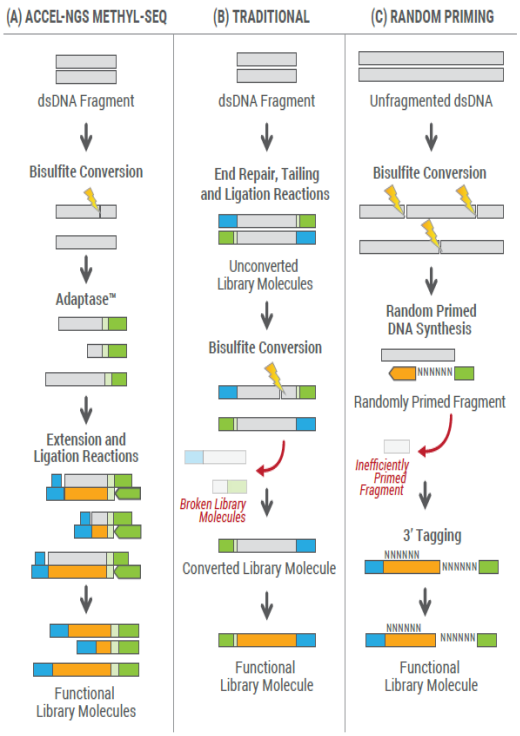
LOW INPUT WGBS

MAIN APPLICATIONS

DNA methylation occurring at CpG dinucleotides is probably the best-studied epigenetic modification due to the extensive mapping of DNA methylation patterns in different diseases. DNA methylation based biomarkers bear the promise to provide valuable information for early diagnosis, prognosis, disease classification and might assist in the prediction of response to therapy. While initially epigenetic research has focused on the analysis of epigenetic alterations in cancer, research in recent years has demonstrated that alterations are also present in nearly all complex diseases including autoimmune and inflammatory diseases, allergic disorders, metabolic as well as neurodegenerative and psychological disorders. Whole-genome bisulfite sequencing (WGBS) or MethylC-seq can be considered as the current gold standard for the genome-wide identification of differentially methylated CpGs (DMCs) and differentially methylated regions (DMRs) at single nucleotide resolution. The whole genome read-out after bisulfite conversion overcomes the limitations of cloning and Sanger sequencing. However, the unprecedented quantitative and spatial resolution that is currently transforming DNA methylation analysis still comes at a high cost requiring substantial sequencing to obtain a proper and even coverage and specialized bioinformatic expertise and resources.

METHODOLOGICAL AND SCIENTIFIC BACKGROUND:

The WGBS method using the Swift Accel-NGS Methyl-Seq DNA Library preparation protocol (Swift Biosciences, Ann Arbor, MI) differs from the conventional WGBS library preparation method as it uses the proprietary Adaptase™ technology. Bisulfite conversion process leads to damage of DNA. The Adaptase™ technology has been designed to overcome the drawbacks of the traditional method by capturing the bulk of single-stranded bisulfite-converted libraries. For the Accel-NGS Methyl Seq method, first bisulfite conversion is performed on fragmented samples and then they undergo library preparation, hence requiring smaller input amounts. In comparison, the traditional method uses large input quantities as libraries are prepared before bisulfite conversion and then undergo a large loss of yield during the conversion process. During the Adaptase step, a short polynucleotide tail along with the first truncated adapter is added at the 3'-termini of the fragmented and single-stranded bisulfite-converted sample. This additional tail needs to be trimmed later from bioinformatics data. Then an extension step is performed followed by a Ligation step to attach the second truncated adapter. Finally, an Indexing PCR is performed that incorporates full length adapters. The Adaptase™ technology also provides more uniform coverage when compared to Random priming method. The Accel-NGS Methyl Seq protocol requires 200ng input of genomic DNA and additionally unmethylated Lambda DNA (Promega) is added as a control to check efficiency of the bisulfite conversion and Sample assisted spike in (SASI) is added as a control to determine cross-contamination between samples. Bisulfite conversion is performed using the Qiagen EpiTect Bisulfite Kit.



Comparison of three WGBS models: (A) describes the Accel-NGS Methyl Seq method using the Adaptase™ technology and has been compared to (B) - Traditional WGBS model and (C) - Random Priming model (Source:https://swiftbiosci.com/wp-content/uploads/2019/02/16-0831-Methylome-AppNote-WEB.pdf).

YIELD AND EXPECTED RESULTS

The fragmentation protocol has been modified to obtain larger fragmented DNA sizes of approximately 600-850bp and an additional size selection step is added after the Extension step. This in turn provides a larger library size and eventually a larger insert size after sequencing on Illumina HiSeq X sequencer. For the PCR reaction, 6 cycles are required. We usually sequence one WGBS library on 1-2 HiSeq X lanes depending on the required output. One HiSeq X lanes results in 23-27-fold coverage of the human genome (2x 300 Mio. reads) with a duplication rate of 20-25%.