Illumina Two-Channel SBS Sequencing Technology

High data accuracy with faster data generation.

Introduction

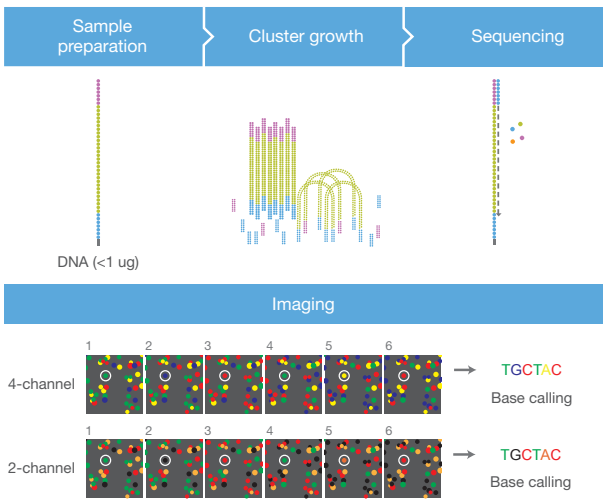
Illumina sequencing platforms leverage a highly accurate and robust sequencing by synthesis (SBS) technology that has become the most successful and widely adopted next-generation sequencing platform worldwide. Illumina SBS technology supports massively parallel sequencing using a proprietary reversible terminator-based method that enables detection of single bases as they are incorporated into growing DNA strands.

Illumina SBS technology is the foundation of Illumina HiSeq® and MiSeq® systems, which employ a four-channel method to detect individual bases. The new NextSeq™ 500 System employs the latest evolution in SBS technology. Its two-channel SBS method supports reduced cycle and data processing times, enabling the NextSeq 500 System to be the first desktop sequencing system to perform high-throughput applications.

SBS Technology

Illumina two- and four-channel SBS sequencing technology uses fluorescently labeled nucleotides to sequence hundreds of millions of clusters on a flow cell surface in parallel (Figure 1). During each sequencing cycle, a single labeled deoxynucleotide triphosphate (dNTP) is added to the nucleic acid chain. The nucleotide label serves as a terminator for polymerization, so after each dNTP incorporation, the fluorescent dye is imaged to identify the base and then chemically cleaved to allow incorporation of the next nucleotide. Since all four reversible terminator-bound dNTPs (A, C, T, G) are present as single, separate molecules, natural competition minimizes incorporation bias. Base calls are made directly from signal intensity measurements during each cycle, greatly reducing raw error rates compared to other technologies. View a video about the SBS technology at www.illumina.com/systems/nextseq-sequencer/technology.ilmn.

Figure 1: Four-Channel vs. Two-Channel SBS Technology Image Detection and Base Calling



In four-channel SBS, four images are necessary to capture the unique fluorescent dyes for each base. In contrast, two-channel SBS requires only two images to determine all four base calls.

Four-Channel SBS Sequencing Process

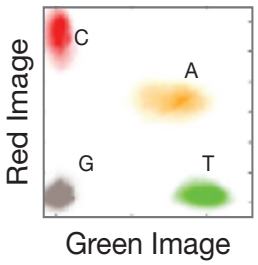
In four-channel SBS, the acquisition of four distinct images enables a cycle-by-cycle observation of which color dye is incorporated into an individual cluster. Cluster detection software algorithms then process the images to determine the individual base calls for each unique cluster. With four-channel sequencing, all four images are required to build up the DNA sequence.

New Two-Channel SBS Sequencing Process

Two-channel SBS sequencing simplifies nucleotide detection, leveraging an innovative data processing approach that requires only two images to determine all four base calls. Rather than a separate dye for each base, two-channel sequencing uses a mix of dyes (Figure 2).

Images are taken of each cluster using red and green filter bands, with the two-fold reduction in image acquisition, reducing sequencing time and accelerating sequence processing. Clusters seen in red or green images are interpreted as C and T bases, respectively. Clusters observed in both red and green images are flagged as A bases (appearing as yellow clusters in Figure 2), while unlabeled clusters are identified as a G base. The standard template generation process is built up over five cycles. In instances where clusters begin with a G base, the G base will be detected in subsequent cycles as A, C, or T bases are observed.

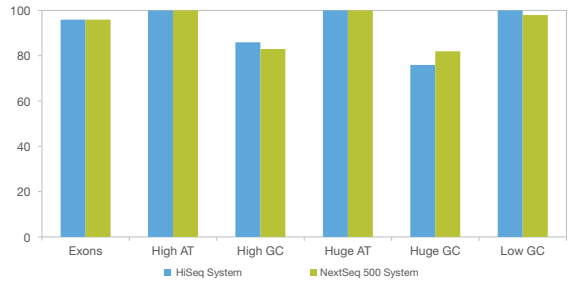
Figure 2: Two-Channel SBS Imaging



Accelerated detection of all four DNA bases is performed on the NextSeq 500 System using only two images to capture red and green filter wavelength bands. A bases will be present in both images (yellow cluster), C bases in red only, T bases in green only, and G bases in neither.

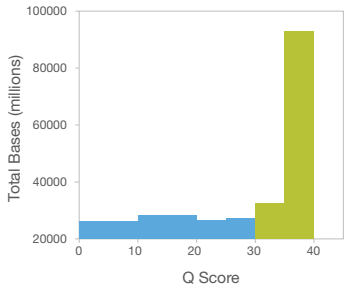
*Q30 = 1 error in 1,000 base calls or an accuracy of 99.9%

Figure 3: Coverage in Hard-to-Sequence Regions



Designed using the same fundamental sequencing chemistry technology, NextSeq and HiSeq Systems exhibit robust performance in difficult-to-sequence regions of the genome.

Figure 4: Base Call Accuracy and Performance



Two-channel SBS technology enables the NextSeq system to deliver high-quality data, with a sequencing accuracy of >75% of sequenced bases over Q30 at 2 x 150 bp.

SBS Accuracy and System Compatibility

Illumina SBS-based sequencing systems deliver the most accurate sequencing data at any coverage, the highest yield of error-free reads, and the highest percentage of base calls above Q30* in the industry, even in difficult-to-sequence regions of the genome [Figure 3]. This high data quality results in low false positive and false negative rates, reducing the need for extensive downstream validation and giving researchers full confidence in the data generated for their genome, exome, transcriptome, or other studies.

Since all Illumina sequencers are based on the same core SBS technology, researchers can confidently transition their research from one Illumina sequencer to another and still access the industry's highest-quality data (Figure 4). Regardless of whether data is generated by a four-channel or two-channel SBS system, it can be seamlessly compared and analyzed in BaseSpace, the Illumina genomics computing environment, or across a wide array of third-party analysis tools.

Summary

Illumina SBS technology offers the highest NGS accuracy, enabling accurate base-by-base sequencing and robust performance across the genome. The latest evolution of this transformational technology can be found in the NextSeq 500 System, where it enables a breakthrough reduction in data generation times, enabling high-throughout sequencing applications to become everyday research tools.

References

1. www.illumina.com/systems/nextseq-sequencer/technology.ilmn

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Pub. No. 770-2013-054 Current as of 30 January 2014

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