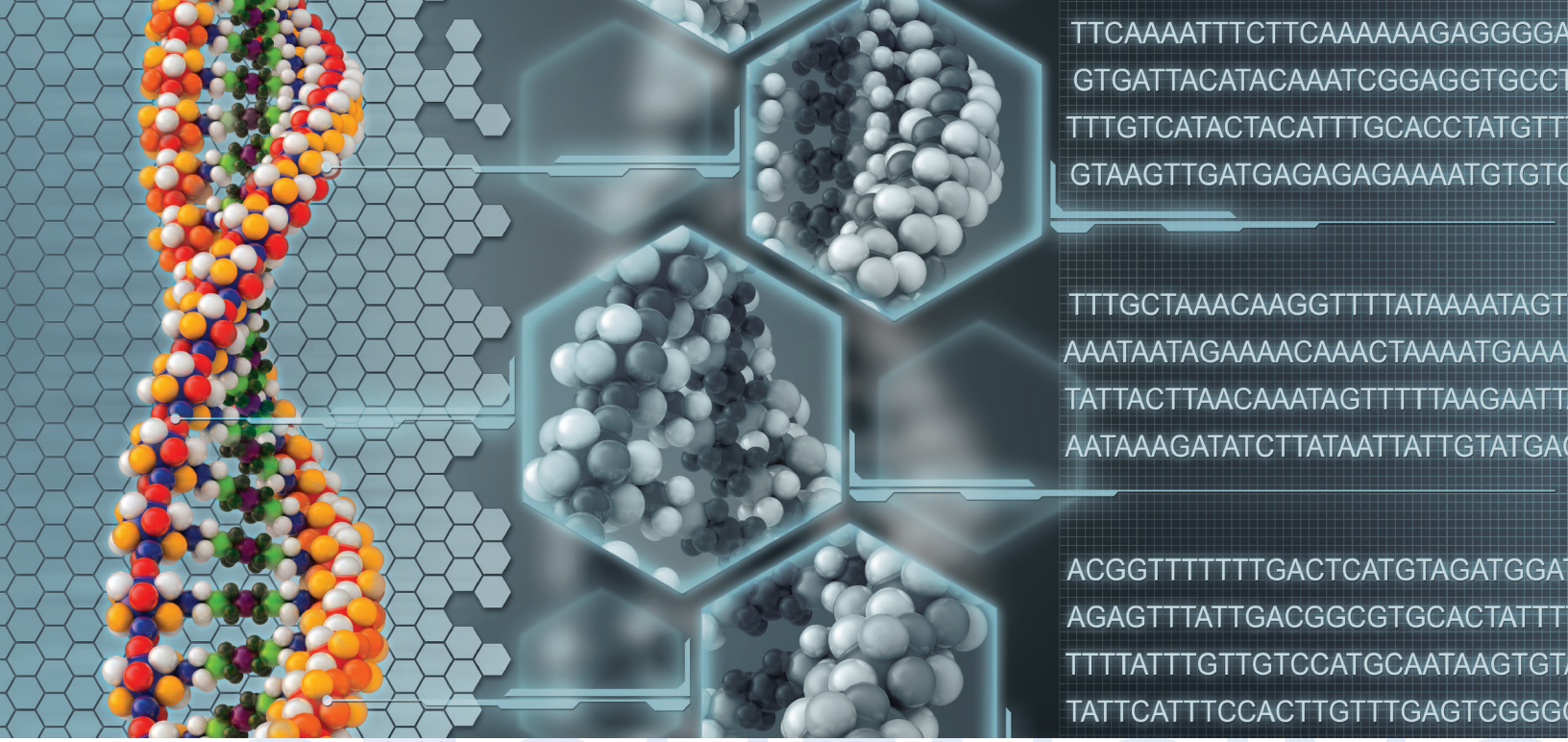


SEQUENCING

ACCESS THE POTENTIAL OF NEXT-GENERATION SEQUENCING

Fluidigm®

THE PROMISE OF NEXT-GENERATION SEQUENCING

Next-Generation Sequencing (NGS) technologies have transformed life science research. In addition to making it possible to sequence entire genomes, NGS makes possible new sequencing-based applications, such as targeted resequencing, to identify sequence variations relevant to cancer, disease research, and population genetics. The Access Array™ System, combined with next-generation resequencing, is a powerful approach for SNP identification, sequence variation and mutation detection, DNA methylation mapping, exon sequencing, and more—across hundreds of samples or more. Resequencing applications require sequencing targeted regions of interest from multiple samples, and are more cost-effective and easier to analyze than sequencing the entire genome of each sample. The true promise of NGS requires that the library preparation of target sequences be fast and easy enough to realize the full throughput potential. Current targeted resequencing technologies focus on the capture of large regions of interest in relatively few DNA samples, require large amounts of sample, and suffer from uneven representation of targeted regions.

ACCESS ARRAY SYSTEM: TARGET ENRICHMENT FOR NEXT-GENERATION SEQUENCING

The Access Array System is the first high-throughput, target-enrichment system designed to work with all of the major next-generation sequencing instruments. It enables the user to enrich hundreds of unique targets (such as exons) from a large number of samples, all at one time. The system combines the cost and throughput benefits of microfluidics with the proven performance and flexibility of PCR.

The Access Array™ System comprises a thermal cycler and a pre-PCR IFC Controller AX for loading samples, and a post-PCR IFC Controller Ax for harvesting amplified products.

LIBRARY GENERATION

Regardless of your choice of sequencer for targeted sequencing, you must create a library—a molecular construct that has the adaptors necessary for clonal amplification and/or sequencing amended to the ends of the region of interest. Because of the specificity and sensitivity of PCR, it is used in a variety of amplicon library creation strategies.

Any of these approaches to amplicon library preparation can easily lead to a bottleneck, because the number of samples and primer pairs escalates if libraries are prepared by conventional methods. The Access Array workflow allows you to conduct targeted resequencing experiments of otherwise unimaginable sample sets.

SCALABILITY

The Access Array System is highly scalable due to its modular design and simple workflow. A library prepared on a single Access Array Integrated Fluidic Circuit (IFC) is an ideal fit for complete experiments on a variety of benchtop sequencers. Multiple chips may be pooled together to generate libraries to match the throughput of the largest next-generation sequencers.

TARGETED RESEQUENCING, SIMPLIFIED

The Access Array System enables high-throughput targeted resequencing of the highest quality while reducing experimental inputs:

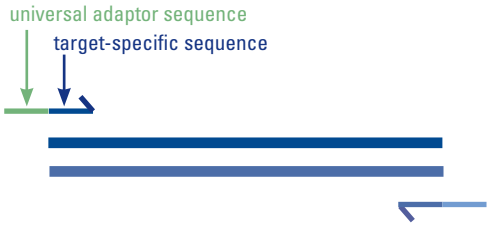
- Only 50 ng of total gDNA per sample are required, and a special FFPE protocol is available
- Targets and primer pairs are mixed on-chip, minimizing pipetting steps and hands-on time, and avoiding pipetting errors
- Reaction volumes are controlled by the microfluidic architecture of the chip, for highly consistent PCR reactions across and between experiments
- The Access Array barcode libraries contain adaptor sequences specific to the sequencing system of choice and come with up to 384 unique 10 position barcodes
- Primers and thermal cycling conditions are designed to minimize primer dimer formation and ensure uniformity across amplicons

In the amplicon tagging protocol, primers are designed to attach sample-specific barcode sequences, and sequencer-specific tags, to each PCR product. Barcode sequences allow up to 384 samples to be pooled and sequenced in one multiplex sequencing reaction.

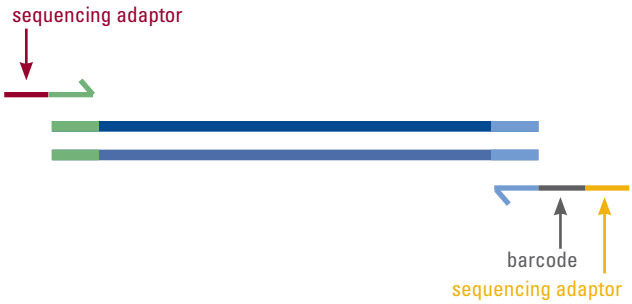
The Fluidigm four-primer amplicon tagging protocol as applied to Illumina sequencing is shown on the right. To apply the protocol to other next-generation sequencers, simply swap the barcode primers with the Illumina-specific adaptor sequences for those compatible with your sequencer of choice. Barcodes for other sequencers may be incorporated into the adaptors at both ends.

AMPLIFICATION, TAGGING, BARCODING

1. Hybridization of sequence-specific primers to appropriate region of genomic DNA. Primers contain universal tag sequences to allow binding of barcode primers.



2. Hybridization of barcode primers, which also contain a capture sequence appropriate for sequencer chemistry



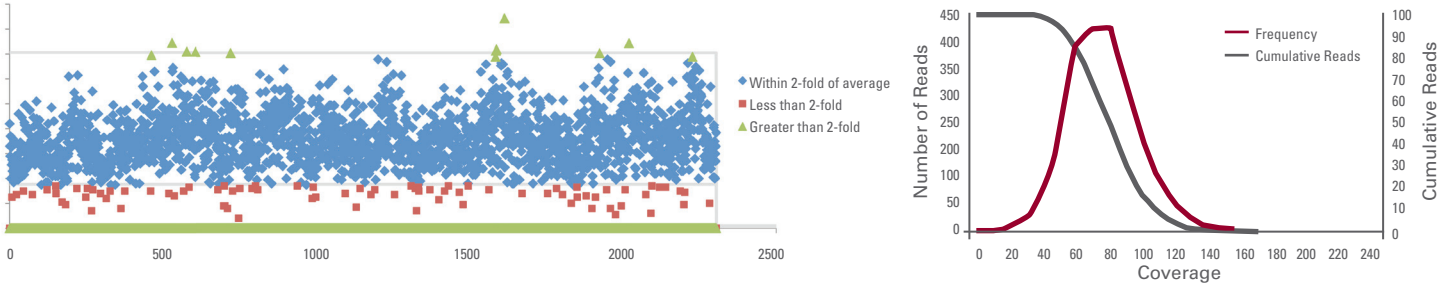
3. Final amplicon contains barcode sequence to identify parent DNA sample, and is tagged for capture and entry into emPCR.

FOUR-PRIMER AMPLICON TAGGING

While the Access Array System can be used for nearly any PCR-based enrichment strategy, four-primer amplicon tagging with Fluidigm protocol and reagents is an ideal method for creating amplicon libraries for any next-generation sequencing platform. In the four-primer amplicon tagging, the inclusion of a short Consensus Sequence (CS) Tag onto the 5′ end of the target-specific primer and the three-prime end of the sequencing adaptor allows the user to design and validate only target-specific primers regardless of the sequencer type or level of sample multiplexing required. The barcode oligos with CS linkers are universal reagents in the four-primer amplicon tagging protocol and can be used with any target-specific primer sequences, greatly reducing the number of unique oligos that are required to conduct multiple targeted resequencing experiments.

EVEN REPRESENTATION OF ENRICHED SEQUENCES

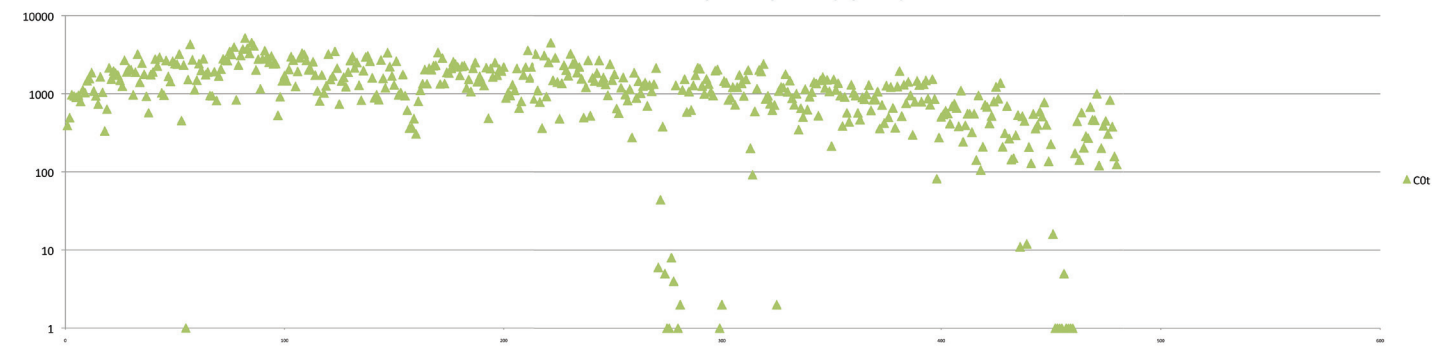
Access Array provides high-quality data with uniform sequence representation across multiple targets and samples, which is important for maximizing the utility of your sequence. The figure below shows the representation of 2,304 amplicons generated from 48 genomic DNA samples and 48 different sequence-specific primer sets. Greater than 95% of the amplicons demonstrate coverage within two-fold of the average. Better consistency of amplicon generation during library preparation means that you get a more even distribution of sequencing reads across all samples and spend less time and money on sequencing.



Uniform sequence representation of 48 unique amplicons from 48 genomic DNA samples

MULTIPLEX PCR FOR INCREASED SEQUENCE COVERAGE

The amount of sequence enriched per sample is highly scalable with multiplex PCR protocols. Each sample can be enriched using as few as 48 primer pairs in singleplex mode, up to as many as 480 primer pairs in 10-plex mode. The unique protocol and specific IFCs developed for the Access Array System results in no loss of data quality in terms of representation or uniformity when used in a 10-plex format.



Coverage achieved for 480 cancer specific exons in a 48 x 10-plex amplicon pool from a single 48.48 Access Array™ IFC

MAKE THE PROMISE OF NEXT-GENERATION TARGETED RESEQUENCING A REALITY

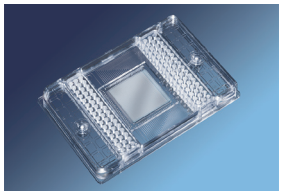
Access Array™ Target-Specific Primers

Access Array Target-Specific Primers allow you to take full advantage of your Access Array System with minimal experiment setup time while producing robust results. The Access Array System provides fast, simple, and inexpensive preparation of sequencing-ready libraries, helping you obtain your next-generation sequencing goals.

Unlike preformatted capture technologies, the Access Array Target-Specific Primers are custom designed to your requirements. When used with the Access Array System, the primers allow for preparation of up to 480 unique amplicons across 48 samples. Simply provide Fluidigm with your regions of interest for the human genome and we will design primer sets to amplify (and tag) PCR products for sequencing.

- 96-well plate(s) containing pooled forward and reverse primers
- Spreadsheet containing amplicon and primer sequences
- Links to the UCSC genome browser displaying mapped amplicon positions
- Electronic copies of the protocol

Access Array™ IFC



48.48 Access Array™ IFC

The Access Array IFC, a Fluidigm patented Integrated Fluidic Circuit, enables researchers to perform nanoliter-volume, high-throughput PCR. Each microfluidic reaction chamber can accommodate up to 10X independent amplicons, enabling the enrichment of up to 480 amplicons across 48 samples in a single run.

- HIGH THROUGHPUT—Simultaneously enrich targets of interest from 48 samples at a time. When used with the Access Array Barcode Library, each library is uniquely tagged so up to 384 samples can be pooled and sequenced in a single multiplex sequencing run with no additional library preparation.
- EASE OF USE—Requires only five manual steps and four hours to produce 48 sequencer-ready libraries (or a single multiplex of 48) from genomic DNA.
- OPEN PLATFORM—Compatible with any PCR-based enrichment technology, including amplicon tagging, long-range PCR and multiplex PCR. Libraries can be tagged to be compatible with all next-generation sequencing platforms, including the 454 and Illumina systems as well as the upcoming third generation sequencers.
- DATA QUALITY—Excellent sample and amplicon uniformity for high-quality data and powerful data analysis.

EASY WORKFLOW

An entire target enrichment experiment from genomic DNA to a finished amplicon library can be carried out with only five hands-on steps, and completed in four hours with minimal hands-on time.

COMPLETE EXPERIMENTS IN FIVE EASY STEPS

1. Samples and primers are loaded onto the 48.48 Access Array IFC, which is mounted on an SBS-compatible carrier, allowing sample loading with an 8-channel pipette or a liquid dispensing robot.
2. In the pre-PCR IFC Controller AX, samples and primers are automatically combined into 2,304 unique PCR reactions.
3. The IFC is placed in the FC1™ Cycler for target amplification.
4. After PCR, products from each sample are pooled on-chip in the post-PCR IFC Controller AX and pumped out for collection.
5. The 48 amplified and tagged products can be collected using a multi-channel pipette.

Five hands-on steps to generate 48 sequencer-ready libraries

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