Access Array™ Target-specific Primers

Access Array Target-specific Primers allow you to take full advantage of your Access Array System with minimal experimental 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 supply Fluidigm with your regions of interest for the human genome and we will design primer sets to amplify (and tag) PCR products for sequencing.

- 86-well plates 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

**DATA QUALITY—**Excellent sample and amplicon uniformity for high-quality data and powerful data analysis. **EASE OF USE—**Requires only five manual steps and four hours to produce 48 sequencer-ready libraries (or a single multiplex of 480 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. **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.

**COMPLETE EXPERIMENTS IN FIVE EASY STEPS**

**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.

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**MAKING THE PROMISE OF NEXT-GENERATION TARGETED RESEQUENCING A REALITY**

**ACCESS THE POTENTIAL OF NEXT-GENERATION SEQUENCING**

**PUBLICATIONS**


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Circuit (IFC) is an ideal fit for complete experiments prepared on a single Access Array Integrated Fluidic: its modular design and simple workflow. A library creation strategy is an excellent example of how to efficiently generate libraries for Next-Generation Sequencing (NGS) technologies, even from a limited amount of sample. Resequencing applications require scaling up of libraries, and 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 enriched using 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 reactions across and between experiments.

The Access Array barcode libraries contain adapter sequences specific to the sequencing system of choice and come with up to 384 unique 10 position barcodes. For other sequencers, simply swap the barcode primers with Illumina-specific adaptor sequences for those barcodes.

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 illum-run-specific adapter sequences for those compatible with your sequencer of choice. Barcodes for other sequencers may be incorporated into the adapters at both ends.

Uniform sequence representation of 48 unique amplicons from 48 genomic DNA samples. The Access Array System is highly scalable for uniformity when used in a 10-plex format. The unique protocol and specific adapters developed for the Access Array System result in no loss of data quality in terms of representation or uniformity when used in a 10-plex format.

**MULTIPLEX PCR FOR INCREASED SEQUENCING COVERAGE**

The amount of sequence enriched per sample is highly scalable with multiple 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 multiplex mode. At least 10 different primers are required for target-specific sequencing. The Fluidigm four-primer amplicon tagging protocol is an ideal fit for complete experiments on a variety of benchtop sequencers. Multiple chips may be prepared together to generate libraries to match the throughput of the largest next-generation sequencers.
Circuit (IFC) is an ideal fit for complete experiments prepared on a single Access Array Integrated Fluidic.
The Access Array System is highly scalable due to its modular design and simple workflow. A library is prepared on a single Access Array Integrated Fluidic Circuit (IFC) is an ideal fit for complete experiments.

TARGETED RESequENCING, SIMPLIFIED
The Access Array System enables high-throughput targeted resequencing of the highest quality while reducing experimental inputs:
- One-to-one ratio of total DNA per sample are required, and a special FFPE protocol is available
- Targets and primer pairs are universally optimized for 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 reaction 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 dimmer formation and ensure uniformity across amplifications.

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

LIBRARY GENERATION
Regardless of your choice of sequence for targeted sequencing, you must create a library—a molecular library that contains the adapters necessary for clonal amplification and/or sequencing extended to the end of the region of interest. Because of the specificity and sensitivity of PCR, it is used in a variety of amplicon library creation strategies.

By any of these approaches to amplicon library preparation, it can be enriched using as few as 48 primer pairs in singleplex mode, up to as many as 480 primer pairs specific primer sets. Greater than 95% of the amplicons demonstrate coverage within two-fold of the average.

The promise of NGS requires that the library preparation of target sequences be fast and easy enough to realize the full potential of the sequencing technology. Current targeted resequencing technologies focus on the capture of large regions of interest in relatively low DNA samples, requiring large amounts of sample, and suffer from uneven representation of targeted regions.

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, at all one time. The system combines the cost and throughput benefits of microfluidics with the proven performance and flexibility of PCR.

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, at all one time. The system combines the cost and throughput benefits of microfluidics with the proven performance and flexibility of PCR.

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

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

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

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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 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.

QUALITY AND YIELD
Regardless of your choice of sequence for targeted sequencing, you must create a library—a molecular library that contains the adapters necessary for clonal amplification and/or sequencing extended to the end 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 be easily loaded into a bottleneck, because the number of samples and primer pairs scales up if libraries prepared by conventional methods. The Access Array workflow allows you to conduct targeted resequencing experiments of otherwise unattainable sample sets.

The Access Array System is highly scalable due to its modular design and simple workflow. A library is prepared on a single Access Array Integrated Fluidic Circuit (IFC) is an ideal fit for complete experiments.

SCALABILITY
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Even Representation of Enriched Sequences
Access Array provides high-quality data with uniform sequence representation across multiple targets and samples, which is required 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 sequences 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.

See more. Resequencing applications require sequencing throughput of the largest next-generation sequencers.

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, exome sequencing, and more—across hundreds of samples or more. Resequencing applications require sequencing throughput of the largest next-generation sequencers.

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 48 ng of total gDNA per sample are required, and a special F9PPE protocol is available
- Targets and primer pairs are amplified using 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 reactions across and between experiments

The Access Array barcode libraries contain adapter sequences specific to the sequencing system of choice and come with up to 384 unique pairs. Barcoded libraries can be pooled together to generate libraries to match the throughput of the largest next-generation sequencers.

In the amplicon tagging protocol, primers are applied to other next-generation sequencers, simply swap the barcode adapters with the Illumina-specific adaptor sequences for those sequencers, simply swap the barcode primers with those specific to the sequencing system of interest in relatively few DNA samples, requiring large amounts of sample, and suffer from uneven representation of targeted regions.

Any of these approaches to amplicon library preparation can easily lead to a bottleneck, because the number of samples and primer pairs scales with the number of unique oligos that are required to conduct the targeted sequencing experiments of otherwise unattainable sample sets.

The Access Array System is highly scalable due to its modular design and simple workflow. A library is prepared on a single Access Array Integrated Fluidic Circuit (IFC) and is ideal for complete experiments 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, requiring large amounts of sample, and suffer from uneven representation of targeted regions.

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

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 sequencing.

DATA QUALITY—Excellent sample and amplicon uniformity for high-quality data and powerful data analysis.

EASY WORKFLOW

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COMPLETE EXPERIMENTS IN FIVE EASY STEPS

1. Samples and primers are loaded into the 48.48 Access Array™ IFC, which is mounted on an 880-compatible carrier, allowing sample loading with an 8-channel dispensing robot.

2. In the post-PCR IFC Controller, all samples are automatically combined into a 2,304 unique PCR reactions.

3. The IFC is placed into the FC1™ Cycler for target amplification.

4. After PCR, products from each sample are pooled on-chip in the post-PCR IFC Controller, and pumped out for collection.

5. The 48 amplified and uniquely tagged products can be collected using a multi-channel pipette.

PUBLICATIONS

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**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 as to produce 384 samples that 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 480 from genomic DNA).

**OPEN PLATFORM**—Compatible with any PCR-based enrichment technology, including amplification 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.

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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. **Collect samples**
   - Samples and primers are loaded into the 48.48 Access Array IFC, which is mounted on an IFC postprocessor. Samples are loaded using a 48-channel pipette or a liquid with an 8-channel SBS-compatible carrier, which is mounted on an 48.48 Access Array IFC.

2. **Primer loading**
   - Samples and primers are loaded into the 48.48 Access Array IFC, which is mounted on an IFC postprocessor. Samples are loaded using a 48-channel pipette or a liquid with an 8-channel SBS-compatible carrier, which is mounted on an 48.48 Access Array IFC.

3. **Post-PCR IFC Controller**
   - After PCR, products from each sample are pooled directly into the 48.48 Access Array IFC postprocessor for collection.

4. **Multiplex PCR**
   - The 48 amplified and tagged products can be collected using a multi-channel dispenser.

5. **Data analysis**
   - The 48 amplified and tagged products can be collected using a multi-channel dispenser.

**APPLICATIONS**

- **Sequencing for diagnostic testing of congenital disorders**
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**PUBLICATIONS**

