WG OX-BS SEQ

SCIENTIFIC BACKGROUND AND MAIN APPLICATIONS

5-hydroxymethylation (5hmC) has attracted substantial interest for its role in gene regulation. 5hmC is an intermediate in an active demethylation process, but also to constitute a distinct layer in the complex process of epigenetic regulation with its own distribution and regulatory functions. 5hmC is most abundant in human brain tissue and embryonic stem cells, but at levels approximately 10-fold lower than those of 5-methylcytosine. 5hmC levels do not correlate with 5mC levels of the respective tissue and 5hmC was found enriched at specific active functional elements of the genome, in particular enhancers, promoters and gene bodies associating 5hmC with open chromatin and transcriptional activity.

TECHNICAL DESCRIPTION AND PROTOCOL

The oxBS protocol is also based on the commercial Ovation® UltraLow Methyl-seq DR Multiplex System (NuGen/ Tecan Genomics, San Carlos, CA), but including an extra oxidation step prior to bisulfite conversion. In a first step sequencing and digestion spike-in controls are added to the sample that allow for quality monitoring of both the sequencing as well as the conversion efficiency. The sample is split into two parts, and one part is oxidized converting hydroxymethylated cytosines to formylcytosines while the other part undergoes a mock reaction in the absence of oxidating reagents. Both aliquots are subsequently bisulfite-converted. In the conventional bisulfite reaction, the remaining cytosine signal reflects a mixture of both methylated and hydroxymethylated cytosines, with the first exceeding largely in most tissues. By comparing the conventional bisulfite-converted sample with the sample that has undergone the additional oxidation step, 5mC and 5hmC can be distinguished. Hydroxymethylated cytosines are thus indirectly determined as the difference in methylation between the two reactions. Sequencing is performed on a HiSeq 4000 or a HiSeq X5 in a multiplex of four to eight samples per lane on multiple lanes to obtain sufficient coverage. The levels of hydroxymethylated cytosines are obtained by subtracting methylated cytosines from the overall DNA methylation levels determined without oxidation for each CpG.

EXPERIMENTAL DESIGN

Please note, that a parallel WGBS sequencing is required for each sample.

YIELD AND EXPECTED RESULTS

With this protocol, we obtain per samples sequenced on e.g. 1/4 of 4 HiSeq lanes ~ 400 M reads, of which 60-70 % can be mapped to a bisulfite treated human genome. Rate of duplicate reads are ~ 25 % resulting in a genome-wide coverage of 7-15 x with generally lower coverage for the oxidized sample.

SAMPLE TECHNICAL REQUIREMENTS

Four hundred nanogramms of genomic DNA is best used as input material, but libraries can be constructed from as little as 100 ng, although duplicate rates will substantially increase