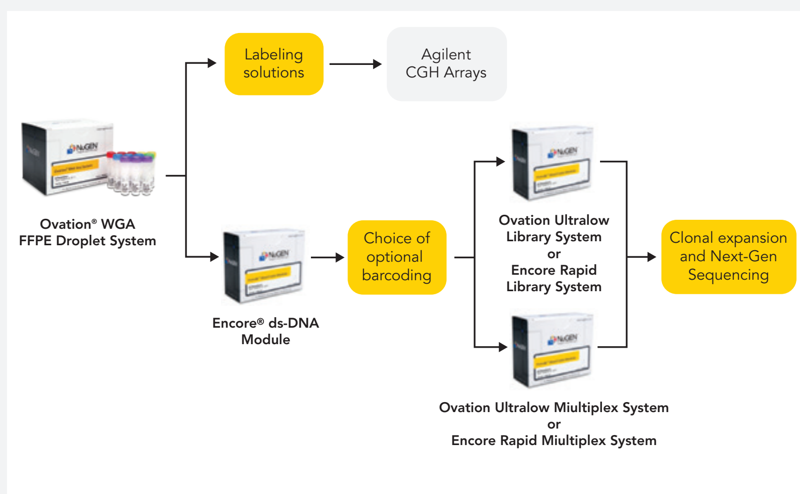
Ovation® WGA FFPE System

The first linear WGA technology for DNA amplification from FFPE samples for use with array CGH and Next-Generation Sequencing workflows

Highlights of the Ovation WGA FFPE System

- **Genomic analysis from previously inaccessible samples:** Leverage the power of SPIA® technology to generate µg amounts of amplified DNA from 100 ng genomic DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue for accurate, unbiased representation of the genome
- **Flexible downstream analysis:** Analyze genomic aberrations from FFPE samples by array-based Comparative Genomic Hybridization (aCGH), or by Next-Generation Sequencing using any of the leading sequencing platforms
- **Superior technical performance suited to a range of DNA sequencing applications:** High reproducibility, low error rates, no positional bias and minimal chimeras facilitates targeted resequencing for variation calls (SNVs), whole genome sequencing and genome partitioning

FIGURE 1 NuGEN's Portfolio of Products for aCGH and NGS Analysis Using gDNA from FFPE Samples



Introduction

Array-based Comparative Genomic Hybridization (aCGH) is a good solution for higher resolution analysis of genomic structural changes including deletions and amplifications. Increased accuracy is seen with Next-Generation Sequencing (NGS), in the detection of both large structural rearrangements as well as single nucleotide variations. Each of these techniques is used for analyzing precious sample types—especially archived material from clinical specimens. However, genomic DNA (gDNA) from FFPE samples, which represents the largest source of archival DNA, and often can be connected to patient data through healthcare, disease and population registries, is frequently not suitable for analysis by aCGH or NGS due to several reasons, such as the small amount of recovered DNA, a high degree of degradation and cross-linking between DNA strands.

NuGEN has developed a whole genome amplification system for FFPE samples, which for the first time provides linear amplification of DNA matched to workflows used in both aCGH and NGS. Powered by NuGEN's proprietary Single Primer Isothermal Amplification (SPIA) technology, The Ovation WGA FFPE System provides all reagents to generate 3–5 µg of amplified SPIA product from 100 ng of genomic DNA in less than 7 hours. The SPIA product can then be labeled for analysis on Agilent aCGH Microarrays, or further processed using the Encore® ds-DNA Module for construction of NGS libraries for the leading platforms. For users of the Illumina sequencing platforms, the Encore ds-DNA Module integrates directly with the Ovation Ultralow Multiplex Systems or Encore Rapid Multiplex Systems, providing a complete end-to-end solution for NGS sample preparation using gDNA from FFPE samples.

With NuGEN's new Ovation WGA FFPE System you can survey the entire genome for structural changes with aCGH, explore detailed sequence variations with NGS, or use these methods together with the same starting DNA for a more complete and accurate understanding of genomic diversity **(Figure 1).**

NuGEN imagine more from less®

TABLE 1 Robust aCGH Performance Metrics from 100 ng gDNA

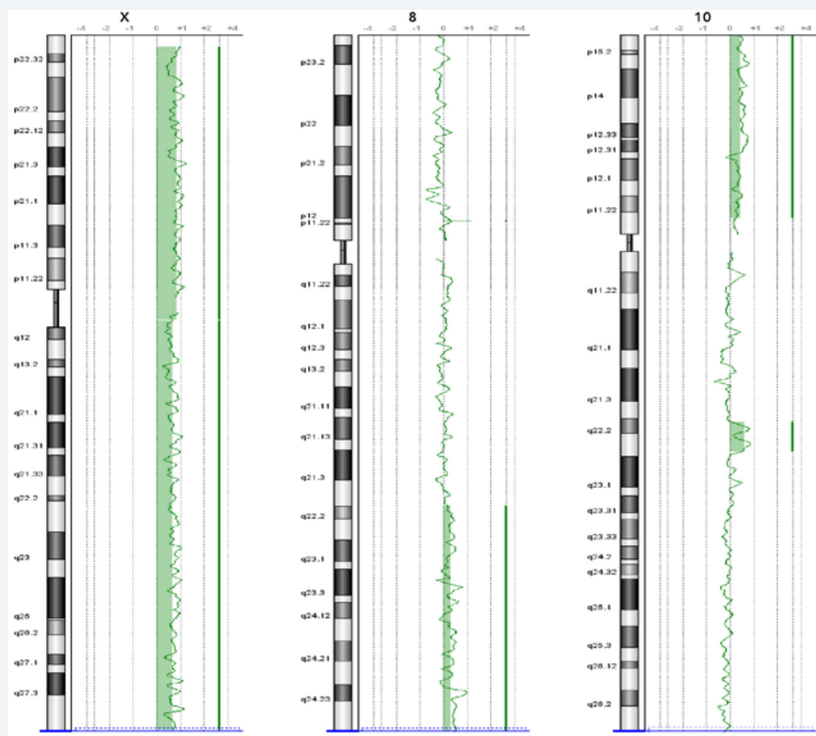
	DLR Spread	Signal To Noise Green	Signal To Noise Red	Signal Intensity Green	Signal Intensity Red	BG Noise Green	BG Noise Red
FFPE Human Breast Tumor-1	0.49	228	202	410	749	1.79	3.71
FFPE Human Breast Tumor-2	0.54	209	323	531	1705	2.54	5.28
FFPE Human Stomach Tumor	0.43	197	287	441	1360	2.24	4.74
Fresh Frozen Human Breast Tumor	0.13	385	340	725	1118	1.88	3.29

Robust aCGH Performance for the Most Challenging Samples

Genomic analysis by aCGH has been a broadly used, high resolution tool to assess genomic architecture and to detect genomic changes relative to a reference control. The utility of this technique is often compromised with FFPE samples due to the high degree of DNA degradation and cross-linking caused by the fixation process. Performance metrics from aCGH experiments using our Ovation WGA FFPE System are summarized in **Table 1**.

One hundred ng of starting material from three different tumor samples derived from FFPE and one fresh frozen (FF) samples were amplified, labeled using BioPrime Total labeling kit (Invitrogen Cat. # A10963-011), hybridized to Agilent 4x44k Human aCGH Microarrays, and processed according to the manufacturer. The DNA from the fixed tumor samples was isolated from five to six year old FFPE blocks while the normal reference DNA was a sex-mismatched sample isolated from a six year old fixed liver block. The Derivative Log Ratio Standard Deviation or DLRSpread value is an estimate of the noise of the log ratios on the array and, therefore, an indicator of confidence in aberration calls using algorithms such as ADM-1 and ADM-2 within Agilent's Genomic Workbench software suite. We use the 2:1 chromosome X ratio as an internal

FIGURE 2 Detection of Chromosomal Aberrations in FFPE Breast Tumor-1



Representative ideograms showing the detection of DNA aberrations in the FFPE breast tumor-1 sample. Distinct amplifications can be seen on the distal half of 8q, 10p, and a ~5Mb interval at 10q22.2. The ADM-2 algorithm was used with thresholds set at 5.0 and 6.7 for calls on chr.8 and chr.10, respectively. Because the reference and test samples were chosen to be sex-mismatched, the 2:1 chr.X gain is used as an internal control for sensitivity of aberration detection. The entire chromosome X shows a gain in the female breast tumor sample and persists to a threshold of 10.7. The trace shown is generated using the triangular moving average algorithm with a window size of 1 Mb.

TABLE 2 Quality Sequencing Results to Enable a Range of NGS Applications

	Reads[1]	Percent Chimera[2]	Percent of Aligned Forward Reads	Percent of Aligned Reverse Reads	Median Insert Length (nt)	Error Rate[3]
gDNA	2.35E+06	1.497%	96.04%	94.77%	174	0.150%
WGA1	1.51E+06	1.38%	97.83%	96.77%	178	0.164%
WGA2	1.59E+06	1.34%	97.99%	97.05%	174	0.160%

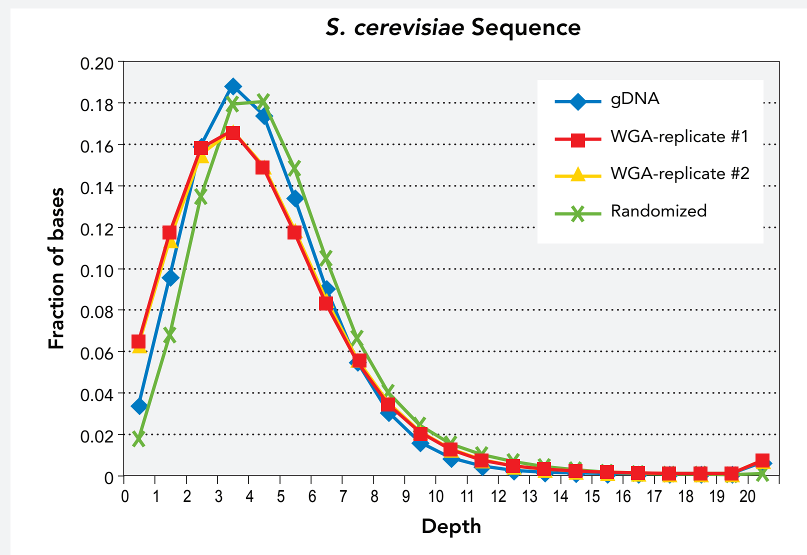
[1] Number of reads is from an 8-plex, paired-end sequencing lane on the Illumina GAIIx.
[2] Estimate based on paired reads that map >3 Kb apart from one another considering all orientations of possible chimeras.
[3] Average of the Forward and Reverse error rate for paired-end sequencing.

control for assessing the sensitivity of the calls in aCGH. The gain of copy number on chromosomes 8 and 10 is routinely seen using relatively high threshold values with DLR values below 0.5. Other metrics, including signal to noise, signal intensity and background noise, are all within a good range indicative of high-quality aCGH results. Specific genomic copy number differences are shown in **Figure 2** for FFPE human breast cancer tumor-1 prepared with the Ovation WGA FFPE System.

The First Linear WGA Technology for FFPE Sample Prep Used in Next-Generation Sequencing

WGA methods used for NGS applications require high fidelity amplification of the genome to allow detection of both large rearrangements seen in human cancers, as well as single nucleotide polymorphisms (SNPs). In order to make an accurate determination of genomic variations, amplification error rates and bias must be minimized. To address these variables, *S. cerevisiae* DNA samples were prepared using the Ovation WGA FFPE System together with the Encore ds-DNA Module to make double-stranded DNA for input into the Ovation Ultralow Library System. Libraries from duplicate amplifications (WGA1 and WGA2) as well as unamplified gDNA were sequenced on the Illumina Genome Analyzer IIx, and results are shown in **Table 2**. The sequencing performance metrics for all samples demonstrated a high percentage of aligned reads and low error rates. Importantly, both the error rates and percent chimeras (large sequences that are replicated during sample preparation) were very similar in both WGA samples and unamplified material. Similar results were obtained in sequencing experiments with human DNA from FFPE samples where nearly identical error rates were obtained with WGA and

FIGURE 3 Deep Sequencing of Libraries Prepared with gDNA



Fragmented genomic DNA was prepared from *S. cerevisiae*, amplified using the Ovation WGA FFPE System (WGA-replicate #1 and #2) and made double-stranded using the Encore ds-DNA Module. Libraries from amplified (red and gold tracks) and unamplified gDNA (blue track) were made using the Encore NGS Multiplex System I, and paired-end sequencing with 40 base-pair reads performed on the Illumina GAIIx. The distribution of 1.5M reads from each sample is plotted to determine the depth of coverage across the genome. The green track represents the theoretical read distribution with randomly picked 40 base-pair sequences mapped to the *S. cerevisiae* genome.

unamplified samples, indicating there is no increase in sequencing errors contributed by amplification. A low frequency of chimeras is critical to use NGS for mapping larger structural changes in the sample DNA. The results obtained for WGA1 and WGA2 were very similar, illustrating the high degree of reproducibility enabled by NuGEN's end-to-end solution for DNA sequencing from FFPE samples.

Among the list of critical factors for NGS sample preparation solutions, including aligned reads, low error rates, and minimal chimeras, is the need to generate uniform read coverage across the reference genome. As shown in **Figure 3**, the distribution of sequencing reads from each sample are plotted to determine the depth of coverage across the *S. cerevisiae* genome, as well as to assess the reproducibility of amplification, library construction and sequencing. The reads from WGA1 and WGA2 are mapped to the reference genome with a normal distribution centered on the theoretical read distribution of a random 40 base-pair sequence. No positional bias was observed, and mapping of these 1.5M reads provides an average depth of 4X. These results demonstrate that the combined use of the Ovation WGA FFPE System, Encore ds-DNA Module and Encore NGS Library Systems provides a high depth of coverage in a reproducible fashion with no bias in the positional mapping of reads.

Conclusions

FFPE samples represent a huge repository of genomic information that has remained untapped due to the lack of sample preparation tools suitable for high-quality aCGH and NGS analyses. The Ovation WGA FFPE System offers a linear amplification technology that enables the generation of a scalable resource for flexible downstream analysis by each of these techniques. Both for archived FFPE samples and prospective studies, the Ovation WGA FFPE System facilitates your discovery and research with this important source of DNA.

ORDERING INFORMATION

Part No.	Product Name
6200-08	Ovation® WGA FFPE System
2500-08	Encore® ds-DNA Module
7102-08	Ovation® RNA-Seq System V2
7150-08	Ovation® RNA-Seq FFPE System
0303-08	Ovation® Ultralow Library System
0330-08	Ovation® Ultralow DR Multiplex System 1–8
0331-08	Ovation® Ultralow DR Multiplex System 9–16
0329-96	Ovation® Ultralow DR Multiplex System 1–96
0316-08	Encore® Rapid Library System
0319-08	Encore® Rapid DR Multiplex System 1–8
0320-08	Encore® Rapid DR Multiplex System 9–16
0328-96	Encore® Rapid DR Multiplex System 1–96

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