

Read Me

Applied Biosystems
DNA Sequencing Analysis Software v5.1.1

March 2004

Why Sequencing Analysis software v5.1.1 and how does it differ from Sequencing Analysis software v5.1?

After releasing **Sequencing Analysis software v5.1**, we discovered three defect areas that we would like to address right away. These two defects and their descriptions are:

1. **Sequencing Analysis v5.1** will lock up when displaying sample files with signal intensity that is extremely low. This defect is isolated only to this type of signal intensity. This defect is addressed in v5.1.1
2. **KB Basecaller v1.1.1**—We found two defects in KB basecaller v1.1 The first defect sometimes causes early truncation of the basecalling analysis resulting in shorter than expected read length. This occurs in sequencing samples that migrate more slowly through the capillary than indicated by the basecaller's calibration data, results that usually arises when users modify the run parameters of the electrophoresis system (e.g., temperature). The second defect leads to a high rate of deletion errors in data with uncommonly low resolution.. More information can be found in the KB Basecaller v1.1.1 FAQ section A.
3. **Files used to connect to Celera Discovery Systems (CDS)**—A file needed to be updated to ensure connection between Sequencing Analysis Software and CDS.

If you have Sequencing Analysis software v5.1 and don't use CDS or modify your run parameters, you do not need Sequencing Analysis software v5.1.1. If your lab has a mixture of Sequencing Analysis software v5.1 regular and **v5.1.1**, use the **v5.1.1** discs to replace the regular version so that your entire lab has the same version.

March 2004

These release notes contain the most current set of information about Sequencing Analysis Software v5.1.1.

The portion of the README file below is identical to the README file for Sequencing Analysis v5.1, written in September, 2003. Please review these notes **prior** to installing the application.

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A. What is Sequencing Analysis Software v5.1? (User manual Chapter 2)

This software is designed to display, edit, basecall, trim and print sequencing sample files generated from ABI PRISM® 310, 377, 3100-Avant, 3100, 3700 Genetic Analysis instruments and Applied Biosystems 3730/3730xl DNA Analyzers.

This software reads ONLY the following file types: AB1, .seq files and .fasta files.
This software generates the following file types: .AB1, .seq, .fasta, .scf and .phd.1 files.

B. New Features in Sequencing Analysis software v5.1 (Chapter 2)

- KB Basecaller v1.1 which identifies bases and assigns basecall quality values
 1. Processes data generated on ABI PRISM® 310, 3100-Avant and 3100 Genetic Analysis instruments
 2. Processes data generated on Applied Biosystems 3730/3730xl DNA Analyzers
 3. Option to assign Ns to bases with Quality values below user-definable threshold
 4. Option to process data with true or flat profiles
- New user interface that is different than that in Sequencing Analysis version 3.x
- New Analysis report displaying quality of data and length of read
- Three levels of user access
- Login security: User name and password is required
- Optional audit trail feature which tracks each time a user insert, delete or modify a base
- Compatible with Windows® 2000 OS sp3 and Windows XP® OS sp1
- Allows chromatograms to be printed up to 15 panels on one page
- Generates .phd.1 and .scf file types in addition to the .seq files in ABI and FASTA formats.
- Contains the feature to create matrix files for ABI PRISM® 310 and 377 data
- Contains a NON-GUI software that is integrated with 310 Data Collection software v3.0 for auto analysis
- Contains a web link to the Celera Discovery™ System online platform page

C. Features removed from Sequencing Analysis software v5.1

- Connectivity with Sequence Collector Software
- Removal of Factura Software that was used to identify mixed bases. The KB basecaller performs this function.

D. Compatible Operating systems and minimum systems requirements

Compatible with Windows 2000 OS sp3 and Windows XP OS sp1

Minimum system requirements: 512MB RAM, 733MHz CPU, 1GB of hard disk space

E. Compatible Data Collection Software

Sequencing Analysis version 5.1 replaces version 3.7 and v5.0. Sequencing Analysis version 5.1 is compatible with the following Data Collection software products:

- 310 Data Collection v3.0
- 3100-Avant Data Collection v2.0
- 3100 Data Collection v2.0
- 3730/3730xl Data Collection v2.0

If you do NOT have the Data Collection software version listed above, you need to obtain the listed versions before using Sequencing Analysis v5.1. If you use previous versions of Data Collection software, you will not have the same basecaller versions as those in Sequencing Analysis v5.1.

F. Installing Sequencing Analysis software (Chapter 1)

Computers with 310 Data Collection v3.0:

- Install 310 Data Collection software v3.0 PRIOR to installing Sequencing Analysis v5.1.
- 310 Data Collection software v3.0 installer will create folders in this location Programs Files: AppliedBio
- **CLOSE** all applications and then install Sequencing Analysis v5.1
- Sequencing Analysis v5.1 installer will create folders in this location Programs Files: Applied Biosystems

NOTE: Both 310 Data Collection software v3.0 and Sequencing Analysis Software v5.1 uses matrix files and mobility files. These files reside under **different** folder locations (Programs Files/AppliedBio and Programs Files/Applied Biosystems). When you create a new matrix file using Sequencing Analysis Software, it is essential that you make a copy of this new matrix file and move it to the 310 Data collection folders (Programs Files: AppliedBio).

Computers with 3100-Avant, 3100 or 3730/3730xl Data Collection software v2.0:

- Install Data Collection software v2.0 PRIOR to installing Sequencing Analysis v5.1.
- **Run the Data Collection software** and keep the software open while installing Sequencing Analysis software v5.1

Computers without Data Collection software:

If you have Sequencing Analysis v3.X, uninstall this application before installing Sequencing Analysis software v5.1

If you have Sequencing Analysis v5.0, the Sequencing Analysis software v5.1 installer will automatically remove v5.0 software.

G. IMPORTANT information about basecalling algorithms

Sequencing Analysis version 1.X, 2.X and 3.X had a single basecaller algorithm, which was referred to as “the basecaller”

In Sequencing Analysis v 5.0, we introduced a novel algorithm called KB basecaller v1.0 and referred to “the basecaller” as the ABI basecaller.

- In version 5.0, both the ABI basecaller and KB basecaller v1.0 supported analysis of data generated on Applied Biosystems 3730/3730xl DNA Analyzer only.

In Sequencing Analysis v5.1, we included both the ABI basecaller and the KB Basecaller v1.1.

- The ABI basecaller can be used to process data generated on ABI PRISM® 310, 377, 3100-Avant, 3100, 3700 Genetic Analysis instruments and Applied Biosystems 3730/3730xl DNA Analyzers.
- The KB basecaller can be used to process data generated on ABI PRISM® 310, 3100-Avant, 3100 Genetic Analysis instruments and Applied Biosystems 3730/3730xl DNA Analyzers.
- The KB basecaller is NOT designed to support analysis of data generated on ABI PRISM® 377 and 3700 Genetic Analysis instruments.

We have rigorously tested the KB Basecaller on thousands of sample files with a known consensus sequence. Several different customer sites have provided us with this large dataset.

In future releases of Sequencing Analysis, we will remove the ABI basecaller support for data generated on ABI PRISM® 310, 3100-Avant, 3100 Genetic Analysis instruments and Applied Biosystems 3730/3730xl DNA Analyzers. We will continue to improve and develop the KB basecaller. No further development will be done with the ABI basecaller. We encourage you to compare and validate the KB basecaller to the ABI basecaller using your own dataset.

Please refer to the KB basecaller documentation for more details on this algorithm.

H. Known Issues

310 Automation

- 310 auto analysis: plate set up does not support mixed Basecaller type.

Printing

- Printed Analysis Report can get truncated.
 - Work around: Set margin to 0.5 in page set up.
- Changes made to the default font and size for analysis report will affect only column header on print out
- Changing page range from print dialog box is not supported.

Sample Manager

- Last base in electropherogram view does not get highlighted.
- The "Add Samples" dialog box may take some time to open if there are mapped network drives on your computer system.
- The default view for unanalyzed sample file is the electropherogram view.
- Opening multiple sample files (greater than 10) via a right mouse click may cause application to exit.
 - Workaround: Use the "Add Samples" dialog box from within the application imports multiple samples.
- The analysis process may be slow, when more than 96 samples are displayed (Show check box selected for all samples) in the Sample Manager, sample view pane.

- The "Add Samples" dialog box may not always be updated when files are added to an opened folder.
 - Workaround: Closing the "Add Samples" dialog box and reopening it should refresh the view for all sample files.
- New matrix created during the session will not be recognized by the application and shown as bold and italic
 - Workaround: Re-launch Sequencing Analysis Application

KB Basecaller v1.1

- **In-del mutations**

Detection of in-del mutations is not supported by KB v1.1. For such samples containing in-del mutations, the results generated by KB v1.1 will appear normal on the 5' side of the mutation and will appear like poor quality data on the 3' side.
- **Selection of mobility files for ABI PRISM 310 Genetic Analyzer**

When analyzing data generated on the ABI PRISM 310 Genetic Analyzer, be sure to select the mobility file corresponding to the correct run module and capillary length. This step is unique to analyzing data from ABI PRISM 310 instruments because, unlike previous basecallers, the KB Basecaller relies on some data, such as the capillary length, that may not be correctly stored in the sample files.

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