Patterned Flow Cell Technology

Fixed spacing of sequencing clusters with defined feature sizes contributes to increased data output, reduced costs, and faster run times.

Introduction

Leveraging the highly accurate and robust sequencing by synthesis (SBS) technology, Illumina sequencing platforms are the most successful and widely adopted next-generation sequencing (NGS) systems worldwide. Patterned flow cell technology significantly increases the throughput of Illumina NGS systems, drastically reducing the sequencing cost per sample. This price decrease enables data-intensive applications such as whole-genome sequencing for population-scale studies, including the $1000 human genome, on the HiSeq X™ Ten and HiSeq X Five Systems.* The HiSeq® 3000/ HiSeq 4000 Systems† take advantage of this technology improvement to offer a cost-effective solution for a broad range of applications.

Higher-Density Clonal Clusters

Two innovative breakthroughs contribute to the boost in sequencing power experienced with patterned flow cell technology: 1) a distinct, ordered nanowell design and 2) a new exclusion amplification chemistry. The result is more efficient use of the flow cell surface area yielding a higher density of sequenceable clusters during cluster generation. Higher cluster density leads to more useable data per flow cell, driving down the cost per gigabase (Gb) of the sequencing run.

Impact of Cluster Generation

The basic Illumina NGS workflow includes 4 steps: 1) library preparation, 2) cluster generation, 3) sequencing, and 4) alignment and data analysis. Historically, during cluster generation, adapter-ligated library elements hybridized to complementary oligonucleotides on the surface of a flow cell. Each attached library fragment acted as a "seed" and, through a process called bridge amplification, was amplified to generate a clonal cluster containing thousands of identical fragments. After cluster generation was complete, the flow cell contained millions to billions of clusters on its surface.

Ideally, clusters are of similar size and spaced well apart from each other to achieve accurate resolution during imaging. In reality, DNA clusters are randomly distributed across the flow cell with many clusters in close proximity to neighboring clusters, especially if the sample is overloaded, making it difficult to discern individual clusters from each other. If a cluster cannot be accurately resolved from an adjacent cluster, the data from that cluster is considered unusable, reducing the amount of information generated during the run. Patterned flow cell technology overcomes these challenges with prearranged nanowells that optimize cluster spacing. Clusters can only form in the nanowells, making the flow cells less susceptible to overloading. New patented chemistry allows simultaneous seeding and amplification during cluster generation to minimize the chances of multiple library fragments amplifying as a single cluster.

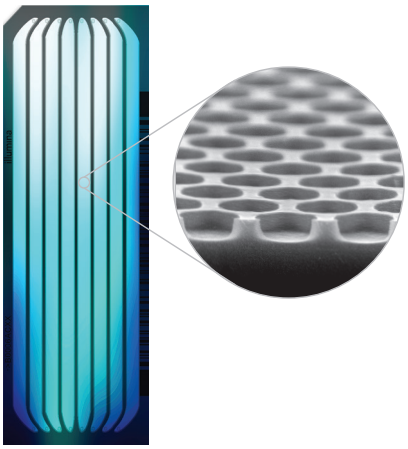

Figure 1: Patterned Flow Cell Design—Patterned flow cells contain billions of nanowells at fixed locations, providing even cluster spacing and uniform feature size to deliver extremely high cluster densities.

Prearranged Cluster Distribution on the Flow Cell

To make more effective use of the flow cell surface space, Illumina created the patterned flow cell with distinct nanowells for cluster generation (Figure 1). Patterned flow cells are produced using semiconductor manufacturing technology. Starting with a glass substrate, patterned nanowells are etched into the surface. Each nanowell contains DNA probes used to capture prepared DNA strands for amplification during cluster generation. The area between the nanowells is devoid of DNA probes, preventing the formation of clusters in the interstitial regions between wells.

This process ensures that DNA clusters only form within the nanowells, providing even, consistent spacing between adjacent clusters and allowing accurate resolution of clusters during imaging. The result is maximal use of the flow cell surface leading to overall higher clustering.

Exclusion Amplification

By enabling simultaneous seeding (landing of the DNA strand in the nanowell) and amplification, exclusion amplification promotes monoclonal cluster generation within the nanowells. This improvement allows the number of monoclonal clusters available for sequencing on a patterned flow cell to exceed the Poisson statistical limit, significantly increasing data output.

* The HiSeq X Ten System is a suite of 10 individual HiSeq X instruments. The HiSeq X Five System is a suite of 5 individual HiSeq X instruments.

† The HiSeq 4000 System is a dual-flow cell instrument with higher throughput than the single-flow cell HiSeq 3000 System.

Less Sensitive to Overclustering

When using nonpatterned flow cells, precise library quantification is required as library concentration greatly impacts cluster density. Overclustering limits performance as the clusters become more difficult to discern. With patterned flow cell technology, updated chemistry combined with prearranged, ordered nanowells spaced to provide optimal cluster density make the process more tolerant to a broader range of library densities. For optimal performance, and to minimize duplicates, it is still important to make sure that the library is loaded at the recommended concentrations.

Eliminate Template Registration

Having an ordered array substrate where clusters only form within wells eliminates time-consuming template registration, an informatics-based mapping of clusters on the flow cell. With patterned flow cells, nanowells are precisely positioned relative to known fiducial locations. There's no need to map cluster sites, saving hours on each sequencing run.

Functional Quality Control

Quality control (QC) occurs throughout the manufacturing process, starting with an initial quality assessment of the glass substrate itself and ending with a functional hybridization-based test of the finished product. This final QC step binds fluorescently labeled DNA to the flow cell. Analyzing the evenness and intensity of the fluorescence across the flow cell surface ensures that DNA strands bind only in the nanowells and that the expected number of DNA probes are immobilized in each well. Each flow cell undergoes > 50 QC checks before it leaves the Illumina manufacturing area.

Advanced Systems Broaden Applications

The HiSeq 3000/HiSeq 4000, HiSeq X Five, and HiSeq X Ten Systems (Figures 2 and 3) use the innovative patterned flow cell technology to generate more usable data than ever before. Achieve new levels of understanding through exome, transcriptome, and genome studies on the HiSeq 3000/HiSeq 4000 Systems. Generate the $1000 genome

Figure 2: The HiSeq 4000 System—The HiSeq 4000 System provides cost-effective, efficient sequencing for large-scale studies across multiple applications.

using the HiSeq X Ten System for large-scale population sequencing studies. Open doors for genomic studies at a scale previously unattainable.

Summary

Illumina SBS technology is the most widely adopted NGS method worldwide. Patterned flow cells and updated chemistry represent the next important step in the continued innovation of Illumina SBS chemistry and NGS technology, generating more data in a single run, effectively reducing the cost per sample. These powerful innovations, employed on the HiSeq 3000/HiSeq 4000, HiSeq X Five, and HiSeq X Ten Systems, enable laboratories to increase the productivity of their sequencing experiments, driving the pace of genetic discovery.

Learn More

Watch a video showing patterned flow cell technology in action at www.illumina.com/PatternedFlowCell.

Figure 3: The HiSeq X Ten System—Using patterned flow cell technology, the HiSeq X Ten System provides maximum throughput and lowest cost for population-scale whole-genome sequencing.

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