

# Increased Productivity For Core Labs Using One Polymer and One Array Length for Multiple Applications

## - Applied Biosystems 3730/3730x/ DNA Analyzers Version 3.0



**Take Ogawa, Steve Berosik, Larry Joe, Allen Swei, Jim Bourey, Jim Burrows, Suresh Pisharody, Lauren Seilnacht, Sreeram Santhanam and Ruben Pingue**  
**Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404**

### ABSTRACT

The flexibility to perform both DNA sequencing and fragment analysis applications without switching polymer and capillary array length is essential for many of today's core laboratories. The ability to analyze both sequencing and fragment analysis samples quickly and accurately provides two important benefits: less hands-on time, and further reduction of sample costs.

The Applied Biosystems 3730 and 3730x/ DNA Analyzers Version 3.0 incorporates new features for improved application flexibility as well as a new analysis software suite that offers expanded application support and superior quality scoring. Utilizing the 50cm capillary array and POP-7™ polymer, the system now offers automatic switching between fragment analysis applications and a choice of sequencing modules with varying run times and lengths of read.

In addition, even shorter run times for sequencing, microsatellite analysis and SNPlex™ system high-throughput genotyping can be performed with the same polymer on the 3730 and 3730x/ systems by simply converting to the 36cm capillary array. This capability offers the flexibility of multiple applications combined with reduced polymer consumption.

The Any4Dye-*HDR* dye set has also been added to benefit core labs running sample plates with high variability. This new dye set offers the advantages of reduced off-scale data and the flexibility to be used with any sequencing or fragment analysis application.

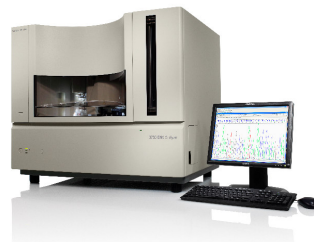
The Applied Biosystems 3730 and 3730x/ DNA Analyzers Version 3.0 can simultaneously deliver high quality DNA sequencing and fragment analysis results on either the 36cm or 50cm array without the need to switch polymer or array length. The choice to perform multiple applications on one polymer and one capillary array length will offer important cost savings and increased versatility for genetic analysis projects in core laboratories.

### INTRODUCTION

The new version 3.0 system is available as a software upgrade from the version 2.0 systems, further expanding the flexibility of the 3730/3730x/ DNA Analyzers. One of the major new features is the addition of a 50-cm array fragment analysis run module and the accompanying 50-cm spectral calibration module. Combined with the existing 50-cm sequencing run modules and 36-cm modules, the v3.0 systems allows complete coverage of applications for both sequencing and fragment analysis applications on the same capillary array.

The benefits of the 50-cm fragment analysis module are the following:

- Throughput: 43 minute run time yields 33 runs/day
- Optimized sizing precision up to 500 bp\*
- Optimized signal strength\*
- Optimized compatibility with GeneMapper® software
- Minimal spectral pull-up\*



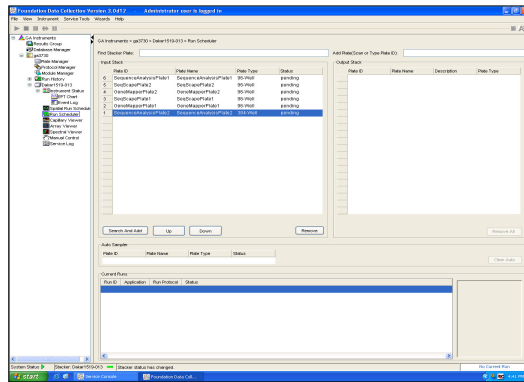
\*As determined by analyzing GeneScan™ Installation Standard DS-33 samples

### Data Collection Software v3.0 New Features for the 3730/3730x/ DNA Analyzers (expected release: March 2005):

- 50-cm fragment analysis module
- Any4Dye-*HDR*
- SNPlex™ system run modules and DyeSet installed by default
- Windows® 2000 and Windows® XP compatible
- Early database full warning message
- New versions of analysis software (GeneMapper®, Sequencing Analysis and SeqScape®)
- E-signature features to support 21CFR Part11 requirements

### METHODS

**Figure 1. Data Collection Software v3.0 Loading Sample Plates for Multiple Applications Within The Same Batch Of Runs**



**Figure 2. Supported Applications On The 3730/3730x/ DNA Analyzers Using POP-7™ polymer and the 50-cm Capillary Array**

Applications	Array Lengths	Polymer Type	ABI Basecaller Phred Q20 Read Length (bases)	KB™ Basecaller QV 20 Read Length (bases)	Run Time	Throughput
Extra Long Sequencing	50-cm	POP-7™	Not Applicable	900	3 hr	8 runs/day
Long Sequencing	50-cm	POP-7™	800	850	2 hr	12 runs/day
Fast Sequencing	50-cm	POP-7™	Not Applicable	700	1 hr	24 runs/day
Fragment Analysis	50-cm	POP-7™	more than 500 bp resolution with 0.15 bp sizing precision	43-min	33 runs/day	
SNPlex™ Genotyping System	50-cm	POP-7™	48-plex format with >99.5% Accuracy, >95% Call Rate, and >99.7% Precision	25-min	57 runs/day	

Above: Length of read based on BigDye® Terminator v3.1 Sequencing Standards. Use of the ABI Basecaller on Fast and Extra Long Sequencing data may result in reduced read lengths. Only the KB™ Basecaller has been optimized for use with these new modules.

**Figure 3. Supported Applications On The 3730/3730x/ DNA Analyzers Using POP-7™ polymer and the 36-cm Capillary Array**

Applications	Array Lengths	Polymer Type	ABI Basecaller Phred Q20 Read Length (bases)	KB™ Basecaller QV 20 Read Length (bases)	Run Time	Throughput
Standard Sequencing	36-cm	POP-7™	650	700	1 hr	24 runs/day
Rapid Sequencing	36-cm	POP-7™	500	550	35-min	40 runs/day
Fragment Analysis	36-cm	POP-7™	up to 500 bp resolution with 0.15 bp sizing precision	33-min	44 runs/day	
SNPlex™ Genotyping System	36-cm	POP-7™	48-plex format with >99.5% Accuracy, >95% Call Rate, and >99.7% Precision	15-min	96 runs/day	

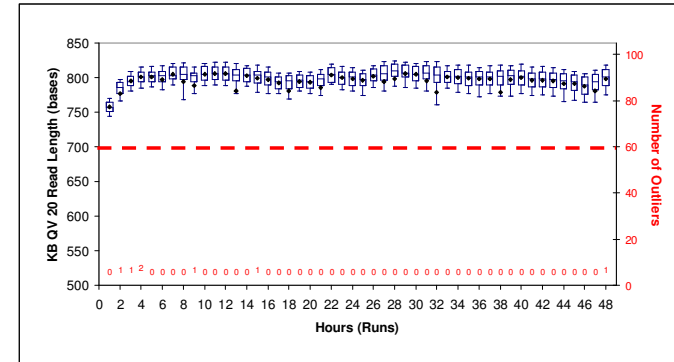
**Figure 4. System-Capable\* Applications On The 3730/3730x/ DNA Analyzers**

AFPL®	LOH (Loss of Heterozygosity)	BAC Fingerprinting	Methylation	RFLP	SAGE™
Resequencing	Large Fragment Sizing	HLA Typing	Conformation Analysis	Comparative Sequencing	SNP Genotyping

\*Certain customers or third parties have demonstrated that the 3730-series analyzers are capable of running these applications without any apparent negative effects on the system. While Applied Biosystems have not performed optimization or testing to fully support these applications, supporting third-party documentation may be available. Please contact your local Applied Biosystems representative to further discuss applications of interest to you.

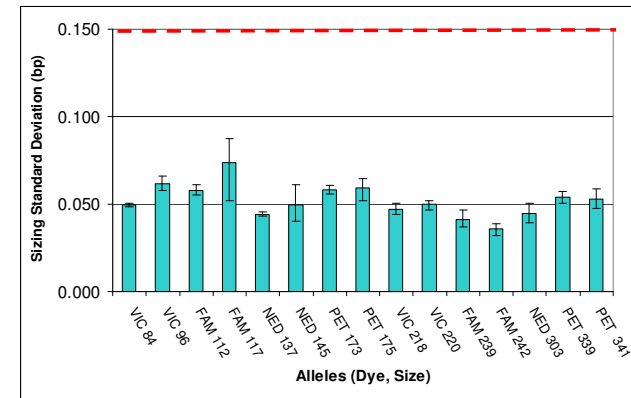
### EXPERIMENTAL RESULTS

**Figure 5. 48 Hour Continuous Operation - Fast Sequencing On The 3730 DNA Analyzer With The 50-cm Capillary Array**



For the experiment presented above, BigDye® Terminator v3.1 Sequencing Standard samples were resuspended in 2-ml Hi-Di™ Formamide per (1) tube of standard. The samples were then aliquoted in 5 micro-liter volumes into 384-well, septa covered, sample plates. POP-7™ polymer was used to fill the capillary arrays and 1x GA with EDTA buffer is placed in the buffer reservoir (60-ml) and buffer jar (67-ml) prior to performing any batch of runs. Both waste and water reservoirs were filled with 60-ml of DI water prior to beginning a 48-hour continuous batch run using the Fast Sequencing module. All runs were performed on a 50-cm/48-capillary array 3730 instrument. Sample files were auto-analyzed using Applied Biosystems' KB Basecaller algorithm via Applied Biosystems' DNA Sequencing Analysis v5.1.1 software. Read lengths are calculated based on base calls with quality values greater than KB QV 20, using the difference between the high end and low end cut-off values for these base calls. The "box-and-whisker" plot summarizes the KB QV 20 length of read for each individual 48-capillary run. The top and bottom of the whiskers represent 95th and 5th percentile of the data respectively for that particular run. The top and bottom of the box represent the 75th and 25th percentile of the data. The line in the box is the median and the dot represents the average. Samples with read lengths of less than 200 bases were omitted from the box plot and noted as outliers. All references to "KB QV 20" in the data plot above and in this caption refer to quality values generated through the use of the Applied Biosystems' KB Basecaller.

**Figure 6. DNA Fragment Sizing Precision Data for The 50-cm Fragment Analysis Module**

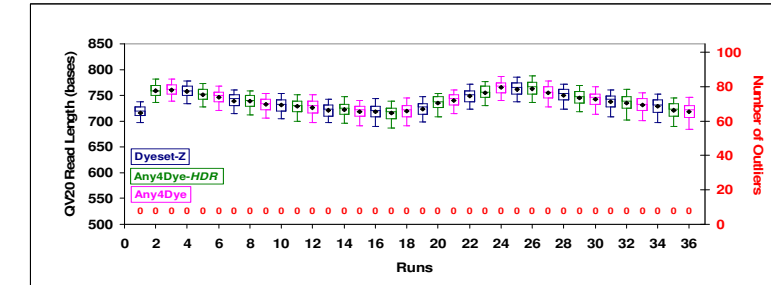


The chart above is the representation of DNA fragment sizing precision using the 50-cm fragment analysis module on the 3730 DNA Analyzer. The data is from 3 runs of the GeneScan™ Installation Standard DS-33 (6FAM™, VIC®, NED™, PET® and LIZ® dyes) using the GeneScan™ 500 LIZ size standard. For each allele, the standard deviation of the size call was calculated within a run and then averaged across the 3 runs. The whiskers represent the maximum and minimum standard deviations for each allele. The specification for sizing precision in this size range is 0.15 bp within a run and this data clearly demonstrated that the 50-cm fragment analysis module has far superior sizing precision.

**Figure 7. New Dye Set Functionality**

Dye Set	Features
Any4Dye- <i>HDR</i>	<p>When to use/Advantages:</p> <ul style="list-style-type: none"> <li>• Sample strength variability – plates containing variable quality of samples and template amounts</li> <li>• High dynamic range – less susceptibility to off-scale data</li> <li>• Resequencing/Mutational Profiling applications</li> <li>• 4-Dye Fragment Analysis applications</li> </ul> <p>Issues:</p> <ul style="list-style-type: none"> <li>• Signal may be reduced compared to data generated using standard dye sets</li> <li>• Essential that spectral calibrations are performed each time the capillary array is replaced or moved within the detection cell</li> </ul>
Any4Dye	<p>When to use/Advantages:</p> <ul style="list-style-type: none"> <li>• Use of unsupported dyes – Provides an open system for system capable applications</li> <li>• Performance of system not tested nor can performance be guaranteed</li> </ul> <p>Issues:</p> <ul style="list-style-type: none"> <li>• De Novo Sequencing – Higher signal relative to the Any4Dye-<i>HDR</i> dye set</li> <li>• Optimized for the highest signal-to-noise ratio</li> </ul>
Standard Z Dye Set	<p>When to use/Advantages:</p> <ul style="list-style-type: none"> <li>• More susceptible to off-scale data relative to the Any4Dye-<i>HDR</i> dye set if sample prep/injections are not fully optimized</li> </ul>

**Figure 8. New Dye Set Performance: Sequencing**



The data above was generated using a 3730x/ DNA Analyzer that was setup with a 50-cm/96-capillary capillary array and POP-7 polymer and then spectrally calibrated with the BigDye® Terminator v3.1 Sequencing Standard. Three spectral calibrations were performed: using the standard Z dye-set option, the Any4dye option, and the Any4dye-*HDR* option. All runs were performed on the 3730x/ instrument using the FastSeq50\_POP7 run module. Sample files were auto-analyzed using Applied Biosystems' KB v1.2 Basecaller algorithm via Applied Biosystems' DNA Sequencing Analysis v5.2 software. Read lengths are calculated based on base calls with quality values greater than KB QV 20, using the difference between the high end and low end cut-off values for these base calls. The "box-and-whisker" plot summarizes the KB QV 20 length of read for each individual run. The results demonstrate equivalent basecalling performance using each of the dye sets within the same batch of runs.

### CONCLUSIONS

The ability to perform both sequencing and fragment analysis applications on the same polymer without switching the capillary array provides the user significant time and cost savings. The new v3.0 system has an added benefit of providing the use of the 50-cm capillary array for both applications, further expanding the flexibility of the Applied Biosystems 3730/3730x/ DNA Analyzers. With the 50-cm capillary array, a user can sequence more than 700 bases in just an hour and then genotype fragments up to 500 bases in under 43 minutes, and vice versa, within the same batch of runs. Other benefits of the v3.0 system includes the Any4Dye-*HDR* feature and SNPlex™ system support.

### ACKNOWLEDGEMENTS

The author wishes to acknowledge the following people for their continued contributions to the development of the Applied Biosystems 3730/3730x/ DNA Analyzers: Eric Nordman, Johan Goudberg, Sue Bay, Ben Johnson, Davis Hershey and Hitachi High Technologies.

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

The Applied Biosystems 3730x/ and 3730 DNA Analyzers include patented technology licensed from Hitachi, Ltd. as part of a strategic partnership between Applied Biosystems and Hitachi, Ltd., as well as patented technology of Applied Biosystems.

Applied Biosystems, LIZ, VIC, PET, GeneMapper, BigDye and SeqScape are registered trademarks and Applied, AB (Design), KB, SNPlex, GeneScan, FAM, Hi-Di, NED, ROX and POP-7 are trademarks of Applied Biosystems or its subsidiaries in the US and/or certain other countries. ©2005 Applied Biosystems. All rights reserved.

AFPL is a registered trademark of Keygene N.V.

SAGE is a registered trademark of Genzyme Corporation.

Windows is a registered trademark of Microsoft Corporation.