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APPLICATION NOTE

Efficient and streamlined solution for high-quality NGS library preparation using the Bioruptor® Pico and JetSeq™ Flex DNA Library Preparation Kit

INTRODUCTION

Next Generation Sequencing workflows include a number of critical steps which can have a significant impact both on the quality of the results and on the overall cost of the project. In particular, DNA fragmentation and library preparation, as the first two steps of the workflow, are crucial, since at these early stages significant bias can be introduced to the sample, compromising the quality of the datasets obtained later.

In this application note we describe how a fully functional, high-quality library for Illumina platforms has been prepared, using the Diagenode Bioruptor® Pico and the Bioline JetSeq™ Flex DNA Library Preparation Kit.

A SEAMLESS PROTOCOL FOR DNA LIBRARY PREPARATION FOR ILLUMINA PLATFORMS

1. Precise DNA shearing

The Bioruptor Pico generates fragments suitable for Next Generation Sequencing library preparation. Human DNA samples (10 ng/ul, sample volume 50 ul) were sheared using the Bioruptor Pico to approximately 160 bp in 0.2 ml Bioruptor tubes. The Bioruptor Pico delivers controlled and consistent shearing, enhancing the productivity by providing a unique sonication water bath for simultaneous processing of 16 samples. Figure 1 shows the size distribution of the prepared input DNA.

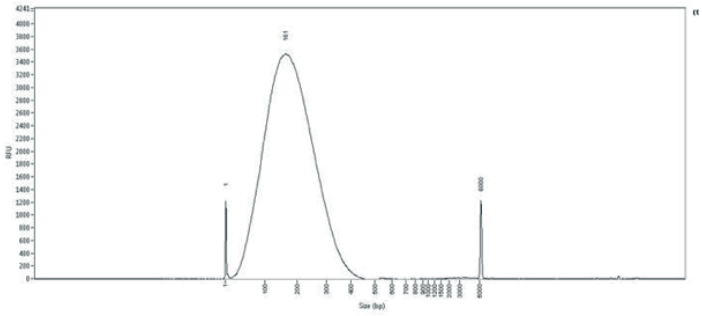

Figure 1. Electropherogram of the sheared human DNA obtained using the Fragment Analyzer. The fragments show a unimodal distribution around 161 bp.

2. High quality library preparation

After fragmentation, 100 ng of sheared DNA was used as starting material, following the Bioline JetSeq Flex DNA Library Preparation Kit protocol summarised in Figure 2.

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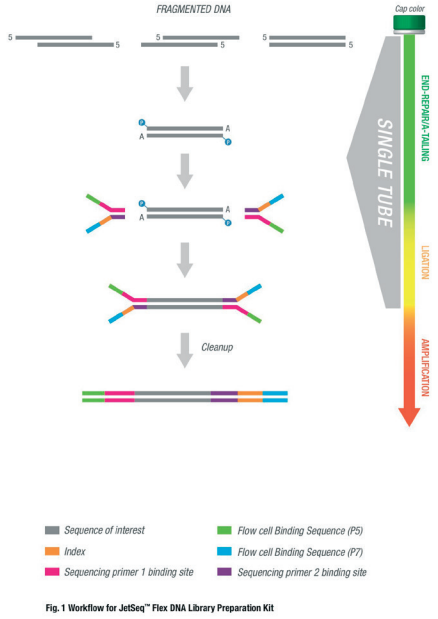

Figure 2. JetSeq Flex DNA Library Preparation Kit workflow. The steps described consist of: fragmentation, end-repair and adenylation, adapter ligation, PCR.

JetSeq Flex DNA Library Preparation Kit provides a single cocktail of enzymes allowing highly efficient end-repair and 3'-end adenylation. The sheared DNA was converted to end-repaired fragments ready to be ligated with adapters in only 35 minutes.

The optimised buffer and enzymes allow the adapter-ligation step to be performed directly from the end repair reaction. This greatly improves the workflow, minimising the risks of cross-contamination. Standard Y-shaped Illumina® TruSeq adapters were used, following the adapter:fragment ratio recommended in the JetSeq Flex DNA Library Preparation Kit protocol.

Following adapter ligation, unbound adapters and adapter-dimers were removed by bead-based clean-up using JetSeq Clean. The effectiveness of the clean-up was assessed with the Bioanalyzer instrument, as shown in Figure 3. Using the amount of JetSeq Clean beads recommended in JetSeq Flex DNA Library Preparation Kit protocol, all undesired oligonucleotides were completely removed.

The adapter-ligated DNA was then amplified and cleaned up once again following JetSeq Flex DNA Library Preparation Kit protocol. This generated a library with unimodal fragment distribution of the expected size, without any carry-over of adapters or primers. The final library was quantified using both the Bioanalyzer analysis and the JetSeq Library Quantification Kit, resulting in 30 µl of a purified library at concentration of 70nM, sufficient for downstream Illumina sequencing and for further quality control analysis, if required.

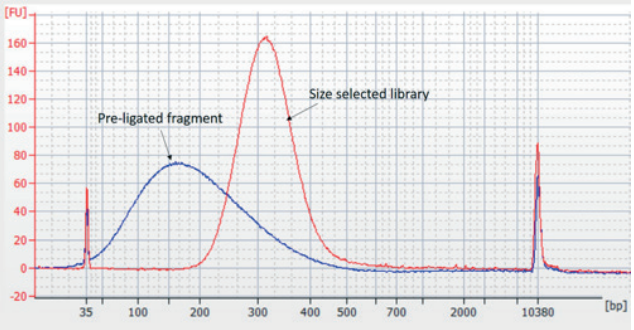

Figure 3. Libraries were prepared with the Bioline JetSeq Flex DNA Library Preparation Kit. The electropherogram of DNA fragments analysed on a Bioanalyzer High Sensitivity Chip shows pre-ligated (blue) and final amplified library (red).

3. Sequencing Data Analysis

The libraries constructed using the JetSeq Flex DNA Library Preparation kit were sequenced on an Illumina HiSeq 2500 instrument with HiSeq SBS Kit v4 chemistry, with 125 bp paired-end reads using the appropriate single index read. To ensure that the JetSeq Flex DNA Library Preparation kit produced high quality libraries, FastQC analysis was performed to assess the quality of the raw sequencing data (FASTQ files). The modules listed below generated by the FastQC reports show good quality scores across all bases at each position, with no bias in the GC composition as well as low sequence duplication level of reads within the libraries. In addition, sequencing reads were mapped to a reference genome and the percentages of unique reads were scored.

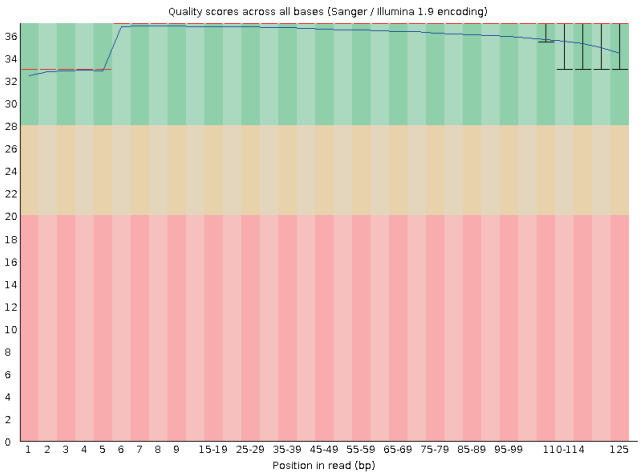

Figure 4. Phred quality score across all bases at each position. The y-axis on the graph showing the quality scores, the x-axis shows the position in the read. Phred score > 28 is considered as very good quality calls (green), between 20 to 28 is considered as reasonable quality (yellow) and Phred score < 20 is considered as poor quality (red). As indicated, the quality score of the sequencing data obtained using JetSeq™ Flex DNA Library Preparation Kit was high, giving complete confidence in the subsequent downstream sequencing analysis.

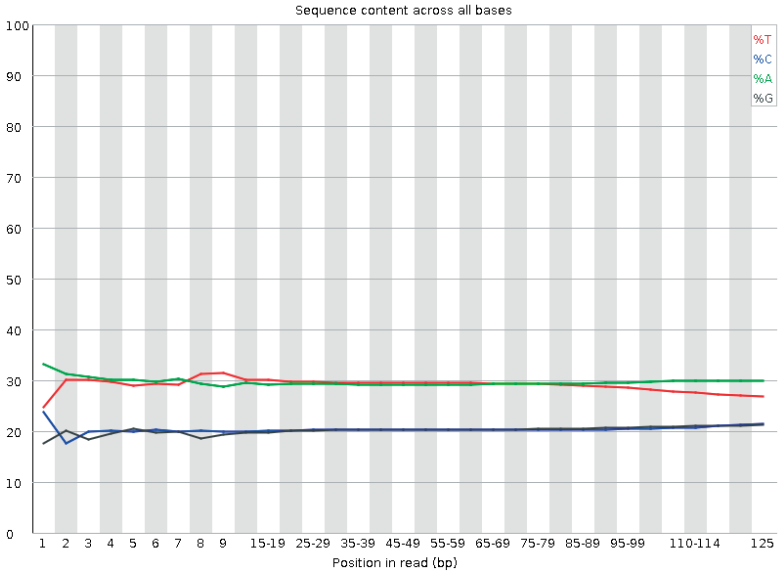

Figure 5. Per base sequence content showing the distribution of A, C, G and T at each position. The y-axis on the graph shows the percentage of occurrence, the x-axis shows the position in the read. The proportion of G and C nucleotide bases in the human genome is approximately 40%, thus the G and C base calling are 20% each.

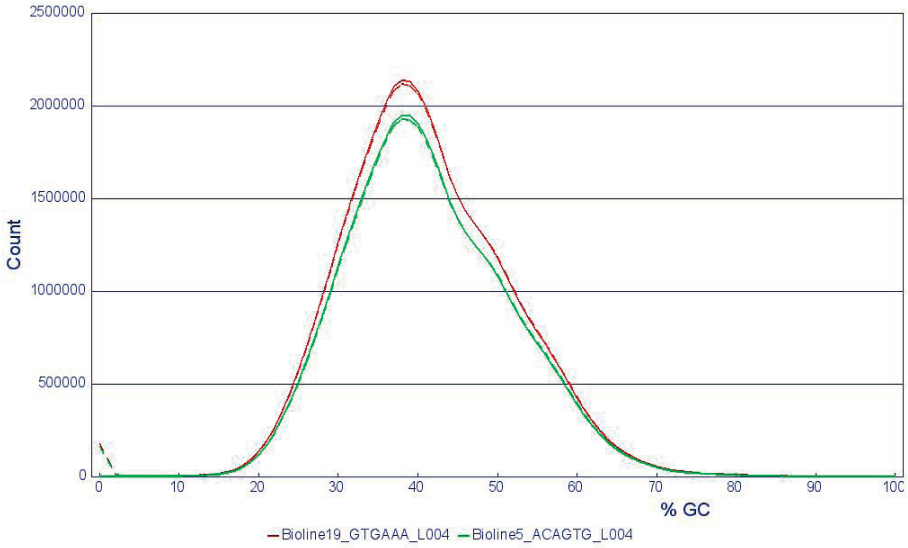

Figure 6. Per sequence GC content graph showing the GC content across the whole length of each sequence. Bioline 19 (red) and Bioline 5 (green) are human genome sequencing data labelled with different indexes. A GC distribution of GC around 40% is consistent to the GC content of a human genome. The JetSeq Flex DNA Library Preparation Kit uses a highly efficient polymerase to ensure uniform amplification of all genomic regions regardless of the GC content, thereby reducing bias and resulting in even coverage in sequencing reactions.

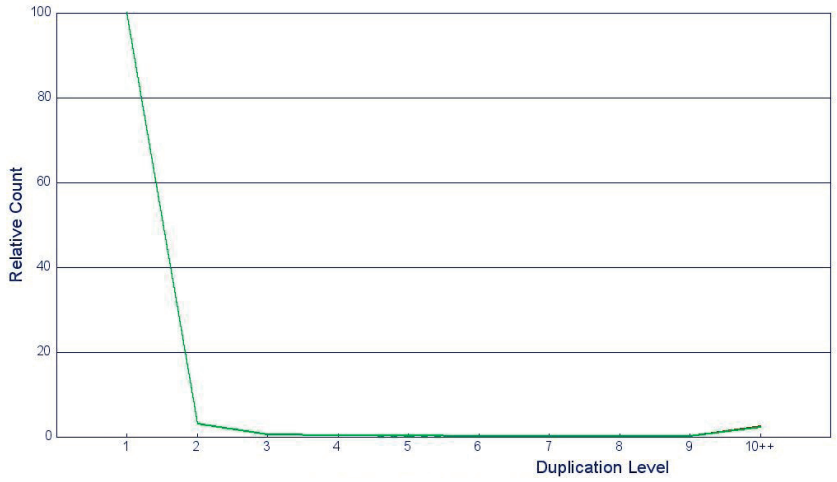

Figure 7. Sequence duplication level showing how unique the sequences are in the library. They counts the degree of duplication for every read and creates a plot to show the relative number of reads with different degrees of duplication. The y-axis on the graph shows the percentage of occurrence, the x-axis shows the duplication level. A high duplication level is likely due to PCR enrichment bias, as indicated above, using the JetSeq Flex DNA Library Preparation Kit majority of reads come from sequences which only occurred once within the library, suggesting that the library has a diverse population.

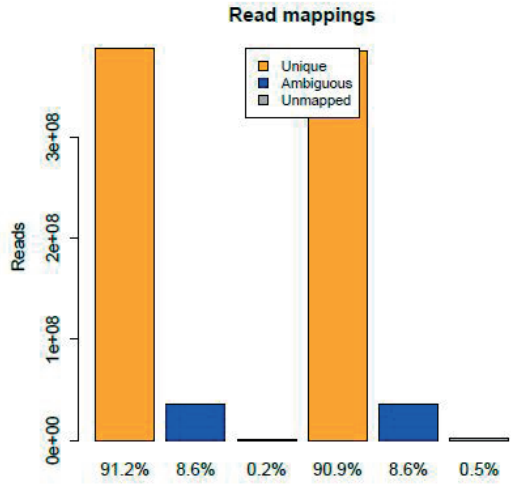

Figure 8. Mapping results showing the number of reads that were uniquely and ambiguously mapped to the reference genome and unmapped reads. 1st mate and 2nd mate refer to forward and reverse paired end reads. As indicated, the JetSeq Flex DNA Library Preparation Kit gives over 90% of reads that are uniquely mapped to the reference genome.

CONCLUSIONS

We have presented a combined protocol utilizing the JetSeq Flex DNA Library Preparation Kit and Bioruptor Pico to produce high quality NGS libraries.

The JetSeq Flex DNA Library Preparation Kit offers an efficient, streamlined solution for the preparation of libraries compatible with Illumina NGS platforms.

The kit has been developed to provide high quality libraries starting from a DNA input ranging between 1 ng and 1 µg, using any Illumina-compatible adapters. In this particular case, the current library was obtained using Universal TruSeq adapters.

End-repair and ligation steps occur in the same tube, reducing the hands-on time and minimising any risk of cross-contamination. Thus, the protocol is very straightforward and efficient, and ready to be sequenced libraries can be prepared in less than 3 hours.

The Bioruptor Pico enables controlled and consistent shearing to produce DNA fragments within the optimal size range.

The libraries obtained with the combined use of Bioruptor Pico and JetSeq Flex DNA Library Preparation kit produced a set of high quality data with low duplication levels, low GC bias, and a high percentage of unique mapping reads.