

What's new

SEQUENCE Pilot^{CE}

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developed by

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Table of Contents

1	Modules SeqNext, SeqPatient and SeqC.....	3
1.1	Operation Sequence - Variation/Mutation table and dialogue Mutation.....	3
1.2	Mutation [master file].....	3
2	Modules SeqNext and SeqHLA454.....	3
2.1	Operation Joining.....	3
2.2	Operation Run.....	3
3	Modules SeqHLA454 and SeqHLA.....	3
3.1	Nomenclature.....	3
4	Modules SeqPatient and SeqHLA.....	3
4.1	Operation Joining/Sequence.....	3
5	Module SeqNext.....	4
5.1	Definition of Multiplicom MASTR assays.....	4
5.2	Operation Enrichment [master file].....	4
5.3	Operation Joining/contet menu settings.....	4
5.4	Operation Run.....	4
5.5	Operation Sequence.....	5
5.5.1	Files, PCRGroups and Genes/Chromosomes.....	5
5.5.2	PCR Products/Locations.....	5
5.5.3	Summary.....	5
5.5.4	Fragments view/context menu.....	5
6	Module SeqHLA454.....	5
6.1	Operation Run.....	5
6.2	Operation Joining.....	5
6.3	Operation Joining/Operation Sequence.....	5
6.4	Operation Sequence.....	5
7	Module SeqPatient.....	6
7.1	Operation Mutation [master file].....	6
7.2	Operation Sequence/Base Caller.....	6
8	Module SeqHLA.....	6
8.1	Operation SeqPrimer [master file].....	6
8.2	Operations Joining and Sequence.....	6
9	Module MLPA.....	6
9.1	Peak calling/Load MLPA Resultfiles.....	6
10	Module Talkmaster.....	6
10.1	All modules.....	6
10.2	Modules SeqHLA and SeqHLA454.....	6

1 Modules **SeqNext**, **SeqPatient** and **SeqC**

1.1 Operation Sequence - Variation/Mutation table and dialogue Mutation

The date a mutation was edited is listed in the dialogue *Mutation* in the field *changed date*. Moreover in the *Variation/Mutation table* there is a tooltip available when the cursor is moved over an entry in the column *mut. Ref*. The following items are shown:

- *Mut Effect*
- *p. mut Effect*
- *p. mut Causality*
- *Ethnicity*
- *Changed date*

1.2 Mutation [master file]

Connections between mutations can be examined. Therefore the new button [*Mut. Query...*] is available in the operation *Mutation [master file]*. For further details have a look at the User Manuals.

2 Modules **SeqNext** and **SeqHLA454**

2.1 Operation Joining

The context menu entry *show settings...* was renamed into *show info...* *Show info* lists information about the file, settings, software and hardware information.

2.2 Operation Run

For file selection the last selected directory is stored and re-opened when the next file is loaded.

3 Modules **SeqHLA454** and **SeqHLA**

3.1 Nomenclature

For G nomenclature the whole exon 2 of the *DRB1* gene is regarded now. For HLA database versions < 3.4.0, *DRB1* was only regarded up to base 256.

4 Modules **SeqPatient** and **SeqHLA**

4.1 Operation Joining/Sequence

For each result file an average phred value is calculated and listed in the operation *Sequence/Positions/Resultfiles table*/column *Avg Phred Value*. In case the quality score is smaller than a value adjustable in the `lis.ini` file, the result files are not joined to the order and remain in the *Upper Table of Joining* with an according hint. A value between 0 and 100 can be entered in the `lis.ini`-file in the section `[SeqPilot]` behind the entry `MinAvgPhredValue=`. By default the value is 0, therefore resultfiles are not filtered by quality score by default.

5 Module SeqNext

5.1 Definition of Multiplicom MASTR assays

Files for **Multiplicom MASTR assays**, allowing direct analysis of sequencing data with **SeqNext**, are now prepared by **Multiplicom**.

The following **MASTR** assays are supported:

- BRCA MASTR
- CFTR MASTR
- FAP MASTR
- HNPCC MASTR

To obtain these definition files, please mail Multiplicom at customerservice@multiplicom.com.

5.2 Operation Enrichment [master file]

Manifest files from **Illumina** can be loaded and defined now. Moreover `gff`-files can be used.

In the enrichment files (`bed`, `gff` or `manifest`) isoforms for the analysed genes can be defined. Therefore enter the information at any position in the file, using the following formate (example for BRCA2, ENST00000380152):

```
#gene=BRCA2 isoform=ENST00000380152
```

There are several new items available:

- *New Settings*:
 - *cut primer*: activate to cut off primer sequence automatically.
 - *build amp. parts*: activate to build amplicon parts automatically.
- Moreover the table entries can be saved as PCR Products in the section *Save as PCR Products*. They then will be available in the operation *PCR Product [master file]*. Several options can be chosen to select the entries that should be saved:
 - *active only*: all active entries are saved
 - *selected*: all selected entries are saved.
 - *all*: all entries are saved.

Then press *[Save]* in this section. A dialogue opens where a prefix name for the PCR Products must be entered. Moreover PCR Products can be activated here.

5.3 Operation Joining/contet menu settings

In case the type (patient or control) of a resultfile is changed it is no longer recalculated automatically.

5.4 Operation Run

- Paired end sequencing data from the **MiSeq** platform can be analyzed now. Therefore select the setting *MiSeq* in the operation *Run*.
- The automatical import and start of a run now also works for Enrichments.

5.5 Operation Sequence

5.5.1 Files, PCRGroups and Genes/Chromosomes

There is the new context menu item *export > variation table...* available in the dialogue parts *Files*, *PCR Groups* and *Genes/Chromosomes*. This item allows to export the selected tab of the *Variation/Mutation table* for several genes in one step.

5.5.2 PCR Products/Locations

The warnings *low*, *dropout* and *nocall* shown in the column *Coverage* are highlighted **orange** now.

5.5.3 Summary

The summary can be exported as a `txt`-file now for tab *Gene* or *Amplicon*. Therefore right-click an entry in the summary table and select *export > table* from the context menu.

5.5.4 Fragments view/context menu

A context menu is available for selected fragments of the fragments view:

- With *save a ignored sequence* single fragments can be ignored and excluded in all following analyses. Therefore right-click the fragment in the *fragments view* and select the item *save as ignore sequence*. The ignored fragment is saved in a `txt`-file in the folder `SeqNPart/IgnoreSequence` of your `SeqPilot` installation. The filename is the PCR product name, all further ignored fragments for the same PCR Product are saved in this file as well.
- With *copy > sequence* the sequence of the selected fragment is copied to the clipboard.

6 Module **SeqHLA454**

6.1 Operation Run

- Several files for the same patient(s) can be loaded in one Run.

6.2 Operation Joining

- The context menu item *settings...* in the *Upper Table* changed: Several files can be selected or removed, moreover projects are listed (projects can not be edited).
- In the operation *Joining* information about a result file (such as file, settings, software and hardware information) can be viewed in the *Upper* and *Lower Table*. Therefore right-click the result file and select the context menu entry *show info....*

6.3 Operation Joining/Operation Sequence

Files are analysed and reanalysed in less time.

6.4 Operation Sequence

Analysed files are shown in the operation *Sequence*. Used settings can be displayed after right-click on a file and selecting *show > info*.

7 Module **SeqPatient**

7.1 Operation Mutation [master file]

A mark is shown in the electropherogram of the dialogue *Mutation Sequences* in case the mutation was edited. (Open the dialogue *Mutation Sequences* by right-clicking an order in the *Orders* table and selecting *show sequence...* from the context menu).

7.2 Operation Sequence/Base Caller

In case there are two identical bases following each other, for base calling no slope is necessary for the second peak. This helps to detect peaks in case the second peak is "linked" with the first peak.

8 Module **SeqHLA**

8.1 Operation SeqPrimer [master file]

There is the new column "*Generic and specific Primer*" available in the operation *SeqPrimer [master file]*. It can be used to distinguish between R.O.S.E. Kits and PROTRANS Kits. When this column is activated the assignment of the haplotypes is done according to the R.O.S.E Kits. For the R.O.S.E Kits that are available for download on our homepage, this column is activated already.

8.2 Operations Joining and Sequence

Files are analysed and reanalysed in less time. Moreover the alignment was improved.

9 Module **MLPA**

9.1 Peak calling/Load MLPA Resultfiles

In version 3.5 the peak calling was changed. Now the old peak calling is used again. Therefore the entry `CheckThresholdSmoothingCurve=no` is `CheckThresholdSmoothingCurve=yes` by default in the `lis.ini`-file section `[MLPA]`.

10 Module Talkmaster

10.1 All modules

- The MV export can be defined for each **SeqPilot** module.
- The export dialogue can be defined for each **SeqPilot** module.

10.2 Modules **SeqHLA** and **SeqHLA454**

Ambiguous Alleles Report is adjustable.