

Accuracy and Performance Breakthroughs with the 5500 Series SOLiD™ Sequencers



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ABSTRACT

The 5500 Series SOLiD™ System incorporates a number of improvements in system design and chemistry to give the most accurate and flexible next generation sequencer. The 5500 series uses a 6-lane FlowChip™ where clonally-amplified beads are deposited. The 5500x1 supports two FlowChips while the 5500 supports one FlowChip. Each FlowChip lane is independently addressed with a proprietary, direct-injection fluidics system which decreases run time, minimizes dead-volume and greatly simplifies the design. Each lane can contain a distinct sample: The system will deliver the required reagents to each lane, independent of the other lanes, using only the reagents required. Imaging time on the 5500 is made faster by incorporating an innovative fast filter wheel, hardware autofocus, and fast camera. Images are processed in real time using a workstation-class computer utilizing state-of-the-art algorithms and GPGPU. The amount of data generated and saved is reduced by an order of magnitude. Chemistry improvements include longer 75 bp reads (forward), 35 bp (reverse paired end), and 2x60 long mate-pair. Accuracy improvements using the Exact Call Chemistry™ system increase accuracy to 99.99% allowing heterogeneous SNP detection at levels requiring much less redundancy and improved confidence levels. Users can expect data rates of 20-30 Gb/day with the 5500x1. Results from several library types and applications will be shown, including run times.

SPECIFICATIONS

Table 1. System Specifications

| | 5500 SOLiD™ Sequencer | 5500x1 SOLiD™ Sequencer |
|--------------------------------|--|--|
| Gb/day ⁽¹⁾ | 10-15 Gb/day | 20-30 Gb/day 30-45 Gb/day (Nano-beads) |
| Samples/run ⁽²⁾ | 1 Genome 12 Exomes 6 Transcriptsomes | 2 Genomes 24 Exomes 12 Transcriptsomes |
| System Accuracy ⁽³⁾ | 99.99% | 99.99% |
| Read Length | MP: 60x60bp PE: 75x35bp Fragment: 75bp | MP: 60x60bp PE: 75x35bp Fragment: 75bp |
| Independent lanes | 1 to 6 | 1 to 12 |
| Multiplexing | 96 for RNA, DNA 7 days for 60x60bp (6 lanes) 6 days for 75x35bp (6 lanes) 1 day for 35bp (1 lane) | 96 for RNA, DNA 7 days for 60x60bp (12 lanes) 6 days for 75x35bp (12 lanes) 1 day for 35bp (1 lane) |

PAY-AS-YOU GO FLEXIBILITY

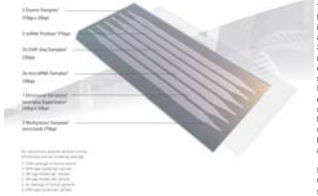


Figure 2. FlowChip Flexibility

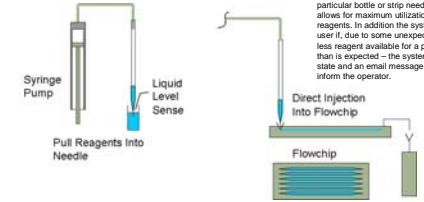


Figure 3. Fluidics Flexibility. Pay per use reagents and fluidic performance.

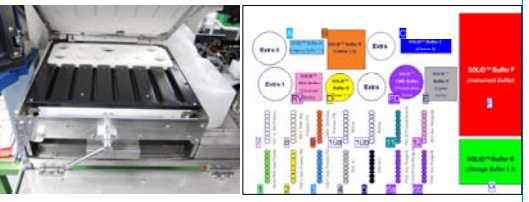


Figure 4. Reagents. Enough reagents for (2) FlowChips, 75x35 Paired End, Unattended

The reagents for the 5500 are packaged more as bulk reagents that are not necessarily specific to any particular application. The reagent block is loaded with pre-packaged reagents and the user can then run any mixture of samples on the system, as specified in the instrument control software, on a per lane basis. If a particular application uses one set of reagents more than another, then a user just needs to replenish that reagent set, not the entire reagent deck. The reagent deck is kept at 4°C and stability of reagents on the reagent deck is guaranteed for several weeks.

ENDOGENOUS CONTROLS



Figure 6. Controls Design

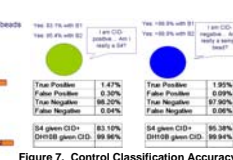


Figure 7. Control Classification Accuracy



Figure 8. Runtime feedback on data quality using controls

The 5500 system incorporates the use of a sophisticated synthetic control system. These synthetic control beads are spiked into every sample at a very low concentration. The control beads are designed in such a fashion as to explore every possible base combination and transition associated with SOLiD™ 4-style chemistry. This information is used to continually monitor run progress and quality providing information to user about sample bead quality and continually comparing to that to expected quality. This will allow users to distinguish between instrument, chemistry, and sample problems quantitatively.

Feedback is provided through the control system user interface, with predictions about individual sample performance. Sliders allow the user to change the read-length of the system dynamically to not continue to utilize precious reagents, to not acquire data that will just be trimmed in filtering, and shorten run time.

FAST PERFORMANCE

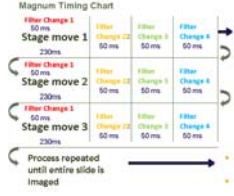
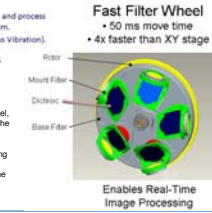


Figure 9. Imaging performance is much faster. Optics are vibration isolated internally (robot) and externally.



Another performance enhancement of the 5500-series systems is a patent pending fast filter wheel. All four colors are interrogated sequentially per panel, making the overall data acquisition time >2.5 times faster than SOLiD™ 4. The FW requires minimal setting time before a picture is taken. The optical system of the 5500 has been optimized compared with SOLiD™ 4 and includes a hardware autofocus system to increase reliability of focus (resulting in fewer picture retakes) and a much faster camera. The optical system combines with a new computer system that does real-time data image processing, greatly accelerating performance and minimizing the amount of hard-disk space allocated to images.

HIGH QUALITY DATA FROM LONG READS



Figure 10. 5500 has equivalent data quality to SOLiD™ 4

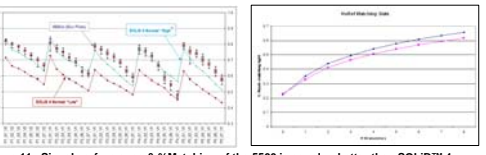


Figure 11. Signal performance & %Matching of the 5500 is equal or better than SOLiD™ 4



Figure 12. 75 bp reads on the 5500

EXCEPTIONAL ACCURACY WITH EXACT CALL CHEMISTRY (ECC)

