

Using a High Performance Capillary Electrophoresis System for Both Fragment Analysis and Sequencing Applications



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ABSTRACT

Genetic analysis researchers often must run a wide variety of applications. Switching between applications can be costly and time-consuming. Therefore, a flexible instrument that easily lends itself to various applications is essential. The instrument must be able to analyze both sequencing and fragment analysis samples quickly and accurately with a single array and polymer, thus minimizing hands-on time. Researchers who choose the 3130 Series Genetic Analyzers can perform multiple applications using just one array and one polymer to generate high-quality sequencing and fragment analysis data. Additionally, they can choose from multiple configuration options that meet their workflow and application needs.

KEY FEATURES OF THE 3130 SERIES SYSTEMS

Automated Polymer Delivery System	<ul style="list-style-type: none"> Ease to use Easy to maintain
New 3130 POP-7™ Polymer	<ul style="list-style-type: none"> One polymer & one array for all applications
Detection Cell Heater and 3130 POP-7 Polymer	<ul style="list-style-type: none"> Faster turn around times Longer Reads Better peak resolution



MAXIMIZES PRODUCTIVITY, REDUCES COSTS

Now, more than ever, researchers have the flexibility to choose one configuration for all their sequencing and fragment analysis needs. The flexible 3130 Series Systems feature 3130 POP-7™ Polymer with the 36-cm, 50-cm, and 80-cm capillary arrays. Another novel feature, the detection cell heater, enables higher run temperatures with all 3130 POP-7 Polymer configurations. Higher run temperatures, in turn, result in highly resolved peaks, less run-to-run variability, and faster electrophoresis times than any other system in today's market. Because there are no array changes, and thus less loss of polymer, the flexibility and performance of the system is increased, reducing the cost per sample. With the easy-to-use, automated polymer delivery system (Figure 1), the 3130 series allows automatic polymer loading, which minimizes hands-on time and maintenance while maximizing performance.

Scientists can choose any configuration to achieve optimal conditions for their research. With the introduction of 3130 POP-7 polymer for all three array lengths, the 36-cm and 50-cm array can be used for standard fragment analysis and sequencing runs, while the 80-cm array for long-read sequencing (Tables 1 and 2). When researchers must switch between polymer type and array lengths, time and reagents are wasted. By offering single configurations for multiple applications, the 3130 Series analyzers conserve reagents, minimize hands-on instrument time, and maximize performance.

AUTOMATED POLYMER DELIVER SYSTEM

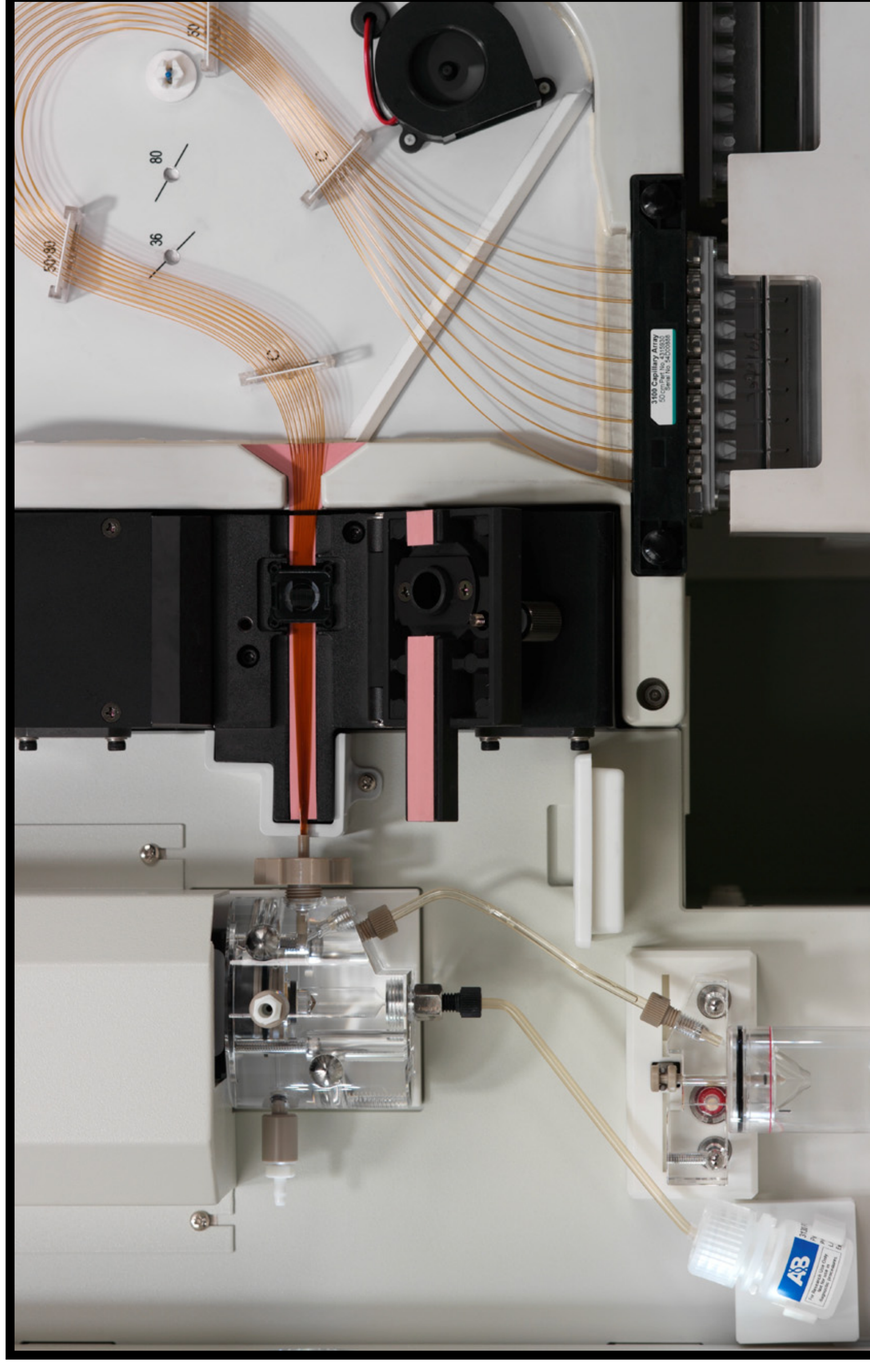


Figure 1. The 3130 automated polymer delivery system allows automatic polymer loading, which minimizes hands-on time and maintenance and maximizing performance.

Table 1. 3130 POP-7 Polymer Sequencing Run Modules

Sequencing Run Modules	Array Length (cm)	Run Time (min)	Samples in 24-hr		KB™ Basecaller QV ₂₀ LOR††
			3130 system	3130x/ system	
UltraSeq36_POP7	36	35	164	656	500
RapidSeq36_POP7	36	60	96	384	600
FastSeq50_POP7	50	60	96	384	700
StdSeq50_POP7	50	120	48	192	850
LongSeq80_POP7	80	170	32	128	950

*Sequencing Analysis Software v5.2 provides a metric Length Of Read (LOR), defined as the usable range of high-quality or high-accuracy bases determined by Quality Values (QV) generated by the KB Basecaller v1.2. The LOR is determined using a sliding window of 20 bases, which has an average QV greater than 20.
†98.5% basecalling accuracy, less than 2% N's

Table 2. 3130 POP-7 Polymer Fragment Analysis Run Modules

Fragment Analysis Run Modules	Array Length (cm)	Run Time (min)	Throughput 24-hr*		Resolution (bp)
			3130 system	3130x/ system	
FragmentAnalysis36_POP7	36	35	3,290	13,170	500
FragmentAnalysis50_POP7	50	50	2,300	9,220	500

*20 Genotypes/injection
**Performance Standard Deviation= 0.15; 1 bp-resolution at 99.99% accuracy

ULTRA-RAPID SEQUENCING

Researchers can now use POP-7™ polymer, the 36-cm capillary array, and the Ultra-Rapid Sequencing module to automatically sequence up to 16 samples that will yield read lengths greater than 500 base pairs (bp). The total run time for the Ultra-Rapid Sequencing module for POP-7 polymer is 35 minutes (Figure 1). The throughput of high-quality data in a 24-hour period is 41 runs, or 656 samples, on a 3130x/ system—a 14% increase for runs that use POP-4™ polymer.

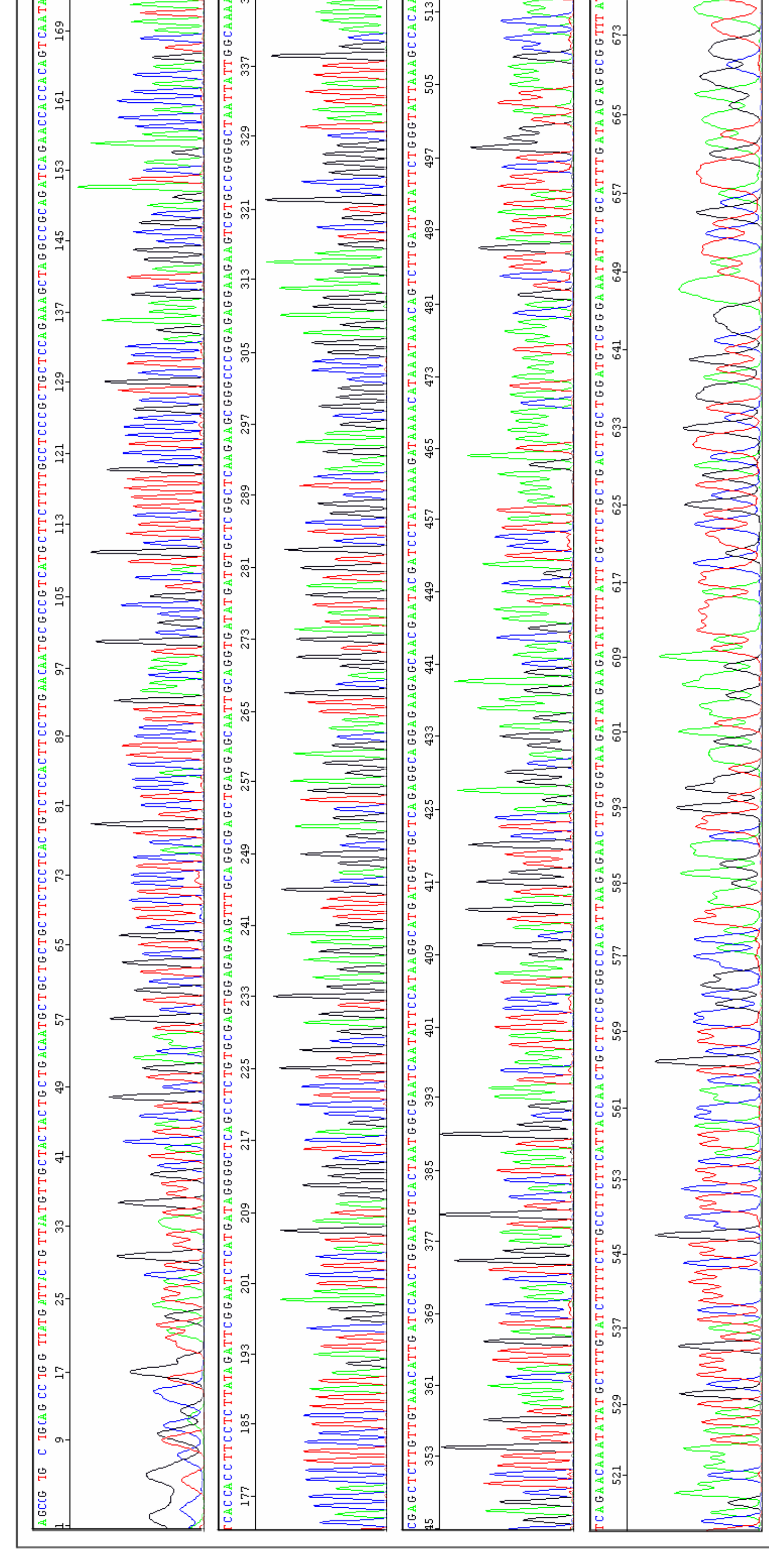


Figure 2. Example of a typical sequencing run using the Ultra-Rapid Sequencing module. The run was performed using BigDye® Terminator Sequencing Standard v 3.1. A length of read of 668-bp was achieved for this sample using 3130 POP-7 polymer and the 36-cm capillary array. The total run time was 35 minutes

FRAGMENT ANALYSIS

By combining the 36-cm capillary array and POP-7 polymer, the 3130 Series Systems provide an ideal method for microsatellite analysis. Microsatellites or Short Tandem Repeats (STRs) are polymorphic DNA loci that contain a repeated nucleotide sequence. STRs can be used for a variety of applications, such as human disease-association studies, genetic mapping, population genetics, and parentage testing. STRs can also be used as a tool for plant and animal breeders. The ABI PRISM® Linkage Mapping Set, GeneMapper® v3.7 Software and the 3130 Series Genetic Analyzers are an example of a complete microsatellite system.

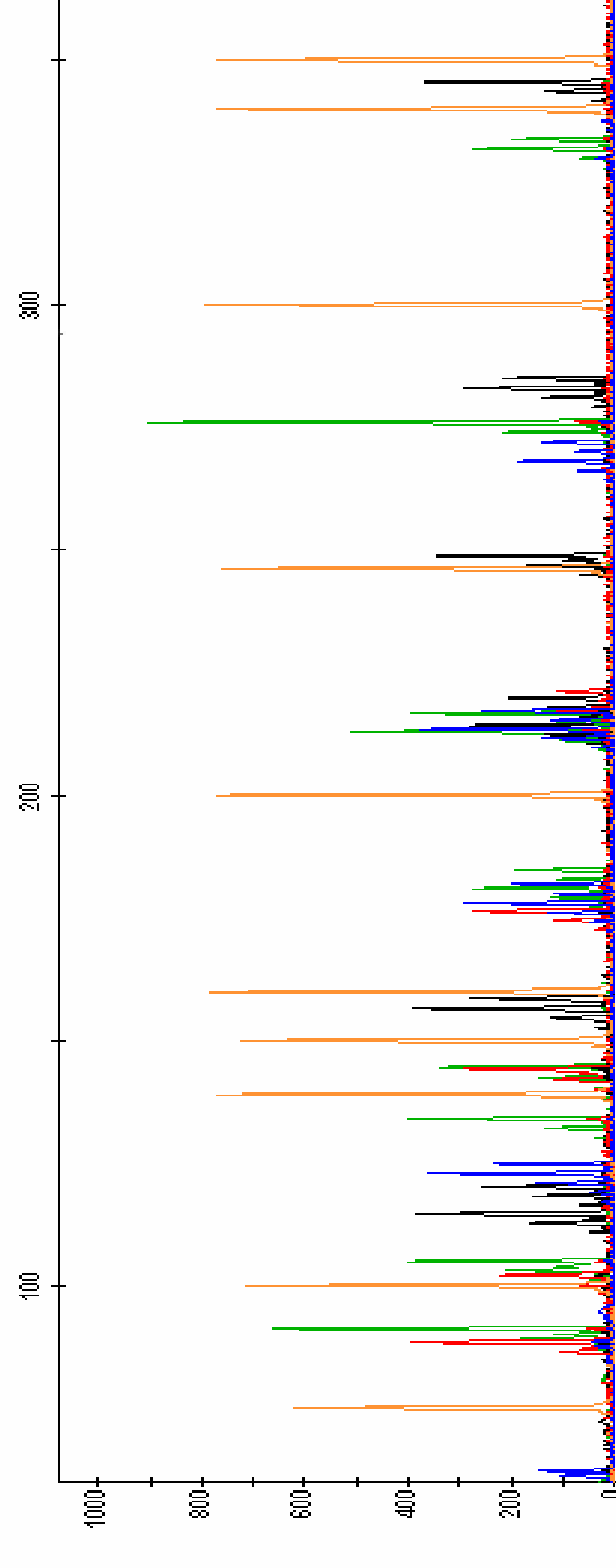


Figure 3. Electropherogram of microsatellite loci from the 3130x/ Genetic Analyzer, ran with the GeneScan™-500LIZ® size standard, a 36-cm array and 3130 POP-7™ Polymer with a run time of 35 minutes.

LONG READ SEQUENCING

For sequencing longer read lengths, researchers need only switch the 36-cm or 50-cm capillary array to an 80-cm capillary array and continue using POP-7 polymer as the separation medium. This system now automatically sequences up to 16 samples with a QV₂₀ LOR greater than 950 bp. The total run time is 2 hours, 50 minutes on a 3130x/ instrument. With this system, the researcher can efficiently sequence a total of 8 runs (128 samples) in a 24-hour period, generating high-quality data in excess of 121 kb with minimal hands-on time.

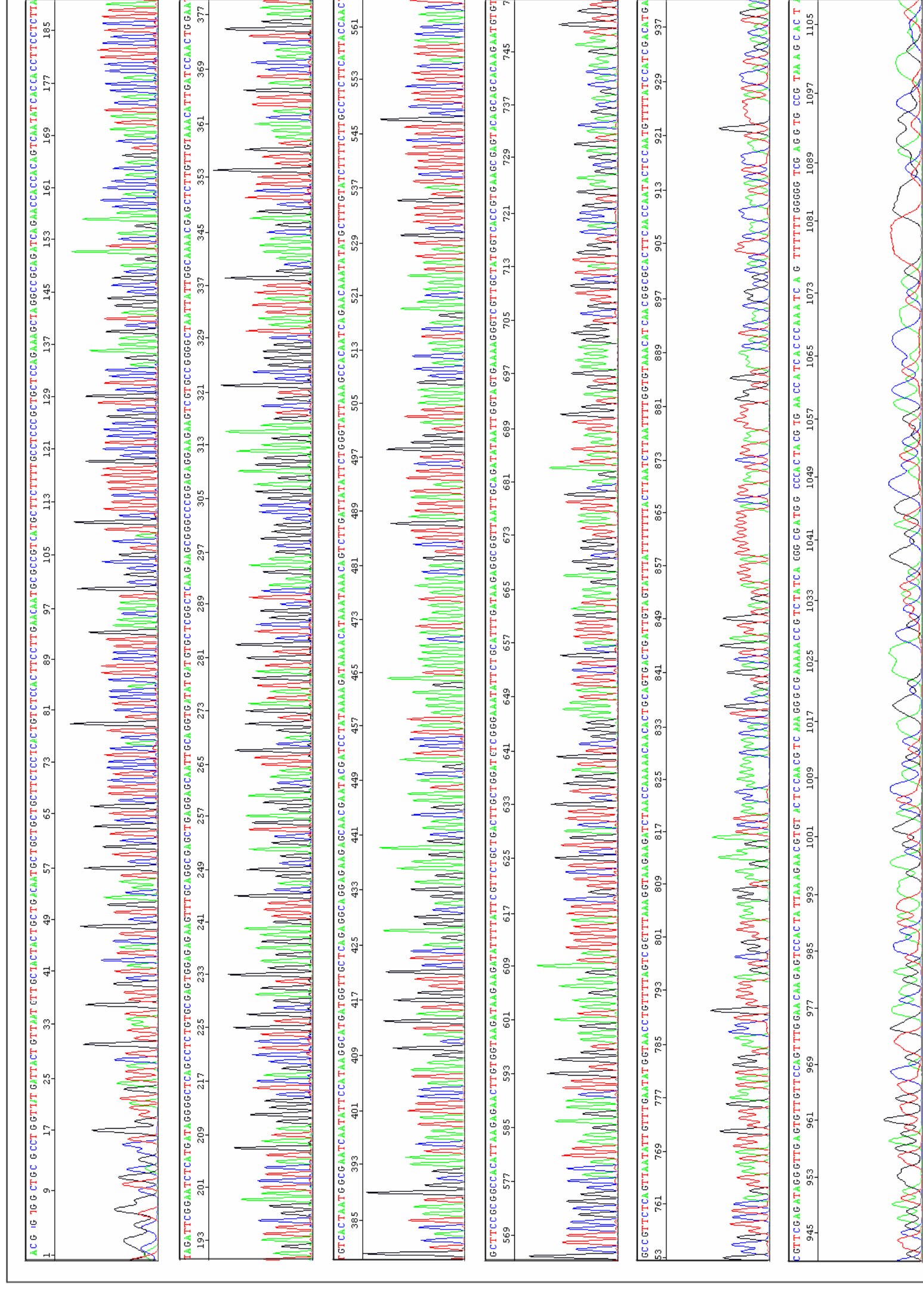


Figure 4. This electropherogram from a 3130x/ Genetic Analyzer is an example of a typical long-read sequence obtained from a BigDye Terminator Sequencing Standard v3.1. A QV₂₀ LOR of 1,090-bp was achieved using 3130 POP-7 polymer. Total run time: 2 hr, 50 min.

ONE POLYMER, ONE ARRAY, MAXIMUM PERFORMANCE

The 3130 Series Systems are fully automated, high-performance, fluorescence-based, multi-capillary, electrophoresis instruments that analyze 4 or 16 samples simultaneously. On both 3130 Series Systems, sample analysis is fully automated from the moment the plate is placed on the instrument and the run is started. Whether for sequencing, fragment analysis, or both applications, the 3130 Series Systems provide continuous, unattended operation, from automated polymer loading and sample injection to separation, detection, and data analysis. Automated polymer delivery through the polymer delivery system eliminates the normal, clean-up steps that must be performed manually.

Furthermore, the detection cell heater provides better environmental control, which, when coupled with POP-7 polymer, yields improved data quality, faster runs, and longer read lengths. The integration of these components in the 3130 Series Systems improves performance, functionality and ensures instrument flexibility. Easy-to-use features maximize laboratory productivity while reducing the overall cost per sample. Compared with other capillary electrophoresis instruments on the market today, the Applied Biosystems 3130 and 3130x/ Genetic Analyzers provide the most cost-effective means of achieving maximum performance.

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