A versatile method for circulating cell-free dna methylome profiling by reduced representation bisulfite sequencing

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Abstract

Background: DNA methylation holds great promise as a biomarker as methylation patterns emerge early in tumorgenesis, are specific to cancer type, tissue-of-origin and are retained in cfDNA unlocking the liquid biopsy diagnosis approach. Although whole genome bisulfite sequencing is the most comprehensive method to assess DNA methylation, it is expensive for clinical use. Since methylation occurs in CpG dinucleotides which tend to form blocks that are co-methylated, investigation of CG rich subpart of the genome is the most cost-effective and performant compromise, in particular for cfDNA.

Objective: Which is the best strategy to subselect the genome? The selection can be achieved through proteins that have affinity for methylated sequences. Hence, unmethylated CpGs are not covered. On the other hand, microarrays have been well established in the field with the MethylationEPIC (EPIC) BeadChip covering 863,904 predefined CpGs4. On contrary, Reduced Representation Bisulfite Sequencing (RRBS) is a probe and hybridization free approach that has the capacity of assessing up to 3 million CpGs. The CpG-rich fraction is enriched by treating genomic DNA with a CG recognition site restriction enzyme (typically MspI), followed by size-selection on agarose gel. However, this approach is not compatible with highly fragmented DNA such as cfDNA, because its size distribution overlaps with the MspI digested fragments. Our lab has successfully adjusted RRBS to be compatible with cfDNA input developing cell free RRBS (cfRRBS).

Methods: The main innovation lays on burning the haystack: looped adaptors are selectively ligated to target fragments forming circular molecules. The circular molecules are resistant to treatment with exonucleases resulting in degradation of only off targets.

Results: The workflow consists of automatable enzymatic steps in a single tube and performs well in cell line DNA, cfDNA and FFPE samples. We achieved over 40-fold target enrichment with cfDNA, which is in the same range as classical RRBS on genomic DNA.

Conclusion: cfRRBS is a well suited method to investigate DNA methylation in the clinic as it is cost effective, time efficient and informative. Clinical studies have already illustrated its utility in subtyping of pediatric tumors and determination of Cancer of Unknown Primary Disease.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.