

# User Bulletin

## Applied Biosystems 3730/3730x/ DNA Analyzer

February 1, 2006

### SUBJECT: TargetSeq™ Resequencing System

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**Overview** The TargetSeq™ Resequencing System now available from Applied Biosystems is a 3730-series software system designed for high-throughput resequencing or medical research sequencing of small exons and other small targeted regions. The TargetSeq System enables a 3730x/ Instrument with a 36-cm array to produce 400 QV20<sup>‡</sup> bases at a run-to-run time of 20 min or less, expanding the daily production capacity of the 3730x/ System to greater than 2800 QV20 Kbases or 6912 samples (72 runs). The TargetSeq System uses existing hardware, software, and reagent components available from Applied Biosystems, including 36-cm arrays (50-cm arrays not supported), Data Collection Software v3.0, POP-7 polymer, and BigDye® Terminator v3.1 chemistry (version 1.1 chemistry not supported).

The TargetSeq Resequencing System, includes the following software components:

- **TargetSeq Run Module** – A fast run module optimized for the TargetSeq System. The run module can be customized for read lengths of 200 to 400 QV20 bases to shorten the run-to-run time and maximize the daily run capacity of the 3730/3730x/ System. See “[Optimizing the Run\\_Time Setting](#)” on [page 4](#) for more information.
- **KB™ Basecaller v1.3** – A new version of the KB Basecaller, featuring improved:
  - Flexibility and decreased susceptibility to basecalling errors when modifying module electrophoresis voltage parameters
  - Heterozygote/mixed-base detection

**Note:** See the *KB™ Basecaller v1.3 FAQ* for a complete list of features. The FAQ is installed with the KB Basecaller v1.3 files.

- **New Pre-batch File** – A pre-batch file designed for the TargetSeq System. The new file differs from the existing pre-batch file in that it causes the 3730/3730x/ System to heat the instrument oven throughout the pre-run process.

**Note:** The pre-batch file can be downgraded for non-TargetSeq System runs. See “[Downgrading the Pre-Batch File](#)” on [page 5](#) for more information.

‡. Quality Value 20 Bases

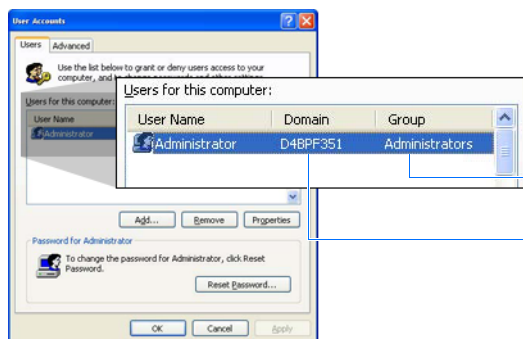
## Installation Requirements

Before installing the TargetSeq Resequencing System, ensure that the:

- Computer contains any *combination* of the following software:
  - Data Collection Software v3.0 for the 3730/3730xl System
  - Sequencing Analysis Software v5.2
  - SeqScape® Software v2.5
- Computer meets the hardware requirements for Data Collection Software v3.0
- User account used to install the software:
  - Belongs to the local computer (not to a network domain)
  - Has administrator privileges (complete and unrestricted access)

To verify that a user account meets the installation requirements:

1. In the desktop, select **Start ▶ Control Panel** (or **Start ▶ Settings ▶ Control Panel**).
2. In the Control Panel window, double-click **User Accounts**.
3. In the Users tab of the User Accounts dialog box, confirm that your user account belongs to the Administrators group of the local computer (domain).



4. Click **OK**.

## Installing or Uninstalling the TargetSeq System

**IMPORTANT!** The Data Collection Software (if installed) must be started and idle before you install the TargetSeq System.


1. Power on and log onto the computer containing the Data Collection Software v3.0.
2. Close all open active software applications.

**IMPORTANT!** All applications, other than the Data Collection Software, must be closed before you install the TargetSeq System.

3. Start the Data Collection Software Services on the local computer:
  - a. Select **Start ▶ All Programs ▶ Applied Biosystems ▶ Data Collection ▶ Run 3730/3730xl Data Collection v3.0**.
  - b. After the Service Console opens, wait until all four symbols change to green squares. If the services do not start automatically, click **Start All**.
  - c. If necessary, type your Login Name and Password in the Login dialog box, then click **OK**.

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4. Insert the TargetSeq Resequencing System Installation CD into the CD drive, then wait for the installer software to start.

If the installer does not start automatically:

- a. Right-click **My Computer**, then select **Explore**.
  - b. In the navigation pane of the window, select the CD drive.
  - c. At the right-side of the window, double-click  **setup.exe**.
5. In the Welcome screen of the TargetSeq System installer, select the appropriate option (**Install TargetSeq** or **Uninstall TargetSeq**), then click **Next**. If you are uninstalling the TargetSeq System, click **Yes** when prompted to confirm the uninstallation.  
  
After the installer completes the installation or removes the TargetSeq System, the software displays the InstallShield Wizard Complete screen.
  6. If you are installing the TargetSeq System, read the release notes displayed in the Readme screen, then click **Next**.
  7. In the InstallShield Wizard Complete screen, click **Finish**.
  8. Remove the TargetSeq Resequencing System Installation CD from the drive.
  9. If the computer contains the Sequencing Analysis Software, configure the software for use with the TargetSeq System, as explained in [“Reconfiguring the Sequencing Analysis Software” on page 6](#).
  10. If performing runs of less than or greater than 435 QV20 bases, optimize the Run\_Time setting of the TargetSeq Run Module for your 3730/3730xl System(s). See [“Optimizing the Run\\_Time Setting” on page 4](#) for more information.

**IMPORTANT!** When you uninstall the TargetSeq System, the software removes the pre-batch file and KB Basecaller v1.3, then restores the original version of each component. The software does not delete the TargetSeq Run Module.

## Guidelines for Use

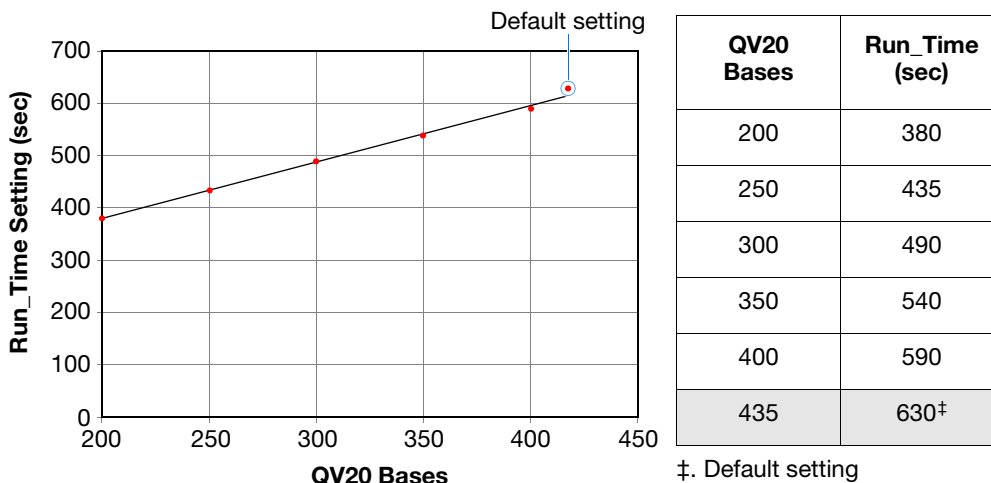
- Do not use the TargetSeq System for runs significantly greater than 500 QV20 bases. For longer reads, use the RapidSeq Run Module.
- Consider modifying the Run\_Time setting of the TargetSeq Run Module to optimize the run-to-run time for your 3730/3730xl System(s). See [“Optimizing the Run\\_Time Setting” on page 4](#) for more information.

**Note:** The shortened run-to-run times of the TargetSeq Run Module decrease the unattended operation of the 3730/3730xl System to less than 21 h, permitting additional runs to be performed during a 24-hour period.

- If you plan to use a run module other than the TargetSeq Run Module, you can downgrade the pre-batch file to an earlier version to optimize the 3730/3730xl System run time for batch runs. See [“Downgrading the Pre-Batch File” on page 5](#) for more information.

## Optimizing the Run\_Time Setting

You can optimize runs performed using the TargetSeq Resequencing System by adjusting the Run\_Time setting of the run module based on the number of QV20 bases that you want to collect during the run (length of read). **Figure 1** shows the correlation between the Run\_Time setting of the TargetSeq Run Module and the corresponding read length achieved by an Applied Biosystems 3730xl DNA Analyzer. For example, under normal operating conditions and using the recommended hardware and chemistry, the 3730xl System requires at least 490 sec to collect data for a minimum of 300 QV20 bases (**Figure 1**).



**Figure 1** Correlation of Run\_Time setting and read length

**IMPORTANT!** **Figure 1** shows the correlation between Run\_Time setting and QV20 bases when the 3730xl System operates at 22 °C. Operating conditions may alter the trend.


**Note:** If you calculate a Run\_Time setting using the equation for the best fit line ( $y = 1.0535x + 171.08$ , where  $x$  is the desired number of QV20 Bases and  $y$  is the Run\_Time setting), note that the correlation between Run\_Time setting and read length becomes nonlinear for read lengths greater than 435 QV20 bases.

After you determine the Run\_Time setting for your system, you can estimate the total run time as follows:

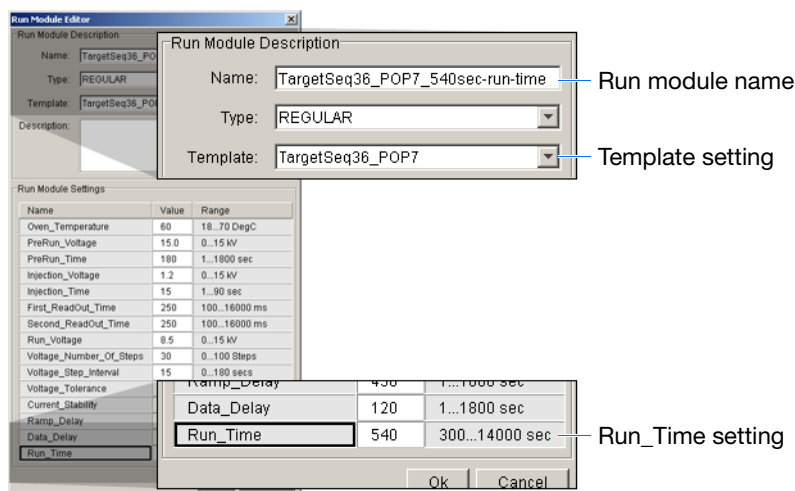
$$\text{total run time} = (\text{Run\_Time setting}) + 9 \text{ min}$$

where 9 min is the time required by the Data Collection Software to prepare the 3730xl System for the next run.

### To optimize the Run\_Time setting for the TargetSeq Resequencing System:

1. Determine the desired read length.
2. Use **Figure 1** to determine the optimal Run\_Time setting for your system.
3. In the Data Collection Software v3.0, click  (Module Manager).
4. Click **New**.
5. In the Name field of the Run Module Editor dialog box, type a name for the new run module (for example, **Custom-TargetSeq36\_POP7**).

6. Select **Type ▶ Regular**.
7. Select **Template ▶ TargetSeq36\_POP7** to use the TargetSeq Run Module as the basis for the new run module.
8. In the Run\_Time field of the Run Module Settings table, type the optimal setting (in seconds) that you determined in [step 2 on page 4](#).



9. Click **OK** to save the custom run module.

To run samples using the optimized TargetSeq Run Module, apply the custom run module to plate records as explained in the *Applied Biosystems 3730/3730xl DNA Analyzer User Guide*.

## Downgrading the Pre-Batch File

If you use another run module to perform runs on a 3730/3730xl System containing the TargetSeq Resequencing System, you may want to downgrade the pre-batch file. Unlike the original pre-batch file, the file installed with the TargetSeq System directs the 3730/3730xl System to heat the instrument oven throughout the pre-run process. The additional time required to heat the oven can delay the first run in a batch and slightly decrease the daily throughput potential of the 3730/3730xl System for non-TargetSeq System runs. To avoid the decrease in throughput, downgrade the file as explained below.

### To downgrade the pre-batch file:

1. Using Windows Explorer, navigate to:  
E:\AppliedBiosystems\UDC\DataCollection\SupportFiles\ga3730\ServiceModules
2. Rename PreBatchModule.txt (the new pre-batch file) to **PreBatchModule.old**.
3. Rename OldPreBatchModule.txt (the original file) to **PreBatchModule.txt**. The system is now configured to use the previous version of the pre-batch file.


**IMPORTANT!** When you resume use of the TargetSeq Run Module, reverse the procedure to restore the pre-batch file to the version installed with the TargetSeq System.

## Reconfiguring the Sequencing Analysis Software

When you install the TargetSeq Resequencing System to a computer that has the Sequencing Analysis Software, you can adjust the Length of Read (LOR) Indicator thresholds in the Sequencing Analysis Software to match the read length supported by the TargetSeq System. The LOR Indicator thresholds define the upper and lower limits that the Sequencing Analysis Software uses to perform the LOR test in the Analysis QC Report. Because the read lengths supported by the TargetSeq Run Module (200 to 400 bases) are approximately half those of default runs (500 to 800 bases), the software may display a red or yellow LOR warning in the report if you do not change the setting.

**Note:** For more information on the Length of Read (LOR) Indicator thresholds, see the *Applied Biosystems DNA Sequencing Analysis Software Version 5.2 for Windows® XP and 2000 Platforms User Guide*.

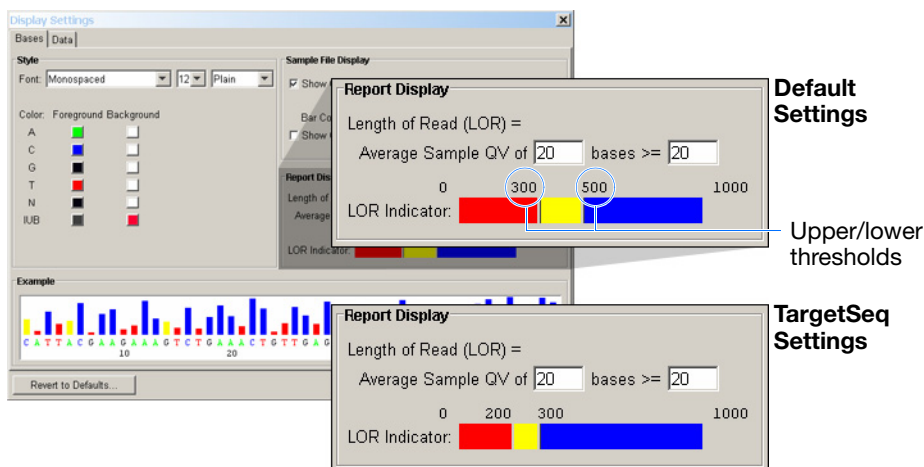
### To adjust the Length of Read (LOR) Indicator color thresholds:

1. Power on and log onto the computer containing the Sequencing Analysis Software.
2. Start and log into the Sequencing Analysis Software (select **Start** ▶ **All Programs** ▶ **Applied Biosystems** ▶ **Sequencing Analysis 5.2** ▶ **Sequencing Analysis 5.2**).
3. In the Sequencing Analysis v5.2 window, click  to open the display settings.
4. In the Display Settings dialog box, drag the upper and lower color thresholds for the LOR Indicator to the settings appropriate for your system.

When using the default Run\_Time setting of the TargetSeq Run Module, Applied Biosystems recommends that you adjust the color thresholds as follows:



If you have modified the Run\_Time setting as described in “[Optimizing the Run\\_Time Setting](#)” on page 4, adjust the LOR Indicator color thresholds according to the modified read length.

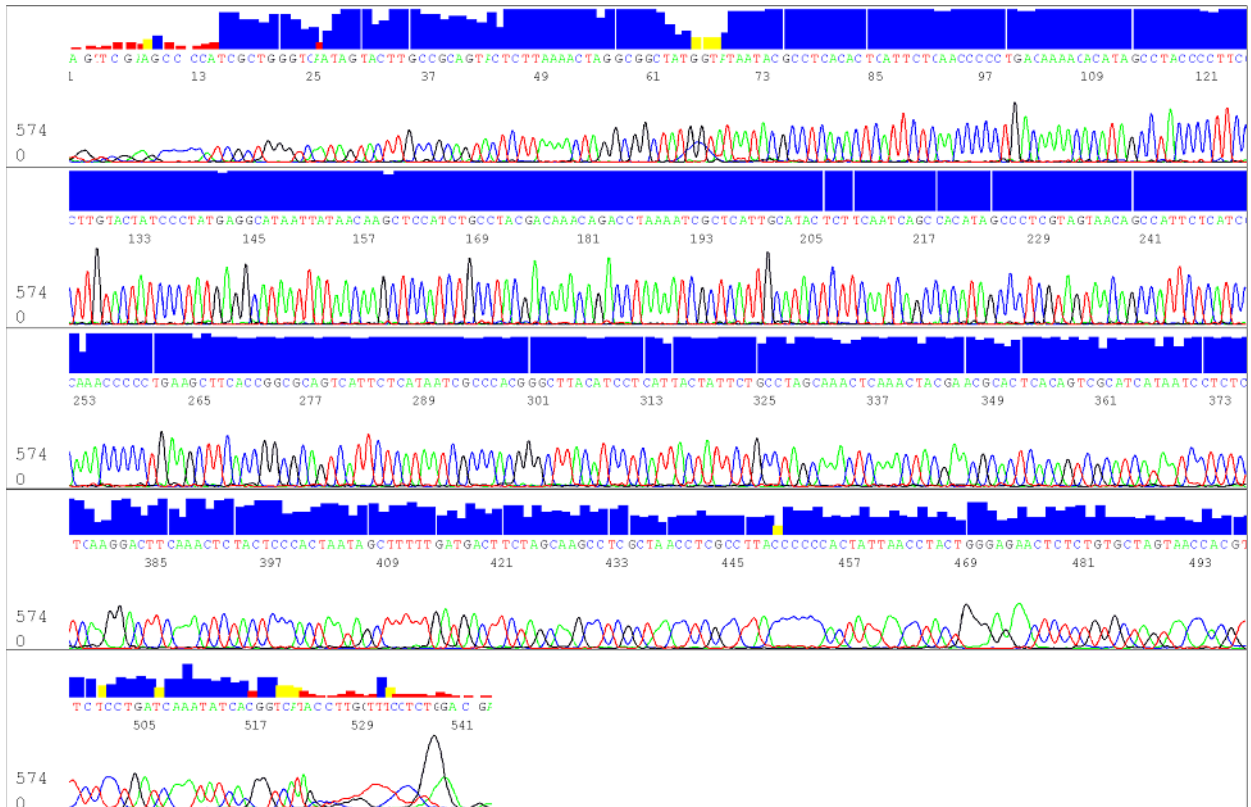


5. Click **OK** to save the Display Settings.

**IMPORTANT!** If you remove the TargetSeq Resequencing System, or analyze samples that have been run using a different run module, you must restore the LOR Indicator color thresholds to the default settings.

**Example Data** Figure 2 displays an electropherogram generated from an example experiment using the TargetSeq Resequencing System. For the example experiment, human genomic DNA was amplified and sequenced using VariantSEQR™ Resequencing System mitochondrial primer (RSA001145192) according to the protocol found in the VariantSEQR™ Resequencing System Quick Reference Card (PN 4350217 Rev D). The BigDye® Terminator v3.1 sequencing reaction was loaded on a 3730xl System using POP-7™, then run using the default settings of the TargetSeq Run Module.

As shown below, 504 bases or 93% of the data are high-quality (QV20) bases. The high-quality data begins at base 15 in the analyzed trace (30 bases from the M13-21 forward sequencing primer) and extends to base 519.



**Figure 2** Electropherogram of human genomic DNA (prepared using the VariantSEQR™ Resequencing System and BigDye® Terminator v3.1 chemistries) generated by an Applied Biosystems 3730xl DNA Analyzer using the TargetSeq Resequencing System

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