ChimerisMonitor RUO

Handbook (HB)

RUO

For research use only. Not for use in diagnostic procedures.

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LOT 3.0.7 and higher (software version)

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Notice of Change

Please note the following adaptions compared to the previous handbook version:

Document code	Changes	Date
CSMHB01v1en	Initial version	02.04.2025
CSMHB01v2en	Change of ordering number Mentype® DIPscreen PCR Amplification Kit	29.04.2025
CSMHB01v3en	Specification of procedure for Mentype® DIPquant, removal of CE import button within Patient Editor	21.08.2025

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for "ChimerisMonitor RUO", herein referred to as SOFTWARE

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October 2024

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Product Description

ChimerisMonitor RUO is a professional software for automated chimerism analysis which combines speed and accuracy with an intuitive and customized interface. The software mediates analysis of chimerism samples from initial genotyping to highly sensitive quantification of residual donor or recipient cells.

ChimerisMonitor RUO enables the analysis of data obtained with Mentype® Chimera® PCR Amplification Kit, Mentype® DIPscreen PCR Amplification Kit or Mentype® DIPquant.

The software is only intended to be used for research purposes, a use for diagnostic purposes is not permitted.

The software should only be used by professional users trained in molecular-genetic techniques.

Summary and Explanation

ChimerisMonitor RUO is an advanced software for an automated data analysis, evaluation of electropherograms and chimerism calculation. The integrated Patient Management system allows to monitor chimerism kinetics in high resolution reports, but also in graphs and tabular visualization. Within each patient the transplantation history and chimerism kinetics over time can be assessed. Informative markers are logged prior the transplantation according to the respective donor profile. After the transplantation, a semi-quantitative analysis of selected informative markers (Mentype® Chimera® or Mentype® DIPscreen PCR Amplification Kits) or relative quantification of allele-specific markers (Mentype® DIPquant) can be carried out and patient or donor chimerism [%] is calculated as mean and for each marker respectively.

Required evaluation templates for fragment length analyses of multiplex PCRs are included in the Test Kit Management system of ChimerisMonitor RUO. Those contain analysis methods as well as linked Bin and Panel templates. The software is performing a general, integrated run and sample validation during the batch import based on the either the assay-specific

requirements of Mentype® Chimera® PCR Amplification Kit and Mentype® DIPscreen PCR Amplification Kit or according to own requirements. In addition, the quality of run and sample data can be assessed visually in 5 panels of electropherograms (6-FAM™, BTG, BTY, BTO, BTR) as well as via size calling regression.
For high sensitive relative quantification of allele-specific DIPs (Deletion-Insertion Polymorphism) after transplantation, qPCR-results from Mentype® DIPquant analyses can be imported and evaluated. After genotyping the donor and patient using the Mentype® DIPscreen PCR Amplification Kit, the DIPselector tool identifies informative and available DIPs to support the selection of suitable marker sets for further analyses. Patient-specific or donor-specific Mentype® DIPquant assays can be used for ongoing monitoring and saved within the same patient record, enabling seamless switching between semi-quantitative monitoring with Mentype® DIPscreen for all chimerism ranges and highly sensitive qPCR analysis with Mentype® DIPquant assays.

Scientific Background

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a treatment option to cure patients with non-malignant and malignant hematological diseases, such as leukemia. Chimerism analysis is used to determine the mixture of donor and recipient hematopoietic cells in allo-HSCT recipients to detect early signs of graft rejection. Human peripheral venous blood is used for genotyping and monitoring. According to the CLSI Guidelines (MM05-A2, 2nd edition) anticoagulants like EDTA and citrate are recommended for blood collection. Depending on the success of transplantation, different forms of hematopoietic chimerism (complete, mixed or loss) can develop. Different approaches are used for chimerism analysis, including fluorescence in situ hybridization (FISH), restriction fragment length polymorphism (RFLP), blood count analysis and PCR-based methods. Currently, PCR-based amplification of short tandem repeat (STR) polymorphisms is the golden standard for chimerism analysis. To detect early signs of graft rejection, chimerism analysis should be done at regular intervals and shortly after the allogeneic HSCT.

Materials provided

The ChimerisMonitor RUO Software is available for download via www.biotype.de/en/products/chimerismonitor.

Material and devices required

License keys

ChimerisMonitor RUO is a license-based software application. Trial licenses, 1-year or 3-year licenses can be ordered via sales@biotype.de (for details see Table 1). The local system-identificator, the order number, desktop or client application and desired type of license must be included when ordering.

For detailed information about how to activate the software with a license key, please refer to chapter Activating software with license.

NOTE

The validity of license keys is displayed in the bottom bar. If the license is expiring within the next two months, the days until expiration are counted down.

Table 1. Ordering information licenses ChimerisMonitor RUO

Licenses	Supplier	Order number
ChimerisMonitor RUO - Trial version - 1-year license - 3-year license	BIOTYPE GmbH	46-14801-0000

Kits supported by ChimerisMonitor RUO

The software ChimerisMonitor RUO is an application that supports the data analysis of the assays Mentype® Chimera® PCR Amplification Kit, Mentype® DIPscreen PCR Amplification Kit and Mentype® DIPquant as described in Table 2.

Table 2. multiplex PCR-Assays supported by ChimerisMonitor RUO

Reagent	Supplier	Order number
Mentype® Chimera® PCR Amplification Kit (CE-IVD)	BIOTYPE GmbH	45-12200-0025 45-12200-0100 45-12200-0400
Mentype® Chimera® PCR Amplification Kit (RUO)	BIOTYPE GmbH	45-12211-0100 45-12211-0400
Mentype® DIPscreen PCR Amplification Kit (CE-IVD)	BIOTYPE GmbH	45-12300-0025 45-12300-0100
Mentype® DIPquant (RUO)	BIOTYPE GmbH	45-015xx-0025* 45-01591-0100

*xx describes locus specific ordering number as detailed in Table in Appendix.

System requirements desktop version/ database computer

Table 3. System requirements desktop version/ database computer

Specification	Requirements
Operating System	Windows 10 or 11
Free Harddisk	1 GB + Database
Processor	2 GHz Dual-Core
RAM	4 GB RAM

System requirements client computer

Table 4. System requirements of client computer

Specification	Requirements
Operating System	Windows 10 or 11
Free Harddisk	1 GB
Processor	2 GHz Dual-Core
RAM	2 GB RAM

Input data

For fragment length analyses, the software uses fsa-files generated on Genetic Analyzers of Thermo Fisher Scientific (Applied Biosystems division). The data import is carried out in batch. In the process the run evaluation is carried out based on the respective requirements of the test kit.

For qPCR analyses, the software uses txt-files, exported from a standard qPCR cycler. Consider cycler-specific file structure and column assignment for patient-specific import.

Warnings and Precautions

- Read the Handbook carefully before using the product.
- Before the first use, check the system requirements. Consult your local IT for installation procedures and refer to chapter Installation. Administrator rights are needed for installation.
- The user is responsible for installing the application in a secure environment with regard to the operating system, network and data backup and for taking appropriate Cybersecurity measures.
- ChimerisMonitor RUO is a license based software application, please include the system-identificator, order number, use with local database or as network database and desired type of license in your order.
- If personalized access to the software is unauthorized or restricted, please contact the software administrator.

Installation

Installation process

The present software can be installed either as a desktop or network version. Decide which version is needed, before the application is installed.

Within the desktop version the database is installed locally on a computer. Other users have no access to this database. Using a network version and a central database for several clients, no separate database is created on the individual computers in the network.

Before installing the software, please close all active applications to prevent potential conflicts or errors during the installation process.

NOTE

You need administrator rights to install the software. The installation of the present software is to be carried out only by IT personnel. For installation, data backup and validation of the software and therefore for the integration of the software into the existing software environment and in the applied quality management system, the user is responsible and accountable.

1. Start the installation by executing the ChimerisMonitor RUO.exe.
2. Choose your preferred installation language (English).
3. The installation assistant will guide you through the setup. Click ***Next*** to go on.
4. Read the license terms carefully and accept them by clicking on ***I Agree*** to continue with the setup.
5. Select a destination folder where the ChimerisMonitor RUO Client program will be installed into.
6. Select the Start Menu folder where the programm shortcuts will be created.
7. Select the type of installation of ChimerisMonitor RUO. The components to be installed will be pre-selected accordingly:
    a. Desktop (default): For single user installations. All components will be installed on the same computer.
    b. Client/-Database: For multi-user installation, if different users are working with separate client PCs and the database will be installed on a central server. Select ***Database*** when installing the database on the central server. Select ***Client*** to install the client application on the user computers.
8. Select ***Install*** to proceed. The installation progress will be shown in detail in the installer's console window.

9. During desktop or database installations, the installer verifies if a former ChimerisMonitor 2.1 database exists locally on the computer. The database can be selected for the import into ChimerisMonitor RUO.
10. After successful installation the installer allows the creation of an additional desktop shortcut.
11. If an error occurs during the installation, the process will be stopped and the installation is terminated by clicking on ***Cancel***. The console content can be copied by right-clicking and may be saved for further analysis.

Import of the ChimerisMonitor 2.1 database

The import of an existing ChimerisMonitor 2.1 database is handled by the installer during the installation process. The import is only possible during the first time installation of ChimerisMonitor RUO. The installer searches only in the ChimerisMonitor 2.1 default database folder C:\ProgramData\Biotype\ChimerisMonitor\database for an existing ChimerisMonitor 2.1 database.

If a ChimerisMonitor RUO or ChimerisMonitor IVD database is already existent, the import option is no longer available and the step is skipped during the installation.

Activating software with license

The software checks on login if a valid license is installed. If there is no valid license found, a dialog is presented showing the System-Identificator for the local system. The System-Identificator is necessary to order a license key. In order to activate the software, the purchased license key must be copied into the field ***License*** (see Figure 1). Click ***OK*** to unlock the application.

Alternatively, the application can be opened in read-only mode, where no changes to patient data is possible. The read-only mode allows only viewing and filtering within the Patient Management, opening the Patient Editor as well as Report generation and CSV export.

Login

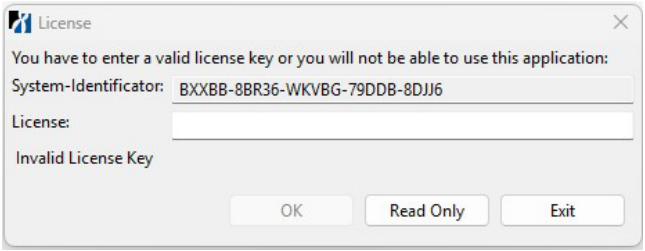


Figure 1. Licence key dialog

ChimerisMonitor RUO is a password protected application. Thereby the software supports the setup of users with optionality of administrator rights.

User specific logins enable the traceabality of Batch Import and Report generation.

NOTE

For creation and management of user profiles please refer to chapter User Management.

It is recommend to add new users after the first login.

For the first login please use the following data:

Table 5. First login data

First user name	admin
First password	admin
Server	localhost resp. IP address*

*Select the server localhost if using the desktop version. If a central database is used in the network, the IP address of the database computer must be entered as server

Click ***Finish*** to login.

NOTE

The administrative user is responsible to change the generic first login password to a personal and secure one.

Overview of the Workflow - Quick Guide

Table 6. Quick guide for automated chimerism analysis

No.	Icon	Working step
1		Sample Import
		***Create new patient***. A database of all created patients is represented in the Patient Management
		***Batch Import:*** - Select the test kit ***Biotype Mentype Chimera*** or ***Biotype Mentype DIPscreen*** - All thresholds for the correct run and sample evaluation are linked to the respective analysis method. - Import a run containing fsa-files of the allelic ladder, positive control, no-template control, and the samples. - Select sample types manually (essential for correct peak assignment and chimerism calculation) - General run evaluation is carried out by the software
		Open the ***Batch Import Management***
		***Assign Sample:*** Select a sample and assign it to the patient
2		Check controls – ChimerisMonitor RUO performs an integrated quality check and a run evaluation based on the test kit requirements
		Check the ***Allelic Ladder Electropherogram*** and ***Size Calling Regression*** Possible quality warnings are displayed… - Within tab ***Run Validation*** during the Batch Import - Within tab ***FSA Import Error and Warnings*** in the Patient editor

No.	Icon	Working step
		Check the ***Positive Control Electropherogram*** and ***Size Calling Regression*** The ***Run Validation*** during the Batch Import displays possible quality warnings.
		Check the ***No Template Control Electropherogram*** and ***Size Calling Regression*** The ***Run Validation*** during the Batch Import displays possible quality warnings
3	Sample evaluation	
		Check the ***Sample Electropherogram*** A correct peak assignment is essential for an accurate definition of informative markers and a robust chimerism calculation. The ***Sample Quality check*** during the Batch Import displays possible quality warnings
		Check the ***Sample's Size Calling Regression*** The ***Sample Quality check*** during the Batch Import displays possible quality warnings
4	Definition of informative markers	
		***Create a new transplantation:*** Predefined markers can be selected for patient monitoring
5	Chimerism Analysis	
		***Calculate Chimerism:*** See preselected markers for chimerism analysis and carry out chimerism calculation (single marker chimerism, total chimerism and standard deviation)

No.	Icon	Working step
6		qPCR Sample Import
		***Create new qPCR sample*** Select all Cp values per patient for import. Chimerism calculation is carried out by software automatically.
7		Report
		***Create Report***: Single values and chimerism kinetics are displayed over time (table and graph, file format pdf or ***Export Patient*** function also in csv)
8		Build a database-driven system for ***Patient Management***

User Interface

The user interface of ChimerisMonitor RUO is organized in several sections. These display detailed patient information, Patient Management and detailed sample or transplantation information. The toolbar includes several general functions for the data and patient management. All sections are defined in Figure 2.

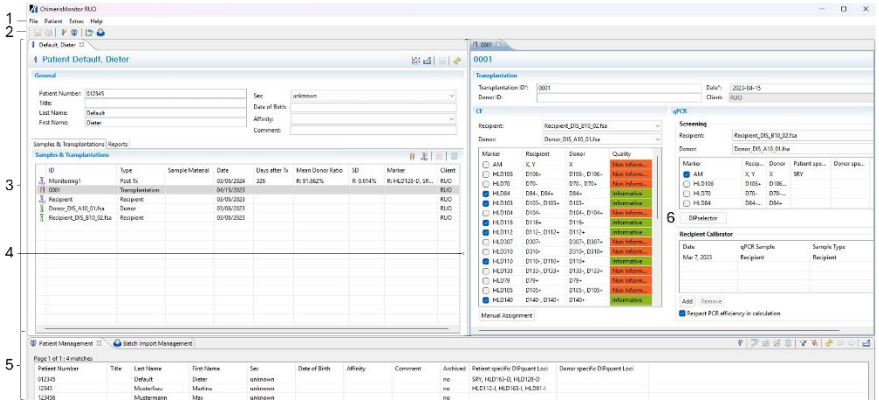


Figure 2. Sections of the user interface

Table 7. Description of the User Interface

No.		Description
1	Menu Bar	The menu bar is situated in the upper range of the main window directly below the title bar. It includes different menus, like ***File*** and ***Extras***, which give access to specific functions.
2	Toolbar	The toolbar consists of several buttons, tagged with icons. These buttons give access to functions of the program and can be active (coloured) or inactive (grey). Toolbars exist in many parts of the software, for instance within specific overviews or editors.
3	Patient Editor	The window Patient Editor can be displayed after a patient was created or opened from the ***Patient Management*** via double click on the selected patient in the table or the icon ***Show Patient***. The Editor displays general information on the patient and a tabular list of samples and transplantations. Within the Patient Editor the ***Sample- and Transplantation Editor*** is accessible via a double click on the respective line in the table.
4	Sample/ Transplantation Editor	Sample- and Transplantation Editor are windows, which display details of the specific dataset. The ***Sample Editor*** displays general information on the sample, a toolbar and depending on the sample type also detailed Chimerism calculation values. The ***Transplantation Editor*** displays general information on the event, an assignment of donor and recipient sample as well as the selection of informative markers. For detailed information please refer to chapter Sample Editor or Transplantation Editor.
5	Management	The Management section displays collections of specific datasets. ***Patient Management*** (see Figure 2) shows a collection of all patients and general information like name, sex or date of birth. ***Batch Import Management*** displays all imported run data. Within this overview single samples can be assigned to the respective patient. ***User Management*** displays an

No.		Description
		overview of all created user accounts with their respective rights and allows editing as well as creation of user accounts. ***DIP Management*** displays the PCR efficiencies for all available Mentype® DIPquant. Assay markers. ***Sample Material*** enables the addition and activation/deactivation of selectable sample material within the ***Sample Editor.***
6	DIPselector	The DIPselector is ranking the informative DIP markers and the respective specific Mentype® DIPquant assays for their suitability to analyse chimerism with qPCR. The Mentype® DIPquant assays that are suited best, appear at the top of the list.

The user interface of ChimerisMonitor RUO offers extensive possiblities for rearrangement, allowing users to make adaptations to their personal preferences. Windows can be rearranged within the software by drag and drop.

Dialogs and Assistants

Dialogs are windows that are detached to the main window. They can be displaced from the main window and moved independently.

Assistants are dialogs with several steps through a workflow. Within this manual both terms will be used synonymously. Dialogs can be used for adding or visualizing data or they assist calculation or reporting procedures.

While a dialog is open or a process is ongoing, the access to the main window is locked.

Windows

Windows show data and enable its editing.

- Closing Windows

  Windows stay active until the window is closed or the program is exited. Individual windows can be closed by clicking the ***Close*** button next to their window title. Alternatively, use the pop-up menu of the window title to close windows by right click. Windows are not closed automatically when a new one is opened. Tabs allow switching between different windows. The order of the tabs can be be changed by dragging and dropping.

Table 8. Functions within windows pop-up

Function	Description
Close	Closes the selected window
Close other	Closes all windows in the editor area but the one selected
Close all	Closes all windows in the editor area

- Adjusting width and height of a window

  To adjust the window size, place the mouse pointer onto the border of the window. A double-headed arrow allows changing of the window size to desired parameters. Use functions like Minimize and Maximize or double-click on on tabs to adapt the window size. After a window is maximized, the whole main window is occupied. This can be reversed by clicking on the Restore button.

- Relocating windows

  To relocate windows, click on the corresponding tab and move it by dragging and dropping.

- Detaching windows/editors

  Editors can also be detached from the main window and the general user interface. To detach an overview, open the pop-up menu and select the item ***Detached***. Repeat the procedure to reverse the display.

Tables and sections

Tables and sections are displayed in different windows. They are used to collect detailed information about data or patients. To analyze your patient data as conveniently and effectively as possible, the following functions can be used:

- Fade in, fade out sections

  Labelled sections in editors show blue arrows. Sections can be faded in and out by clicking on the arrow icon.

- Adjusting width of table columns

  To adjust a column width, place the mouse pointer onto the border of the column. A double-headed arrow allows to change the column width.

- Selecting elements

- Several elements can be selected at once by clicking and holding the CTRL key while clicking on the desired elements. Within the Batch Import Mangement only single files can be assigned to each patient.Shortcuts

  Several shortcuts can be used to access several functions of ChimerisMonitor RUO. They are based on Windows standards and are listed in Table 9.

Table 9. Shortcuts for ChimerisMonitor RUO

Shortcut	Function
CTRL + A	Select all
CTRL + S	Save
CTRL + Shift + S	Save all
CTRL + F7	Change view

Functions of ChimerisMonitor RUO

Basic functions of the menu bar

File

Within ***File*** basic functions of the software can be controlled.

Table 10. Functions for menu bar - *File*

Function	Description
Logout	To log off the current user
Login	To log in a specific user
Exit	To terminate the program
Save	To save modifications
Save all	To save all modifications

Patient

Within the ***Patient*** menu, new patients can be created or existing patients can be displayed and edited within the ***Patient Management***. To create a new patient, go to ***Patient > Create New Patient*** within the menu bar or click on the icon  ***Create New Patient*** in the tool bar. A dialog to create a new patient data sheet will open (see Figure 3).

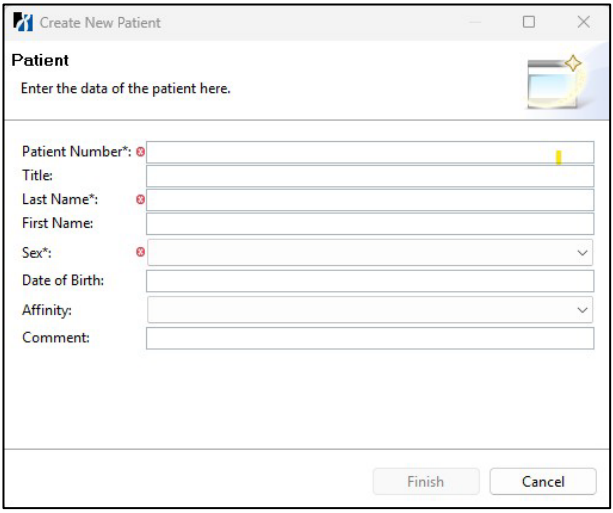


Figure 3. Create new Patient dialog

For detailed instructions for the Patient Management refer to chapter Functions within the Patient Management.

NOTE

Calculated mean chimerism values and standard deviation are displayed within **Patient Editor** with a letter in front of the value to indicate whether chimerism was calculated patient specicifc (“R”) or donor specific (“D”).

Extras

This menu provides basic information and software settings which can be modified if necessary.

Sample Material

The sample material specifies an existing sample, for example peripheral blood (PB) or bone marrow (BM). A selection of commonly used sample

materials is predefined and active. Generating a catalogue of different sample materials supports the standardization of used terms. Open the sample material view by selecting ***Extras > Sample Material***. The view contains a list of all sample materials as well as a specific tool bar with functions to edit sample material data.

Table 11. Functions within Sample Material section

Icon	Function	Description
	Create new sample material	Create a new type of sample material
	Edit sample material	Edit a specific sample material
	Delete sample material	Delete the selected sample material from the list

DIP Management

All qPCR efficiencies for Mentype® DIPquant qPCR assays are displayed by marker.

Reference data

Open ***Extras > Reference*** data to obtain information on Test Kit and Size Standard Management.

Test Kit Management

The Test Kit Management includes all Bin and Panel data for supported BIOTYPE test kits. These are important features to realize allele calling and to validate positive and no-template controls during the batch import according to the requirements of the test kit.

Details can be displayed by clicking on ***View***. Information about included markers, stutter limits, allele sizes, and tolerances are summarized within the overview. The history of test kits can be opened by clicking ***History***.

Open the Test Kit Management within the menu bar under ***Extras > Reference data > Test Kit Management.***

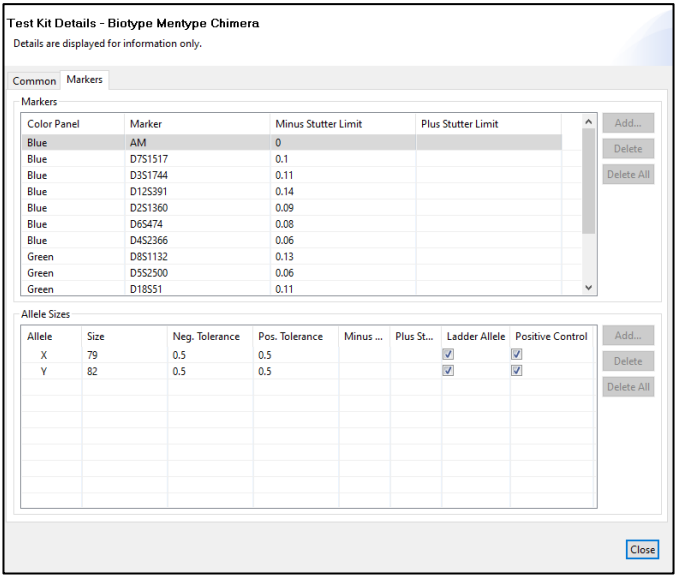


Figure 4. Test Kit Management details of e. g. Mentype® Chimera® PCR Amplification Kit

Size Standard Management

Size standards are required for the exact size calling of raw data. Open the Size Standard Management within the menu item ***Extras > Reference data > Size Standard Management***. All required definitions for the application of Mentype® Chimera® PCR Amplification Kit and Mentype® DIPscreen PCR Amplification Kit are listed and can be displayed in detail by clicking on ***View***.

Overwrite Password

Users with administrator rights within the application can change passwords and assign new ones. Please enter the new password. Then please retype the new password. Click ***Finish*** to save or ***Cancel*** to reject the changes.

User Management

To open User Management select the menu item ***Extras > User Management.*** It contains a table showing the user names of all user accounts including their role (e. g. administrator). All functions required for user administration by an admin can be accessed using the buttons in the tool bar (see Table 12).

NOTE

User accounts can only be edited by administrators

Table 12. Functions within the User Management

Icon	Function	Description
	Create User	Specify information to set up a new user
	Edit User	Change the status or information about the selected user
	Overwrite Password	Change the password of the selected users by an admin
	Delete	Delete the selected user

NOTE

The user, who is currently logged in is displayed in the bottom bar. 

License Management

To open the License Management select the menu item ***Extras > License Management***. The overview contains information of all installed licenses for ChimerisMonitor IVD. The active license is displayed with a  golden key icon, whereas inactive licenses are marked with a  silver key icon.

The installation and expiry date can be tracked in the displayed table. If required, new license keys can be added after the first login. Click ***Add License*** and enter the new license key.

NOTE

New licenses can be orderd via sales@biotype.de with the following order number 46-14801-0000. Please include the validity period, when ordering - trial versions, 1 year or 3 year licenses can be purchased. The local system identifier and desktop or client application must also be specified.

NOTE

Within the bottom bar notes for expiring licenses are displayed. License expires in 24 days

Preferences

To open the user preferences select the menu item ***Extras > Preferences*** (see Figure 5). Within the preferences users can specify their preferred settings for the bioinformatic analyses, resulting reports and visualization of electropherograms.

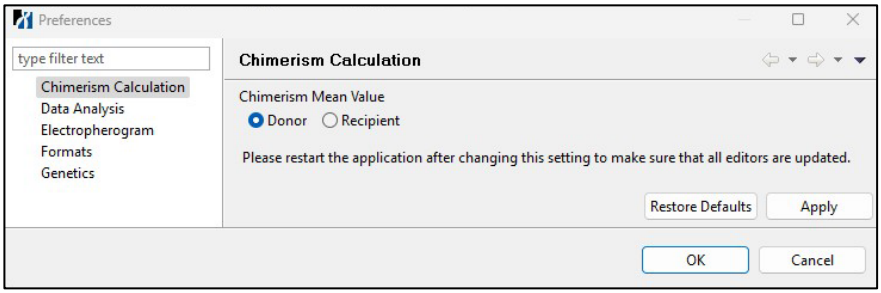


Figure 5. Preferences that can be adapted by users

- **Chimerism calculation**

The output for chimerism calculations can be specified within Chimerism Calculation (***Extras > Preferences > Chimerism Calculation***). The output option for chimerism mean and single values in % Donor or % Recipient can be selected.

- **Electropherogram**

Users can specify their preferred display settings for the electropherogram within this dialog.

Use the button ***Restore Defaults*** to reset all modifications to default settings. Click ***Apply*** to save changes and keep preferences menu open or ***OK*** to save settings and close preferences. Click ***Cancel*** to close preferences and discard the changes.

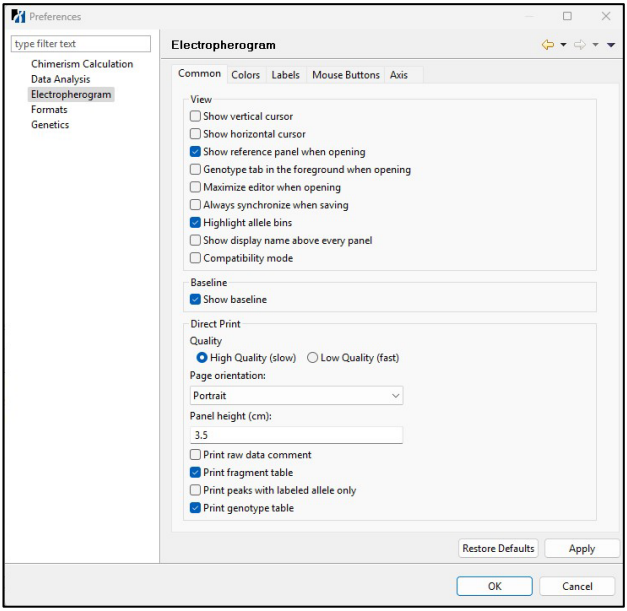


Figure 6. Electropherogram display settings

The settings can be modified within different tabs (see Table 13).

Table 13. Functions for editing electropherograms

Tab	Function	Description
Common	Show vertical/ horizontal cursor	By activating these functions vertical and/or horizontal cursors will indicate the position of the mouse pointer within the coordinate system. Select the color of the cursors within the tab ***Colors.***
	Show reference panel when opening	Select this option to fade in or out the reference color panel (color of the size standard) when opening an electropherogram.
	Genotype tab in the foreground when opening	Select this option to see the genotype tab in front instead of the fragment tab when opening an electropherogram.
	Maximize editor when opening	Check this box to maximize electropherogram and size standard regression views while opening.
	Always synchronize when saving	Select this option for automatic synchronization of changes within the electropherogram-coordinate system and the fragment/genotype table.
	Highlight allele bins	Deactivate this option if allele bins should not be highlighted in grey within the electropherogram
	Show display name above every panel	Select this option to display the name of the raw data file above every panel within the electropherogram. This setting might be important when printing the electropherogram.
	Compatibility mode	Activate it, if problems occur while viewing an electropherogram. In most cases problems will be fixed by this option

Tab	Function	Description
	Show baseline	This option determines whether or not the baseline is displayed.
	Quality	Select if direct prints of electropherograms should be done with high (slow printing) or low/medium quality (fast printing).
	Page orientation	This setting affects the orientation of the electropherograms within the print. Choose between ***Portrait*** or ***Landscape.***
	Panel height (cm)	Defines the height of the panel (cm) within the print.
	Print raw data comment	Select this option if comments imported from raw data should be included in the print (displayed above the coordinate system).
	Print fragment table	Select this option to print the electropherogram and the corresponding fragment table.
	Print peaks with labeled alleles only	Select this option if the print of the table information should be restricted to labeled alleles only.
	Print genotype table	Select this option to print electropherogram and the corresponding genotype table.
Color	Specify colors for cursors, peak height, size and area as well as for artefacts.	
Labels	Choose individual colors for the labels. Specify fonts for different elements and select to which sample type labels should be assigned. Furthermore you can decide which peak information should be indicated in a coordinate system.	
Mouse Buttons	Different functions to mouse buttons can be assigned to work within electropherograms. By choice, a mouse click could assign or delete an allele, open the pop-up menu of a specific peak or select a peak.	

Tab	Function	Description
	All four options could be assigned to a desired mouse button and the operation mode could be switched between single or double click. Please notice that changes made here will become active only for electropherograms opened after saving.	
Axes	Specify axes and scaling of axes within the electropherogram.	
	Position of the X/Y-axis scale	Select where axes will be displayed in the coordinate system (bottom or/and top or left or/and right of the coordinate system).
	Unit of the X-axis:	Choose the unit of the X-axis: data point or (calculated) base pairs. By default, base pairs will be displayed.
	Use equal RFU scale for all panels by default	Select this option to decide whether RFU scale of all panels is in the same range by default or RFU scale of panels is adapted to the respective peak heights. Equal scaling can also be directly switched on and off by clicking ***Equalize Zoom*** within the electropherogram editor.
	Show this range of the electropherogram	Select this option to define which range is displayed in the electropherogram. The X-axis will be adjusted to this range. By default whole area of data points above the cut off will be displayed. Please notice that changes made here will become active only for electropherograms opened after saving.

- **Data Analysis**

ChimerisMonitor RUO supports the import and evaluation of raw data of different sequencer formats (fsa files) and qPCR Cyclers (txt files). The program identifies peaks and artefacts as part of the process and is also capable of assigning peaks to alleles and, thus, to generate DNA profiles. For a detailed description of raw data analysis of fragment analysis data,

please refer to chapter Analysis of Electropherograms and Procedure for chimerism analysis. Find all preinstalled and user cerated Analysis Methods within the path ***Extras > Preferences > FSA File Import > Analysis Methods*.**

Analysis Methods (CE)

Detailed parameters of the preinstalled Analysis Methods ***Chimera RUO*** and ***DIPscreen RUO*** can be displayed by clicking ***Show***. ***Chimera RUO*** and ***DIPscreen RUO*** are the basic methods for data analysis of Mentype® Chimera® PCR Amplification Kit and Mentype® DIPscreen PCR Amplification Kit. The data evaluation is only intended to be used for research purposes, a use for diagnostic purposes is not permitted. By selection of the check box, the default analysis method within the ***CE Import*** or ***Batch Import*** is chosen.

For creation of a new analysis method, click ***Add***. To customize an existing method select it in the list and click ***Edit***. In both cases an editor mask with four tabs will open.

Adapt values within the four tabs to adjust the analysis method to user specific needs. Select ***OK*** to save settings and close the window. Click ***Cancel*** to close window and discard the changes.

NOTE

Adaptions within ***Chimera RUO*** and ***DIPscreen RUO*** analysis method are not recommendet. Select the button ***Restore defaults*** to reset all modifications to default settings.

Attention: All user-created methods will be deleted using the restore button.

The sections listed in Table 14 explain the parameters of the Analysis Methods by the ChimerisMonitor RUO software.

Table 14. Parameters of the Analysis Method

Tab	Function	Setting	Description
General		Name	Add a name to be displayed for the Analysis Method.
		Description	Optionally add a description for the Analysis Method.
	Size Calling		Size Calling is used to calculate the fragment sizes in other color panels with the help of the regression line. Therefore, one needs to choose between two algorithms in the Algorithm drop-down menu of the General tab.
		Algorithm	**Local Southern:** This algorithm generates interpolation curves for each peak using the peak itself and the neighboring peaks before and after it. The two resulting fragment sizes for the peak are averaged. **Cubic Spline:** This algorithm interpolates a curve for all peaks assigned to sizes of the size standard. Hence, this algorithm is more exact, but also more susceptible to outliers.
		Cut-off	This Cut-off describes the maximum heigth for peaks of the size standard. By enabling ***Manual cut-off*** the default setting can be adjusted.
		Baselining window size	Baselining normalizes the signal values in their heights. It improves the signal-to-noise ratio, and is the distinction between peaks and background noise. First, the program calculates a baseline for each individual data point. In this process, the lowest RFU value, found in a

Tab	Function	Setting	Description
			window of 25 data points before and after a given data point, is subtracted from each data point. The Baselining Window Size can be adjusted in the General tab. It should neither be too large, resulting in dwindling peaks, nor too small, causing a decrease of contrasting effects. The default size is 51 data points (the data point itself plus the 25 points before and after it).
		Smoothing	Smoothing represses the high-frequency signal noise and optimizes the peak shape in its width and height. Thus, the number of falsely detected peaks is reduced. The greater (coarser) the smoothing is set the rounder the peaks will appear. Indeed, they lose in height in the process. Therefore, only a fine smoothing is recommended for analysis of standard data. A selection between Fine Smoothing, Rough Smoothing or No Smoothing is available.
		Criteria for the Rating of the Size Calling	In size matching, peaks of reference color panels are assigned to sizes of size standards. The assignment can also be adjusted manually. The Chimeria RUO and DIPscreen RUO methodology finds the best solution for size matching with the help of an optimization function with the following characteristics: inclusion of all available fragments and consideration of the heuristic parameters: distance and equal heights of the fragments. Even early terminated runs are correctly identified. In the next step, a regression line for the identified peaks

Tab	Function	Setting	Description
			and their respective sizes is generated. The quality of the regression is calculated by the coefficient of determination. Use the field Minimum Regression Quality within the General tab to specify a minimum value for the coefficient. You can also state how many fragments of the size standard have to be assigned to peaks. If all fragments are assigned, the fraction will be 100 %, otherwise lower. Use the field Minimum Percentage of Assigned Sizes within the General tab to specify a minimum value for the assignment. Another warning can be displayed by the parameter Maximum Ratio of Unassigned Peaks. If the ratio between the total number of peaks and the number of peaks assigned to size standard sizes is larger than the value given here, a quality warning will appear instead of a green hook.
Peak detection	Peak detection		The processed signals are grouped into two classes: fragments (peaks) and noise signals (baseline). For the assignment of signals to peaks, several criteria have to be fulfilled. These criteria can be adjusted in the Peak Detection tab.
		Minimum Peak Heigth	Peaks have to reach a Minimum Peak Height, depending on the particular fluorescent dye.
		Polynomal Degree/ Peak Window Size	The actual peak detection in the Chimeria RUO and DIPscreen RUO analysis method is achieved with the help of a polynomial function that is created in a window of predetermined

Tab	Function	Setting	Description
			width for the respective data points. The higher the polynomial degree and the smaller the window size, the finer peaks can be separated. The lower the polynomial degree, the more noise can be suppressed. However, close shoulder peaks could be united in this case.
	Limits	Minimum peak width	Peaks should not be too narrow. The value can be entered in data points.
		Starting point	The field indicates from which data point peaks should be detected. This ensures that primer peaks are ignored.
		End point	The field specifies up to which data point peaks should be assigned.
		Background Noise Limit	The value sets the limit until background noise is recognized.
Allele Assign ment	Allele Calling		In allele calling peaks are assigned to alleles on the base of their fragment size with the help of the respective test kit or allelic ladder. Within the section additional peak assignement rules can be selected or deselected as well as the minimum peak heigth for allele calling defined.
	Allelic Ladder		In case of analyzing an allelic ladder, the fragment sizes (listed in the particular test kit) are assigned to the peak positions of the allelic ladder. For this purpose the respective peak for every allele is identified and the offset regarding to the predefined length is determined (allele matching). As in size matching, Chimera RUO and DIPscreen RUO considers the heuristic parameters distance and

Tab	Function	Setting	Description
			equal height of fragments for the allele matching process. This procedure is repeated with every marker.
		Offset	Definition of the maximum Offset of the fragment length of the first allele in a marker.
Matrix			After a first capillary electrophoresis, the spectral overlaps of fluorescent dyes have to be corrected by calculation. Sequencers of the 3100 generation and beyond already clear these overlaps internally. Devices as the 310 sequencer, however, require a revision with help of ChimerisMonitor RUO and so-called matrices. Matrices are applied on RFU signals across all colors. If there is a matrix available, it will be read out from the .fsa file. Otherwise, you can select an external matrix via the tab ***Extras > Preferences > FSA File Import > Matrices (ABI 310).***

Matrices (ABI 310)

Within the path ***Extras > Preferences > FSA File Import > Matrices (ABI 310)*** specific matrix files as result of spectral calibration of ABI Prism 310 instruments can be added. These matrix files could be used in data analysis. Click ***Add*** and select the respective .mxt file. To edit or delete a matrix, select the table entry and click the respective button ***Edit*** or ***Remove***.

- **Formats**

The preferred date format can be edited: e. g. yyyy-mm-dd

NOTE

Coherent dates are important to create transplantations and to define sample types.

- **Genetics**

Artifacts can lead to misinterpretation of data. For this reason, there are multiple algorithms integrated into ChimerisMonitor RUO that serve the identification of different artifact types. All artifact controls are performed for every peak. The artifact types are listed in Table 15. Artifacts will be shown in the coordinate system, too. Though no allele will be assigned to them, artifacts can be manually labeled with an abbreviation for the specific artifact type.

The results of the particular artifact analyses are indicated in the fragment table by icons. The result can either indicate that the artifact has not been found, the artifact has been detected or the identification is not safe – please review. The results are summarized in the Quality column in the fragment table. A good quality here means that the program has not detected any artifact for the peak at all.

Table 15. Artifacts identifiable with ChimerisMonitor RUO

Artifact	Description
Stutter (ST):	Stutters are the result of a wrong PCR amplification when the polymerase adds or leaves out a repetitive unit of the marker. Thus, stutter fragments are approximately one repetitive unit (usually 4 bp) larger or smaller than the actual allele fragment. The area of a stutter peak is smaller than the one of the allele peak; normally, it is 15 % of the allele peak area or less. However, the exact maximum height of the stutter peak - the stutter limit - depends on the locus and the allele. Stutter limits for markers within the test kits are already applied.
Shoulder- und Split-	Shoulder and split peaks are double peaks with maxima, that are approximately one base pair apart. These peaks can be the result of inconsistent non-template addition, a consequence of the

Peaks (SH):	incomplete adenylation of PCR products by the polymerase during the PCR reaction.
Off-ladder (OL)	Off-ladder peaks are located outside of allele bins. They can result either from very rare alleles or from other artifacts. Off-ladder peaks can be assigned to alleles (new or user-defined ones).
Off-scale (OS)	The fluorescence (RFU) of off-scale peaks excesses the measurement range. Such peaks exhibit a flat maximum.
Tri-Band (Tri):	Sometimes, an analysis apparatus might detect three signals, although there are only two PCR-fragments. This phenomenon is called tri-band pattern. The peaks of a tri-band pattern are all of the same height or two of the peaks cumulate to the height of the third one. Tri-band patterns are specific for analysis apparatuses and kits. A number of known tri-band patterns are already deposited in the reference database. However, more tri-band patterns can be added.
Spectral Overlap (SO	Spectral overlap artifacts are peaks resulting from overlapping emission spectra of fluorescent dyes, i.e. the signal of one dye is mistakenly detected in another color panel, too. Hence, these 'pull-ups' are situated at the same position (data point) as the larger causing peak, only in another color panel.
Peak height and width	Too large RFU values can indicate pull-ups. Furthermore, too wide peaks should also not be regarded as allele fragments. Hence, you can set maximum peak heights and widths.

Artifact Thresholds

The applied artifact threshold are displayed in ***Extras > Preferences > Genetics > Artifact Thresholds.***

Artifact Filter

In ***Preferences > Genetics >Artifact Filter*** the user can select which artifacts should be filtered and displayed within the electropherogram and how the detected artifacts should be labeled.

Table 16. Artifacts identifiable with ChimerisMonitor RUO

Tab	Function	Description
Filter Settings		Specify the artifact types which should be filtered in the electropherogram. No allele label will be assigned to a filtered artifact peak within the coordinate system of the electropherogram.
	Allowed	If this option is deactivated, filter for this specific artifact will not be used within the electropherogram view, meaning: label of these peaks cannot be faded out. Note: To label peaks within the coordinate system, users have to activate labeling of artifact peaks in general. Find the option to do this within Preferences > Electropherogram in the tab Labels
	In Summary	Selected artifacts will be become summarized under the button All artifacts in the electropherogram view.
	Show label	Select this box to label artifacts with the respective abbreviation (if labeling for peaks is active; see above).
	Acivate filter by default	Select this option to activate filters by default after opening the electropherogram.
Filter Rating		Select to see how the appearance of each artifact type influences the peak quality.
		Peak quality should be validated as bad.
		Peak quality should be validated as questionable. Manual review of the peak is necessary.

Use the button ***Restore defaults*** to reset all modifications to default settings. Click ***Apply*** within the preference window to save changes and keep preferences menu open or ***OK***, to save settings and close preferences. Click ***Cancel*** to close preferences and discard the changes.

Functions within editors

Patient Editor

The ***Patient Editor*** represents the central overview for patient-specific data management and subsequent calculations within the software.

All ***Samples & Transplantations*** of the patient are displayed in tabular form. In addition, results of conducted chimerism calculations can be reviewed within the overview or reports can be generated and will be collected in the tab ***Reports***. All possible functions within the ***Patient Editor*** are summarized in Table 17.

Table 17. Functions within the Patient Editor

Icon	Function	Description
	Create Report	Create a monitoring report for chimerism analysis
	Export Patient	Detailed patient information is exported in csv
	Save	Save changes
	Delete	Delete a sample or transplantation. To delete a transplantation no recipient or donor files should be selected within the transplantation
	Open Electropherogram	Open the Panel Editor: All electropherograms of selected CE-samples are displayed
	Create new Transplantation	Create a new transplantation. For details refer to chapter Transplantation Editor.
	Create new qPCR sample	Import of qPCR results for selected patient.

- **Delete**

Samples and transplantations within the ***Samples & Transplantations*** section in the ***Patient Editor*** can be deleted via the icon  ***Delete***.

Multiselection is available via CTRL key and left-click. Positive Control and No Template Controls cannot be deleted. Deletion of all entries within is necessary prior to delete a patient.

- **Panel Editor**

By selection of multiple samples within the ***Patient Editor*** and click on icon  ***Open Electropherogram***, all selected samples are displayed in one screen within the Panel Editor. For details on the available functions refer to chapter Analysis of Electropherograms. Transplantations cannot be displayed within the Panel Editor.

NOTE

Selection of minimum one sample of client “IVD” or “TRANSFER” lead to reduction of functions within the Panel Editor as samples of these clients are read-only and no changes within the electropherogram are allowed.

- **Create new qPCR sample**

Prior to the processing of qPCR data for the chimerism quantification, raw data of amplification curves have to be analyzed with the appropriate software from the real-time instrument manufacturer. Please follow the instructions of the instrument specific manual to analyze and export data as tab.delimited txt file. Please export at least the following data (sample name, calculated Cp/Ct values).

By using an flexible, user-defined import algorithm ChimerisMonitor RUO provides the opportunity to import and process pre-analyzed data from many different instruments.

Import of qPCR data

1. Open the ***Patient Editor*** (see chapter Functions within the Patient) and select ***Create new qPCR sample***. The import assistant is displayed.

2. Click ***Browse*** in the section “Import File” and select the respective .txt file. By click on the button ***Open***, the file content appears within the table at the lower area of the import assistant (“Content”).

3. For the first import of specific instrument data several “Import Settings” have to be specified to recognize single measurements within the file. Click the ***Import Settings*** section header to expand the “Import Settings” section. A menu will appear to assist the import adjustments for a specific instrument export format.

- Select from the “Separator” group the separator that is used in the file to separate the columns
- Select from the “Header” group one of the following:
    a. Select “First Line”, if the header is located in the first line of the file.
    b. Select “After last empty line”, if the file starts with comments and the comments are finished with an empty line.
    c. Select “At specified line”, to specify the header line number. Insert the line number in the text field located beneath.

The file contents will be displayed in the area “Content” as soon as the correct “Separator” and “Header” settings have been set.

Specify the name of the imported columns for Sample Name and Cp/Ct values by using the combo boxes in the “Import Columns” group. Only these data will be imported.

Click the ***Save*** button in the “Import settings” section to save the setting. Enter a name for the setting (e.g. the name of the qPCR device) and click ***OK*** to save the setting. The setting can from this timepoint on chosen from the ***Import Template*** dropdown menu.

NOTE	
	Subsequent data processing is simplified if the sample name also contains the name of the used Mentype® DIPquant assay.

4. Select all samplings that belong to one measurement from the “Samplings” table. To select multiple samples hold down the CTRL key and left-click with the mouse the appropriate sampling rows.

5. Click Finish to finalize the import. The Sample ID dialog is displayed. Insert a Sample ID in the text field to describe all samplings and click OK or click Cancel to abort the import and close the assistant.

6. After a successful data import, samples will appear within the Samples & Transplantations overview of the Patient Editor.

NOTE

If a sample was analyzed with recipient and donor specific Mentype® DIPquant loci, it is recommended to import them as two separate samples (one for recipient specific marker, one for donor specific marker).

- **Create Report**

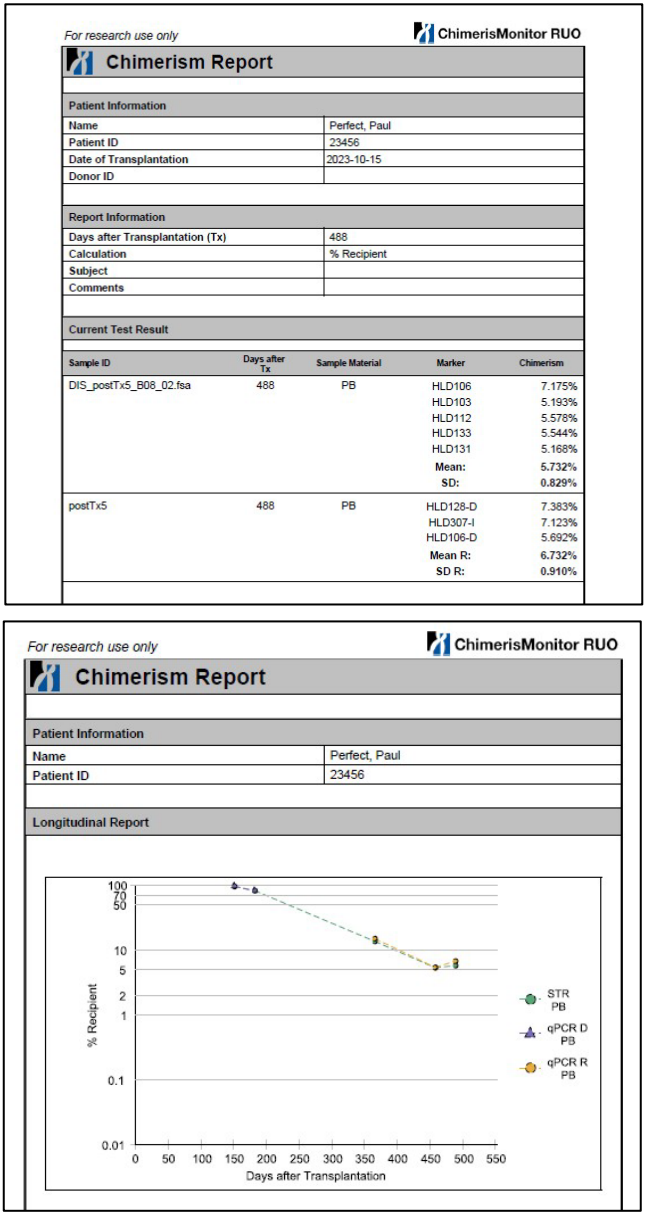
To record the monitoring of a patient, a report function is available. This report contains results of the most recently performed calculations that are depicted in tabular form (see Figure 7, a) and are sorted with respect to the used method. Moreover, the report displays the overall monitoring course of the patient in a graph (see Figure 7, b).

To create a report, click on the icon  ***Create Report*** in the upper rigth part of the ***Patient Editor***.

You may fill in respective free text fields (Subject, Comments). This information will be incorporated in the report.

Click ***OK*** to start automated compiling.

The report will subsequently be converted to PDF format for printing or saving the results. Already compiled reports will be saved in the tab ***Reports*** in the ***Patient Editor*** and can be opened with a double click or deleted with a click on the icon  ***Delete File***.



For research use only

ChimerisMonitor RUO

a

Chimerism Report

Patient Information	
Name	Perfect, Paul
Patient ID	23456
Date of Transplantation	2023-10-15
Donor ID	

Report Information	
Days after Transplantation (Tx)	488
Calculation	% Recipient
Subject	
Comments	

Current Test Result

Sample ID	Days after Tx	Sample Material	Marker	Chimerism
DIS_postTx5_B08_02.fsa	488	PB	HLD106	7.175%
			HLD103	5.193%
			HLD112	5.578%
			HLD133	5.544%
			HLD131	5.168%
			Mean:	**5.732%**
			SD:	**0.829%**
postTx5	488	PB	HLD128-D	7.383%
			HLD307-I	7.123%
			HLD106-D	5.692%
			Mean R:	**6.732%**
			SD R:	**0.910%**

For research use only

ChimerisMonitor RUO

b

Chimerism Report

Patient Information	
Name	Perfect, Paul
Patient ID	23456

Longitudinal Report

Figure 7. Chimerism report in (a) tabular form and (b) as longitudinal report

- **Export Patient**

To generate the monitoring of a patient as a processable csv file, an export function is available. This export contains the general patient information as well as all information displayed in the ***Samples & Transplantations*** table. The export will be generated as a csv file via file explorer in a selectable location.

NOTE

Please note that depending on the settings of the operating system or used spreadsheet software, additional steps to separate information in different cells might be necessary.

Sample Editor

The ***Sample Editor*** (see Figure 8) can be opened by double-clicking on single samples in the tabular display of the ***Patient Editor***. The overview contains general sample information and a table of chimerism values for each marker after the calculation has been performed.

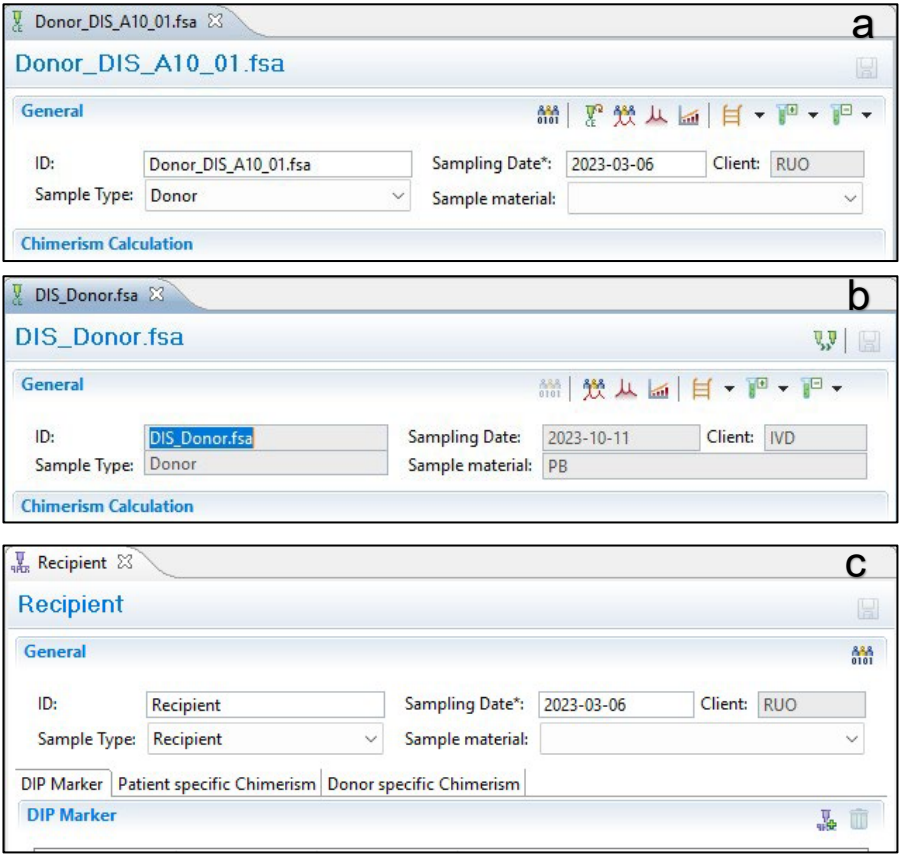


Figure 8. Section of the Sample Editor with CE samples imported in ChimerisMonitor RUO (a) or ChimerisMonitor IVD (b) and qPCR sample to ChimerisMonitor RUO (c)

The ***Sample Editor*** is divided into three sections. Within the ***General*** section further parameters (see Table 18) are defined for each sample. Define respective parameters as they are necessary for successful transplantation setups or chimerism calculations.

Table 18. Information defined within the general tab of the Sample Editor

Information	Description
Sample Type	Defining the ***Sample Type*** is essential for setting up transplantations or chimerism calculation, choose between ***Recipient*** (prior allo-HSCT), ***Donor*** or ***PostTx*** (Monitoring sample after allo-HSCT)
Sampling date	Define the date of sampling. Samples with the type ***Recipient*** and ***Donor*** must have a date prior to transplantation date. Samples with type ***PostTx*** must have a date after transplantation date
Sample material	Defines tissue or origin of the sample. For the application of Mentype® Chimera® PCR Amplification Kit and Mentype® DIPscreen PCR Amplification Kit ***PB*** as peripheral blood is preselected for the IVD Client and cannot be changed
Client	Describes the software status. Samples processed in the ChimerisMonitor RUO application display *RUO* as client. Database entries imported from ChimerisMonitor 2.1 application display the Client *TRANSFER*. Database entries imported by ChimerisMonitor IVD application display *IVD* as client.

Confirm all modifications within the Sample Editor by clicking  ***Save*** within the menu bar of the main window. Otherwise, you will be asked to confirm your changes when closing the ***Sample Editor*** or when starting certain calculations. To confirm, click ***Yes***. To close the Sample Editor without saving the changes click ***No*** or click ***Cancel*** to return to the ***Sample Editor***.

NOTE

Changes to samples will not be automatically applied to already finished calculations or transplantations which are associated with these samples. Therefore all calculations and transplantation settings have to be repeated or reset if used retrospectively.

The section ***Chimerism Calculation*** within the ***Sample Editor*** serves as a tabular overview for the calculation results of monitoring samples. The section is active after a successful calculation. Subsequently, the calculated values will also be summarized within the ***Samples & Transplantations*** view of the ***Patient Editor***.

The section ***FSA Import Errors and Warnings*** shows all possible warnings regarding sample or run quality. Before further processing check electropherograms and size calling regressions.

Several functions of the Sample Editor can be selected via the toolbar. For detailed descriptions see Table 19.

NOTE

Only samples with displayed client “RUO” enable all functions for editing. Samples with displayed client “IVD” or “TRANSFER” can be cloned to client “RUO” or will remain read-only.

Table 19. Functions within the Sample Editor

Icon	Function	Description
	Calculate Chimerism	Guided process with display of detected markers, marker selection for chimerism analysis and chimerism calculation (single marker chimerism, total chimerism and standard deviation)
	Reprocess Sample	Reprocess import of the selected sample with the possibilty to edit import settings. Function is

Icon	Function	Description
		available for CE samples with displayed client “RUO” only. Plausibility check is not executed using the function.
	Add qPCR Sampling	Add qPCR result to selected sample. Function only available for qPCR samples.
	Open Chimerism Panel	Display of electropherograms from postTx- samples, donor and recipient profiles. Compare profiles and check possible positions for recipient-specific alleles
	Open Electropherogram Editor	Display of the Sample Electropherogram Sample validity is checked within the batch import, but always perform a plausibility check. A correct peak assignment is essential for an accurate definition of informative markers and a robust chimerism calculation
	Open Size Calling Regression	Sample’s Size Calling Regression Display of the size calling regression line and quality value. Additionally a table with details on the peaks and their quality is shown. The Sample Quality Check during the Batch Import displays possible quality warnings
	Open Allelic Ladder Electropherogram	***Allelic Ladder Electropherogram*** and its ***Size Calling Regression*** Possible quality warnings are displayed… Within tab ***Run Validation*** during the Batch Import Within tab ***FSA Import Warnings*** in the Patient View
	Open Positive Control Electropherogram	***Positive Control Electropherogram and Size Calling Regression*** The Evaluation of Positive Controls during the Batch Import displays possible quality warnings
	Open No Template Control Electropherogram	***No Template Control Electropherogram*** and ***Size Calling Regression***

Icon	Function	Description
		The Evaluation of No Template Controls during the Batch Import displays possible quality warnings.
	Clone to RUO	Clone sample with client “IVD” or “TRANSFER” to client “RUO” to enable editing functions of ChimerisMonitor RUO.
	Save	Save all modifications made

NOTE

Formulas for chimerism calculation can be reviewed within chapter Semi-quantitative analysis – chimerism analysis

NOTE

The function “Reprocess sample” should be used to change import setting. Changing the sample itself via the function is not recommended.

Analysis of Electropherograms

The analysis of sample and control electropherograms is an important part for a reliant quality assessment.

NOTE

Even though a general run and sample vaidation is performed automaticially during the Batch Import, always check results for plausibility. Reviewing electropherograms is an important part to assess sample and device performance

All electropherograms represent a graphical output of analysed raw data from capillary gel electrophoresis. ChimerisMonitor IVD offers a special

graphic user interface for the visualization of electropherograms. For the assessment of the selected sample electropherogram click on ***Open Electropherogram Editor***, for the Positive Control choose ***Open Positive Control Electropherogram*** via the icon and for the No Template Control ***Open No Template Control Electropherogram*** via the icon .

For possible adaptions of the visualization according to the users preferences please select ***Extras > Preferences > Electropherogram.***

The Electropherogram Editor (see Figure 9) contains a menu bar including several functions (see Table 20) and analyzed raw data of the capillary gel electrophoresis displayed in preselected panels. The unit of the Y-axis of the coordinate system is RFU, whereas the shared X-axis uses *base pairs* or *data points*.The coordinate systems do always display the same range of the X-axis. Additionally, detailed information about ***Fragments***, that includes absolute values like data points, alleles, marker, size, height, area and QC flags, but also the ***Genotype*** are displayed in tables below the electropherograms.

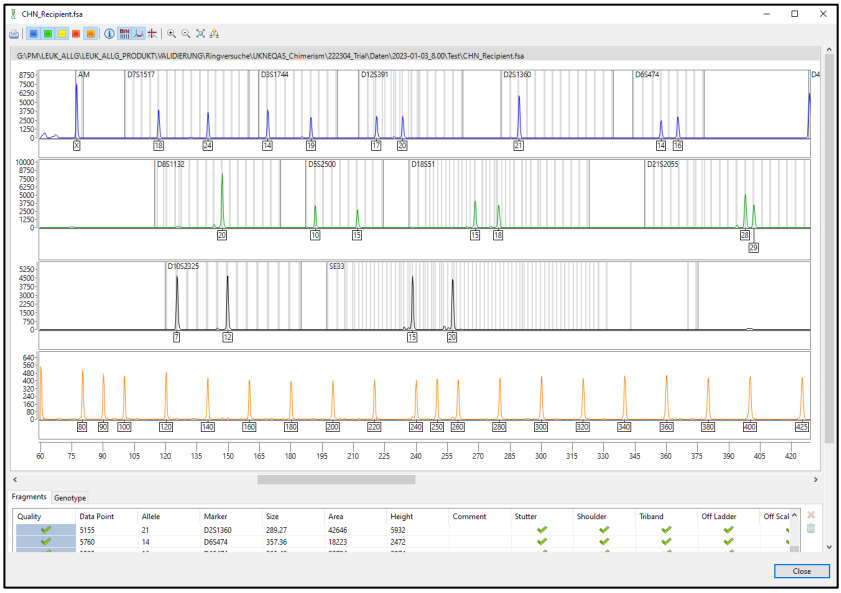


Figure 9. Electropherogram Editor, e.g. sample from Mentype® Chimera® PCR Amplification Kit Analysis

The following functions can be used, when working with the ***Electropherogram Editor*** (see Table 20).

Table 20. Function within Electropherogram Editor

Icon	Function	Description
	Print	Print the electropherogram in its current state, including the current scaling, the panel and label selection as well as the two tables. To change the printing preferences, go to Extras > Preferences > Electropherogram > Direct Print.
	Dye Selection	Select any desired color to show or hide the referring color panels.
	Display Name Above Every Panel	The sample name can be displayed permanently above the panels. This has no impact on the printing preferences.
	Show/Hide Allele Bins	Deactivate or activate allele bins, that are represented by grey stripes in the coordinate systems.
	Show/Hide Baseline	For a simplified analysis the baseline can be displayed or not.
	Show/Hide Cursor	The position of the mouse pointer can be indicated by cursors in a color of choice (see preferences).
	Zoom In	Change the Panel view by zooming in.
	Zoom Out	Change the Panel view by zooming out.
	Fit	Come back to the complete representation of the active panel.
	Equalize Zoom	Determine, whether the Y-range of all panels shall always be the same, meaning if you zoom into one panel, the other ones will be magnified as well or

Icon	Function	Description
		whether the zoom into one panel should work independently.

In the lower part of the representation, there are two tables on separated tabs. The fragment table on the tab ***Fragments*** displays the information displayed in Table 21. The second tab, ***Genotype***, is also located in the lower part of the electropherogram representation. The table displays the overall genotype of the sample and its raw data. All changes made here are directly transferred to the sample genotype. The table contains the following columns displayed in Table 21.

Table 21. Information within Electropherogram Editor table

Header	Description
Quality	Summarizes, whether there are artifacts detected for this peak or not or if the identification is not safe and need to be checked. Besides, it indicates the color of the peak.
Data point	The data point the peak is located at.
Allele	The allele assigned to the peak (if applicable).
Marker	The marker of this peak (if applicable).
Size	The size of the peak when calculated with the current regression.
Area	The peak area
Height	The RFU value of the peak maximum.
Manually edited	The icon is displayed, when the peak assignment was manually edited.
Comment	A comment entered manually.

Header	Description
Stutter, Shoulder, Tri-Band, Off-ladder, Off-scale and Spectral Overlap	Icons indicate whether the referring artifact type has been detected for this peak or not or if the identification is not safe and need to be checked .
MPH and MPW	Icons indicate, whether the admissible maximum peaks heights (MPH) and width (MPW) were overstepped or not . Quality settings can be changed within the preferences.
Marker	The STR locus investigated.
Allele	The allele determined for the given locus.
Manual	Shows if the allele assignment has been edited manually (yes/no). Peaks can be selected by either clicking on the peak in the coordinate system or by selecting the relevant line of the fragment table. Selected peaks become black and will be highlighted in the fragment table. A right click on a peak will open a pop-up menu.

- **Editing allele assignments**

The allele assignment of a peak can be edited manually. To do so, select the corresponding peak by clicking on it in the coordinate system or by selecting the respective line in the fragment table. Do a right click on the selected peak in the electropherogram or select the cell within the column Allele in the respective line and click on the displayed button . A peak specific menu is displayed which enables either to ***Edit allele assignement*** or ***Delete allele assignment.***

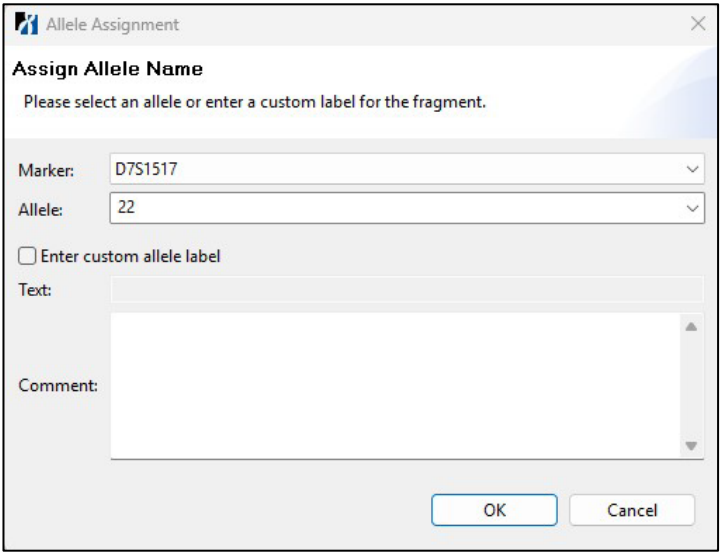


Figure 10. Allele Assignment display

After selection of ***Edit allele assignement*** within the menu, an input mask opens up (see Figure 10) Choose marker and allele in the drop-down list.

NOTE

The choice is limited to the markers of the active color panel only.

Choose an allele from the drop-down list or type a name. There is also the possibility to use a user-defined label. To assign an own label, activate the check box ***Enter custom allele label*** and enter the desired label into the Text field. This added label will be displayed in both, the coordinate system and the Allele field of the ***Fragment*** table. Notes entered into the Comment field will be displayed in the respective field in the fragment table. Select ***OK*** to confirm your input or discard it with ***Cancel***.

- **Remove allele assignment**

If you want to remove the allele assignment of a peak without replacing the assignment by another one, press after selection of the respective line in the table the button  ***Remove Assigned Allele*** next to the fragment table or select the menu item ***Remove Allele Assignment*** in the peak menu. Use the button  **Remove All Allele Assignments** next to the fragment table, if you want to remove all allele assignments (for the raw data item) at once.

NOTE

All changes will be directly synchronized with the fragment- and genotype table. Please consider that you have to save your changes before they are adopted permanently by the system.

Changes that have not been saved yet, are indicated by a * in front of the tab title. Select ***OK*** to confirm your input or discard it with ***Cancel***.

Analysis of Size Calling Regression

The Size Calling Regression is an important method for an exact length assignment of amplicons in each panel. Next to the allelic ladder performance, it is one of the prerequisites for exact allele assignments.

The analysis of size standards can be assessed within each sample, allelic ladder, positive and no template control by clicking the respective icons  (see Table 20) within the ***Sample Editor***. The ***Quality of Regression*** should not exceed 0.995 for the application of the DNA Size Standard 550 (BTO) within the Mentype® Chimera® PCR Amplification kit or Mentype® DIPscreen PCR Amplification Kit analysis.

NOTE

The size standard validity is checked as part of the sample and run validity test within the batch import.

Similar functions as for the analysis of electropherograms (see Table 20) can be used within the tool bar of the Size Calling Regression Editor (see Figure 11).

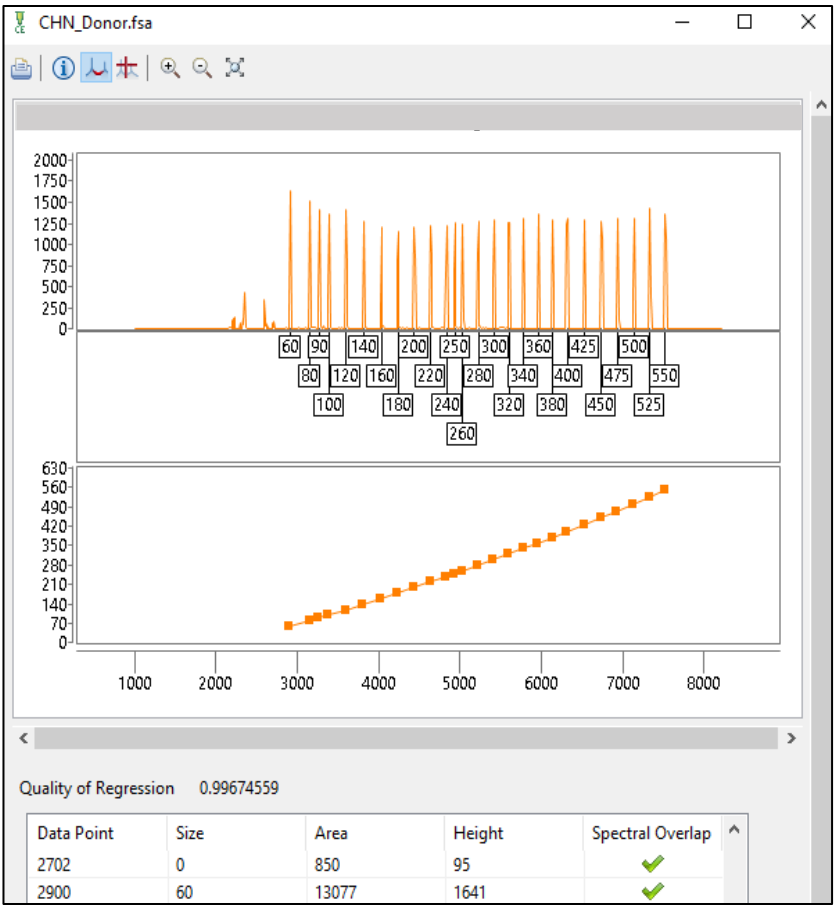


Figure 11. Size Calling Regression for DNA Size Standard 550 (BTO)

Transplantation Editor

After having successfully defined donor and recipient samples, a transplantation can be set up to which these can be assigned.

To assign the transplantation to a patient, click on the icon  ***Create new Transplantation*** in the ***Sample Editor***. In Figure 12 an exemplary transplantation setup is shown. Perform the following steps to create a transplantation:

1. Assign a ***Transplantation ID***.
2. Assign the ***Date*** of the transplantation. This entry is mandatory in order to select recipient and donor samples.
3. Optional: Assign a ***Donor ID*** for an unambiguous definition of the transplantation.
4. Choose respective donor and recipient (Pre Tx) samples in the ***CE***- or ***qPCR***-section. Donor and recipient samples within the ***qPCR***-section should be imported with Testkit Biotype Mentype DIPscreen.
5. After the selection of the reference data, the genotype of all markers for donors and recipients enclosed in the kit will appear in the ***Transplantation Editor*** (see Figure 12).
6. Determine which markers should be chosen as standard to calculate the chimerism status of the respective transplantation. Please refer to the instructions for use of Mentype® Chimera® PCR Amplification Kit, Mentype® DIPscreen PCR Amplification Kit or handbook of Mentype® DIPquant. Please refer also to chapter Setting up a transplantation.

NOTE

Only sample types defined as Recipient and Donor and with a date of sampling prior to the date of transplantation are depicted.

Within the ***CE***-section it is also possible to choose ***Manual Assigment*** if no donor or recipient sample is available, By selecting the respective button it is possible to choose the assay and add the known genotype.

Within the ***qPCR***-section it is also possible to choose DIPselector to get more detealled information on the informativity of the marker. Please refer also to chapter Setting up a transplantation for more details.

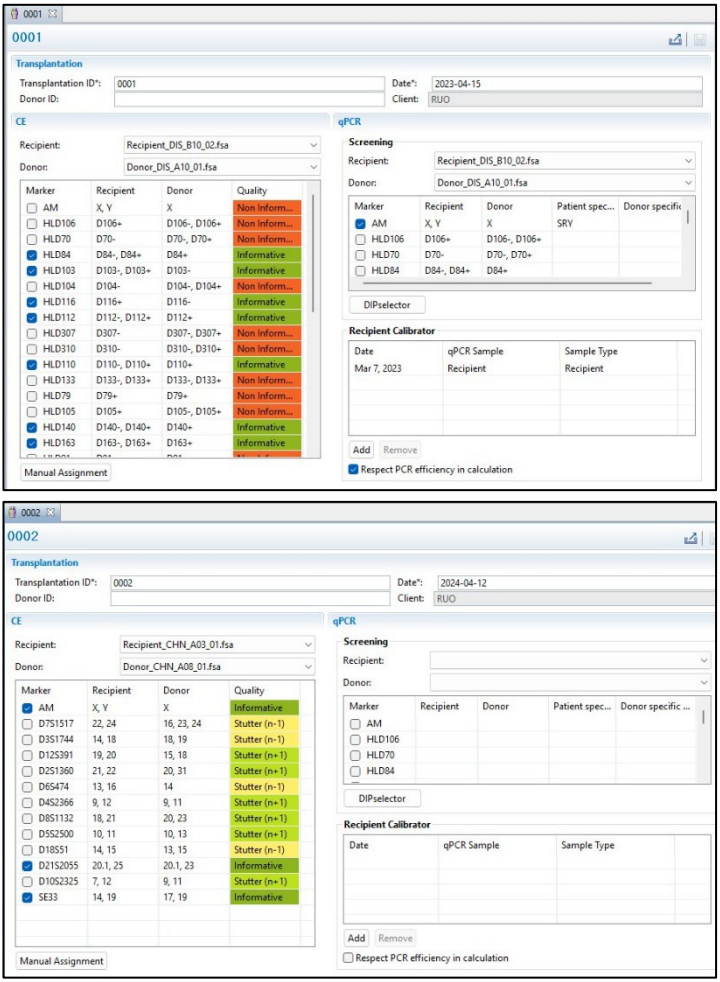


Figure 12. Transplantation Editor with the exemplary application of Mentype® DIPscreen PCR Amplification Kit and Mentype® DIPquant (a) as well as Mentype® Chimera® PCR Amplification Kit (b)

To export a Transplantation as .csv file, click the icon . Transplantations with displayed client “IVD” or “TRANSFER” are read-only. To enable editing,

the transplantation can be cloned to RUO via selection of icon  . Save all entries or changes by selection of icon  .

NOTE

Marker selection is solely in the responsibility of the user.

NOTE

Transplantations with displayed client “TRANSFER” which contain qPCR samples cannot be cloned to RUO.

Calculate Chimerism

Before starting the calculation, please determine whether the results shall appear as % recipient or % donor chimerism in ***Extras > Preferences > Chimerism Calculation***. After the transplantation is set up and informative markers are preselected the chimerism calculation can be started within the ***Sample Editor***. Click on  ***Calculate Chimerism.***

- **CE samples**

A dialog appears. First a window containing all detected alleles of the monitoring sample assigned to the donor and the recipient of the respective transplantation is displayed. Alleles of unknown origin (neither donor nor recipient) will likewise be depicted but can be faded out by a click on the button ***Hide alleles of unknown origin.***

NOTE

Only alleles that clearly originate from donor or recipient will be included in the calculation.

Click ***Next*** to confirm the donor and recipient assignment or click ***Cancel*** to return to the ***Sample Editor***.

The next window shows the input values for the chimerism calculation (see Figure 13). Markers preselected within the Transplantation Editor are shown in the table. These can either be used or deselected while others can be added. This function is especially helpful, when excluding single outliers from one analysis. Furthermore, the for the calculation used parameter heigth or area can be selected.

NOTE

Settings for the marker selection will only be active for the current calculation. The pre-settings of the transplantation will not be changed.

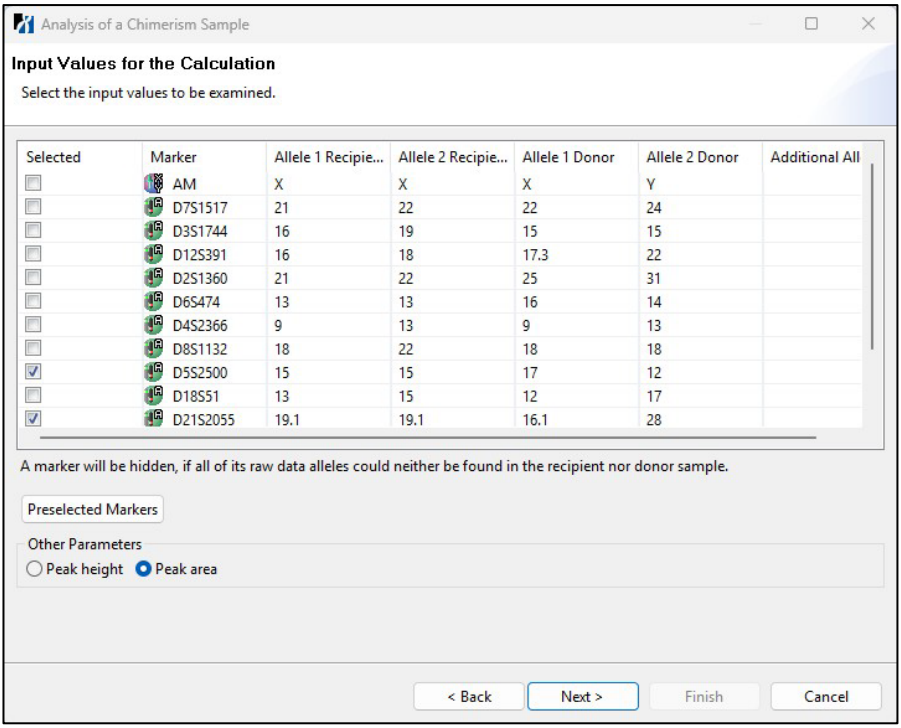


Figure 13. Input values for chimerism calculation, e. g. STR- alleles with Mentype® Chimera® PCR Amplification Kit

NOTE

After the chimerism calculation is successfully completed, a report can be issued within the ***Patient Editor***.

Click ***Next*** to confirm the input values and to see an overview of all detected alleles (see Figure 14). By clicking ***Finish*** all single marker chimerism values and the mean chimerism with standard deviation is displayed within the ***Sample Editor***.

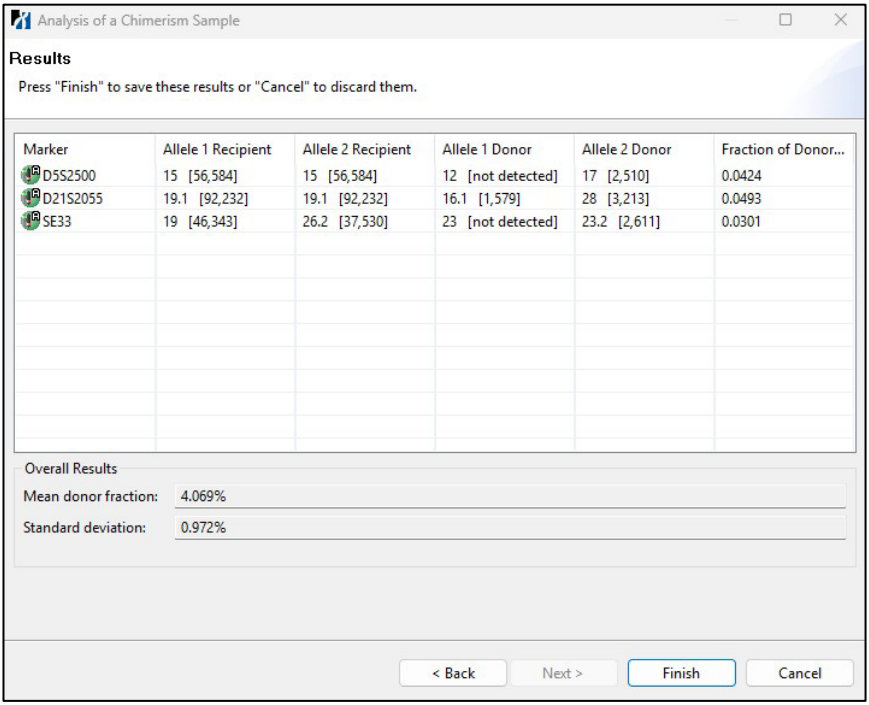


Figure 14. Result window

- **qPCR samples**

Select whithin the opened chimerism calculation settings whether PCR efficiencies of the respective Mentype® DIPquant assays should be considered. Select ***OK*** to perform the calculation with the chosen parameters. The result view (tab ***Patient specific Chimerism*** or ***Donor specific Chimerism***) is displayed. (see Figure 15)

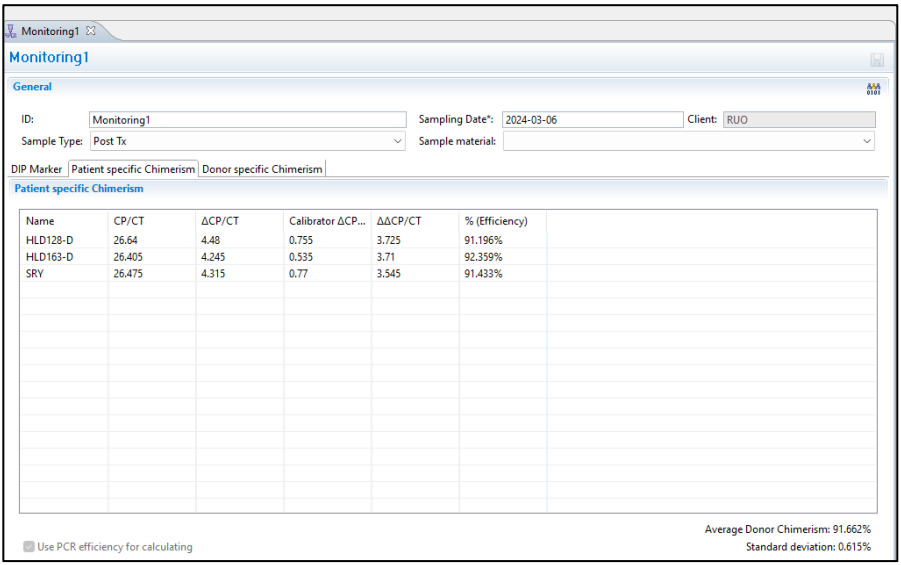


Figure 15. Result view of the chimerism calculated from a patient specific Mentype® DIPquant monitoring sample

The tabular result view shows all individual measurements for the selected Mentype® DIPquant assays (markers). For example, if patient specific loci were chosen for chimerism monitoring, results will be depicted in tab patient specific chimerism. The average value and the corresponding standard deviation of all markers are depicted in the lower part of the table. See Table 22 for details on the evaluation values. For details concerning the calculation please see chapter Chimerism Calculation.

Table 22. Values within qPCR chimerism calculation result display

Header	Description
Name	Displays the term of applied DIPquant assays.
CP/CT	Average value of multiple-measurements performed with one marker in a sample.
ΔCP/CT	Difference of the Cp/Ct average value of a specific DIP marker to Cp/Ct average value of the reference gene in a sample.
Calibrator ΔCP/CT	Difference between the Cp/Ct average value of a specific DIP marker and the Cp/Ct average value of the reference value deposited in the calibration sample (“Pre Tx”) in the transplantation.
ΔΔCP/CT	Difference between ΔCP of the monitoring sample and ΔCP of the calibrator.
% (Efficiency)	% chimerism, depending on the user’s preferences, either recipient – or donor chimerism.

NOTE

Calculated mean chimerism values and standard deviation are displayed within **Patient Editor** with a letter in front of the value to indicate whether chimerism was calculated patient specicifc (“R”) or donor specific (“D”).

Functions within the Patient Management

The Patient Management represents a database of all created patients. The tabular overview contains a list of all patients as well as a specific tool bar with functions to edit them. The functions within Patient Management are listed in Table 23. The tabular visualization can be edited according to the requirements of the user. To add or delete single columns, click the right mouse button.

Table 23. Function of the Patient Management

Icon	Function	Description
	Create new patient	Create a new patient
	Show patient	Open the patient editor
	Archive Patient	The patient is archived and not listed within the active Patient Management. Archived patients can be displayed after setting the filter respectively
	Reopen Patient	The archived patient is reopened within the active Patient Management
	Delete Patient	Delete a patient from the database
	Filter	Filter for specific attributes (detailed patient information) within the patient data base.
	Reset Filter	Reset the filter to display all active patients.
	Turn Pages	Show previous or next page
	Export Patients	Detailed patient information is exported as csv file
	Refresh	Refresh the page to show all recent adaptions

Create new patient

To create a new patient, go to ***Patient > Create new Patient*** within the menu bar, click on the icon ***Create new Patient*** in the tool bar or in the Patient Management. A dialog to create a new patient data sheet will open (see Figure 16). Information on Patient Number, Last Name and Sex are mandatory. Sex hereby refers to the biological sex determination.

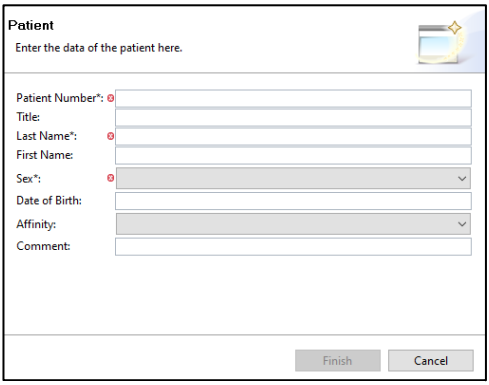


Figure 16. Create new Patient wizard

Show Patient

To open the Patient Editor select a patient within the Patient Management by double-clicking or select the icon ***Show Patient***. The Patient Editor contains all detailed patient information and assigned samples and transplantations.

Delete Patient

Select the respective patient from the Patient Management and choose the icon ***Delete Patient***. In order to delete patients all connected transplantations must be deleted within the ***Patient Editor***. A window will ask to confirm the deletion. Press ***Finish*** to delete the patient from the list or ***Cancel*** to abort the procedure.

Filter

The function ***Filter*** is helpful to search for patients from the data base. Click on the icon  ***Filter*** to open a filter mask (see Figure 17). Choose your respective criteria within the mask. Use the filter function to enter the archive (Status: archived), because the default Patient Management shows active patients only.

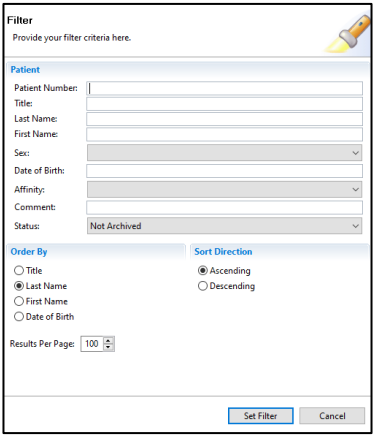


Figure 17. Filter mask within *Patient Management*

Archive

For an easy access and simplified overview within the data base, patients can be archived by clicking the icon  ***Archive Patient***. To reopen a patient and change the status to active, select the icon  ***Reopen Patient***.

Archived patients can be opened in the Patient Editor read-only as well as to generate a csv export.

Functions within the Batch Import Management

Fsa-files of a capillary gel electrophoresis run are imported in batch, meaning a whole folder is selected for the primary run validation, including sample files and control files (positive control, no-template control, allelic ladder).The

Batch Import Management provides an overview of all performed Batch Imports. Therby information about the processing user, date, time, and type or amount of imported samples is summarized within the management (see Figure 18). The table can be sorted by import date by clicking on the date column.

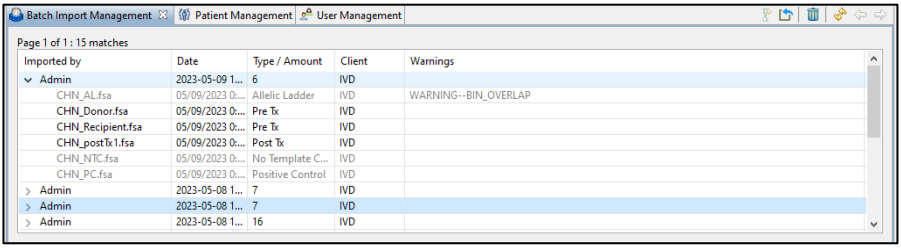


Figure 18. Batch Import Management

After the successful Batch Import, single files can be assigned to the desired ***Patient Editor***. See functions of the ***Batch Import Management*** below in Table 24.

Table 24. Functions of the Batch Import Management

Icon	Function	Description
	Assign CE sample	Assign the selected sample file to the recently opened ***Patient Editor. Allelic ladders, Positiv and No template Controls cannot be assigned.***
	Batch Import	Start batch import.
	Delete Batch	Delete a Batch from the database
	Refresh	Refresh the page to show all recent adaptions
	Turn Pages	Show previous or next page

NOTE

Control files are a mandatory part for the run validation process during the ***Batch Import.*** They are assessed based on the requirements of the test kit used. The CE-files of controls remain within the ***Batch Import Management***: they are already assigned to the specific ***Sample Editor*** and available for visual analysis within the toolbar.

Procedure for chimerism analysis

Run/Batch Import

ChimerisMonitor RUO supports the import and evaluation of raw data of different sequencer formats (fsa files). The program identifies peaks and artifacts as part of the process and is also capable of assigning peaks to alleles and, thus, to generate DNA profiles. During import an automated validation process is started based on the requirements of the applied kit.

To start the Batch Import click  ***Batch Import*** within the menu bar or ***Batch Import Management***. After defining the specific properties of the imported files like the applied ***Size Standard*** and ***Test Kit***, select ***Add Folder*** to search for saved runs (see Figure 19). Now adapt single sample types for the imported files by clicking in the respective table cell and select from the drop down menu.

Click ***Next*** to proceed.

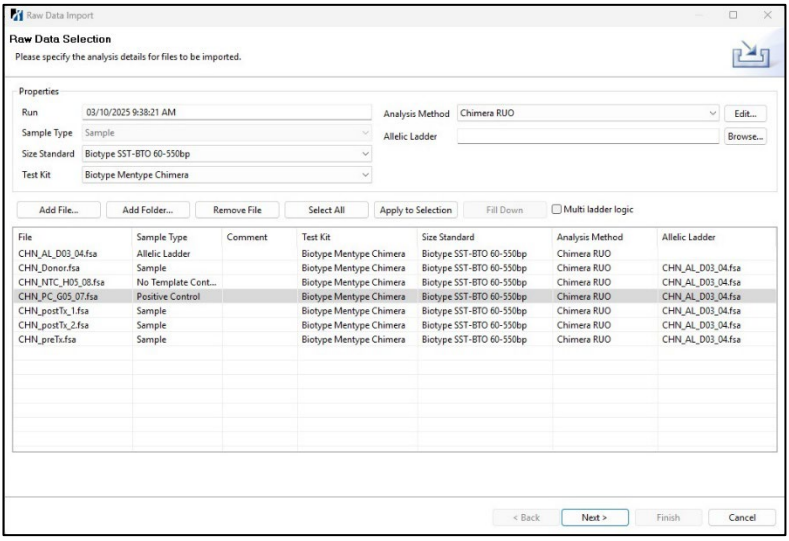


Figure 19. Raw Data Selection within the Batch Import

The window ***Run Validation*** provides a status of the Batch Import (Figure 20). The quality of all run controls, including the ***Allelic Ladder***, ***Positive Control*** and ***No Template Control*** is assessed.

NOTE

All passed quality criteria are marked with ☑

Possible warnings are marked with ⚠. Some criteria are not fulfilled. However, an analysis of the raw data is possible. Please review sample warnings within the ***Sample Editor***.

If quality criteria are not fullfilled, messages are marked with ✖

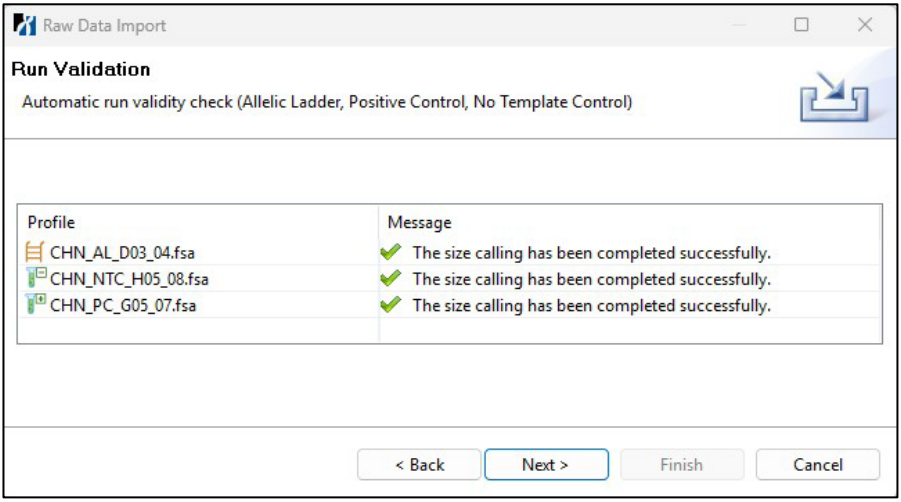


Figure 20. Run Validation within the Batch Import

The following criteria are checked within the Run Validation according to the selected anaylsis method:

1. Valid DNA Size Standard (BTO) of all controls
2. Valid peak heights in all control samples
3. Suitable number of allele peaks per loci, or no peak detection for No Template Control.

NOTE

For detailed information about the validation requirementents and criteria of Mentype® Chimera® PCR Amplification Kit and Mentype® DIPscreen PCR Amplification Kit, please refer to the kit specific instructions for use. Settings for the applied analysis method can be displayed via ***Extras > Preferences > Data Analysis > Analysis Methods.***

Click ***Next*** to proceed with the assessment of the ***Sample Quality***. The same general criteria as described above are used for the validity evaluation.

Make sure, that the correct sample type is selected for each sample (see Figure 21). In order to change the sample type, select the respective sample and click either in the tabel cell and select from the drop down menu or change by selecting the button ***preTx Sample*** or ***postTx Sample*** below the table.

An error explanation can be expanded for samples assigned as failed by clicking on the arrow in the Sample/Warnings column. In case of failed sample validity the respective sample needs to be deleted from the file table on page 1 of the wizard to finish the import.

Click ***Finish*** to complete the process. Click ***Cancel*** to abort.

The ***Batch Import Management*** will now open to assign validated samples to the ***Patient Editor***. Please refer to chapter Functions within the Batch Import Management

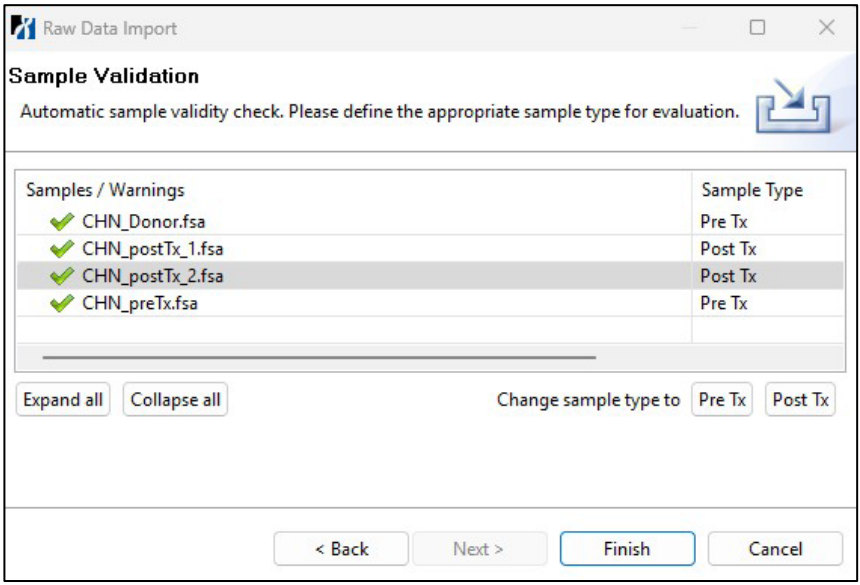


Figure 21. Sample Quality assessment within the Batch Import. e. g. sample type for postTX-samples must be changed.

Analysis of Electropherograms

ChimerisMonitor RUO is performing a general run and sample validation according to kit specific parameters and requirements. Nevertheless, each sample electropherogram should be evaluated by the user.

For detailed information about single functions within the Sample or Electropherogram Editors, please refer to chapter Sample Editor.

To assess the quality of a capillary gel electrophoresis run, please check the following parameters:

1. Correct allele assignments:

   Only alleles that are assigned are available for marker selection for preTx samples and only they are integrated within the chimerism calculation for postTx samples. A visual assessment of all electropherograms is higly recommended.

2. Evaluate all control samples:

   Including allelic ladder, positive control and no template control as well as their sizing quality. Check peak heights, correct allele assignment and peak shape based on the instructions for use of the respective test kit.

NOTE

Day to day fluctuations, environmental conditions, consumable status and DNA quality can effect the results of a capillary gel electrophoresis run. These effects include changing peak shapes with alterations like tailing, fronting, shoulder peaks or broadened peak basis, but also reduced or unbalanced peak heights.

3. Evaluate sample quality:

   By checking peak heights, allele assignments, peak shape and sizing quality according to the instructions for use of the respective test kit.

The number of called alleles should be plausible. When analysing postTx samples, DNA profiles should be comparable to donor or recipient samples. The software supports in filtering plausible donor- or patient-specific alleles. Any other additional peaks might be artifacts or contaminations, please refer to chapter Troubleshooting.

4. Evaluation of possible artifacts:

   Artifacts can be detected by ChimerisMonitor RUO. Please evaluate the quality section of each fragment table within the Electropherogram Editor.

   Stutters: The occurrence of stutter peaks depends on the sequence of the repeat structure and the number of alleles. n-4 peaks are caused by a loss of a repeat unit during amplification of tetranucleotide STR motives, caused by slippage effects of the Multi Taq 2 DNA Polymerase. Stutters are only relevant for the STR kit Mentype® Chimera® PCR Amplification Kit.

   Shoulder- und Split-Peaks: Shoulder and split peaks are double peaks with maxima, that are approximately one base pair apart. These peaks can be the result of inconsistent non-template addition, a consequence of the incomplete adenylation of PCR products by the polymerase during the PCR reaction.

   Off-ladder: Off-ladder peaks are located outside of allele bins. They can result either from very rare alleles or from other artifacts. Off-ladder peaks can be assigned to alleles (new or user-defined ones).

   Off-scale: The fluorescence (RFU) of off-scale peaks excesses the measurement range (up to 32,000 RFU).

   Tri-Band: Sometimes, an analysis apparatus might detect three signals, although there are only two PCR-fragments. This phenomenon is called tri-band pattern. The peaks of a tri-band pattern are all of the same height or two of the peaks cumulate to the height of the third one. Tri-band patterns are specific for analysis apparatuses and kits. A number of known tri-band patterns are already deposited in the reference database.

   Spectral Overlap: Spectral overlap artifacts are peaks resulting from overlapping emission spectra of fluorescent dyes, i. e. the signal of

one dye is mistakenly detected in another color panel, too. Hence, these pull-ups are situated at the same position (data point) as the larger causing peak, only in another color panel.

Peak height and width: Too large RFU values can indicate pull-ups. Furthermore, too wide peaks should also not be regarded as allele fragments. Hence, you can set maximum peak heights and widths.

Setting up a transplantation

In order to define informative loci via the ***Transplantation Editor***, all sample types must be assigned for both ***Donor*** and ***Recipient*** within each ***Sample Editor***. For detailed information about all functions within the Transplantation Editor please refer to chapter Transplantation Editor. Click  ***Create new Transplantation*** Within the ***Patient Editor*** and define Date and Transplantation ID. After assigning Donor and Recipient sample to the Transplantation, informative loci can be selected as a general preselection for upcoming chimerism calculations.

Qualitative analysis – identification of informative loci

- **CE**

In the following, the identification and differentiation of patient specific loci is explained. Therefore, donor specific loci are defined as non-informative. The identification of informative loci is performed using data from patient and donor before the transplantation.

Informative Loci: At least one allele in the patient sample cannot be detected in the donor sample.

Only for Mentype® Chimera® PCR Amplification Kit: This allele shall not be in the stutter area of the donor sample.

Non-informative loci: Loci where the patient specific peak overlap with the donor specific peak, or donor-specific loci.

Only relevant for **Mentype® Chimera® PCR Amplification Kit:**

Stutter (n+1) loci: The patient specific peak is overlaping with the n+1 stutter of the donor specific peak. Such loci can be used if few informative loci are available.

Stutter (n-1) loci: The patient specific peak is overlaping with the n-1 stutter of the donor specific peak. Such loci should only be used if no other informative or stutter (n+1) loci are available.

The evaluation of the locus informativity is performed according to the published formulas described in: Nollet, F.; Billiet, J.; Selleslag, D.; Criel, A. (2001) *Standardisation of multiplex fluorescent short tandem repeat analysis for chimerism testing*, Bone Marrow Transplantation 28 (5), p. 511-518.[1]

NOTE

Using stutter (n-1) loci for the semi-quantitative monitoring, the sensitivity is decreased due to overlap of the patient specific allele and the stutter from the donor allele.

- **qPCR**

To enable calibration within the chimerism calculation to the recipient, a qPCR sample of the recipient with minimum the previously selected markers should be selected in the section Recipient Calibrator via the button ***Add***. Only chronoligical relevant samples with the necessary settings (qPCR, Sample Type: preTX) are displayed in the opened dialog. Select the respective sample and add the sampling date by manual input or via the date selection. Click ***OK*** to add the selection to the Recipient Calibrator table or ***Cancel*** to close the window without saving. By selection of the button ***Remove*** the selected sample is deleted from the table.

By selection of the check box ***Respect PCR efficiency in calculation,*** the default for the inclusion of the marker specific PCR efficiences within the chimerism calculation is set respectivly.

The ***DIPselector*** is a function within the ***Transplantation Editor*** to determine marker informativity donor- or recipient specific. It supports the correct selection of Mentype® DIPquant assays to the respective preTX samples.

After selection of the ***DIPselector,*** a Genotype Overview with the particular DIP markers for the donor and recipient is displayed. By selection of ***Next*** the DIP Selection is summarized in a Patient Specific Loci- and a Donor Specific Loci table. Here all DIP markers together with the available specific Mentype® DIPquant assays are listed.

In addition, the type of the allelic constellation between donor and recipient is provided. Type 1 describes the homozygote allelic constellation of DIP markers (two copies per cell). Type 2 describes the heterozygote allelic constellation of DIP markers (one copy per cell).

The DIPselector is ranking the informative DIP markers and the respective specific Mentype® DIPquant assays for their suitability to quantitatively address chimerism. The Mentype® DIPquant assays that are suited best, appear at the top of the list. Type 1 constellations (homozygote) are more sensitive and thus superior to Type 2 constellations (heterozygote,). Furthermore, qPCR assays with the highest efficiency within one specific type are preferred. (see Figure 22).

Choose DIP markers and Mentype® DIPquant assays to use for chimerism calculation by selection of the respective check box. Select ***Finish*** to confirm the selection or ***Cancel*** to return to the ***Transplanation Editor*** without saving***.***

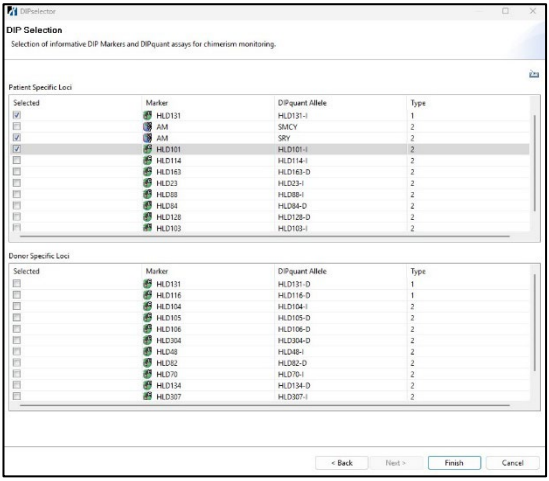


Figure 22. Selection of informative DIP markers by the DIPselector

Chimerism Calculation

After the transplantation is set up and informative markers are preselected the chimerism calculation can be started within the ***Sample Editor***. Click on ***Calculate Chimerism.*** For detailed information on how to perform the chimerism calculation, please refer to chapter Calculate Chimerism.

NOTE

Only alleles that are assigned and selected within the transplantation or within the window Input Values for Transplantation will be included in the calculation.

Semi-quantitative analysis – chimerism analysis CE

A chimerism analysis is performed to monitor the success of the allo-HSCT. After transplantation, some of the patient's cells show the patient's native genotype **P** and some cells show the genotype of the donor's cells **D**. The patient shows characteristics of a chimerism. Ideally, the ratio of the native patient cells will lower to zero in comparison to the donor cell ratio **D** that will steadily increase.

Chimerism analysis identifies the ratio of donor - and recipient genotype **F(D)** in a patient sample.

To analyse chimerism, the native genotypes **D** and **P**, thus the donor's and recipient's genotype, need to be known before the transplantation.

Chimerism analysis can either be performed on the peak area (recommended) or the peak height. Only peaks of alleles are considered for the calculation that can clearly be assigned to either the donor's **D** or the recipient's **P** genotype. All loci, in which the donor's or recipient's genotypes differ in at least one allele, can be included in the analysis.

The following formula is used to calculate the % donor ratio:

$$\boldsymbol{F(D) = 100\,\% * \frac{A(D)}{A(D) + A(P)}}$$

To calculate the % recipient ration F(P), the value of the donor chimerism will be subtracted from 100 %.

$$\boldsymbol{F(P) = 100\ \% - F(D)}$$

F(D) cell ratio of donor-genotype D in percent,

A(D) total peak area of donor alleles,

A(P) total peak area of native patient alleles,

For the overall evaluation of the sample, the average value of all marker-percentages that were included in the analysis, will be calculated. Moreover, the standard deviation will be assessed.

The evaluation of the chimerism calculation is performed according to the published formulas described in *Nollet et al. (2001).*[1]

Relative quantification – chimerism anaylsis qPCR

The quantification of qPCR data is performed by the relative quantification method. As an example calculation with a patient specific assays is depicted.

- **Quantification of the calibration sample (C) – before transplantation (Pre Tx)**

1. Individual CP/CT values of the reference (REF) and the patient specific DIPquant assays “Alleles of Interest” (AOI) of the patient before transplantation (pre Tx) will be imported.

2. In case of multi-measurements (replicates) the average value for each marker will be calculated first.

3. Following, the ΔCP/CT value for each AOI in reference to the REF will be calculated:

ΔCP/CT(C) = CP/CT(AOI) – CP/CT(REF)

4. ΔCP/CT(C) depicts the value of the calibrator for the following calculation and relates to 100 % recipient.

- **Quantification of the monitoring sample (P) – after transplantation (Post Tx)**

1. Individual CP/CT values of the reference (REF) and the recipient specific DIPquant assays „Alleles of Interest“ (AOI) of the patient sample (P) will be imported.

2. In case of multi-measurements (replicates) the average value for each marker will be calculated first.

3. Afterwards the ΔCP/CT value for each AOI in reference to the REF within a monitoring sample will be calculated:

ΔCP/CT(P) = CP/CT(AOI) – CP/CT(REF)

4. ΔCP/CT(P) will be used to calculate the unknown monitoring status.

5. Now, the ΔΔCP/CT for the quantification of the chimerism will be calculated; the ΔCP/CT value of the calibrator (C) will be subtracted from the ΔCP/CT value of the patient sample (P):

ΔΔCP/CT = ΔCP/CT(P) – ΔCP/CT(C)

5. The calculation of the % recipient ratio can only be performed in dependency of the qPCR efficiency:

F(P)% = ((1+E)-(ΔΔCP/CT)) ∗ 100

or with a generally assumed qPCR value of 100 % efficiency:

(2-(ΔΔCp)) x 100

To calculate % donor ration F(D), the value of the recipient chimerism will be subtracted from 100 %.

F(P)= 100 % - F(D)

For the overall evaluation of the sample, the average value of all marker-percentages included in the analysis will be calculated. Moreover, the standard deviation will be assessed.

Troubleshooting

Installation

Symptom	Answer
Installer shows a dialog requesting username and password.	Installation requires administrative permissions. Request an administrators assistance or to provide credentials of a privileged user.
An error shows up during installation.	Check the Installer console window for details. If necessary console output can be copied by right-click and may be saved into an file for further analysis.
Installation aborts before completion.	Check the Installer console window for details. If necessary console output can be copied by right-click and may be saved into an file for further analysis.
Installer doesn't prompt to import existing ChimerisMonitor 2.1 database.	The installer searches only in the ChimerisMonitor 2.1 *default database folder* C:\ProgramData\Biotype\ChimerisMonitor\database for an ChimerisMonitor 2.1 database. An import prompt is only shown, if the directory exists and the file PG_RELEASE is found there.
After canceling the database import dialog, the dialog is no longer shown.	Import of ChimerisMonitor 2.1 data is only possible on first time installations if no ChimerisMonitor IVD database exists already. Canceling the import dialog will create a new ChimerisMonitor IVD database. As the import into an existing ChimerisMonitor IVD database is not supported at all, the dialog is no longer shown.

Application

Symptom	Answer
User “admin” cannot be edited.	The user “admin” is built-in by the software and does not allow any modification.
The license is invalid.	The license key is incorrect or the license has timed out.
A problem occurred accessing the database	This can be caused by serveral problems, check: the server setting in the login dialog if the ChimerisMonitor IVD database service is running
The client won't start.	Try to start the client in “Safe mode” by selecting the relevant menu entry in Windows Start-Menu. Alternatively check the client logfile.
It is not possible to create patients, change samples or transplantations.	The software may be in read-only mode. To switch mode enter a valid license.

Batch Import

Symptom	Answer
The UUID of the Run is not unique.	Selected samples seem to belong to different analyzer runs. For quality reasons it is recommended that all samples should origin from the same run.
There are additional peaks after the assigned size standard peaks. Check the size standard.	More peaks than expected have been detected in the size standard. It is solely possible to use the in the Batch Import selected Size Standard. If artefacts are interfering with the size calling regression please repeat the capillary

Symptom	Answer
	gel electrophoresis. Make sure to use fresh consumables.
Ladder Calibration failed: No alleles detected.	Calibration of allelic ladder file seems unsuccesful as no alleles had been assigned to peaks. Please repeat the analysis. Make sure that the correct allelic ladder is added to the respective well and that all components are mixed sufficiently.
Could not detect an X allele for amelogenin in the marker.	The file contained no allele marked as gender determinand allele X. As the Genotype is not obtained completely, the analysis should be repeated. Allelic dropout or insufficient amplification could be a reason, if all other markers are detected.
The profile requires a Positive/ No Template Control associated with it.	There was no No Template Control or Positive Control in the list of files to import. It is strongly recommended for a successful run validation.
The profile needs an allelic ladder associated with it.	The imported file contains no reference about the ladder file it was called with. Please repeat the analysis with an Allelic Ladder included.
Not all expected ladder alleles were found in raw data.	The imported allelic ladder file doesn't contain all expected alleles of the selected test kit. Make sure that correct allelic ladder is added to the respective well and that all components are mixed sufficiently.
Additional alleles were found that are not in ladder.	Imported allelic ladder file contains more alleles than expected by testkit. If artefacts are interfering with the Allelic Ladder please repeat the capillary gel electrophoresis. Make sure to use fresh consumables.

Symptom	Answer
Ladder calibration warning : Bins are overlapping {0}	Bins of neighboring alleles are overlapping. Because of day-to-day interferences or different Genetic Analyzer performances it can happen, that peak bases of neighboring alleles merge, because of broader peak bases. Please visually assess the Allelic Ladder for the respective alleles.
Too many alleles found in marker	Imported profile contains more alleles than expected. The expected allele count is determined by sample type reference. The detection of additional alleles in preTx samples or more than 4 alleles in postTx samples might be caused by contaminations or CE-artefacts. Repeat PCR or capillary electrophoresis and ensure a clean working environment. The detection of 3-4 alleles in postTx samples could be caused by mixed chimerism and will be checked for plausibility during sample assignement to the patient.
Not enough alleles found in marker	Imported profile has less alleles than expected. The expected allele count is determined by sample type: reference or chimerism. Alleles below the test kit specific thresholds are not assigned. Please refer to the test kits instructions to ensure the correct setup of the PCR reaction
Unable to assign sample from “Batch Import Management” to patient	Check selected sample type. Only reference- or chimerism samples can be assigned to a patient.

References

[1] Nollet, F., Billiet, J., Selleslag, D., & Criel, A. (2001). Standardisation of multiplex fluorescent short tandem repeat analysis for chimerism testing. Bone marrow transplantation, 28(5), 511-518.

[**2**] Clark, J. R., Scott, S. D., Jack, A. L., Lee, H., Mason, J., Carter, G. I., ... & Barnett, D. (2015). Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): technical recommendations for the use of short tandem repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. British journal of haematology, 168(1), 26-37.

[**3**] Thiede, C., Florek, M., Bornhäuser, M., Ritter, M., Mohr, B., Brendel, C., ... & Neubauer, A. (1999). Rapid quantification of mixed chimerism using multiplex amplification of short tandem repeat markers and fluorescence detection. Bone Marrow Transplantation, 23(10), 1055-1060.

Cybersecurity

This chapter outlines necessary cybersecurity measures and guidelines to ensure the integrity and confidentiality of data handled by ChimerisMonitor RUO. As this software is employed in sensitive medical applications, protecting patient information and ensuring the reliability of data is our outmost concern.

System Requirements

Operating Systems: ChimerisMonitor RUO is compatible with the operating systems Windows 10 or 11.

Antivirus Software: Up-to-date antivirus software must be installed and running.

Firewall Settings: A configured firewall to protect data inflow and outflow.

Configure firewall rules to restrict access to only authorized IP addresses, protocols, and ports required for system functionality.

Implement rate-limiting to control the number of requests allowed per user or system within a specified time frame, preventing abuse or denial-of-service attempts.

Secure Installation and Configuration

Ensure that ChimerisMonitor RUO is installed by a qualified technician or an authorized representative. Set strong, unique passwords for accessing the software, and change them periodically.

Data Privacy

All patient data should be treated as confidential. Access should be restricted to authorized personnel only. This also applies to exported data and system backups. Ensure compliance with local and international data protection regulations (e. g., GDPR, HIPAA).

Regular Updates and Maintenance

Regularly update ChimerisMonitor RUOto the latest version to protect against vulnerabilities. Apply security patches and updates as soon as they are available. Maintain and regularly review system and access logs to monitor for any unauthorized access or anomalies.

Server Logs

Server logs are generated periodically and can be found at C:\ProgramData\Biotype\ChimerisMonitor 3\database\log. Regularly check these logs for any unusual activity or errors. These logs are crucial for troubleshooting and understanding the health of the server components.

Client Logs

Client application logs are incrementally generated and located in the directory %USERPROFILE%\ChimerisMonitor RUO\.metadata. Client logs provide insights into user operations and should be reviewed in response to user-reported issues or unexpected client behavior. These logs are essential for tracking user interactions and potential application issues.

Backups

Make sure, that backup routines are regularly performed for crucial data. Follow these steps:

Stop the "ChimerisMonitor-RUO-Database" service via the Windows Services Manager. Access the Database Folder: Open the Windows Explorer. Navigate to the ‘View’ tab and check the option for ‘Hidden Items’. Go to the directory C:\ProgramData\Biotype\ChimerisMonitor 3. Copy the database folder: Locate and select the ‘database’ folder within the directory. Right-click and choose ‘Copy’. Securely paste the copied folder to your designated backup location, ensuring it is outside the local drive for redundancy. Restart the "ChimerisMonitor-RUO-Database" service to resume normal operations.

Incident Response

If suspicious behavior is observed, users should consult their IT administrator or IT security officer immediately. Form an incident response team capable of responding to cybersecurity threats. Develop a comprehensive disaster recovery plan to restore functionality and data in the event of a cybersecurity incident.

Decommissioning

If the software needs to be taken out of operation, it is crucial not only to uninstall the application but also to ensure that all sensitive data is either securely erased from storage devices or archived according to the requirements of your organization. This process should follow a standard, verified data destruction protocol.

Training and Awareness

Conduct regular training sessions for all users to understand the cybersecurity policies and procedures related to ChimerisMonitor RUO.

Implement ongoing awareness campaigns to keep security at the forefront of operations.

Technical Assistance

For technical advice or assistance with cybersecurity issues please contact our Technical Support:

e-mail: support@biotype.de

phone: +49 (0)351 8838 400

Limitations of Use

- The procedures in this handbook must be followed, as described. Any deviations may result in error messages.
- ChimerisMonitor RUO is a software solution for automated analysis of capillary electrophoresis-data (fsa-files) using the Mentype® Chimera® PCR AmpIfication Kit, Mentype® DIPscreen PCR Amplification Kit and of qPCR data (txt-files) using Mentype® DIPquant.assays only.
- Use of this product is limited to professional laboratory users trained on molecular-genetic techniques, multiplex PCR, and the handling of Genetic Analyzers of Thermo Fisher Scientific (Applied Biosystems division).
- The software is only intended to be used for research purposes, a use for diagnostic purposes is not permitted.
- Chimerism monitoring of patients whose donor is their identical twin is not possible.

Trademarks and Disclaimers

Mentype® and Chimera® are registered trademarks of BIOTYPE GmbH.

Other trademarks: Applied Biosystems® (Applied Biosystems LLC group)

The PCR is covered by patents. Patentees are Hoffmann-La Roche Inc. and F. Hoffmann-La Roche (Roche).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

Not available in all countries.

This product is for Research Use Only (RUO) and is not intended for diagnostic applications. While the manual may reference terms such as ‘patient’, ‘donor’ or ‘transplantation’ among others, these references do not imply clinical use. Research Use Only products must be validated by the customer with clinically relevant material for diagnostic purposes. The user assumes full responsibility for adjusting assay-specific parameters to meet their specific requirements.

Explanation of Symbols

	Manufacturer
	Batch code
	Reference to eIFU
	Catalogue number
RUO	In-Vitro-Diagnostics

Further marking used in this Instruction for Use:

	Useful tips
	Attention, be sure to follow this notice!
blue underlined text	Links leading to external content like homepages, e-mail adresses
black underlined text	Cross-links in the document for easy navigation

Appendix

Mentype® DIPquant qPCR assays supported by ChimerisMonitor RUO

Product	Packaging size	Order number
Mentype® DIPquant Reference	25 reactions	45-01591-0025
Mentype® DIPquant Reference	100 reactions	45-01591-0100
Mentype® DIPquant SRY	25 reactions	45-01590-0025
Mentype® DIPquant SMCY	25 reactions	45-01589-0025
Mentype® DIPquant HLD23-I	25 reactions	45-01538-0025
Mentype® DIPquant HLD38-I	25 reactions	45-01558-0025
Mentype® DIPquant HLD48-I	25 reactions	45-01560-0025
Mentype® DIPquant HLD53-D	25 reactions	45-01561-0025
Mentype® DIPquant HLD53-I	25 reactions	45-01562-0025
Mentype® DIPquant HLD67-D	25 reactions	45-01567-0025
Mentype® DIPquant HLD67-I	25 reactions	45-01568-0025
Mentype® DIPquant HLD70-D	25 reactions	45-01569-0025
Mentype® DIPquant HLD70-I	25 reactions	45-01570-0025
Mentype® DIPquant HLD79-I	25 reactions	45-01576-0025
Mentype® DIPquant HLD82-D	25 reactions	45-01577-0025
Mentype® DIPquant HLD82-I	25 reactions	45-01578-0025
Mentype® DIPquant HLD84-D	25 reactions	45-01579-0025
Mentype® DIPquant HLD84-I	25 reactions	45-01580-0025
Mentype® DIPquant HLD88-D	25 reactions	45-01581-0025
Mentype® DIPquant HLD88-I	25 reactions	45-01582-0025
Mentype® DIPquant HLD91-D	25 reactions	45-01585-0025
Mentype® DIPquant HLD91-I	25 reactions	45-01586-0025
Mentype® DIPquant HLD97-I	25 reactions	45-01588-0025

Product	Packaging size	Order number
Mentype® DIPquant HLD101-D	25 reactions	45-01501-0025
Mentype® DIPquant HLD101-I	25 reactions	45-01502-0025
Mentype® DIPquant HLD103-D	25 reactions	45-01505-0025
Mentype® DIPquant HLD103-I	25 reactions	45-01506-0025
Mentype® DIPquant HLD104-D	25 reactions	45-01507-0025
Mentype® DIPquant HLD104-I	25 reactions	45-01508-0025
Mentype® DIPquant HLD105-D	25 reactions	45-01509-0025
Mentype® DIPquant HLD105-I	25 reactions	45-01510-0025
Mentype® DIPquant HLD106-D	25 reactions	45-01511-0025
Mentype® DIPquant HLD106-I	25 reactions	45-01512-0025
Mentype® DIPquant HLD110-I	25 reactions	45-01514-0025
Mentype® DIPquant HLD112-I	25 reactions	45-01516-0025
Mentype® DIPquant HLD114-D	25 reactions	45-01517-0025
Mentype® DIPquant HLD114-I	25 reactions	45-01518-0025
Mentype® DIPquant HLD116-D	25 reactions	45-01519-0025
Mentype® DIPquant HLD116-I	25 reactions	45-01520-0025
Mentype® DIPquant HLD128-D	25 reactions	45-01523-0025
Mentype® DIPquant HLD128-I	25 reactions	45-01524-0025
Mentype® DIPquant HLD131-D	25 reactions	45-01525-0025
Mentype® DIPquant HLD131-I	25 reactions	45-01526-0025
Mentype® DIPquant HLD133-I	25 reactions	45-01528-0025
Mentype® DIPquant HLD134-D	25 reactions	45-01529-0025
Mentype® DIPquant HLD134-I	25 reactions	45-01530-0025
Mentype® DIPquant HLD140-I	25 reactions	45-01532-0025
Mentype® DIPquant HLD152-D	25 reactions	45-01533-0025
Mentype® DIPquant HLD163-D	25 reactions	45-01535-0025

Product	Packaging size	Order number
Mentype® DIPquant HLD163-I	25 reactions	45-01536-0025
Mentype® DIPquant HLD301-D	25 reactions	45-01539-0025
Mentype® DIPquant HLD301-I	25 reactions	45-01540-0025
Mentype® DIPquant HLD304-D	25 reactions	45-01541-0025
Mentype® DIPquant HLD305-D	25 reactions	45-01543-0025
Mentype® DIPquant HLD305-I	25 reactions	45-01544-0025
Mentype® DIPquant HLD307-D	25 reactions	45-01545-0025
Mentype® DIPquant HLD307-I	25 reactions	45-01546-0025
Mentype® DIPquant HLD310-D	25 reactions	45-01549-0025

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