --chimJunctionOverhangMin 8 \
   --chimOutJunctionFormat 1 \
   --alignSJDBoverhangMin 10 \
   --alignMatesGapMax 100000 \
   --alignIntronMax 100000 \
   --alignSJstitchMismatchNmax 5 -1 5 5 \
   --outSAMattrRGline ID:GRPundef \
   --chimMultimapScoreRange 3 \
   --chimScoreJunctionNonGTAG -4 \
   --chimMultimapNmax 20 \
   --chimNonchimScoreDropMin 10 \
   --peOverlapNbasesMin 12 \
   --peOverlapMMp 0.1 \
   --alignInsertionFlush Right \
   --alignSplicedMateMapLminOverLmate 0 \
   --alignSplicedMateMapLmin 30 \
   --outFilterMultimapNmax 200 \
   --outSAMtype BAM SortedByCoordinate
```

Where **--genomeDir**, **--readFilesIn**, **--runThreadN** and **--readFilesCommand** should be adjusted depending on file locations, file formats and the number of available threads. The STAR output file Aligned.sortedByCoord.out.bam is used as input to Qlucore Diagnostics. The file Chimeric.out.junction is also created by STAR and is used by STAR-Fusion in the next section.

18.6.2. Running STAR-Fusion

```
STAR-Fusion \
   --genome_lib_dir path/to/GRCh37_gencode_v19_CTAT_lib_Mar012021.plug-n-play/ctat_genome_lib_build_dir/ \
   --CPU 40 \
   --examine_coding_effect \
   -J path/to/Chimeric.out.junction \
   --output_dir star_fusion
```

Where the --genome_lib_dir should be adjusted depending on file location, **-J** refers to the file created by STAR in the previous section and the **--CPU** number is the number of available threads. The STAR Fusion output file, saved to **star_fusion/star-fusion.fusion_predictions.abridged.coding_effect.tsv** contains gene fusion information and is used as input to Qlucore Diagnostics.

18.6.3. Running FusionCatcher

Run FusionCatcher using the default commands, starting from the unaligned FASTQ files.

```
fusioncatcher.py \
   -p 5 \
   -d /path/to/references/human_v102 \
   -i /path/to/fastq/ \
   -o /path/to/out
```

Where **-p** is the number of processes for parallelization. Set this value to the number of available threads. The default value is 0. The FusionCatcher output file, saved to final-list_candidate-fusion-genes.hg19.txt, contains gene fusion information and is used as input to Qlucore Diagnostics.

18.6.4. Running Arriba

Running Arriba requires a second alignment using STAR for compatibility with the genomic coordinates of the blacklist of gene fusions provided by Arriba, in order to disregard known false positive predictions. Run STAR with the commands shown below, recommended for compatibility with Arriba.

Download the genome assembly, gene annotation and STAR index for GRCh37 using the **download_references.sh** script provided by Arriba by running:

```
/arriba_v2.4.0/download_references.sh GRCh37+GENCODE19
```

Then align the FASTQ files with STAR and pipe output to Arriba:

```
STAR \
   --runThreadN 40 \
   --genomeDir /path/to/STAR_index_GRCh37_GENCODE19 \
   --genomeLoad NoSharedMemory \
   --readFilesIn R1.fastq.gz R2.fastq.gz \
   --readFilesCommand zcat \
   --outStd BAM_Unsorted \
   --outSAMtype BAM Unsorted \
   --outSAMunmapped Within \
   --outBAMcompression 0 \
   --outFilterMultimapNmax 50 \
   --peOverlapNbasesMin 10 \
   --alignSplicedMateMapLminOverLmate 0.5 \
   --alignSJstitchMismatchNmax 5 -1 5 5 \
   --chimSegmentMin 10 \
   --chimOutType WithinBAM HardClip \
   --chimJunctionOverhangMin 10 \
   --chimScoreDropMax 30 \
   --chimScoreJunctionNonGTAG 0 \
   --chimScoreSeparation 1 \
   --chimSegmentReadGapMax 3 \
   --chimMultimapNmax 50 | \
/arriba_v2.4.0/arriba \
   -x /dev/stdin \
   -o /output/fusions.tsv -O /output/fusions.discarded.tsv \
   -a /path/to/GRCh37.fa \
   -g /path/to/GENCODE19.gtf \
   -b /path/to/database/blacklist_hg19_hs37d5_GRCh37_v2.4.0.tsv.gz \
   -k /path/to/database/known_fusions_hg19_hs37d5_GRCh37_v2.4.0.tsv.gz \
   -t /path/to/database/known_fusions_hg19_hs37d5_GRCh37_v2.4.0.tsv.gz \
   -p /path/to/database/protein_domains_hg19_hs37d5_GRCh37_v2.4.0.gff3
```

Where --genomeDir, --readFilesIn, --runThreadN and –readFilesCommand in STAR should be adjusted depending on file locations, file formats and the number of available threads. The path/to/genome/ file path should be adjusted based on the location of Arriba. The Arriba output file, saved to **output/fusions.tsv**, contains the gene fusion information used as input to Qlucore Diagnostics.

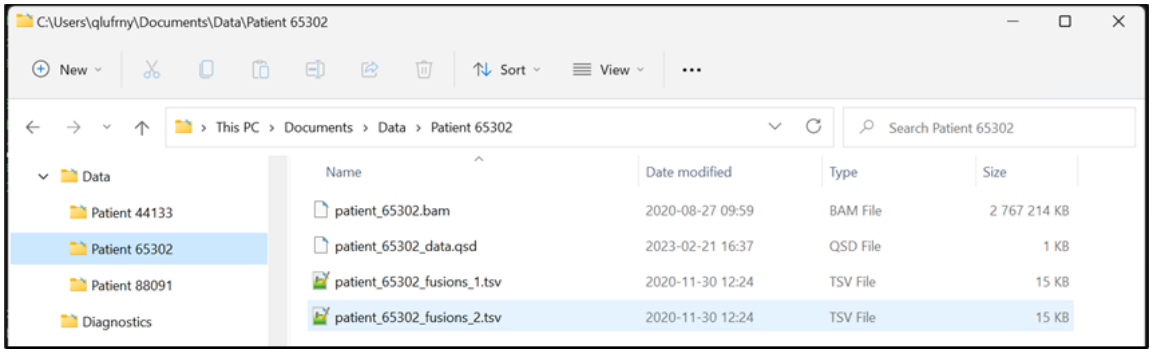
18.7. Performing the analysis

At least one model file needs to be loaded, as described in **17.1. Installing a model** above, before Qlucore Diagnostics is ready to perform the first analysis in GUI mode. For command-line mode, model installation is not necessary.

For input files to be analyzed correctly, they must first have been prepared according to the steps described in **18.2. Sample preparation and RNA extraction—18.5. Sequencing** above. Refer to this section for details about the steps preceding the analysis.

18.7.1. Recommended file naming and folder structure

It is recommended to create one folder per patient, containing all files related to that patient, naming the folder and all files using the same patient or sample ID, as illustrated below:



18.7.2. Preparing QSD and QCSLS files

The QSD sample data file

Qlucore Diagnostics requires QSD sample files as input. QSD (short for 'Qlucore Sample Data') is an XML format saved with a .qsd file extension, and it must have the following structure:

```
<?xml version="1.0"?>
<QFF Producer='Qlucore' Format='QlucoreSampleData' FormatVersion='1.0' QFFVersion='1.1'>
	<SampleData>
		<SubjectId>19790525-1234</SubjectId>
		<SubjectName>Ellen Ripley</SubjectName>
		<SampleDateTime>2023-Mar-14 03:14:15</SampleDateTime>
		<SampleId>d5sdfew7f8x</SampleId>
		<SampleTissue>Blood sample</SampleTissue>
	</SampleData>
</QFF>
```

The "Format" property of the root must be 'QlucoreSampleData'. The root tag must be 'SampleData'. The mandatory child tags are:

- SubjectId: the patient's ID
- SubjectName: the patient's name
- SampleDateTime: the timestamp of the sample, given as a text string – it is strongly recommended to use the QD Platform time format, "yyyy-MMM-dd hh:mm:ss", to align to timestamps produced by the QD Platform
- SampleId: the sample's ID
- SampleTissue: what type of tissue the data was extracted from.

The QCSLS custom labels file

The optional labels file is also an XML file. QCSLS (short for 'Qlucore Custom Sample Label Specification) files must be saved with a QCSLS extension and the structure of the file must be as in the example below:

```
<?xml version="1.0"?>
<QFF Producer='Qlucore' Format='QlucoreCustomSampleLabelSpecification' FormatVersion='1.0' QFFVersion='1.1'>
	<CustomSampleLabelSpecification>
		<CustomLabel>
			<Tag>InsuranceNumber</Tag>
			<Label>Insurance Number</Label>
		</CustomLabel>
		<CustomLabel>
			<Tag>SecondName</Tag>
			<Label>Second Name</Label>
		</CustomLabel>
```

```
        </CustomSampleLabelSpecification>
</QFF>
```

This example QCSLS file would require all sample data (QSD) files to have the following structure:

```
<?xml version="1.0"?>
<QFF Producer='Qlucore' Format='QlucoreSampleData' FormatVersion='1.0' QFFVersion='1.1'>
        <SampleData>
                <SubjectId>19790525-1234</SubjectId>
                <SubjectName>Ellen Ripley</SubjectName>
                <SampleDateTime>2023-Mar-14 03:14:15</SampleDateTime>
                <SampleId>d5sdfew7f8x</SampleId>
                <SampleTissue>Blood sample</SampleTissue>
                <InsuranceNumber>123456789-0-ABC</InsuranceNumber>
                <SecondName>Weaver</SecondName>
        </SampleData>
</QFF>
```

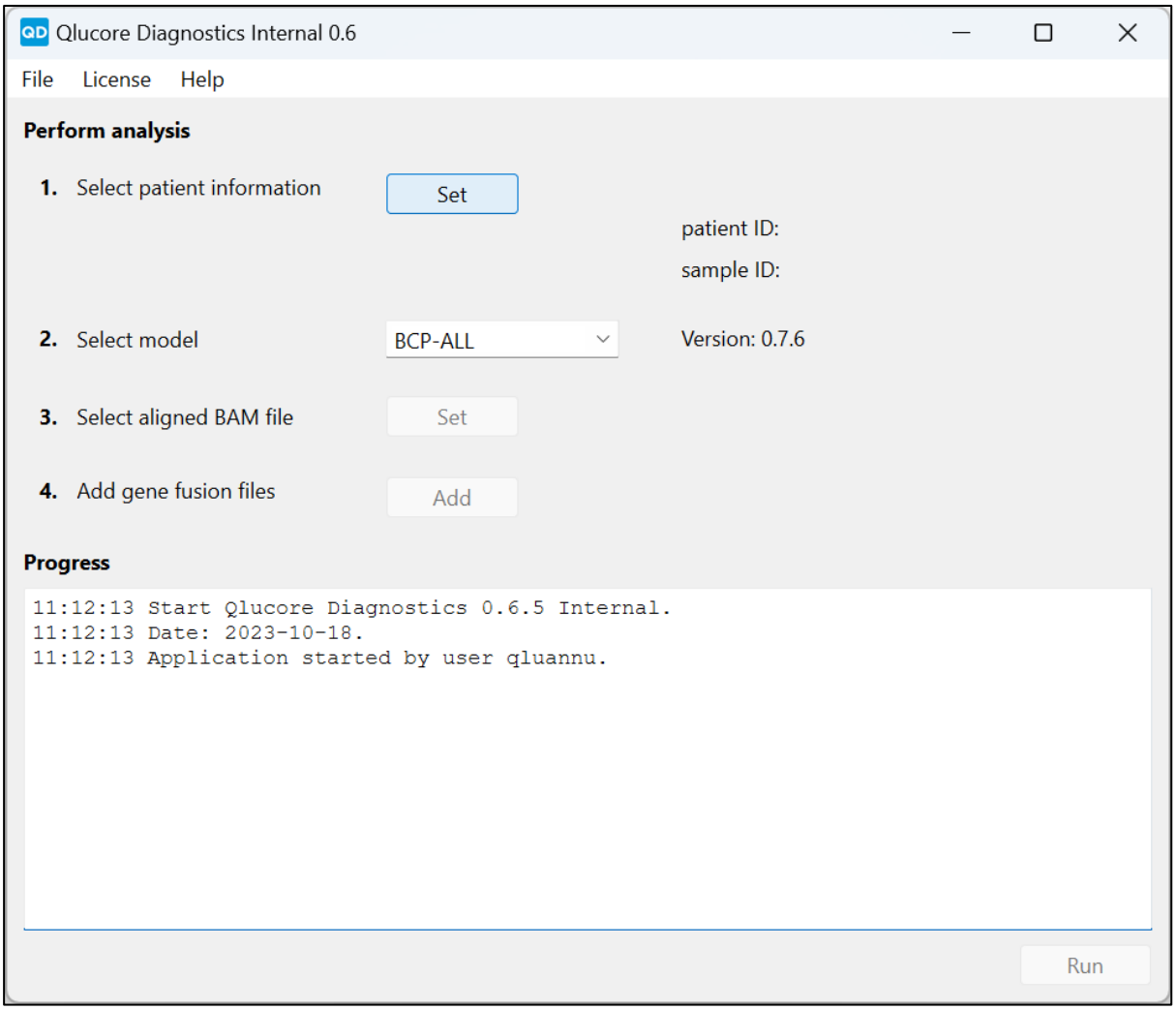
There may be up to six optional fields. If more than six custom labels are specified, the platform will fail to parse the file.

All TagName and DisplayName values have a maximum allowed length of 64 Unicode characters. If any value exceeds this, the file is invalid.

18.7.3. Loading the analysis files

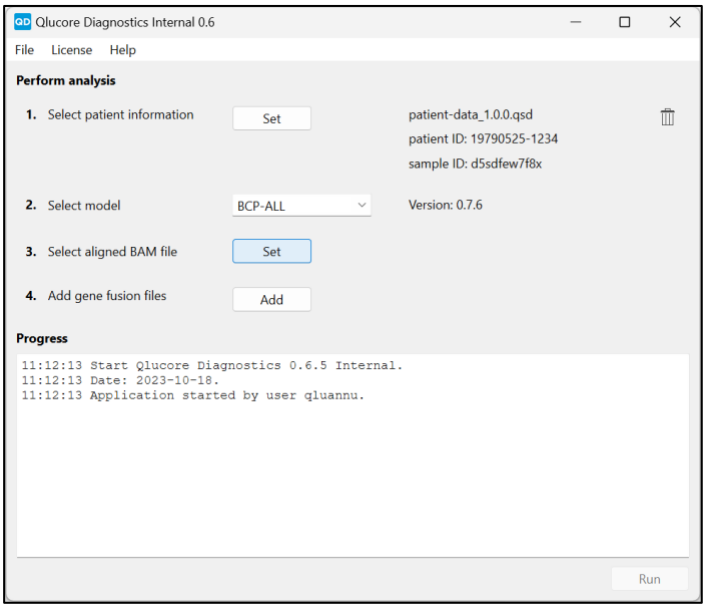
To process a case, the files associated with the case must first be loaded. This is done as a first step in the **Perform Analysis** dialog.

1. On the main window, Click the **Set** button, next to **Select patient information**, to select the relevant patient data file.



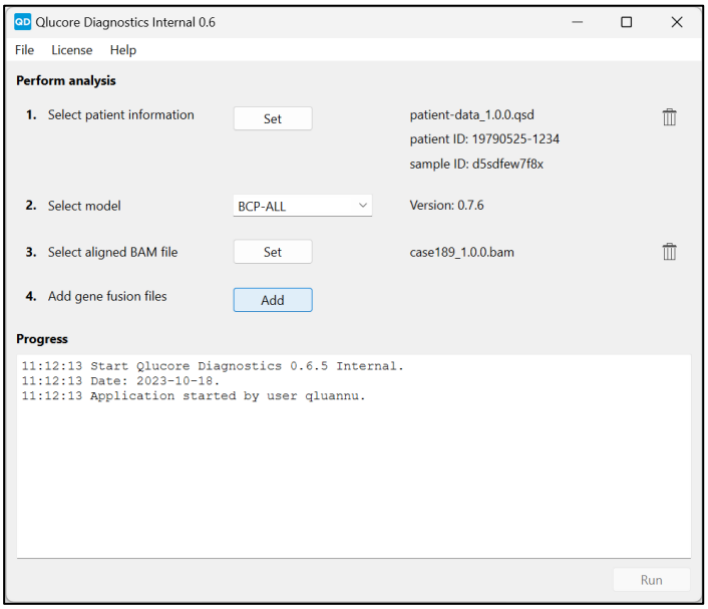
This brings up a file selection window.

2. Locate and select the relevant QSD sample file. The patient data file must be formatted as described in **18.7.2. Preparing QSD and QCSLS files** above.
3. Click **Open**.
4. Select the model to use from the **Select model** dropdown list which contains all models that are currently installed.
5. Click the **Set** button next to **Select aligned BAM file**.



This brings up a file selection window.

6. Locate and select the relevant BAM file. The aligned BAM file must be prepared according to the description in **18.6.1. Running STAR** to use for the analysis.
7. Click **Open**.
8. Click the **Add** button next to add gene fusion files. A minimum of two, and up to three fusion files can be added for a case.

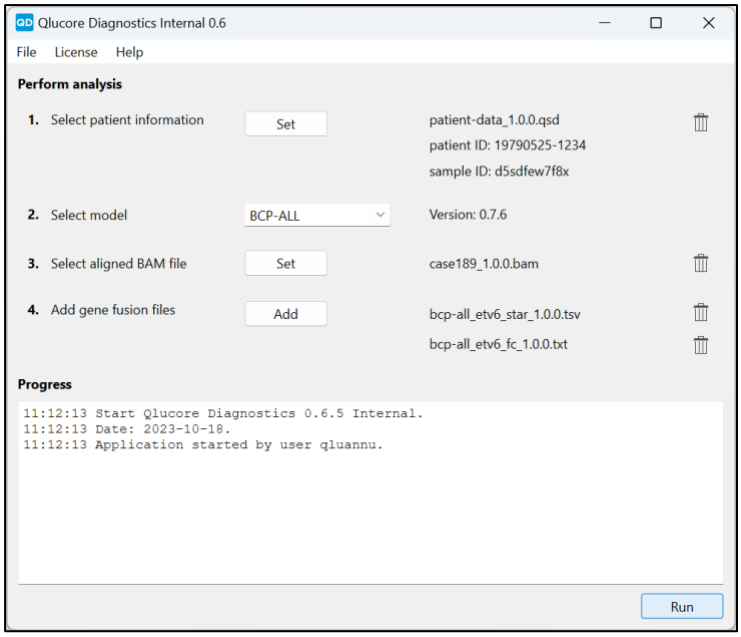


A file selection window will be displayed.

9. Locate and select the gene fusion files, which can have either a TSV or TXT extension. The gene fusion file(s) must be prepared according to **18.6.2. Running STAR-Fusion**—**18.6.4. Running Arriba**.
10. Click **Open**.

Note! It is not possible to select multiple files in the file selection window, so to add more than one fusion file, you will have to click **Add** multiple times.

Now, all the required files have been loaded, and the analysis can be performed. Click **Run** to process the case and perform the analysis.



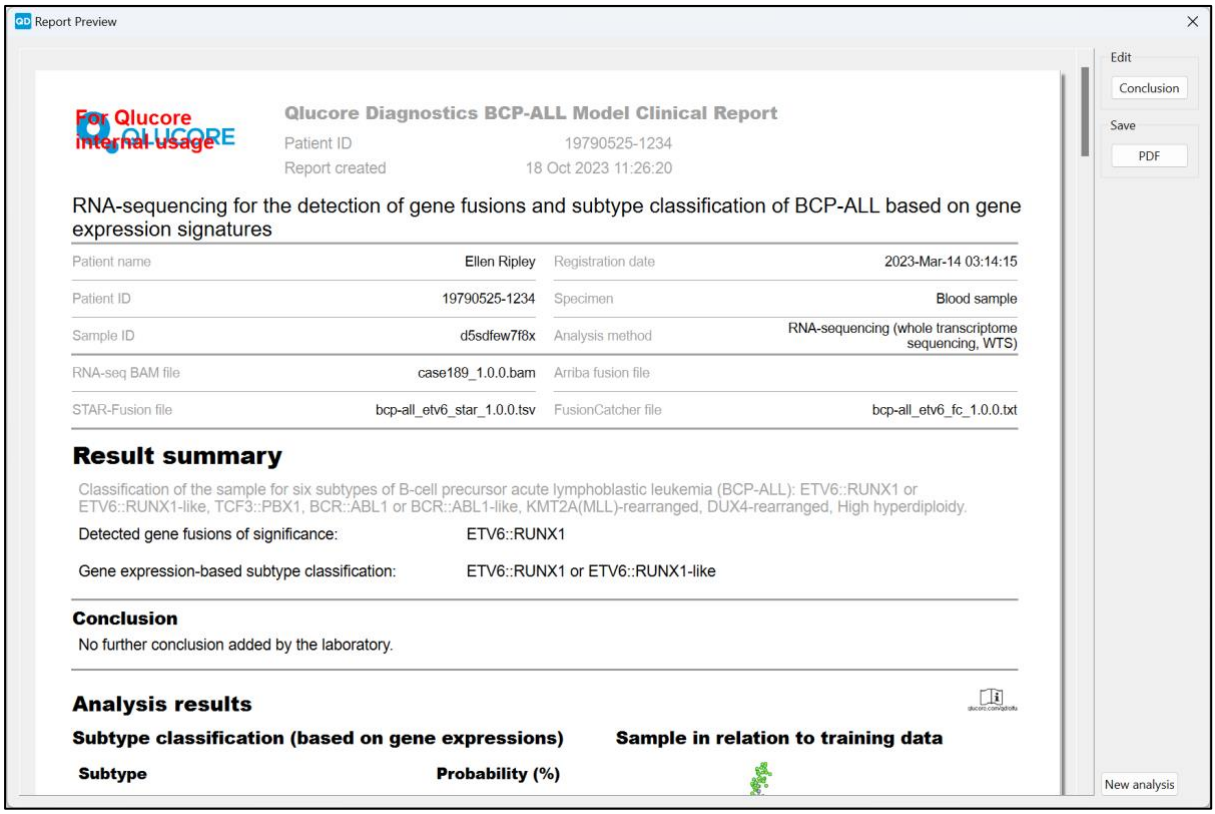
The files will be analyzed, and a preview of the results will be displayed.

Note! Running the first analysis may trigger a local firewall warning. Refer to **13. Security considerations** for more information regarding IT security.

The progress section provides information about important events occurring on the platform, to show that the expected action(s) are performed because of user interaction, as well as errors and warning messages in case something goes wrong.

18.8. Previewing results

When the analysis is complete, a preview of the results is displayed:



In the preview, click the **Conclusion** button to add comments or conclusions regarding the analysis. Any text written as a comment in this text box will be displayed under the **Conclusion** heading in the final report exported from Qlucore Diagnostics.

Note that the option to add comments is not available if Qlucore Diagnostics is run in command-line mode.

18.9. Exporting the report

After reviewing the contents of the report preview, click the **PDF** button to export a PDF version of the analysis result:

A file selection window will be displayed, where you can select where to save the exported report file.

Note! If you do not close the preview window or click the **New Analysis** button, the main window will still display the files you have loaded, so that you can double-check what files have been used in the analysis.

19. Interpreting the report

The report exported from Qlucore Diagnostics presents the result of the analysis, along with descriptions of the methods used in the analysis and information about the patient, the input files and the model version used. The report contains the following sections:

19.1. Result summary

The top section of the report displays data concerning the patient, the type of sample that has been used for the analysis, the analysis method, the model version and the date that the analysis was performed. The **Result summary** presents the most important findings of the analysis, which are then described in detail in subsequent sections of the report.

19.2. Conclusion

If the operator has added any comments in the report preview (see **18.8 Previewing results** above), they will be displayed under this heading.

19.3. Analysis results

19.3.1. Subtype classification

The **Analysis results** section shows the result of the BCP-ALL subtype classification. Each sample is analyzed for the following six subtypes:

- *BCR*::*ABL1* or *BCR*::*ABL1*-like
- *DUX4*-rearranged
- *ETV6*::*RUNX1* or *ETV6*::*RUNX1*-like
- High hyperdiploidy
- *KMT2A*(MLL)-rearranged
- *TCF3*::*PBX1*

The **Analysis Results** table displays all detected subtypes with their respective probability percentage numbers, sorted from higher to lower percentage numbers.

The model uses a separate classifier for each subtype, which means that the total number shown in the table may exceed 100 %.

If the analysis shows that *none* of the subtypes have a probability score of over 50 per cent, the **Subtype classification** table will be replaced by a text message that says the classification is inconclusive. This means that, based on the sample analyzed, the software was not able to detect any of the listed subtypes with a high enough degree of certainty.

19.3.2. Sample in relation to training data

The PCA (Principal Component Analysis) plot shown in the **Analysis results** section shows the relation of the analyzed sample to the training data.

19.3.3. Gene fusions of significance

A minimum of two, and no more than three input files containing gene fusion information can be loaded for analysis in Qlucore Diagnostics. The result of the gene fusion analysis is presented in three tiers, beginning with those that are considered most significant.

Each table displays information about which gene fusion has been detected, the positions of the fusion, the number of spanning fragments and breakpoint reads, and whether the fusion was predicted by the fusion caller to be in-frame. It also shows if the gene fusion has been mapped to the Mitelman database and which fusion caller has reported the gene fusion. If more than one fusion caller has reported the same instance of that gene fusion, these are listed underneath each other. The listed gene fusions are sorted by the number of breakpoint reads.

Table header	Format	Description
Gene fusion	Gene A::Gene B	Gene symbol for the first and second gene in the fusion.

Table header	Format	Description
Position A/B	[Chromosome name] [position]	Chromosomal position of the breakpoint in gene A and gene B.
Spanning fragments	Integer number	The number of spanning reads across the junction.
Breakpoint reads	Integer number	The number of reads across the junction.
Fusion frameshift	'In-frame'; 'Out-of-frame'; '-'	Whether the fusion is in-frame or not.
Database	'Mitelman'; '-'	Whether the fusion is mapped to Mitelman database or not.
Fusion caller	'Arriba'; 'FusionCatcher'; 'STAR-Fusion'	The fusion caller which detected the fusion.

Tier 1 gene fusions are listed in the classification of BCP-ALL according to the 5th edition of the WHO Classification of Haematolymphoid Tumours [Ref. 1] and/or the International Consensus Classification (ICC) of Myeloid Neoplasms and Acute Leukemia [Ref. 2].

In addition, to be listed in Tier 1 the gene fusion must fulfil the following criteria:

- have at least 1 spanning fragment and 1 breakpoint read detected by at least one fusion caller.
- predicted as in-frame by at least one fusion caller.

However, if a second fusion caller was used, and the same instance of the Tier 1 gene fusion was reported by the second fusion caller, but the Tier 1 criteria listed above were not fulfilled, the Tier 1 table in the report will display the result for this instance of the gene fusion for the second fusion caller as well.

Tier 1 gene fusions table may include gene fusions not relevant for BCP-ALL as the definitions in guidelines include wildcards.

The **Tier 2** table contains detected gene fusions which are considered less significant than the Tier 1 matches, but which may still have an influence on the interpretation of the report. A Tier 2 gene fusion must map to the Mitelman database as detected in BCP-ALL and have at least 1 spanning fragment and 1 breakpoint read detected by at least one fusion caller. There are no in-frame prediction requirements on the Tier 2 table. This means that a gene fusion listed in the WHO and ICC guidelines that has solely been detected as out-of-frame or unknown ("-") by all fusion callers will be listed in the Tier 2 table.

An example of such a gene fusion is the *DUX4*-rearrangement, which is difficult to map, due to the repetitive nature of the *DUX4* region, and in the instance of a fusion of *DUX4* with the *IGH* locus, which is highly polymorphic. It is very likely that the gene fusion caller will predict a *DUX4*-rearrangement as out-of-frame or unknown, and therefore the gene fusion will be listed in the Tier 2 table, even though the *DUX4*-rearrangement is included in the WHO and ICC guidelines.

Tier 3 gene fusions are listed in the Appendix.

Three-way gene fusions are not detectable by any of the callers and therefore never listed in any of the tables. The Mitelman database lists one three-way fusion of relevance to *BCP-ALL: BCR*::*RALGPS1*::*ABL1*.

FusionCatcher includes alternative scaffolds of chromosomes when performing alignment to reference genome GRCh37 (hg19). Some transcripts cannot be properly mapped and will instead be assigned to alternative scaffolds, such as 'Un_gl000228', or simply 'not converted'. These data are copied as-is to the 'Position A/B' fields in gene fusion tables in the report. The data is not flawed, but the mapping to chromosomes is ambiguous.

19.3.4. Quality metrics

The Quality metrics section presents information on the quality of the BAM file which was used as input for the analysis process.

The **Aligned read pairs** value measures the number of read pairs for paired-end sequencing in the sample that was mapped to the reference genome. A read-pair is mapped if there is at least one region of the reference genome which has a sequence similar to the reads.

Read pairs mapping to features measures the number of read pairs which are unambiguously assigned to a feature (i.e. within a gene in the reference genome). The features are formed by taking the union of all exons of all transcripts of a gene and a read-pair is assigned to a feature if it is completely contained in the feature. Reads assigned to multiple features are not counted.

Fraction of read pairs mapping to features (%) presents the "Read pairs mapping to features" divided by "Aligned read pairs", i.e., the proportion of read pairs providing gene expression information. It is a global indicator of the overall sequencing accuracy, and it is expected that approximately 70—90% of reads are mapped for the human genome.

The **Normal cell score** is a proxy for the tumor cell content of a sample. It is calculated from genes that are differentially expressed between three normal bone marrow samples and the samples in the BCP-ALL training dataset. The first principal component of the expression levels for these genes in the training data set are calculated. This value is then normalized, to a sample mean of 0 and a variance of 1. The same linear combination of gene expression values is calculated for the analyzed sample. The normal cell score should be larger for samples with higher normal cell content and lower for samples with lower normal cell content. But it does not provide absolute values such as 30% normal cell content.

The **Local outlier factor** is a way to assess whether a sample is an outlier or not. Local densities are computed for the sample as well as its neighbors. The density is higher if there are more samples close by. The Local Outlier Factor is calculated by comparing the density of the sample to the average densities of its neighbors. Values close to 1 mean that the sample has a density comparable to its neighbors, which means the sample is not an outlier, by this measure. A value below 1, which is rare, also indicates that the sample is not an outlier. Large values indicate that the sample is different from the neighboring samples.

The local outlier factor does not give any information on why the sample is different, the reasons behind this may be biological or technical.

The **Paired reads inner distance** indicates the distance between the end of the first read and the beginning of the second read in a fragment. The value can be negative if the two reads overlap. Since the nucleotides between the first and second reads are unknown, the inner distance must be estimated from the distance in

the reference genome sequence. Introns are excluded when estimating the inner distance and outlier fragments (fragments with an estimated inner distance below -250 or above 250) are ignored.

The **Reads fractions** values indicate how reads are assigned to strands. The values depend on the strand of the feature, the strand of the read and whether the read is the first or second read in the pair. Strand-specific library protocols preserve the information about on which strand the mRNA originated, while, for un-stranded protocols, this information is lost. First strand synthesis protocols amplify only the template cDNA strand, meaning that almost all reads should be assigned to the reverse strand. Second strand protocols mark and degrade the template cDNA strand, meaning that almost all reads should be assigned to the forward strand. For un-stranded library protocols, roughly half the reads should be assigned to each strand. Read pairs are considered ambiguous if they cannot be assigned to either strand (because of overlapping genes on different strands). Only read pairs within features are included when computing the read fractions.

19.3.5. Input

The Input section provides information about the files used with Qlucore Diagnostics for this particular analysis. The Sample file section displays the name of the BAM file, the Reference Genome section displays the version of the reference genome, and the Gene fusion file(s) section lists the name(s) of the loaded gene fusion files.

19.3.6. Appendix

The Appendix lists gene fusions that are not included in the classification of BCP-ALL according to WHO or ICC and have not been reported as detected in BCP-ALL in the Mitelman database, but that may still have an influence on the final evaluation of the analysis.

An example of such a gene fusion is the *DUX4*-rearrangement, which is difficult to map, due to the repetitive nature of the *DUX4* region, and in the instance of a fusion of *DUX4* with the *IGH* locus, which is highly polymorphic. Arriba and STAR-Fusion can report a *DUX4*-rearrangement as *DUX4L** (* can be any of the *DUX4*-Like pseudogenes in the human genome) which will not be mapped to *DUX4* and BCP-ALL in the Mitelman database and therefore will be included in the Appendix.

20. Command-line mode

Using Qlucore Diagnostics in command-line mode enables the batch-processing of several samples without graphical user interaction. When running the program from the command-line, all the input-files are passed as arguments to the program without any graphical user interaction.

Running the Qlucore Diagnostics application in command-line mode requires knowledge of using the terminal application on your system.

20.1. Prerequisites

Before running the Qlucore Diagnostics application in command-line mode you must first set up a valid license and select a language. This is done from within the normal Qlucore Diagnostics application using graphical user interaction as described in **17. Running Qlucore Diagnostics for the first time.**

After license and language configuration, close the application's graphical user interface, to ensure that the settings are saved.

To run the program in command-line mode, you need to know where the application's executable is located on your system. Check with your local IT administrator if you are unsure. You must also know the location of all your input files and the location of the model which you intend to use.

The model used in command-line mode refers to a model file stored on disk and is completely independent from the models installed in the application using graphical user interaction. Always make sure to specify the correct version of the model when running in command-line mode.

20.2. Setting up PATH on Windows

On Windows the default path to the executable is:

C:\Program Files\Qlucore\Qlucore Diagnostics 1.0\Bin\QlucoreDiagnostics.exe.

To make it easier to run the program in command-line mode, it is a good idea to set the PATH environment variable on your system to include the path to Qlucore Diagnostics. Check with your local IT administrator if you are unsure how to do this. If you do not do this, the full path to the executable must be entered each time you run the command.

20.3. Starting terminal on Windows

On Windows, the two most common terminal applications are the **Command Prompt** which is found under **Start Menu/Windows System/Command Prompt** and **PowerShell** which is found under **Start Menu/Windows PowerShell/PowerShell**.

20.4. Runing a case on Windows

In the following example we assume that there exists a folder on your disk named **C:\data** where the input data is stored. We also assume that a folder called **C:\output** exists, where output files can be stored, and that a valid model file is present in **C:\models**.

The paths to the files are passed as arguments to the program. Each path is preceded by a hyphen and a letter indicating what type of file the path that follows represents. See **20.8 List of command-line arguments** for a list of valid arguments.

Example using Windows PowerShell:

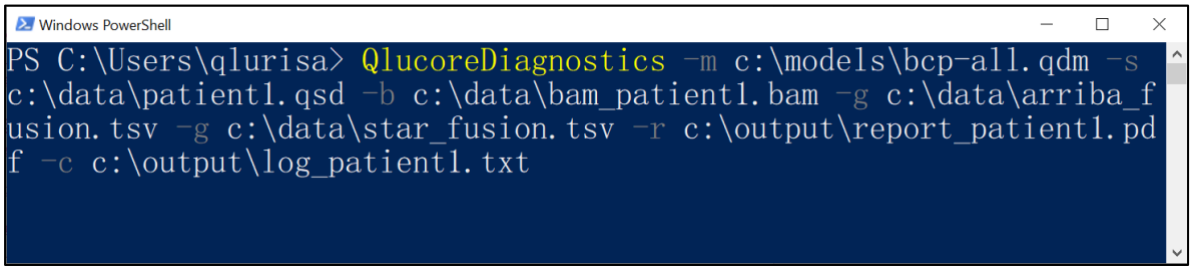


Figure 2. Running a case with PowerShell.

The command typed above is **QlucoreDiagnostics -m c:\models\bcp-all.qdm -s c:\data\patient1.qsd -b c:\data\bam_patient1.bam -g c:\data\arriba_fusion.tsv -g c:\data\star_fusion.tsv -r c:\output\report_patient1.pdf -c c:\output\log_patient1.txt**.

In the above examples we have assumed that the PATH variable for the executable name QlucoreDiagnostics has been set up correctly as described above. If this has not been done, the full path will have to be entered for the executable name and the syntax will be slightly different between the **Command Prompt** and **PowerShell**.

20.5. Setting up PATH on Mac

On Mac the path to the executable is usually

/Application/Qlucore Diagnostics.app/Contents/MacOS/Qlucore Diagnostics.

To make it easier to run the program in command-line mode, it is a good idea to set the PATH environment variable on your system to include the path to Qlucore Diagnostics. Check with your local IT administrator if you are unsure.

20.6. Starting terminal on Mac

On Mac, the terminal application is found under **/Applications/Utilities/Terminal**.

20.7. Runing a case on Mac

In the following example we assume that there exists a folder on your disk called **/Users/abc/models** where models are stored, and a folder named **/Users/abc/data** where input data is stored. We also assume that there exists a folder called **/Users/abc/output** for storing output files.

The paths to the files are passed as arguments to the program, each path is preceded by a hyphen and a letter indicating what type of file the following path represents. See **20.8 List of command-line arguments** for a list of valid arguments.

Example using Mac Terminal:

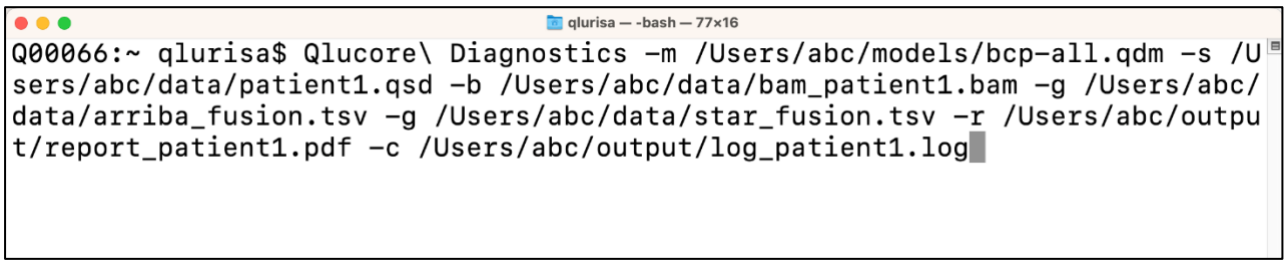


Figure 3. Running a case using Mac Terminal

The command typed above is **Qlucore\ Diagnostics -m /Users/abc/models/bcp-all.qdm -s /Users/abc/data/patient1.qsd -b /Users/abc/data/bam_patient1.bam -g /Users/abc/data/arriba_fusion.tsv -g /Users/abc/data/star_fusion.tsv -r /Users/abc/output/report_patient1.pdf -c /Users/abc/output/log_patient1.log**.

In the above example we have assumed that the PATH variable has been set up correctly as described above. If this has not been done, the full path will have to be entered for the executable name.

Note that on Mac the executable name **Qlucore Diagnostics** contains a space and must therefore be written as **Qlucore\ Diagnostics**.

20.8. List of command-line arguments

Option	Argument	Description
-s, --sample-file	filepath	Complete path to sample data file (mandatory).
-m, --model-file	filepath	Complete path to model file (mandatory).
-b, --bam-file	filepath	Complete path to BAM file. (Mandatory)
-g, --gene-fusions-file	filepath	Complete path to gene fusion file. This option must be given at least twice and can be given multiple times to specify more gene fusions file. (Mandatory)
-r, --report-file	filepath	Complete path to generated report file. The file should have the extension .pdf. (Mandatory)
-c, --case-log-file	filepath	Complete path to generated case log file (optional).
-h, --help	N/A	Returns a help message.
-v, --version	N/A	Returns the platform version.

21. Updating Qlucore Diagnostics

Whenever an updated version of Qlucore Diagnostics becomes available, an email will be sent to all registered users, to the email address that was used when registering for downloading the software when it was purchased. Make sure to update your account information so that its email address is up to date.

Download the update by clicking the link included in the update email. The downloaded software will use the standard update feature of the operating system to update Qlucore Diagnostics.

Some Qlucore Diagnostics updates are published to address safety or security related issues. A residual risk exists that the update information is missed or not acted upon.

Action

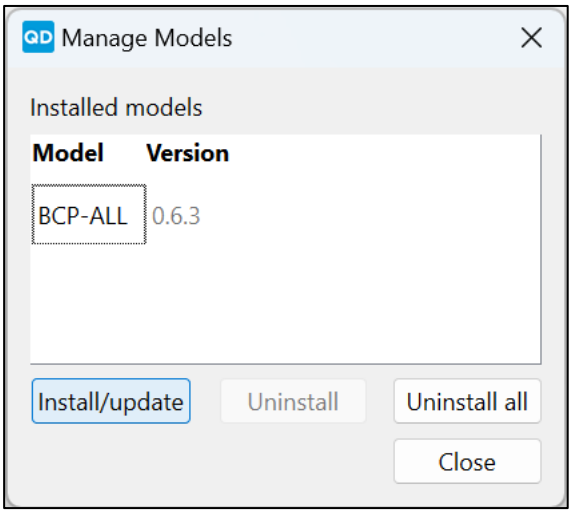
Monitor the registered email to which information about product updates will be sent, paying particular attention to whether the update is required for safety or security concerns.

22. Managing Qlucore Diagnostics models

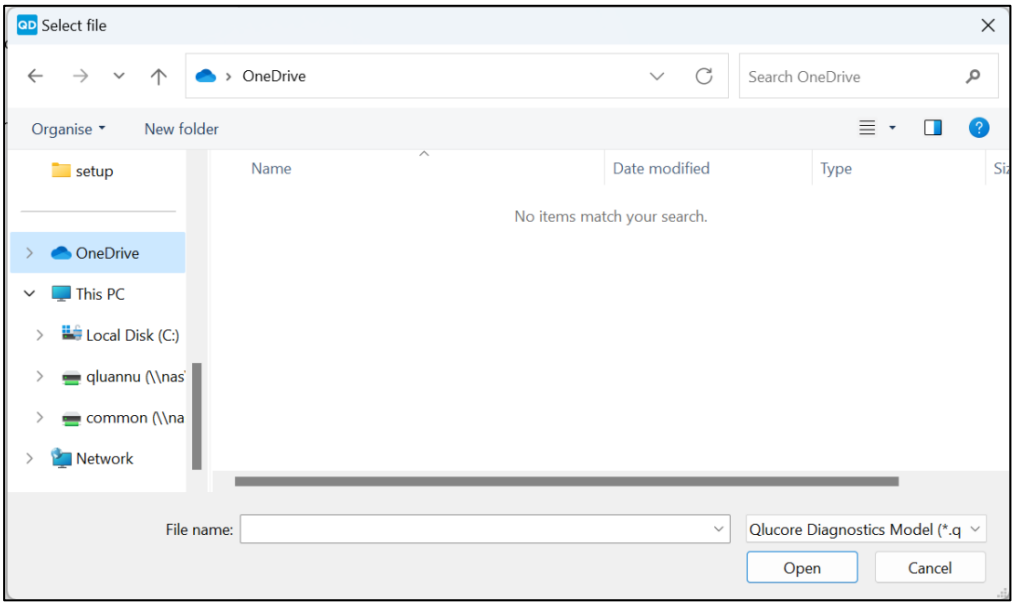
Use the Manage models dialog, accessed via the **File** menu, to install or remove Qlucore Diagnostics models.

22.1. Updating a model

1. Select **File**>**Manage models**. In the dialog that is displayed, click the **Install/update** button.



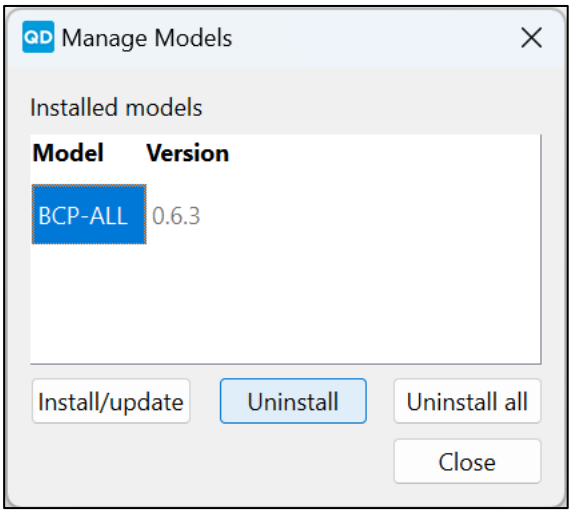
This will display a file selection window.



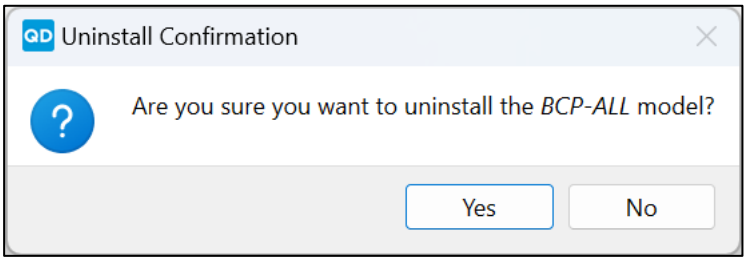
2. Locate and select the relevant model file.
3. Click Open to add the file to the Installed models list.

22.1.1. Uninstalling a model

1. To uninstall a model, select **File**>**Manage models**. In the dialog that is displayed, click **Uninstall**.

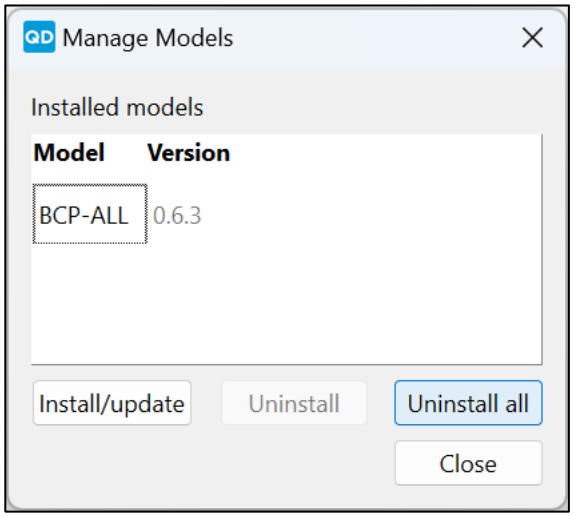


2. This brings up a confirmation dialog. Click **Yes** to confirm the uninstallation of the model.



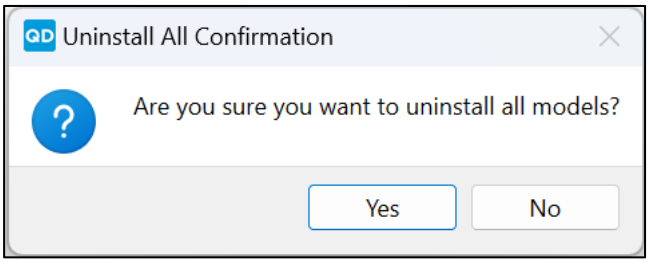
22.1.2. Uninstalling all models

1. To uninstall all models, select **File**>**Manage models**. In the dialog that is displayed, click **Uninstall all**.



This brings up a confirmation dialog.

2. Click **Yes** to confirm the uninstallation of all models.



23. Maintenance

The application does not require any maintenance.

24. Uninstalling Qlucore Diagnostics

24.1. Windows

To uninstall Qlucore Diagnostics on a Windows system, follow the instructions provided by Microsoft for uninstalling or removing software. Under **C:\ProgramData\Qlucore\Diagnostics**, a settings XML-file (persistent_attributes.xml) and three folders; License, Models, system_log will remain. If you choose to remove these manually, future installations will need to be reconfigured, will require a new license activation and model installations, and the logs will be lost.

24.2. Mac

To uninstall Qlucore Diagnostics on a Mac system, follow the instructions provided by Apple for uninstalling or removing Apps. Under **/Users/Shared/Qlucore/Diagnostics**, a settings XML-file (persistent_attributes.xml) and three folders: License, Models and system_log will remain. If you choose to remove these manually, future installations will need to be reconfigured, will require a new license activation and model installations, and the logs will be lost.

25. Troubleshooting

25.1. The Platform Does Not Open

If you cannot access the platform and there is no response, it may be because the platform is already open in another session. The platform allows only one active session at a time amongst all users on the same computer. To resolve this issue, ensure no other user has an active session open.

25.2. Error Messages

The following tables list the error messages that may be displayed in the Qlucore Diagnostics interface. The signs “%1”, “%2” and “%3” are wildcards, that can take on and display different values depending on the context in which the message is displayed.

Table 12. Error messages.

Error message	Description	Action
A case cannot be run without a patient data file.	No patient data file has been loaded.	Make sure all required files have been loaded before running a case.

Error message	Description	Action
A maximum of 0 BAM files allowed, but 1 was provided.	You provided one too many BAM files.	Do not provide any BAM files.
A minimum of 1 BAM file required, but 0 were provided.	At least one BAM file is required to run a case.	Make sure to load an aligned BAM file for the case.
The path '%1' does not point to a valid file.	No file with the correct file type can be found at the specified location.	Point to a location where the correct file can be found.
Incorrect file '%1'. Expected a BAM file.	The specified file is not a BAM file.	Select a BAM file where this is expected.
The BAM file is aligned with '%1' version %2 which is not supported by the model, or the alignment command line used differs from the model instructions.	The alignment of the BAM file has been made with the wrong version of an alignment software, or the commands given do not work with the model used.	The BAM file should be aligned using STAR v2.7.8a and the commands specified in the IFU.
Incorrect file '%1'. Expected a gene fusion file.	The file type is wrong.	Select a gene fusion file, with the. TSV or TXT extension.
The path '%1' does not point to a valid file.	No valid file found in the specified location.	Point to a location that contains the correct file type.
Only one fusion file from each caller is allowed.	Multiple fusion files from the same caller have been added.	Make sure to load no more than one single fusion file from each fusion caller.
A minimum of %1 fusion files required, but %2 provided.	Too few fusion files have been loaded.	Add at least the minimum number of fusion files.
A maximum of %1 fusion files allowed, but %2 provided.	Too many fusion files have been loaded.	Only provide up to the maximum number of fusion files.
A file of type %1 is required by this model.	The wrong file type has been selected.	Add the required file type to the case run.
%1 is the only tag allowed below the root tag in a QSD file.	An incorrect tag type has been added to the root tag of the QSD file.	Change the QSD file so that it only contains the allowed tag.
The QSD file must not contain unknown tags. Found: %1, expected: %2.	An unknown tag has been found in the QSD file.	Follow the QSD example given in 18.7.2. Preparing QSD and QCSLS files.
The QSD value '%2' is longer than the max length of %1.	A value exceeds the maximum number of characters.	Only provide strings that do not exceed the maximum length.

Error message	Description	Action
The file '%1' does not exist.	There is no file at this location.	Only provide file paths for existing locations.
The file '%1' is not a QSD file.	The wrong file type has been selected.	Select a QSD file.
Trying to add a QSD file. The format name should be '%1' but is '%2'.	The wrong file format has been selected.	Select a QSD file.
Trying to add a QSD file. The version of '%1' should be %2 but is %3.	The version number of the QSD file is incorrect.	Locate and select a QSD file with the correct version number.
Please specify a valid path to a report file (*.pdf).	No path to a report file was given.	Enter a path to a report (*pdf) file.
The report file '%1' is missing a PDF extension (*.pdf).	The report path given did not end with .pdf.	End the report path with .pdf.
A report named %1 already exists.	A report with the name entered has already been saved.	Provide a file path that does not exist yet.
Failed to create directory for report %1.	The folder for the report could not be created.	Select a different location or manually create the folder.
Failed to create directory for log %1.	The folder for the log file could not be created.	Select a different location or manually create the folder.
Failed to create log file %1.	The log file could not be created.	If a restart does not solve this issue, contact Qlucore Support.
Unknown option in the command.	A command-line option that is not allowed has been used.	Check the syntax of the commands.
Multiple values are not allowed for this option.	More than one value has been entered.	Remove all but one value for this option.
Error parsing command-line arguments.	The program arguments were not entered correctly.	Enter the program arguments correctly, according to the instructions in section **20. Command-line mode**.
Error parsing the command-line arguments. A parameter without an option is not allowed.	The program arguments were not entered correctly.	Enter the program arguments correctly, according to the instructions in section **20. Command-line mode**.

Error message	Description	Action
Error parsing the command-line arguments. Help and version options are not allowed together.	The program arguments were not entered correctly.	Enter the program arguments correctly, according to the instructions in section **20. Command-line mode**.
The custom logotype file, \"%1\", does not exist.	There is no custom (PNG) logotype file at the specified location.	Locate the correct path to a logotype file (PNG format).
The file \"%1\" is not a PNG file.	The selected logotype file has the wrong format.	Select a PNG file.
The image file must not be bigger than %1 x %1 pixels.	The image file is too big.	Make sure the image file does not exceed the maximum size specified.
Not more than %1 custom labels are allowed.	Too many custom labels have been added.	Remove any unexpected labels.
There must not be duplicate tags.	Duplicate tags have been found.	Remove any duplicate tags.
The custom QSD label '%2' is longer than the max length of %1.	A label is too long.	Shorten the label so that it does not exceed the maximum length.
The file \"%1\" does not exist.	A file is missing in the specified location.	Select an existing file path.
The file \"%1\" is not a custom label file.	A file added as a custom label file is the wrong file type.	Select a QCSLS file.
Failed to add a custom-label file. The format name should be %1 but is %2.	The wrong file format has been entered.	Use the QCSLS file format for adding a custom label file.
Failed to add a custom-label file. The version of %1 should be %2 but is %3.	The wrong version of the QCSLS format has been used.	Use the correct version of the QCSLS format.
The platform cannot handle more than %1 models.	More than the maximum number of models has been added.	Do not attempt to load more than the maximum number of models that can be handled by the platform.
The file %1 was not found.	The file was not found.	Select an existing file.
The file %1 could not be opened.	The file could not be opened.	Select an existing, non-corrupt, file.

Error message	Description	Action
Failed to remove a folder, please restart your computer.	A folder could not be removed.	If a restart does not solve this issue, contact Qlucore Support.
Failed to create a folder, please restart your computer.	A folder could not be created.	If a restart does not solve this issue, contact Qlucore Support.
The model file path '%1' does not exist.	The path that was selected to point to the model does not exist.	Select a path that points to an existing location for the model.
The file '%1' is not a model file. Please select a valid model file (*.qdm).	A file with the wrong file extension has been selected.	Locate and select a file with the QDM extension.
The model is missing the language '%1'.	When the case run was started, it was detected that the model does not support the currently selected language.	Change language or select a model that supports the currently selected language.
The model (%1, version %2) requires platform version %3 or higher. The current platform version is %4.	The model is not compatible with the current platform.	Choose another model that is compatible with this platform release or update the platform.
This model (%1, version %2) does not meet the platform requirements, please choose a more recent model that supports platform versions %3 or higher.	The model is not compatible with the current platform.	Choose a more recent model.
The platform is Performance marked and therefore not compatible with a model that is marked as %1.	The model is not compatible with the current platform.	Only use a CE marked platform and CE marked models.
The platform is CE marked and therefore not compatible with a model that is marked as %1.	The model is not compatible with the current platform.	Only use a CE marked platform and CE marked models.
The file '%1' does not exist. Please select a valid model file (*.qdm).	The selected file path does not point to a file that is valid for the current model.	Choose an existing model file.
The file '%1' is not a model file. Please select a valid model file (*.qdm).	The wrong type of file has been selected.	Select a valid file, that has the QDM file extension.
The file '%1' does not exist. Please select a valid model file (*.qdm).	A file path that does not exist has been chosen for the model.	Select a valid file, that has the QDM file extension.

Error message	Description	Action
Trying to load a wrongly formatted file. This file expects that the tag %1 has a child tag with name %2.	The selected file has tag errors.	Check that the file tags are correct, as described in **18.7.2. Preparing QSD and QCSLS files**.
Trying to load a wrongly formatted file. This file expects the '%1' tag.	The selected file has tag errors.	Check that the file tags are correct, as described in **18.7.2. Preparing QSD and QCSLS files**.
Trying to load a wrongly formatted file. All content in this file format must be preceded by a tag.	The selected file has tag errors.	Check that the file tags are correct, as described in **18.7.2. Preparing QSD and QCSLS files**.
Trying to load a wrongly formatted file, it must not end with content.	The selected file has content errors.	Check that the file is correctly formatted, as described in **18.7.2. Preparing QSD and QCSLS files**.
Trying to load a file with a wrongly formatted version. The version must be two integers separated by a dot.	The selected file has a version format error.	Make sure that the version number is correctly formatted, as two integers separated by a dot.
Trying to load a file with a wrongly formatted version. The part of a version before the dot (.) must be a valid integer.	The selected file has a version format error.	Make sure that the version number is correctly formatted, as two integers separated by a dot.
Trying to load a file with a wrongly formatted version. The part of a version after the dot (.) must be a valid integer.	The selected file has a version format error.	Make sure that the version number is correctly formatted, as two integers separated by a dot.
Failed to copy case log file to %1.	The case log file could not be created in the specified location.	If a restart does not solve this issue, contact Qlucore Support.
Error while creating case setup.	Something went wrong in the case setup.	Restart the platform.
No license found.	No active license was found.	Activate a new license.
License (%1) has already been activated.	The license key entered is already in use.	Use a fresh license key.
Not a license key.	The license key entered has the wrong format.	Enter a valid license key.

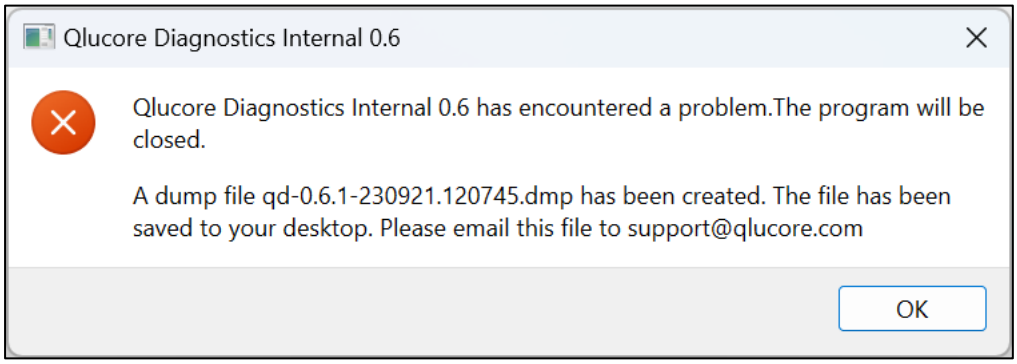
Error message	Description	Action
This license has already been activated.	The license key entered is already in use.	Use a fresh license key.
This license has already been activated before.	The license key entered is already in use.	Use a fresh license key.
The license has no case runs left.	All case runs associated with this license have been used.	Activate a new license.
The provided activation key is not valid. Make sure that you have copied the whole key. The format should be: XXXXX-XXXXX-XXXXX-XXXXX.	The license key entered may be incomplete.	Make sure that the license key entered has the correct format, XXXXX-XXXXX-XXXXX-XXXXX.
This license seems to have been activated on another computer. If this is not the case, please contact the support.	The license key is already in use.	Contact Qlucore Support if the license key has not been activated already.
Make sure that your computer has a working internet connection and try again. If the computer must stay offline, please contact the support for a manual license activation.	There is no Internet connection.	Make sure there is a working Internet connection to activate the license online, or contact Qlucore Support for manual, off-line license activation.
The License has expired.	The active license is no longer valid.	Activate a new license.
The license is out of case runs.	The maximum number of case runs has been reached for this license.	Activate a new license.
There are no case runs left and the license has expired.	The maximum number of case runs has been reached for this license, and it is no longer valid.	Activate a new license.
The license expires in %n day(s).	The active license will soon become invalid.	Activate a new license.
There is/are only %n case run(s) left.	The active license will soon reach its maximum number of case runs.	Activate a new license.

Error message	Description	Action
The license expires in %1 days and there are only %2 case runs left.	The maximum number of case runs will soon be reached for this license, and it will no longer be valid.	Activate a new license.
The custom logotype file, \"%1\", does not exist.	The custom logotype file previously selected cannot be found.	Under Preferences, select a new logotype file.
A valid path and a PDF extension is required for PDF report export.	The report path does not end in ".pdf"	Add ".pdf" to the report name.
No language selected, please select a language in the GUI application.	A language has to be set.	Select a language from the drop-down menu in the Preferences dialog.
Technical error	Internal technical error.	Contact Qlucore Support for further information.

25.3. Application crash

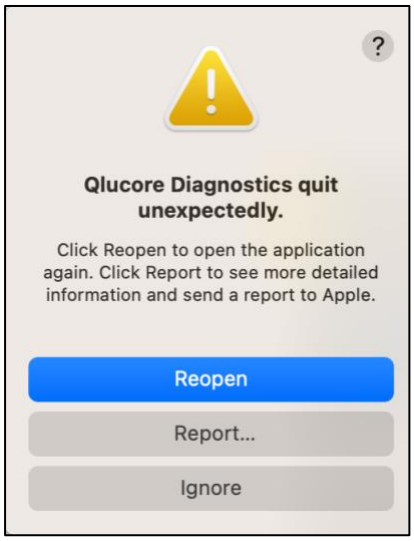
25.3.1. Windows system

If the Qlucore Diagnostics platform should crash, a crash dump file will be created and saved to the computer desktop. Send this file to the Qlucore Support email address shown in the dialogue box that is displayed.



25.3.2. MacOS system

If the Qlucore Diagnostics platform crashes, the following dialog box will be displayed:



The following steps are recommended:

1. Select **Ignore**.
2. Ask the system administrator to locate the crash report file in **~/Library/Logs/DiagnosticReports/**
3. Email the crash report file to Qlucore Support at support@qlucore.com.

26. Related documentation

Table 13. List of related documentation.

Document ID	Title	Type
	RNeasy Mini Handbook [October 2019]	
	TruSeq RNA Library Prep Kit v2 guide [March 2014]	
	TruSeq Stranded mRNA Library Preparation guide [October 2017]	
	Illumina Stranded mRNA Library Preparation kit [June 2022]	

27. References

Table 14. List of references.

Reference	Document ID	Title	Type
1		Alaggio, R., Amador, C., Anagnostopoulos, I. et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Leukemia 36, 1720–1748 (2022).	

Reference	Document ID	Title	Type
2		Arber, DA., Orazi, A., Hasserjian, RP. et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood 140 (11), 1200–1228 (2022).	

28. Manufacturer information

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28.1. Summary of Safety and Performance (SSP)

You can obtain a copy of the *Summary of Safety and Performance (SSP)* document by visiting the Downloads section of Qlucore.com. Alternatively, contact your nearest Qlucore office using the contact details provided above.

28.2. Support

For support, visit the Support section on Qlucore.com or send an e-mail to support@qlucore.com.