

### **FAQ for FFPE**

### 1. What are the requirements for sending FFPE tissue or slices?

BGI recommends that collaborators send FFPE slices instead of Macrodissection given our expertise. To ensure that we obtain enough nucleic acid from the FFPE tissue, send at least four pieces of unstained sections, each of an area of about 250 square mm (approximately the area of a thumb). Tumor regions should be more than 90% of the sample.

### 2. What is the extraction yield for FFPE samples?

Differences in the FFPE quality affect the extraction yield. Use this information as a reference.

For DNA, 500 ng-1.5 ug/slice For RNA, 200 ng-2 ug/slice

#### 3. What method do you use for DNA/RNA extraction?

Based on testing different kits, we developed an extraction method that combines in-house modification with a special FFPE extraction kit. The kits we use are as follow:

- DNA is extracted using the QIAamp® DNA FFPE Tissue Kit. 50USD/sample
- RNA is extracted using the Ambion® RecoverAll™ Total Nucleic Acid Isolation Kit; 50USD/sample.

### 4. What insert size of FFPE samples and fresh frozen (FF) samples is used for library construction?

Based on our project experience, sizes are:

- DNA FFPE samples: about 100bp
- DNA FF samples: about 120bp
- RNA FFPE samples: about 150bp
- RNA FF samples: about 200bp

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### 5. When I send FFPE samples for DNA and RNA extraction, is there a special team at BGI that performs the extraction? How long does the process take?

The same team that extracts normal samples does the FFPE samples using a special kit. Depending on the situation, the time varies. Typically, one day is sufficient for 20 to 30 samples, not including the time to order the kit.

### 6. What is the recommended band size of nucleic acid for FFPE samples and the length of the insert size?

Based on our experience the band size of FFPE nucleic acid is a smear that ranges from 300bp to 1kb. The degree of degradation of different FFPE samples can affect the insert size. We have found that the insert size is about 100 (DNA) and 150 (RNA) as indicated above.





## 7. Should we use slides or sections for extraction of the DNA/RNA from FFPE samples?

We can accept both but prefer sections. We recommend that you send sections in which the tumor region constitutes at least 80% of the sample after removing the non-tumor region. You can mark the tumor regions on slides and send them to us to scrape them.

## 8. Will DSN normalization affect downstream bioinformatics, such as quantification?

DSN has a little influence on mRNA and the quantification of genes. We have conducted tests to validate the quantification. After RPKM calculations were aligned to RefSeq annotation, the correlation of gene expression between FF and FFPE samples with DSN method was performed. It showed a high correlation between frozen controls and FFPE samples for gene expression quantification (Pearson R>0.84). We also compared the sequencing results with and without DSN treatment on FF samples and observed acceptable concordance (the Spearman R is approximately 0.8). See our brochure for additional details.

### 9. If we can extract more RNA from FFPE, can you use the normal pipeline?

No, no matter how much total RNA can be extracted, we use the FFPE pipeline because the mRNA may lose 3'-end information due to degradation. Additionally, we cannot obtain mRNA information using the normal pipeline (poly-A enrichment mRNA method).

#### 10. Are you able to perform 200ng FFPE sequencing, and how do the results look?

Although we have projects involving 200ng FFPE DNA sequencing we are unable to share any details due to non-disclosure agreements.

We are still modifying the protocol and informatics. When we've refined the techniques of 200ng FFPE DNA sequencing, and they are mature enough for commercial projects, we will publish the details and let you know.

# 11. For a 50 human genomes reseq FFPE sample, with about 200ng DNA, what is the coverage per genome for 10x and 30x?

For WGRS FFPE samples, the mapping rate is around 60%.

10x:  $3G \times 10/60\% = 50G$  (raw) 30x:  $3G \times 30/60\% = 150G$  (raw)

As such, for human genomes reseq FFPE samples, we estimate 50G of data for 10X and 150G data for 30X.

# 12. For FFPE transcriptome, how much raw data are needed to yield 4G of clean data?

For RIN=2.4 samples, the genome mapping rate is about 70%.

4G clean data: 4G/70%=7G raw





Therefore, we recommend 7G raw of data to yield 4G of clean data.

## 13. With small fragments in total RNA can you determine if they are from degradation or real, small RNA?

We can distinguish degraded extrons and introns from real small RNA through annotation.

## 14. What bioinformatics results do you provide for exome sequencing, and what is the cost?

The bioinformatics results include:

- 1) Somatic SNP/InDel detection for paired normal-tumor samples
- 2) SNV detection, annotation, and statistics for paired normal-tumor samples
- 3) Filtering with the known databases such as cosmic, dbSNP, etc.
- 4) Amino acid substitution prediction (SIFT, Polyphon-2)
- 5) GO enrichment analysis for selected genes and pathway enrichment analysis Pricing is about 1.3 times the standard price.

# 15. Is the coverage you report the average or a percentage of the target (i.e., 80% of target covered to 30X, 50X, etc.).

Based on the statistics of FFPE exome sequencing projects, we see similar performance between FFPE samples and normal samples. For exome sequencing of FFPE samples the clean rate > 0.88 and the mapping rate > 0.7.

We recommend 100X for FFPE samples, as well for cancer analysis. Please be advised that we normally do not guarantee sequencing depth of FFPE samples, because of unpredictable sample quality that can affect sequencing depth.

However, based on our results:

If we sequence at 100X, 90%-95% will be covered at 10X, 80%-92% will be covered at 20X, and at least 75% will be covered at 30X.

If we sequence at 50X, at least 80% will be covered at 10X, at least 70% will be covered at 20X, and at least 60% will be covered at 30X.

#### 16. What is required for BGI to capture a library that we want to send?

We need to confirm if the adaptor sequence and index primer sequence are compatible with Hiseq 2000. If so, the library size required must be greater than 500ng and the band size needs to be from 250bp to 420bp. Both Qubit and Agilent 2100 formats are acceptable. We do prefer that you send samples to BGI for library construction.

#### 17. Does BGI offer RRBS for FFPE samples?

RRBS is still in the R&D stage and not appropriate yet to be used commercially. We are actively pursuing this endeavor but know of only one research paper using NGS and FFPE samples for methylation research. However, we are open to





collaboration projects.



