Next Generation Sequencing Application of DNA from FFPE Samples

Comprehensive Analysis of Your Stored Samples

1. Introduction

Formalin-Fixed Paraffin-Embedded (FFPE) samples are common biological material for disease diagnoses and scientific research. This technology allows samples to be stored for several years. It is however challenging to get intact information from such samples, since severe degradation, damage and molecular or biological modification could appear during sample preparation. Although there are several kinds of array-based methods for FFPE tissues, they are in general limited by capacity and fixed targets. Next Generation Sequencing (NGS) for FFPE samples will substantially facilitate our understanding of undefined pathological mechanism and help to broaden our insights into biomedical research.

As the premier scientific partner of scientists and researchers, BGI works relentlessly in applying NGS to FFPE samples to unveil the information concealed in FFPE samples.

2. Tests for FFPE Samples

Among various NGS applications, Whole Genome Resequencing (WGRS) and Whole Exome Sequencing (WES) are both commonly used genomic research methods. Plenty of biomedical and drug R&D work have already proved the substantial power of these NGS methods in locating key disease related genes, profiling global mutations, biomarker discovery, *etc*. There is a large source of useful data available on FFPE samples. NGS is the premier choice for a comprehensive utilization of these precious resources.

2.1 Workflow of FFPE Sample Sequencing

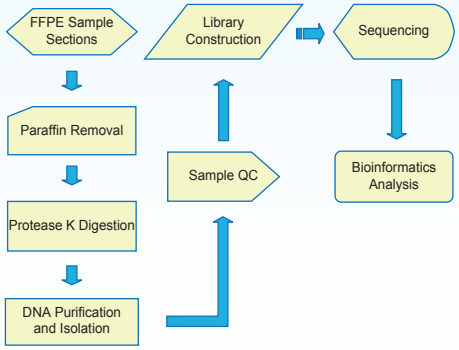

Figure 1 Workflow of FFPE Sample Sequencing

2.2 Whole Genome Resequencing

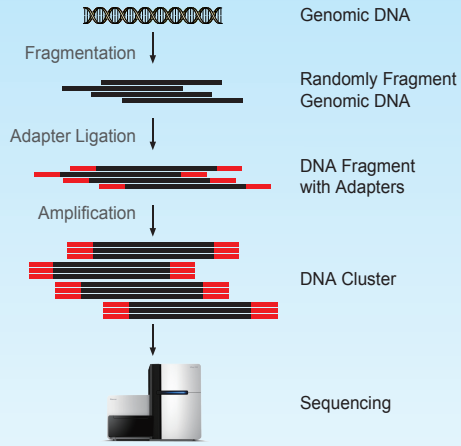

Figure 2 WGRS Workflow

2.2.1 DNA Extraction and Library Construction

The QIAamp® DNA FFPE Tissue Kit (QIAGEN) with in-house modification was used to extract total DNA from cervical tumor tissue FFPE samples. Although DNA degradation existed, we successfully constructed WGRS libraries (data not shown).

2.2.2 Sequencing Results

WGRS libraries were assayed for sequencing using Illumina HiSeq™ 2000. The effective average sequencing depth of each sample was 25~37×. As a measure of how successful the sequencing of total DNA extracted from FFPE samples was, we compared the conversion rate, mapped rate and coverage between the non-FFPE and the FFPE samples as shown in Table 1. Results show that FFPE-derived and non-FFPE-derived sequencing data have high conversion rate (clean data/raw data), indicating the total DNA isolated with the extraction kits tested was of sufficient quantity and quality for sequencing using Illumina HiSeq™ 2000. In general, slightly more mapped rate and coverage were detected in the non-FFPE samples than in FFPE samples, indicating that better retention of the DNA during storage and total DNA extraction was achieved.

Table 1 Comparison of WGRS Data Outcomes between FFPE and Non-FFPE* Samples

Sample	Raw Data (G)	Clean Data (G)	Conversion Rate (%)	Sequencing Fold (X)	Mapped Rate (%)	Depth (X)	Coverage >=10X (%)
FFPE-1	212.28	194.21	91.49%	63.77	72.43%	25.17	88.47%
FFPE-2	130.71	120.18	91.94%	37.63	73.71%	24.84	92.61%
FFPE-3	175.94	162.18	92.18%	51.61	79.38%	37.35	95.80%
Average	-	-	91.87%	-	75.17%	29.12	92.29%
Non-FFPEs*	-	-	90%	-	85%	30	95%

Note:

[1] Non-FFPE* means normal sample without FFPE process, the data of non-FFPEs are conservative empirical data based on BGI's finished projects.

[2] Clean data comes from raw data after filtering low quality reads and reads with adaptors.

[3] Conversion rate is the ratio between clean data and raw data.

[4] Sequencing fold is the ratio of clean data to the effective genome size.

[5] Mapped rate is the percent of clean data mapped to the effective genome size.

[6] Depth is the ratio of clean data filtered out PCR duplication data to the effective genome size.

[7] Coverage ≥10× is the percent of effective genome size, which is covered by at least ten reads.

2.3 Whole Exome Sequencing

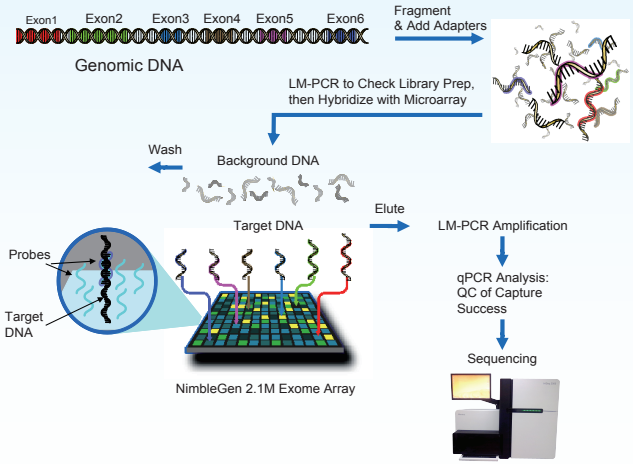

Figure 3 WES Workflow

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2.3.1 DNA Extraction and Library Construction

The QIAamp® DNA FFPE Tissue Kit (QIAGEN) with in-house modification was used to extract the total DNA from gastric tumor-adjacent tissue FFPE samples, while the QIAamp® DNA mini Kit was used to extract total DNA from matched Fresh Frozen (FF) samples. The degree of extracted DNA degradation was evaluated by agarose gel electrophoresis. As expected, analysis of DNA isolated from FF and FFPE tissues for the matched tumor samples shows that genomic DNA from FF samples was of much higher molecular weight and less degraded than that from FFPE tissues (Figure 4). We successfully constructed exome capture libraries from FFPE sample using NimbleGen 2.1 M array, which was similar to FF sample showed in Figure 5.

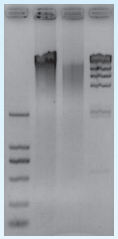

Figure 4 Total DNA Extracted from FF and FFPE Samples of Gastric Paracancerous Tissue

Lane 1: D2000 DNA marker;
Lane 2: Total DNA extraction from gastric paracancerous tissue FF sample;
Lane 3: Total DNA extraction from gastric paracancerous tissue FFPE sample;
Lane 4: λHind III DNA marker.
The figure shows that the DNA band from FFPE sample moved further down compared to the band from FF sample, which indicates some degradation.

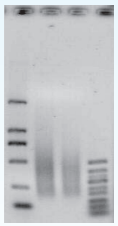

Figure 5 Exome Capture Library from FF and FFPE Samples of Gastric Paracancerous Tissue

Lane 1: D2000 DNA marker
Lane 2: Exome capture library constructed from FF sample
Lane 3: Exome capture library constructed from FFPE sample
Lane 4: 50 bp Ladder DNA marker
The figure shows the result of library construction with high-similarity between FFPE sample and FF sample.

2.3.2 Sequencing Results

WES libraries were assayed for sequencing using Illumina HiSeq™ 2000. The mean depth of target region of each sample was 18.6× and 23.3× respectively. To measure of how successful the sequencing of total DNA extracted from FFPE samples was, we compared the mapped rate and coverage between the FF and the FFPE samples as shown in Table 2. Results show that sequencing data generated from FFPE and FF samples had high mapping rate (mapped data / raw data), indicating that the total DNA isolated with the extraction kits tested was of sufficient quantity and quality for sequencing using Illumina HiSeq™ 2000. In general, slightly more coverage was detected in the FF samples than in FFPE samples, indicating that better retention of the DNA during storage and total DNA extraction was achieved.

Table 2 Comparison of WES Data between FFPE and FF Samples

Samples	Raw Data (M)	Mapped Data (M)	Mapped Rate (%)	Mean Depth of Target Region (×)	Coverage of Target Region (%)	Coverage of Target Region ≥10× (%)	Capture Specificity (%)
FFPE	1419.41	1165.91	82.14	18.61	93.31	65.52	65.22%
FF	1763.11	1490.43	84.53	23.33	94.84	74.14	64.07%

Note:

[1] Mapped data represents data that was mapped to reference genome.
[2] Mapped rate is the percentage of raw data mapped with respect to the effective genome size.
[3] Mean depth of target region is the ratio of mapped data to the target region size.
[4] Coverage of target region is the percentage of target region covered by at least one read.
[5] Coverage of target region ≥10× is the percentage of target region covered by at least ten reads.
[6] Capture specificity is the percentage of raw data mapped to the target region.

2.3.3 SNP Calling Results

Sequencing data was analyzed and evaluated for sequence coverage and uniformity (Table 2), along with Single Nucleotide Polymorphism (SNP) detection and concordance between the FF and FFPE samples (Figure 6 and Figure 7). Concordance for normal SNPs (with both allele depth higher than 4×) and high quality SNPs (with both allele depth higher than 20× and quality score > 20) was approximately 98%, which indicated that we can get reliable genome variation from FFPE samples that is comparable with FF samples.

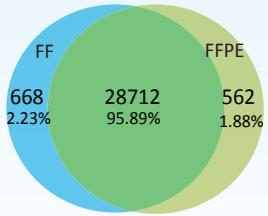

Figure 6 Concordance of 'Normal' SNPs between FF and FFPE Samples

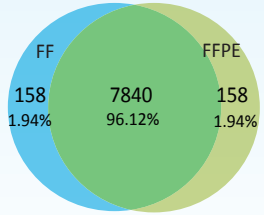

Figure 7 Concordance of 'High Quality' SNPs between FF and FFPE Samples

3. Conclusions

Most tumor tissue samples are preserved in the form of FFPE blocks, which in general present several challenges, including variability of fixation methods, diverse ages and store environment of samples, and several possible damages that may occurs to the DNA during the FFPE process.

The results presented in this study demonstrate that DNA amplified from degraded FFPE DNA can be successfully used for WGRS and WES studies while maintaining acceptable levels of performance and allowing the exploitation of FFPE samples by NGS technologies. These results enable the use of a vast amount of FFPE samples available for biomedical researches and applications.

Sample Requirement

Both the original FFPE samples and the DNA materials extracted are acceptable. We recommend clients to extract DNA themselves.

FFPE Samples

Please make sure that each FFPE sample has no less than 20 sections, each of about 10-20 μm thick. About 100 μm sections are required for DNA extraction each time. For example, if the FFPE section has a thickness of 20 μm, 5 sections are needed for DNA extraction. Please transfer tissue sections to clean centrifuge tubes by clean nippers after section processing. To avoid cross contamination, be sure to change different blades and nippers when transferring different samples. BGI does not accept stained FFPE sections.
FFPE tissue sections provided by our collaborator are required to be unstained; this can be sent under room temperature.

DNA Samples

• For Whole Genome Resequencing
Amount: 3×(N+1) μg; Concentration: ≥ 50 ng/μL; Purity: $OD_{260/280}$= 1.8~2.0
N represents the number of library construction.

• For Whole Exome Sequencing
Amount: 6 μg; Concentration: ≥ 50 ng/μL; Purity: $OD_{260/280}$ = 1.8~2.0

Contact Us

China (Mainland)
BGI-Shenzhen, Beishan Industrial Zone, Yantian District, Shenzhen, 518083, China
Tel: 400-706-6615
+86-755-25281960
Email: bgi-PGx@genomics.cn
www.genomics.cn

Asia Pacific（Hong Kong）
BGI Hong Kong Co. Limited, 16th Dai Fu Street, Tai Po Industrial Estate, Tai Po, Hong Kong
Tel: +852-3610-3510
Email: bgihk.enquiry@genomics.cn
www.bgisequence.com

Europe (Copenhagen)
Ole Maaløes Vej 3, DK-2200 Copenhagen N, Denmark
Tel: +45-7026 0806
Email: bgieurope@genomics.cn
www.bgisequence.com

North America (Boston)
BGI Americas Corporation, One Broadway, 14th Floor, Cambridge, MA 02142, USA
Tel: +1-617-500-2741
Email: info@bgiamericas.com
www.bgiamericas.com
www.bgisequence.com