AlloSeq Software Installation Guide

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1. Introduction and Intended Use

CareDx AlloSeq software is the companion software for the CareDx AlloSeq lab products (assays). We recommend reading this Installer Guide before proceeding to the product-specific Instruction For Use (IFU).

AlloSeq Software is intended to assist labs and clinicians who work with post-transplant testing data. It provides a means of tracking recipients, donors, transplants, treatments, samples, batches, and tests over time. The software can be configured to support either or both solid organ transplants and HCT (hematopoietic stem cell transplants).

The AlloSeq assays are NGS (Next Generation Sequencing) based tests, and AlloSeq software provides algorithms for analyzing the NGS data and providing simple test results for post-transplant surveillance.

AlloSeq software displays results for multiple indicators for multiple samples taken from a single recipient over time in a single graph per recipient, to provide a comprehensive overview of all the testing performed on that recipient. AlloSeq software can also display results for single samples that have been tested multiple times, for control samples or samples used repeatedly in proficiency testing.

AlloSeq software can also display results for other test types - beyond those supplied by CareDx - which can also be included in the surveillance visualization, including STR tests (for HCT validation), creatinine and proteinuria (for kidneys) and HLA antibody data.

2. Installation

There are two components to the AlloSeq software - the Server and the Client component. While both can be installed on a single computer, the Server is usually only installed once per lab, on a centrally available server machine. The Client is usually installed multiple times, once per user of the software.

The server component performs the analysis of NGS data and contains a small database, which holds the results of the AlloSeq analyses and metadata for the recipients and other transplant-related items. The database also holds the login information for the users of the AlloSeq software.

To get the installation programs for the AlloSeq software, please contact techsupport-labproducts@caredx.com.

Please contact CareDx should you have any issues with the Installation process.

2.1 Uninstallation

The AlloSeq installers come with an uninstallation program, which can be found in the folder in which the AlloSeq software has been installed.

2.2 System Requirements

The server is available for computers with 64-bit Windows operating systems only.

Minimum hardware requirements:
16-Gb installed memory (RAM)
4 CPU cores, 1.7 GHz
64-bit operating system required
150-GB hard drive space
Encrypted hard drive (recommended but not required)
Software requirements:
Windows 10

The Client is available for computers with 64-bit Windows operating systems.

After installation of the server version, an additional configuration step is needed. This configuration step is usually performed by an IT Support Engineer or Network Administrator, only needs to be done once, and is described at the end of the server installation section below.

2.3 Server Installation - Overview

The server installer creates a 'service' which will always run in the background, and which will automatically start if the server is restarted. Because of this service creation, **you will need administrator access** to the server machine to install the server software. If you don't have this administrator access, you will need to ask for assistance from your IT Support team.

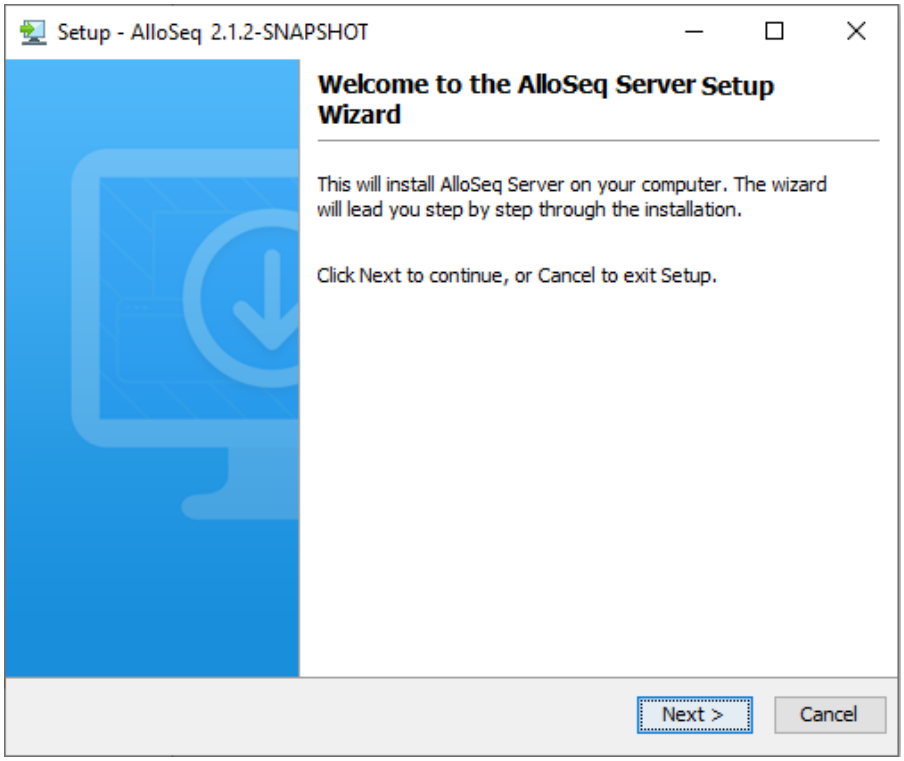
2.4 Client Installation - Overview

The AlloSeq client installer does not have any special access or privileges. It is also recommended to install a client on the server computer.

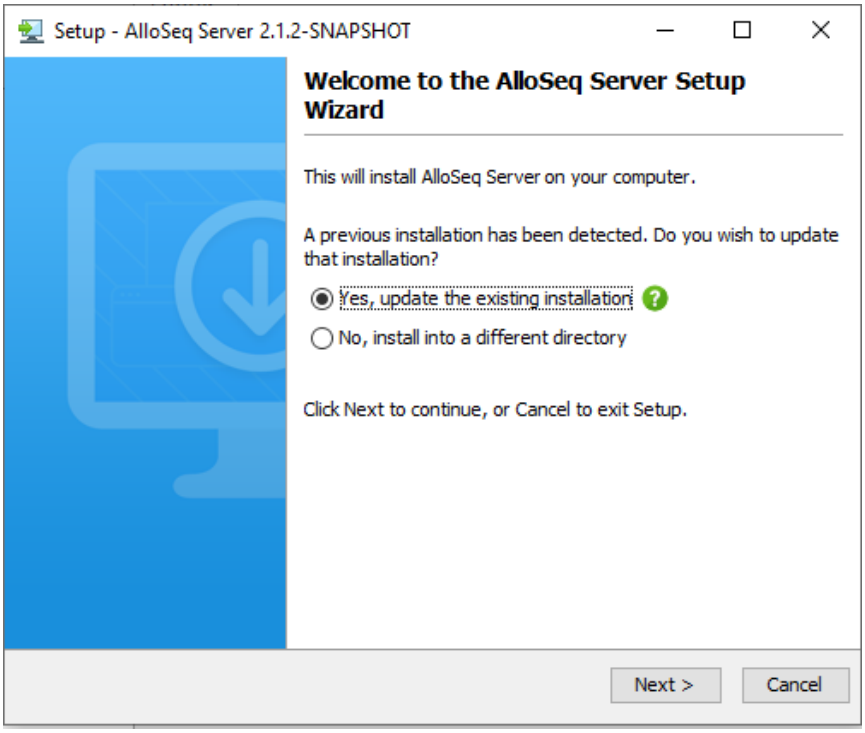
2.5 Server Installation - Windows

The CareDx support team will send you a link to download the Windows installer for the AlloSeq server. This file is usually called `alloseqserver_windows-x64_2_0_0-a2cc84.exe`, where the `2_0_0` will be replaced by the latest version number if there is a new release of the software. The last part `a2cc84` is the 'build number', this will also be displayed in the software (in the Settings screen) and CareDx support may ask for this if you run into any issues.

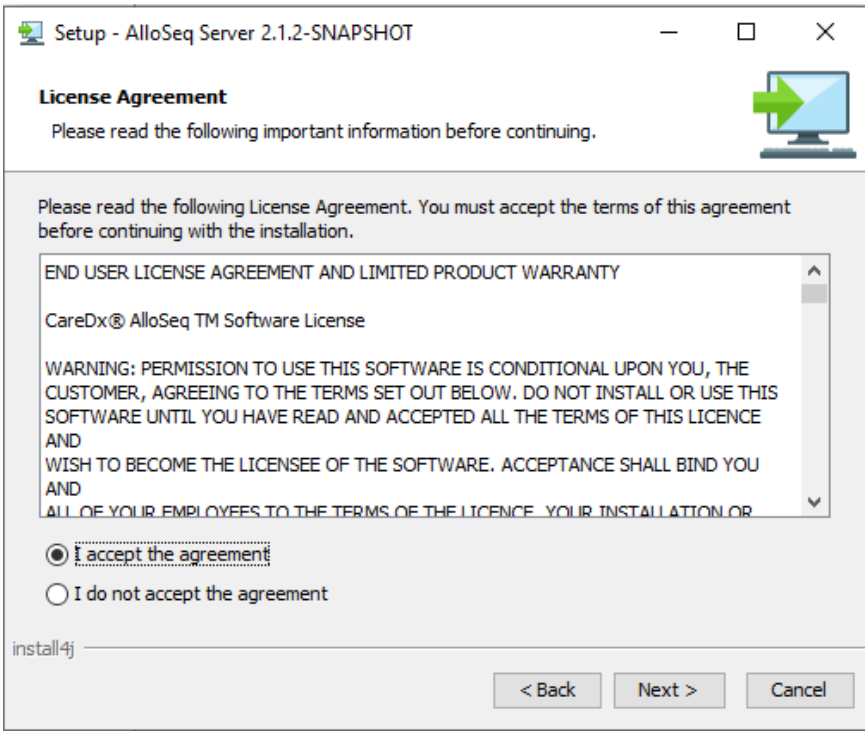
Once you have downloaded the software, please run the exe file by **right-clicking on it and selecting 'Run as administrator'**. The installer GUI will appear and will guide you through the rest of the installation process. If this is the first time installing the AlloSeq server, a Welcome screen will be displayed. Click Next to continue to the License Agreement screen.



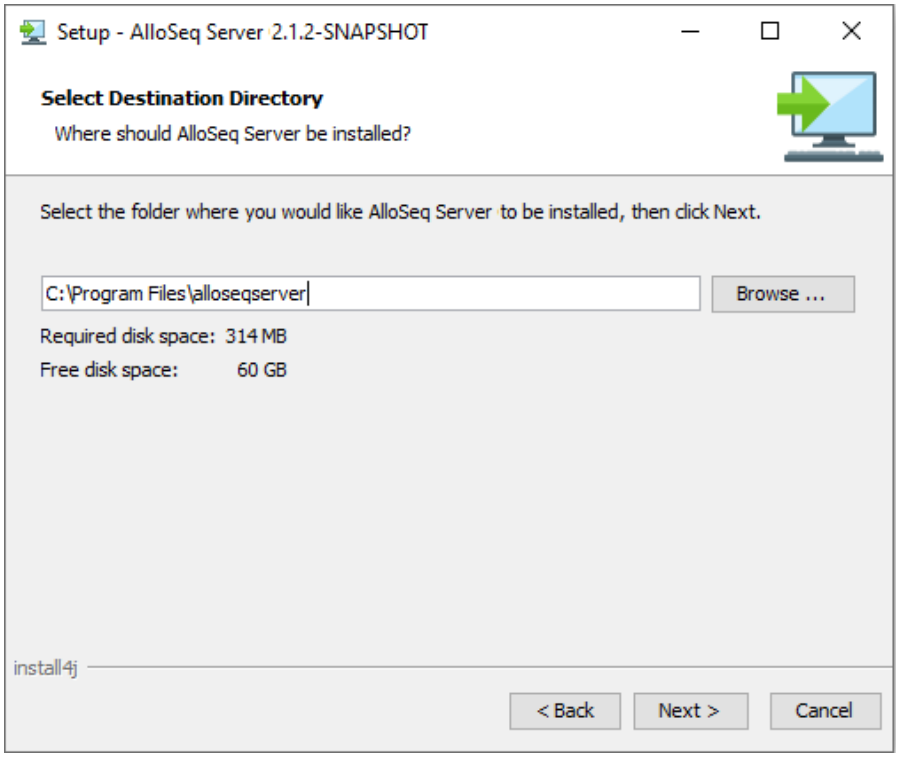
If you already have the AlloSeq Server installed and are upgrading to a newer version of the software, then you will be presented with a Choice Screen instead of the Welcome Screen.



If you wish to test the new version independently of the old version, then you can choose to install into a different directory, otherwise it is safe to choose the 'update the existing installation' option as all your data from the old version will be retained. Click Next to continue to the License Agreement screen.

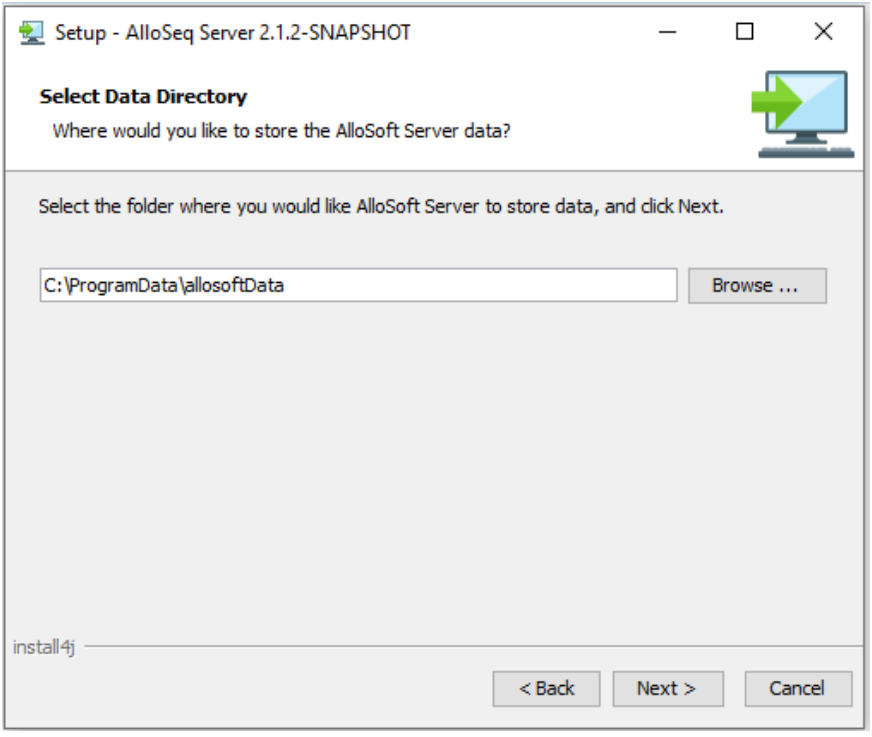


Select 'I accept the agreement' and click Next to continue to the Destination Directory screen.



There shouldn't be any reason to change this destination, unless you wish to have two versions of the AlloSeq server installed for testing a software upgrade. Please note that only one instance of the AlloSeq server service can be running at any one time.

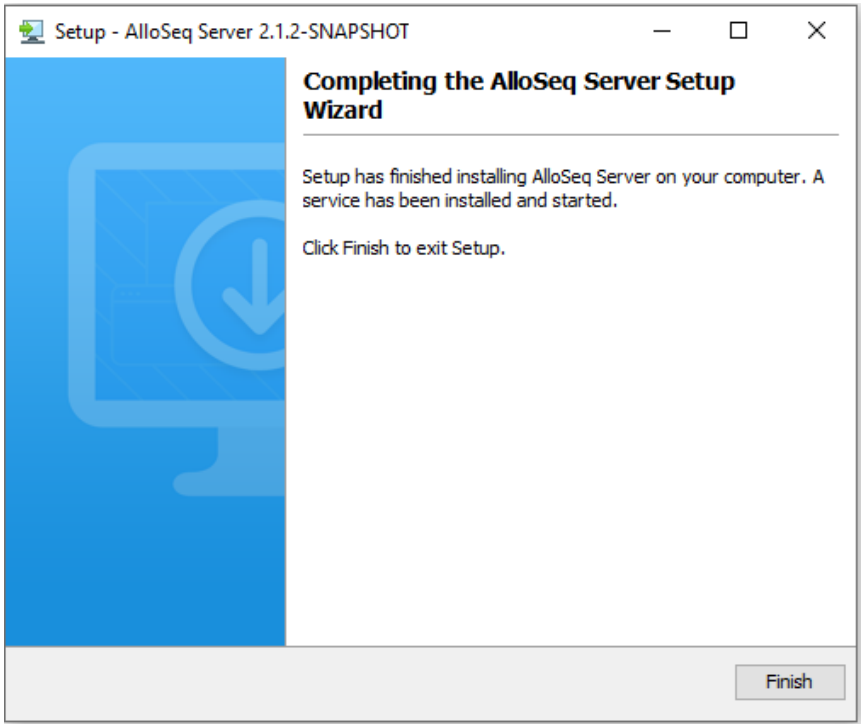
Click 'Next' to continue to the Select Data Directory screen.



Choose where you want to store the AlloSeq server data. This will hold all the data you enter into AlloSeq - i.e., the recipient/sample/batch metadata (in a small database), the results files for the NGS data analysis, and some other program metadata. Typically, you will want to keep this data from one version of the software to the next.

Important Note: This location must be a folder that is accessible by all logged in users of the server.

It is possible to point two versions of the AlloSeq server to a single Data Directory, however, only one version of the AlloSeq server service can run at any one time. It is usually sufficient to accept the default location in this wizard and select 'Next' to continue to the Finish screen, however if the computer has multiple users, you should ensure that all users have access to the chosen location.



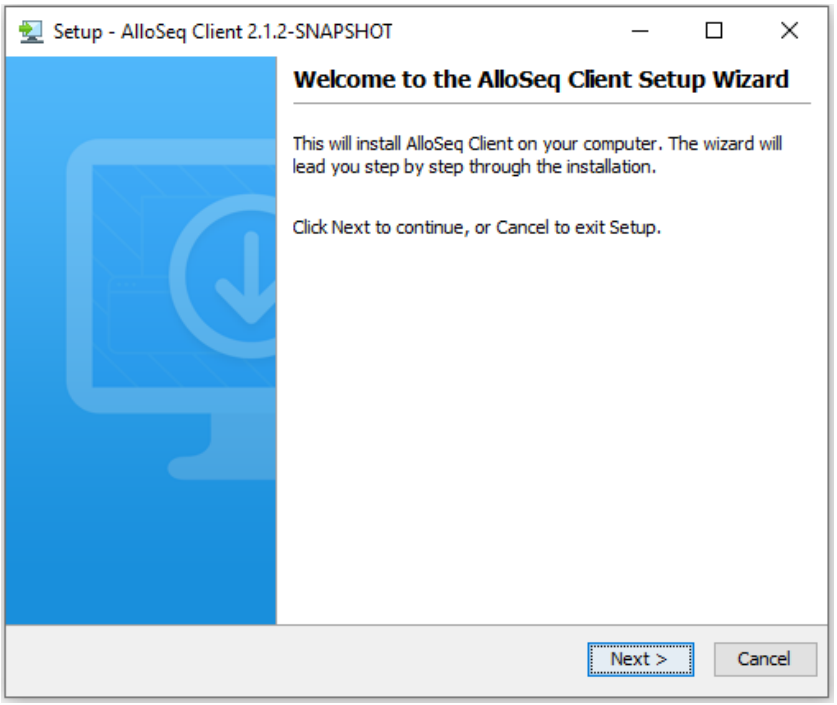
The service will automatically be started and is configured to also start automatically if the server machine is restarted.

After installation of the server an additional configuration step is needed. This configuration step is usually performed by an IT Support Engineer or Network Administrator, and only needs to be done once. The client communicates to the server via a single port (port 6606) on the server machine, so the server machine must be visible across the network to the clients and port 6606 must be opened to allow the client machines TCP access. This may also require some firewall configuration.

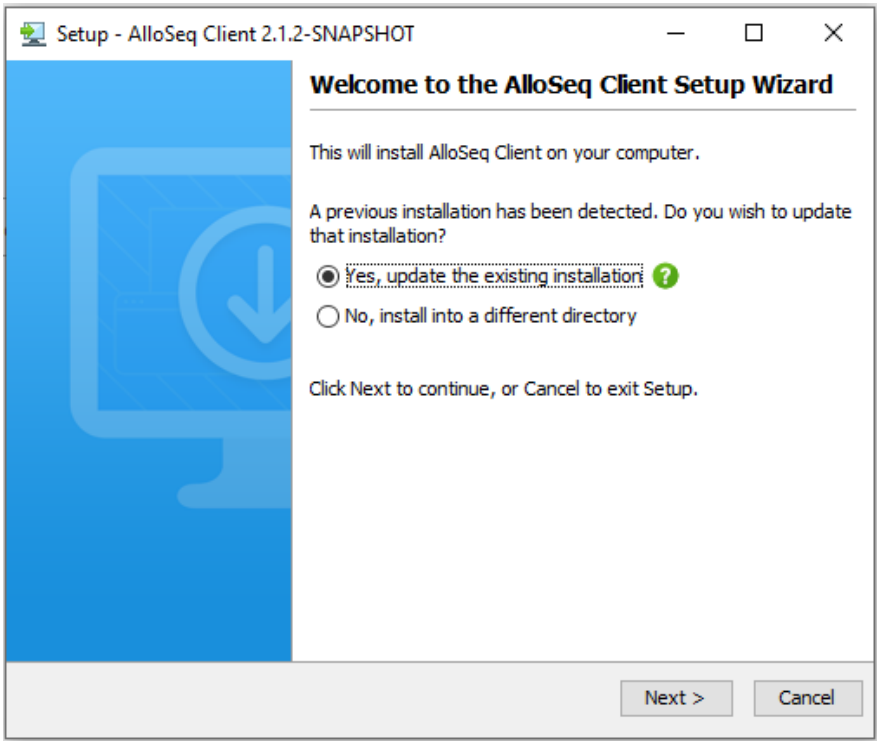
2.6 Client Installation - Windows

The CareDx support team will send you a link to download the Windows installer for the AlloSeq client. This file is usually called `alloseqclient64_windows-x64_2_0_0-a2cc84.exe`, where the `2_0_0` will be replaced by the latest version number if there is a new release of the software. The last part `a2cc84` is the 'build number', this will also be displayed in the software (in the Settings screen) and CareDx support may ask for this if you run into any issues. This build number should match for the Client and Server.

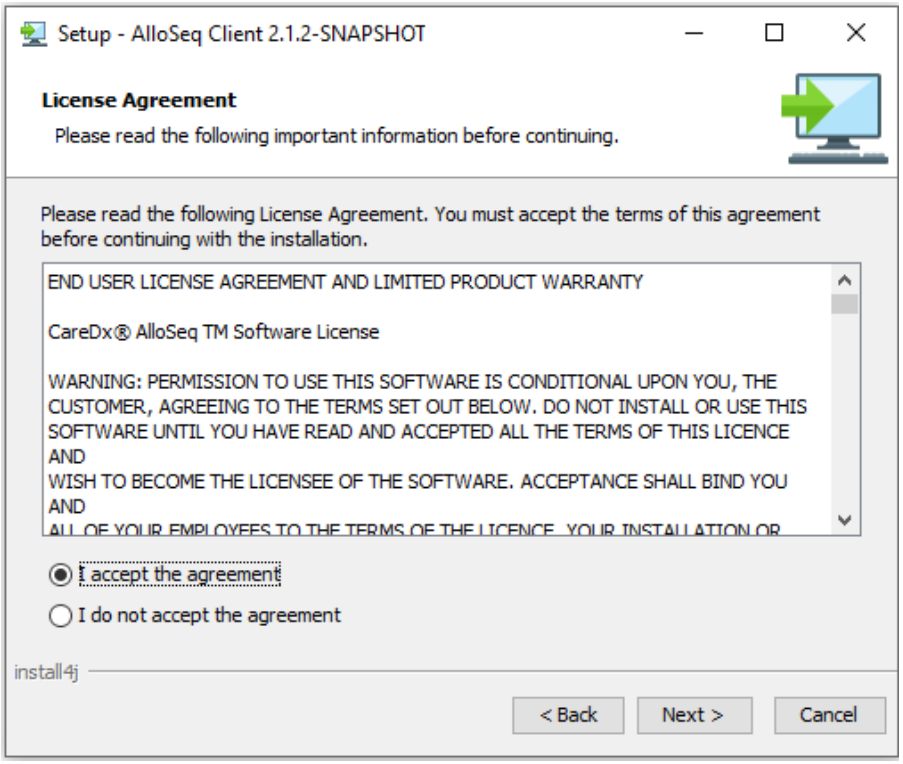
Once you have downloaded the software, please run the exe file by double-clicking on it. The installer GUI will appear and will guide you through the rest of the installation process. If this is the first time installing the AlloSeq client, a Welcome screen will be displayed. Click Next to continue to the License Agreement screen.



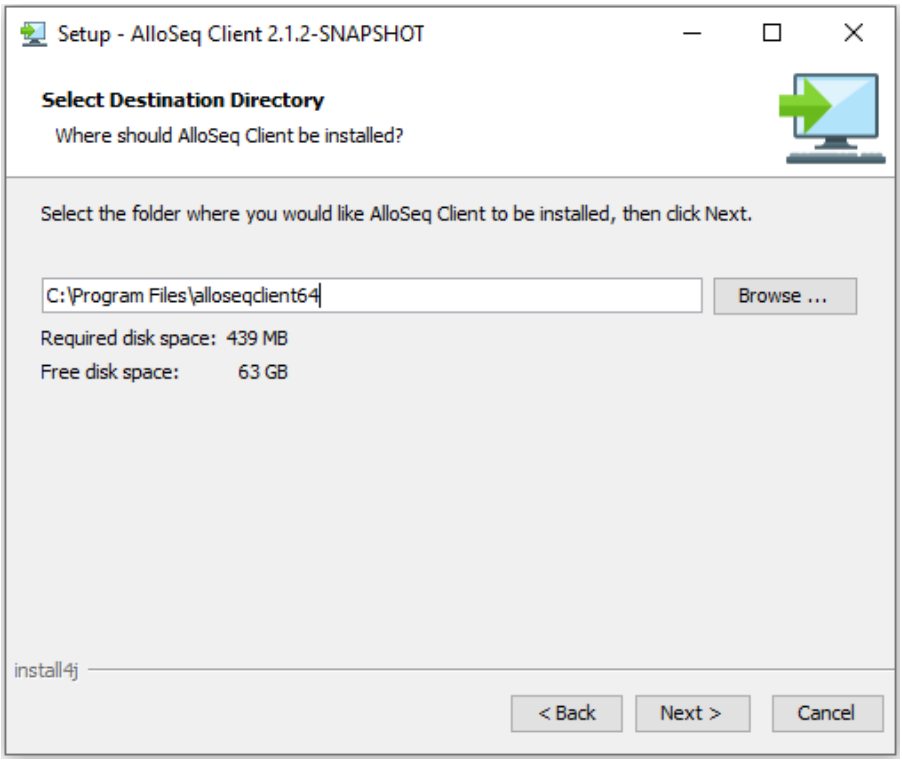
If you already have the AlloSeq Client installed and are upgrading to a newer version of the software, then you will be presented with a Choice Screen instead of the Welcome Screen.



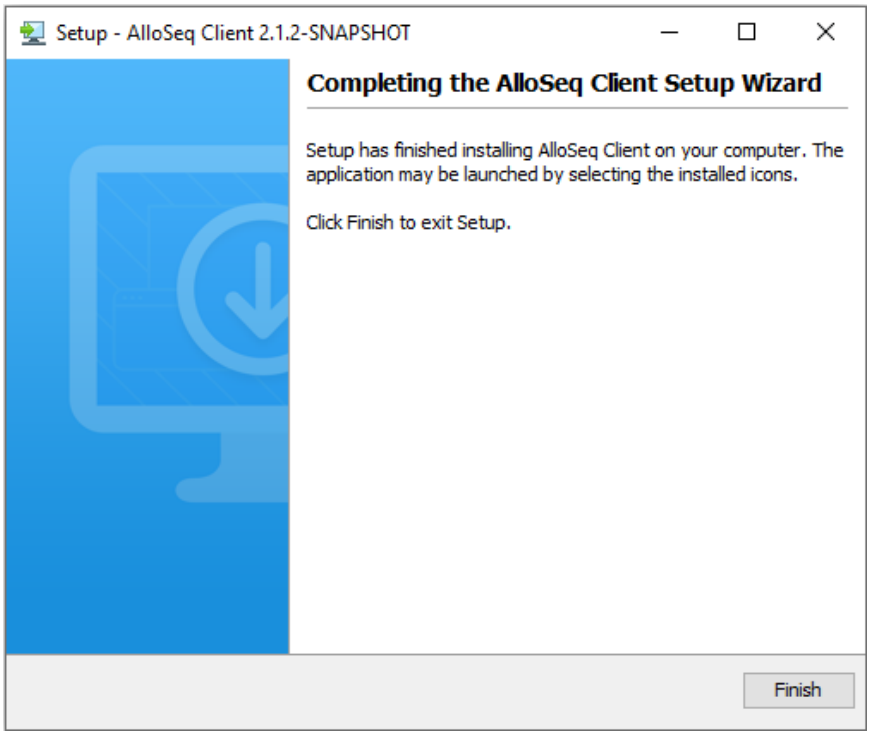
If you wish to test the new version independently of the old version, then you can choose to install into a different directory. Click Next to continue to the License Agreement screen.



Select 'I accept the agreement' and click Next to continue to the Destination Directory screen.



There shouldn't be any reason to change this destination, unless you wish to have two versions of the AlloSeq client installed for testing a software upgrade. Select 'Next' to continue to the Finish screen.



3. Operation

3.1 Connecting to the Server

This step only needs to be performed once per client installation.

When the AlloSeq client is started, it will try to connect to a AlloSeq server. If the AlloSeq server is installed and running on the same machine as the AlloSeq client, this should happen automatically. If the AlloSeq server is installed elsewhere, then the first step is for the client to be connected to the server.

This step is managed via the 'Server Setup' screen, which is accessible from the 'Login' screen on the client. The 'IP address' and 'port number' (6606) that have been configured for the server must be entered here. You will get this information from your IT Support or Network Administrator. Once you have entered this information into the screen, you can test the connection. If the AlloSeq server is found, then you will see a success message and can now login to the application.

3.2 Logging in (All Users)

You will need to login every time you access AlloSeq. If you are not the Lab Director, you should ask your Lab Director or IT Support to create a User account for you and give you the details for accessing this account. If you have forgotten your user details or password, you can ask the Lab Director or IT Support and they can reset these for you.

Step 3.2a - First time use (Lab Directors / IT Support only)

These steps are only required the first time you use AlloSeq in the lab. We recommend performing this step and step 3.2b below before allowing your lab staff to access AlloSeq. If you are the Lab Director and this is the first time you are accessing the AlloSeq application, you need to use the default Superuser account for the first login (only). You should log in to the AlloSeq server using these login details:

```
Username: superuser
Password: admin
```

This will log you into the AlloSeq server for the first time.

The next recommended step is to create a 'Lab Director' user, and then switch to using this user. At this point, you should also change the default password for the 'superuser' user.
To do these you should open the 'Settings - Users' panel. Select the superuser (it should be the only user in the list at this point) and choose the 'change password' function. Enter the new password twice and press OK. **Write this password down somewhere safe.** Note - we recommend giving the superuser login to your IT support team so they can keep it safe and help you with any issues you may have with the AlloSeq software.

Next, add a new user using the 'Add New' function. If you are the lab director, enter your details here, otherwise, enter the details of your lab director. This will include the username and password you wish to use to log in to AlloSeq.

Once the Lab Director user has been created, log out from AlloSeq, and then login again using your new user account details.

Step 3.2b - Configure AlloSeq (Lab Directors / IT Support only)

These steps need to be performed by the Lab Director and will only need to be repeated if the setup of the Lab changes.

Part 1 - Create New Users
Create one user for each member of lab staff you wish to allow to use the AlloSeq software. Follow the guidelines in step 2a above for creating users. You can ask your staff to come over and enter their own passwords or create passwords for them.

Part 2 - Perform Lab Setup
Navigate to the Lab Setup tab in the Settings. In here, you can tell the AlloSeq software about what your lab does, what equipment you have available, and how you want to use the software.

By default, the software assumes you have an Illumina MiSeq. If you have a different sequencer (or combination of sequencers) please enter these here.

By default, the software assumes you are working with data from both solid organ transplants and HCT. If you are only doing one or the other, please choose that option here.

Part 3 - Import License
The software comes pre-configured with an evaluation license file. This can be extended by requesting a new license file or license key from techsupport-labproducts@caredx.com.

Either download the file to your computer or copy the license key from the email. Open the 'License' section of the 'Settings'. Either browse to the location where you downloaded the license file, select the file and press 'Upload', or paste the license key into the box. If the license is valid and has not expired, you should see the details in the screen in front of you. If there is an issue, contact Technical Support.

3.3 Configure Preferences (All Users)

This step is optional. AlloSeq can be configured to use another language, and visual menu options can also be configured here to match your preferences. Go to the settings screen, the first tab allows you to choose the language and menu setup for AlloSeq.

3.4 Create Recipients and Samples

Once you have logged in, you can now start working with Recipients, Samples and Batches.

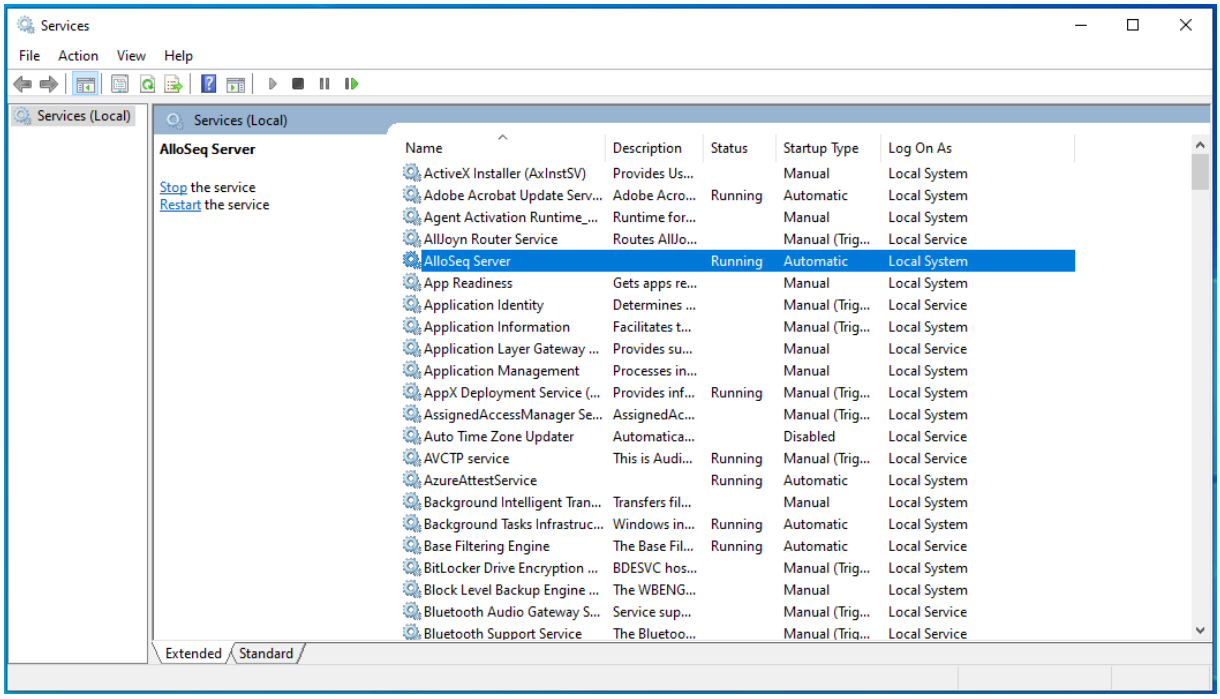
The steps to add new information in AlloSeq are very intuitive. There is a single round button 'shortcuts' menu on the main dashboard, that provides useful shortcuts to most of the day-to-day functions of the software. You can also use the main menu to the left of the screen to navigate to the Recipients screen and other main screens.

You can enter Recipient details at any time. You can add Samples to Recipients or Donors - or just add Control Samples - at any time. We recommend using the Batches function to batch all the samples you wish to run through the sequencer together.

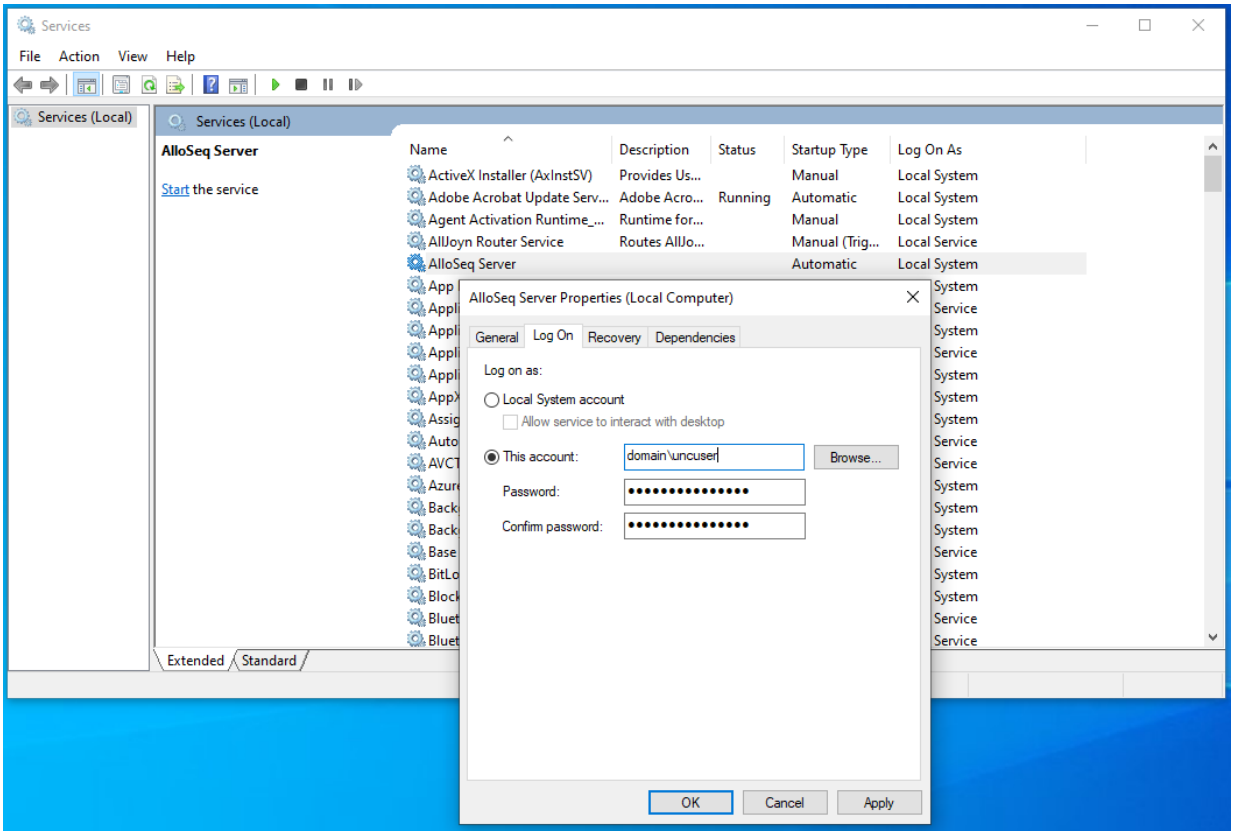
3.5 Configuring Mapped Network Drives

The AlloSeq Server is installed as a Windows service. Network shared drives are not visible to Windows services by default on start up, so It is recommended to create a new domain user who can reach the shared drives ('UNC shares') and start the AlloSeq Server with this domain user.

1. Create a new domain user with the proper permissions to access the UNC shares
    a. You may need the assistance of your IT support team for this and the next three steps
2. Stop the AlloSeq Server
    a. Open Services screen (e.g., services.msc in command line or start typing 'services' in the Windows search box)
    b. Right click on AlloSeq Server and choose 'Stop'



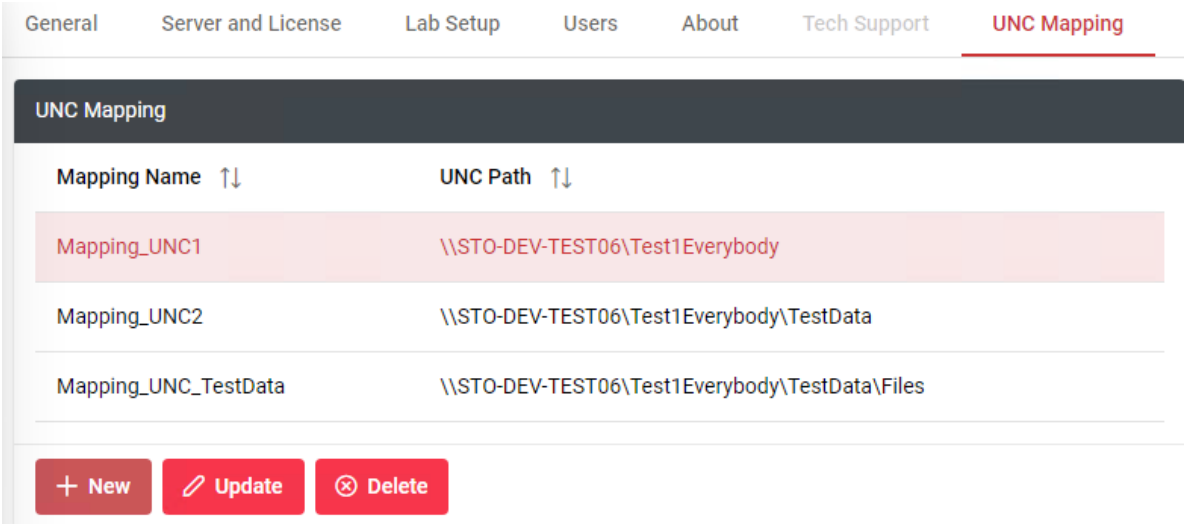
3. Configure the AlloSeq Server to be started by this new domain user
    a. Right click again on AlloSeq Server and choose 'Properties'
    b. Choose the 'Log on' tab
    c. Choose the 'This account' option and set username and password (twice) for the new domain user
    d. Click 'Apply' to save
    e. **Important note**: The AlloSeq server will be started with the given domain user - if this user does not have permissions to reach the AlloSeq data folder (see section 2.5 above) the server will not start!



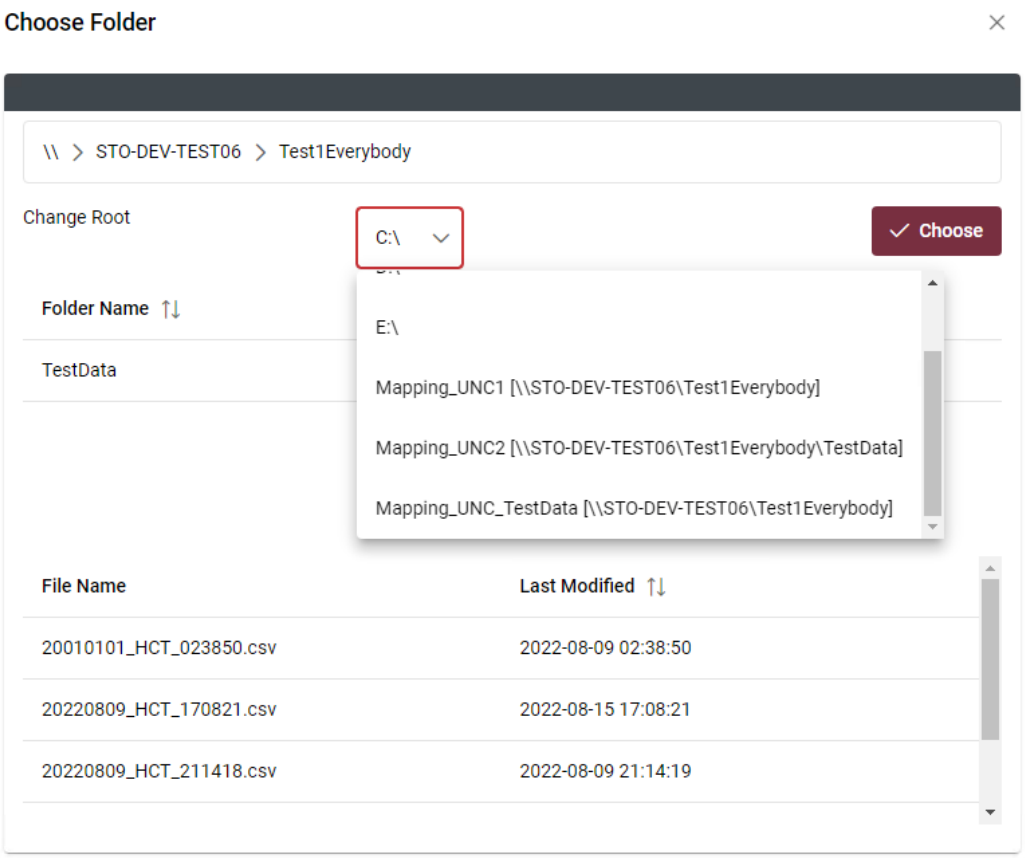
4. Start the AlloSeq Server
    a. Open Services screen
    b. Right click on AlloSeq Server and choose ‘Start’

Once the AlloSeq Server has been started with the correct domain user the UNC shares can be mapped in the AlloSeq software.

1. Open the AlloSeq Client, log in
2. Navigate to the Settings/UNC screen
3. Choose option - ‘New UNC mapping’
4. Enter Details:
    a. Name: my-shared-data (this is an example; you can choose the name)
    b. UNC path: \\my-server\my-shared\data
        i. You can copy the UNC path from Windows Explorer or from Total Commander.



5. You can now see your UNC share in the "Drives" dropdown in the file browser screen (for example in the Generate Run Sheet or Analyse Results functions from the Batch screen)



The approach outlined above is the recommended approach. Please see the Appendix if this approach is not suitable.

4. Usage Notes

4.1 Lifecyles

There are two small lifecycles in the software - the Batch lifecycle and the Approval (Task) lifecycle.

4.1.1 Batch Lifecycle

The Batch lifecycle consists of three steps - 'scheduled', 'protocol' and 'results'.

Each Batch that is created starts off being 'scheduled'. Once you are happy that all the samples you need have been included in this Batch, you can generate the run sheets (csv file for loading onto the Illumina sequencer) for all the Samples in the Batch, which will move the Batch into the 'protocol' step. The Batch details can be changed, and the run sheet can be regenerated if necessary. Finally, you can analyze and import results for all the Samples in the Batch together, which will move the whole Batch into the 'results' step, and the Batch details will no longer be editable. This phase will also trigger the 'approval' lifecycle for each sample tested in the Batch.

Note: If logged in as a Lab Director or Super User it is possible to trigger a reanalysis of the same batch.

Note: If logged in as a Super User it is possible to 'Rename Fastq' files to analyze samples from the version 1 HCT software or from another lab. Please ask CareDx support for guidance in using this function.

4.1.2 Approval Lifecycle

The Approval lifecycle applies to each test of a particular sample. The Approval lifecycle is initiated at the end of the Batch lifecycle (see above), i.e., once we have results for a particular test of a sample. At this point these test results become 'Tasks' and are assigned to users to be handled.

The Approval/Task lifecycle consists of five steps - 'scheduled', 'assigned', 'ready', 'approved', and 'exported'.

When the results of a Batch are analyzed, the software automatically moves all the Tasks from 'scheduled' to 'assigned' and assigns the Tasks to the user who performed the results analysis.

These Tasks can be 'assigned' to other users for checking. Once the results have been checked, they can be set to 'ready' (i.e., 'ready for approval'), which will automatically re-assign the Tasks to the Lab Director. When the Lab Director logs in they will be able to see all the tasks that are 'ready' for approval and check these, moving them to 'approved' once they are happy, and then finally 'exporting' the results from AlloSeq for filing and/or for entry into a LIMS system.

4.2 Results Review

There are two main surveillance analysis algorithms built into AlloSeq, called the 'blind' and 'targeted' analysis.

4.2.1 Blind Analysis

This is automatically run for every sample. This analysis calculates the second strongest 'percent DNA' value as a proportion of the strongest signal present.

Limitations of the blind analysis

The blind analysis cannot distinguish which of the signals belongs to the donor or recipient.

- For HCT testing, there could have been a major relapse and the stronger signal could now be the recipient. Because of this, it is safest to genotype the recipient and/or donor (preferably both) before starting surveillance.

The blind analysis works best with lower 'percent DNA' values.

- If the percent DNA rises above 30% (which can be seen with a relapse) the blind analysis will start to struggle to distinguish background heterozygosity and actual signal and the accuracy of the analysis will decrease.

4.2.2 Targeted Analysis

The targeted analysis will only be run if either or (preferably) both recipient and donor have prior genotyping information entered into AlloSeq. In this case, the targeted analysis is run in addition to blind analysis, and the targeted analysis results will **override** the blind analysis results and become the percent DNA and coverage results displayed. The targeted analysis uses the information provided by the genotyping and will only analyze the 'informative' markers – those that are unique (either homozygous or heterozygous) for one party and homozygous identical for all other parties.

5. FAQs

This section contains frequently asked questions and their answers.

The AlloSeq Client can't connect to the AlloSeq Server?

Check that the server is running (check the service), and that both the server machine and port 6606 on the server machine are visible and accessible across the network to the client (you may need to ask for help from IT support for this).

The AlloSeq Client is connected but I can't log in?

This is likely due to an incorrect combination of username and password. If you can't remember your username, ask your lab director or IT support to check it for you and if necessary, reset the password for you.

Can AlloSeq be run on multiple computers?
Yes. There needs to be a single server computer where the analyses will be run, and this will need to be 64-bit Windows. The client software can be installed and run on any computer that is on the same network as the server.

Do I need a license to run AlloSeq?
Yes. You will be sent a license file by the CareDx support team when you get started. The license file expires once a year, and a new license needs to be imported. The license import is in the Settings within AlloSeq.

What's the maximum number of users that can use AlloSeq at the same time?
There is no theoretical limit to this. In practice, if a lot of users (more than 10) are using AlloSeq at the same time you may experience some slowing down of the user interface.

Can I export my results to a LIMS system?
Results can be exported in csv or tsv format. If this is not sufficient or you would like to see this feature extended within AlloSeq for a particular LIMS system, then please contact techsupport-labproducts@caredx.com.

Can I import my patient data from a LIMS system?
This feature is envisaged for a future AlloSeq version. If you would like to see this feature implemented within AlloSeq for a particular LIMS system, then please contact techsupport-labproducts@caredx.com.

6. Advanced Tip and Tricks

6.1 Navigation

There is usually more than one way to move around or trigger functions in AlloSeq.

- Most of the tables have 'right click' pop-up menus with advanced options.
- Many of the display items can be clicked on to see more detail about that item.
- The buttons often have menus attached with more advanced options.

Clicking on the CareDx logo in the top left corner will take you back to the main Dashboard.
There are 'back' and 'forward' buttons on the top task bar.

7. Contact Information

Manufacturer:

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Website: http://www.caredx.com

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Fax: +46-8-717 88 18
E-mail: orders-se@caredx.com
Website: http://www.caredx.com/

Americas
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901 S. Bolmar St., Suite R, West Chester, PA 19382
Tel: 1-877-OLERUP1
Fax: 610-344-7989
Email: orders-us@caredx.com
Website: http://www.caredx.com

Technical Support:
Email: techsupport-global@caredx.com

For more information, please refer to the CareDx website (https://www.caredx.com/contact-us/).

Related Products:
AlloSeq HCT

8. Appendix

There is more than one method for allowing mapped network drives to be visible in the software. The older, alternative procedure below still works and can be used if the recommended method from Section 3.5 above is not possible for some reason.

8.1 Configuring Mapped Network Drives (Alternative Procedure)

The AlloSeq Server is installed as a Windows service. Network shared drives are not visible to Windows services by default on start up, so this procedure needs to be run to make these shared drives visible to a Windows service permanently. We recommend asking your IT support team to run this procedure, this only needs to be performed once.

1. Login as the NT AUTHORITY\SYSTEM account.

2. Download the Sysinternals Suite from Microsoft and unzip it to a directory say C:\SYSTEMP.

The following steps assume that you've unzipped SysInternals to C:\SYSTEMP and all the executables are in there.

3. Launch a command prompt as Administrator.

Start typing 'command prompt' into your windows search and select 'Run as Administrator' option

4. Open the folder where the files were unzipped, type into the command prompt:

CD \SYSTEMP

5. Then type in the following:

PsExec64 -i -s cmd.exe

This launches a new command prompt window and step 6 should be performed in that new window.

6. Type in the following in the new command prompt window:

net use Z: \\servername_OR_IP\sharedfolder /u:username /persistent:yes

Changing the drive letter, server name and shared folder name to match your needs, using the username of a user having access to the shared folder.

7. Restart the AlloSeq Server service

Open the Services list, find the 'AlloSeq Server' in the list, right click and choose the 'restart' option.

8. Create a script

Create a script that runs on computer start-up that has only the full 'net use' command from step 6 in it, according to Microsoft's article: Assign Computer Startup scripts.

This will ensure that the mapped drives are still visible to the Windows services once the server is restarted.

Method history

Version	Date	Modification
1.0	01Dec21	First version of the AlloSeq Software Installation Guide – refer to CR-2021-090. ISSUED on 01Dec21
2.0	05Feb22	v2.1 release, new instructions for mapped drives. Refer to CR-2022-004. Reissued 11 Feb 22
3.0	02May22	Add Section 2" Please contact CareDx should you have any issues with the Installation process" Reissued on 04May22
4.0	30Aug22	V2.1.2 patch release, added new UNC mapping approach for mapped drives, moved older approach to appendix Reissued on 21Sep22.