The first low input, three-day NGS Ion AmpliSeq™ Methylation Panel and protocol (Poster #142)

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ABSTRACT

Of critical importance to a variety of cancer types is the identification of reliable diagnostic and prognostic DNA methylation biomarkers. Impeding this mission is the absence of streamlined bioinformatics pipelines combined with painless, resolved, flexible multiplexed assays with low input requirements. The recently released Ion AmpliSeq™ Methylation Panel for Cancer Research is the first to kit and demonstrate the feasibility to create a targeted panel leveraging the Ion AmpliSeq™ technology on the Ion NS5 sequencing platform. The Ion AmpliSeq™ Methylation Panel for Cancer Research combines an easy 3-day protocol with manual or automated library options, low input (~10-20 ng starting), and flexible multiplexed approach with quantitative information at single base pair resolution. The bioinformatics analysis has been streamlined into a downloadable plugin providing DNA methylation calls on both Watson and Crick strands and methylated unmethylated ratios for each CpG. Samples tested to date include standard fresh frozen and FFPE.

RESULTS

A complete workflow has been developed starting from bisulfite conversion and progressing through library construction, template preparation, sequencing, and the final data analysis. The entire end-to-end workflow can be completed in three days (Fig. 2). The Ion AmpliSeq™ Methylation Panel for Cancer Research was created to be comprised of clinically relevant markers from the BLUEPRINT consortium4. Amplions were targeted to design both original strands, but only one of the two was selected for the final panel, which contains 40 amplions, 31 of which are cancer markers associated with colon cancer, prostate cancer, leukemia and lymphoma (Fig.3). The panel was evaluated using two control DNA samples. The first had an expected average methylation state across all CpGs of >98% and the second <5%. An equal mixture of these two samples was also tested (Fig.4). The analysis report provides the total number of reads covering the target CpGs, and the percentage of those reads that are methylated at both the sample and amplicon level (Fig. 6). The AmpliSeq™ methylation technology provided quantitative information with FFPE cancer versus benign samples at base pair resolution, independent of methylation status, compared orthogonally to MSP assay output (Fig.5).

The AmpliSeq™ Methylation Panel for Cancer Research offers a low DNA input, three-day end-to-end workflow with high resolution, targeted and quantitative methylation analysis of clinically relevant targets at low DNA input. The potential exists to design custom methylation panels.

MATERIALS AND METHODS

Consult the bisulfite methylation library construction protocol using the Ion AmpliSeq™ Library Kit & parser and the protocol using the Ion AmpliSeq™ Kit for ChIP or for detailed material and methods. The community AmpliSeq™ Methylation Panel for Cancer Research contains one pool at 5x concentration. Sample protocol: 16 per 500 chip for ChIP E7 template (12 samples + 2 NT), Genomic DNA or FFPE DNA (Bisulfite converted DNA required for library production). (Fig. 2) This protocol provides available control gDNA samples of average methylation state across all CpGs of approximately >98%, <5%, and an equal mixture of the two, equaling ~52% methylation states were run (Fig. 4). The DNA standards are purified from HCT116 DNA cells that contain genetic knockouts of DNA methyltransferases. DNMT1, 2, and 3, and the unmethylated standard has less than 5% DNA methylation (Fig. 4). The human methylation standards has been methylated enzymatically at all CG dinucleotides by M.SssI methyltransferase (Fig. 4). Per SOP, unmethylated Lambda DNA was spiked into each sample prior to bisulfite conversion and primers exist in the panel to determine conversion efficiency using separate output files. The methylation-analysis plugin and alignment calling.

Figure 5. Biomarker and therapeutic target discovery for bladder and prostate cancer

CONCLUSIONS

The first low input, three-day, end-to-end NGS workflow for Ion AmpliSeq™ Methylation Panel was showcased here. The streamlined protocol starts at bisulfite conversion and progressing through flexible multiplexed AmpliSeq™ library construction (both manual and automated), template preparation, sequencing, and simplified data analysis. The Ion AmpliSeq™ Methylation Panel for Cancer Research was initially tested with commercially available methylation controls and showed high correlation with known truth at low (~<5%), middle (~52%) and high (>88%) methylation status. A subset of the panel provided quantitative information with FFPE cancer versus benign samples at base pair resolution, independent of methylation status, and compared orthogonally to MSP assay output. The potential also exists to design custom methylation panels.

REFERENCES


ACKNOWLEDGEMENTS

1. David Berman, MD, PhD, Director Queen’s Cancer Research Institute. Division of Cancer Biology and Genetics, Queen’s University, Canada. Early access manuscript was provided by Thermo Fisher Scientific in support of this project, but not for remuneration.
2. Kun Zhang (BLUEPRINT Consortium), Professor Director of Bioinformatics, University of California at San Diego (UCSD).

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Figure 6. Ion AmpliSeq™ Methylation Panel - Methylation Analysis Plugin

Figure 7. Ion AmpliSeq™ Methylation Panel - Methylation Analysis Outline

The methylation-analysis plugin is executed after standard Torrent Suite™ analysis pipeline. Two bisulfite converted reference files are created during the 5x use of the plugin only. Bisulfite converted reference files are created during the first use of the plugin only. The reference files are converted into a sequence and then compared against a set of DNA sequences. The conversion pipeline is executed sequentially on the Watson (W) and Crick (C) strands for each biomarker.

The pipeline incorporates a modified version of the bisulfite alignment program Bismark: TADAF with isothon.