

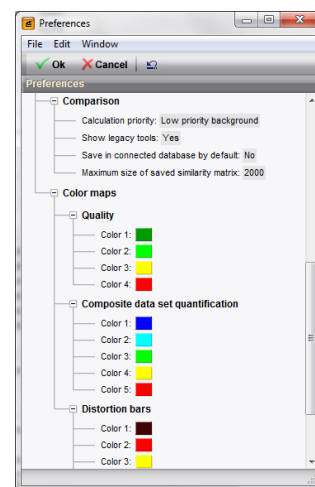
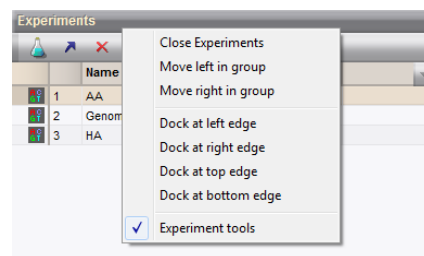
RELEASE NOTE: BIONUMERICS VERSION 6.1

Ten months after the impressive major upgrade to BioNumerics version 6.0, Applied Maths proudly presents BioNumerics version 6.1. Honoring a good old Applied Maths tradition of supplying its “minor” upgrades with a list of new features worth the label “major”, we have provided an impressive number of novel and improved features in this upgrade, covering many aspects of databasing, analysis and the user interface.

Below is a list of the new features.

INTERFACE

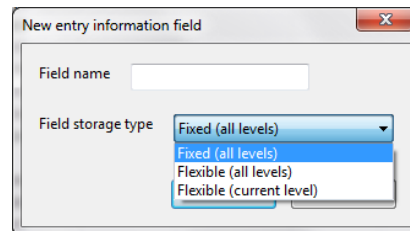
- Context menu in panel headers: right-click on header (some extra, convenient panel re-arrangement functions).
- Columns in a grid panel can now be enabled or disabled using a menu “Set active fields”, which opens a dialog box where fields can be selected conveniently.
- More comprehensive logging to file BNLOG.TXT.
- In the *Main window*, the active (selected) experiment is highlighted in the *Experiment grid panel*.
- For *experiment types*, any custom field can now be selected as the *display field*, and the content of this field will be used to label that experiment type in the comparison window, entry window, etc. This option is more flexible and less intrusive than renaming the experiment types. The software now produces a warning if a user tries to rename an experiment type, recommending the *display field* as an alternative.
- The panel structure of the contig assembly window (Assembler) has been improved; panels can now be re-arranged by the user.
- The software now supports printing with Eastern character sets (e.g. Kanji).
- The *Preferences box* has been redesigned, using a more flexible user interface in the form of an editable tree. Some new preferences have been added:
 - Most important feature is the “*Color maps*” setting, that can be used to customize the color ranges used for the following items:
 - Quality scores.
 - Distortion bars on a gel.
 - Composite data set quantification colors.
 - The new setting “*Windows > Font > Dialog box font*”, with the options “*Use system font*” (=previous situation and still the default), and “*Use software font*”. The latter option uses the same font for dialog boxes as set for the



windows in BioNumerics. It allows users to have better control over the size of the dialog boxes, particularly useful with extremely low or high screen resolutions.

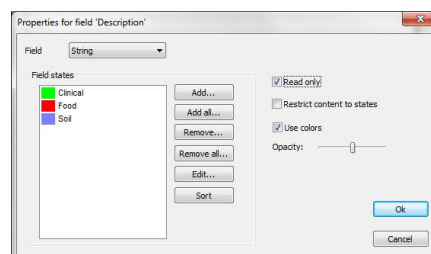
DATABASE

- A new type of entry information is introduced: *Flexible entry fields*. For querying, displaying and all other features, they behave just like the traditional *Fixed fields*, however they have a number of advantages:
 - They are stored in a separate, dedicated table in a normalized way.
 - They can be attached to a specific *level*, so that only entries belonging to this level possess this field.
 - They are easier to create, rename or remove, because no change in the table structure is required.



Both types of fields have their logical place. For example, when working with levels, the traditional fields can be thought of as the "base" set of fields that apply to every kind of entry, whereas flexible fields can be used to add specific information to specific levels.

- A new database field property "*Read only*". This property causes BioNumerics not to update the field and the user cannot edit the field either. It is useful for computed fields or field data that should not be altered after creation.
- The maximum number of entry information fields has been increased to 500 (previously 150). This maximum includes fixed and flexible fields.
- When a new experiment type is created, an error is reported if the invalid characters %/[are present in the name.
- The use of the local file DBINFO.BNF has been discontinued in *connected databases*. As a result, in distributed connected databases, removing and adding information fields is now automatically adopted in all client users.
- New connected database settings option "*Do not use stored procedures*". This option is implemented to make BN 6.1 compatible with PostgreSQL databases.



FINGERPRINT TYPES

- The maximum resolution of fingerprints has been increased to 100 000 points (previously 40 000). The increase allows BioNumerics to handle high-resolution profiles such as from MALDI and similar techniques.
- The maximum number of reference lanes in a gel or sequencer run has been increased from 150 to 300. This allows the import of high-throughput multi-dye based sequencer electrophoresis runs.

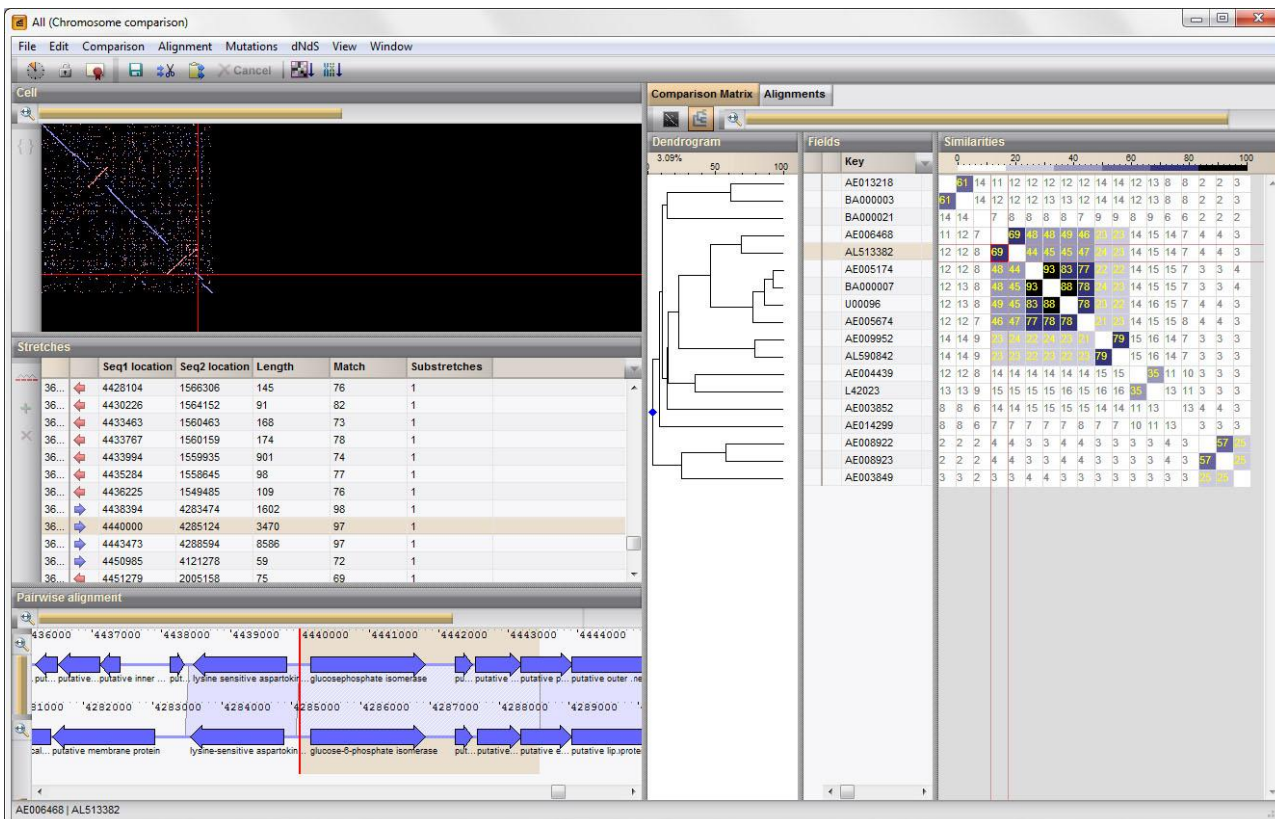
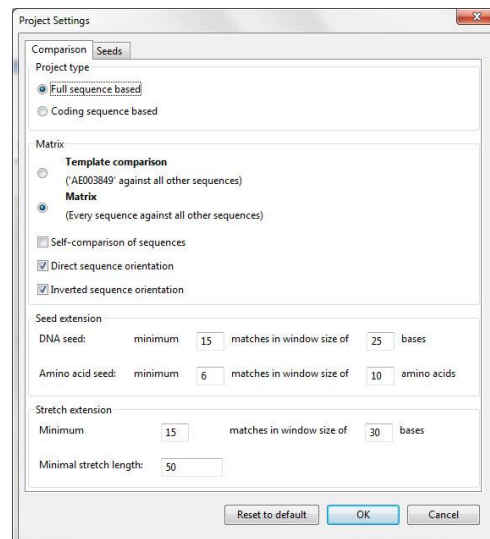
SEQUENCE TYPES

- A new major application has been added: the ***Chromosome comparison and alignment tools***, offering side-by-side comparison of genomes and chromosomes, analysis of organization and functional behavior of genomes, alignment of multiple chromosomes and chromosome-based SNP analysis. This functionality

is available in a new module **Chromosome comparison tools**, and involves the creation of a new project type *Chromosome comparisons*. Some of the main features:

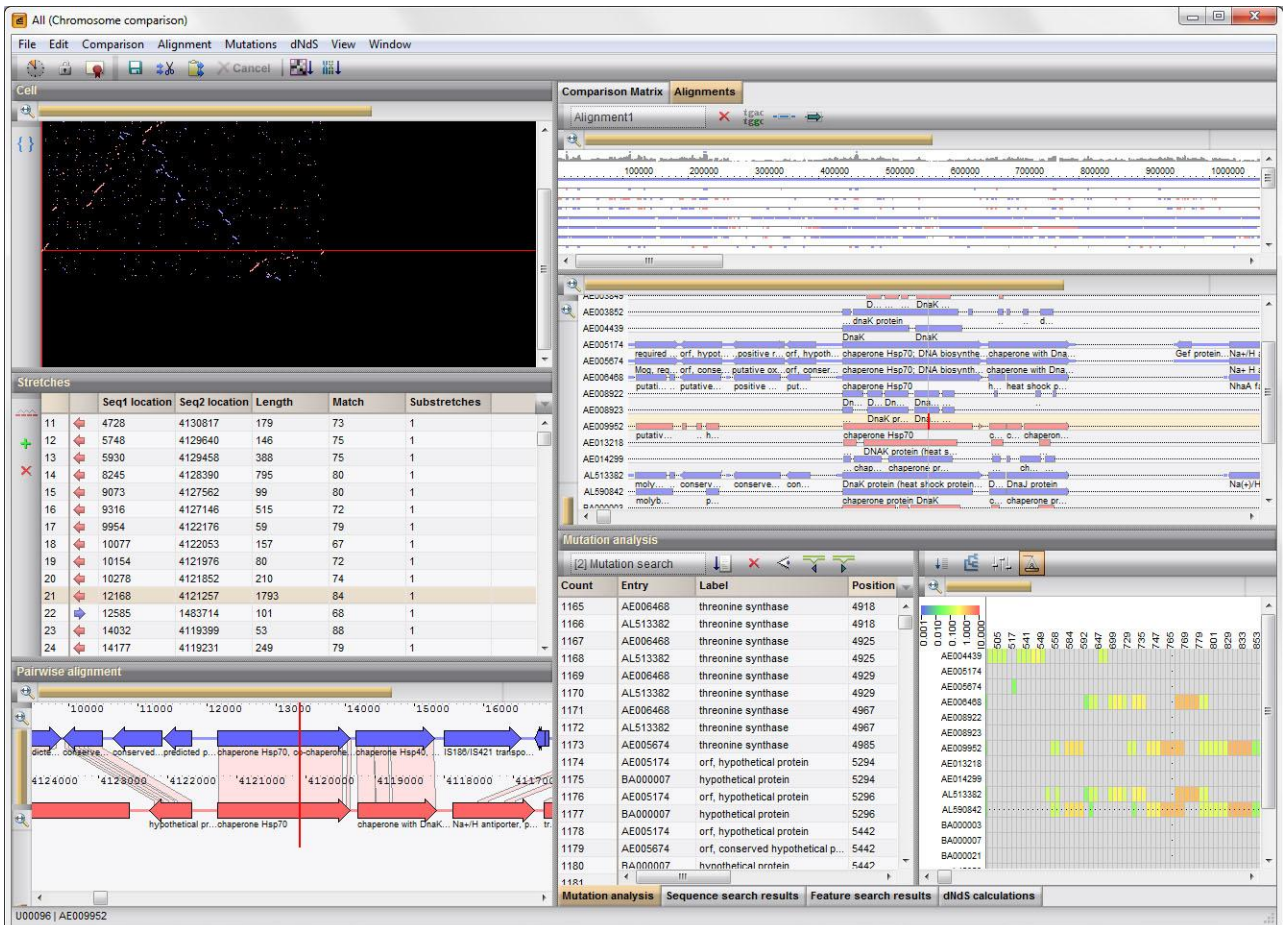
1. *nxn* pairwise chromosome comparisons

- Extremely fast pairwise matching and alignment of *nxn* sequences of up to full chromosome size.
- Seed and stretch based sequence matching, allowing for discontinuous alignments, including inversions, swaps, duplications, insertions and deletions.
- Full-sequence based or coding sequence based alignment, using user-defined nucleic acid seeds and/or amino acid seeds.
- Display of clickable *nxn* chromosome similarity matrix and associated clustering. The selected pair of chromosomes is displayed in the dot plot *Cell panel*. Direct and inverted matches are displayed in different color.
- Clickable dot plot matrix, updating (1) currently selected stretch in the *Stretches panel* and (2) associated alignment in the *Pairwise alignment panel*.
- Calculation of superstretches, i.e. larger clusters of stretches that contain no major discontinuities and that have a minimal overall homology.



2. Template-based multiple chromosome alignment

- Alignment of multiple chromosomes based upon selected template chromosome.
- Display of overview panel and detailed multiple alignment panel.
- Synchronized selection in *overview, multiple alignment, dot plot, stretches* and *pairwise alignment* panels.
- Versatile search and display functions for features and subsequences on multiple chromosomes.
- dNdS analysis based on ratio synonymous/nonsynonymous mutations within gene clusters to predict evolutionary selection pressure on genes.



3. Mutation and SNP analysis

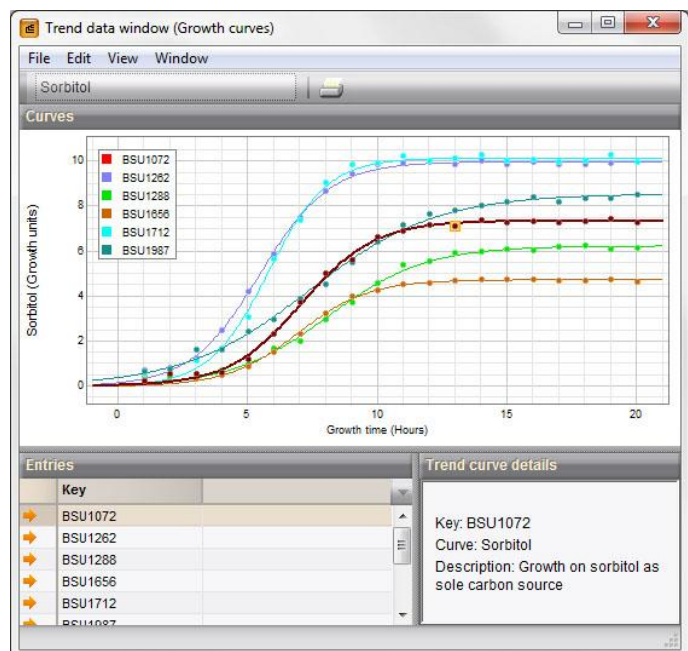
- Search and display of mutations within multiple alignments, with discrimination between intergenic, synonymous, non-synonymous and Indel mutations.
 - Additional filtering based on SNP quality scores.
 - Display colors based on mutation type or quality; sorting based on position, gene, NA change, AA change, quality.
 - Direct clustering based upon mutations or export of mutation list for further analysis.
- New features in contig assembler:
 - Base numbering is shown on consensus.

- Base numbering is shown on individual trace files ("raw traces").
- "Trimming" and "Assembly" tabs on top of window (to avoid confusion with "raw trace" and "aligned traces" tabs).
- Panels are dockable and can be rearranged by the user.
- Viewing window is automatically offsetted to avoid blind spot on the left.



TREND DATA TYPES

- A description field is available for trend curves.
- A set of new parameters (based on fitted model): *Minimum*, *Maximum* and x-positions of *Minimum* and *Maximum* over a user-defined range and inverted value.
- New option "Maximum interpolation distance" in the *Linear interpolation* and *Cubic spline* curve models. This can be used to avoid the interpolation of blocks of missing data.
- *Michaelis–Menten* kinetics model implemented.
- The X axis name and the X & Y units can be entered in trend data type properties.
- The *parameters* are labeled using correct units.
- New interface features in the *Trend data display window*:
 - Labels & units are displayed on the axes.
 - Possibility to edit points.



- Selected curve is highlighted.
- New synced list panel with entries.
- New curve details report panel.

PLUGINS

General:

The URL tools in plugins that offer synchronization with data servers (MLST, spa, MIRU) have been made compatible with most proxy servers. Furthermore, in case of remaining problems, the connection settings in BioNumerics can be tuned by a system administrator.

Import plugin

New option "*Do not create absent experiments*" causes the software not to create character experiments if a line in the import file is empty.

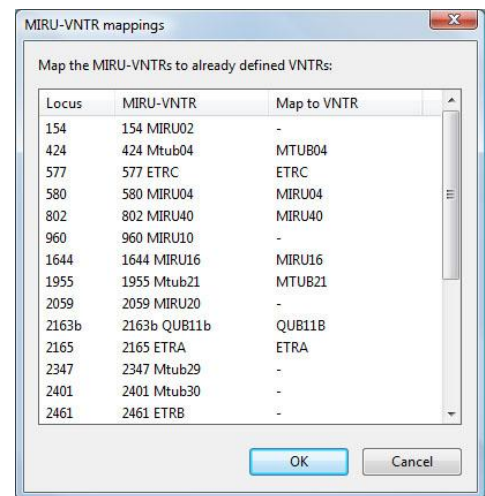
In case new characters need to be created the plugin now performs a proper check to see if the user has sufficient privileges and reports an error if not.

HDA plugin.

- A *Left* and *Right* exclude region for *Secondary* peak.
- Settings can optionally be saved in the *Analysis settings dialog box*.
- The *Pools* list is wider in the *Analyze current selection* function.
- The maximum length of the pool name is increased to 60 characters.

New plugin: MIRU-VNTR

The MIRU-VNTR plugin is based on the successful MLVA plugin and provides specific functionality for *Mycobacterium* interspersed repetitive units typing. The plugin automatically processes sequencer trace files, interprets and analyzes MIRU-VNTR data and interacts with the MIRU-VNTRplus website (<http://www.miru-vntrplus.org>) for typing and global epidemiology of mycobacteria. The synchronization with the MIRU-VNTRplus server allows types to be assigned automatically and new types to be submitted.



New plugin: SNP genotyping

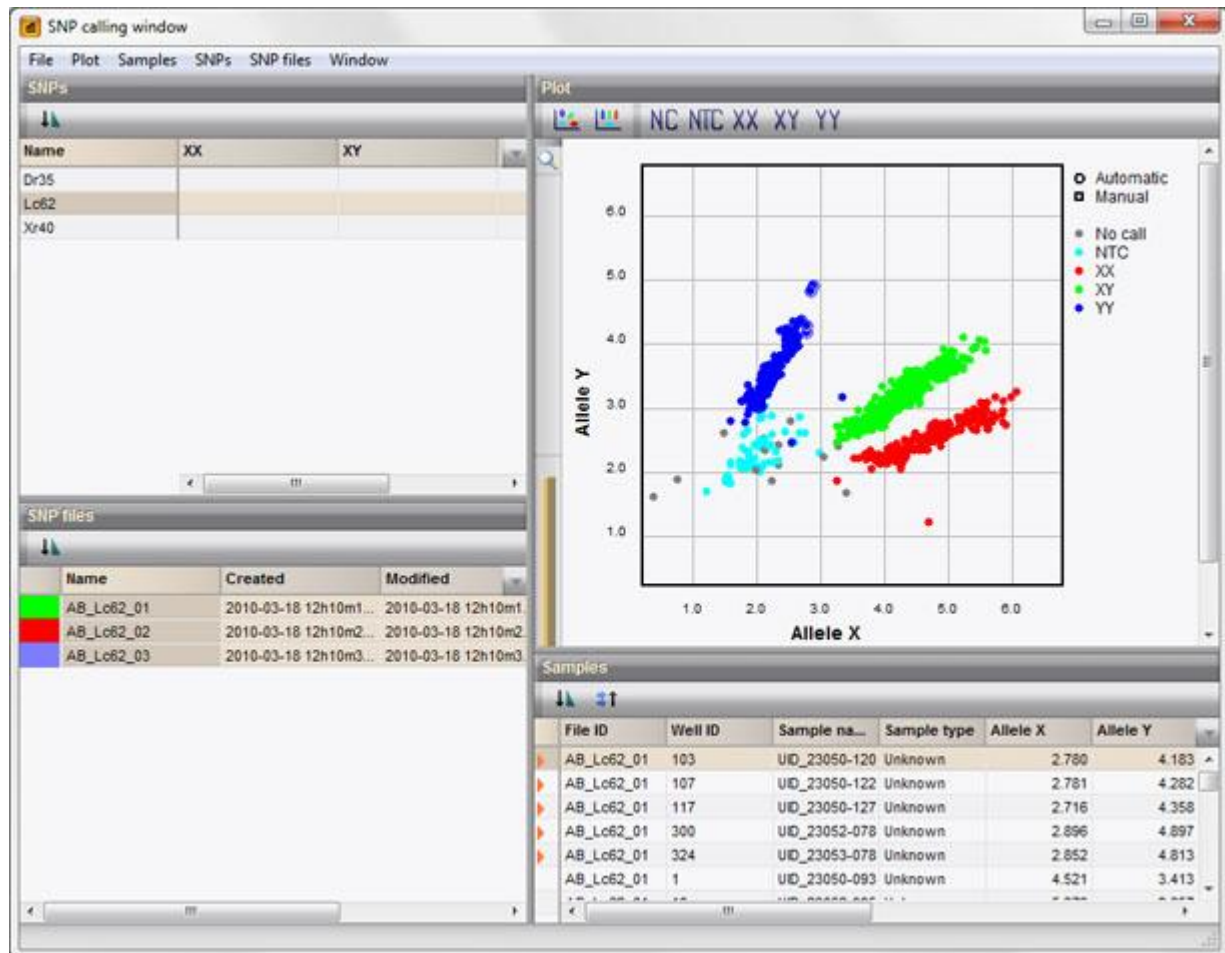
The BioNumerics SNP genotyping plugin provides a fully automated platform for reliable SNP calling and genotyping of TaqMan® based SNP genotyping technology such as Applied Biosystems 7900HT, BMG Labtech 384-well microplate readers, and the Fluidigm Dynamic Array system. The plugin is very flexible and accommodates for different workflows for SNP genotyping data analysis:

- Perform an auto-calling in other software and import the calls and their corresponding confidence values in BioNumerics.
- Perform an auto-calling in BioNumerics during import.
- Import data as "No call" and perform an auto-calling in the SNP calling window.

Through its rich and integrated environment, BioNumerics offers a number of features that no other software tool can deliver:

- Powerful databasing including user and security features and optional audit trails compliant with the highest standards.

- One platform to analyze SNP genotyping data and numerous other techniques such as HDA/CSCE, MLPA, microsatellites, sequence analysis etc.
- Unparalleled degree of automation from import of raw data to reporting of results and problem samples. In addition the software is fully scriptable in Python to achieve any degree of customization. BioNumerics is therefore extremely suitable for high throughput analysis.
- BioNumerics offers a myriad of data mining, analysis, and statistical tools, enabling clustering, statistical analysis and hypothesis testing on large data sets.



New plugin: Import and analysis of Diversilab patterns

In collaboration with bioMérieux (www.biomerieux.com) Applied Maths has developed a plugin for import and analysis of patterns generated using the Diversilab™ system. The web-based Diversilab software from bioMérieux allows XML files to be exported from sets of patterns, which can be imported in BioNumerics. BioNumerics stores the patterns as fingerprints in a database, offering the advantages of creating libraries of thousands of patterns and analyzing Diversilab patterns in combination with other techniques. The BioNumerics Diversilab plugin also provides smart on-the-fly normalization algorithms for automatically aligning the Diversilab patterns within a comparison.

New plugin: User Management

This plugin provides easy export and import of groups of users and is particularly useful in multi-user environments.