illumina®

cBot

Fully automated clonal cluster generation for Illumina sequencing.

Illumina cBot Highlights

- **Fast, Efficient Workflow:**
  Amplify > 96 samples in ~4–5 hours with < 10 minutes of hands-on time
- **Easiest to Use:**
  Pre-packaged 96-well TruSeq™ reagents, and simple touch screen interface simplifies operation
- **Innovative System Design:**
  Real-time fluidic monitoring, integrated system sensors and remote monitoring ensure robust instrument operation
- **Highest Quality Results:**
  Improved chemistry generates higher density clusters and sequencing accuracy

The Best Next-Gen Sequencing Workflow Just Got Better

cBot is a revolutionary automated clonal amplification system at the core of the Illumina sequencing workflow (Figure 1, upper panel). cBot replaces a lab full of equipment with a single compact device, delivering unsurpassed efficiency and ease of use for the highest quality sequencing results.

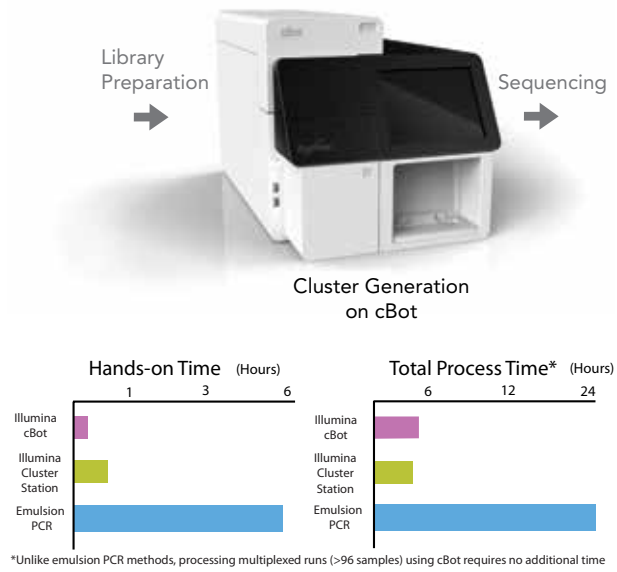
With cBot, hands-on time is reduced to less than 10 minutes, compared to more than six hours of hands-on effort for emulsion PCR methods. The process of creating sequencing templates is complete in about four hours, compared to more than 24 hours for emulsion PCR-based protocols (Figure 1, lower panel).

Breakthrough System for Cluster Generation

The Illumina sequencing workflow is based on three simple steps: libraries are prepared from virtually any nucleic acid sample, amplified to produce clonal clusters, and sequenced using massively parallel synthesis. The cBot clonal amplification system has innovative features that eliminate user intervention, reduce potential failure points, and increase sequencing productivity.

TruSeq Cluster Generation reagents are packaged in ready-to-use 96-well plates, completely removing reagent preparation errors, potential sources of contamination, and decreasing storage requirements. cBot features a single unique, plate-piercing manifold for intervention-free runs. Cluster generation occurs within the sealed, eight-channel Illumina flow cell, bypassing the frequent handling and contamination issues inherent to emulsion PCR-based protocols. cBot is capable of processing > 96 samples within a single flow cell, resulting in substantial cost savings without incremental effort and wasted reagents. Innovative instrument features ensure seamless operation for your sequencing workflow (Figure 2).

Figure 1: Cluster Generation is Streamlined and Efficient on cBot



*Unlike emulsion PCR methods, processing multiplexed runs (>96 samples) using cBot requires no additional time

cBot performs automated simultaneous clonal amplification of hundreds of millions of single-molecule DNA templates, dramatically reducing hands-on and total preparation time required to produce sequencing template, compared to emulsion PCR methods.

Better Results with Less Effort

cBot software enhancements and user interaction features ensure high productivity:

- **Integrated 8-inch touch screen provides simplified operation in a small, lab-friendly footprint**
- **On-screen, step-by-step instructions with embedded multimedia help enable user operation with no prior training**
- **Real-time progress indicators provide at-a-glance monitoring**
- **Remote monitoring allows a single user to manage multiple systems from any web browser or phone**
- **Status emails are sent when the run is complete or when intervention is required**

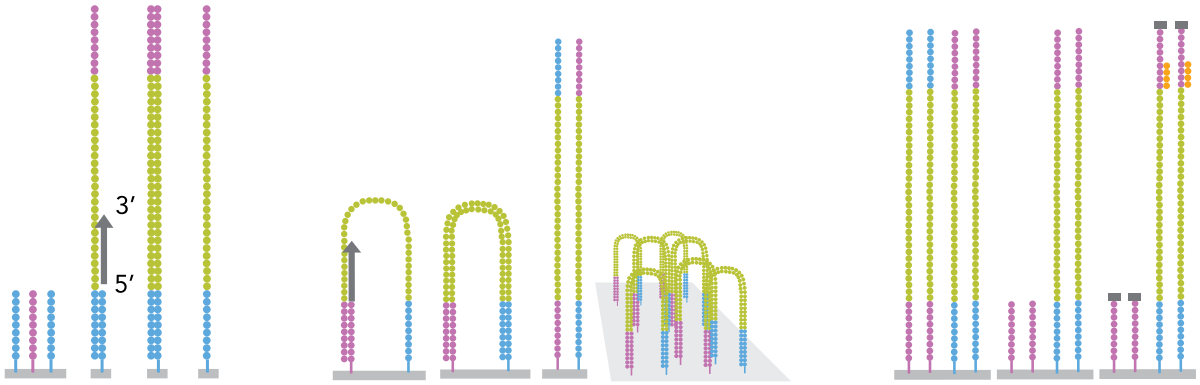
cBot Cluster Generation Process

Prior to sequencing, single-molecule DNA templates are bridge amplified to form clonal clusters inside the flow cell. (Figure 3).

Figure 2: New cBot Features Enables Rapid and Streamlined Cluster Generation

The cBot cluster generation system is the next generation of workflow improvements for Illumina sequencing. Novel innovations include pre-packaged reagents, a single manifold, advanced fluidics and thermal stage features, integrated sensors, remote monitoring capabilities, and simplified data entry and tracking with the touch screen and barcode scanner.

Figure 3: 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.

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

Immobilized DNA template copies are amplified by isothermal bridge amplification. 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 two ssDNA strands. These two strands loop over and hybridize to adjacent oligonucleotides and are extended again to form two 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 is 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

Illumina sequencing with cBot automated cluster generation sets the new standard for simplified next- generation sequencing. Ready-to-use reagents, smart instrumentation improvements, and new cluster generation chemistry offers significant advantages over emulsion PCR-based workflows and promotes even higher data density and sequencing accuracy. By streamlining the critical clonal amplification step in the next-generation sequencing workflow, Illumina continues to accelerate your landmark discoveries and publications.

Ordering Information

Description	Catalog No.		
	cBot	HiSeq System	Genome Analyzer
cBot Instrument Includes cBot, flow cell adapter plate, one year warranty, user manual	SY-301-2002		
cBot Flow Cell Manifold (Optional)	SY-301-2014		
TruSeq Single-Read Cluster Generation Kits include flow cell, reagent plate, manifold, user instructions		GD-401-3001	GD-300-2001
TruSeq Paired-End Cluster Generation Kits include flow cell, reagent plate, manifold, PE reagents, user instructions		PE-401-3001	PE-300-2001

cBot System Specifications

Catalog No.
SY-301-2002
Instrument Configuration
CE Marked and ETL Listed instrument, Installation, setup, and accessories
Instrument Control Computer
Mini-ITX Board with Celeron M Processor
1 GB RAM, 80 GB Hard Drive Windows Embedded OS
Integrated 8” Touch Screen Monitor
Operating Environment
Temperature: 22°C ± 3°C
Humidity: Non-Condensing 20%–80%
Altitude: Less than 2,000 m (6,500 ft)
Air Quality: Pollution Degree Rating of II
For Indoor Use Only
Laser
Class 2 Laser: 630–650 nm
Dimensions
W×D×H: 38 cm × 62 cm × 40 cm
Weight: 34 kg
Crated Weight: 36 kg
Power Requirements
100–240V AC 50/60 Hz, 4A, 400 Watts

ADDITIONAL INFORMATION

For more information about
Illumina sequencing, visit www.illumina.com/sequencing or contact us at the address below.

Laser radiation

Do not stare into the visible-light beam of the barcode scanner. The barcode scanner is a Class 2 laser product.

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