Ovation® Target Enrichment System

A complete solution for custom targeted sequencing of genomic DNA

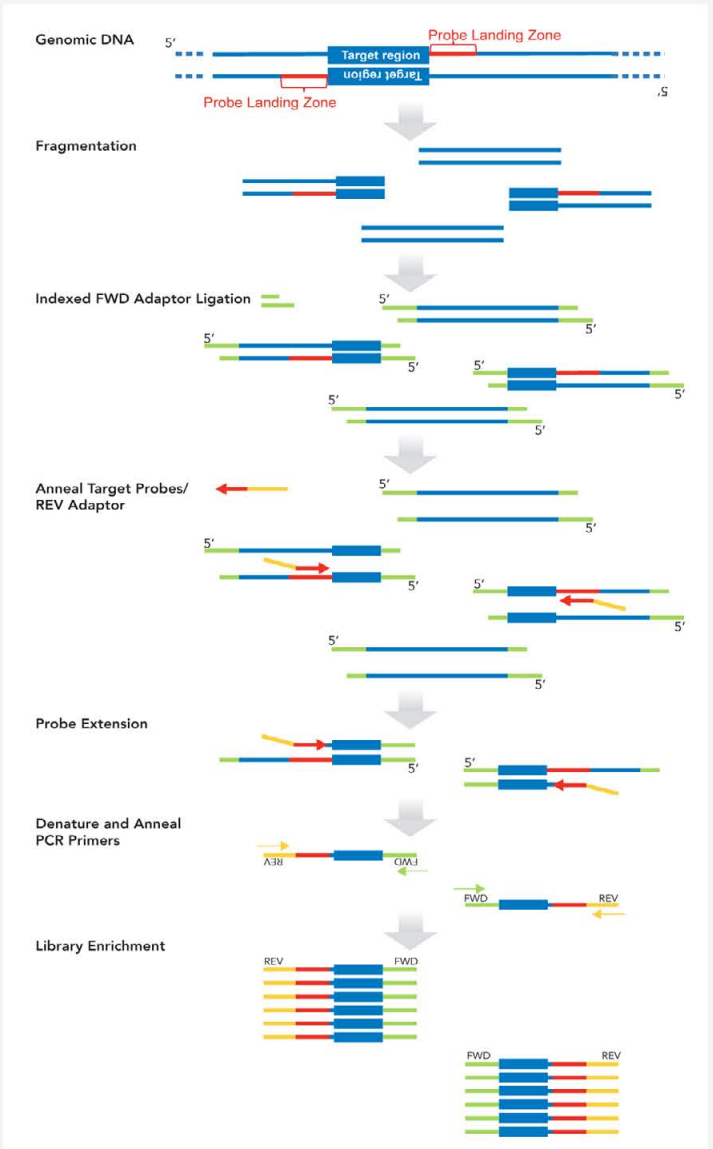
Highlights of the Ovation Target Enrichment System

- **Flexible probe design:** Create custom targeted enrichment panels for genomic regions of interest from a few kb up to 10 Mb with a broad range of samples and as little as 10 ng genomic DNA input
- **Unique technology:** Single Primer Enrichment Technology (SPET) independently interrogates both strands of the target, enabling robust variant verification with only single-end sequencing
- **Simple and fast:** Add and incubate workflow speeds up time to results by minimizing hands-on time and eliminating laborious capture steps and time-consuming multi-day hybridizations
- **Superior results:** Generate high value targeted sequence data with excellent specificity (>80% bases on-target), minimal dropouts and even coverage.

Introduction

The core of the Ovation Target Enrichment System is a proprietary method developed by NuGEN called Single Primer Enrichment Technology (SPET). As shown in **Figure 1**, probes are placed adjacent to the target region of interest on both strands. The flexible probe design algorithm allows for tiling

FIGURE 1 Single Primer Enrichment Technology (SPET) in the Ovation Target Enrichment System.



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of probes throughout large contiguous regions and for closer spacing of probes to accommodate samples that are compromised in quality, such as FFPE DNA.

The first steps in the target enrichment process are fragmentation and end repair of the DNA. Following end repair, indexed forward sequencing adaptors are ligated to the 5′ ends of the randomly fragmented DNA. Once individual samples are barcoded by the ligation of the indexed adaptors, the samples are pooled together for probe annealing and extension. The probe annealing and extension process can be completed in a short 3 hours or be allowed to proceed overnight to facilitate more flexibility in the workflow.

The probe sequences are comprised of a 3′ sequence uniquely complementary to the probe landing zone and a 5′ region that contains a portion of the reverse sequencing adaptor. During the annealing and extension process, primers hybridize to the probe landing zone and are extended by DNA polymerase through the target region and the ligated forward adaptor, resulting in the target sequence being flanked by forward and reverse adaptor sequences. PCR amplification with primers specific to the forward and reverse adaptors completes the library construction process by enriching for molecules containing the target region. The entire process can be completed in a single day or two short half-days. The amplified library is ready for quantification and sequencing.

The novel forward sequencing adaptor structure is illustrated in **Figure 2**. Relocating the index to the forward adaptor allows for the unambiguous tagging of individual samples prior to multiplex probe hybridization and extension. The index is an 8-base edit-3 distance index followed by six random nucleotides (N6). The purpose of the N6 sequence is to identify duplicate reads generated by PCR without requiring paired-end sequencing. The combination of the mapped forward read sequence and the N6 sequence allows for unambiguous marking of PCR duplicates. A simple script (provided by NuGEN) analyzes the sequence and N6 data, marking PCR duplicates and generating a sequence file that removes all PCR duplicates. This powerful capability to unambiguously identify true PCR duplicates greatly enhances variant identification and verification.

Table 1 contains basic sequence metric data derived from a broad variety of custom Ovation Target Enrichment System panels. The table shows data from custom target panels ranging from 16 Kb to 7.1 Mb in size. NuGEN'S pre-defined Ovation Cancer Panel Target Enrichment System and Ovation Mitochondrial Genome Panel Target Enrichment System are readily available catalog products and are valuable for evaluating the system. NuGEN's novel adaptor design and high efficiency ligation minimize adaptor dimer formation and allows an exceptionally

FIGURE 2 Adaptor Structure with gDNA Target Insert.

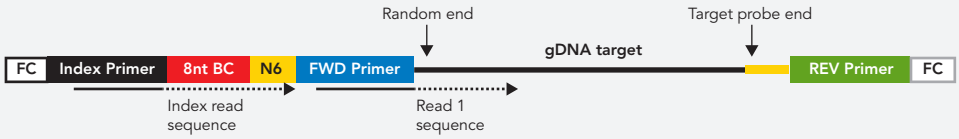


TABLE 1 Target enrichment alignment metrics.

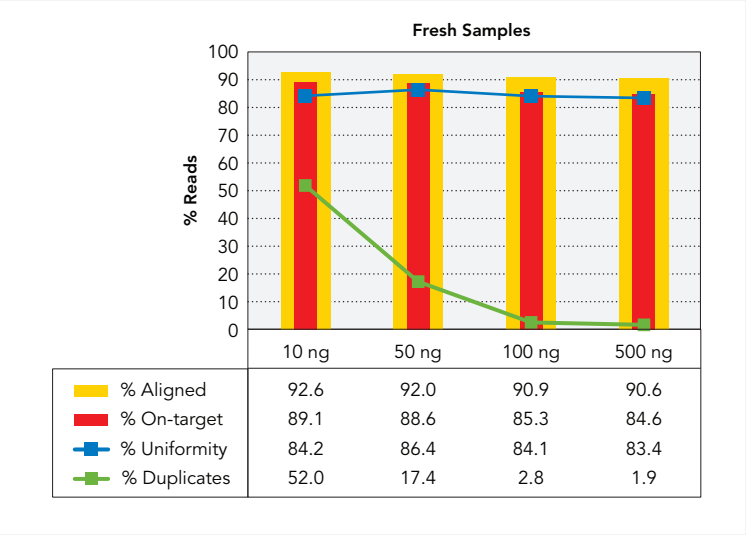
Target	Reads	% Aligned	% On-Target	% Uniformity
Large Test Panel (7.1 Mb)	7.1M	94.1	88.8	80
ABRF Test Panel (3.5 Mb)	1.0M	87.8	97.9	69
Ovation Cancer Panel (1 Mb)	3.5M	95.4	86.8	85
Custom (0.6 Mb)	3.2M	95.8	84.8	91
Ovation Mitochondrial Genome Panel (16 Kb)	1.3M	99	93	99

high percentage of read alignment to be achieved. The high read alignment coupled with a high percentage of bases on-target (>80% on-target across the board) increases both the value and depth of targeted sequencing studies. Consistency of target coverage (Uniformity) ensures that maximum valuable information can be obtained from minimum sequence.

The graph in **Figure 3** illustrates the effect of input mass on target enrichment metrics. From 10 ng up to 500 ng input, the alignment, on-target and uniformity metrics remain essentially unchanged. However, PCR duplicates increase as the input decreased due to a reduction in the complexity of the sample in terms of the number of independent genomes and therefore the number of potential independent fragments that start at the same breakpoint. Despite the elevated number of PCR duplicates, the on-target and uniformity of coverage remains high, indicating a minimum degree of PCR-induced bias.

Variant detection in clinical samples is a primary application of targeted resequencing. In order to assess the ability of the Ovation Target Enrichment System to properly detect low frequency variants in a mixed sample we created specific mixtures of genomic DNA from two HapMap samples with known variants at specific locations within the target range of the cancel panel. Genomic DNAs from two samples, NA12878 & NA19238, were mixed together in specific mass ratios (1/99, 5/95, 20/80 and 50/50). Target enriched libraries from 100 ng of each gDNA mixture were sequenced and NA12878 SNPs were measured in each mixed sample. As the graph in **Figure 4** illustrates, the frequency of NA12878 detection was concordant with the percentage of the NA12878 gDNA in the mixed sample. In a 1% mixture, over 70% of the SNPs were detected demonstrating the sensitivity of the system in faithfully representing the content of the target sequences in the sample.

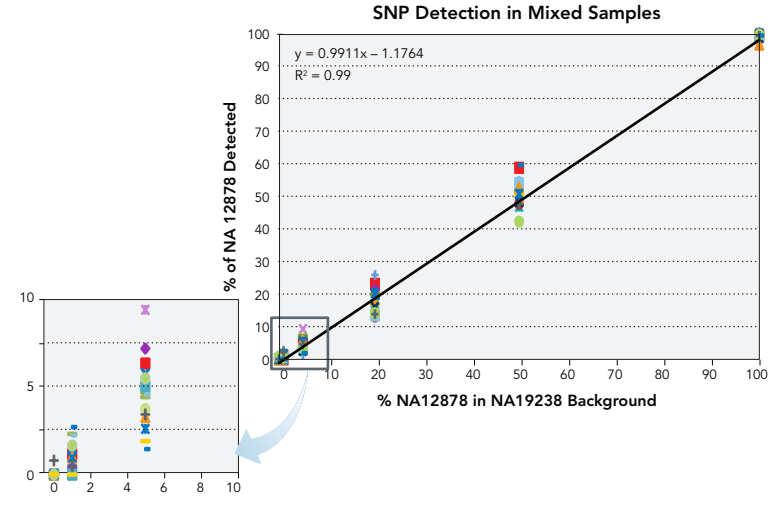
FIGURE 3 Effect of Input Mass on Sequence Metrics.



	10 ng	50 ng	100 ng	500 ng
% Aligned	92.6	92.0	90.9	90.6
% On-target	89.1	88.6	85.3	84.6
% Uniformity	84.2	86.4	84.1	83.4
% Duplicates	52.0	17.4	2.8	1.9

Normalized to 1M reads per library.

FIGURE 4 Variant Detection in Mixed HapMap Samples.



Most samples of interest to clinical researchers have been preserved prior to extraction of the genomic material. DNA derived from formalin-fixed paraffin-embedded (FFPE) tissue is generally compromised in quality and therefore resistant to many genomic assays due to the fragmented nature of the material as well as the presence of DNA-DNA and DNA-protein cross-links. The dynamic probe design algorithm employed by the Ovation Target Enrichment System accommodates the expected smaller size fragments of FFPE genomic DNA by placing probes at a higher density within target regions and closer to the target edges to ensure that any available fragments containing target can be captured and enriched. **Table 2** shows the target enrichment sequence metrics on a set of FFPE genomic DNA samples that were processed using the Ovation Cancer Panel FFPE Target Enrichment System. Despite the compromised state of the FFPE samples, the data demonstrate that significant enrichment and even coverage can be achieved using this valuable resource.

Conclusion

The Ovation Target Enrichment System is a powerful and flexible tool for targeted resequencing, combining the low input capabilities of amplicon-based strategies with the custom target flexibility of traditional capture methodologies in a simple, short, single-tube assay.

TABLE 2 Ovation Cancer Panel FFPE Target Enrichment System Alignment Metrics.

Input (FFPE gDNA)	Reads	% Aligned	% On-Target	% Uniformity
100 ng Liver	0.4M	93.5	81.6	76.1
100 ng Lung	0.7M	95.2	82.9	74.2
500 ng Colon Normal	0.7M	93.4	86.8	70.1
500 ng Colon Tumor	1.8M	94.5	84.8	82.2

ORDERING INFORMATION

Part No.	No. of Reactions
Ovation Cancer Panel Target Enrichment System	
9079-08	8 reactions
9079-32	32 reactions
9079-96	96 reactions
Ovation Custom Target Enrichment System	
9###-32	32 reactions
9###-96	96 reactions

Part No.	No. of Reactions
Ovation Cancer Panel FFPE Target Enrichment System	
9099-08	8 reactions
9099-32	32 reactions
9099-96	96 reactions
Ovation Human Mitochondrion Target Enrichment System	
9087-08	8 reactions
9087-32	32 reactions
9087-96	96 reactions

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