



# User guide

Omixon HLA Twin CE  
Version 3.1.1

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## 2 Introduction

### 2.1 Company information

This product is manufactured by Omixon Biocomputing Ltd.

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### 2.2 General information

Omixon HLA Twin provides two independent algorithms for genotyping next generation sequencing data: Statistical Genotyping (SG) and Consensus Genotyping (CG). The algorithms were co-developed with the Omixon Holotype HLA sequencing assay. The two algorithms can be run simultaneously and results can be examined in a single table. Alongside this high-level overview table, detailed statistics and quality control measures are provided for each sample.

Omixon HLA Twin offers time based licensing, which allows unlimited genotyping in a given period of time. Please

contact [sales@omixon.com](mailto:sales@omixon.com)<sup>3</sup> for a quote. A license valid for 90 days is included in the evaluation version.

Each software version is fully supported for 13 months from its release. When a software version reaches its end of support, bug fixes will no longer be implemented for it and it will not be validated with new IMGT databases. It is strongly advised to upgrade to the newest software version before end of support.

### 2.3 Sequencing technologies

Omixon HLA Twin supports sequencing Illumina data.

### 2.4 Principle of method

#### 2.4.1 Consensus Genotyping (CG) algorithm

The consensus genotyping algorithm is a de novo assembly based method. The assembler method is receiving data prefiltered to genes based on the IMGT database. The output of the assembly is one or more contigs, each contig consists of one or more phased regions. The generated consensus sequences are compared to the allele sequences in the IMGT/HLA database and the allele pair or pairs with minimal key-exon, other exon and non-exon mismatch counts are reported. The consensus genotyping algorithm reports full (4-field) resolution genotyping results.

#### 2.4.2 Statistical Genotyping (SG) algorithm

The statistical genotyping algorithm is an alignment based method. Reads and read pairs are aligned to all exonic sequences defined in the IMGT database then assigned to alleles with the highest alignment score. Alleles are pre-filtered and paired. Allele pairs are compared and ordered based on the combined amount of supporting reads in the pair. All allele pairs that are considered equally good results based on the comparison results are reported. The statistical genotyping algorithm reports exon-based (3-field) resolution genotyping results.

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## 2.5 System requirements

The recommended minimum hardware requirements for the tool are the following:

	<b>Desktop</b>	<b>Server</b>	<b>Client</b>
CPU	64 bit multi-core	64 bit multi-core	64 bit multi-core
Memory (minimum/recommended)	12GB/16GB	12GB/16GB	4GB/8GB
Operating system	64 bit Windows/Linux or OS X	64 bit Windows or Linux (OS X not supported)	64 bit Windows/Linux or OS X

Storage space requirements depend on the size of the samples and need to be calculated with regards to legal requirements for storing the data, minimum level of backup and redundancy, as well as on the expected annual volume. Omixon can assist with calculating the storage space requirements, please contact [support@omixon.com](mailto:support@omixon.com)<sup>4</sup> if you need any help.

## 2.6 Intended use

Omixon HLA Twin is intended for the interpretation of Next Generation Sequencing (NGS) data generated on the Illumina sequencers by the Omixon Hologroup HLA sequencing assay. This results in highly accurate, single-pass allele level HLA typing with very low ambiguity rate at the 2 field level. The software provides human histocompatibility information of HLA Class I (HLA-A, B, and C) and Class II (HLA-DPA1, DPB1, DQA1, DQB1 and DRB1/3/4/5) genes by two independent algorithms: Statistical Genotyping (SG) and Consensus Genotyping (CG). The two algorithms can be run simultaneously and the concordance between the algorithms is always displayed next to the primary genotyping algorithm result when both methods have been running. Alongside this high-level overview table, detailed statistics and quality control measures are provided for each sample.

The Omixon HLA Twin software is intended for in vitro diagnostic use by professional health care personnel, such as laboratory technicians and physicians, who have been trained in HLA typing in diagnostic laboratories and work at either EFI or ASHI accredited labs (or labs that are able to work according to EFI or ASHI specifications). The results generated by the software must not be used as the sole basis for making clinical decisions.

## 2.7 Warning and precaution:

### 2.7.1 Product use limitations

The algorithms were co-developed and extensively validated together with the Omixon Hologroup HLA sequencing assay. For the best performance, please use the software together with Omixon Hologroup HLA assay for HLA typing by NGS on the Illumina MiSeq system. Use of any other HLA sequencing assays or NGS platforms other than those specified above must be extensively verified and validated by the user! For a list of known assay and algorithmic limitations, see the Known product limitations document!

## 2.8 Validation methods and performance characteristics

The performance statistics presented below were generated using Omixon HLA Twin version 3.1.1 and IMGT database version 3.31.0\_5. Performance measures were calculated using the method described by Ng et al. (1993)<sup>1</sup>. Genotyping results were compared to available reference genotyping information on a two-field level resolution.

### 2.8.1 Hologroup HLA v1

Altogether 416 samples (originating from 197 reference cell lines) were analyzed. Sequencing data was generated using Hologroup HLA version 1.

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<sup>4</sup> <mailto:support@omixon.com>



Measure	HLA-A	HLA-B	HLA-C	HLA-DPB1	HLA-DQA1	HLA-DQB1	HLA-DRB1	Total
Sensitivity	99.52%	98.56%	98.68%	99.04%	99.52%	95.55%	98.32%	98.45%
Specificity	99.98%	99.97%	99.95%	99.96%	99.95%	99.80%	99.96%	99.95%
Precision	99.52%	98.56%	98.68%	99.04%	99.52%	95.55%	98.32%	98.45%
Negative Predictive Value	99.98%	99.97%	99.95%	99.96%	99.95%	99.80%	99.96%	99.95%
Type Correctly Classified	99.97%	99.94%	99.91%	99.92%	99.91%	99.61%	99.92%	99.90%

## 2.8.2 Holotype HLA v2

Altogether 176 samples were analyzed. Sequencing data was generated using Holotype HLA version 2.

Measure	HLA-A	HLA-B	HLA-C	HLA-DPA1	HLA-DPB1	HLA-DQA1	HLA-DQB1	HLA-DRB1	HLA-DRB3	HLA-DRB4	HLA-DRB5	Total
Sensitivity	100.00%	98.58%	97.73%	99.71%	98.86%	95.71%	96.88%	99.43%	98.21%	89.71%	98.28%	97.58%
Specificity	100.00%	99.98%	99.93%	99.97%	99.96%	99.76%	99.84%	99.99%	99.64%	96.57%	99.43%	99.90%
Precision	100.00%	98.58%	97.73%	99.71%	98.86%	95.71%	96.88%	99.43%	98.21%	89.71%	98.28%	97.58%
Negative Predictive Value	100.00%	99.98%	99.93%	99.97%	99.96%	99.76%	99.84%	99.99%	99.64%	96.57%	99.43%	99.90%
Type Correctly Classified	100.00%	99.96%	99.87%	99.94%	99.92%	99.55%	99.69%	99.98%	99.40%	94.85%	99.14%	99.82%

## 2.9 Release notes

For the list of new features and bug fixes see the section *Release Notes* at <https://www.omixon.com/support-and-resources/hla-twin/>

## 2.10 References

<sup>1</sup>Ng J, Nurlay CK, Baxter-Lowe LA, Chepak M, Cappe PA, Hagland J, KaKuraya D, Manes D, Rosner G, Schmeckpaper B, Yang SY, Dupont B and Hartzman RJ (1993), Large-scale oligonucleotide typing for HLA-DRB1/3/4 and HLA-DQB1 is highly accurate, specific, and reliable. *Tissue Antigens*, 42: 473–479.





## 3 Quick start guide

### 3.1 Log in

After initializing the run of the HLA software, the user must log in to the application. Enter user name and password to the fields and click login. At first login, a superuser is created.

### 3.2 Genotyping dashboard

After logging in to the software, the Genotyping dashboard will be visible. This is the home dashboard of the software. All genotyping functions are available from here.

The dashboard consists of the following subscreens:

- Information Panel: this contains all the main functions and some high-level information about the current folder and selected files. It also shows information about the current user and active IMGT database and provides some navigation functions.
- File Browser: this part of the screen can be used for navigation between all accessible folders.

The Information Panel is located on the top of the screen. It has 3 different sections:

- The top part shows:
  - the ID of the current user,
  - the memory usage widget panel,
  - the status panel of the process manager,
  - the welcome tutorial button,
  - the logout button,
  - and the exit button.
- The middle part shows:
  - the navigation buttons: Back, Forward, Up and Home (Home takes you back to the Genotyping dashboard.)
  - the available information about the used IMGT database right from the navigation buttons
  - the bookmark and context specific help buttons on the right side of the screen
- The lower part presents a series of buttons that enables you to choose between the main functions of this dashboard:
  - the typing and data analysis options to submit genotyping
  - the typing and data analysis options to visualize results
  - the file browser functions to navigate between samples and folders
  - Application settings

The main section of the display is the File Browser where you can navigate by clicking on drives and folder names - just like in a regular file browser. Use the browser to navigate to the folder where your genotyping samples are stored. Each sample file is marked with a small DNA sign and has its own unique name identical to the name that was generated by the sample sheet. For paired reads, the software automatically pairs files based on the file names and only the "R1" FASTQ file is displayed in the file browser to decrease redundancy and help navigation. By hovering the mouse over the sample file, you can see the size of the individual FASTQ files.

After a sample is analysed, a separate file, the genotyping result file will be displayed in the file browser. The result file has .htr extension. By default, all result files are placed automatically into the same folder where the sample data is. Each analysis file is marked with a small table sign. The name of the analysis file consists of the sample name and a timestamp that belongs to the time when the analysis was submitted. By hovering the mouse over the analysis file, you can see all available information about the analysis. When looking at an .htr file, on the very right you can see the high level quality summary of the typing results. Results are annotated using a "Traffic light" system. Similarly to a traffic light, three different colors are used with different meanings. Unlike in a real traffic light, "mixed colors" are available.

The genotyping functions are disabled - buttons grayed out - as long as there are no samples displayed on the screen. As soon as you locate a FASTQ file (or any other supported format) - the genotyping buttons become active.

To submit an analysis, follow the Analysis section.

You can find detailed information about this screen and all available functions in this User Manual in the Omixon Handbook/Genotyping Dashboard section.

### 3.3 Analysis

When at least one sample is located, you have several options to start genotyping.

### 3.3.1 Simple Genotyping - recommended for Holotype samples

Genotyping with the default parameters can be initiated by a single click on the "Analyse" button displayed in each row for each sample. You can type multiple samples with the Holotype protocol by selecting them with mouse click and the Ctrl or the Shift button and pressing the Simple Genotyping button in the top menu. You can check that the typing is running in the Process manager displayed in the top right corner. You can find detailed information about this and other related functions, such as genotyping with custom parameters and re-analyzing samples in this User Manual in the User Guide/Genotyping Dashboard section.

### 3.3.2 Results

As the progress reaches 100% in the Process manager a new result file will appear in the file browser. In case multiple samples are typed at once a result for each will be presented as soon as it's ready - you can start viewing the first results while the rest is still in progress. The result files display a Traffic light result for quick overview and you can display further details by clicking the "View" button at the end of each row. To display multiple results use the Ctrl or Shift button to select them and then the "View results" button in the top menu. When working with analysis results in Omixon HLA software please be aware that primer sequences do not affect the results since they are trimmed before the analysis. You can find detailed information about interpreting the results in this User manual in the User guide/Genotyping Analysis result and User guide/Genotyping Sample result sections.

## 3.4 Genotyping Analysis result

As described in the Results section, you can visualize your results by highlighting one or multiple samples and clicking to the "View results" button. This will navigate you to the next section of HLA Twin: The Genotyping analysis results.

**Note**  
If more than 50 best matching results can be found on a locus, LD will not be calculated. If you press the Show LD details button, no information will be displayed.

In the Genotyping analysis results section you can observe that this is broken down into two bigger parts:

- Information Panel - note that the structure of this panel is largely identical to the Genotyping dashboard Information Panel (see above for details)
- Results Table, that has the overview table consists of the results of the previously highlighted samples.

The lower part of the Information Panel contains a series of buttons that enables you to choose between the main functions of this dashboard.

- Sample details and browse alignment
- Display setup buttons
- Assignment buttons
- Export table
- Further options to comment sample/approve sample/cancel the approval of a sample/ show Linkage Disequilibrium

In the Results Table of the Genotyping analysis results, you can see a high-level overview of your results for each locus of each sample. The table has the following columns:

- Approval
  - Indicates if a sample is approved, ready for approval or still being worked on
- Sample
  - Contains the name of the .htr file that is generated based on the name of the sample and the timestamp that refers to the time when the analysis was submitted.
  - If one sample has been analyzed multiple times, the displaying of the samples follows the order of the analysis. You can use the timestamp to track different analysis times.
- Allele
  - Shows the two alleles Allele 1 and Allele 2 in different rows
- Separate columns for the analyzed loci








- On the very left of the displayed result you can see a small "tick" sign that is indicating if you see the "Best matching allele". You can assign this result by simply clicking to the tick mark - the sign will become green, indicating that the result is assigned.

Next to the tick, you can see the traffic lights.

- Concordance traffic light:  
The concordance traffic light system has the following outputs:

- (green): results of SG and CG are fully concordant (at the 3rd field),
- (yellow): results of SG and CG are concordant up to 4-digits (2nd field),
- (red/yellow): results of SG and CG are concordant up to 2-digits (1st field),
- (red): results of SG and CG are discordant.

Note, that concordance traffic lights are only presented for best matching alleles.  
The second traffic light is based on locus level quality control (QC) measures.

- Quality control traffic light:  
These lights are based on the locus level quality control measures.
  - (green) - PASSED: the locus passed all QC tests,
  - (yellow/green) - INFO: one or more QC tests produced lower than average results,
  - (yellow) - INSPECT: one or more QC tests produced concerning results, manual inspection of the results needed,
  - (red/yellow) - INVESTIGATE: one or more QC tests showed low result quality, manual inspection and possibly reanalysis is needed,
  - (red) - FAILED: one or more QC tests showed very low result quality, manual inspection is needed to determine the cause and the locus or sample likely needs re-sequencing or re-typed by alternative methods.
- Some other markups can also be presented for alleles:
  - Alleles displayed with the blue font are homozygous.
  - Rare alleles are marked with an exclamation point icon .
  - Novel alleles containing exonic (or exonic and intronic) novelties are marked with , while novel alleles containing only intronic novelties are marked with .
  - Imbalanced alleles are displayed with italic font.
  - Alleles with extended allele sequence are marked with a *plus sign* .
  - If a minor allele with well-known low amplification is present in the imbalanced minor allele list, the allele is marked with . In this case validation of the homozygous result using an alternate genotyping method (e.g. SSO) is strongly suggested.
  - Hemizygous alleles are marked with !. In case a locus is hemizygous only one allele is displayed and the other cell is left empty. In case the zygosity of a locus can't be determined based on the data available, the alleles are marked with .

By hovering the mouse over the different sections of the table a pop-up window will be displayed containing further available information on the sections.

From the Genotyping Analysis result dashboard, you can enter the Genotyping Sample Result or the Genome Browser directly.

In the Genotyping Sample Results you can observe the detailed quality metrics of the analysed sample. To enter here, highlight the sample you wish to see and click on the "Sample Details" button on the bottom row of the Top section.

In the Genome Browser you are able to visualize the detailed results for each of the typed loci. To enter here, highlight the sample you wish to see and click on the "Browse Alignment" button on the bottom row of the Top section.

For further information, see the Genotyping Analysis result section of the Omixon Handbook.

### 3.4.1 Troubleshooting missing results

When no alleles could be reported for a targeted gene, a markup describing the possible reason for the missing allele call is shown. For additional information, hover over the info icon next to the markup and read the tooltip. The following cases can be reported:

For non DRB3/4/5 loci:



- *No data present* - No data present means that either the locus was not targeted in the sample, or it has dropped out during sequencing and should be re-sequenced.
- *Insufficient or low quality data* - There is insufficient data or the data is of low quality in the sample. Quality control results should be checked for more detail.

For DRB3/4/5:

- *Allele not expected* - There is no allele expected at this locus based on known linkage disequilibrium with HLA-DRB1 and HLA-DQB1.
- *Expected allele not found* - This markup means that based on known linkage disequilibrium information, data was expected for the locus/allele but was not found.
- *Unexpected allele found* - Data was found for a locus/allele, which was not expected based on known linkage disequilibrium information.
- *Insufficient or low quality data* - There is insufficient data or the data is of low quality in the sample. Quality control results should be checked for more detail.

When no alleles are reported for a targeted gene it is suggested to rerun the sample in question using a higher number of reads. (The number of processed reads can be set in the *Advanced Genotyping wizard*.) The reasons behind the missing allele level results can be that the coverage does not reach the minimum threshold on the allele or on the exons, or the coverage depth is too small. Processing more reads can help making the signals that support the correct alleles stronger.

## 3.5 Genotyping Sample result

In the Genotyping Sample result section, you can inspect the details of the genotyping results, the quality metrics and the data statistics for each locus of a selected sample.

The Genotyping Sample results section can be broken down into two bigger parts:

- Information Panel - note that the structure of this panel is largely identical to the Genotyping dashboard Information Panel (see above for details)
- Details Panel, that can show the genotyping result, quality metrics and data statistics

The lower part of the Information Panel displays a series of buttons that enables you to choose between the main functions of this dashboard.

- Opening the browser
- Detailed genotyping information
- Customizing displayed results
- Assigning alleles
- Commenting

In the Details Panel you can choose from 3 different tabs to decide what would you like to display:

- Genotype
  - In the Genotype section, you can observe the genotype that is selected by the software.
  - You can add/remove alleles manually
- Quality control
  - Several quality control measures are calculated for every locus. Each measure for each locus is marked with a traffic light system.
  - The Quality control table has one column for all of the metrics and separate columns for each of the displayed loci.
  - The "Overall" row indicates the overall result for each of the individual loci based on the traffic light system.
  - Each metric has its own row in the table. Right next to the name of the metric, a small "i" mark is displayed. By hovering the mouse over the "i" mark, a pop-up window will be displayed, containing more detailed description of the selected metric.
  - For each of the metrics, you can see the traffic light, the value of the metric and a small "i" mark with the information about the specific thresholds of the metric. By hovering the mouse over the "i" mark, a pop-up window will be displayed, containing more detailed information about the thresholds of the selected metric.
- Data Statistics
  - Overview section - Read counts and proportions are available for several different steps of the analysis.
  - Allele imbalance section - This figure shows the per-region allelic imbalance for all the genes.
  - Fragment size section - This histogram shows the fragment size distribution of paired reads.
  - Read quality section - On this graph, the base quality per 5 bases is shown for the processed reads. Read positions are on the x-axis while on the y-axis quality values are shown.

Different loci can be selected on the left side of the bottom section.

For the selected loci, you can enter to the genome browser by clicking to the "Browse Alignment", "Browse Allele 1", "Browse Allele 2" buttons.

For further information, please see the Genotyping Sample result section of the Omixon Handbook.

## 3.6 Gene Browser

**The Gene Browser allows visual inspection of genomics data. Multiple allele candidates can be browsed together.**

With default settings, the following tracks are available in the browser:

- *Position track* - Shows the coordinates for all visible tracks. Numbering starts from one.
- *Phasing track group*:

*Phasing track* - This track contains annotations for continuously phased regions (aka Phasing regions).

*Variants track* - Shows the number of overlapping read pairs between two consecutive heterozygous positions (i.e. two position where the two consensus sequences differ from each other). The 'Straight' label shows the number of reads for each consensus that supports the phasing shown in the browser, while the 'Cross' label shows the number of supporting reads for the other possible phasing of the two positions.

- *Consensus sequence 1* - The generated consensus sequence for one of the chromosomes.
- *Coverage depth for consensus 1* - Shows the depth of coverage for every position of the consensus sequence 1 *assembly*.
- *Consensus sequence 2* - The generated consensus sequence for the other chromosome.
- *Coverage depth for consensus 2* - Shows the depth of coverage for every position of the consensus sequence 2 *assembly*.
- *Allele 1 sequence* - Nucleotide sequence of the allele that matches the first consensus the best.
- *Region annotation for allele 1* - Annotations for exons, introns and UTRs are shown for allele 1.
- *Coverage depth track for allele 1* - Shows the depth of coverage for every position of the allele 1 *alignment*.
- *Allele 2 sequence* - Nucleotide sequence of the allele that matches the second consensus the best.
- *Region annotation for allele 2* - Annotations for exons, introns and UTRs are shown for allele 2.
- *Coverage depth track for allele 2* - Shows the depth of coverage for every position of the allele 2 *alignment*.

For novel alleles, two reference tracks are shown: the reference sequence of the novel allele (*Novel ref*) and the reference sequence of the closely related allele (*Rel ref*) from which the novel allele was derived.

Note that consensus sequences and the corresponding short reads can be viewed in the browser, even when no allele match pairs are found.

### **Additional tracks:**

- *Noise track* - Shows systematic noise filtered out during consensus assembly. The noise consensus contains the major nucleotide for every position.
- *Amino acid track* - Shows the amino acid sequence for all allele and consensus sequences, including novel alleles, colored based on amino acid hydrophobicity.

By default, detailed coverage tracks are displayed for the allele alignments, alongside region annotations. The coverage track has a built-in base statistics visualization support: for bases in reads different from the actual consensus/reference base the corresponding coverage depth is shown with the associated nucleotide base color proportionally.

### **Additional modes for short read tracks**

Other than the default *coverage depth mode*, the following alternative short read visualization modes are available for the short read track:

- *Short read mode* - Shows short reads displayed in a stranded fashion, so that forward strand reads (pink) and reverse strand reads (yellow) can be easily distinguished within the display.
- *Fragment mode* - Paired visualization mode that shows the corresponding forward and reverse reads in pairs in the same line. Overlapping sections between read pairs are marked with blue, while non-overlapping reads are connected with a thin line.

In both of the above modes, the short reads track can be *collapsed* which gives a summary view of the short reads (and does not allow each read to be inspected in detail).

## 3.7 Settings dashboard

Reachable from the *Genotyping dashboard* using the *Application settings* button, the *Settings dashboard* displays an overview of the settings in the tool, allows access to administration features and display configurations. Some general information about the current version of the software and the current user is also available on this dashboard.

## 3.8 General information

There are three blocks of information on the Settings Dashboard:

- **Omixon HLA edition**: this part contains the name and version of the software, the build identifier with a dedicated copy to clipboard button and some contact and copyright information.



- Omixon HLA edition: this part contains the name, version and reference number of the software, the build identifier with a dedicated copy to clipboard button and some contact and copyright information.
- User info: this part contains the login name, first and last name of the current user.
- License info: this part shows the number of available credits and the expiration date of the license.

## 3.9 Sidebar

The left sidebar contains the following function sets:

### 3.9.1 General

In this function group you can set where analysis data and result files are stored, create and manage protocols, set targeted genes for analysis and select the assay version to be used for analysis. For details about protocols see the *Analysis Protocols* help page.

### 3.9.2 Database

With the *Install New Database* function, you can set up one or multiple versions of the IMGT database used for genotyping. With the *Select Active Database* function, you can specify the active version of the database. Genotyping will always be initiated using the active version. In case you want to remove an installed database you can utilize the *Remove Database* function which allows you to delete any installed database that is not currently selected as active. You can set whether or not to use database extensions in the *Configure Database Extensions* menu.

### 3.9.3 Administration

With the *User management* option, you can create, edit and remove users. With the *Display Hardware Key* option, you can display an alphanumeric identifier for your computer which can be used for generating a license for that specific machine. The *Upload Licence* option can be used for manually importing a license file into the software.

### 3.9.4 Automation

This function group allows you to configure automatic analysis on server-client configurations.

### 3.9.5 Export Settings

Here you can configure LIMS export.

### 3.9.6 Screen Settings

In this function group you can change the display configurations for the Gene Browser and result screens. Note, that these changes will modify the default behavior and appearance of the software. If you only want to temporarily change browser settings the *Display settings* option on the browser screen should be used. You can also modify the default filters of the Genotyping result screens. Be aware, that if you unselect a locus in this wizard, results for that locus won't be shown, regardless of the typing results. For both configuration sets, you can set all the parameters back to the default values using the *Restore defaults* function. For details about these settings, please see the following help pages: *Analysis Result Screens* and *Gene Browser*.



## 4 Installation guide

Our software can be installed on the following operation systems

- Windows
- Linux
- Mac OS X

### 4.1 Windows

We provide an installer package for Windows operating systems bundled with a Java Runtime Environment (JRE). We have tested Omixon HLA software with 64-bit Windows 7, Windows 8/8.1 and Windows 10.

#### 4.1.1 Installation steps

- Launch the executable - Welcome the setup wizard appears
  - If you have previously installed HLA Twin, choose "Yes, update the existing installation" in the installation wizard
  - If you wish to perform a clean installation, choose "No, install to a different directory" in the setup wizard
  - After choosing according to your wish, click next
- Accept the software license agreement to proceed with the installation, click next
- Select the path where you would like the application to be installed, click next
- Select data directory, the permanent data files will be stored here.
  - If you have previously installed HLA Twin, please do not change the proposed path - it has to point to the previously used database folder.
  - After choosing the directory, click next
- Select temporary files directory, click next
- Set maximum amount of memory to be used by the application
- Omixon HLA Typer Server installation only: Set IP address and port of the *Omixon HLA Server*
- Select Start menu folder
  - If you want to create Start menu folder, use "Create Start Menu folder" checkbox and name our folder
  - If you want to make the folder visible for all users, click "Create shortcuts for all users" checkbox
  - Click next, installation will start now
- Installation is finished, the Completing the Omixon HLA Setup dialog indicates the success

The application can be started by clicking on this startup icon or running the executable from application directory. For further information and detailed steps of uninstallation, see the Extended Installation Guide in the Omixon Handbook.

### 4.2 Linux

We provide an installer package for Linux operating systems bundled with a Java Runtime Environment (JRE):

The install packages are single file shell scripts, suitable to install on various Linux distributions.

The downloaded installer does not have the permissions to run directly. Open a terminal window to make it executable with the following command:

```
chmod +x installer_name
```

After that, it can be started with the following command:

```
./installer_name
```

#### 4.2.1 Installation steps

- Launch the installer shell script - Welcome the setup wizard appears
  - If you have previously installed HLA Twin, select "Yes, update the existing installation" in the installation wizard
  - If you wish to perform a clean installation, select "No, install to a different directory" in the setup wizard
  - After choosing according to your wish, click next
- Accept the software license agreement to proceed with the installation, click next



- Select the path where you would like the application to be installed, click next
- Select data directory, the permanent data files will be stored here.
  - If you have previously installed HLA Twin, please do not change the proposed path - it has to point to the previously used database folder.
  - After choosing the directory, click next
- Select temporary files directory, click next
- Set maximum amount of memory to be used by the application
- Omixon HLA Typewriter Server installation only: Set IP address and port of the *Omixon HLA Server*
- Select Directory for Symlinks, click next - Installation will start now
- Installation is finished, the Completing the Omixon HLA Setup dialog indicates the success

An application icon is placed into the System menu. By clicking on this icon, the application starts and is ready for use. For further information and detailed steps of uninstallation, see the Extended Installation Guide in the Omixon Handbook.

## 4.3 Mac OS X

As Mac OS X is an exclusively 64-bit operating system and the Java Runtime Environment is updated regularly the installer does not contain a Java Runtime Environment (JRE).

This avoids any conflicts among the different JREs but may cause incompatibility problems. The required JRE version is: 1.8  
If you don't have Java installed on your Mac, it will be automatically downloaded during installation.

### 4.3.1 Installation steps

- Open the installer which is packed within a DMG archive. Click on the *Omixon HLA Installer.app* icon
  - If you have previously installed HLA Twin, choose "Yes, update the existing installation" in the installation wizard
  - If you wish to perform a clean installation, choose "No, install to a different directory" in the setup wizard
  - After choosing according to your wish, click next
- Accept the software license agreement to proceed with the installation, click next
- Use the provided default path to install the application, click next
- Use the default location for the data directory where the permanent data - eg. results - will be stored
- Select temporary files directory, click next - Installation will start now
- Set maximum amount of memory to be used by the application
- Installation is finished, the Completing the Omixon HLA Setup dialog indicates the success

In Application list, *Omixon HLA* is available now. By clicking the *Omixon-HLA* icon, application starts and is ready for use. For further information and detailed steps of uninstallation, see the Extended Installation Guide in the Omixon Handbook.





## 5 Omixon HLA Server

Omixon HLA Server accepts multiple client connections simultaneously. It allows collaborative work and sharing of analysis results between users.

The client-server version also features an automation service which allows the automated typing of samples as they are produced. Protocol details, processing frequency, sample identification and various other parameters need to be defined before the service can start working - please contact [support@omixon.com](mailto:support@omixon.com)<sup>5</sup> for assistance.

### 5.1 Installation

Omixon provides three separate installers for the server version:

- Server installer - containing the *Omixon HLA Server*, the *Omixon HLA Typer Server* and a single client
- Client installer - containing only the client
- Typer server installer - containing only the *Omixon HLA Typer Server*

The server is bundled together with a client, so there is no need to install a client separately on the server computer.

To run the server installers, system administrator rights are required. The server installers can also be run from the command line, if no GUI is available (please contact [support@omixon.com](mailto:support@omixon.com)<sup>6</sup> for details). The installation of the client doesn't need system administrator rights. For the steps of the Setup Wizard please check the Installation Guides of your operating system found in the preceding sections of this manual.

Please stop any other Omixon software before starting the installation of the server, and make sure you select the appropriate Installation directory either for a clean installation or for an upgrade.

You can copy and use the client installer on multiple PCs. If you already have a desktop version installed please make sure you select a different 'installation location' to make sure that your existing installation is not overwritten.

### 5.2 Launching The Servers

#### 5.2.1 Important Note

*The server automatically starts up after installation. In case it has been installed previously the server usually starts up when the computer is switched on.*

*The server needs to be configured before the first client can connect to it. The new settings are applied when the server is started/restarted after the configuration file is saved.*

#### 5.2.2 Linux

Enter the installation directory and start the server daemon by running the server executable from the command line with one of the following input arguments. The available arguments get listed when the server is launched without any.

*Usage*

*omixon hla server executable {start|stop|status|restart|force-reload}*

<b>start</b>	<b>stop</b>	<b>status</b>	<b>restart or force-reload</b>
Starts server if status is stopped.	Shuts server down if status is running.	Returns running status.	Restarts server or starts if isn't running.

Automatic startup is not set by default. To set up automatic startup and specify in which runlevel to start the service, use a service configuration utility like *chkconfig* or *update-rc.d*.

---

<sup>5</sup> <mailto:support@omixon.com>

<sup>6</sup> <mailto:support@omixon.com>



## 5.2.3 Windows

To start, stop and set startup type for the *Omixon HLA Server* and the *Omixon HLA Typer Server*, launch 'Control panel → Administrative tools → Services' and modify the 'Omixon HLA Server' or the 'Omixon HLA Typer Server' service's properties. By default, startup type is set to automatic and the service is run under the System user account.

## 5.3 Configuring The Server Environment

### 5.3.1 Client side settings

In order to get started with default settings and connect to the server with a client the only necessary configuration is to provide the *Omixon HLA Server* host's IP address or host name and access port for the client.

This can be done by starting up the client and clicking the "Switch server" button at the bottom of the popup window. Proceed to "Add new server" and provide the IP address in the "Server host" field. Click "Add" to finish editing the connection and click "Connect" on the new server card in order to connect with the client.

#### *IMPORTANT*

*Only the same edition (CE or RUO) and version (2.5.1, 2.6.0, etc.) of server and client can be connected. If these details are different - for example because the server has been upgraded and the client hasn't - an error message will be displayed when a connection is attempted.*

### 5.3.2 Server side configuration

The following configuration files are the placeholders of the servers

- *omixon-hla-[twin | explore]-server.vmoptions* for the *Omixon HLA Server*
- *omixon-hla-[twin | explore]-typer-server.vmoptions* for the *Omixon HLA Typer Server*.

### Modifying the configuration

If manual modification of the configuration is necessary it can be done by editing the server configuration file after the installation has finished. The configuration files are placed in the installation directory and have a "vmoptions" extension. Since both servers and also the bundled client have configuration files with the same extension it must be ensured that the configuration file belonging to the right server is customized.

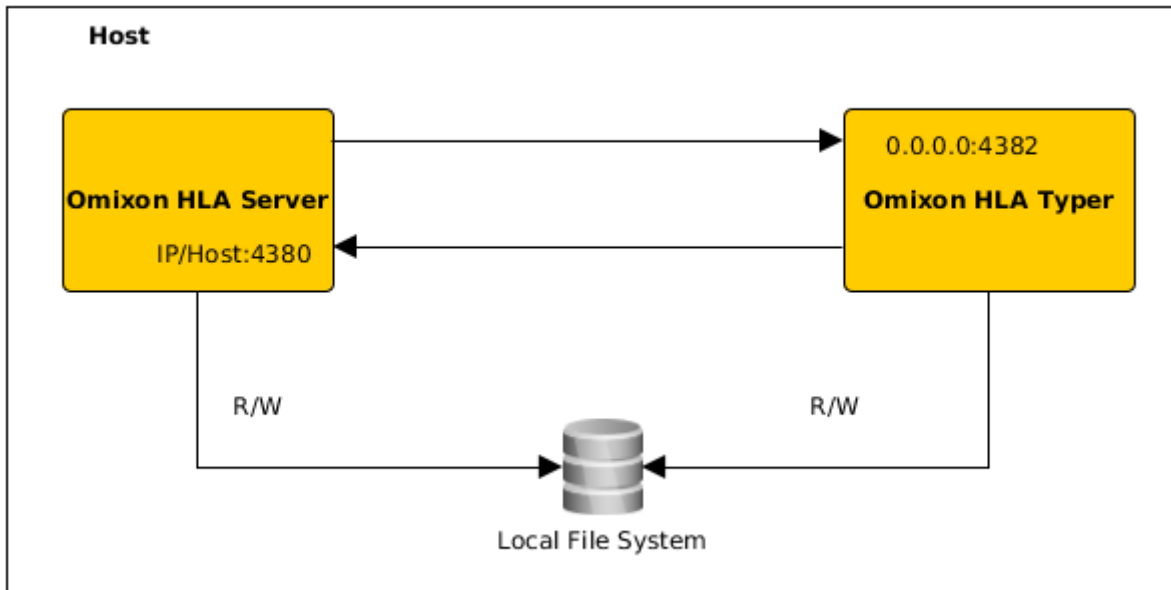
Modifications take effect only after restarting the server.

#### *IMPORTANT*

*The last line in the vmoptions file must be followed by a line feed.*

### Installation types

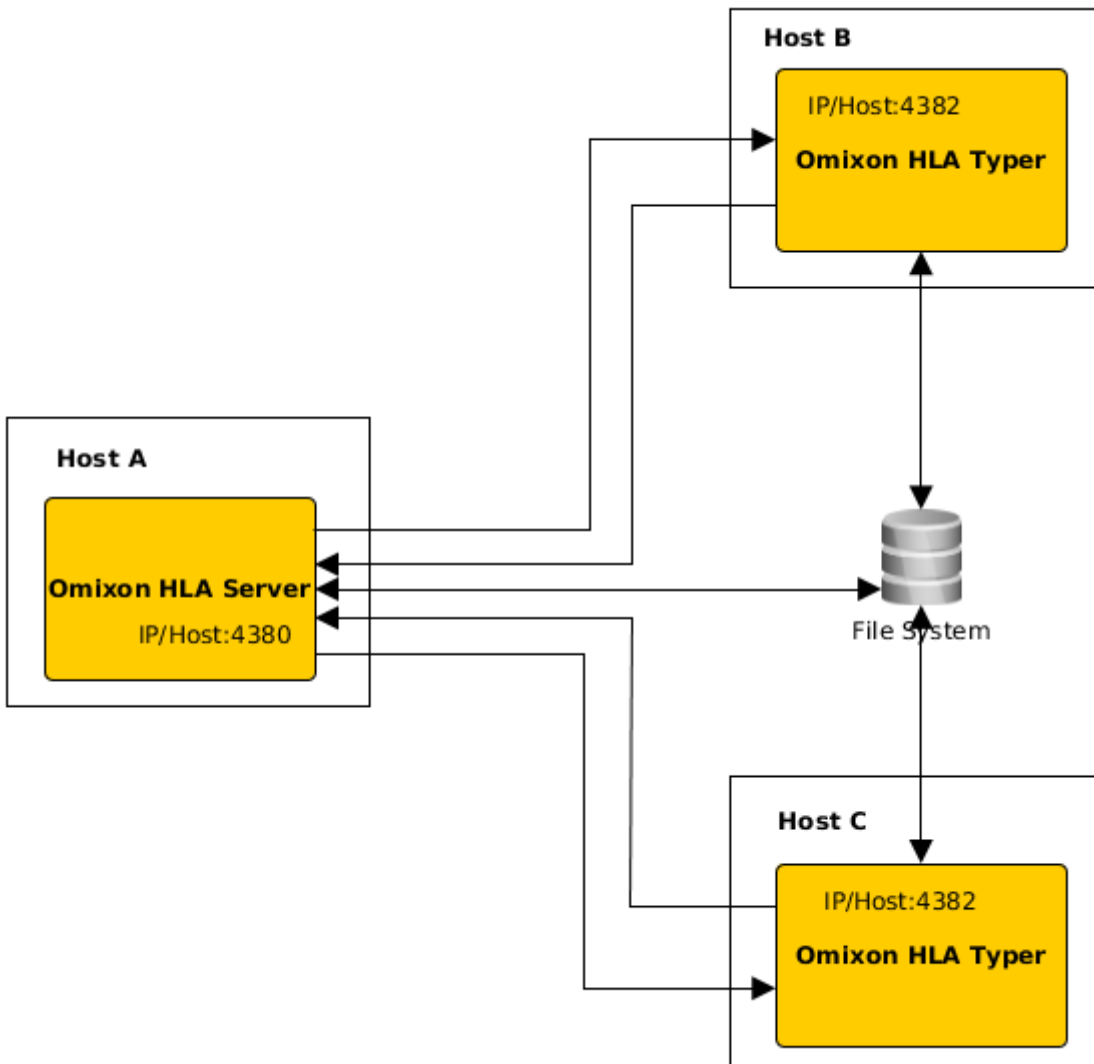
**Default installation:** *Omixon HLA Server* and *Omixon HLA Typer Server* are installed on the same host. This can be achieved by simply running the server installer since it includes both servers.



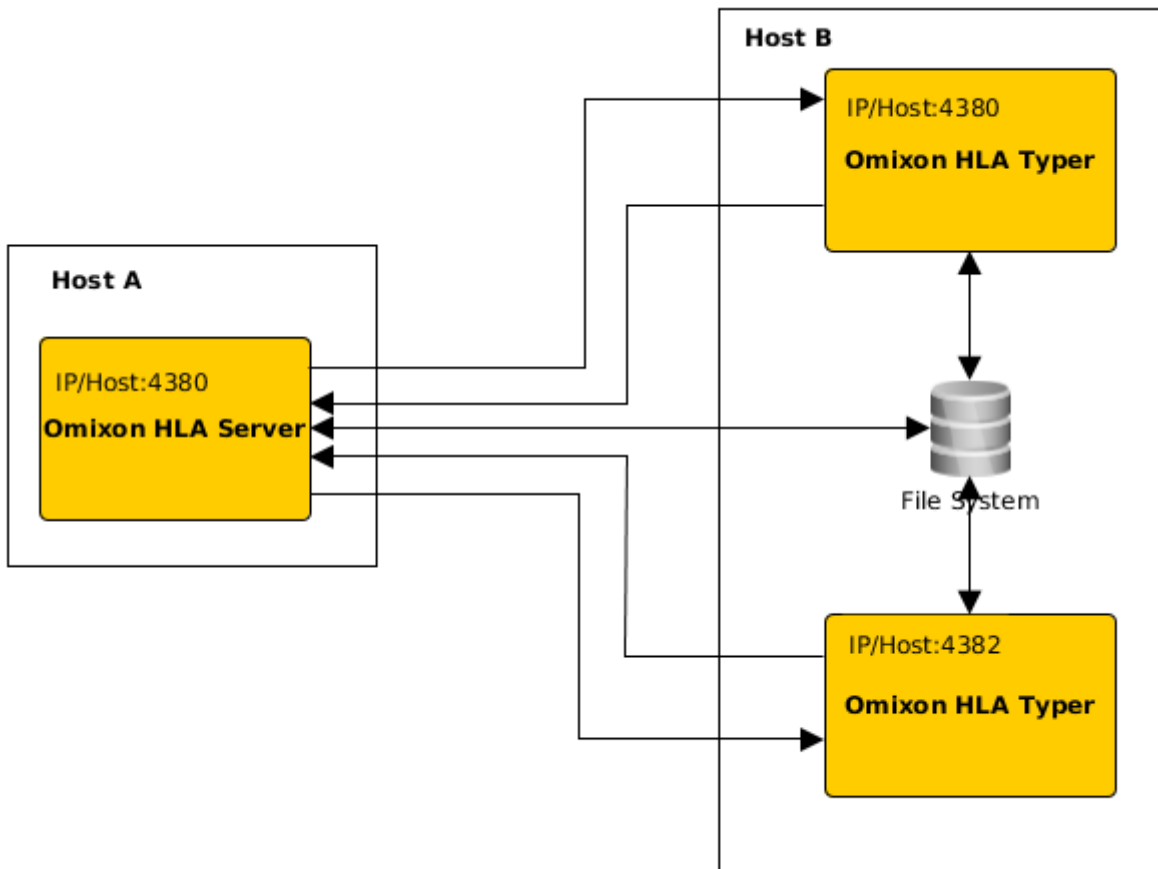
*Note*

*Omixon Typer Server cannot be accessed directly by the clients only through the Omixon HLA Server.*

**Separate typer server(s):** Because the *Omixon HLA Typer Server* needs more hardware resources (CPU, RAM) it can be installed on separate hosts for better performance. To run the *Omixon HLA Typer Server* on a separate host the *Omixon HLA Server* must be installed on one host using the default installation - see above - and the *Omixon HLA Typer Server* on a different one with the separate typer server installer. In case of batch genotyping task the analysis of the samples can be distributed between the configured typer sever instances. They can be deployed on different hosts - see below



or - if more hardware resources are available on the host - the same machine:



In this deployment type the *omixon-hla-[twin | explore]-typer-server.vmoptions* files must contain the following entries

- Domixon.server.host=*Omixon HLA Server ip/hostname*
- Domixon.server.port=*Omixon HLA Server port (default 4380)*
- Dtyper.server.host=*Omixon HLA Typer Server ip/host*
- Dtyper.server.port=*Omixon HLA Typer Server port*

**IMPORTANT**

Currently all servers must be installed on hosts with the same operation system (Windows or Linux), heterogeneous systems are not supported.

## File system preparation

If *Omixon HLA Server* and *Omixon HLA Typer Server* run on the same host (default installation) no additional file system settings are required. If this is not the case, the following preparations must be made:

*Omixon HLA Server* and *Omixon HLA Typer Server* must have read access to the folder your sample files are stored in.

Additionally the sample storage must be mounted to the same path on both hosts in order for the sample files to be accessible for both servers.

**IMPORTANT**

If you would like to change the sample files (for example copy, move or delete them) then *Omixon HLA Server* must have write access too to the given storage.

Moreover both the *Omixon HLA Server* and *Omixon HLA Typer Server* use a dedicated working folder for file exchange - for example where the reference database, genotyping results, etc. are being extracted or written out. This folder must be accessible for both nodes with read and write permission.



You must set the path to this working folder in the *omixon-hla-[edition]-server.vmoptions* and *omixon-hla-[edition]-typer-server.vmoptions* files in the variables *-Dogve.temp.dir* and *-Djava.io<sup>7</sup>.tmpdir*.

## Omixon HLA Server settings

The *Omixon HLA Server* can be configured by modifying the *omixon-hla-[edition]-server.vmoptions* file which can be found in the installation directory of the software.

### Set communication host and port

It might be necessary to set host and port parameters. Modify the following default parameters to do so:

*-Domixon.server.host=0.0.0.0*

*-Domixon.server.port=4380*

#### Host

The value of the parameter *-Domixon.server.host* can be the hostname (e.g.: *omixon-server*), the full domain name (e.g.: *omixon-server.mycompany.com<sup>8</sup>*) or the IP address of the machine running the *Omixon HLA Server*.

#### IMPORTANT

*In the client's connection configuration, 'Server host' must be set the exact same value as '-Domixon.server.host' in the Omixon HLA Server configuration.*

*Make sure that the server host name resolves to the same IP on the server and client machines otherwise the clients will get a connection refused error. It is a common configuration problem that the host name resolves to a different IP on the server (e.g. to 127.0.0.1 via an internal network interface) which causes connection deny.*

*It is always safe to specify an exact IP address both for the server and clients which is reachable from all the related machines across the network.*

#### Port

Leaving the default port setting for *-Domixon.server.port* is recommended but in case it needs modification - see the above indications for this - it can be changed to any port. Make sure that both the configured port and the next one are free and accessible since they will be used for communication between the server and the client(s).

#### IMPORTANT

*Verify that the set port and the next one are both free for use on the server and client machines.*

## Omixon HLA Typer Server settings

The *Omixon HLA Typer Server* can be configured by modifying the *omixon-hla-[edition]-typer-server.vmoptions* file which can be found in the installation directory of the software.

In case *Omixon HLA Server* and *Omixon HLA Typer Server* are installed on the same host (default server installation), the default setting is sufficient for the HTTP communication between the two servers.

In case the *Omixon HLA Typer Server* is installed on a different host, you must modify the configuration values accordingly. Additionally the typer server ip address/hostname must be changed too:

*-Domixon.server.host=Omixon HLA Server ip/hostname*

*-Domixon.server.port=Omixon HLA Server port (default 4380)*

*-Dtyper.server.host=Omixon HLA Typer Server ip/host*

*-Dtyper.server.port=Omixon HLA Typer Server port*

## Examples for server setup

### **Omixon HLA Server and first Omixon HLA Typer Server on one host, second Omixon HLA Typer Server on a different host**

- Install the *Omixon HLA Server* on a host with the server installer and the *Omixon HLA Typer Server* on a different one with the typer server installer

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<sup>7</sup> <http://Djava.io>

<sup>8</sup> <http://twin-server.mycompany.com>



- *omixon-hla-[edition]-server.vmoptions* and *omixon-hla-[edition]-typer-server.vmoptions* on the *Omixon HLA Server* host must contain:

- Domixon.server.host*=<IP or host name of Omixon Server host>
  - Domixon.server.port*=4380

- *omixon-hla-[edition]-typer-server.vmoptions* on the *Omixon HLA Typer Server* host must contain:

- Domixon.server.host*=<IP or host name of Omixon Server host>
  - Domixon.server.port*=4380
  - Dtyper.server.host*=<IP or host name of Typer Server host>
  - Dtyper.server.port*=4382

- Make sure that both chosen ports and *omixon.server.port* +1 are free and accessible (think of other processes, firewall settings etc.)
- Prepare storage and working folder. The following criteria must be met:

- The user account(s) running the two servers must have read and write access to the working folder and at least read access to the storage folder
- The access path of the storage folder needs to be exactly the same on both hosts
- The path to the working folder must be set in *omixon-hla-[edition]-server.vmoptions* as well as in *omixon-hla-[edition]-typer-server.vmoptions* as follows:

- Dogve.temp.dir*=<path to working folder>
  - Djava.io.tmpdir*=<path to working folder>

- Restart both servers after completing *vmoptions* settings
- Configure server access on the client side by adding the exact same IP/host name and port as defined in *-Domixon.server.host* and *-Domixon.server.port* on the servers
- Connect to the *Omixon HLA Server* with a client

#### **Omixon HLA Server and Omixon HLA Typing Server on same host (default server installation)**

- Install *Omixon HLA Server* and *Omixon HLA Typer Server* with the server installer on a host
- *omixon-hla-[twin|explore]-server.vmoptions* and *omixon-hla-[twin|explore]-typer-server.vmoptions* in the *Omixon Server* installation directory must contain:

- Domixon.server.host*=<IP or host name of Omixon Server host>
  - Domixon.server.port*=4380

- Make sure that *omixon.server.port* and *omixon.server.port* + 1 are free and accessible (think of other processes, firewall settings etc.)
- Restart both servers after completing *vmoptions* settings
- Configure server access on the client side by adding the exact same IP/host name and port as defined in *-Domixon.server.host* and *-Domixon.server.port* in *omixon-hla-[edition]-server.vmoptions*
- Connect to the *Omixon HLA Server* with a client

## 5.4 Logging Configuration

### 5.4.1 Overview

The *Omixon HLA Server* and the *Omixon HLA Typer Server* run in different JVMs and both of them have their own logging configuration. The installed application contains default logging settings, this section is about the custom logging configuration.

### 5.4.2 Default Settings

The application logging is based on the Simple Logging Facade for Java (SLF4J) framework, the configurations of the servers can be found under



- `${install_dir}/conf/omixon/logback.xml` for *Omixon HLA Server*
- `${install_dir}/conf/typer/logback.xml` for *Omixon HLA Typer Server*

The configurations have the following format and content:





```
<?xml version="1.0" encoding="UTF-8" ?>
<!-- ===== -->
<!-- Logback configuration file for Omixon Server (PRODUCTION) -->
<!-- ===== -->

<configuration scan="true" debug="false">
  <contextName>server</contextName>

  <appender name="OMIXON-SERVER"
class="ch.qos.logback.core.rolling.RollingFileAppender">
    <file>logs/server.log</file>
    <rollingPolicy class="ch.qos.logback.core.rolling.TimeBasedRollingPolicy">
      <fileNamePattern>logs/server.%d{yyyy-MM-dd}.log</fileNamePattern>
      <maxHistory>30</maxHistory>
    </rollingPolicy>
    <encoder>
      <pattern>[%d{yyyy-MM-dd - HH:mm:ss.SSS}] [%contextName] [%thread]
[%c] %5p - %m %n</pattern>
    </encoder>
  </appender>

  <appender name="AUTOMATION" class="ch.qos.logback.core.FileAppender">
    <file>logs/automation.log</file>
    <rollingPolicy class="ch.qos.logback.core.rolling.TimeBasedRollingPolicy">
      <fileNamePattern>logs/automation.%d{yyyy-MM-dd}.log</fileNamePattern>
      <maxHistory>30</maxHistory>
    </rollingPolicy>
    <encoder>
      <pattern>[%d{yyyy-MM-dd - HH:mm:ss.SSS}] [%contextName] [%thread]
[%c] %5p - %m %n</pattern>
    </encoder>
  </appender>

  <logger name="org.springframework" level="WARN" /> <!-- explicitly removes these from
logs -->
  <logger name="ch.qos.logback" level="WARN" />

  <logger name="com.omixon" level="INFO" additivity="false">
    <appender-ref ref="OMIXON-SERVER" />
  </logger>
  <logger name="omixon.automation" level="INFO" additivity="false">
    <appender-ref ref="AUTOMATION" />
  </logger>

  <root level="INFO">
    <appender-ref ref="OMIXON-SERVER" />
  </root>
</configuration>
```

The default log folder is  $\$[install\_dir]/logs$ . Modify the `<file>` tag of the configuration to create the log files in a different directory. If you have more servers this gives you the possibility to configure them to create their log files in a common directory for easy access.

### 5.4.3 Garbage Collection Log

To diagnose any memory problems, the Java Garbage Collection (GC) log file is the best place to start. It provides several statistics about the Java Virtual Machine (JVM) while the application is running.

The *vmoptions* files of the *Omixon HLA Server* and *Omixon HLA Typer Server* contain the following settings:

```
-Xloggc:$omixon_install_dir/logs/app-gcmonitor.log
-XX:+PrintGCDetails
-XX:+PrintGCDateStamps
-XX:+PrintGCTimeStamps
-XX:+UseGCLogFileRotation
-XX:NumberOfGCLogFiles=5
-XX:GCLogFileSize=20M
```

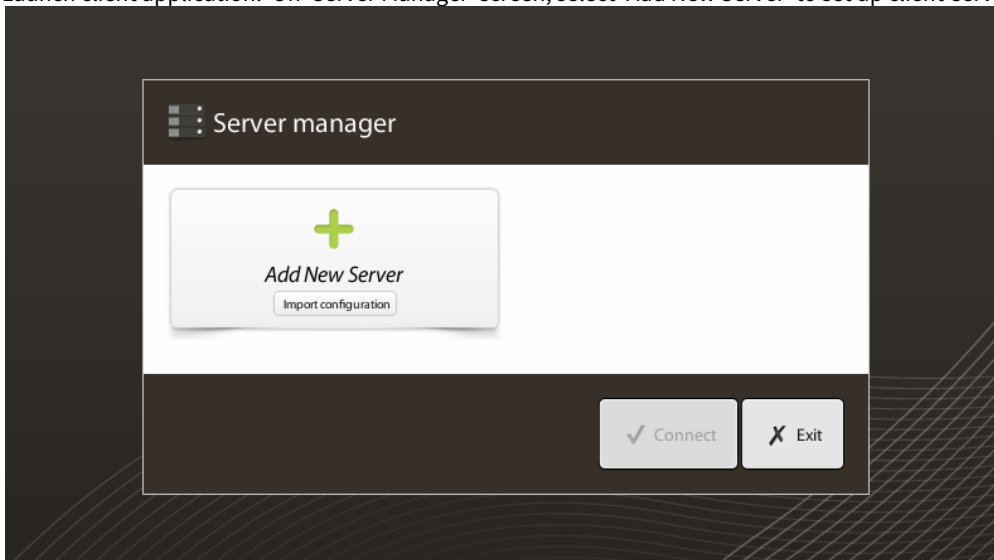
To disable GC logging, comment out or remove the above settings from the *vmoptions* file. Modify the *-Xloggc* configuration value to create the log files in a different directory.

## 5.5 Accepting Client Connections

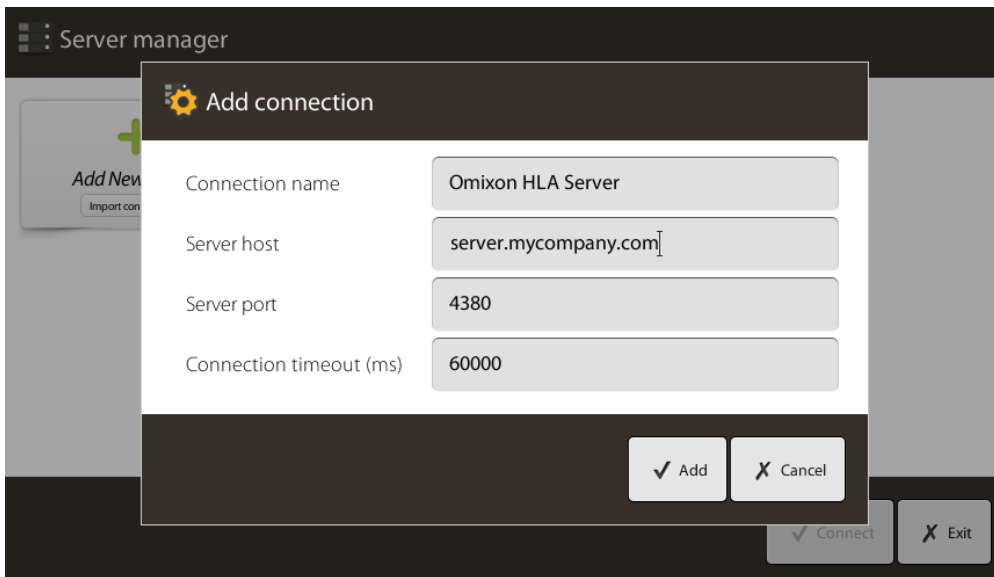
After setting up and starting the server, it is listening to incoming client connection requests.

### 5.5.1 Connecting the client

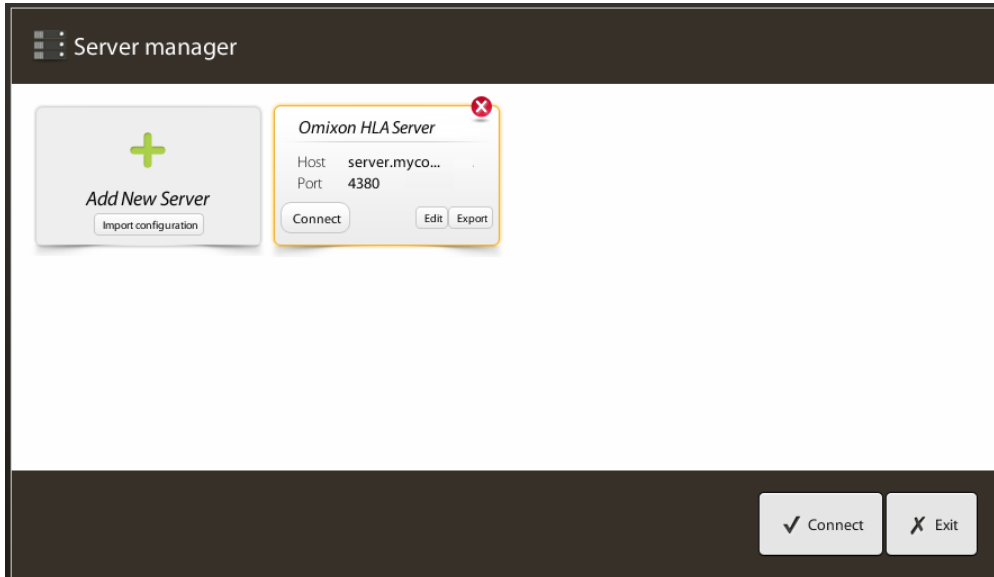
Launch client application. On 'Server Manager' screen, select 'Add New Server' to set up client-server connection.



Name your connection and enter exactly the same host and port settings which were set for server.



Select server connection and do connect.



**IMPORTANT**

Check your firewall settings on the server computer. Set up your firewall to let Omixon HLA Server accept incoming connections.

## 5.5.2 Exporting and importing connection configuration

Instead of configuring the connection manually, the users can choose to import a configuration file by clicking 'Import configuration' button on 'Add New Server' card in 'Server Manager' dialogue. The file is provided by the system administrator, who needs to export the connection settings into a file, by clicking 'Export' on the selected connection in 'Server Manager'.

## 5.6 The Super User Role

The very first user to register becomes a 'Superuser' by default.

This can not be changed later however other users can also be granted Superuser rights.

It is recommended that the System Administrator registers first to complete the setup. The Superuser has the permission to create and manage other User Accounts which can be used to login via the clients.



Please consult the User Management chapter of the User Manual for further information on User roles and permissions.

## 5.7 Data Management

All the analyses are done by the server application and the results are stored on the server side. Results can be viewed - and exported - through the client.

Browsing the remote server filesystem means that the data doesn't need to be transferred through the network between the client and the server; the task starts immediately.