H(O)TA: estimation of DNA methylation and hydroxylation levels and efficiencies from time course data

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Abstract

Motivation: Methylation and hydroxylation of cytosines to form 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) belong to the most important epigenetic modifications and their vital role in the regulation of gene expression has been widely recognized. Recent experimental techniques allow to infer methylation and hydroxylation levels at CpG dinucleotides but require a sophisticated statistical analysis to achieve accurate estimates.

Results: We present H(O)TA, a software tool based on a stochastic modeling approach, which simultaneously analyzes time course data from hairpin bisulfite sequencing and hairpin oxidative bisulfite sequencing.

Availability and additional information: https://mosi.uni-saarland.de/HOTA

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1 Introduction

DNA methylation refers to the transfer of a methyl group to the C-5 position of cytosine (C) to produce 5-methylcytosine (5mC). In mammals it is predominantly found in the symmetrical CpG context and, as a major epigenetic modification, it plays an essential role in the regulation of gene expression. Moreover, DNA methylation contributes to a wide range of cellular processes such as development, X-inactivation, and imprinting (Brudo and Besenbacher 2002) and aberrant methylation patterns have been linked to several human diseases including cancer (Brambilla 1999). The oxidized form, 5-hydroxymethylcytosine (5hmC), has recently gained attention as it is not only involved in gene regulation but also seems to play a major role in active and passive DNA demethylation. It is hypothesized that CpGs can traverse an iterative cycle of methylation and demethylation through oxidation and base excision repair (Meissner et al. 2008).

DNA methylation is commonly measured by using bisulfite genomic sequencing (BS-seq) during which C is converted to uracil (Frommer et al. 1992) while both 5mC and 5hmC are read as Cs and can therefore not be discriminated (see Fig 1). As opposed to this, oxidative bisulfite sequencing (oxBS-seq) converts 5hmC to 5-formylcytosine (5fC) and conversion of the newly formed 5fC to uracil allows to discriminate between 5hmC and 5mC but not between C and 5hmC (Booth et al. 2012). Hence 5hmC levels must be inferred by a simultaneous estimation based on both BS-seq and oxBS-seq data. Standard BS-seq or oxBS-seq can only capture the modification state of one individual DNA strand at a time. To overcome this limitation hairpin BS-seq has been developed, which allows to determine the state of both cytosines of a CpG dyad. Thus, nine different possible states (pairs of the three possible states C, 5mC and 5hmC) can be implicitly measured (Laird et al. 2004). Moreover, while most cell types display relatively stable DNA methylation patterns, the dynamically changing gene expression program during mammalian development is accompanied by an alternation of methylation patterns. Given measurements at different times, (time-dependent) methylation efficiencies can be inferred and provide useful information about the mechanisms that control the developmental program (Arand et al. 2012).

Here, we present Hairpin (Oxidative) bisulfite sequencing Time course Analyzer (H(O)TA) - a tool that accurately infers (hydroxycytosine)methylation levels and determines the efficiencies of the involved enzymes at a certain DNA locus. The procedure for estimating levels and efficiencies is based on the construction of two coupled...
Hidden Markov Models (HMMs) and gets as input time course measurements from hairpin BS-seq and oxBS-seq, respectively. Using the ML approach proposed in [Giehr et al. (2016)], the evolution of the HMMs is determined by time-dependent methylation and (hydroxy-)methylation efficiencies and takes into account all relevant conversion errors (see dashed arrows in Fig. 1).

2 H(O)TA Software

H(O)TA has been developed in MATLAB and its execution requires the installation of the free Matlab runtime environment (MRE). The tool and the MRE can be downloaded as a single installation file available for Linux, MacOS, and Windows operating systems. As opposed to methods for single time point data, H(O)TA performs an analysis that considers the transient probability distribution over the set \{u, m, h\}^2 of nine hidden states of the two cytosines of a CpG dyad, where u, m and h describe C, 5mC and 5hmC, respectively. Thus, besides the states uu and mm, which correspond to the blue and red bars in the bar plots of the hidden states’ probabilities in Fig. 1, the model considers hemimethylated sites (states uu, mm, green bars) as well as fully hydroxylated sites (state hh) and hemihydroxylated sites (states uh, hu) and combinations of 5mC and 5hmC (states uh, hu), whose levels are given by orange bars and refined in detailed plots on the right of each bar plot in Fig. 1. The observable states reflect the possible outcomes of hairpin BS-seq and hairpin oxBS-seq, respectively, that is, \{T, C\}^2 (cf. last line in Fig. 1 and upper middle line plots). Users can provide BS-seq and oxBS-seq time course data. For each observation time point, estimates of the methylation and hydroxylation levels are computed, as well as, linear functions for the methylation (maintenance or de novo) and hydroxylation efficiencies, i.e., the probability of a methylation or a hydroxylation event between two cell divisions. In addition, an estimation is provided for the probability that no maintenance is performed when the current state is mm or uu, which hints on the existence of a passive demethylation mechanism induced by hydroxylation. The user must specify the number of cell divisions between two observation time points and provide conversion errors of BS-seq and oxBS-seq (see dashed arrows in Fig. 1). For all the estimated parameters confidence intervals are computed and a statistical test is carried out in order to verify certain hypotheses about the efficiencies. For a derivation of the likelihood and details about the optimization as well as the statistical validation of the results, we refer to [Giehr et al. (2016)].

The graphical user interface of H(O)TA consists of two windows: a dialogue window for loading the input files of a DNA locus and running the analysis and the main window (Fig. 2) for visualizing the output. The tool can automatically aggregate data of different CpGs of a locus and compute average (hydroxy-)methylation levels as well as average efficiencies. In addition, the analysis can be performed for each CpG individually.

Users can provide three input .txt files. The first file contains BS-seq data, the second one oxBS-seq data and the third file contains the conversion errors of the two experiments as well as a string that describes how many cell divisions take place between two observation time points. Only the file with the BS-seq data is mandatory and the other two are optional. If only BS-seq data is given, then the tool will predict only the methylation levels of the region (merged with the unknown hydroxylation levels). For a detailed documentation of the input files we refer to the tool webpage.

The main window has two subpanels. The right panel shows the output of the analysis either for the aggregated data or for each of the previously chosen CpG sites. The upper left and middle plots show the fit between the data and the model prediction for the observable states TT, TC, CT, CC for BS and oxBS experiments. The upper right plot presents the efficiencies of the enzymes responsible for maintenance methylation (dark red), de novo methylation (blue) and hydroxylation (yellow) as well as the total methylation (light red) on hemimethylated CpGs (see the tool webpage). The lower left plot shows the (hydroxy-)methylation levels of the current region and the lower right plot shows the exact distribution of the different hydroxylation states. In the upper left corner of the right panel the user can choose to focus on the plots of different CpG sites (or the aggregated data). In the lower right corner there are several options for exporting the estimation results in a desirable format. In the left panel of the main window the (hydroxy-)methylation levels and the efficiencies of all individual CpGs are plotted such that they can be compared with each other and with the chosen plots in the right panel.

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