ABSTRACT

In the course of designing more than 200,000 primer pairs and generating more than 18 million sequence reads, bioinformatics specialists and R&D scientists who worked on the Applera Genome Initiative gained valuable experience in primer design, as well as insight into the genomic complexities and technical challenges associated with them. The 3130 and 3130xl Genetic Analyzers, when combined with the Applied Biosystems VariantSEQr™ Resequencing System and SeqScape® Software v2.5 for seamless data analysis, provide the most robust and efficient resequencing system for customers who require low to medium throughput. The components of this integrated system simplify the rate-limiting steps found in current resequencing protocols, and offer a complete, cost-effective solution for laboratories performing either large or small resequencing studies, thus, revolutionizing DNA sequencing as a tool for the detection of human gene mutations.

A HIGH PERFORMANCE SYSTEM FOR SEQUENCING APPLICATIONS

The 3130 series Genetic Analyzers are fully automated, high-performance, fluorescence-based, capillary electrophoresis systems that can analyze multiple samples simultaneously. Samples can be resequenced in 35 minutes using the 16-capillary 3130xl system or the 4-capillary 3130 system with 3130 POP-7™ polymer and the 39-capillary 3130 system. After the sequence data is collected, the KB Basecaller will automatically process the data and provide a length of read (LOR) greater than 500 base pairs (bp). Furthermore, the throughput of the 3130 system, using the 3130x POP-7™ run mode, can efficiently sequence up to 41 runs (656 samples) in a 24-hour period, generating high quality, high resolution data with minimal hands-on-time. The 3130 series system is designed for ease-of-use to maximize laboratory productivity while reducing the overall cost per sample. Now more than ever, researchers have the flexibility to choose one configuration for all their resequencing needs. The Automated Polymer Delivery System in the 3130 series system allows automatic polymer loading, which minimizes hands-on time and maintenance while maximizing performance. The system enables the use of 3130 POP-7 polymer, not only for the 36-cm capillary array, but also for the 50-cm and 80-cm capillary arrays. The run configurations, specific for POP-7 polymer, incorporate a higher temperature through the detection cell heater, which yields peaks of greater resolution, less run-to-run variability, and faster electrophoresis times than any other capillary electrophoresis system available on the market today.

KEY FEATURES OF THE 3130 SERIES SYSTEMS

- Automated Polymer Delivery System
  - Ease to use
  - Easy to maintain
- New 3130 POP-7™ Polymer
  - One polymer or one array for all applications
- Detection Cell Header and 3130 POP-7 Polymer
  - Faster turn around times
  - Longer Reads
  - Better peak resolution

3130 POP-7™ Polymer Sequencing Run Modules for Variant Identification

The table below lists Data Collection Software v3.0 run modules that were used for the variant identification experiment.

| Run Modules | Array Length (cm) | Run Time (min) | Samples in 24 hr | KB Basecaller
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<tr>
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<tbody>
<tr>
<td>Ultraseq Pop-7 POP-7</td>
<td>30</td>
<td>35</td>
<td>164</td>
<td>656</td>
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<tr>
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<td>98</td>
<td>384</td>
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<tr>
<td>Rapidseq36 POP-7 POP-7</td>
<td>36</td>
<td>60</td>
<td>98</td>
<td>384</td>
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The process is completely automated providing fast and accurate data analysis. After the primers have generated sequences, the sequence files and project template (part of the resequencing primer product) are analyzed by SeqScape software (Figure 2).

The process provides project templates for each Resequencing Set (RGS). Each project template provides a reference sequence (Reference Data Group) to which all the specimens in a project are compared, settings that are used to analyze the data (Analysis Defaults) and settings that are used to display the data (Display Settings). By directly importing the RGS project template into the SeqScape Manager within the software, a project can be created. After the project is analyzed, the quality of the results can be reviewed and the variants can be examined (Figure 3).