Application Note

QIAseq™ SPE technology for Illumina®: Redefining amplicon sequencing

Amplicon-based enrichment and sequencing takes advantage of PCR workflows to turn amplicons that represent regions of interest into library fragments that can be sequenced. It is more advantageous than other enrichment approaches for biomarker profiling of low-quality and low-quantity DNA samples, and has numerous benefits including:

- Requirement for low DNA input
- Tolerance to low-quality DNA samples
- Fast library construction protocols
- Efficient coverage of hotspots

However, current amplicon-based enrichment and sequencing methods that rely on either two-primer designs or nested PCR approaches suffer from the following inefficiencies:

- Low uniformity and lack of coverage due to primer dropouts
- Requirement for multiple primer pools resulting in increased DNA input per sample (see Figure 1)
- Need of a large contiguous stretch of the targeted regions to cover two primers for a nested PCR design (see Figure 2)
- Inflexibility with regards to adding primers to increase panel content
- Low library complexity as both start and stop positions are defined by primers (see Figure 3)
- Inefficiency to cover entire CDS regions

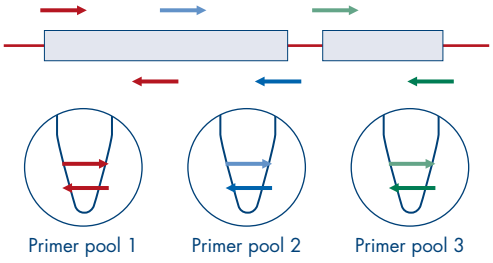

Figure 1. Current amplicon approaches require multiple primer pools and increase the risk of primer dropouts.

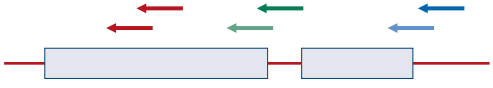

Figure 2. A large contiguous region is required in nested PCR approaches, limiting the flexibility in primer design.

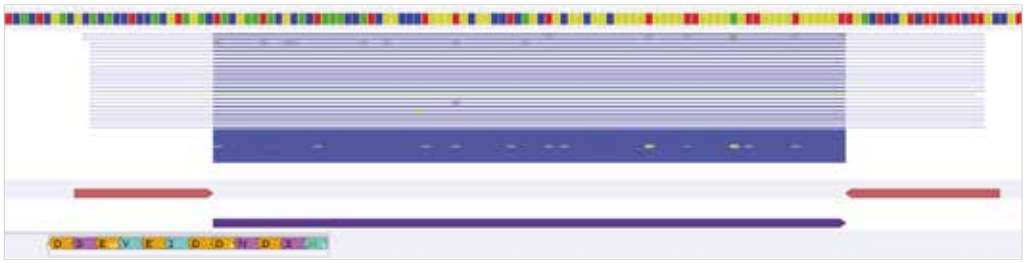

Figure 3. Libraries generated by traditional two-primer amplicon-based enrichment and sequencing methods show low complexity.

Single primer extension (SPE) is a revolutionary approach that redefines amplicon-based enrichment and sequencing. With SPE, each genomic region is targeted by only one region-specific primer plus a universal adapter primer that binds to sequences introduced through library adapters (Figure 4).

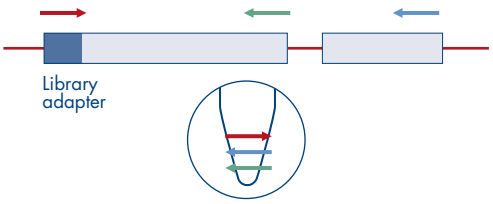

Figure 4. Primer design using single primer extension (SPE). Amplicon-based enrichment is performed in a single reaction regardless of the size of targeted regions.

SPE overcomes the challenges of traditional amplicon-based approaches of two-primer and nested PCR designs to deliver the following advantages:

Uniform coverage by reducing the number of required primers and pools

SPE utilizes a reduced number of required primers resulting in highly uniform enrichment and sequencing due to a reduction in primer dropouts (Figure 5).

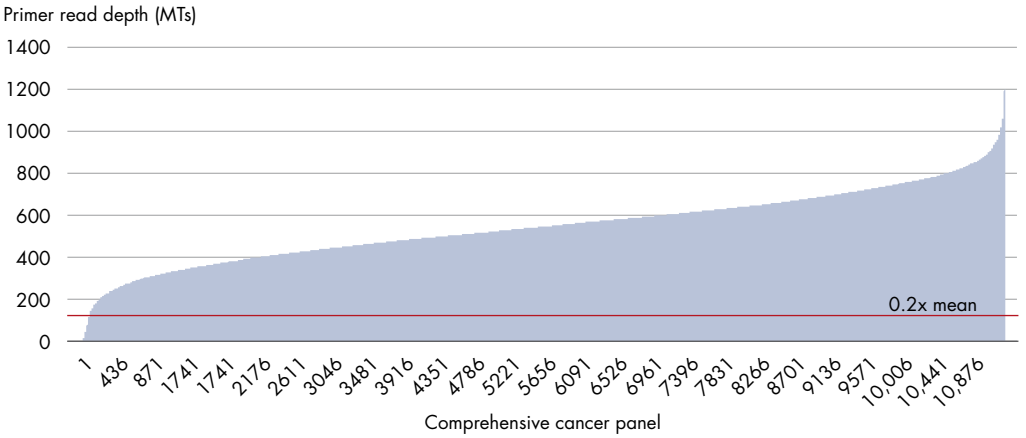

Figure 5. Highly uniform enrichment and sequencing. In this experiment, 6000 SNPs were enriched from 20 ng of NA12878 DNA. Library was constructed for sequencing on a MiSeq®, with 4,000,000 reads generated. The panel achieved a uniformity of 99.5% at 0.2x of mean coverage, and 96% at 0.5x of mean coverage.

Comprehensive coverage due to flexibility in primer design

As opposed to traditional two-primer amplicon and nested PCR approaches, SPE provides a high degree of primer design flexibility, allowing for primers to be aggressively tiled across targeted regions (Figure 6).

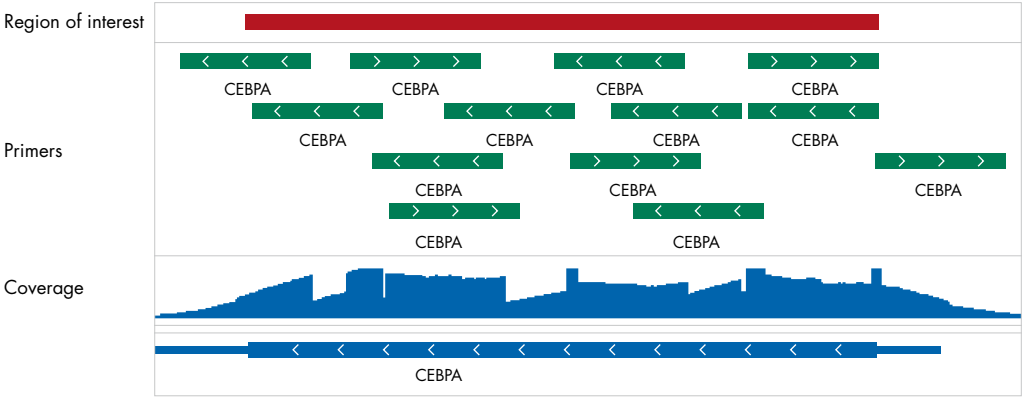

Figure 6. Comprehensive coverage of CEBPA is facilitated by the flexibility in primer design with SPE. Thirteen primers are designed against CEBPA to ensure complete coverage.

Ability to easily increase panel content

Because SPE requires only one region-specific primer, panel content can be easily increased to accommodate new scientific and biological findings (Figure 8).

Generation of complex libraries

Since each targeted region is defined by one fixed, region-specific primer, and one universal primer, the resulting library is more complex than libraries generated with traditional two-primer amplicon approaches (Figure 7).

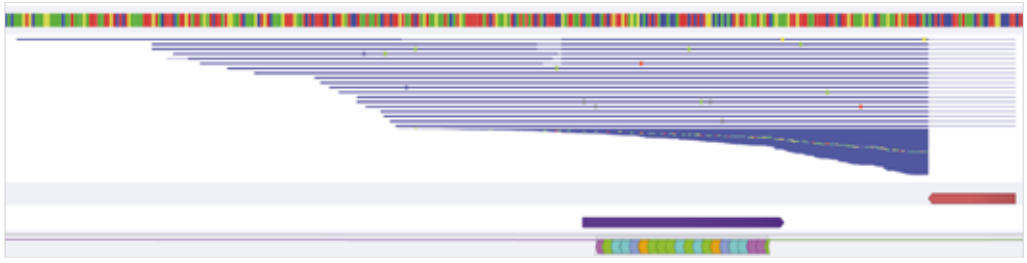

Figure 7. Complexity of a library generated with SPE.

Efficient coverage of both hotspots and entire CDS region

With SPE, you do not need to settle for hotspots. Instead, up to 20,000 primers can be pooled in an individual pool of primers, resulting in maximum coverage of around 500 genes, regardless of whether you are targeting hotspots, CDS, intronic regions, promoters, etc.

Customization made easy

The robustness of SPE simplifies the designing and building of custom panels. The QIAseq™ Targeted DNA Custom Panel Builder takes your input of genes and/or genomic coordinates to design SPE primers, delivering custom panels that reliably work at the first attempt.

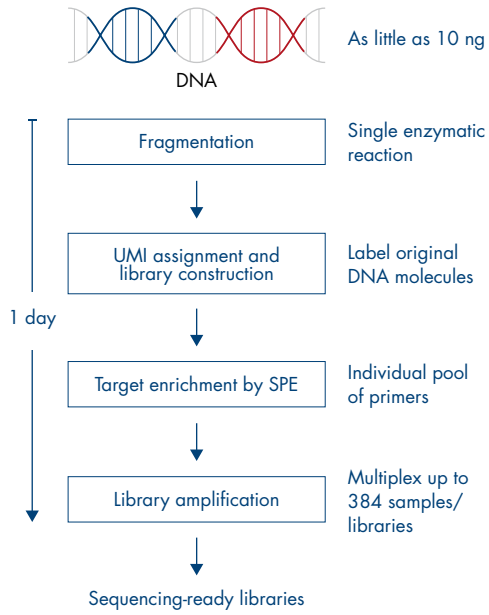

Figure 8. QIAseq SPE procedure.

Ordering Information

Product	Contents	Cat. no.
Human Breast Cancer Panel	Kit containing all reagents (except indices) for targeted DNA sequencing; fixed panel for 12 or 96 samples	DHS-001Z 333502 (12 samples) 333505 (96 samples)
Human Colorectal Cancer Panel	Kit containing all reagents (except indices) for targeted DNA sequencing; fixed panel for 12 or 96 samples	DHS-002Z 333502 (12 samples) 333505 (96 samples)
Human Myeloid Neoplasms Panel	Kit containing all reagents (except indices) for targeted DNA sequencing; fixed panel for 12 or 96 samples	DHS-003Z 333502 (12 samples) 333505 (96 samples)
Human Lung Cancer Panel	Kit containing all reagents (except indices) for targeted DNA sequencing; fixed panel for 12 or 96 samples	DHS-005Z 333502 (12 samples) 333505 (96 samples)
Human Actionable Solid Tumor Panel	Kit containing all reagents (except indices) for targeted DNA sequencing; fixed panel for 12 or 96 samples	DHS-101Z 333502 (12 samples) 333505 (96 samples)
Human BRCA1 and BRCA2 Panel	Kit containing all reagents (except indices) for targeted DNA sequencing; fixed panel for 12 or 96 samples	DHS-102Z 333502 (12 samples) 333505 (96 samples)
Human BRCA1 and BRCA2 Plus Panel	Kit containing all reagents (except indices) for targeted DNA sequencing; fixed panel for 12 or 96 samples	DHS-103Z 333502 (12 samples) 333505 (96 samples)
Human Pharmacogenomics Panel	Kit containing all reagents (except indices) for targeted DNA sequencing; fixed panel for 12 or 96 samples	DHS-104Z 333502 (12 samples) 333505 (96 samples)
Human Mitochondria Panel	Kit containing all reagents (except indices) for targeted DNA sequencing; fixed panel for 12 or 96 samples	DHS-105Z 333502 (12 samples) 333505 (96 samples)
Human Inherited Disease Panel	Kit containing all reagents (except indices) for targeted DNA sequencing; fixed panel for 12 or 96 samples	DHS-3011Z 333512 (12 samples) 333515 (96 samples)
Human Comprehensive Cancer Panel	Kit containing all reagents (except indices) for targeted DNA sequencing; fixed panel for 12 or 96 samples	DHS-3501Z 333512 (12 samples) 333515 (96 samples)

Ordering Information

Product	Contents	Cat. no.
QIAseq Targeted DNA Custom Panels	Kit containing all reagents (except indices) for targeted DNA sequencing; custom panel for 96 samples	Varies
QIAseq 12-Index I (48)	Box containing oligos, enough for a total of 48 samples, for indexing up to 12 samples for targeted panel sequencing on Illumina platforms	333714
QIAseq 96-Index I Set A (384)	Box containing oligos, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; one of four sets required for multiplexing 384 samples	333727
QIAseq 96-Index I Set B (384)	Box containing oligos, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; two of four sets required for multiplexing 384 samples	333737
QIAseq 96-Index I Set C (384)	Box containing oligos, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; three of four sets required for multiplexing 384 samples	333747
QIAseq 96-Index I Set D (384)	Box containing oligos, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Illumina platforms; four of four sets required for multiplexing 384 samples	333757
QIAseq 12-Index L (48)	Box containing oligos, enough for a total of 48 samples, for indexing up to 12 samples for targeted panel sequencing on Ion Torrent™ platforms	333764
QIAseq 96-Index L (384)	Box containing oligos in arrays, enough for a total of 384 samples, for indexing up to 96 samples for targeted panel sequencing on Ion Torrent platforms	333777

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Explore the QIAseq Targeted DNA Custom Panel Builder at **www.qiagen.com/QIAseqDNAcustom**