User Manual

uTYPE™ HLA Sequence Analysis Software (Research)

Version 7.x Rev 03
Catalog #: 539991

For Research Use Only. Not for use in diagnostic procedures.

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Product is for research use only and should not be used for clinical test results. The software design does not mitigate hazards associated with the software. Please note that this document was prepared in advance of the software release. Therefore, you may notice slight differences in the content of the actual application screens.

For Research Use Only. Not for use in diagnostic procedures.

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Purpose of this guide

This guide provides instruction for the use of the uTYPE7.x Sequencing Software, which will be referred to as uTYPE7.x Software throughout this guide. This guide includes:

- Procedures that show how to perform basic sequence-based trace (SBT) editing
- Procedures for administration and maintenance tasks
- A Reference section that illustrates and describes the software interface elements
- A Troubleshooting section

Audience

This guide is for laboratory technicians analyzing sequencing data or HLA allele alignment.

Assumptions

This guide assumes the user:

- Has installed uTYPE7.x Software
- Is processing .ab1 sequence files generated by the Applied Biosystems Genetic Analyzers
- Is experienced (or being trained) in resolving trace data discrepancies

Safety alert words

Four safety alert words appear in Invitrogen user documentation at points in the document where the user needs to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper system operation or accurate chemistry kit use.

⚠ **CAUTION! –** Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to

alert against unsafe practices**.**

⚠ **WARNING! –** Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to

alert against unsafe practices.

⚠ **DANGER! –** Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be

limited to the most extreme situations**.**

Except for IMPORTANTs, each safety alert word in an Invitrogen document appears with an open triangle figure that contains a hazard symbol.

ProductInformation

Overview of uTYPE7.x Sequencing Software

uTYPE7.x Software processes DNA sample data files of Class I and Class II Human Leukocyte Antigens (HLA) for analysis and HLA allele assignment by comparison to the IMGT/HLA database. The sample data files are generated from samples processed with SeCore® Sequencing or GSSP kits and Applied Biosystems Genetic Analyzers.

uTYPE7.x Software can be used to analyze:

Locus	Exons
HLA A	1,2,3,4,5
HLA B	1,2,3,4,5
HLA C	1,2,3,4,5,6,7
HLA DRB1	1,2,3
HLA DRB3,4,5	2,3
HLA DQA1	1,2, 3, 4
HLA DQB1	2,3
HLA DPA1	2
HLA DPB1	2,3,4
MICA	2,3,4,5
MICB	2,3,4,5

Intended Use

uTYPE Software is intended to interpret and match sequencing data generated on the genetic analyzer with data collection software using the HLA sequencing kits to known HLA type sequences.

Initial Setup

System requirements

uTYPE 7.x Software has the following minimum computer and operating system requirements:

- PC with CD-ROM drive or network connection.
- A minimum of 1 GB free disk space in the software install directory.
- Compatible operating
  systems: Windows 10

Input file requirements

uTYPE 7.x Software requires .ab1 sequence files generated from ABI Genetic Analyzer Software. The files need to follow the uTYPE 7.x Software naming conventions.

Assign names to the samples in the uTYPE 7.x Software according to the following criteria:

- Maximum 24 characters
- No special characters ('space', / : \ * ? " < > |)
- Sample ID includes all of the characters before the first hyphen or underscore

The software examines the file name for the locus designations and exon directions in the sample file, and uses the information to fill in fields in the **Load Files** dialog box.

If sample names contain errors, they will not load and a red block will appear in the **Load Files** dialog box, showing the missing information. For example, in the following figure, 'Locus' needs to be edited in the file names.

Sample	Locus	Special	Exon	File Name	Path
☐ 406		SPZ3	0F	406_Z3_G02.ab1	C:\Documents and Set1
☐ 406			2F	406_2F_A02.ab1	C:\Documents and Set1
☐ 406			2R	406_2R_H02.ab1	C:\Documents and Set1
☐ 406			3F	406_3F_C02.ab1	C:\Documents and Set1
☐ 406			3R	406_3R_D02.ab1	C:\Documents and Set1
☐ 406			4F	406_4F_E02.ab1	C:\Documents and Set1
☐ 406			4R	406_4R_F02.ab1	C:\Documents and Set1

The sample ID can contain hyphens to separate the sample name from the DRB1 group when using the DRB Group Kit. Starting with the locus name, all other data needs to be separated by underscored spaces.

The standard format is:

```
[SampleID]_[locus]_[exon and direction]
```

Note: A genetic analyzer assigned sequence ID will be appended to all data files, for example SampleID_A_2F entered by the user will become SampleID_A_2F_C01_1085.ab1.

Kit	Locus designation	Specific naming conventions	Example
A Locus	_A_	None	SampleID_A_2F
B Locus	_B_	None	SampleID_B_2F
C Locus	_C_	None	SampleID_C_3F
DPA1	_DPA_orDPA1	None	SampleID_DPA_2R
DPB1 Locus	_DPB_ or _DPB1_	Z38 indicates codon 85 Z39 indicates codon 8	SampleID_DPB_2R SampleID_DPB_Z38 SampleID_DPB_Z39
DQA1 Locus	_DQA_	None	SampleID_DQA_2R
DQB1 Two Amp	_DQB_ or _DQB1_	None	SampleID_DQB1_2F
DRB1 Locus	_DRB1_	TG indicates codon 86.	SampleID_DRB1_2F SampleID_DRB1_TG
DRB Group	_DRB_	Use the part of the sample name in front of the loci designation to indicate specific DRB1 group. See Example for DRB1*01, DRB1*08,DRB 3,DRB 4 and DRB5.	SampleID-01_DRB1_2F SampleID-08_DRB1_2F SampleID_DRB3_2F SampleID_DRB4_2F SampleID_DRB5_2F
GSSP	[locus]_Z#_	Include locus name followed by the Z primer number	SampleID_A_Z5 SampleID_A_Z13
MICA	_MICA_	None	SampleID_MICA_2F
MICB	_MICB_	None	SampleID_MICB_2F

Back up existing files

IMPORTANT! Perform data backup before installing a new version of the software. At a minimum, backup the uTYPE 7.x Software installation folder and its storage folder. The storage folder can be in the installation folder or in a designated network folder.

Uninstall previous versions of the software before installation of uTYPE 7.x Software.

Get Started

Suggested workflow

```
┌─────────────────────────────────────┐
│   Load and process sequence files    │
└─────────────────────────────────────┘
                 ↓
┌─────────────────────────────────────┐
│   Select sequence file in Sample List │
└─────────────────────────────────────┘
                 ↓
┌─────────────────────────────────────┐
│   Review marked positions in sequence │
│ (discrepant sites, software calls,    │
│  and/or mismatches)                   │
└─────────────────────────────────────┘
                 ↓
┌─────────────────────────────────────┐
│       Edit sequences (if necessary)   │
└─────────────────────────────────────┘
                 ↓
┌─────────────────────────────────────┐
│          Examine Match List           │
└─────────────────────────────────────┘
                 ↓
┌─────────────────────────────────────┐
│ Filter the Match List or "check"      │
│ marked positions in the sequence      │
│ (need at least one allele pair with   │
│ "0" mismatches against the library)   │
└─────────────────────────────────────┘
                 ↓
┌─────────────────────────────────────┐
│ Resolvable ambiguous allele           │
│ combination present?                  │
│   Yes                  No             │
└─────────────────────────────────────┘
       ↓                    ↓
     Save                  Save
       ↓                    ↓
   Run GSSP          Review/approve sequence
       ↓                    ↓
 Load saved sequence       Save
 file and GSSP              ↓
 sequence file        Generate report
```

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Log on to software

Note: Each user will need a unique username and password. Users can be created only by a Supervisor. See Add and remove users.

1. Start the software by selecting **Start > Programs > SBT > HLA SBT uTYPE 7.x** or by double-clicking the HLA SBT uTYPE 7.x Software   icon
on the desktop.

2. Select your username in the dropdown menu, type in your password, and click **Logon**.

1. Warning screen appears. All .ab1 files must be analyzed using mixed base identification. Click 'Exit' on this screen.
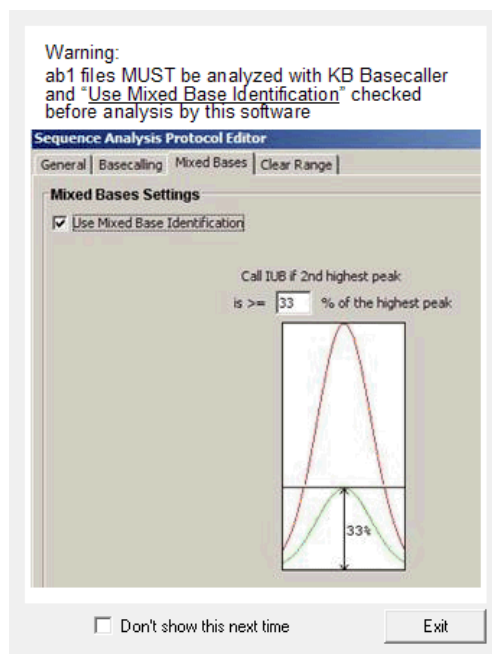
The uTYPE 7.x Software main window appears.
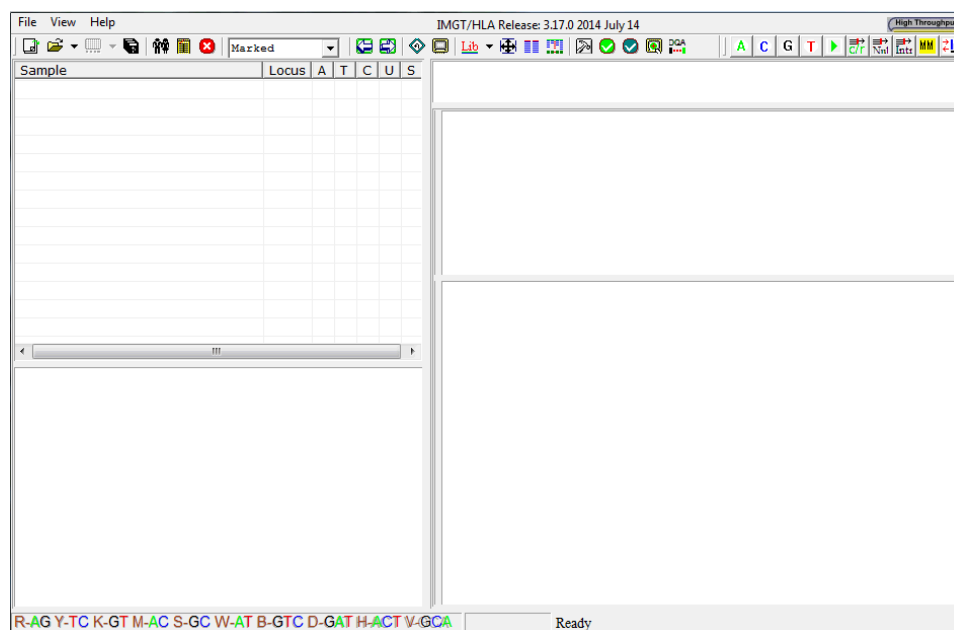
For an overview of the uTYPE 7.x Software main window toolbar, and functions of the different panes in the window, see Toolbar Overview and Main Window Overview.

IMPORTANT! If your library files are older than 120 days, a warning will be displayed on your logon screen. For instructions for ordering the database library, see Update Library.

Specify storage location for saved results

The software creates a storage folder in the default location:

C:\Program   Files\Invitrogen\SBT\storage

If you wish to change the location of the database storage folder, you must be logged on as a Supervisor-level user. In addition, you cannot have any files loaded at the time you change storage location.

1. Log in as a Supervisor-level user.
2. Before loading any files, click the **Settings** icon **> Database Options tab > Set to new location** in the toolbar.
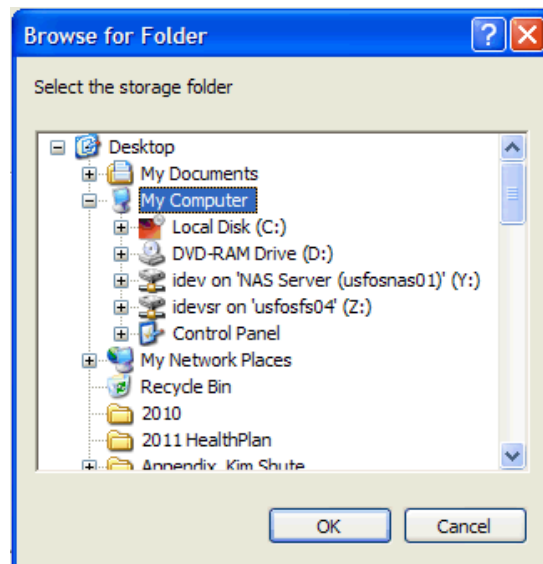
3. Choose the new location for the database storage folder and click **OK**.

    If you wish to restore the database storage folder to its default location, click **Reset to default.**

Load sequence files

1. Select **File > Load Files** or click in the toolbar. Alternatively .ab1 files can be dragged from the Windows interface and dropped onto the main window.
2. When the **Load Files** window opens, click **Flexible Load**.
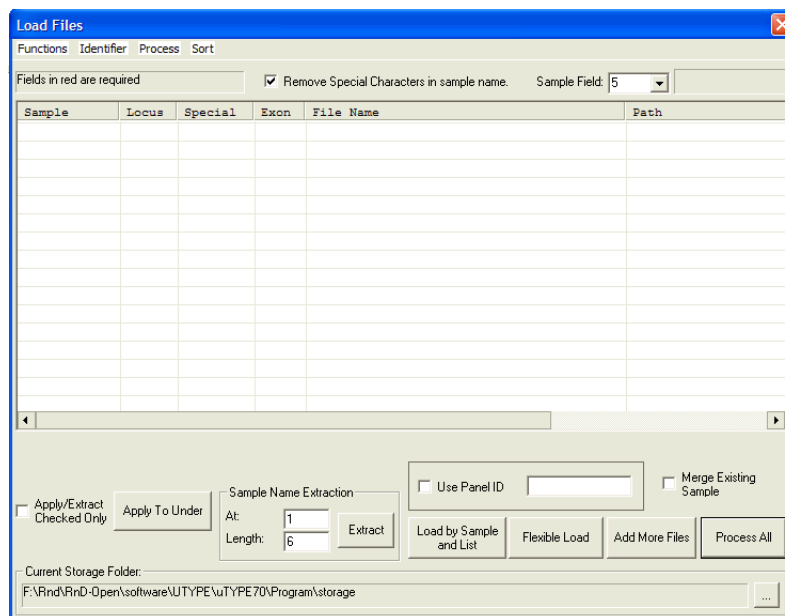
3. Navigate to the location of your files.
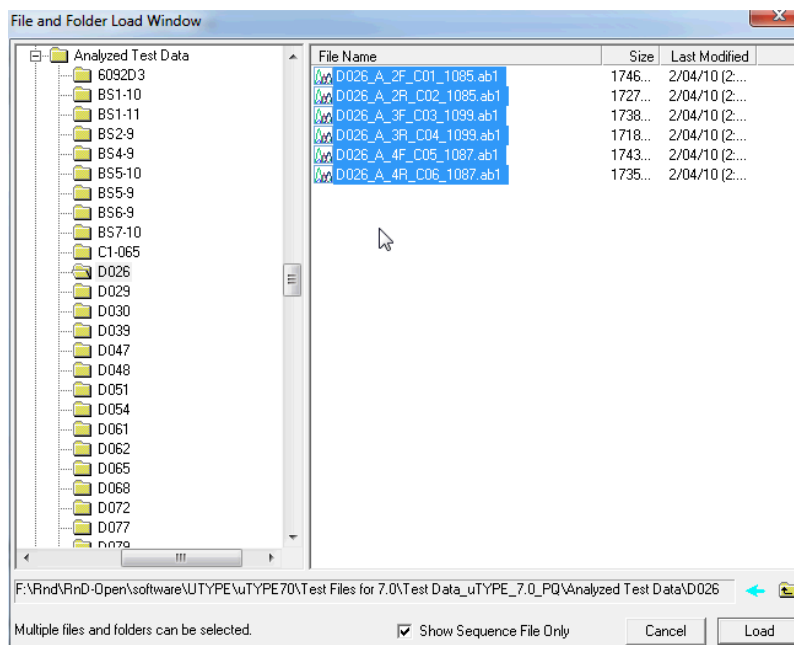
4. Check **Show Sequence File Only**, to show only .ab1 files.

5. Select the files or folders you wish to work with and click **Load**. Individual files or multiple folders may be selected and loaded.

    The software will automatically default to this folder the next time you load files.
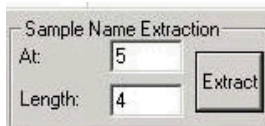
6. In the **Load Files** window, click **'Process All'** to process all of the files**.** The files load and appear in the **Sample List.** For more information see .
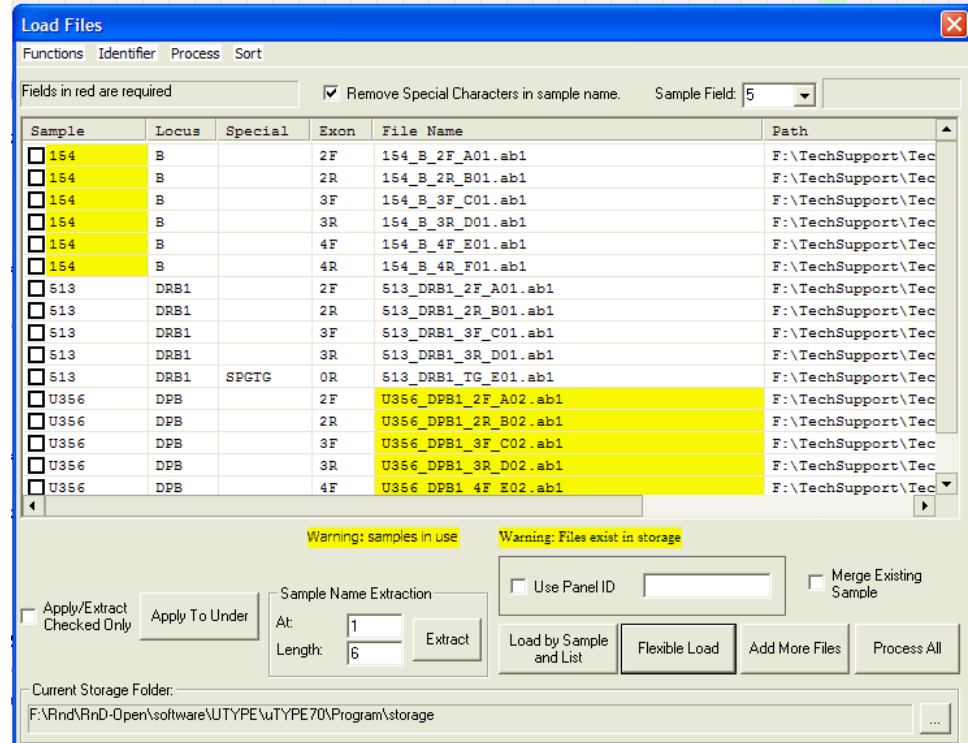
The software provides a visual indication when sample files are currently in use (yellow shading of sample name) or when sample files already exist in storage (yellow shading of file name). This functionality provides notification to users to help prevent two users from editing the same files or overwriting previously analyzed data and is limited to terminals/users accessing the same networked storage folder.

Load Files dialog box

Columns and buttons	Description and use
Sample column	The **Sample** column shows the sample name being extracted. The sample name can be edited by selecting the name and entering changes.

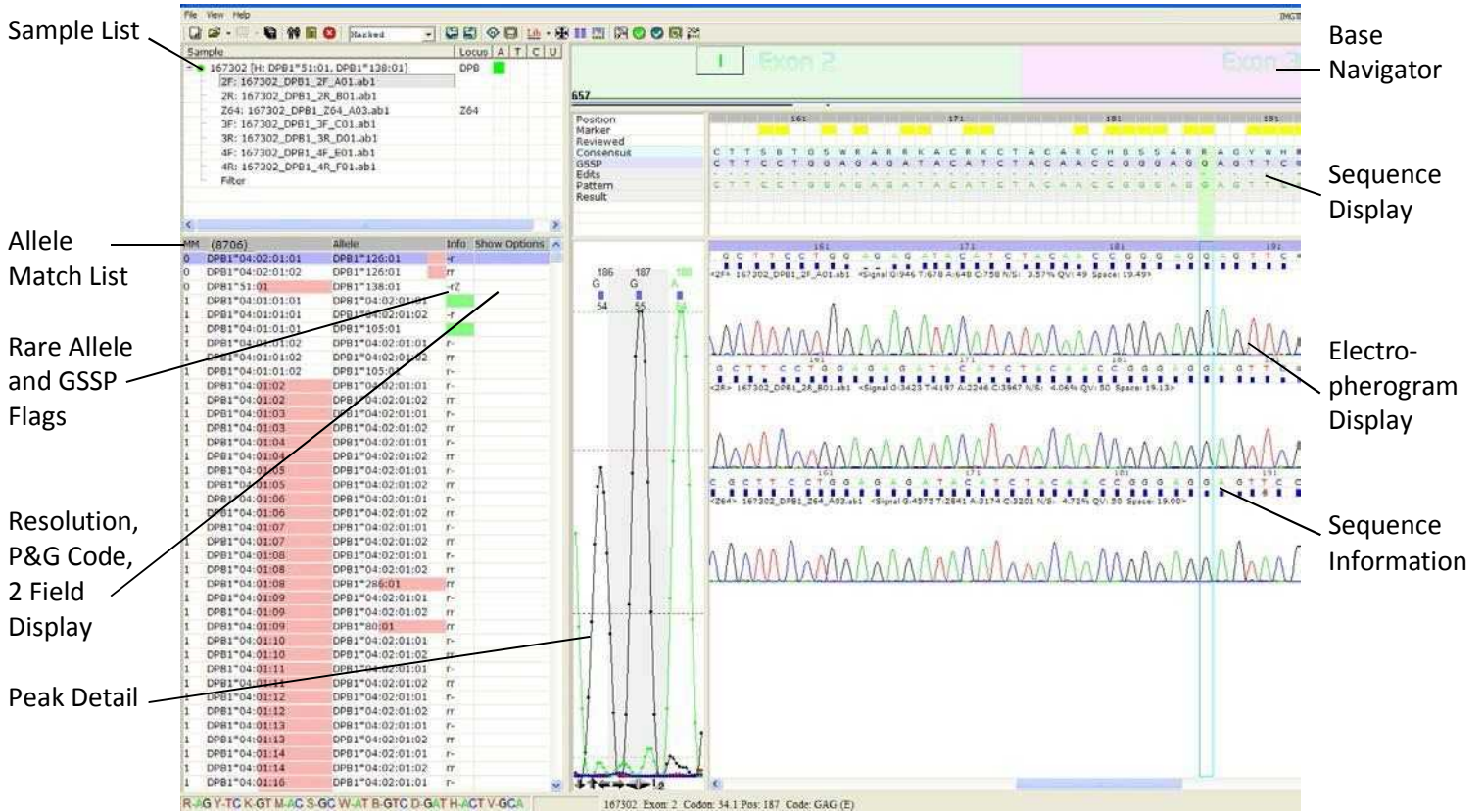|	
Locus column	The **Locus** column is filled in automatically by the uTYPE7.x Software or defined by the user. A dropdown list is accessible by selecting the entry field. The locus can be selected by double-clicking. The **Apply To Under** function fills down the locus for multiple files after the first row has been selected. Red boxes indicate a missing locus; a locus must be entered to perform analysis.
Special column	The **Special** column is used to define GSSP data files. This enables the software to combine Class I and Class II files with a specific "Z" primer and/or codon 86 files. See Input file requirements for the naming convention. Codon 86 and "Z" primers can be selected from the drop-down list. Z# is prefixed by "SP" notation for program use.
Exon column	The **Exon** column lists the exon and direction associated with the file name. An exon and direction must be associated with each file for software analysis. 0F is used for GSSP files.
File Name column	The **File Name** column shows the file names.
Path column	The **Path** column shows the directory path to the file location.
Sample Name Extraction	The **Sample Name Extraction** function may be used to define the sample ID embedded in the file name. Enter the sample name start position in the "At" field. Enter the length of the sample name in the "Length" field. "Extract" will find the sample name. *Note: the original file names are not altered by analysis.*
Apply/Extract Checked Only	Check this box to have other functions in the Load Files window apply only to files that you have 'checked'.
	Extract will extract sample names from only the checked rows.
	Apply to Under will apply the current locus to all checked samples.
Function drop-down menu	The **Function** drop-down list allows for the inclusion or removal of files loaded into the File Load window. The user can select "Check All" and "Uncheck All" to specify files for a specific action. "Remove Checked", "Remove All", and "Remove Files in Yellow" will remove specified files.

Identifier drop-down menu	The **Identifier** drop-down list applies to the file naming convention. The user can select "Use Underscore _" or "Use Dot ." for the name delimiter. The default setting is an underscore "_".
Process drop-down menu	The **Process** drop-down list enables "Process All" or "Process Checked Files".
Sort drop-down menu	The **Sort** drop-down list enables sorting of the sample files by "Sample Name" or "Locus".
Use Panel ID	**Use Panel ID** checkbox will allow manual assignment of a panel ID to all samples within the Load Files window for saving and retrieving files as a panel. Once checked, Use Panel ID will remain selected until the user removes the check mark. If Use Panel ID is checked and no name is entered, the software will auto populate this field with the folder name when saving the sample.
Merge Existing Sample	**Merge Existing Sample** checkbox will automatically retrieve and load analyzed and saved standard sequence files in the Sample List for analysis with corresponding GSSP data file after selecting Process All. Functionality limited to terminals/users accessing the same networked storage folder.
Load by Sample and List	**Load by Sample and List** will enable query function for a sample search.
Flexible Load	**Flexible Load** will display a directory browser. Multiple folders can be selected and loaded at one time by holding the control key and selecting folders in the window on the right side. Once a folder is selected in the directory, the folder contents will be displayed in the right column for selection.
Add More Files	**Add More Files** opens a browse window to load sequence files individually.
Process All	Select **Process All** to analyze data.
Sample Field	The **Sample Field** can be set to define an imbedded sample name. Changes must be made before loading files. For example, in Well#_SampleName_Locus_XX, the sample field setting is 2 because the sample name is in the second delimited position.
Remove Special Characters in Sample Name	**Remove Special Characters in sample name** automatically removes any special characters that are imbedded in the sample name. Only the double-quote characters can be removed, "and ".

Main Window Overview

After loading the sample files, the main window of the uTYPE 7.x software appears:

Sample List

Allele
Match List

Rare Allele
and GSSP
Flags

Resolution,
P&G Code,
2 Field
Display

Peak Detail

Base
Navigator

Sequence
Display

Electro-
pherogram
Display

Sequence
Information

The main display window contains the **Sample List**, **Base Navigator**, **Sequence Display**, **Electropherogram Trace Display**, Peak Detail view, and allele **Match List**. Each pane is adjustable.

If the peak detail view pane is not open, click on the upper left corner of the Sequence Display window or click on the left edge of the **Electropherogram Trace Display** pane and drag to the right.
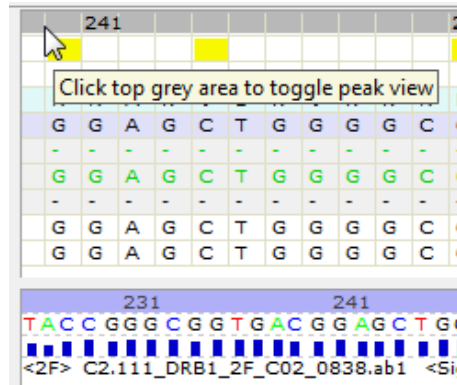
Base Navigator Pane

The base navigator background shows the exons available for analysis depending on the locus loaded. If "UN" appears in the **Sample List**, the background display will change to "Exon Unknown". The base navigator has two levels, the **Upper Navigator** area and the **Lower Navigator** area.

Upper Navigator area
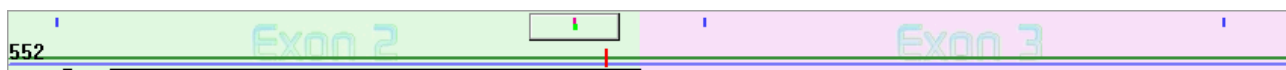

The **Upper Navigator** area displays discrepancies between the forward and reverse sequence as well as discrepancies between sample sequence and consensus sequence. It also tracks edited and filtered sites.

Navigate to each position by clicking ⬅ or ➡ in the toolbar. The rectangular viewer box tracks the location of the green cursor that aligns with the **Sequence Display** pane and **Electropherogram Trace Curve** pane.

Viewer box (can be in either upper or lower area)

Color	Definition
	Mismatch with consensus sequence or allele pair in **Match List**
	Discrepant between forward & reverse sequence
	Closest match mismatch positions. Displays only if MM is selected in the toolbar
	Filtered position
	Manually edited position
	Software edited position
	Indicates position "checked"
	User defined Advanced Base Review

Lower Navigator area

To move between the upper and lower navigator areas, click on the upper or lower navigator areas.

The **Lower Navigator** area shows mismatches with the closest allele match. The lower area appears when an allele pair in the **Match List** is selected. All the mismatches associated with that particular combination appear as a red point in the viewer box. A green horizontal line indicates forward sequence, blue is for reverse sequence and black indicates GSSP sequence. The length of the line indicates the coverage for each sequence.

Color codes are the same as for the Upper Navigator on the previous page.

Sequence Display Pane

To open and close the **Sequence Display** legend pane, click the top grey bar in the lower navigator window.

The **Sequence Display** pane aligns the Pattern sequence (the sequence determined by the software) with the Consensus sequence to identify the closest allele matches. The Pattern sequence is a composite of forward and reverse sequence data.

Color codes for colored squares are the same as for the Upper Navigator on the previous page.
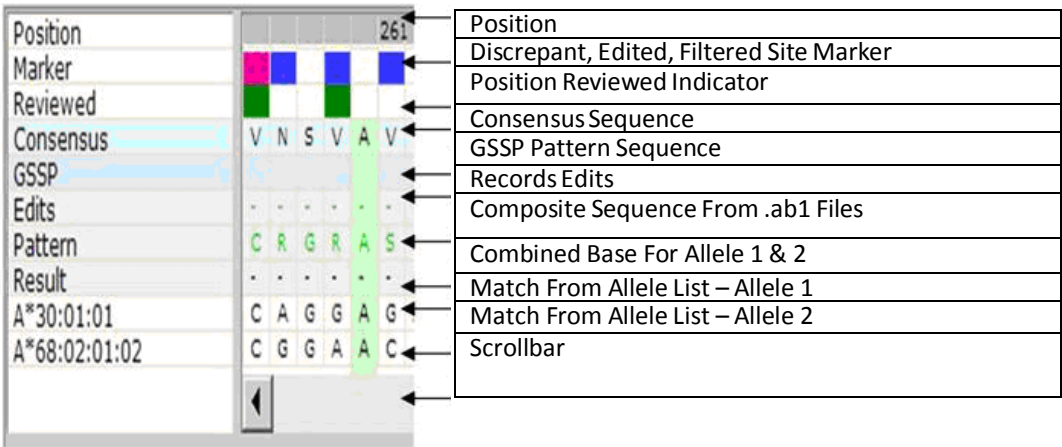
Alleles from the **Match List** will only be displayed when an allele pair from the **Match List** is selected.

To align the sequence in the **Sequence Display** pane to the same position in the **Electropherogram Trace Display** pane and **Navigator** pane, click on a base in the **Sequence Display**. The bases highlighted in green will align with the alignment indicator in the **Electropherogram Trace Display** pane.

If alleles that have undefined sequences are selected in the **Match List** (the alleles will be highlighted in pink), the allele sequence in the **Sequence Display** will display '*'s
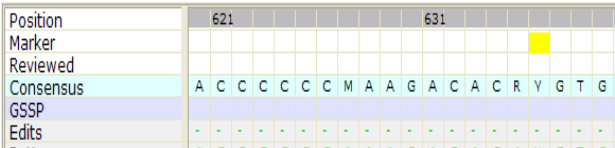
for all bases.

When the Pattern contains a mismatch to an allele pair, the position will be highlighted in pink in the Result sequence.

Edits	-	-	-
Pattern	A	G	G
Result	-	R	-
A*30:01:01	A	G	G
A*68:02:04	A	A	G

If an edit is made to the electropherogram, the change will be reflected in the Pattern sequence and recorded in the Edits row.

Edited bases are displayed in lower case (g, a, c, t) in the Edits line.

Electropherogram Trace Display Pane

Aligned forward and reverse electropherograms (traces) are shown in the **Electropherogram Trace Display** pane.

Base position

sample name and signal

Forward trace

Basecalls

Quality Value histograms

Alignment indicator

Reverse trace

Sample Name, Exon, Codon,
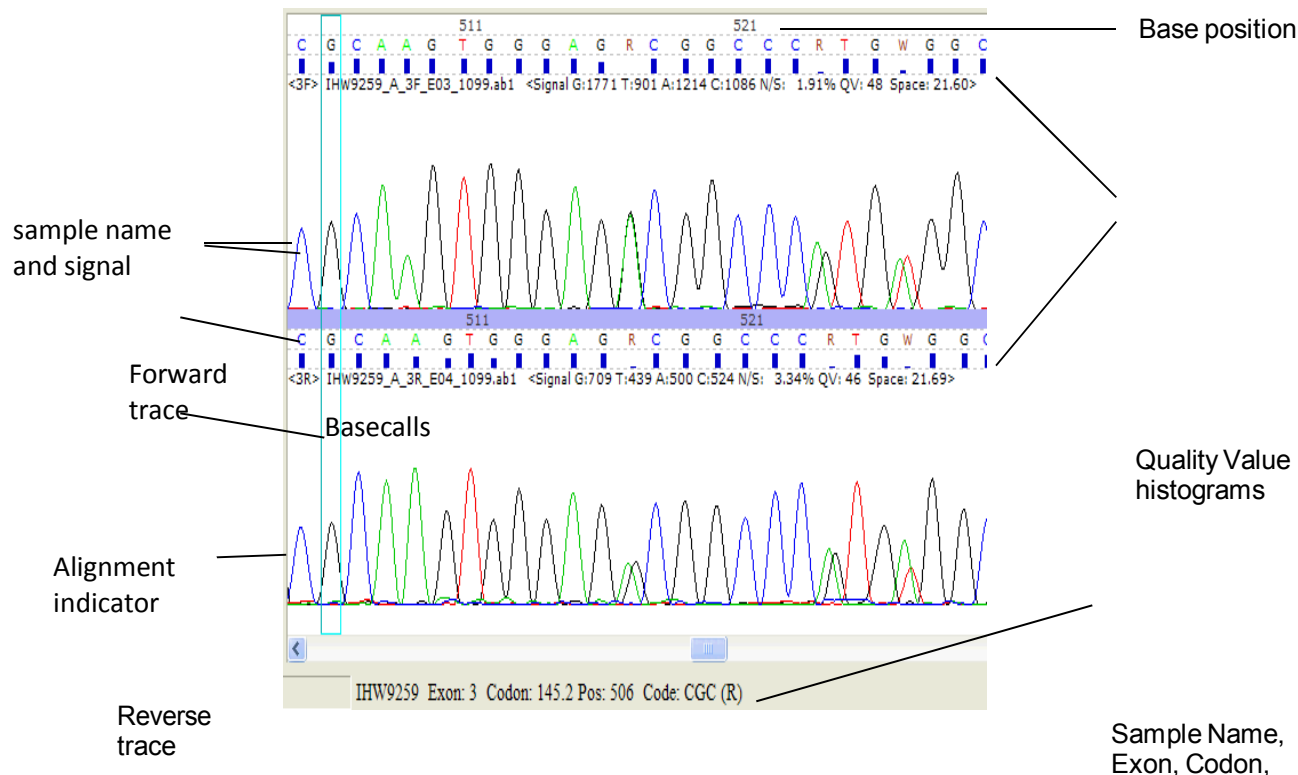
Positio
n and
Amino
Acid
Code

Trace curves

The forward trace curve is aligned above the reverse trace curve. Click on the edited bases area under the basecalls to align the sequence display pane and navigator pane to the trace curves.

Peaks in a gray background are excluded from analysis. The gray background indicates the end of an exon or sequence.
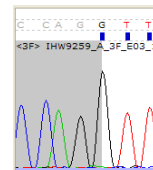
To make another trace active, click anywhere on the desired trace. Click on the area below the bases to display the sample name, exon, codon, position, amino acid code and base quality score (QV) at the bottom of the screen.

167302 Exon: 3 Codon: 145.3 Pos: 522 Code: CTG (L) OV: 54

The base position row will be highlighted in blue to show the active trace.
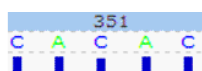
     Blue highlight shows active trace

Basecalls

The colored basecalls are used to obtain sequence results. Gray colored bases are excluded from analysis.

The space below the basecalls will show the original basecall if the base above had been edited. The space also shows individual base Quality Value histograms. The corresponding numerical value is displayed in the peak detail window.

Sample Name and Signal Data

The current sample name is displayed in the upper left corner of the pane. The numbers to the right of the sample name contain the sample's signal data, including signal for each base, Noise/Signal (N/S) Ratio, Quality Value (QV) and Spacing. Position numbers are listed by tens across the top of the pane.

Select the Display QV/Seq Info   icon   on the toolbar to toggle display of QV histogram and electropherogram information.

Sequence Overview

To view the overall electropherogram sequence, select Ctrl-D.
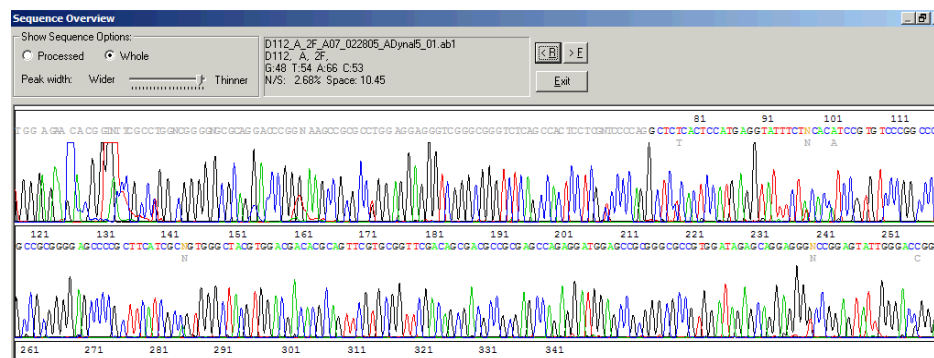
Screen items	Description and use
	Adjusts the view of raw and processed data. Peak width can also be adjusted to preference.
	Use to scroll through all sample sequences

Navigation and Review Panel

The **Navigation Panel** within the toolbar controls the display parameters of the electropherogram. It allows you to navigate through the electropherogram and check marked positions. Navigation to marked positions can also be achieved by using the browse arrows on the toolbar or arrow keys on the computer keyboard.

Electropherogram Menu

Right-click on the **Electropherogram Trace Display** window to display the Electropherogram Menu.

Insertion or Deletion Alleles
Insert Base N

Filter by Current Base
Remove Last Base Filter
Remove All Base Filters
Remove Last Null Allele Filter

Exclude Left   (I)
Exclude Right (O)
Reset Exclusion

Exclude Middle Start
Exclude Middle End
Reset Middle Exclusion

Set to DRB1*01 Start Position
Reset to SeCore DRB1 Start Position

Change Current Sequence's Exon/Direction
Remove Current Sequence (Alt-X)
Reset Current Sequence
Toggle Activation of Current Sequence
Show Sequences in Mixed Groups

Electropherogram Menu Items	Description and use

Allele	Position	Ins/Del	Sequence
☐ A*03:36N	265	insertion	ACGGAATATGAAGGCCCACTCACA..
☐ A*24:36N	253	insertion	GG
☐ A*02:125N	262	insertion	G

Current set:

Allele	

List All Nulls
Unset
Set
Cancel

Insertion or Deletion Alleles	If an In/Del is suspected within the sequenced data this tool can be used by right clicking on the position that the In/Del starts and selecting **Insertion or Deletion Alleles.** The window will display known alleles caused by In/Dels in close proximity to the selectedregion. This information

can be used to determine which alleles may be present and the allele can be assigned by clicking on 'Set'. The allele can be removed from the final typing by selecting 'Unset'. The Data must be edited to obtain as close to a 0 mismatch result as possible to use this function.

List All Nulls will list all known current locus nulls caused by insertions or deletions.

Electropherogram Menu Items	Description and use
Insert Base N	To insert a base to force the sequence into alignment, select a base as if to edit, right-click, and select **Insert Base N**.
Filter by Current Base	Filters out base pair mismatches between the Match List and trace curves.
Remove Last Base Filters	Removes last filter applied.
Remove all Base Filters	Removes all filters applied.
Remove Last Null Allele Filter	Removes the filter applied to a null allele.
Exclude Left (I) Exclude Right (O)	Removes the sequence to the left or right of the highlighted base. The trimmed sequences are indicated with a gray background in the trace display.
Reset Exclusion	Resets to the previous user defined excluded sequence.
Exclude Middle Start Exclude Middle End	Removes the user's selected area from analysis. The sequence will be grayed out on the Electropherogram.
Reset Middle Exclusion	Resets any middle exclusion.
Set to DRB1*01 Start Reset to SeCore DRB1 Start Position	If a result contains the DRB1*01 allele group, this will resolve the DRB1*01:01/07 ambiguity located in codon 10 (not applicable to SeCore DRB1 Exon 2 and 3 Sequencing Kit). Select Reset to revert back to the default start position.
Change Current Sequence's Exon/Direction	- Manually assign sequence exon and/or direction. - Manually assign GSSP specificity. - Select the exon and direction, and provide the number of bases excluded (if needed) from the beginning or ending of the sequence. - Exon and direction are required fields for GSSP analysis.
Remove Current Sequence (Alt-X)	Removes a trace file. Select the electropherogram to be eliminated, right-click, and select Remove Current Sequence (Alt-X).
Reset Current Sequence	Removes edits to current trace.
Toggle Activation of Current Sequence	Inactivates/activates selected trace. An inactive trace is grayed-out.

Electropherogram Menu Items	Description and use
Show Sequences in Mixed Groups	Combines electropherograms in single view for a DRB1 group sample that is positive for two mixes. Reverts back to default display when the next sample is selected.

Match List Pane

The **Match List** displays the closest allele matches when the Pattern Sequence is compared to the Consensus Sequence. The left column (MM) shows the number of mismatches associated with a particular allele combination.

Mismatches

Number of Closest Matches

Filterable In/Del Alleles

Toggle for view of resolution, location, P and G codes, 2 field display

Resolvable from top pairs with AmbiSolv™ (S)

Rare Alleles (r)

Resolvable from top pairs with GSSP (Z)

Match List codes	Description and Use
Z	The ambiguity is resolvable by GSSPs. "Z" indicates a specific Group Specific Sequencing Primer, i.e., Z12, Z18. See Resolve Ambiguities for more detail.
*	Indicates the presence of a filterable null allele in the allele pair. Yellow and blue highlighting gives the user additional information on filterable null alleles. Allele pairs with yellow shading in the MM column of the Match List indicate the presence of null alleles, caused either by point mutations or insertions/deletions. These can be ruled out with additional regions of data not covered by the SeCore product line, or by needed data that is not currently included in the analysis. Allele pairs with blue shading in the MM column of the Match List indicate that, for Class I tests only, incorporation of exon 4 sequence into the analysis will provide the data needed to rule the null allele in or out.
-	Please Holder. Marks a space, so that if only one of the alleles is rare, you can tell which one it is.
r	Rare alleles. Alleles notated with an 'r' are not contained within the Common and Well Documented (CWD) allele list.
S	Resolvable with AmbiSolv™ SSP mix.
Color codes	Allele has an undefined sequence. Both alleles are common and/or well-documented alleles.
Show Options	Toggle **Show Options** to display: • Sequence region required to resolve a highlighted allele pair (Exon 1 or 5 in screen shot above). • Display P Code for the result, if applicable (Download from http://hla.alleles.org/wmda/ See Preferences for instructions) • Display G Code for the result, if applicable (Download from http://hla.alleles.org/wmda/ See Preferences for instructions) 'Show in 2 fields' displays only the first two fields of each allele within the Match List.

Match List Menu

Right-click in the **Match List** to open the menu.

```
Run Next Mismatch Block
Reset Mismatch Block

Show Mutation and GSSP Info

Show Unsequenced Exon
Show All Pair Alignments
Show With Current Pair Alignments
```

Menu item	Description and use
Run Next or Reset Mismatch Block	Expands the Match List to display additional allele pairs with mismatches. Reset Mismatch Block returns the Match List to the original state. If there is no next block, use the re-run match button on the tool bar to reset the block.
Show Mutations and GSSP Info	Provides detailed information on the GSSPs that can be used to isolate and sequence novel mutations if they are suspected. See Show Mutations and GSSP Info.
Show Unsequenced Exon	Provides details on the location and bases that are undefined.
Show All Pair Alignments	Opens the Allele Alignment Study Tool for all alleles listed in the 0 MM
Show with Current Pair Alignments	To display pair alignment for a selected pair of alleles, left-click on the first allele pair and right-click on the second allele pair, then select **Show Current Pair Alignment**. Alignments can be sorted numerically by allele group and printed.

Run Next Mismatch Block

The match list will display all allele pairs for a sample without limitation. Those with '0' mismatches display first, followed by '1' mismatch, and then '2' mismatches. This constitutes the first block.

Each block will accommodate up to 3 mismatch levels only (e.g., {1,2,3}, {4,5,6}, etc.). When the count of allele pairs on the list reaches 2000, the software will complete that level and then go on to the next block. For example, if the count reaches 2000 but there are still 10 more matches for level 3, the software will include those 10 before beginning a new block.

MM	(8706)		Allele		Info	Show Options	
0	C*06:02:01:01		C*08:01:01				
0	C*06:02:01:01		C*08:22			Exon 6	
0*	C*06:02:01:01		C*08:89N		-r		
0	C*06:02:01:01		C*08:99		-r	Exon 5	
0	C*06:02:01:01		C*08:102		-r	Exon 6	
0	C*06:02:01:02		C*08:01:01				
0	C*06:02:01:02		C*08:22			Exon 6	
0*	C*06:02:01:02		C*08:89N		-r		
0	C*06:02:01:02		C*08:99		-r	Exon 5	
0	C*06:02:01:02		C*08:102		-r	Exon 6	
0	C*06:02:01:03		C*08:01:01		r-		
0	C*06:02:01:03		C*08:22		r-	Exon 6	
0*	C*06:02:01:03		C*08:89N		rr		

Right-click and select "Run Next Mismatch Block." The title bar for the list becomes shaded in dark teal as an indicator that the list is not displaying the first block.

The next block of allele pairs displays, with the same guidelines in place – no more than 3 mismatch levels per block, cutoff will be ~2000. There is no limitation on the overall count; you may therefore continuing to run the next block as needed.

MM	(3572)		Allele		Info	Show Options	
3	C*05:11		C*06:11		rr		
3	C*05:12		C*06:11		rr		
3	C*05:18:01		C*06:11		rr		
3	C*05:68		C*06:82		rr		
3	C*05:68		C*06:122		rr		
3	C*05:76		C*06:11		rr		
3	C*05:79		C*06:82		rr		
3	C*05:79		C*06:122		rr		
3	C*06:02:01:01		C*08:04:01				
3	C*06:02:01:01		C*08:09		-r		
3	C*06:02:01:01		C*08:16:02		-r		
3	C*06:02:01:01		C*08:33:03		-r		
3	C*06:02:01:01		C*08:54		-r		
3	C*06:02:01:01		C*08:61		-r		

Show Mutations and GSSP Info

Select **Show Mutations and GSSP Info**, to open the
Mutation Confirmation and GSSP Info dialog box.

Screen Items	Description and Use
Allele 1 and Allele 2 entry fields	Allele 1 and Allele 2 fields will auto-populate with the allele pair selected within the Match List or Allele 1 and Allele 2 can be manually entered. When an allele pair is highlighted in the Match List, the Positive GSSPs and Exclusive list will be automatically populated.
Positive GSSPs	When **Positive GSSPs** is selected all GSSPs positive for the allele will be listed with the Code, Exon, and coverage.
Exclusive List	When **Exclusive List** is selected, GSSPs positive only for Allele 1 or Allele 2 will populate the GSSPs for allele 1 or allele 2 only windows.

Match List and Trace Display

When a result with 1 or more MM is selected, mismatches appear as a
pink marker in the **Sequence Display** pane. The left   and right
browsers can be used to navigate to those positions for editing in
the **Electropherogram Trace Display**, when "Marked" is selected
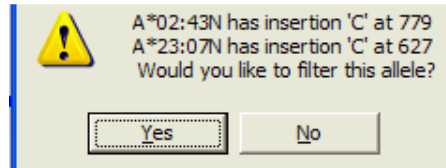
under Navigation Selection.

Mismatch

Filter null allele mismatches from Match List

Matches containing a null allele are designated with an asterisk (*) in the MM
column. They can be filtered directly from the **Match List**, and do not require
editing in the **Electropherogram Trace Display**.

1. Click in the MM column of an allele pair that contains a null allele ('N') and is marked with '*' in the **Match List**.

| 0* | A*02:43N | A*23:07N | rr |

The software will automatically display the location of the In/Del or point mutation corresponding to the null allele in the trace curve and gives the option to filter the null allele.


⚠ A*02:43N has insertion 'C' at 779
A*23:07N has insertion 'C' at 627
Would you like to filter this allele?

[Yes]   [No]

2. If you wish to filter the allele, click **Yes**.

The null allele is filtered from the **Match List**.

Sample List and Audit Pane

The **Sample List** shows the names of the sequence samples and the descriptive codes.

The currently selected sample is highlighted in gray.

Sample List pane

Audit pane
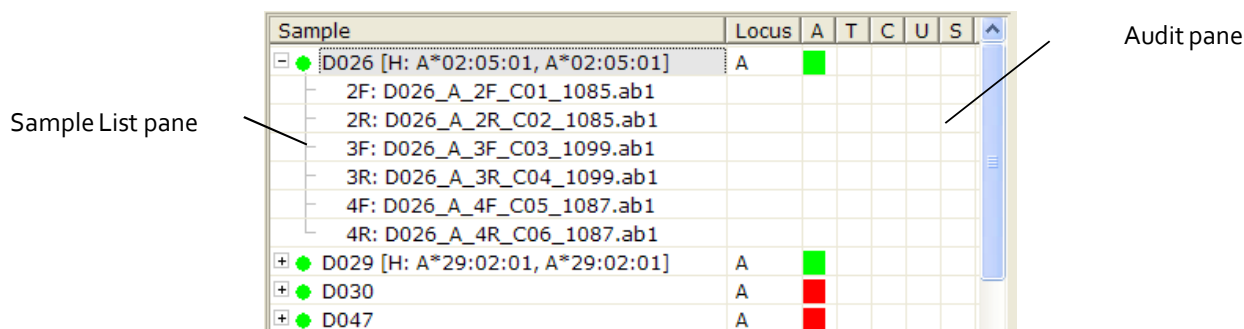
The **Sample List** can be expanded to display the list of data files grouped for each sample. A typing result without ambiguities is displayed next to the locus for quick review.

Sample List Codes	Description and Use
2F, 2R, 3F, 3R, 4F, 4R	The exon and direction.
UN	The software is unable to analyze the sequence
H	Indicates unambiguous typing.
M	Indicates an unambiguous NMDP code typing, unless it crosses multiple groups.
L	Indicates unambiguous typing of the same allele group.
GTG	Codon 86 sequence.
Z#	GSSP sequence.
Filter	Codon 86 and/or GSSP filtered result.
DRB Group	DRB1 combined result.

Sample Status (Assigned by software)	Bullet Point	Definition
		100% Match
		Partial Match
		Sample has Sequence(s) with >10% Background

		Top Match with Rare Allele
		Unidentified Sequence Exists
		Sequence has > 10% Background

Sample Status (Assigned Manually)	Sample has been reviewed. To mark sample as reviewed, click in toolbar or 'U' field.
	
	  Sample has been approved by supervisor. To mark sample as approved, click in toolbar or 'S' field.
	  Sample is pending review. Right click **Locus > Mark Pending Review**.
	  Sample is to be retested. Right click **Locus > Mark Repeat**.
	  Sample has failed. Right click **Locus > Mark Failed.**
	
	 The user can also right click on the sample name to access these commands.

Audit Pane codes	Description and Use

'A' Column	Audit column
	
	  Indicates a sample with discrepancies and marked positions have not been "checked". Requires user review.
	
	 Indicates a sample that has been manually edited, and marked positions have not been "checked".
	
	   ▢ When sample is first opened, indicates that there are no marked positions present.
	  ▢ Indicates that all marked positions have been "checked".

'T' Column	Click for shortcut to **Sample Final Type Settings** pane. See Sample Final Type Settings. Indicates that the final type has been set.
'C' Column	Click for shortcut to **Sample User Comments** pane.

	■ Indicates that comments have been entered.
'U' Column	Review column
	■ If the sample status is ✔, indicates that the sample is pending review. Status is assigned manually, not by software.
	■ If the sample status is ❌, indicates that the sample has failed. Status is assigned manually, not by software.
	■ Indicates that the sample has been reviewed.
'S' Column	Approval column
	■ Indicates that the sample has been approved.

Sample List Menu

To launch the **Sample List Menu**, right click the Locus or the sample name in the Sample List.
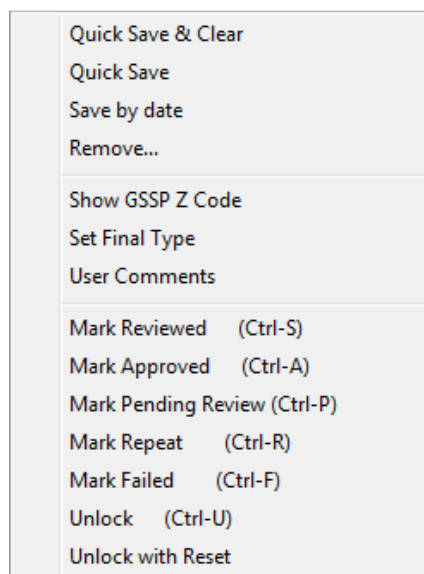
Menu Items	Description and Use
Quick Save & Clear	Removes the sample name from the sample list after saving.
Quick Save	Saves individual samples without going through the Save menu.
Save by Date	Links the date of analysis to the sample name. This feature distinguishes analysis of the same sample on different days.
Remove	Removes the trace from the study. It will not delete the original file.

Show GSSP Z Code	Opens the GSSP Selection and Information window which displays detailed sample information on the GSSP Z code for a particular allele combination. See GSSP Selection and Information.
Set Final Type	Opens the **Sample Final Type Settings** window. See Sample Final Type Settings
User Comments	Comments can be added and linked to a sample, and will appear on the sample report.
Mark Reviewed Mark Approved Mark Pending Review Mark Repeat Mark Failed Unlock Unlock with Reset	Reviewed , Approved , Pending , Repeat , and Failed status can be applied and removed from individual samples. **Unlock with Reset** removes the designated final type set with the **Final Type** tool. **Unlock with Reset** will require two executions to remove the final type designation if the sample is at "Approved" status. For batch approval and more information on the approval process, see Review and Approve Genotypes. **Unlock** removes Supervisor approval. Note: **Unlock** and **Unlock with Reset** can only be performed by technician that assigned the status or by a supervisor.

Sequence Menu

To Launch the Sequence Menu, right click under the locus column next to each sequence.

Menu Items	Description and Use
Remove	Removes the selected sequence information.
Filter by Current GSSP	Updates the Match List based on the data from the selected GSSP.

Edit Traces

Edit a trace

A trace can be edited when the "Pattern" sequence in the **Sequence Display** pane is discordant with the **Electropherogram Trace Display** pane. Valid bases are A, C, G, T, R, Y, K, M, S, W, and N.

Pressing the spacebar will remove a base from being recognized as part of the sequence. This is shown by changing the base color to a grey color. If an unacceptable base is inserted, a beep sounds.

When a base is changed, the software will automatically reanalyze the data. If editing requires insertions or deletions, reanalysis will only occur if the number of inserted bases equals the number of deleted bases. Click the **Rerun Match** icon in the toolbar to re-run the analysis if there are unequal insertion/deletion edits.

> Note: If this is the first time you are editing a trace, you may wish to turn off **Auto Run**. Otherwise, the software will automatically shift to the next discrepant base pair after an edit.
>
> To toggle **Auto Run**, click the green triangle in the tool bar.

For the purpose of showing how a trace is edited we will change the 'C', to an 'S'.

1. Click on 'C' in the electropherogram , then type 'S'.

   The trace updates and shows an 'S' for the upper trace.

    The 'C' is shown under the 'S', to indicate the edit.

2. Move to the next discrepant position by clicking and edit as appropriate. (If **Auto Run** is on, the software will move to the next position automatically).

   Note: In general, all discrepant positions in an electropherogram must be reviewed and edited, if required, before filtering the possible matches.

3. Edit the trace until you have a least one '0' mismatch in the **Match List**.

Note: Base edits in the printed report, and the original electropherogram, are displayed by sequence location. The bases in the electropherogram and mismatch/polymorphic sites in the printed report are displayed in consensus location.

Edit a trace with the IUB bar

Bases may also be edited through the IUB bar (International Union of Biochemistry, see reference in Bibliography). Select a base in the electropherogram or navigate to a specific position and then position the mouse over a base in the IUB bar. Double-click on the base to make the base change.

R-AG Y-TC K-GT M-AC S-GC W-AT B-GTC D-GAT H-ACT V-GCA

Undo edits to a trace

To undo edits to a trace, click the **Reset Changes to Current Locus** icon in the toolbar.

Filter the Closest Matches

After editing the sequence-based traces until you have at least 1 allele pair with 0 mismatches, you will still see allele pairs with the closest matches in the **Match List**.

Key polymorphic positions of allele pairs with the closest match can be reviewed and filtered from the **Match List**.

1. Click on the first mismatch (1) in the **Match List**.

The possible mismatch is shown above the electropherogram.

Possible mismatch 'M'. Underneath it are the 'A' and 'C' bases that need to be present for this to be an 'M'.

The electropherogram shows that the actual bases are 'A'.

The 'M' outlined in pink indicates the possible mismatch.

Because an 'M' would be an 'A' and a 'C', and the peaks are clearly 'A', we can filter out the 'M'.

1. Press 'f' on the keyboard, which is the shortcut key for 'filter'.

   (Alternatively, right-click on the green box outlining the 'A' peaks and select **Filter by Current Base**)

   The base is filtered and no longer appears in the list.

   In addition, any other mismatches that depend on the 'M' mismatch are updated as well.

2. Repeat this procedure for as many possible mismatches as you wish.

In some cases the sites will be clearly polymorphic, and you will filter out the possibility of the site being a single base.
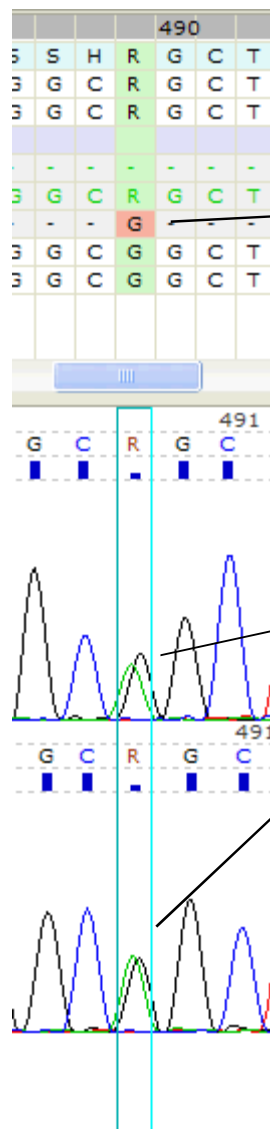
Possible mismatch 'G'. Underneath it are shown the 'G' bases that need to be present for this to be a 'G'.
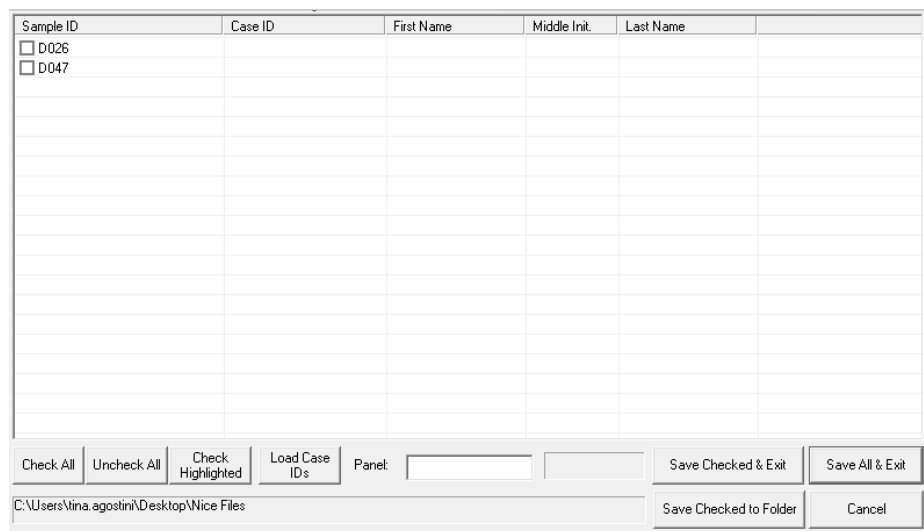
The electropherogram shows that the actual bases are polymorphic, 'G' and 'A' bases, symbolized by 'R'.

Save, Retrieve and Report Results

Save Sample and Case Information

To save sample data, select the **Save All Results** icon ![icon] in the toolbar. Samples are saved with the original trace data files in the uTYPE 7.x Software folder database and subdirectory.

By importing files, you can assign Case ID and additional sample information to each sample before saving.

Save Options	Description and Use
Load Case IDs	The relevant information can be entered into the corresponding columns (Case ID, First Name, Middle Init., and Last Name) manually or auto populated by selecting **Load Case IDs**. Upload must be in .csv file format with the csv file Sample ID identical to the software Sample ID. Fields are limited to 24 characters. csv file format: • Column A: Sample ID • Column B: Case ID • Column C: First Name • Column D: Middle Initial • Column E: Last Name
Check All Uncheck All	Checks or unchecks all sample checkboxes.
Check Highlighted	Samples can be highlighted and saved.

| Panel | You can save multiple files together as a panel. Enter an ID for the panel here.

If the Panel ID was selected in the Load Files window, the Panel ID entered will be displayed. If no name was entered upon sample loading the Panel ID will be the sample's folder name, however the name can be changed at this time. |
|---|---|
| Save Checked and Exit | Saves checked samples to storage folder set in Preferences. |
| Save All and Exit | Saves all listed samples and exits the window. |
| Save checked to Folder | Saves checked samples to a storage folder other than the default location set in Preferences. |

⚠ Samples may be overwritten when separate users modify the same sample at the same time. A laboratory procedure must be developed to prevent overwriting existing sample files.

Review saved data

To review saved data, select the **Review Stored Results** icon 🖼 in the toolbar. When the **Data Management and Review** pane opens, enter your search

parameters and click 🔍 to retrieve the files.

Review Options	Description and Use
Search from database	Retrieves processed data by date. The search is filterable by locus. Click 🔍 or enter to retrieve files.
Search by Sample ID	Searches by sample ID; a wild card (*) may also be used. Enter the ID, then click 🔍 or enter to retrieve files. Wildcard searches are not case sensitive. Note: The search will be appended to the sample list.
Check All Uncheck All	Selects and deselects samples in a batch.
Clear All	Removes samples from the list.
Delete Checked	Deletes samples from database.
Search by Case Name	Searches samples using the first and last name entered during sample save; a wild card (*) may also be used.
Search by Case ID	Searches by Case ID assigned during sample save.
Search by Panel ID	Searches by Panel ID assigned during sample save.
Search by Status	Searches by Owned, Reviewed, and Approved sample status. Owned – sample data that has been opened, analyzed and saved. Reviewed – samples that have been marked as reviewed. ✔ Approved – samples that have been approved by the supervisor. ✔
Check Highlighted	Checks highlighted samples.
Change Database	Enables software to retrieve data from a different storage folder when multiple storage locations are in use. When software is closed and re-launched, storage folder will reset to original location designated in Preferences.
List to xls	Retrieved data can be sorted by column. "List to xls" is an exportable table of search results.
Load Unchecked Samples Load Checked Samples	Loads only those samples with selected or de-selected checkboxes.

Print and customize report

The **Report** window allows for customization of report formats. To open the **Report** window, click 📋 in the toolbar.

You can also 👀 view the report as a .pdf which can be saved or printed.   🔍

Report Items on General Tab	Description and Use
Sample Count	Displays the number of samples loaded for reporting.
Current Locus Current Sample All Samples	Creates a report for the selected locus, a single sample including all loci, or all samples included in the current analysis.
QC Features Q Value Peak Balance	For troubleshooting, provides reports of statistical information for the loaded data.
Export Detail (xml)	Generates detailed report in .xml file format.
Summary Report (pdf)	Displayed assigned type report in .pdf file format including Sample Information and Assigned Type.
Detail Report (pdf)	Displays detailed report in .pdf file format.
All Samples Match Export (xls)	Provides condensed print options for (xls) report. ▫ Assigned Type displays only single allele pair or NMDP code match. ▫ Perfect Match displays all perfect match allele pairs.
Automated backup copy report to following specific folder	Designates the report storage folder by selecting the search icon. Use X to clear selection.

Report Items on CMDP Tab	Description and Use
Serologic File:	Path to location of Serologic file. This will be used in the creation of CMDP Report.
Template File:	Path to location of template to create CMDP report
Tester:	Text box to enter the name of the tester
Report	Button to create CMDP report

Report Preview Options

Report Items	Description and Use
Number of allele pairs in the report	Defines the number of pairs to be printed on the report, including those with mismatches.

PDF Page Break Options	Provides print options for PDF reports. • No page break – Each test sample and locus will be printed without page breaks. The first sample's name will appear on the footer of each page. • Each Locus – A new page will be created for each test. The sample name will be displayed on the footer of each page.
Show Pattern Sequence	Includes combined trimmed sequence in the report.
Hide Mismatches	Lists only alleles with '0' mismatches on the report.
Show Polymorphic Position	Displays polymorphic positions of the alleles listed in the report.
Show Signal	Displays the .ab1 file signal values.
Show G Code Show P Code	Displays typing result with G Code and/or P Code which applicable. Download from http://hla.alleles.org/wmda/. See Preferences for instructions.
Show Barcode	Displays barcode on the report with an embedded sample ID and unambiguous typing results.
Show Ambiguity List	Displays ambiguities resolvable by GSSP and/or AmbiSolv™ SSP mixes.
Hide Edits	Hides edits and filtering information table.
Show Case Info	Displays Case ID information incorporated during sample saving, see Save Sample and Case Information.
2 Fields Only	Displays alleles using only 2 fields.
Institution Info	Open fields allow entry for report customization including institution address and phone number. Institution logo can be loaded through the **Logo File Path** field (.bmp only).
Save to PDF	Saved PDF Report file.

CMDP Report Output–

The report generated based on CMDP Excel Template, includes serological code, NMDP codes, ambiguous genotype, and final typing

Figure 1: CMDP report headings A to K

Columns	Description and Use
A	Sample ID
B to F	Assigned Type in Final Type settings, cell is colored red if an ambiguous result is present; otherwise, cell has no color
G to K	MM = 0 Allele Pairs

Figure 2: CMDP report headings L to U

Columns	Description and Use
L to P	NMDP, P, or G Code displays a. When G code present, have NULL allele that is part of the G group but not present in the genotype results, and G Code Date is included in the second row. b. When NMDP code present, code and definition is displayed
Q to U	Serology displays. c. When 2 serologic codes are present, cell has no color. d. When fewer than 2 or more than 2 serologic codes are present, cell color is red.

Figure 3: CMDP report headings V to AK

Columns	Description and Use

V to Z	Locus-designated high-resolution allele. Displays if Resolution is "High Resolution"
	
	   a. If greater than 4 total types, the cells with more than 1 type are shaded yellow.
	   b. If fewer than 4 or equal to 4 total types, then cells with more than 1 type are shaded green.
	   c. If no types are present, no color is displayed in the cell.

AA	Tester Name
AB	Test Date
AC	Role
AD	Review Date.
AE	Resolution comment. The lowest resolution is displayed in the order of L (low), M (medium), A (accepted high resolution), or H (High).
AF to AJ	GSSP Recommendations. Right-click on locus and select "Show GSSP Z Code." Look for "GSSP List."
AK	Comments: User Comments

Resolve Ambiguities

Resolve ambiguities with GSSPs

SeCore® GSSP's (Group Specific Sequencing Products) are designed to resolve ambiguous HLA heterozygous combinations, and are used in conjunction with SeCore® Sequencing Kits.

If an allele pair in the **Match List** has a 'Z' designation in the Info column, the software has determined that the ambiguity is resolvable with GSSPs.

Select "Z" on the **Match List for ambiguity resolution**.

MM	(8706)	Allele	Info	Show Options
0	DPB1*04:02:01:01	DPB1*126:01	-r	
0	DPB1*04:02:01:02	DPB1*126:01	rr	
0	DPB1*51:01	DPB1*138:01	-rZ	
1	DPB1*04:01:01:01	DPB1*04:02:01:01		

The GSSP code(s) that will resolve the selected ambiguity will be displayed in the lower right corner of the sequence information display.

[167302] DPB1*04:02:01:01, DPB1*126:01 = DPB1*51:01, DPB1*138:01 GSSP: Z64 or Z65   at 194

'S' indicates an allele pair that can be resolved by a SSP AmbiSolv™ mix.

MM	(8706)	Allele	Info
0	A*11:01:01	A*26:01:01	
0*	A*11:01:01	A*26:25N	-r
0	A*11:01:07	A*26:01:17	rr
0	A*11:19	A*26:13	ZS
0*	A*11:21N	A*26:01:01	r-
0*	A*11:21N	A*26:25N	rr
0	A*11:40	A*66:01:01	r-ZS

The software recommended GSSPs are also displayed within the Sample List, next to the sample name. In the example below the ambiguities in the sample can be resolved with GSSP Z64.

Sample
- 167302 (Z64)
  - 2F: 167302_DPB1_2F_A01.ab1
  - 2R: 167302_DPB1_2R_B01.ab1
  - 3F: 167302_DPB1_3F_C01.ab1
  - 3R: 167302_DPB1_3R_D01.ab1
  - 4F: 167302_DPB1_4F_E01.ab1
  - 4R: 167302_DPB1_4R_F01.ab1

The GSSPs displayed in the Sample List represents the minimum required GSSPs for ambiguity resolution.

GSSP Selection and Information

The user can also view the GSSPs for the ambiguities by right clicking the sample name or locus in the sample column of the Sample List Menu and selecting Show GSSP Z Code. See Sample List Menu.

GSSP Selection and Information Option	Description and Use
Sample	Displays selected sample name
GSSP List	Shows original software -recommended GSSP list, even when GSSPs have been loaded.
GSSPs Recommended by Software	Lists the GSSPs that are required to resolve all ambiguities. If only one GSSP is required but more than one can resolve the ambiguity, the GSSP with the lowest numerical value will be displayed.
GSSPs Set by User	Use this window to define a GSSP other than the ones displayed in the window above.
Save Software- or User - set and Exit	When clicked, the GSSPs listed by the software or by the User will be included in the final typing report.
Top Window	A list of each allele pair ambiguity that can be found and the GSSPs that will resolve the ambiguity. If no GSSPs are listed, then there are none available to resolve the listed ambiguity.
Bottom Window	When one ambiguity is selected, the base displayed under each allele is the base at the 'Position' listed. The 'Target' and 'Seq' shows the base and position the 'GSSP' listed needs to bind for resolution. For example, DPB1*04:02 contains a T at position 194, GSSP Z64 will bind to the sequence at position 251 if a C is present to resolve the ambiguity.
View Alignment	Will open the Allele Alignment Study window for the selected allele pairs.
Save List	Will open the top window in a text document.

GSSP analysis

After sequencing DNA with GSSPs, load the original sequence files and the GSSP files for analysis. Alternatively, if the sequencing data were already analyzed and saved, load the GSSP and check the ☑ Merge Existing Sample checkbox. The original sequence files with automatically populate the Sample List after Process All is selected. Merge Existing Sample function will function only when default storage folder contains original sequences.

The sample list shows the standard sequences and special sequences under the same sample name. The GSSP sequences automatically align under the standard sequences of the appropriate exon in the **Electropherogram Trace Display** window.

1. Analyze the forward and reverse sequences for each exon before analyzing the sequence for GSSP.
2. Click on 'Filter' to combine the GSSP files (Z64 in the figure) with the exon trace files. If 'Filter' is not available, the ambiguity was not resolved by the GSSP.

The allele list contains the combined results. The mismatch column indicates if mismatches are still present in the forward, reverse or GSSP sequence.
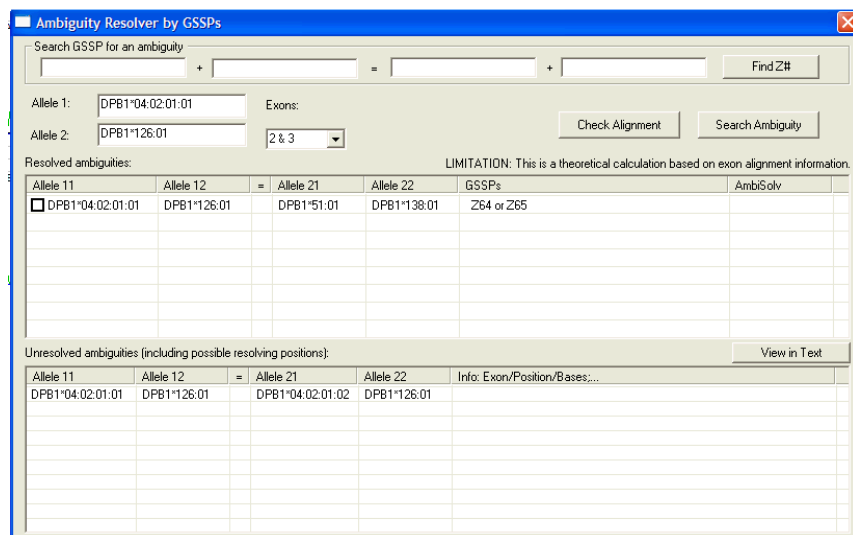
Resolve allele ambiguity by GSSPs tool

The **Ambiguity Resolver by GSSPs** dialog box will suggest GSSPs that can resolve allele ambiguities before any sequencing data has been loaded. If the User has a low resolution result or is aware of an ambiguity that they need resolved, GSSPs that will resolve any known ambiguities for a single allele pair or between two pairs of alleles can be determined before setting up the sequencing run using this tool. The GSSP(s) can then be run upfront rather than after the initial sequencing run.

1. Select the **Ambiguity Solver** icon to open the **Ambiguity Resolver by GSSP** window.

   Once the **Ambiguity Resolver by GSSP** is open, you can manually enter alleles, designated by either group or full allele name. You can also enter exons to limit the range of the search.

2. Click **Search Ambiguity**.

   The applicable GSSP to resolve ambiguities is listed in the GSSPs column. If any ambiguities remain that cannot be resolved by existing GSSPs, they will be listed under Unresolved ambiguities.

3. If you wish to launch the **Allele Alignment Study Tool**, select an allele pair in the **Resolved Ambiguities** list and click **Check Alignment**.

4. The Search GSSP for an ambiguity fields enable entry of specific allele pairs to determine if the ambiguity is resolvable with an existing GSSP by selecting **Find Z#**.
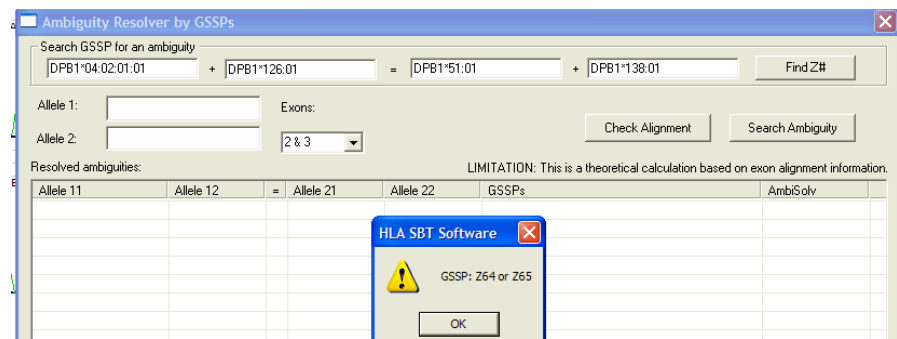
5. Click View in Text to open a text version of the Resolved and Unresolved ambiguities lists.

⚠ **CAUTION!** – This is a theoretical calculation based on alignment information. Some null alleles may be listed as an unresolved ambiguity when they can be manually filtered.

Review and Approve Genotypes

Sample review and submission

Anyone given 'User' level permission can mark samples as reviewed, but only a 'Supervisor' level user can approve samples.

1. The User can review and submit all open samples with a batch review or review samples individually.

For Batch Review:

On the tool bar, click the **Technician Final Review and Submission** icon for batch final review and submission of all samples.

Would you like to submit all samples as reviewed (except those marked as pending)?

[Yes]   [No]
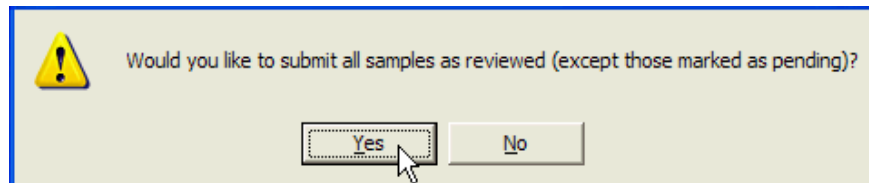
Click **Yes.**
Click **OK**.

For Individual Sample Review:

Select a sample and type **Ctrl-S,** or right click the Locus or sample name > **Sample Lis**t > **Mark Reviewed**. Alternatively, click within the "U" column of the Sample List window.

2. The reviewed sample in the sample list will display a check next to the light green bullet point.

⊞···● D139

3. To remove reviewed status from all samples in sample list, select **File > Unlock All** or **File > Unlock All with Reset**. **Unlock All with Reset** removes the final type designation set in the **Final Type** tool.

4. To re-analyze all unlocked samples, select **File > Re-Run All.**

5. To remove reviewed status from an individual sample, select a sample and type **Ctrl-U,** or right click the Locus or sample name in the **Sample Lis**t menu, select **Unlock.**

6. After review, submit and save samples. Click **File > Save All.**

7. In the **Save Sample and Case Information** pane, click **Save All and Exit.** See Save Sample and Case Information for additional details.

Sample approval

A user with 'Supervisor' level permission can both review and approve samples.

Samples can be approved and submitted together in a batch review or individually.

To mark all samples as approved or unapproved:

For Batch Approval:

1. On the tool bar, click the supervisor **Final Supervisor Approval** icon .
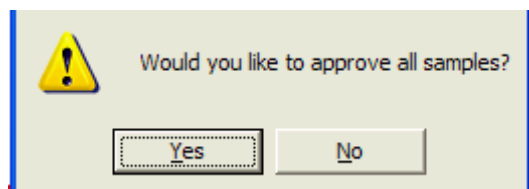
2. Click **Yes.**

   The status is changed to 'approved'.

3. Click **OK.**

   For Individual Sample Approval:

4. Select a sample and type **Ctrl-A**, or right click the Locus or sample name in the **Sample List** menu, select **Mark Approved.**

5. The approved sample in the sample list will display a check next to the dark green bullet point.
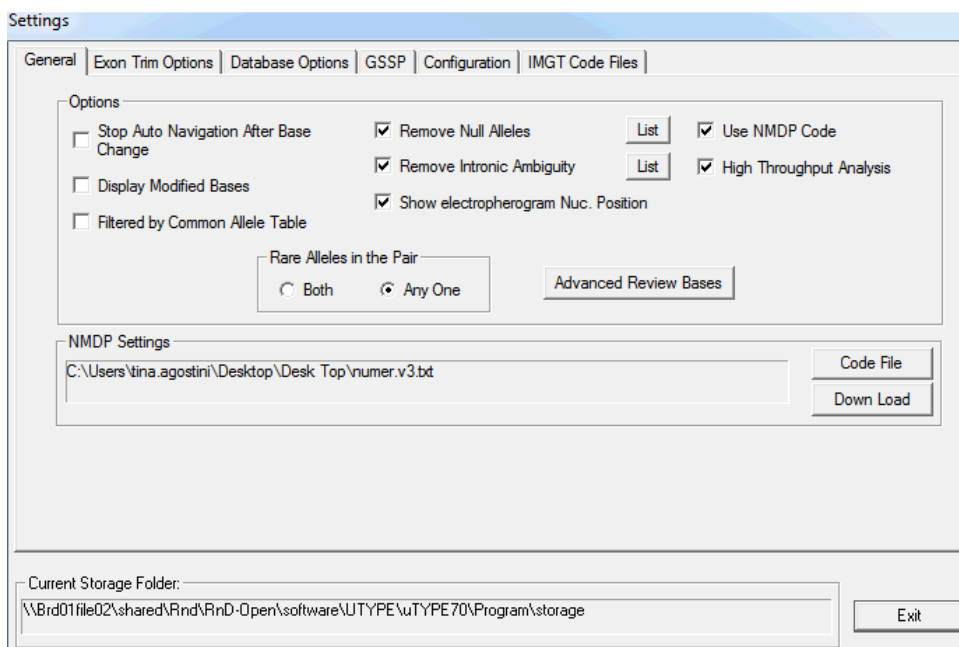
6. To remove approved status from all samples loaded, select **File > Unlock All** or **File > Unlock All with Reset**. **Unlock All with Reset** removes the final type designation set in the **Final Type** tool.

7. To remove approvals individually, select a sample and type **Ctrl-U** or right click the Locus or sample name in the **Sample List** menu, select **Unlock. Unlock with Reset** needs to be executed twice to remove final type.

8. Click **File > Save All.** See Save Sample and Case Information for additional details.

Settings
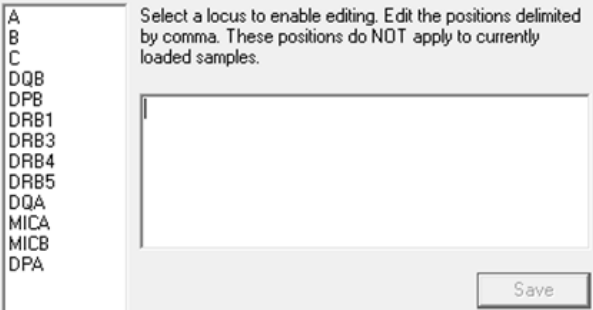
Open the **Settings** dialog box by clicking the **Preferences** icon   in the toolbar.

General tab
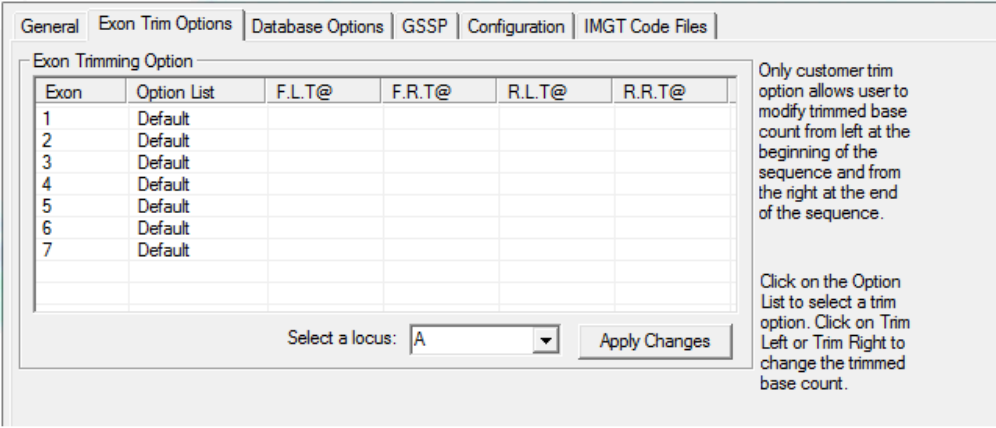
General Options	Description and Use
Stop Auto Navigation After Base Change	Stops the software from automatically skipping to the next discrepant position for review after a base edit.
Display Modified Bases	Shows inactive bases that act as place holders.
Filtered by Common Allele Table	Check box to eliminate alleles not contained within the CWD list from **Match List**.
Rare Alleles in the Pair	**Any One:** When Filtered by Common Allele Table is checked, allele pairs with at least one rare allele will be filtered from the Match List.
	Both: When Filtered by Common Allele Table is checked, allele pairs with two rare alleles will be filtered from the Match List.

Remove Null Alleles	Check box to remove known alleles caused by insertions of deletions within the coding region from the Match List. If unchecked, Null Alleles can be removed manually from the **Match List.**
Remove Intronic Ambiguity	Check box to analyze intronic ambiguities that are just outside the coding region with existing sequencing data. If unchecked, alleles with Intronic Ambiguities can be removed manually from the **Match List.**
List	Click to display a list of null alleles and alleles defined by intronic polymorphisms that can be filtered by the software when the Null Allele filter and/or the Intronic Ambiguity filters are enabled.
Show Electropherogram Nuc. Position	Displays in the electropherogram the actual HLA position number rather than the ABI imbedded base number.
Use NMDP code	Prints the NMDP code or 4 -digit type on report.
High Throughput Analysis	Allows the software to make more base call decisions (for reference laboratories that run a large volume of samples). All software changes are indicated to the user by the addition of pink marked positions in the Upper Navigator window. When High Throughput Mode is on, the Common Allele Table is referenced to make automatic edits. The edits made are dependent on one of the selected "Rare Alleles in the Pair" options. The below will describe how the software utilizes the 2 options available "Any One" or "Both". 1) High Throughput Mode On + "Any One" selected: The software will automatically edit as needed to find a common/common result. If Any One of the alleles in a pair is rare, the software is biased against that typing merely as a function of allele frequency. Automatic edits or changes are shown on the Base Navigator Pane with pink color indicating the Software Edited position. Users are advised to review those positions. 2) High Throughput Mode On + "Both" selected: The software will automatically edit as needed to find either a common/common OR common/rare result. If Both of the alleles in a pair are rare, the software is only biased against the double rare result. 3) User will have to use "Standard" mode (enabled by toggling off High Throughput analysis) if they would like to manually make edits different than what the consensus sequence shown. "Rare Alleles in the Pair" setting has no effect on suggested allele pair results when in "Standard" Mode.

Advanced Review Bases	Select a locus to enable editing. Edit the positions delimited by comma. These positions do NOT apply to currently loaded samples.	Type the position number within the open window and select "Save". The position entered in the window will automatically be marked in subsequent analysis of future samples.
NMDP Settings	If Use NMDP Code is checked, the NMDP files must be added. Down Load : Automatically downloads the numeric.zip file from NMDP website (http://bioinformatics.nmdp.org/HLA/numeric_allele_code_list.html). Save it to a folder. Code File: Sets the path for the numer.v3.txt file from download.	

Exon Trim Options tab

Allows for the definition of Exon start and end positions by the user. Once the Exon Trim Options are set, the start and end positions will apply to all subsequent samples for the locus selected.

Exon Trim Settings	Description and Use

Options List	Choose "Default", "Full Exon", and "Customer" by selecting the "Option List" column. Default - Vendor-defined exon trim positions.
	Full Exon – Exon start and stop positions correspond to the HLA-designated start and end positions for each locus
	Customer – Customer/User-defined start and end positions. If "Customer" is selected, then the trim setting for the beginning or end of each direction in the exon can be modified. Only the loci and exons supported by the software are adjustable when the "Customer" option is selected.
FLT/FRT/RLT/RRT	FLT- forward trace, left side trim position FRT- forward trace, right side trim position RLT- reverse trace, left side trim position RRT- reverse trace, right side trim position Input value for these columns is a base position number.
Locus	Select the locus in which the exon trim options need to be set.

Database Options tab

Database Options Settings	Description and use
Use Default DB	Select radio button to set to default database storage location. The default storage database is a Microsoft Access database.
Use Oracle DB	Select radio button to enable the use of an Oracle database. Use Set DSN to browse to the Oracle Database. The Database Administrator is responsible for configuration of the database connections.
Use SQL Server DB	Select radio button to enable the use of a SQL Server database. Use Set DSN to browse to the SQL Server database. The Database Administrator is responsible for configuration the database connections.
Set to new location	Allows selection of a new storage location. A new storage location can only be designated when no files are loaded into the software. Only a supervisor can change the storage location.

GSSP tab

Users can enter GSSPs of their own design into the uTYPE 7.x Software.

To add a GSSP, follow the naming convention listed below:

GSSP Settings	Example	Description and Use
Code	Code: 1	Customer Assigned Code, user created GSSPs will be designated by an X preceding the GSSP name.
Select a Locus		Primer targeted locus.
Position		Site of primer specificity
Bases	Bases: G	Defining base(s) at the site of primer specificity
Skip Count	Skip Count: 20	Number of bases at the start of the sequence read that should be excluded from analysis (the primer length is not included in this value). 20 bases is suggested for POP6 polymer and 30 for POP7 polymer.
Direction of Primer	L R	L for a Reverse Primer R for a Forward
Exon	Exon: 3	Exon targeted by primer
Cross (F23/R23)	Cross (F23/R23)	**F23**: GSSP crosses from exon 2 to exon 3. **R23**: GSSP crosses from exon 3 to exon 2

Select **Add GSSP** to list the custom GSSP in the table. Select **Save** for the new GSSP to be activated in the software. Select **List All GSSPs** to display GSSPs in the

database. Select Remove Selected to remove selected GSSPs.

Configuration tab

Configuration Settings	Description and use
Global Settings Guidelines	Global Settings enables multiple installed instances of uTYPE 7.x Software to utilize mirrored setting profiles. Global setting is only applicable to software that is accessible by multiple users launched from a shared networked location. The uTYPE 7.x Software should be installed on a network location which is accessible for all users from this location for the Global Settings to be in effect. Modifications to the Global Settings can only be made by users with a Supervisor role. Updates can be made at any time. It is recommended that users enable global settings upon installation of the uTYPE 7.x Software. Users can change individual preferences for an analysis session while Use Global Settings is checked, but Global Setting Preference is restored when the user logs out and logs back in to the uTYPE 7.x Software.

Global Settings Preference	Global Settings can be created for the following items: 1. Save Log Info 2. Use NMDP Code 3. Filtered by Common Allele Table 4. Stop Auto Navigation After Base Change 5. High Throughput Analysis 6. Remove Null Alleles 7. Remove Intronic Ambiguity 8. Rare Alleles in the Pair 9. Database to use 10. Data Storage Folder 11. NMDP Settings 12. IMGT Code Files (P and G code files) 13. Exon Trim Options 14. Advanced Review Bases

Use Global Settings Checkbox	Enables Global Setting Preference when checked.
Export Settings	When a change to a Global Setting is made, the Global Settings are updated by clicking the Export Settings button.
View Current Settings	Allows the user to view current Global Settings preference selections. Settings are not global until Export Settings is selected.
Change Logon User	Allows multiple users to log on and track changes without closing out the software.
Curve Display	Adjusts the peak thickness in the electropherograms.
Information Log and View	**Save to Log File –** Toggles saving to the Activity Log. **Activity History –** Returns a report from a specified date range. **Activity Log –** Returns the activity from the current day.

IMGT Code Files tab

IMGT Code Files Settings	Description and use
Set G File	Use **Set G File** to navigate to g code text file "hla_nom_g.txt" which can be downloaded from http://hla.alleles.org/wmda/. Once the file is downloaded and linked to the software, G code functionality will be enabled.
Set P File	Use **Set P File** to navigate to p code text file "hla_nom_p.txt" which can be downloaded from http://hla.alleles.org/wmda/. Once the file is downloaded and linked to the software, P code functionality will be enabled.
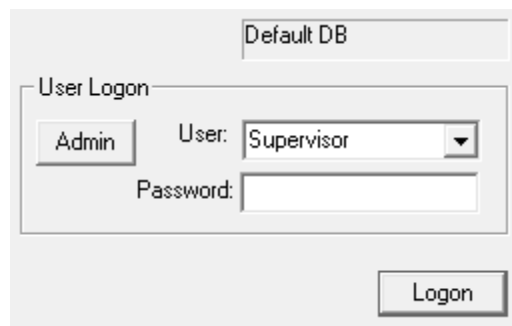
Software Administration and Maintenance

Add and remove users

There are 2 permission levels, 'User' and 'Supervisor'.
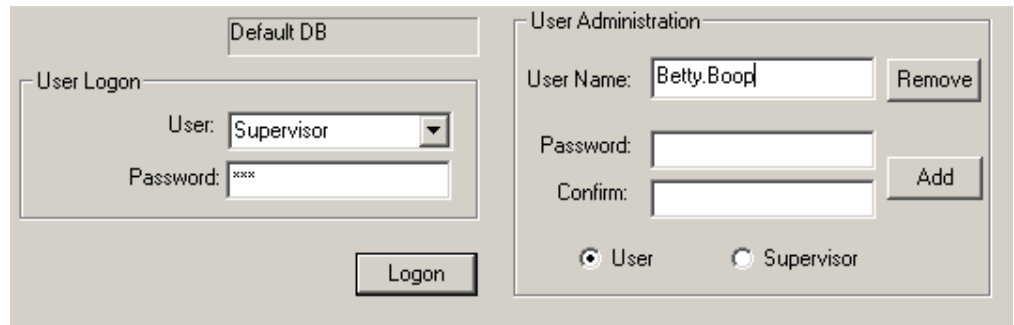
Only a Supervisor can initiate password changes for existing users, and add and remove users. If you are the laboratory administrator, contact your uTYPE 7.x Software Technical Sales Specialist or Field Service Engineer for the Supervisor password.

1. Start the software.
2. Choose "Supervisor" from the drop down in the "User" field.
3. Enter the Supervisor password supplied by your uTYPE 7.x Software Technical Sales Specialist or Field Service Engineer.
4. Click the **Admin** button.

The **User Administration dialog** box appears.

5. Enter the name of the new user and the password.

   Note: User name length is limited to 20 characters and the password is limited to 10 characters.

6. Select the permission level for the new user, either 'User' or 'Supervisor'.
7. Click **Add**.
8. Repeat steps 5-7 for any additional users.
9. To remove a user, type in the user name as listed in the User drop-down menu, and click **Remove**.
10. When you are finished, click **Logon**.

Change password

1. Log on as Supervisor as described in Add and remove users.
2. Type in the username of the user whose password you wish to change.
3. Fill in the **Password** and **Confirm** fields and click **Add**.

Update library

1. In the application window, click the icon in the toolbar.

2. The IMGT/HLA database release version appears.

   ```
   ⚠  HLA-A IMGT/HLA Release: 3.17.0 2014 July 14
       HLA-B IMGT/HLA Release: 3.17.0 2014 July 14
       HLA-C IMGT/HLA Release: 3.17.0 2014 July 14
       HLA-DQB1 IMGT/HLA Release: 3.17.0 2014 July 14
       HLA-DPB1 IMGT/HLA Release: 3.17.0 2014 July 14
       HLA-DRB IMGT/HLA Release: 3.17.0 2014 July 14
       HLA-DQA1 IMGT/HLA Release: 3.17.0 2014 July 14
       HLA-MICA IMGT/HLA Release: 3.17.0 2014 July 14
       MICB IMGT/HLA Release: 3.17.0 2014 July 14
       HLA-DPA1 IMGT/HLA Release: 3.17.0 2014 July 14
       NMDP code: LASTUPDATED:12/17/14

                                              [ OK ]
   ```

3. New releases come out every 3 months.

   If your database is not current, order the latest version of the uTYPE Allele Library through the company website or contact your Technical Sales Specialist for more information.

4. Click the library features dropdown in the toolbar, then choose "Update Library".
5. Navigate to the downloaded .zip file.
6. The update loads into the program directory.
7. Exit the uTYPE 7.x Software and restart to incorporate the library update.
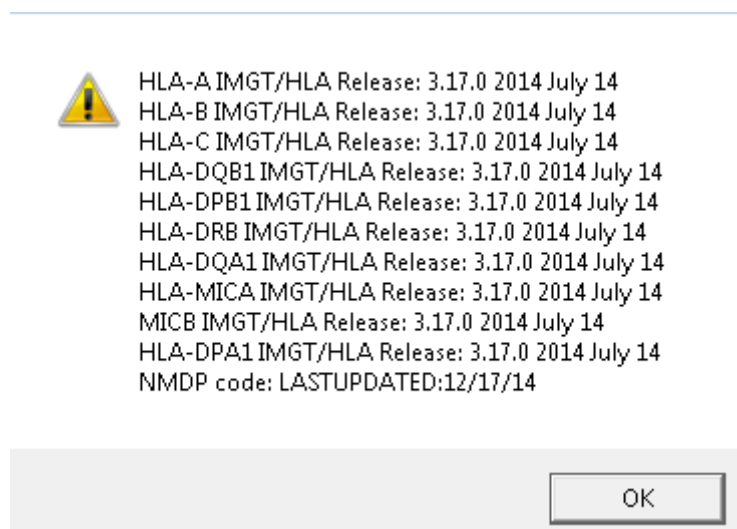
8. Click the icon to confirm that the library has updated.

Check that library has updated

The version of the IMGT/HLA database that is being used by the software appears above the tool bar.

`IMGT/HLA Release: 3.17.0 2014 July 14`

You can also check that the library has updated by checking it against a FASTA file. See Confirm that Library Update has Loaded . Please download the latest reference files from the One Lambda website.

Tools

Allele Alignment Study Tool

The **Allele Alignment** tool can be accessed by selecting **Allele Alignment** in the **Lib** drop-down menu in the toolbar.
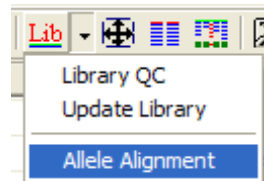
Select the locus and allele combination of interest from the drop-down window on the left. The allele alignment appears as the alleles are selected.
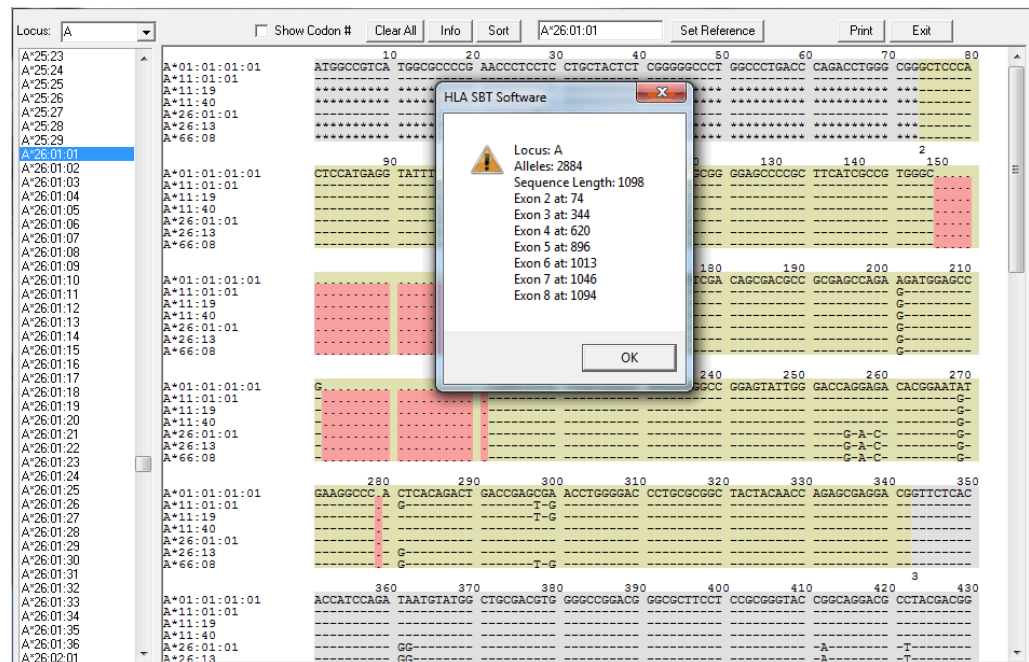
Settings Options	Description and use
Show Codon #	Checking or unchecking allows for toggling of sequence position identification by either codon or position number.
Info	Opens a pop-up display with locus-specific information (as shown above).
Sort	Sorts selected alleles in numerical order.
Set Reference	This function allows the reference to be designated to an allele other than the default by typing the allele in the adjacent window. The allele at the top of the allele list will be used as the default reference.

Allele Comparator tool

The **Allele Comparator** compares the sequence from a selected allele pair directly to sample data. It can be accessed by selecting the **Allele Comparator** icon in the tool bar. You can compare any alleles available in the library, not just the ones that you have loaded into the sample list.

To use the tool, select the alleles that you wish to compare from the drop-down menus in the Allele Pair Selection Dialog box.

The selected alleles appear in the bottom two rows in the sequence display pane.

Selected allele pair

Any discrepant sites between the manually loaded allele pair and the trace curve data appear marked in red in the lower navigator window for review. In the Sequence display, the discrepant base is also marked in red.

Magnifier tool

The **Magnifier** tool acts like a magnifying lens. You can use it to provide a magnified view of an electropherogram detail.

1. Double-click the magnifier icon in the tool bar On the second click, keep holding down on the mouse button.

    The **Magnifier** tool opens.

2. Keep holding down on the mouse button as you drag the magnifier tool to the location you want to magnify. When using dual screens, magnifier will only function on screen 1.
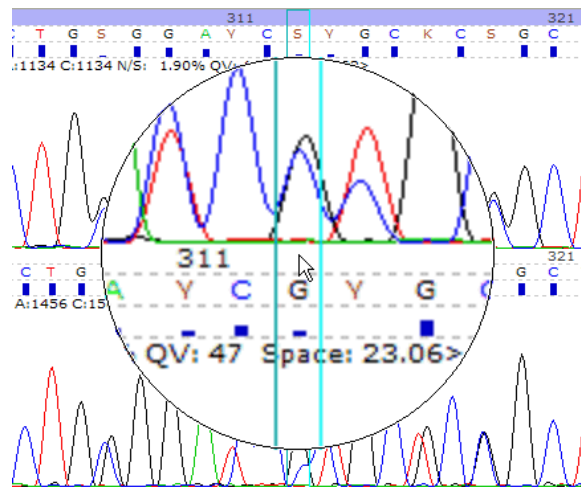
3. Release the mouse button to close the **Magnifier** tool.

Library QC by Sequence in FASTA and Nomenclature Lookup tool

The **Library QC by Sequence in FASTA and Nomenclature Lookup** tool allows you to check that the library has updated by checking it against a FASTA file.

You can also convert an allele from the old nomenclature to the new nomenclature, or from the new nomenclature to the old nomenclature.

To access it, clickin the toolbar and select **QC Library.**
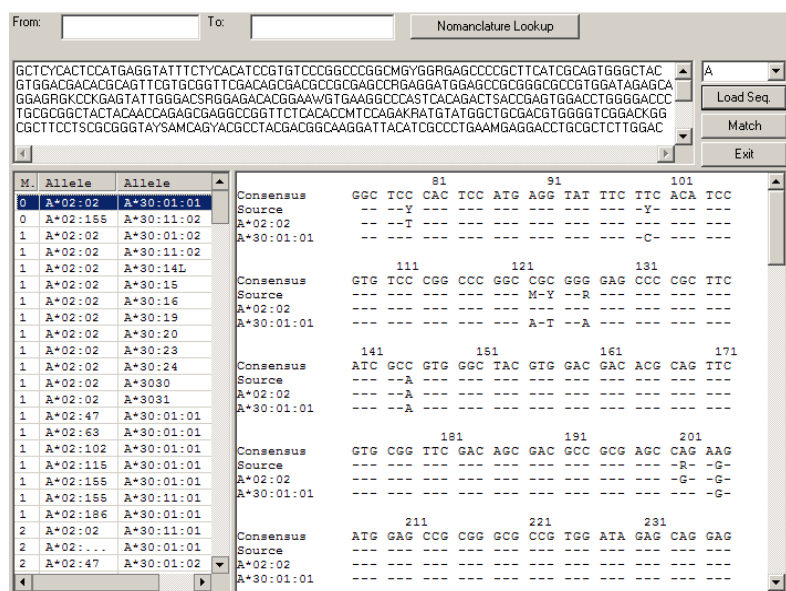
Confirm that library update loaded

To confirm that a library update was loaded:

1. Click in the toolbar and select **QC Library.**

   The **Library QC by Sequence in FASTA and Nomenclature Lookup** pane opens.

2. Click **Load Seq.** to navigate to a known HLA allele FASTA file and load it, or paste a known HLA allele FASTA file into the middle window.

3. Select a locus in the dropdown menu and click **Match.**

   An allele or allele pairs displays.

4. Select the allele(s) to show the alignment.

Nomenclature Lookup

To convert an allele from the old to new naming convention or from the new to old naming convention (when an old name is available):

1. Click ![Lib] in the toolbar and select **QC Library.**

   The **Library QC by Sequence in FASTA and Nomenclature Lookup** tool opens.

2. Enter either old or new nomenclature allele information in the **From:** field and click **Nomenclature Lookup**.

3. The **To:** field shows the nomenclature in the opposite format.

For information on the new and old nomenclature, please see:

hla.alleles.org/announcement.html

hla.alleles.org/nomenclature/naming_prev.html

Reference
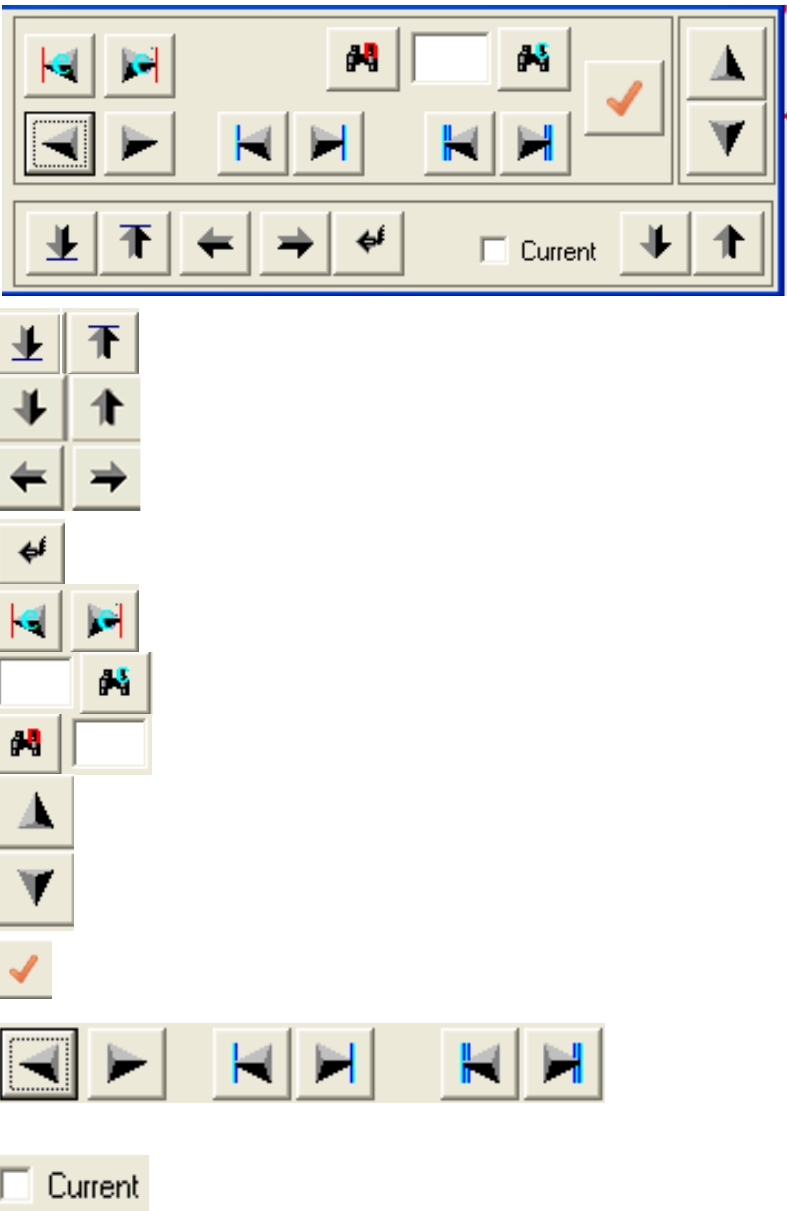
Toolbar Overview

Icon	Description
	Review Stored Results-Opens **Data Management and Review** pane.
	Open/Load Files-Opens **Load Files** pane.
	High throughput user-Call Technical Support for more information.
	Save All Results-Opens **Save Sample and Case Information** pane.
	Re-Run Match.
	Report Function-Opens **Report** pane.
	Clear All Samples.
	Browse Previous or Next Target Base. Using one of these four drop down settings: • **Marked** setting allows software to navigate to all marked positions. • **Polymorphic** setting allows software to navigate to all polymorphic positions. • **Each base** allows software to navigate through each position. • **Type in** a codon location, such as '30.1' or a bp position, such as '253' to navigate to a specified position.
	Reset Changes to Current Locus-Remove changes from current trace set data.
	Print Screen Snap.
	Library Features-**Opens Library QC by Sequence in FASTA & Nomenclature Lookup, Update library** and **Allele Alignment Study** panes. You can apply FASTA files to test library update.
	Allele Comparator-Use to compare selected allele pair to sample data. Opens **Allele Pair Selection Dialog**.
	Ambiguity Solver-Opens **Ambiguity Resolver by GSSPs** pane.
	Preferences-Set software preferences.
	Technician Final Review and Submission.
	Final Supervisor Approval.
	Magnifier-Double-click and hold to open magnifier.
	DQA1 In/Del manual trim-Applies or removes trim function for In/Del sample.
	Turns off trace color in electropherogram display.
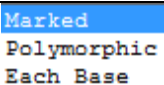
	Turn on/off auto run after base edit.
	C/R Filter Toggles filtering of rare alleles in Match List.

		Show Navigation Panel	
		Increase and Decrease electropherogram display height. Decrease and Increase peak height. Increase and Decrease number of peaks in display window.	
		Reset the display to default.	
		Move to end and to start of exon.	
		Move to codon position in electropherogram.	
		Move to base pair position in electropherogram.	
		Jump to previous or next sample in the Sample List window.	
		Mark a position as reviewed. A green bar appears in the Base Navigator window.	
		Browse each base or polymorphic position or marked base in the electropherogram.	
		Check to make changes to selected trace curve.	

	Use Null Allele (Nul) or Intronic Filter (Intr)-Toggle on/off null and intronic allele filter for all samples loaded.
MM	MM Positions-Select to display yellow in Base Navigator window for the alleles listed in the Match List.
	Display QV/Seq Info-Toggle for electropherogram display options: • No Information • Histogram Only • Histogram, .ab1 File Name, Signal, Background, QV, Spacing • .ab1 File Name, Signal, Background, QV, Spacing • Histogram, .ab1 File Name • .ab1 File Name Only

Short cut keys

Short Cut/Key Entries	Toolbar	Function
F		Filters Basecalls
Alt-L		Show Load Files window
Alt-R		Show Data Management and Review window
Alt-S		Show Save Sample and Case Information window
Z	Marked Polymorphic Each Base	Toggle between Marked, Polymorphic and Each Base
←→ (left, right arrow key)		Browse sequence based on Marked, Polymorphic or each Base selection (above)
ACGTRYKMSWN		In Electropherogram window, change displayed base to another call.
Space		Delete selected base in Electropherogram window
Ctrl-D		Opens Sequence Overview to show whole trace at once.

Sample Final Type Settings

The **Sample Final Type Settings** dialog box allows you to manually designate a final type and save it.

To access the **Sample Final Type Settings** dialog box, open the **Sample List Menu** by right clicking the Locus or sample name of a sample in the **Sample List**, and select **Set Final Type**.

Options	Description and use
NMDP Code Reverse Lookup	NMDP code can be entered to view full allele list. NMDP code functionality set up within Preferences.
Type in Detail	Software automatically combines typing ambiguities into NMDP codes where able.
Use 2 Fields Checkbox	Trims assigned final type to HLA subtype, when Show P or G code is selected.
Use Allele String	Assigned Type box will contain full allele string when Use Allele String is checked.

List of Perfect Match	Displays full list of Perfect Match allele pairs.
Combine Selected	Allows uTYPE 7.x Software to designate the final typing result. The software will combine selected allele pairs into NMDP code if one exists.
Assign Type & Exit	Sets the final type and exits the window.
Type by Software	Allows uTYPE 7.x Software to designate the final typing result. The uTYPE 7.x Software can determine the NMDP code and how the ambiguities are grouped. The user cannot specify specific alleles when this option is used.
Show G Code	Type in detail window displays typing results with G codes where applicable. G code functionality is set up within Preferences. The user has the ability to set the G code as the Assigned Type.
Show P Code	Type in detail window displays typing results with P codes where applicable. P code functionality is set up within Preferences. The user has the ability to set the P code as the Assigned Type.
Show Pair Alignment	Launches the **Allele Alignment Tool** and compares all alleles checked in the **Manual Ambiguity Combination** list.
Assigned Type "R"	The **R** button within the Assigned Type window will convert the NMDP code within Allele 1 and/or Allele 2 fields to the full allele string.
Assigned Type "V"	The **V** button within the Assigned Type window will display the complete allele string in two rows above the Type in Detail window if the allele string is too long to visualize in the Allele 1 and Allele 2 fields.
"X"	To clear Assigned Type alleles.

Plate Record Generator

To access the Plate Record Generator, select **File > Plate Record.**

Options	Description and use
Information for plate record	Fields must be entered manually the first time. The information will be stored by the program. The software will automatically fill in the required entry fields with the previous entry if possible.
Select a model	Dropdown displays genetic analyzer models and data collection software versions.
Auto Fill Vertical	Software will automatically fill in the appropriate exon and direction for each locus. Vertical will auto fill the plate in the vertical direction.
Create Records	Display plate record in format that can be imported in to collection software. Select Save As when storing the plate record before import.
Print Work Sheet	Select to print a 96 well worksheet to facilitate plate setup.
Load Plate	Select to load previously generated plate record to view, review, or edit plate contents.

Print an electropherogram

To print an electropherogram, select **File > Print Sequences.**
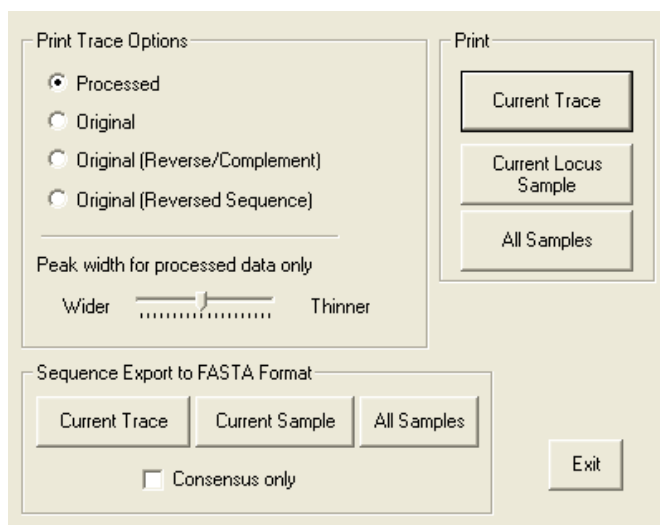
Quality Reports

Quality reports are accessible from the **Load Files** drop-down. Choose **QC View**. You will need to scroll down the **QC View** screen to see all three of the reports.

Note: Print reports only after supervisor review.

QC Report Items	Description and use
Select Data from: ⬚ Current Samples ⬚ From Database	Sets location to select data from. Samples currently loaded. The database folder location is set within **Settings**.
Search from database	Sets date parameters for searching in the database.
Signal Cutoff Value Settings	Sets the low and high signal values to use when creating the reports.

The **Signal Report** includes signal strengths for each base in each electropherogram. The graph also includes the mean and standard deviation Quality Value for reference. High and Low signals are set to laboratory preference.
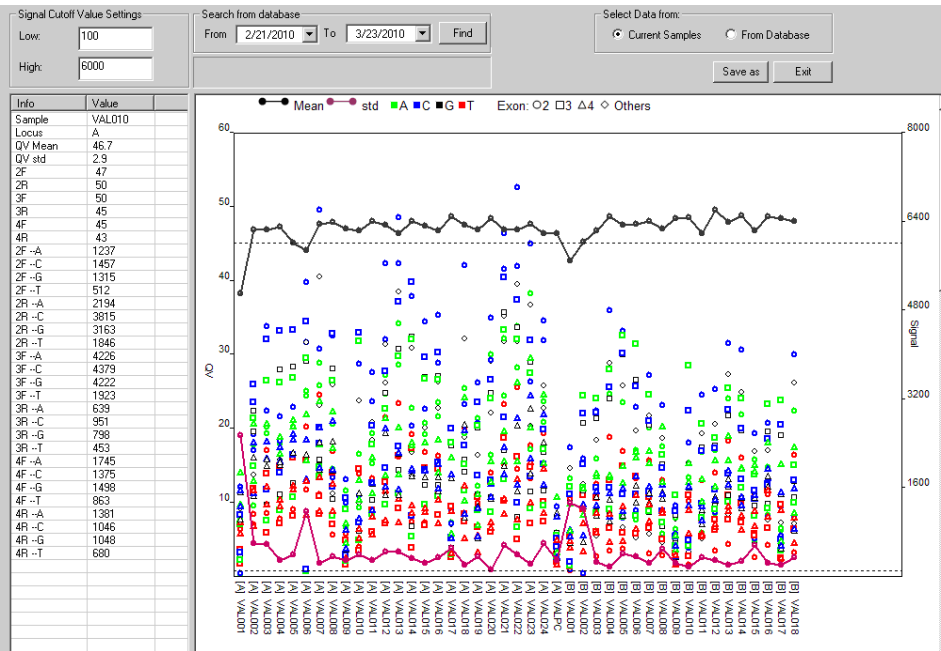
The **Edit Report** includes the number of edits per sample in relation to the Quality Value mean and Quality
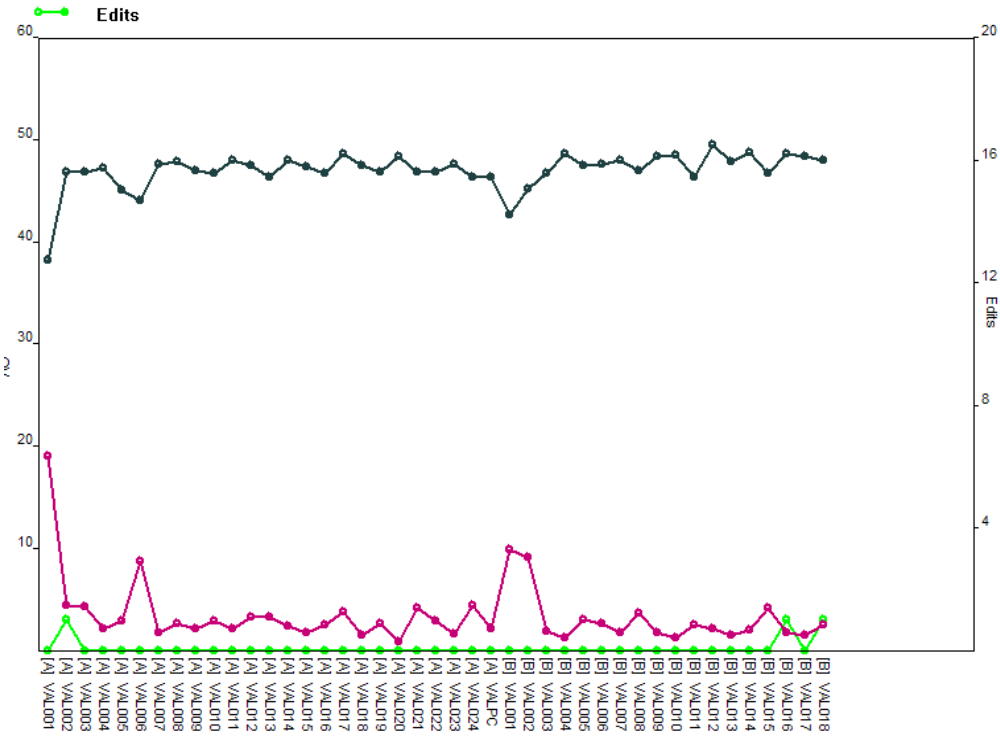
Value standard deviation.

The **Edit per Quality Value** report displays the distribution of bases that scored a specific Quality Value and the associated edits per Quality Value score.
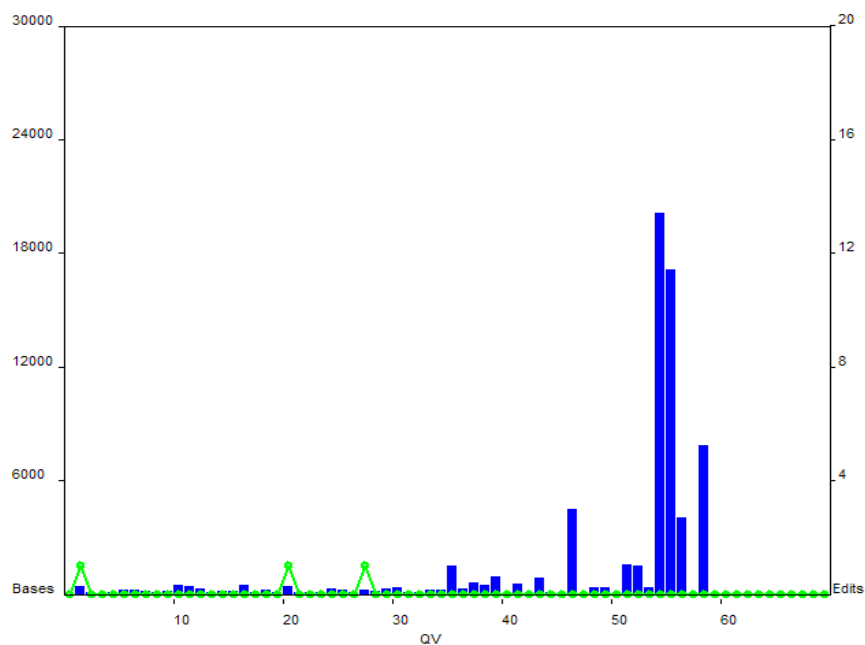
Troubleshooting

Error messages

Message	Cause
Login	
Invalid Password.	Too many characters in password.
You must be a supervisor user.	Only the "Supervisor" user can add or remove users.
Can't login. Please check username and password.	Incorrect password or invalid user selected.
Loading Window	
Error 2: failed to read.	A non-.ab1 file was loaded into the loading window and cannot be processed.
Sample Name can't have "*?" characters.	Sample name contains special characters "*" and/or "?".
Exon and Direction Reanalysis	
Still can't identify the sequence.	A sequence was manually assigned another exon and/or direction, but the software is still unable to recognize the sequence.
Saving Window	
Failed to Save.	Storage subdirectory changed to "read only".
Loading Window	
Fail to load F:	.lgn file renamed in database folder.
Reporting	
Can't open template report file.	Incorrect path to report template.

Problems and solutions

Problem	Possible Causes	Recommended Action
The trace curve contains many mismatches to consensus sequence.	Wrong library loaded.	Reassign correct library.
	Low signal or poor sequence quality.	Re-sequence sample.
Forward and reverse sequences are out of alignment.	Spacing between peaks has caused software to insert an incorrect base.	For sequences that are out of alignment in an isolated area at the end of an exon, trim the incorrect bases.
	After editing a base, the sequence goes out of alignment.	Select the trace curve that was edited and select "Reset Changes" in the toolbar. Locate the position where each sequence goes out of alignment and trim the poorer quality sequence. Alternatively, edit the sequence working toward the end of the exon.
	After adding a base, the sequence goes out of alignment.	Ensure that the number of bases added equals the number removed.
	Low signal or poor sequence quality.	Re-sequence data.
When filtering, all alleles disappear from the Match List.	Before filtering, there must be at least one exact match in the Match List.	Check all discrepant sites to verify software calls or edit to 100% concordance with a possible match.
When filtering a null allele, the trace curve display goes blank.	The filtered null allele is present in an exon that was not loaded. There is no sequence to display.	Re-analyze with the missing exon. For B locus Set1 and Set2 positive samples, nulls located in exon 4 will be filtered when the data is combined.
The sample name did not extract correctly in the loading window.	Samples of the same group have different sample names.	Reload files into the loading window. To extract a sample name from a file in the sample column, use the sample name extraction function to fill in the starting position and length of the correct sample name. The sample name can also be edited in the sample column.

Problem	Possible Causes	Recommended Action
An edited base is not incorporated into the pattern sequence.	Trimmed data is still incorporated into the pattern results.	Select the match button in the toolbar. A trimmed sequence may be remembered by the pattern sequence even after the match button is selected. Edit a base to match the trace curve to trigger the software into auto analysis.
	The pattern is frozen.	Edit a base anywhere in the sequence to trigger the software into auto analysis.
	Multiple files were loaded.	Check to see if multiple files were loaded for the same exon and direction in the sample list in the trace curve window. Correct if necessary.
	The forward and reverse sequences do not match.	The pattern call will default to a heterozygous call. In some instances the forward and reverse sequence will need to be edited to have the change incorporated by the pattern sequence.
	Approved or Reviewed Sample.	Approved or Reviewed status samples cannot be edited unless unlocked.
Expected allele is not identified.	A polymorphic position was overlooked or results are discordant.	Use the allele comparator tool to verify result vs. expected result by selecting expected results from the drop-down list. Discrepant sites will be displayed in the lower navigator window for review.
	Poor data quality.	Re-sequence sample.
No matches are listed in allele list.	Too many discrepant sites between sequence and consensus.	Re-analysis occurs after each change made to the trace curve data. Match List will update automatically as discrepant sites are corrected.
	Codon 86 sequence is being incorporated incorrectly into results.	Do not include codon 86 sequence if it is not needed for typing.
	Poor data quality.	Re-sequence sample.

Problem	Possible Causes	Recommended Action
The software-determined exon and direction is different from what was expected.	File name incorrect.	File was named incorrectly, but the software is able to identify the sequence exon and direction automatically.
	Poor data quality. Sequence may be too long.	Re-assign exon and direction within software. Re-sequence sample.
The sample has a yellow bullet point next to the name in the sample list.	A file cannot be identified by the software.	Highlight to select the undefined sequence and right-click. Try to re-assign the exon and direction. Try to exclude a poor section of data and try to re-assign the exon and direction.
	A file cannot be identified by the software.	Open the sample tree and delete the unidentified file. The bullet will change from yellow to red/green or green.
	Incorrect library.	Load sample into loading window and select alternate analysis library.
	Poor data quality.	Re-sequence sample.
The samples were not grouped as expected.	Naming convention not followed.	Load samples into loading window and scan sample column. Samples with the same name will be grouped together. See section 'Input File requirements' for naming convention.
After an edit, the software does not automatically move to the next discrepant site.	The "polymorphic" setting is selected in the toolbar.	Select "marked" setting from drop-down list in toolbar.
	Under "Preferences", the "Stop Auto Navigation After Base Change" is selected.	Open "Preferences" in the toolbar and uncheck "Stop Auto Navigation After Base Change".
After an edit, the software automatically moves to the next discrepant site.	The "marked" setting is selected in the toolbar.	Select "polymorphic" setting from drop-down list in toolbar.
	Under "Preferences", the "Stop Auto Navigation After Base Change" is not selected.	Open "Preferences" and select to stop auto navigation.
DRB1 data has extra polymorphisms at the start of the sequence.	The SeCore® DRB1 kit contains primers within the exon.	Open "Preferences" and uncheck any exon trimming options that are not the "Default" settings.

Anomalies

- ➢ There are no known anomalies at this time.

Limitations and Precautions

- uTYPE 7.0 Software is intended for Research Use Only.
- uTYPE 7.0 Software is intended only for HLA analysis of HLA Loci A and B Exons 1, 2, 3, 4, 5; HLA C Exons 1, 2, 3, 4, 5, 6, 7; HLA DRB3, DRB4, DRB5,
- DRB1, and DQB1 Exons 2, 3; HLA DPB1 Exons 2, 3, 4; HLA DQA1 Exons 1, 2,
- 3, 4; HLA DPA1 Exon2, MICB Exons 2, 3, 4, 5; and MICA Exons 2,3,4,5 .
- uTYPE 7.0 Software library updates are provided by Invitrogen by user subscription. The newest library update version should be used. Before implementing a library update, the laboratory should perform quality assurance and quality control testing using a panel of known molecularly typed samples/files. Such samples can be obtained from the International Workshop Reference Cell Panel and the UCLA DNA Reference Panel.
- Use the newest NMDP code database. Not all alleles have been assigned NMDP codes.
- To ensure typing accuracy, review all discrepant, manually edited, and software- edited positions, in addition to the closest matched polymorphic sites.
- Perform bi-directional sequencing to ensure typing accuracy.
- Editing a base to "N" may result in additional ambiguities.
- Perform HLA typing in the presence of a qualified Director, Technical Supervisor, and/or general Supervisor following accepted laboratory accreditation standards.
- Confirm all typing results using another typing method.
- The decision as to whether a trace should be edited to indicate a polymorphic or a particular base is beyond the scope of this user guide. Your laboratory policy will dictate whether you edit the identifications made by the software.
- The Allele Alignment tool does not show defined insertion or deletion sequence.
- Users should utilize an alternative reference if this information is required.

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IUB Codes are derived from:

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