illumına®

cBot™ 2 Cluster Generation System

Enabling streamlined cluster generation, end-to-end sample traceability, and seamless integration with Illumina HiSeq® and HiSeq X™ Sequencing Systems.

Highlights

- **Positive Sample Tracking**
  Barcode traceability maintains clear, controlled sample identification throughout the entire sequencing workflow

- **Improved Laboratory Efficiency**
  Automated workflow with keyed tube strips ensures proper sample orientation and loading, minimizing human error and potential rework

- **Streamlined Workflow**
  Precise tracking integrates seamlessly into the Illumina sequencing workflow, increasing data confidence

Introduction

As the price decreases and the workflows improve, next-generation sequencing (NGS) is more accessible to laboratories than ever before. With this accessibility comes an increase in the number of samples a laboratory processes. Efficient, accurate tracking of samples as they progress through the sequencing workflow is critical to establishing a successful experiment. Errors in sample identification can delay results and cause time-consuming rework or potential loss of valuable samples. The cBot 2 Cluster Generation System (Figure 1) overcomes this challenge with seamless integration into the Illumina sequencing workflow, while maintaining positive sample tracking of prepared libraries through cluster generation on a specified flow cell.

Advanced Sample Traceability

The Illumina sequencing workflow consists of 4 basic steps: (1) sample preparation, (2) clonal amplification, (3) sequencing, and (4) downstream analysis. Maintaining sample traceability throughout these 4 steps is critical. To trace samples properly, the instruments in each step must consistently use the same identifiers. As part of the Illumina sequencing workflow, the cBot 2 Cluster Generation System provides an efficient means for tracking samples, reagents, and flow cells as they move through the cluster generation workflow.

The cBot 2 System incorporates 2 internal barcode readers for tracking samples. Unique barcodes on the keyed 8-tube strip identify the position of each sample as it moves through the sequencing workflow. The cBot 2 System uses these barcodes to determine which samples are loaded in a given flow cell lane, maintaining sample traceability throughout the cluster generation process. This information can be integrated directly with a laboratory information management system (LIMS) for the SeqLab workflow and is compatible with non-Clarity LIMS via XML.

Figure 1: cBot 2 Cluster Generation System—Combining positive sample tracking with automated clonal amplification, the cBot 2 System is an integral part of providing complete traceability throughout the sequencing workflow.

Improved Laboratory Efficiency

The cBot 2 System automates clonal amplification of libraries. It includes innovative features that eliminate user intervention, reduce potential failure points, and increase sequencing productivity. Keyed 8-tube strips ensure proper orientation of samples loaded on to the cBot 2 System. Ready-to-use reagents prepacked in 96-well plates remove reagent preparation errors, potential sources of contamination, and decrease storage requirements.

On-instrument features, including a unique, plate-piercing manifold, enable intervention-free runs. Cluster generation occurs within the sealed Illumina flow cell, minimizing sources of error and contamination. The cBot 2 System further reduces potential error by seamlessly tracking genomic samples as they are loaded on the flow cell. Taken together, these features result in a substantial improvement in efficiency during clonal amplification.

Streamlined Sequencing Workflow

The cBot 2 Cluster Generation System is part of a comprehensive sequencing workflow from Illumina. The system is compatible with all HiSeq Systems (HiSeq 2500, 3000, 4000, and X) and integrates seamlessly with the Illumina SeqLab solution for the HiSeq X System.
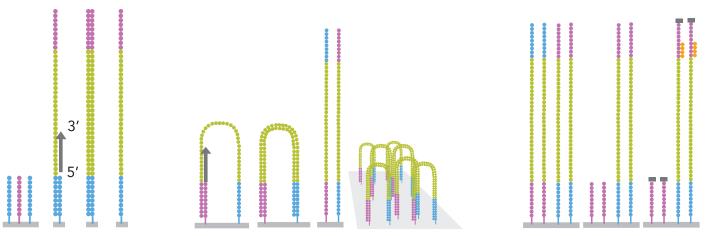
Figure 2: Cluster Generation by Isothermal Bridge Amplification—Cluster generation from single-molecule DNA templates occurs within the sealed Illumina flow cell on the cBot instrument, and involves immobilization and 3' extension, bridge amplification, linearization, and hybridization.

Software enhancements further streamline communication between the sample preparation and HiSeq sequencing instruments, providing better results with less effort. For higher productivity, the cBot 2 System offers the following enhancements:

- Integrated 8-inch touch screen for simplified operation in a small, lab-friendly footprint
- Onscreen, step-by-step instructions with embedded multimedia help for simplified user operation with no prior training
- Real-time progress indicators for at-a-glance monitoring
- Remote monitoring of multiple systems from any web browser or smartphone
- Positive sample tracking throughout the entire workflow for reliable results
- Compatible with external LIMS for a streamlined sequencing workflow from library preparation to data analysis

Monoclonal Cluster Generation

Clonal amplification is the second step in the 4-step Illumina sequencing workflow. During clonal amplification, clusters are generated on a flow cell. For the most efficient and accurate sequencing, it is critical that each cluster represent a single DNA fragment. Automated protocols and optimized reagents enable the cBot 2 Cluster Generation System to produce the highest density of monoclonal clusters for the greatest sequencing efficiency.

Immobilization of Single-Molecule DNA Templates

Hundreds of millions of templates are hybridized to a lawn of oligonucleotides immobilized on the flow cell surface. The templates are copied from the hybridized primers by 3' extension using a high-fidelity DNA polymerase to prevent misincorporation errors. The original templates are denatured, leaving the copies immobilized on the flow cell surface.

Isothermal Bridge Amplification

Isothermal bridge amplification amplifies the immobilized DNA template copies (Figure 2). The templates loop over to hybridize to adjacent lawn oligonucleotides. DNA polymerase copies the templates from the hybridized oligonucleotides, forming dsDNA bridges, which are denatured to form 2 ssDNA strands. These 2 strands loop over and hybridize to adjacent oligonucleotides and are extended again to form 2 new dsDNA loops. The process is repeated on each template by cycles of isothermal denaturation and amplification to create millions of individual, dense clonal clusters containing ~2,000 molecules.

Linearization, Blocking, and Primer Hybridization

Each cluster of dsDNA bridges is denatured, and the reverse strand removed by specific base cleavage, leaving the forward DNA strand. The 3' ends of the DNA strands and flow cell-bound oligonucleotides are blocked to prevent interference with the sequencing reaction. The sequencing primer is hybridized to the complementary sequence on the Illumina adapter on unbound ends of the templates in the clusters. The flow cell now contains > 200 million clusters with ~1,000 molecules/cluster, and is ready for sequencing.

Summary

The cBot 2 Cluster Generation System provides many useful features, including positive tracking of samples and reagents throughout the cluster generation workflow, in addition to high-efficiency monoclonal cluster generation. The cBot 2 System integrates seamlessly into the complete Illumina sequencing workflow for accurate results with high confidence.

Learn More

To learn more about Illumina sequencing, visit www.illumina.com/sequencing.

cBot 2 Cluster Generation System Specifications

Parameter	Specification
Instrument Configuration	CE Marked and ETL Listed instrument, installation, setup, and accessories
Instrument Control Computer	Single Board Computer, Mini-ITX ATOM D2550, 1G Memory Windows Embedded 7 Operating System, 64-bit Integrated 8" touch screen monitor
Operating Environment	Temperature: 22°C ± 3°C Humidity: Noncondensing 20–80% Altitude: < 2000 m (6500 ft) Air quality: Pollution degree rating of II For Indoor Use Only
Laser	Class 1 and 2 laser product
Dimensions	L × W × H: 62 cm × 38 cm × 46 cm Weight: 31 kg Crated weight: ~ 45 kg
Power Requirements	100-240V AC 50/60 Hz, 4A, 400 watts

Laser Radiation

The barcode scanner used in the cBot 2 Cluster Generation System is a Class 1 and 2 laser product. Do not stare into the visible light beam of the barcode scanner.

LASER RADIATION
DO NOT STARE INTO BEAM
CLASS 2 LASER PRODUCT
630-680nm, 1mW
IEC 60825-1 Edition 1,2 2001-08
LABEL P/N: 15007775

Ordering Information

Product	Catalog No.
cBot 2 Cluster Generation System	SY-312-2001
cBot 2 Barcoded Strip Tubes (8-well)	20005160
HiSeq X Ten Sequencing System[a]	SY-412-1001
HiSeq X Ten Reagent Kit v2.5	FC-501-2501
HiSeq X Ten Reagent Kit v2.5 - 10 pack	FC-501-2521
HiSeq X Five Sequencing System[b]	SY-412-4011
HiSeq X Five Reagent Kit v2.5	FC-502-2501
HiSeq X Five Reagent Kit v2.5 - 10 pack	FC-502-2521
HiSeq 4000 Sequencing System	SY-401-4001
HiSeq 3000 Sequencing System	SY-401-3001
HiSeq 4000 System Upgrade	SY-401-4002
HiSeq 3000/4000 SR Cluster Kit	GD-410-1001
HiSeq 3000/4000 PE Cluster Kit	PE-410-1001
HiSeq 2500 Sequencing System	SY–401–2501
HiSeq SR Cluster Kit v4–cBot–HS	GD–401–4001
HiSeq PE Cluster Kit v4–cBot–HS	PE–401–4001
TruSeq PE Cluster Kit v3–cBot–HS	PE–401–3001
TruSeq SR Cluster Kit v3–cBot–HS	GD–401–3001
HiSeq SR Rapid Cluster Kit v2	GD–402–4002
HiSeq PE Rapid Cluster Kit v2	PE–402–4002

a. Catalog numbers refers to an individual system. Minimum order for the HiSeq X Ten is 10 systems.
b. Catalog numbers refers to an individual system. Minimum order for the HiSeq X Five is 5 systems.