

User guide

Omixon HLA Twin CE Version 4.8.1

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Website: https://www.omixon.com Technical contact: support@omixon.com Sales contact: sales@omixon.com



Omixon Biocomputing Ltd. H-1117 Budapest, Kaposvár u. 14-18., Hungary, EU





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1 Revision and Change History

Version	Approval date	Summary of changes
3.0.0	🖻 29 Jun 2018	Introduced ABO- and MIC-related modifications in genotyping and result display. Removed the word "HLA" where ABO or MIC could be meant as well. Miscellaneous minor corrections in the text.
3.1.0	💼 31 Aug 2018	Company Information section was added. Minor corrections in phrasing and formatting.
3.1.1	: 08 Nov 2018	Performance measurements updated for Holotype HLA v1 and added for Holotype HLA v2. Minor corrections in phrasing and formatting.
3.1.2	13 Nov 2018	Added IMGT database support definition to support period description. Minor corrections in references to the Handbook.
3.1.3	💼 13 Feb 2019	Added Twin genotyping description including SG execution logic Modified temporary folder setting recommendations for server setup
4.0.0	i 06 Aug 2019	Updated Quick Start Guide with: • New result table structure • Locus level markups • New placement of the assignment history function • Collapse/Expand table functionality • Event Log • Typer Manager • Bottom information panel on Dashboard Removed Omixon HLA Server section Refactored the Installation Guide section by adding: • Available configurations • Desktop Installation • Standalone Server Installation • First use guide: • Creating the first user • Connecting to the server



Version	Approval date	Summary of changes
4.0.0	🔅 09 Aug 2019	Moved System Requirements page from Introduction to Installation Guide and updated it with actual values
		Added pages to Installation Guide:
		Introduction
		Client installation Data migration
4.0.1	💼 15 Oct 2019	Updated Installation Guide:
		H2 was removed, MySQL is referenced instead
		MySQL configuration was added
		• Direct links to software installation Manual were replaced by textual references
		Moved Installation Guide up in page hierarchy to precede Quick Start Guide
4.1.0	💼 09 Jan 2020	Updated Quick Start Guide: added the serological equivalent antigen icon to the list of markups.
		Performance measurements updated for Holotype HLA v1 and V2 and added for
		Holotype HLA v3.
4.2.0	💼 17 Mar 2020	Performance measurements updated for Holotype HLA v1, v2 and v3
4.2.2	💼 01 Jul 2020	Performance measurements updated for Holotype HLA v2, v3, and v1 was
		removed.



Version	Approval date	Summary of changes
4.3.0	💼 25 Aug 2020	Performance measurements updated for Holotype HLA v2, v3, and Omnitype v1 was added.
		Minor corrections in phrasing and formatting.
		Updated Quick Start Guide with:
		Settings dashboard - General information - Company info
		Removed from Quick Start Guide:
		Settings dashboard - Export settings section
		Refactored/removed from Quick Start Guide
		 Settings dashboard - Database section (remove database function is no more available for users)
		 Settings dashboard - Administration section (changed the remove function to disable)
		 Settings dashboard - Screen settings (configuration of the result screen is no more available for users)
		Updated User Guide with:
		 Desktop installation - Migration related sections were removed, screenshots were actualized. Page breaking and links are corrected. Standalone Server Installation - Migration related sections were removed, screenshots were actualized. Page breaking and links are corrected. Client Installation - Migration related sections were removed, screenshots were actualized. Page breaking and links are corrected. Client Installation - Migration related sections were removed, screenshots were actualized. Page breaking and links are corrected. Installing MySQL - Windows and OSX were updated: use the latest installer, the screenshots are only examples. Database Migration page was removed.
		Updated Software Installation Guide with:
		 Desktop installation - Migration related sections were removed, screenshots were actualized. Page breaking and links are corrected. Server (standalone) - Migration related sections were removed, screenshots were actualized. Page breaking and links are corrected. Server (distributed) - Migration related sections were removed, screenshots were actualized. Page breaking and links are corrected. Client - Migration related sections were removed, screenshots were actualized. Page breaking and links are corrected. Client - Migration related sections were removed, screenshots were actualized. Page breaking and links are corrected. Database Migration page was removed. Adding new Typers to HLA Twin Server page moved to Typer chapter
		Updating from version 4.x page extended with Omnitype EAP restrictions



Version	Approval date	Summary of changes
		 Installing MySQL - Windows and OSX were updated: use the latest installer, the screenshots are only examples.
4.4.0	💼 10 Dec 2020	Updated User Guide with:
		 Introduction - "General symbols used" was updated Performance measurements updated for Holotype HLA v2, v3, and Omnitype
		 File Search - Allows locating sample and analysis files in the whole database of HLA Twin
		Hardware requirements - RAM changes
		Update Software Installation Guide with:
		Hardware requirements - RAM changes
		Update the Handbook with:
		 Export table section - XLS file format related text was removed Overview with P/G group option is introduced
4.4.1	💼 16 Mar 2021	Updated User Guide with:
		 CE symbol - "Conformité Européenne" European Conformity IVD symbol - In vitro diagnostic medical device
4.5.0	💼 22 Jun 2021	Updated User Guide with:
		GTIN number symbol
		Version symbol Build ID symbol
		 CE symbol with Notified Body Number- "Conformité Européenne" European
		Conformity Reference number symbol
		Production date symbol
		Use by date symbol
		Company information symbol
		Company website symbol Company e-mail addresses symbol
		Analytical and clinical performance characteristics



Version	Approval date	Summary of changes
4.6.0	💼 03 Mar 2022	Updated User Guide and Handbook with:
		 the new address of Omixon Biocomputing Ltd.: H-1117 Budapest, Kaposvár u. 14-18., Hungary, EU, better descriptions in Warnings and precautions section of the CE guide, added instruction about what to do in the event of a serious incident during use, a new description of the Settings dashboard that includes the new look and feel, description of the new Strand bias track, description of the new multi-selection and bulk action functionality in the Genotyping analysis result screen, Performance measurements updated for Holotype HLA assay version 2 with protocol version 3.0.1, and Omnitype v1. we extend the support of 4.4.1 until 21/06/2022.
4.8.0	💼 29 Mar 2023	Updated User Guide with:Analytical and clinical performance characteristics
4.8.1	💼 12 Jun 2023	 Updated User Guide with: Analytical and clinical performance characteristics



2 Warnings and precautions

	In this case	Do this:
1	Before using the software	Before use read the Known Product Limitations (KPL) document authored by Omixon.
2	Choosing the type of assay	 For the best performance, use the Omixon Holotype HLA assay on the Illumina MiSeq system. Omixon developed and validated the software for this assay and in this configuration. Omixon cannot guarantee the quality of the analysis results if you used any other assay to obtain the .fastq files that you process with the software.
3	Serious incidents during use	 Report all serious incidents with regards to Omixon HLA Twin CE to: Omixon Biocomputing Ltd. through support@omixon.com¹, the competent authority in your country.

3 Introduction

3.1 Company information

This product is manufactured by Omixon Biocomputing Ltd.

Address: H-1117 Budapest, Kaposvár u. 14-18., Hungary, EU

Website: http://www.omixon.com

Technical contact: support@omixon.com²

Sales contact: sales@omixon.com³

3.2 General symbols used

The following symbols are used in the Omixon Twin product (for Twin specific symbols please read the dedicated chapter).

1 mailto:support@omixon.com

2 mailto:support@omixon.com

3 mailto:sales@omixon.com

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Label	Name	This indicates
GTIN	Global Trade Item Number	The GTIN number, which is a globally unique 14-digit number used to identify trade items, products, or services.
Version	Device version	The current version of the software that you are using.
Build ID	Build identifier	The unique reference string that Omixon generated for the software when we built it from the codebase. Use this string as a reference when you report a problem with the software. This can help us find and fix the problem more quickly.
REF	Reference	The Omixon reference number of the software.
\sim	Production date	The date when Omixon generated the installers for the software.
$\sum_{i=1}^{n}$	Supported by date	The date until when Omixon supports this version of the software. After this date, we will not provide bug fixes and we will not validate new IMGT/HLA databases for it.
www.omixon.com	Consult instructions for use	That you should read the instructions for use (IFU) to learn how to use the product in a safe and efficient way.
IVD	In vitro diagnostic medical device	That this product is an in vitro diagnostic medical device.
CE	Conformité Européenne (fr) European Conformity (en)	The European Conformity (en) or Conformité Européenne (fr) mark indicates compliance with 98/79/ EC European Directive on in vitro diagnostic medical devices.
	Manufacturer of the device	The company name of the manufacturer of the device, Omixon Biocomputing Ltd., and our address: H-1117 Budapest, Kaposvár u. 14-18., Hungary, EU
www	Website of the manufacturer	The website address of our company, https:// www.omixon.com/



Label	Name	This indicates
	E-mail contacts of the manufacturer	 The e-mail addresses that you can use to get into contact with us if you have a question or problem: support@omixon.com⁴ for technical questions and problems, sales@omixon.com⁵ for commercial or general questions.

3.3 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.

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 the end of support.

3.4 Sequencing technologies

Omixon HLA Twin supports sequencing Illumina data.

3.5 Intended use

Omixon HLA Twin is intended for the interpretation of Next Generation Sequencing (NGS) data generated on the Illumina sequencers by the Omixon Holotype HLA CE sequencing assay. This results in highly accurate HLA typing reaching from a single-pass allele to a 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

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⁴ mailto:support@omixon.com

⁵ mailto:sales@omixon.com



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.

3.6 Warning and precaution:

3.6.1 Product use limitations

The algorithms were co-developed and extensively validated together with the Omixon Holotype HLA sequencing assay. For the best performance, please use the software together with Omixon Holotype 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!

3.7 Principle of method

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.

3.7.1 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.

3.7.2 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 prefiltered 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.



3.7.3 Twin Genotyping

Twin genotyping is a combination of the CG and SG algorithms described above. When performing Twin genotyping, the CG algorithm is run for all targeted loci. Subsequently, the SG algorithm is run for loci with CG results fulfilling a set of predefined conditions. In addition to the user-defined execution conditions, the SG algorithm is programmed to always run for loci with novel alleles and never for the locus HLA-DRB3.

3.8 Validation methods and performance characteristics

The performance statistics presented below were generated using Omixon HLA Twin version 4.8.1 (fast mode) and IMGT database version 3.51.0_9. Performance measures were calculated using the method described by Ng et al. (1993) ¹. Genotyping results were compared to available reference genotyping information on a two-field level resolution.

3.8.1 Holotype HLA

Altogether 249 samples were analyzed. Sequencing data was generated using Holotype HLA assay version 2 with protocol version 3.0.1.

Measure	HLA-A	HLA-B	HLA-C	HLA- DPA1	HLA- DPB1	HLA- DQA1	HLA- DQB1	HLA- DRB1	HLA- DRB3	HLA- DRB4	HLA- DRB5
Sensitivity	99.40 %	99.40 %	98.79 %	97.38%	96.17%	96.77%	97.38%	98.19%	99.24%	96.69%	97.66%
Specificity	99.99 %	99.99 %	99.97 %	99.83%	99.91%	99.85%	99.87%	99.97%	99.81%	98.90%	99.61%
Precision	99.40 %	99.40 %	98.79 %	97.38%	96.17%	96.77%	97.38%	98.19%	99.24%	96.69%	97.66%
Negative Predictive Value	99.99 %	99.99 %	99.97 %	99.83%	99.91%	99.85%	99.87%	99.97%	99.81%	98.90%	99.61%
Type Correctly Classified	99.98 %	99.99 %	99.95 %	99.67%	99.83%	99.72%	99.75%	99.95%	99.70%	98.34%	99.33%

3.9 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/



3.10 References

¹Ng 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.

4 Installation Guide

4.1 Introduction

4.1.1 General information

This is an excerpt from the *Software Installation Guide*. If you couldn't find the information you were looking for in this document, please consult the extended *Software Installation Guide*. For further information and assistance, please contact us at support@omixon.com.⁶

4.1.2 Disclaimer

The concurrent installation and usage of more than one version of HLA Twin is not supported by Omixon.

4.1.3 Updating from HLA Twin 3.1.3 or below

Please note that the update process is different than usual. To ensure a safe update without any data loss, please take the time to read the *Installation Guide* chapter that is relevant to you.

4.2 Available Configurations

4.2.1 General information

This chapter explains the three possible configurations HLA Twin can be used in. Each of them is suitable for laboratories with different throughputs.

Each configuration will require a MySQL 8 database server to be installed either locally or remotely.

This User Guide contains instructions for installing the Desktop and the Standalone Server as well as the Client. For detailed information on installing the Distributed Server please consult the extended *Software Installation Guide*.

4.2.2 Desktop

- Suitable for smaller laboratories
- One user can be logged in at a time

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⁶ mailto:support@omixon.com.



• One sample can be analyzed at a time

The software runs on one computer, the users have to share the same computer to work with the software. HLA Twin has its own user management system, so it does not matter who is logged in in Windows, the user can work under their own identity in HLA Twin (this is important for audit, workflow and commenting features). If the same software is installed on another computer, the two pieces of software are unable to communicate, so the mentioned user information will be unavailable. We advise against it.



Workstation (GUI client n)

HLA Twin Server (standalone)

Workstation (GUI client 1)

4.2.3 Server (standalone)

- Suitable for medium-throughput laboratories
- Multiple users can work at the same time
- One sample can be analyzed at a time

The HLA Twin Server (standalone):

- runs sample analyses
- prepares the information for the HLA Twin Client
- holds all user data

The HLA Twin Client

- controls the HLA Twin Server
- · shows what the HLA Twin Server sends to it
- is "an empty shell"

Multiple HLA Twin Client software can connect to the HLA Twin Server at the same time.

The license is bound to the HLA Twin Server so the number of HLA Twin Clients is not limited.

4.2.4 Server (distributed)

- Suitable for high-throughput laboratories
- Multiple users can work at the same time
- Multiple samples can be analyzed at the same time (depending on the number of Typers)

The HLA Twin Server (distributed):

- does not run sample analyses
- controls the HLA Twin Typer instances
- prepares the information for the HLA Twin Client
- holds all user data

The HLA Twin Typer:

- runs sample analyses
- sends the analysis results to the HLA Twin Server

The HLA Twin Client







- controls the HLA Twin Server
- shows what the HLA Twin Server sends to it
- is "an empty shell"

Multiple HLA Twin Typers can be connected to one HLA Twin Server. An HLA Twin Server and an HLA Twin Typer instance can run on the same server.

4.3 System Requirements

HLA Twin Desktop

- **CPU**: 64bit CPU with at least 4 physical cores (8 threads or vCPUs)
- **OS**: Any 64bit operating system
- RAM: At least 12 GB for the software but 16 GB is recommended
- Video: OpenGL 2.0 compatible video card

HLA Twin Client

- CPU: 64bit CPU with at least 2 physical cores (4 is recommended)
- OS: Any 64bit operating system
- RAM: At least 4 GB for the software but 6 GB is recommended
- Video: OpenGL 2.0 compatible video card
- Network: At least a 100/1000 Mbps connection

HLA Twin Server (standalone)

- **CPU**: 64bit CPU with at least 4 physical cores (8 threads or vCPUs)
- **OS**: Any 64bit operating system (OSX not supported)
- RAM: At least 18 GB for the software but 26.5 GB is recommended
- Network: At least a 100/1000 Mbps connection
- Video: OpenGL 2.0 compatible video card (optional*)

HLA Twin Server (distributed)

- CPU: 64bit CPU with at least 4 physical cores (8 threads or vCPUs)
- **OS**: Any 64bit operating system (OSX not supported)
- RAM: At least 6 GB for the software but 8 GB is recommended
- Network: At least a 100/1000 Mbps connection
- Video: OpenGL 2.0 compatible video card (optional*)



HLA Twin Typer (distributed)

- **CPU**: 64bit CPU with at least 4 physical cores (8 threads or vCPUs)
- **OS**: Any 64bit operating system
- RAM: At least 16 GB for the software but 22 GB is recommended
- Network: At least a 100/1000 Mbps connection
- Video: OpenGL 2.0 compatible video card (optional*)

*The Twin Graphical User Interface (Client or Desktop) requires an OpenGL 2.0 compatible hardware and driver for the visualisation, so if you would like to use the Graphical interface on the server computer, an OpenGL 2.0 compatible hardware and driver are required.

Storage space

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.com⁷ if you need any help.

4.4 Installing MySQL

All editions of HLA Twin will rely on an external **MySQL 8** database that <u>you need to set up before installing HLA</u> <u>Twin</u>. This is a new improvement in HLA Twin to provide a more robust and more responsive user experience.

Please follow the instructions in this chapter prior to the installation of HLA Twin.

4.4.1 Windows

If you have a pre-existing **MySQL 8** server in your environment that you would like to use, please see Configuring a pre-existing MySQL database (see page 38). We suggest using a local instance of MySQL for HLA Twin Desktop users.

Please follow these steps to download and install MySQL 8 for Windows.

- 1. Go to https://dev.mysql.com/downloads/installer/
- 2. Download the actual version of "Windows (x86, 32-bit), MSI Installer" package. For example:

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⁷ mailto:support@omixon.com



MySQL Installer 8.0.17			
Select Operating System:		Looking for preventions?	vious GA
	•		
Windows (x86, 32-bit), MSI Installer	8.0.17	18.5M	Download
(mysql-installer-web-community-8.0.17.0.msi)	MD5: 5677	07887fc0d1fad7fc848a87	8a0da2 Signature
Windows (x86, 32-bit), MSI Installer	8.0.17	393.4M	Download
(mysql-installer-community-8.0.17.0.msi)	MD5: 3aa8	d6470fb6b58f517d3efb46	e5472b Signature
• We suggest that you use the MD5 checksum you download.	is and GnuPG signatures to	o verify the integrity of	f the packages

3. Once downloaded, start the installer

4. Accept the license agreement, and click "Next"



5. Select "Server only" and click "Next"

6. Click "Execute" (please note, that the installer might state that MySQL Server is not in "Ready to Install" status. Please click on it to see how to resolve the issue)

MySQL Installer			—		×
MySQL. Installer Adding Community	Installation The following products will be installed.				
	Product	Status	Progress	Notes	
License Agreement	MySQL Server 8.0.17	Ready to Install			
Choosing a Setup Type					
Installation					
Product Configuration					
Installation Complete					
	Click [Execute] to install the following pac	kages.			
		< <u>B</u> ack	Execute	<u>C</u> ance	el

7. When the installation is done, you will see a green tick next to the MySQL icon. Click "Next", **and when the** "Product Configuration" window appears, click "Next" again.

MySQL Installer			—		×
MySQL. Installer Adding Community	Installation The following products will be installed.				
	Product	Status	Progress	Notes	\square
License Agreement	🖉 💽 MySQL Server 8.0.17	Complete			
Choosing a Setup Type					
Installation					
Product Configuration					
Installation Complete					
	<u>S</u> how Details >				
		< <u>B</u> ack	<u>N</u> ext >	<u>C</u> ance	el

8. Select the default "Standalone MySQL Server / Classic MySQL Replication" option and click "Next"

9. Please use the default values on the "Type and Networking" screen, then click "Next"

MySQL Installer						×
MySQL. Installer MySQL Server 8.0.17 High Availability	Type and Networl Server Configuration Type Choose the correct server co define how much system re Config Type: Developmen	king onfiguration type fo sources are assigned t Computer	r this MySQL Serve d to the MySQL Se	er installation. This rver instance.	setting w	āll -]
Type and Networking	Connectivity					1
Authentication Method Accounts and Roles	Use the following controls to TCP/IP Open Windows	o select how you we Port: Firewall ports for ne	3306 etwork access	ct to this server. X Protocol Port:	33060]
Windows Service Apply Configuration	Shared Memory	Pipe Name: Memory Name:	MYSQL			
	Advanced Configuration Select the check box below and logging options for this Show Advanced an	to get additional co : server instance. d Logging Options	nfiguration pages	where you can set	advanced	I
			< <u>B</u> ack	Next >	<u>C</u> ance	el

10. Select "Use Legacy Authentication Method (Retain MySQL 5.x Compatibility), then click "Next"

11. Specify the MySQL Root Password (please make a note of this password), then click on "Add User"

omi

DN

12. Type in "omixon" as the username **and the password** as well, then click "Ok" and "Next"

🔝 MySQL User Ac	count		×
Please specify	the user nar	ne, password, and database role.	
8	User Name:	omixon	
Server	Host:	<all (%)="" hosts=""> V</all>	
	Role:	DB Admin v	
A	Authentication:	MySQL	
MySQL user cr	edentials		
	Password:	•••••	
Confi	rm Password:	•••••	
		Password strength: Weak	
		<u>O</u> K <u>C</u> ance	:I

13. Please use the default values, and click "Next", then "Execute".

MySQL Installer

High Availability

Type and Networking

Authentication Method

Accounts and Roles

Windows Service

Apply Configuration

Windows Service

Configure MySQL Server as a Windows Service

Windows Service Details

Please specify a Windows Service name to be used for this MySQL Server instance. A unique name is required for each instance.

Windows Service Name: MySQL80

☑ Start the MySQL Server at System Startup

Run Windows Service as ...

The MySQL Server needs to run under a given user account. Based on the security requirements of your system you need to pick one of the options below.

Standard System Account

Recommended for most scenarios.

O Custom User

An existing user account can be selected for advanced scenarios.

< <u>B</u>ack

 $\underline{N}ext >$

om

<u>C</u>ancel

×

14. Click Finish when the configuration is complete, then click "Next" then "Finish".

MySQL is now configured properly. You can proceed to install HLA Twin.

4.4.2 OSX

If you have a pre-existing **MySQL 8** server in your environment that you would like to use, please see Configuring a pre-existing MySQL database (see page 38). We suggest using a local instance of MySQL for HLA Twin Desktop users. Please follow these steps to download and install MySQL 8 for OSX.

- 1. Go to https://dev.mysql.com/downloads/mysql/
- 2. Download the actual version of "macOS 10.14 (x86, 64-bit), DMG Archive" package. For example:

MySQL Community Server 8.0.18			
elect Operating System: macOS	¥	Looking for previous (versions?	3A
Packages for Mojave (10.14) are compatible with H	ligh Sierra (10.13)		
macOS 10.14 (x86, 64-bit), DMG Archive	8.0.18	3	wnload
(mysql-8.0.18-macos10.14-x86_64.dmg)	MD5: 6eff	5f8b4a06546b72c .041d3d70bd	Signatur
macOS 10.14 (x86, 64-bit). Compressed TAR Archive	8.0.18	158.3M Do	wnload
interest in the set with a subset with the set			
(mysql-8.0.18-macos10.14-x86_64.tar.gz)	MD5: 5ded	97d03db45374e77e35d7f3a5f56	Signatur
(mysql-8.0.18-macos10.14-x86_64.tar.gz) macOS 10.14 (x86, 64-bit), Compressed TAR Archive	MD5: 5ded	97d03db45374e77e35d7f3a5f56	Signatur wnload
(mysql-8.0.18-macos10.14-x86_64.tar.gz) macOS 10.14 (x86, 64-bit), Compressed TAR Archive Test Suite (mysql-test-8.0.18-macos10.14-x86_64.tar.gz)	MD5: 5ded 8.0.18 MD5: 8e72	97d03db45374e77e35d7f3a5f56 152.0M Do 5fe930a11b34baaaa312aba3ee3	Signatur wnload Signatur
(mysql-8.0.18-macos10.14-x86_64.tar.gz) macOS 10.14 (x86, 64-bit), Compressed TAR Archive Test Suite (mysql-test-8.0.18-macos10.14-x86_64.tar.gz) macOS 10.14 (x86, 64-bit), TAR	MD5: 5ded 8.0.18 MD5: 8e72 8.0.18	97d03db45374e77e35d7f3a5f56 152.0M Do 5fe930a11b34baaaa312aba3ee3 327.7M Do	Signatur wnload Signatur wnload

3. Once downloaded, open the DMG archive and open the PKG file in it

mysql-8.0.17-macos10.14-x86_64
mysql-8.0.1/-macos10.14- x86 64.pkg

4. If you see this message, select "Continue"

0 • 0	Install MySQL 8	.0.17-community		
	This package will run a program to determine if the software can be installed. To keep your computer secure, you should only run programs or install software from a trusted source. If you're not sure about this software's source, click Cancel to stop the program and the installation.			
		Cancel	Continue	
E				
MySQL			Go Back	Continue

5. After going through the introduction and the license, select "Install" on the "Installation Type" window

6. OSX might require authentication to continue

Introduc Licence	Installer is trying to install new software. Touch ID or enter your password to allow this. Use Password Cancel	
 Destination Select Installation Type Installation Configuration Summary 	Preparing for installation	
MySQL	Go Back Continue	

7. Select "Use Legacy Password Encryption" and click next

		-
	😺 Install MySQL 8.0.17-community	
	Configure MySQL Server	
Introduction	OUse Strong Password Encryption	
 Licence 	MySQL 8 supports a new, stronger authentication method	
 Destination Select 	based on SHA256. All new installations of MySQL Server	
 Installation Type 	should use this method.	
 Installation 	Connectors and clients that don't support this method will	
Configuration	connectors and community drivers that use libmysqlclient	
Summary	8.0 support the new method.	
	Use Legacy Password Encryption	
5	The legacy authentication method should only be used when compatibility with MySQL 5.x connectors or clients is required and a client upgrade is not feasible.	
Z N	Next	
MySQL.	Go Back Continue	

8. Specify the MySQL Root Password (**please make a note of this password**) and make sure that "Start MySQL Server once the installation is complete" is set, and click Finish

	😜 Install MySQL 8.0.17-community	
 Introduction Licence Destination Select Installation Type Installation Configuration Summary 	Configure MySQL Server Please enter a password for the "root" user. A reasonable password consists of at least eight characters that are a mix of letters, numbers, and other characters. Start MySQL Server once the installation is complete.	
MySQL	Finish Go Back Continue	

9. After the installation is done, open System Preferences (Apple menu on the top left, System Preferences option). Open MySQL in the bottom row

10. Make sure that MySQL is running (green dot next to it in the list to the left) and that the "Start MySQL when your computer starts up" box in checked to the right.

11. If everything is in order, please close this window and open Terminal in OSX (Finder - Applications - Utilities)

	🔤 Applicat	ions		
$\langle \rangle$		🐵 🗸 🗱 🗸 🗊 🗸	Q Search	
Favourites	Name	Date Modified	Size	Kind
Dronbox (O	IIme Machine	2019. May 27. 9:02	1.3 MB	Application
	M Tunnelblick	2019. September 20. 17:40	31.7 MB	Application
(@) AirDrop	▼ 🔯 Utilities	2018. November 30. 6:49		Folder
Recents	Karaka Activity Monitor	2019. August 9. 0:59		Application
-A- Applications	AirPort Utility	2019. August 9. 0:59		Application
	Mudio MIDI Setup	2019. August 9. 0:59		Application
🕒 Downloads	All Bluetooth File Exchange	2019. August 9. 0:59		Application
Shared	📓 Boot Camp Assistant	2019. August 9. 0:59		Application
A	述 ColorSync Utility	2019. May 27. 9:02		Application
iii janostoth	Console	2019. August 9. 0:59		Application
SXFUSE V	🙆 Digital Colour Meter	2019. May 27. 9:02		Application
- Macintach	🕺 Disk Utility	2019. August 9. 0:59		Application
	🦲 Grapher	2019. August 9. 0:59		Application
iCloud	🕺 Keychain Access	2019. August 9. 0:59		Application
	🌆 Migration Assistant	2019. May 27. 9:02		Application
	🔞 Screenshot	2019. May 27. 9:02		Application
Documents	闅 Script Editor	2019. May 27. 9:02		Application
iCloud D 🕐	🚖 System Information	2019. August 9. 0:59		Application
	Terminal	2019. August 9. 0:59		Application
Locations	🐻 VoiceOver Utility	2019. August 9. 0:59		Application
🧾 ВООТСАМР	刘 Visual Studio Code	2019. August 30. 15:50	227.6 MB	Application
mvsal-8		2019. September 3. 19:02	136.4 MB	Application
	🛂 VMware Fusion	2018. November 21. 10:53	936.8 MB	Application
MySQL ≜	b Voice Memos	2019. August 9. 0:59	3.1 MB	Application
Remote Disc	🕨 🚞 Webex	2019. October 1. 9:15		Folder
	🕑 WebTorrent	2018. April 27. 4:21	142.3 MB	Application

- 12. Run the following commands in sequential order
 - a. cd /usr/local/mysql/bin
 - b. ./mysql -u root -p
 - c. type in the MySQL root password you have set in the installer
 - d. CREATE USER 'omixon'@'localhost' IDENTIFIED BY 'omixon';
 - e. GRANT ALL PRIVILEGES ON omixon_database . * TO 'omixon'@'localhost';
 - f. FLUSH PRIVILEGES;
 - g. quit




If the output looks the same as the screenshot, everything is configured properly. You can proceed to install HLA Twin now.



4.4.3 Linux

If you have a pre-existing **MySQL 8** server in your environment that you would like to use, please see Configuring a pre-existing MySQL database (see page 38). We suggest using a local instance of MySQL for HLA Twin Desktop users.

Since there are a plethora of Linux repositories with different MySQL packages, this document will only provide a list of settings you will need to look out for during configuration:

- HLA Twin only works with version 8 of MySQL
- MySQL needs to use Legacy Password Encryption
- You might need to change the password policy in MySQL for allowing HLA Twin to connect

After you have installed the MySQL 8 server, make sure that you create a new user named omixon with the following commands typed in the terminal:

- 1. mysql -u root -p
- 2. CREATE USER 'omixon'@'localhost' IDENTIFIED BY 'omixon';
- 3. GRANT ALL PRIVILEGES ON omixon_database . * TO 'omixon'@'localhost';
- 4. FLUSH PRIVILEGES;

After setting the MySQL server up, you can proceed to install HLA Twin.

4.5 Configuring a Pre-existing MySQL Database

HLA Twin Server has the ability to store its internal database (containing user data, reference databases, and audit information) in an already existing **MySQL 8** database. This way you would not need to set up a separate MySQL server for HLA Twin.

Please note that the responsiveness of the HLA Twin UI will depend on the network speed between MySQL and HLA Twin.

Your MySQL server needs to use Legacy Password Encryption for HLA Twin to interface with it.

You need to create a new user in your pre-existing database to allow HLA Twin to use it. For this, run the following commands:

- CREATE USER 'omixon'@'localhost' IDENTIFIED BY 'omixon';
- 2. GRANT ALL PRIVILEGES ON omixon_database . * TO 'omixon'@'localhost';
- 3. FLUSH PRIVILEGES;

Now HLA Twin will be able to create its own database in MySQL.



4.6 Desktop Installation

4.6.1 Updating from HLA Twin 3.1.3 or below

- You will not be able to upgrade your previous version of HLA Twin 3.1.3 Desktop as you could in previous versions. Also, the installer will not allow you to install the new HLA Twin in the same folder where an older version was installed.
- If you would like to migrate the internal database of your previous Twin installation in order to keep your user data and audit information, please contact us at support@omixon.com⁸. We will arrange an online session, where we can migrate the previous database to MySQL.
- After a successful installation (and migration, if it was required) you can uninstall previous versions of HLA Twin Desktop from your computer.

4.6.2 Installing HLA Twin Desktop

You will need to install a MySQL 8 database server before being able to install HLA Twin! Please refer to chapter *Installing MySQL* for more information.

- 1. This step depends on the operating system you are using.
- Windows users: Open the installer
 (omixon_hla_twin_XXX_windows-x64_with_jre-desktop.exe)
- Linux users: Open a terminal window, get permissions to the installer (chmod +x omixon_hla_twin_xxx_unix_with_jredesktop.sh) then run the installer.
- OSX users: Open the installer

 (omixon_hla_twin_xxx_macos_with_jre-desktop.dmg)
 (if you are using OSX 10.14.6
 Mojave or above, you might get an error message. If so, please contact us at support@omixon.com⁹)



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⁸ mailto:support@omixon.com



2. Accept the license agreement.

-	Setup - Omixon HLA Twin Desktop 4. —		×
	License Agreement Please read the following important information before continuing.		
	Please read the following License Agreement. You must accept the terms of this agreement before continuing with the in	stallatior	ı.
	SOFTWARE LICENSE AGREEMENT		^
	PLEASE READ THIS SOFTWARE LICENSE AGREEMENT CAREFULLY BEFORE DOWNLOADING OR USING THE SOFTWARE		
	BY CLICKING ON THE "ACCEPT" BUTTON, OPENING THE PACKAGE, DOWNLOADING THE PRODUCT, OR USING THE EQUIPMENT THAT CONTAINS THIS PRODUCT, YOU ARE CONSENTING TO BE BOUND BY THIS AGREEMENT. IF YOU DO NOT AGREE TO ALL OF THE TERMS OF THIS AGREEMENT, CLICK THE "DO NOT ACCEPT" BUTTON AND THE INSTALLATION PROCESS WILL NOT CONTINUE, RETURN THE PRODUCT TO THE PLACE OF PURCHASE FOR A FULL REFUND, OR DO NOT DOWNLOAD THE PRODUCT.		
	Single User License Grant: Omixon Biocomputing Ltd. ("Omixon") and its suppliers grant to Customer ("Customer") a nonexclusive and nontransferable license to use the Omixon software ("Software") in object code form solely on a single central processing unit owned or leased by Customer or otherwise embedded in equipment provided by Omixon.		
	Multiple-Users License Grant: Omixon Biocomputing Ltd. ("Omixon") and its suppliers grant to		¥
	I accept the agreement		
	○ I do not accept the agreement		
ins	stall4j < Back Next :	>	Cancel



3. Select an installation folder.

Windows users, please keep in mind that you might want to change the destination directory so other users in Windows can access the software (the same applies to the other installation folders in the next steps as well).

🛃 Setup - Omixon HLA Twin Desktop 4.	_		Х
Select Destination Directory Where should Omixon HLA Twin Desktop be installed?			
Select the folder where you would like Omixon HLA Twin Desktop to be installed, then click Next.			
C:\Users\Omixon\OmixonHlaTwin		Browse	
Required disk space: 430 MB Free disk space: 31 GB			
install4j	lext >	Can	cel



4. Select a folder for reference database files.

🛃 Setup - Omixon HLA Twin Desktop 4.		_		×
Data directory The directory where the application will store its permanent data files.				
This directory should be readable and writable by the user who will be running the application.				
C:\Users\Omixon\.omixon-hla_twin_RUOv4			Browse	
install4i				
ino cui ij	< Back	Next >	C	Cancel



5. Select a folder for temporary files.

👱 Setup - Omixon HLA Twin Desktop 4. 👘 🗧	_		×
Temp directory The directory where the application will store its temporary data files.			
This directory should be readable and writable by the user who will be running the application.			
C:\Users\Omixon\AppData\Local\Temp		Browse	
r			
<pre>Install4) <back <="" nex="" pre=""></back></pre>	d >	Ca	ncel



6. Specify the IP address and the port number for the MySQL database (the default settings should work fine if you have installed MySQL locally). Please refer to Installing MySQL chapter for installation guides. (see page 18)

🛃 Setup - Omixon HLA Twin Desktop 4.		_		×
Persistence provider MySQL settings				
Host: localhost				
Port: 3306 Test Connection				
Success! Please proceed with the installation.				
install4i				
	< Back	Next >	C	ancel

You cannot proceed until you get a successful connection test!



7. Configure the memory settings.

🛃 Setup - Omixon HLA Twin Desktop 4.	_	
Memory setup You can set how much memory the application will use		
Omixon Application memory [Mb]: [16384 Minimum required: 12288 Maximum available: 32660 Recommended: 16384		
install4j < Back	Next >	Cancel



8. Select Start Menu folder.

👱 Setup - Omixon HLA Twin Desktop 4.	_		×
Select Start Menu Folder			
Where should Setup place the program's shortcuts?			
Select the Start Menu folder in which you would like Setup to create the program's shortcuts, then click Next.			
Create a Start Menu folder			
Omixon HLA Twin Desktop			
7-Zip			^
Accessibility			
Accessories			
Administrative Tools			
AnyDesk			
Blackmagic Design			
DragonDisk			
Dropbox			
Git			
Java			
Java Development Kit			
JetBrains			~
✓ Create shortcuts for all users			
instal 4i			
in is can ij		_	
< Back	Next >	C	ancel



9. Click Finish.

👱 Setup - Omixon HLA Twin	Desktop 4. —		×
	Completing the Omixon HLA Twin Desktop Setup Wizard		
	Setup has finished installing Omixon HLA Twin Desktop on your computer. The application by selecting the installed icons.	1 may be la	aunched
	Click Finish to exit Setup.		
	_	1	Finish



4.7 Standalone Server Installation

4.7.1 Updating from HLA Twin 3.1.3 or below

- You will not be able to update your previous version of HLA Twin 3.1.3 Server as you could in previous versions. Also, the installer will not allow you to install the new HLA Twin in the same folder where an older version was installed.
- If you would like to migrate the internal database of your previous Twin installation in order to keep your user data and audit information, please contact us at support@omixon.com¹⁰. We will arrange an online session, where we can migrate the previous database to MySQL.
- After a successful installation (and migration, if it was required) you can uninstall previous versions of HLA Twin Server from your computer.
- Please keep in mind that the version of the HLA Twin Client and HLA Twin Server software needs to match.
- There is no HLA Twin Typer Server NG service in the new version of HLA Twin Server, one service will handle the analyses and the Clients.

4.7.2 Notes before installation

Database: You will need to install a MySQL 8 database server **before being able to install HLA Twin**! Please refer to chapter *Installing MySQL* for more information.

Networking: HLA Twin Server will communicate with HLA Twin Clients on ports 4380 and 4381 by default, so please make sure to allow them on your firewall.

Windows Service: The HLA Twin Server will run as the Omixon HLA Twin NG Server service in Windows, which is set to automatic startup by default.

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¹⁰ mailto:support@omixon.com



4.7.3 Installing HLA Twin Server

- 1. This step depends on the operating system you are using.
 - Windows users: Open the installer (omixon_hla_twin_XXX_windows-x64_with_jre-serverclient.exe)
 - Linux users: Open a terminal window, get permissions to the installer (chmod
 +x omixon_hla_twin_xxx_unix_with_jre-serverclient.sh) then run the installer.

👻 Setup - Omixon HLA Twin Server 4				×
	Welcome to the Omixon HLA Twin Server Setup Wizard			
	This will install Omixon HLA Twin Server on your computer. The wizard will lead you the installation.	step by	step throu	Jgh
	Click Next to continue, or Cancel to exit Setup.			
		Next >	Ca	ncel



2. Accept the license agreement.

🛃 Setup - Omixon HLA Twin Server 4 👘 🦳 —		×
License Agreement Please read the following important information before continuing.		
Please read the following License Agreement. You must accept the terms of this agreement before continuing with the inst	allation.	
SOFTWARE LICENSE AGREEMENT		^
PLEASE READ THIS SOFTWARE LICENSE AGREEMENT CAREFULLY BEFORE DOWNLOADING OR USING THE SOFTWARE.		
BY CLICKING ON THE "ACCEPT" BUTTON, OPENING THE PACKAGE, DOWNLOADING THE PRODUCT, OR USING THE EQUIPMENT THAT CONTAINS THIS PRODUCT, YOU ARE CONSENTING TO BE BOUND BY THIS AGREEMENT. IF YOU DO NOT AGREE TO ALL OF THE TERMS OF THIS AGREEMENT, CLICK THE "DO NOT ACCEPT" BUTTON AND THE INSTALLATION PROCESS WILL NOT CONTINUE, RETURN THE PRODUCT TO THE PLACE OF PURCHASE FOR A FULL REFUND, OR DO NOT DOWNLOAD THE PRODUCT.		
Single User License Grant: Omixon Biocomputing Ltd. ("Omixon") and its suppliers grant to Customer ("Customer") a nonexclusive and nontransferable license to use the Omixon software ("Software") in object code form solely on a single central processing unit owned or leased by Customer or otherwise embedded in equipment provided by Omixon.		
Multiple-Users License Grant: Omixon Biocomputing Ltd. ("Omixon") and its suppliers grant to		~
I accept the agreement		
○ I do not accept the agreement		
install4j < Back Next >	(Cancel



3. Select an installation folder.

Windows users, please keep in mind that you might want to change the destination directory so other users in Windows can access the software (the same applies to the other installation folders in the next steps as well).

🛃 Setup - Omixon HLA Twin Server 4	_		×
Select Destination Directory Where should Omixon HLA Twin Server be installed?			
Select the folder where you would like Omixon HLA Twin Server to be installed, then click Next.			
C:\Users\Omixon\OmixonHlaTwin		Browse	
Required disk space: 510 MB			
Free disk space: 32 GB			
(Install+) < Back	Next >	Ca	incel



4. Select a folder for reference database files.

🛃 Setup - Omixon HLA Twin Server 4		_		Х
Data directory The directory where the application will store its permanent data files.				
This directory should be readable and writable by the user who will be running the application.				
C:\Users\Omixon\.omixon-hla_twin_RUOv4			Browse .	
install4j				
<	Back	Next >	С	ancel



5. Select a folder for temporary files.

👱 Setup - Omixon HLA Twin Server 4	_		×
Temp directory The directory where the application will store its temporary data files.			
This directory should be readable and writable by the user who will be running the application.			
C:\Users\Omixon\AppData\Local\Temp		Browse .	
install4i			
< Back N	ext >	С	ancel



6. Configure the IP address and port number that the HLA Twin Server will use for communication (local IP).

射 Setup - Omixon H	ILA Twin Server 4		_		×
Network connection You can set the Om	on setup xon Server connection parameters				
Clients and Omixon T Please configure Omi	yper Server must connect to Omixon Server. con Server connection parameters.				
Omixon Server host:	0.0.0.0				
Omixon Server port:	4380				
install4i					
		< Back	Next >	С	ancel



7. Specify the IP address and the port number for the MySQL database (the default settings should work fine if you have installed MySQL locally). Please refer to Installing MySQL chapter for installation guides.

🛃 Setup - Omixon HLA Twin Server 4		_	
Persistence provider MySQL settings			
Host: localhost Port: 3306 Test Connection Success! Please proceed with the installation.			
install4j	< Back	Next >	Cancel

You cannot proceed until you get a successful connection test!



8. Select the standalone architecture (for the distributed configuration with multiple HLA Twin Typers on separate servers, please follow the Server (distributed) chapter in the Software Installation Guide).

🛃 Setup - Omixon HLA Twin Server 4		_		\times
Typer architecture Select your typer architecture				
Standalone				
◯ Distributed				
install4j				
	< Back	Next >	С	ancel



9. Configure the memory settings.

🛃 Setup - Omixon HLA Twin Server 4		_		Х
Memory setup You can set how much memory the application will use				
Omixon Typer Server memory [Mb]: 26624 Minimum required: 18432 Maximum available: 32660 Recommended: 26624				
install4j	< Back	Next >	C	ancel



10. Select Start Menu folder.

🛃 Setup - Omixon HLA Twin Server 4	_		×
Select Start Menu Folder			
Where should Setup place the program's shortcuts?			
Select the Start Menu folder in which you would like Setup to create the program's shortcuts, then click Next.			
☑ Create a Start Menu folder			
Omixon HLA Twin Server			
7-Zip			^
Accessibility			
Accessories			
Administrative Tools			
AnyDesk			
Blackmagic Design			
DragonDisk			
Dropbox			
Git			
Java			
Java Development Kit			
JetBrains			~
Create shortcuts for all users			
in stal Ai			
Install+j			
< Back	Next >	C	ancel



11. Click Finish.

👮 Setup - Omixon HLA Twin	Server 4	_		×
	Completing the Omixon HLA Twin Server Setup Wizard			
	Setup has finished installing Omixon HLA Twin Server on your computer. The applica by selecting the installed icons. Click Finish to exit Setup.	tion may	/ be laund	.hed
			Fir	nish



4.8 Client Installation

4.8.1 Updating from HLA Twin 3.1.3 or below

- You will not be able to update your previous version of HLA Twin 3.1.3 Client as you could in previous versions. Also, the installer will not allow you to install the new HLA Twin in the same folder where an older version was installed.
- Please keep in mind that the version of the HLA Twin Client and HLA Twin Server software needs to match.

4.8.2 Notes before installation

Networking: HLA Twin Server will communicate with HLA Twin Clients on ports 4380 and 4381 by default, so please make sure to allow them on your firewall.



4.8.3 Installing HLA Twin Client

- 1. This step depends on the operating system you are running.
 - Windows users: Open the installer (omixon_hla_twin_XXX_windowsx64_with_jre-client.exe)
 - Linux users: Open a terminal window, get permissions to the installer (chmod
 +x omixon_hla_twin_xxx_unix_with_jre-client.sh) then run the installer.
 - OSX users: Open the installer (omixon_hla_twin_xxx_macos_with_jre-client.dmg) (if you are using OSX 10.14.6 Mojave or above, you might get an error message. If so, please contact us at support@omixon.com¹¹)

👮 Setup - Omixon HLA Twin	Olient 4	_		Х
	Welcome to the Omixon HLA Twin Client Setup Wizard			
	This will install Omixon HLA Twin Client on your computer. The wizard will lead you installation.	step by st	tep throu	igh the
	Click Next to continue, or Cancel to exit Setup.			
		Next >	Ca	ancel

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¹¹ mailto:support@omixon.com



2. Accept the license agreement.

🛃 Setup - Omixon HLA Twin Client 4 👘 🦳 —		×
License Agreement Please read the following important information before continuing.		
Please read the following License Agreement. You must accept the terms of this agreement before continuing with the inst	allation.	
SOFTWARE LICENSE AGREEMENT		^
PLEASE READ THIS SOFTWARE LICENSE AGREEMENT CAREFULLY BEFORE DOWNLOADING OR USING THE SOFTWARE.		
BY CLICKING ON THE "ACCEPT" BUTTON, OPENING THE PACKAGE, DOWNLOADING THE PRODUCT, OR USING THE EQUIPMENT THAT CONTAINS THIS PRODUCT, YOU ARE CONSENTING TO BE BOUND BY THIS AGREEMENT. IF YOU DO NOT AGREE TO ALL OF THE TERMS OF THIS AGREEMENT, CLICK THE "DO NOT ACCEPT" BUTTON AND THE INSTALLATION PROCESS WILL NOT CONTINUE, RETURN THE PRODUCT TO THE PLACE OF PURCHASE FOR A FULL REFUND, OR DO NOT DOWNLOAD THE PRODUCT.		
Single User License Grant: Omixon Biocomputing Ltd. ("Omixon") and its suppliers grant to Customer ("Customer") a nonexclusive and nontransferable license to use the Omixon software ("Software") in object code form solely on a single central processing unit owned or leased by Customer or otherwise embedded in equipment provided by Omixon.		
Multiple-Users License Grant: Omixon Biocomputing Ltd. ("Omixon") and its suppliers grant to		~
 I accept the agreement 		
○ I do not accept the agreement		
install4j		
< Back Next >	(Cancel



3. Select an installation folder.

Windows users, please keep in mind that you might want to change the destination directory so other users in Windows can access the software (the same applies to the other installation folders in the next steps as well).

🛃 Setup - Omixon HLA Twin Client 4	_		(
Select Destination Directory Where should Omixon HLA Twin Client be installed?			
Select the folder where you would like Omixon HLA Twin Client to be installed, then click Next.			
C:\Users\Omixon\OmixonHlaTwin		Browse	
Required disk space: 281 MB			
Free disk space: 32 GB			
Install4)	Next >	Cancel	



4. Select a folder for temporary files.

👱 Setup - Omixon HLA Twin Client 4 👘 –	-		×
Temp directory The directory where the application will store its temporary data files.			
This directory should be readable and writable by the user who will be running the application.			
C: \Users \Omixon \AppData \Local \Temp	Br	owse	
install4j < Back Nex	t>	Car	ncel



5. Configure the memory settings.

🛃 Setup - Omixon HLA Twin Client 4		_		×
Memory setup You can set how much memory the application will use				
Application memory [Mb]: 5144				
Minimum required: 4096				
Maximum available: 32660				
Recommended: 6144				
install4i				
	< Back	Next >	Ca	ancel



6. Select Start Menu folder.

🛃 Setup - Omixon HLA Twin Client 4	—		\times
Select Start Menu Folder Where should Setup place the program's shortcuts?			
Select the Start Menu folder in which you would like Setup to create the program's shortcuts, then click Next.			
☑ Create a Start Menu folder			
Omixon HLA Twin Client			
7-Zip			~
Accessibility			
Accessories			
Administrative Tools			
AnyDesk			
Blackmagic Design			
DragonDisk			
Dropbox			
Git			
Java			
Java Development Kit			
JetBrains			
Jubula			
Maintenance			
Microsoft Office 2013			*
install4j			
< Back	Next >	C	ancel



7. After the installation finishes, click on Finish.

🛃 Setup - Omixon HLA Twin	Client 4	_		×
	Completing the Omixon HLA Twin Client Setup Wizard			
	Setup has finished installing Omixon HLA Twin Client on your computer. The applica selecting the installed icons. Click Finish to exit Setup.	tion may	be laund	ned by
			Fi	nish



4.9 First Use Instructions

4.9.1 Connecting to the server

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

4.9.1.1 Connecting the client

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

Server manager	
Add New Server	
	✓ Connect X Exit

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

Server n	nanager		
	🔯 Add connection		
Add New	Connection name	Omixon HLA Server	
	Server host	server.mycompany.com	
	Server port	4380	
	Connection timeout (ms)	60000	
		✓ Add X Cancel	
		√ Conne	ect X Exit

Select server connection and press Connect.

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Server manager		
Add New Server Import configuration	Omixon HLA Server Host server.myco Port 4380 Connect Edit	
	✓ Connect X Exit	

IMPORTANT

Check your firewall settings on the server computer. Set up your firewall to let Omixon HLA Server accept incoming connections. Also make sure that outbound connections are enabled for the Omixon HLA Server in order for connected clients to receive status updates (eg. for displaying task progress information).

4.9.1.2 Exporting and importing connection configuration

Instead of configuring the connection manually, you can choose to import a configuration file by clicking the *Import configuration* button on the *Add New Server* card in the *Server Manager* dialogue. If you wish to choose this option, ask your System Administrator to provide you with a connection file. Connection settings can be exported to a file by clicking *Export* on the selected connection in the *Server Manager* dialogue.

4.9.2 Creating the first user

When starting the desktop application or connecting to the Omixon Server for the first time, you will be prompted to create the very first application user. The first user to register becomes a *Superuser* by default meaning that he/ she has access to the full functionality of the software, including creating further user accounts. It is recommended that the System Administrator registers first to complete the setup.

Please consult the *Settings Dashboard/User Management* chapter of the *Omixon Handbook* for further information on user roles and permissions.

5 Quick Start Guide

5.1 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.



5.2 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.
- Bottom Panel:

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
 - the Typer Manager for deploying and undeploying typer nodes in distributed server configurations
 - 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 or selecting the analysis file, you can see all available information about the analysis in the bottom information panel. 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.

If you opened multiple results, you can select all, or some of them and perform the following bulk actions:

- bulk submission for approval, bulk approval, bulk reject/revoke approval,
- bulk table export,
- bulk HPRIM export.

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

5.3 Analysis

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

5.3.1 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 the *Genotyping Dashboard* section.

5.3.2 Results

As the progress reaches 100% in the *Typer Scheduler*, 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 the *Genotyping Analysis Result* and *Genotyping Sample Result* sections.



5.4 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.

This screen consists of three main parts:

- Information panel: the structure of this panel is largely identical to the Genotyping dashboard Information Panel (see above for details)
- Function panel: function buttons for filtering, assigning, approving etc.
- Result table: an overview table displaying the analysis results

The Function panel has the following buttons:

- 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:

- 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.
 - In the top right corner you find the clock shaped Assignment history icon and the approval state indicator icon
- Separate columns for the analyzed loci:
 - Result alleles are displayed in two rows signalizing the two chromosomes
 - A variety of locus and allele level markups are displayed in these columns

In the topmost row for each locus, the locus specific markups are displayed. These include:

• Quality control traffic lights:

These lights are based on the locus level quality control measures and can be one of the following:

- (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.

- Zigosity markups: Heterozygous loci have the ^M/₂ markup while homozygous loci are marked by ^M/₂. Hemizygous loci are marked with ^I/₂!. 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, it is marked with ^I/₂?.
- Novelty markups: loci with alleles containing exonic (or exonic and intronic) novelties are marked with \$, while loci with novel alleles containing only intronic novelties are marked with \$.

Also allele results can have a variety of markups, including:

- Best match and assignment state: On the very left of the displayed allele result you can see the small "tick" icon indicating whether or not the allele result is a "Best matching allele". The icon is blue for best matching alleles and grey for remainder allele candidates. You can assign the allele result by simply clicking the tick mark the sign will become green, indicating that the result is assigned.
- Concordance traffic lights: 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 and only if the result was produced with the Twin genotyping algorithm and the Statistical algorithm ran for the specific locus.

- Homozygosity: Alleles displayed with the blue font are homozygous.
- Serological equivalent antigens: If information regarding the associated serological equivalent antigens is available for the locus, the tooltip of the *antigen icon* **H** will contain that information.
- Rare alleles are marked with an *exclamation point* icon \bigcirc .
- Novel alleles containing exonic (or exonic and intronic) novelties are marked with δ_+ , while novel alleles containing only intronic novelties are marked with δ_+ .
- Imbalanced alleles are displayed with *italic* font.
- Allele extensions: Alleles with extended allele sequence are marked with a plus sign igoplus .
- Low amplification: 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.

By hovering the mouse over the different parts of the table a tooltip will be displayed containing further available information on the sections.

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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.

From the Genoyping 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 analyzed 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.

5.4.1 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

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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.

5.5 Genotyping Sample result

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

This screen can be divided into four main sections:

- Information panel note that the structure of this panel is largely identical to the Genotyping dashboard Information Panel (see above for details)
- Function panel: function buttons for filtering, assigning, approving etc.
- Result table: the contents of the result table are identical to that of the table on the Genotyping Analysis result screen, however in this screen the table is displayed in collapsed mode in order to leave more space for the Details Panel.
- Details Panel: displaying the genotyping result, quality metrics and data statistics

The Function panel has the following buttons:

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

The collapsed result table shows the first best matching allele pair for each locus. If there are multiple best matching allele results, the level of ambiguity is indicated by coloring the affected fields red in the allele name, while leaving matching fields black.

In the Details Panel you can choose from three different tabs:

- 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.

5.6 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.

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• 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.
- *Strand bias track* Shows whether reads are overrepresented in a read direction compared with the opposite direction. A balanced result will have a mostly flat strand bias graph that has a height roughly at the middle of the Strand bias track.

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).

5.7 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.

5.7.1 General information

There are three blocks of information on the Settings Dashboard:

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- 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.
- Company info: this part contains the name, address, website, technical, and sales contact of the company.
- License info: this part shows the number of available credits and the expiration date of the license.

5.7.2 Sidebar

The left sidebar contains the following function sets:

5.7.2.1 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.

5.7.2.2 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. You can set whether or not to use database extensions in the *Configure Database Extensions* menu.

5.7.2.3 Administration

With the *User management* option, you can create, edit and disable 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.

5.7.2.4 Automation

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

5.7.2.5 Screen Settings

In this function group, you can change the display configurations for the Gene Browser. 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 configuration* option on the browser screen should be used. 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 page: *Gene Browser*.