LymphoTrack®
MRD Software

Instructions for Use

LymphoTrack® MRD Software v2.0.x

The Research Use Only (RUO) LymphoTrack MRD Software v2.0.x is a bioinformatics tool that runs on supported Microsoft Windows® platforms. This software is intended to detect the presence of *IGHV* Leader, *IGH* FR1/2/3, *TRG* and *TRB* clonal sequences within the output files generated using the Invivoscribe® LymphoTrack Assays and accompanying LymphoTrack bioinformatics software. This software is not intended to define the significance of finding such sequences.

For an MRD **Detected** result, the software will report the number of reads and cumulative frequencies of exact sequence matches and similar sequences (up to two mismatched nucleotides).

For an MRD **Not Detected** result, the software will report the number of reads and cumulative frequencies of exact sequence matches and similar sequences (up to two mismatched nucleotides). In addition, the software will report the confidence level at 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} sensitivity for the searched sequence based upon the sample DNA input and read depth obtained.

For additional information related to sample setup for Minimal Residual Disease (MRD) studies, please refer to the technical bulletin *Study of MRD - Using LymphoTrack Assays* (REF M-0036). This technical bulletin and the *MRD Software* technical bulletin (REF M-0031) can be provided upon request by emailing marketing@invivoscribe.com.

Minimum System Requirements

- Processor: Intel Core 2 Duo or newer CPU recommended.
- Hard Drive: At least 1 GB of free disk space is required; 2 GB recommended.
- RAM: 4 GB required; 8 GB or more recommended.
- Operating System: Windows 10 (64-bit) is required.
- A CD-ROM drive.

Warnings and Precautions

- **Instructions for Use**. Please read the Instructions for Use carefully prior to running the LymphoTrack MRD Software and follow each step closely.
- **Sleep or hibernate settings**. If the computer is set to enter sleep or hibernate modes after a period of inactivity, consider disabling this feature before executing the LymphoTrack MRD Software. If the computer enters sleep or hibernate mode, the software analysis may terminate prematurely.
- **File Naming.** Be certain to use very descriptive file names that can be easily identified, as multiple files are generated by the LymphoTrack MRD Software output.
- **Characters in pathname and file name.** 1) Avoid spaces in the pathname for the files (pathnames include file folders and file names) to be analyzed. 2) The filenames can only contain the following characters (A-Z, a-z, 0-9, _ (underscore), - (hyphen)). If the software encounters a character not within this set, it may fail to complete analysis.

Product Compatibility

The RUO LymphoTrack MRD software v2.0.x is intended to be used with the following Invivoscribe LymphoTrack Assays and Controls to track clonal sequences identified by the corresponding LymphoTrack Software (for Ion S5/PGM REF 75000007 or MiSeq REF 75000009).

Catalog #	Description	Quantity
REF 40880098	LymphoTrack® B-cell Low Positive Control	1 Tube – 5 reactions
REF 40880118	LymphoQuant® B-cell Internal Control	1 Tube – 120 reactions
REF 40880108	LymphoTrack® T-cell Low Positive Control	1 Tube – 5 reactions
REF 40880128	LymphoQuant® T-cell Internal Control	1 Tube – 120 reactions
REF 71210009	LymphoTrack® *IGH* FR1 Assay Kit A - MiSeq	8 indices – 5 reactions each
REF 71210039	LymphoTrack® *IGH* FR1 Assay Panel - MiSeq	24 indices – 5 reactions each
REF 71210149	LymphoTrack® *IGH* FR1 Assay Panel B - MiSeq	24 indices – 5 reactions each
REF 71210089	LymphoTrack® *IGH* FR2 Assay Kit A - MiSeq	8 indices – 5 reactions each
REF 71210099	LymphoTrack® *IGH* FR2 Assay Panel - MiSeq	24 indices – 5 reactions each
REF 71210109	LymphoTrack® *IGH* FR3 Assay Kit A - MiSeq	8 indices – 5 reactions each
REF 71210119	LymphoTrack® *IGH* FR3 Assay Panel - MiSeq	24 indices – 5 reactions each
REF 71210129	LymphoTrack® *IGH* FR1/2/3 Assay Kit A - MiSeq	8 indices per FR region – 5 reactions each
REF 71210139	LymphoTrack® *IGH* FR1/2/3 Assay Panel - MiSeq	24 indices per FR region – 5 reactions each
REF 71210059	LymphoTrack® *IGHV* Leader Somatic Hypermutation Assay Kit A - MiSeq	8 indices – 5 reactions each
REF 71210069	LymphoTrack® *IGHV* Leader Somatic Hypermutation Assay Panel - MiSeq	24 indices – 5 reactions each
REF 72250009	LymphoTrack® *TRB* Assay Kit A – MiSeq	8 indices – 5 reactions each
REF 72250019	LymphoTrack® *TRB* Assay Panel – MiSeq	24 indices – 5 reactions each
REF 72270009	LymphoTrack® *TRG* Assay Kit A – MiSeq	8 indices – 5 reactions each
REF 72270019	LymphoTrack® *TRG* Assay Panel – MiSeq	24 indices – 5 reactions each
REF 71210007	LymphoTrack® *IGH* FR1 Assay – S5/PGM	12 indices – 5 reactions each
REF 71210037	LymphoTrack® *IGH* FR2 Assay – S5/PGM	12 indices – 5 reactions each
REF 71210047	LymphoTrack® *IGH* FR3 Assay – S5/PGM	12 indices – 5 reactions each
REF 71210057	LymphoTrack® *IGH* FR1/2/3 Assay – S5/PGM	12 indices per FR region – 5 reactions each
REF 72270007	LymphoTrack® *TRG* Assay – S5/PGM	12 indices – 5 reactions each

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1. Glossary

Table 1. Commonly used terms and phrases.

Term	Definition
Low Positive Control (LPC)*	Designed specifically for MRD testing, the LymphoTrack Low Positive Controls are optimized to work in concert with the LymphoQuant Internal Controls. When run together as intended, these controls ensure that MRD levels of sensitivity are confidently interrogated in samples where the LymphoQuant Internal Control is being used.
LymphoQuant Internal Control (LQIC)*	LymphoQuant Internal Controls may be spiked into sample PCRs to estimate the respective number of clonal equivalents and calculate the percent clonal present. Addition of a spike-in internal control to the sample PCR facilitates clonal tracking over time without additional sequencing run cost. Consistent use of a spike-in internal control enables objective monitoring of a clonotype over time with a highly standardized, sensitive method.
# of Replicates	The number of independent PCR amplifications per sample. A **PCR replicate** for a sample is defined as an independent PCR **amplification** with a unique LymphoTrack master mix (index). • For example, if Sample A is tested in three separate PCRs by *TRG* MiSeq 01, *TRG* MiSeq 02, and *TRG* MiSeq 03, respectively, Sample A would have three different PCR replicates. • Each PCR replicate sequenced will have a **.unique_reads* file generated by the LymphoTrack software.
# of Resequences	The number of times the same PCR replicate is sequenced on a different sequencing run, potentially because a sample did not reach adequate read depth the first time it was sequenced.
# of Reads per Sequencing	The desired read depth for each sequenced PCR replicate provided as a guide for planning experiments using the Project Planner; this number is assigned to each sequenced replicate, including each planned resequencing run or sample.
Read Depth	The number of sequencing reads generated per run per sample or sample replicate. The Project Planner refers to sample Read Depth as *# of Reads per Sequencing*.
Amount of DNA	The amount of DNA used in each PCR replicate, **NOT** the total amount of DNA interrogated across all replicates.
Run	An entire workflow as it pertains to the set of samples and controls per assay target, including resequences.
Index	A unique oligo sequence used to identify samples or PCR replicates of the same sample.
Confidence	The probability of all sequences resulting in a true negative (calculated using the *# of Replicates, # of Resequences*, *# Reads per Sequencing*, and *Amount of DNA*) at a given threshold.
Cumulative Read Count	In the context of this software, the sum of exact match read count plus 1 mismatch, plus 2 mismatch read counts.
Cumulative Read Frequency (CRF)	The read count generated by a specific sample sequence in the LymphoTrack Software divided by the total number of reads generated by that sample.
Estimated Clonal Cell Equivalents	An approximate calculation of the number of cells containing the interrogated clonal sequence in the sample.
Bi-allelic Clonal	Two unique clonal sequences present on two alleles of the same gene; although it is possible that two detected alleles may arise from the presence of two mono-allelic clonotypes.
Semantic versioning	A software version scheme consisting of 3 numbers (Major.Minor.Patch), aligned with the risk factor of the update.

*The LymphoTrack B-cell Low Positive Control and LymphoQuant B-cell Internal Controls cannot be used with LymphoTrack *IGK* Assays.

2. MRD Sequence Selection

The LymphoTrack Software enables a sensitive, objective approach to MRD tracking of *IGHV* Leader, *IGH* FR1/2/3, *TRG* and *TRB* clonal sequences over time. When the LymphoTrack Assays are used with the respective Low Positive Control (LPC) and LymphoQuant Internal Control (LQIC), they provide a streamlined, standardized MRD workflow. The LymphoTrack Software can help identify clonal sequences for MRD tracking, but due to the unique nature of each locus, special considerations must be made when determining if a clonal sequence is suitable for MRD tracking.

2.1 Immunoglobulin Heavy Chain (*IGH*)

Due to the high genetic diversity of *IGH*, there are typically no special considerations that need to be considered when selecting sequences for MRD tracking.

2.2 Immunoglobulin Kappa Chain (*IGK*)

The use of an *IGK* target for MRD testing is not ideal, and caution is warranted due to the low genetic diversity of this locus. The LymphoTrack B-cell Low Positive Control and LymphoQuant B-cell Internal Control cannot be used to with LymphoTrack *IGK* Assays. If alternate *IGK* controls are utilized, verify that any clonal *IGK* sequences identified are absent in negative control data before using the sequence for MRD tracking. There are many common *IGK* rearrangement sequences that are likely unsuitable for MRD analysis due to the high frequency in which they are observed in polyclonal negative samples. For example, any of the 3 clonal sequences listed below may not be suitable for MRD analyses:

- Intron-K_{del}
- V3D-20 with any J or K_{del}
- V3-11 with any J or K_{del}

2.3 T-Cell Receptor Gamma (*TRG*)

The *TRG* locus is composed of fewer gene segments than *IGH*, *IGK*, and *TRB*, and thus is far less diverse. Due to this lack of diversity, *TRG* gene rearrangements of the same sequence are found between cells at a higher prevalence, increasing the likelihood of false positive MRD results. To minimize this risk, the MRD Software only uses **exact matches** for *TRG* calculations. Likewise, it is also recommended to:

- Track **two clonal sequences** to reduce the probability of a false positive match. The *TRG* locus has a strong propensity for bi-allelic rearrangements and will display as two clonal sequences in the LymphoTrack output. Use both of these sequences for MRD tracking.
- In cases of a bi-clonal sample, the output will likely include more than two clonal sequences and provide multiple trackable sequences that can improve the specificity of the assay.

2.4 T-Cell Receptor Beta (*TRB*)

When tracking *TRB* gene rearrangements, please note that **D-J rearrangements** are less suitable for MRD testing due to the reduced diversity found in these immature rearrangements. To minimize the risk of false positive MRD results, track **two clonal sequences** using only **exact match sequences** for *TRB* clonal tracking.

3. Installing the Software

The LymphoTrack MRD Software package can be downloaded from the Invivoscribe Software Portal or copied from the provided CD.

Note: Administrator permission is required to Install the software

3.1 Load the LymphoTrack MRD Software CD into the disk drive; ***OR***

3.2 Download the LymphoTrack MRD Software from Invivoscribe Software Portal

3.2.1 Using any web browser, navigate to https://catalog.invivoscribe.com/softwareportal/

3.2.2 Complete the following text fields:

3.2.2.1. Email: Enter a valid email address. A link to the software download will be sent to this address.

3.2.2.2. Customer Account Number: Enter your unique ID used when placing orders with Invivoscribe.

3.2.2.3. Software Code: Enter the software code at the bottom of your sales order.

3.2.3 Check the *Terms and Conditions* box to proceed.

3.2.4 Click the **Request Software** icon.

- Following valid input text into the above fields, a link to the software download will be sent to the provided email address.

3.2.5 Click the link or copy+paste into a web browser; the software will automatically download.

3.3 Install the LymphoTrack MRD Software

3.3.1 Double click the **LymphoTrack_MRD-2.0.x.RUO.msi** file.

3.3.1.1. If a message appears, stating *Windows protected your PC,* click **More info** and verify the publisher is Invivoscribe, Inc., then click **Run anyway.**

- The software installer will open and allow the software to be installed to a local drive on the computer.
- The default install location is *C:\Invivoscribe\ LymphoTrack_MRD-2.0.x.RUO.*

3.3.2 Follow the Windows Setup Wizard installation prompts, select the directory file path to save the software and click **Install**.

- Install software only on a local drive (not a network drive); the software might not function properly if run across a network connection.

3.3.2.1. If the User Account Control dialog appears, click **Yes.**

3.3.3 Once installation is complete, click **Finish**.

- The software is now ready to use and can be accessed by navigating to the *Start Menu* or the directory file path selected in step 3.3.2.

4. Creation of a Project Plan

MRD testing requires greater sensitivity, thus greater DNA input, than baseline testing, due to the lower probability that the sample will contain cells with the sequence(s) which are being tracked. The **Project Planner** is a tool within the LymphoTrack MRD Software that aids in the experimental design and features a confidence interval calculator to determine the confidence level of a true MRD negative sample using the number of Replicates, number of Resequences, number of Reads per Sequencing, and Amount of (input) DNA to estimate the confidence level of a true MRD negative sample. The Project Planner tool is useful to ensure the experimental design meets the user-defined requirement for combined sensitivity and confidence necessary for MRD monitoring.

4.1 Open the start menu and type **LymphoTrack_MRD** to locate and open the software.

4.2 Read the license agreement. To accept the terms, click the **Accept** button to proceed.

4.3 From the main window, click the **Projects** tab from the toolbar on the top left of the window, then select the **Create New Project Plan** from the drop-down menu.

- A new window will appear which will allow the user to calculate the confidence of a true negative MRD result depending on the variables of the experimental setup.

4.4 In the new window, enter the following information:

- # of Replicates. Please note that subsequent values (# of Resequences/# of Reads/Amount of DNA) pertain to **EACH** PCR replicate, not the total of all replicates combined.
- # of Resequences. The typical value here is **1** (meaning only a *single* sequencing run).
  - Resequences, by definition, will have the same sample index, and the selected unique reads files are assigned the same PCR replicate number in the Replicate field of the software, as described in section 5.7.
- # of Reads per Sequencing. Estimated read depth based on the read capacity of the flow cell or chip divided by the number of samples and sample replicates run on the flow cell or chip.
  - If a value of *100,000* is assigned to a single replicate with only the initial sequencing, the total reads will be *100,000*.
  - If a value of *100,000* is assigned to a single replicate with the initial sequencing plus a resequenced run, (a value of **2** for *# of Resequences*), the total reads will be *200,000*.
- Amount of DNA. This is the amount of DNA input to each PCR replicate. For example, if the experiment setup was to sequence 5 replicates of 1000 ng of DNA, input the value *1000*, **NOT** *5000*.

4.5 Adjust the confidence levels accordingly, then click **Calculate Confidence**. The lower right quadrant of the window will display the percent confidence of a negative result at each sensitivity level when considering the # of PCR Replicates, # of Resequences, Amount of DNA, and # of Reads per Sequencing.

- For example, to achieve a sensitivity level of 10^{-4} with >95% confidence, sequence 200 ng of DNA with at least 500,000 reads.
- To achieve a sensitivity level of 10^{-5} with >95% confidence, sequence at least 4 µg of DNA.
- To have >95% confidence that 1 target cell in 1 million cells (10^{-6}) can be detected, sequence at least 20 µg of DNA (~3.1 million cells).

4.6 Once the experiment is planned, click **X** in the upper right-hand corner to close the **Project Planner** window.

5. Add Subjects and Samples

Prior to analyzing sample and control data with the LymphoTrack MRD Software, identify the clonal sequence(s) to be tracked using an initial highly clonal sample analyzed with the appropriate LymphoTrack Assay and the corresponding LymphoTrack Software (for Ion S5/PGM REF 75000007 or MiSeq REF 75000009). Once clonal sequences are identified they can be tracked using the LymphoTrack MRD Software. Refer to Figure 1 for a summary of the sample processing workflow. Follow the subsequent steps to analyze subject samples with or without LymphoQuant Internal Control.

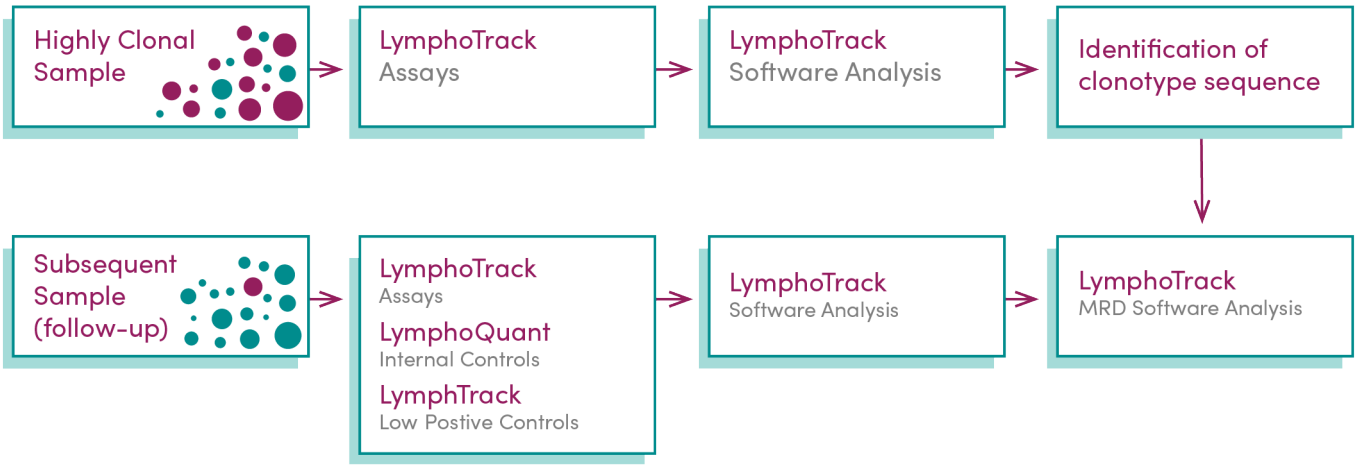

Figure 1. MRD sample testing workflow begins with identification of the clonal sequence(s) from highly clonal samples with the LymphoTrack Assay kits and software. To track the clonal sequence(s) over time, subsequent sample testing is performed using the LymphoTrack Assay kit with the respective B- or T-cell LymphoQuant Internal Control and LymphoTrack Low Positive Control. Lastly, the LymphoTrack MRD Software is used to estimate and track clonal frequencies over time.

5.1 Click **Add/Edit Subjects** to open the main subject table window.

5.2 Enter a *Subject ID* using only the characters (A-Z, a-z, 0-9, _ , -) and no more than one consecutive space in the field labeled *Subject ID*.

5.3 Click the **Add Subject** button. The newly added subject will appear in a list below the field labeled *Subject ID*.

5.4 Click on the *Subject* in the list to edit the subject fields.

5.5 Select a *Gene Target* from the drop-down menu.

- The selected gene target must reflect the LymphoTrack assay used to assay the sample.

5.6 Enter the name of the clonal sequence to be tracked using only the characters (A-Z, a-z, 0-9, _ , -) and no more than one consecutive space in the field labeled *Sequence 1 Name*. Copy and paste the desired sample clonal sequence from the LymphoTrack Report or output file to be tracked into the text box below the sequence name.

- The sequence must only contain capital letters (*e.g.,* ACGT] without any spaces.
- Be sure to copy and paste the full clonal sequence(s); as any missing nucleotides will be treated as mismatches and could generate inaccurate results.

5.7 If tracking two (2) or more **clonal sequences**, select the subsequent *Sequence* tab(s) and repeat the previous step.

- Up to five (5) unique sequences can be tracked simultaneously.
- Click **Save** after all sequences are entered.

IMPORTANT! The clonal sequence name is used in the output file names. Windows has a built-in limit of 255 characters for file pathnames and owing to the verbose naming conventions of NGS data, please consider this limitation.

5.8 To add new time points to a subject, click **Add Sample** on the left of the *Subject Setup* window. Select the subject that will be associated with the new time point from the *Subject ID* drop down menu.

5.8.1 Enter the name of the *Sample Unique Identifier* (*e.g.,* First Follow-up) and *Sample Type* (*e.g.,* Bone Marrow, Peripheral Blood, etc…) in the corresponding fields using only the characters (A-Z, a-z, 0-9, _, -) and no more than one consecutive space.

5.8.2 Enter or select the *Collection Date* in the corresponding field using YYYY/MM/DD format.

5.9 Click the **0 Replicates** button to open the *Replicate Setup* window.

5.9.1 For each replicate, add the *Unique Reads Files* generated by the corresponding LymphoTrack Assay Software.

5.9.2 Add files via *Drag and Drop* or by clicking the **Select Unique Reads Files** button and navigating to the correct file folder that contains the **.fastq_unique_reads.tsv* files.

- These files are generated using the LymphoTrack Software (for Ion S5/PGM REF 75000007 or MiSeq REF 75000009) and may have a TSV extension and will be located within an **._output run* folder created by the LymphoTrack Software after the initial analysis of the FASTQ data from either the MiSeq , Ion S5, or Ion PGM instruments.
- Note that the **.CDR3_unique_reads* file can be analyzed to track only the CDR3 sequence. If this file is chosen, ensure to use a CDR3 sequence on the corresponding *Sequence* tab in the main window.

IMPORTANT! The clonal sequence and **.unique_reads* file must both come from the same version of LymphoTrack software. Analyzing sequences or files from mismatched software versions can result in a false negative report. To check the version of the LymphoTrack software used, open the log file present in the **_output* run folder. The software version will appear immediately after the ****System info**** tag.

IMPORTANT! Each PCR replicate sequenced will have a **.unique_reads* file generated by the LymphoTrack software. When clicking *Select a Unique Reads File*, each file is associated with a different replicate number from the *Replicate* dropdown list.

5.10 Click on **Enter DNA Amount** to enter the *Amount of DNA (ng)* used in the corresponding *Replicate* row and click **Save**.

- This value can range from 50 ng to 7000 ng.
- Do not use decimal places.
- When analyzing the LymphoTrack Low Positive Control, the *Amount of DNA* is **200 ng.**
- If the box is checked next to *LymphoQuant* the user will not need to enter any indicated additional information.

5.11 The LymphoQuant Internal Control is selected by default. Unselect (✓) next to **LymphoQuant** box if the LymphoQuant Internal Control was not added to the sample, and click **Create Sample**.

6. Add Low Positive Control

6.1 To open the *Low Positive Control Setup* window, click the **Add Low Positive Control** button.

6.2 Enter a *Subject ID* and *Sample Unique Identifier* (*e.g.,* B-cell LPC) in the corresponding fields using only the characters (A-Z, a-z, 0-9, _, -) and no more than one consecutive space in the field labeled *Subject ID*.

IMPORTANT! The *Subject ID* must be unique for each run.

6.3 Select the *Gene Target* used from the drop-down menu and click the **0 Replicates** button to open the *Replicate Setup* window.

6.4 Add **.fastq_unique_reads* files for the Low Positive Control via *Drag and Drop* or by clicking the **Select Unique Reads Files** button and navigating to the correct file folder that contains corresponding files generated by the LymphoTrack Assay Software.

6.5 Enter **200** for the *Amount of DNA (ng)* tested in the corresponding Low Positive Control *Replicate* row and click **Save**.

7. Save and Load Projects

7.1 Subjects and samples can be saved as **.mrd* files to be loaded at a later time (*e.g.,* to add new time points to a Subject ID). To save a Project for future use, select **Projects**, then select **Save…** from the drop-down menu.

7.2 Browse to a folder where the **.mrd* files will be saved and click **Select Folder**. A **.mrd* file will be created for each *Subject* in the Project.

7.3 To load subjects into the current *Project*, select **Projects**, then select **Load…** from the drop-down menu.

7.4 Browse to the location of the save files then select the desired **.mrd* file(s) and click **Open**. All subjects will load into the current project as long as the *Subject IDs* are unique.

8. Run MRD Analysis

8.1 Verify all *Replicate* information is correct for each sample and subject.

- If any information is incorrect it can be edited by selecting the row and clicking the **Edit Replicates** button or by clicking directly on the information in the row to be changed. To remove a *Replicate*, select the corresponding row and click **Delete**.

8.2 Click the **Perform MRD Analysis** button to start the analysis.

- Error messages will need to be corrected in order to continue. For additional details regarding error messages, please refer to section 11: *Troubleshooting* and the Frequently Asked Questions (FAQ) which can be accessed at www.invivoscribe.com/mrd-software-help.

8.2.1 If Warning messages appear, either click **Cancel** to address the issue or click **OK** to continue.

- Once all Errors and Warnings have been addressed, a new window will open allowing an output location to be selected.

8.2.2 Select the desired folder location to save output files generated by the LymphoTrack MRD software.

- The analysis begins processing automatically.
- Once the analysis is complete, the output destination folder will open in a new window.

IMPORTANT! The run time of the analysis will depend on the size of the sample file being analyzed, as well as the computer processor speed. A typical sample containing 100,000 unique reads will generally take less than three (3) minutes to analyze. This run time may vary greatly depending on the complexity of the unique reads.

8.3 In the MRD software interface click **Finish** and **X** to close the software.

8.4 Review the *Raw Data*, *Sample*, and corresponding *Summary Report(s)* in the output folder.

9. Software Results

When the analysis completes the folder where the output files were saved will automatically open.

- The files created for each sequence are listed in Table 2, below.

Table 2. Output files

Output Files Created	Description of File
SampleUniqueID_SequenceName_ GeneTarget_output_table	A comma-separated-value (CSV) formatted file that contains intermediate data values used to generate the full output.
SampleUniqueID_SequenceName_ GeneTarget_one_mismatch_ sequences	A FASTA formatted file that contains all sequences exactly 1 mismatch from the target sequence.
SampleUniqueID_SequenceName_ GeneTarget_two_mismatch_ sequences	A FASTA formatted file that contains all sequences exactly 2 mismatches from the target sequence.
SampleUniqueID_Sample_Report	A PDF file that contains the project and sequence information as well as displays the results containing an average of the observed cumulative read frequencies for exact sequence matches and 1 or 2 nucleotide mismatch sequences for each run.
SubjectID_Summary_Report	A PDF file that contains an overview of all sequences and all time points tested for a particular Subject ID. It provides a table and a graph for each sequence analyzed.
SubjectID_Date.mrd	A MRD file format can only be opened by selecting **Load…** from the **Project** menu in the LymphoTrack MRD Software. If the Project was manually saved, this file contains all of the MRD Project information.

10. Data Analysis

Determine if the run was valid based on the established run criteria and the following information:

10.1 If Low Positive Control was run, open the *Low Positive Control Sample Report*.

- If a sufficient number of reads was detected for the Low Positive Control, the run status will be indicated as either ***Passed*** or ***Failed*** if an insufficient number of reads were detected. If Low Positive Control was not used, ***N/A*** will be indicated.
- If LymphoQuant Internal Control was spiked into the Low Positive Control the LymphoQuant Status will indicate ***DETECTED*** if sufficient reads were detected or ***NOT DETECTED*** if an insufficient number of reads were detected. If LymphoQuant Internal Control was not used, it will be listed as ***N/A***.

10.2 Open a Subject MRD Sample Report.

- The Low Positive Control Status will always indicate ***N/A*** for a Subject Sample Report because the Low Positive Control is not directly associated with a Subject ID. ***N/A*** is the expected result and does not indicate that the Low Positive Control failed.
- If LymphoQuant Internal Control was added to the PCR replicate the LymphoQuant Status will indicate ***DETECTED*** if sufficient reads were detected or ***NOT DETECTED*** if an insufficient number of reads were detected. If LymphoQuant Internal Control was not used, ***N/A*** will be indicated.

IMPORTANT! The B-cell Low Positive Control and B-cell LymphoQuant Internal Control cannot be used with *IGK*.

11. Troubleshooting

The following error messages will appear in the application of the input screen if any input parameters are either missing or incompatible.

Table 3. Input parameter error messages and corrective actions

Error Message	Cause	Resolution
Subject is incomplete	The selected *Subject ID* has no gene target assigned or no sequences assigned.	Verify the following information is entered: • a DNA sequence in the **Sequence** box, using only capitalized A, C, G, and T; • a sequence name in the **Sequence Name** box; and • the correct **Target** is selected from the dropdown.
Subject contains max number of samples allowed	The selected *Subject ID* has 11 or more samples assigned -- no more samples can be added.	Only 11 samples can be added to one *Subject ID*.
Exceeded max number of samples allowed. 50 samples can be analyzed at one time	More than 50 samples were created and are present in the **Sample Table.**	A maximum of 50 samples can be added to the **Sample Table**.
The Subject ID contains illegal characters	The *Subject ID* includes one of these illegal characters: ~@*+%?:{}<>/\"^	Use only letters, numbers, characters, and a single space as identified in the *Warnings and Precautions* section.
A subject with this name already exists	The **Add Subject** button was clicked, and a *Subject ID* with same name already exists.	A new *Subject ID* must be unique.
<SUBJECT> is missing a gene target	No gene **Target** was assigned for the *Subject ID*; analysis could not be performed.	Select the correct gene **Target** from the dropdown.
<SUBJECT> is missing sequences	No gene **Sequences** were assigned to the *Subject ID;* analysis could not be performed.	Add DNA sequence(s) to the **Sequence** box, using only capitalized A, C, G, and T.
Sequences must have unique names	Two (2) or more identical sequences are assigned to a *Subject ID,* attempt to save the *Subject ID* failed.	The user cannot enter different DNA amounts for samples that share a *Replicate Number*. *Replicates* that share *Replicate Numbers* are identified as *Resequences* and can be obtained from the initial PCR replicate library.
Sample is missing a name	The *Sample* was not assigned a unique identifier *(Sample ID);* analysis could not be performed.	A unique **Sample ID** must be assigned for each sample before performing the analysis.
<SAMPLE> is missing replicates	The *Sample ID* was not assigned any replicates; analysis could not be performed.	Ensure all data for a replicate has been entered and the replicate has been saved before leaving the **Edit Replicate** window.
Unique reads file malformed	The uploaded *Unique Reads* file is in an invalid format.	Ensure that the unique reads file was not manually edited.
Encountered missing read from unique reads file	The uploaded *Unique Reads* file is in an invalid format.	Ensure that the unique reads file was not manually edited.
Replicates are missing DNA amounts	The **DNA amount** was not assigned in the *Replicate Table;* attempt to save replicate failed.	Ensure all data for a replicate has been entered and the replicate has been saved before leaving the **Edit Replicate** window.
Re-sequenced replicates, indicated by matching replicate number, must have equal DNA amounts	The **DNA amount** assigned to re-sequenced replicates do not match.	Ensure all data for a replicate has been entered and the replicate has been saved before leaving the **Edit Replicate** window.

12. Technical and Customer Service

We appreciate your business. We are happy to assist you with use of this software, and will provide ongoing technical assistance Monday through Friday to keep the assays performing efficiently in your laboratory.

Contact Information

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13. Symbols

The following symbols are used in Invivoscribe NGS product labeling.

REF	Catalog Number		Manufacturer
LOT	Lot Number		Consult Instructions for Use
	Storage Conditions		Expiration Date

14. Legal Notice

This product is covered by one or more patents and patent applications owned by or exclusively licensed to Invivoscribe, Inc., including United States Patent Number 7785783, United States Patent Number 8859748, United States Patent Number 10280462, European Patent Number EP 1549764B1 (validated in 16 countries, and augmented by related European Patents Numbered EP2418287A3 and EP 2460889A3), Japanese Patent Number JP04708029B2, Japanese Patent Application Number 2006-529437, Brazil Patent Application Number PI0410283.5, Canadian Patent Number CA2525122, Indian Patent Number IN243620, Mexican Patent Number MX286493, Chinese Patent Number CN1806051, and Korean Patent Number 101215194.

Use of this product may require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). Any necessary license to practice amplification methods or to use reagents, amplification enzymes or equipment covered by third party patents is the responsibility of the user and no such license is granted by Invivoscribe, Inc., expressly or by implication.

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