TOWARD EPIGENETIC MAPPING OF HUMAN CHROMOSOMES IN NANOCHANNEL ARRAYS

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ABSTRACT

We have recently develop a method for optical detection of the epigenetic modification 5-hydroxymethylcytosine (5hmC) at the single-molecule level. We use a chemo-enzymatic reaction to covalently attach a fluorescent reporter molecule to 5hmC nucleotides. Labeled DNA is electro-kinetically squeezed into an array of silicon nanochannels and imaged on a fluorescence microscope. 5hmC residues are visible as fluorescent spots along the DNA contour. Single-molecule imaging of extended lambda phage genomes that are hydroxymethylated at specific sites revealed individual fluorescent labels at expected positions along the genome. We show first results of single-molecule epigenetic mapping of chromosomal DNA extracted from human peripheral blood cells.

KEYWORDS: Hydroxymethylcytosine, Single-molecule, Nanochannels, Genome mapping

INTRODUCTION

5-hydroxymethyl-cytosine (5hmC) is a recently rediscovered epigenetic modification of DNA with tissue and cell type specific distribution in mammalian genomes.[1] Recent studies of genomic DNA found that a substantial fraction of 5-methyl-cytosine (5mC) in CpG dinucleotides is converted to 5hmC and its role in transcription regulation is in the focus of extensive research. Information regarding quantity and distribution of 5hmC is critical for advancing this research. Moreover, the dynamic nature of this modification implies that 5hmC patterns may exhibit a high degree of cell to cell variation and should be studied by single-cell or single-molecule analysis. To achieve this, we use chemo-enzymatic labeling to highlight the distribution of 5hmC along genomic DNA molecules extended in nanochannels arrays.

EXPERIMENTAL

We used T4 β-glucosyltransferase (β-GT) to tag 5hmC sites with a fluorescent reporter molecule. The enzyme was fed with a synthetic cofactor UDP-6-N3-Glu, resulting in covalent attachment of a functional azide at the 5hmC site. This azide was further reacted with a DBCO functionalized fluorophore via a copper-free "click" chemistry reaction to generate the fluorescently labeled 5hmC (Figure 1 Left). [2]

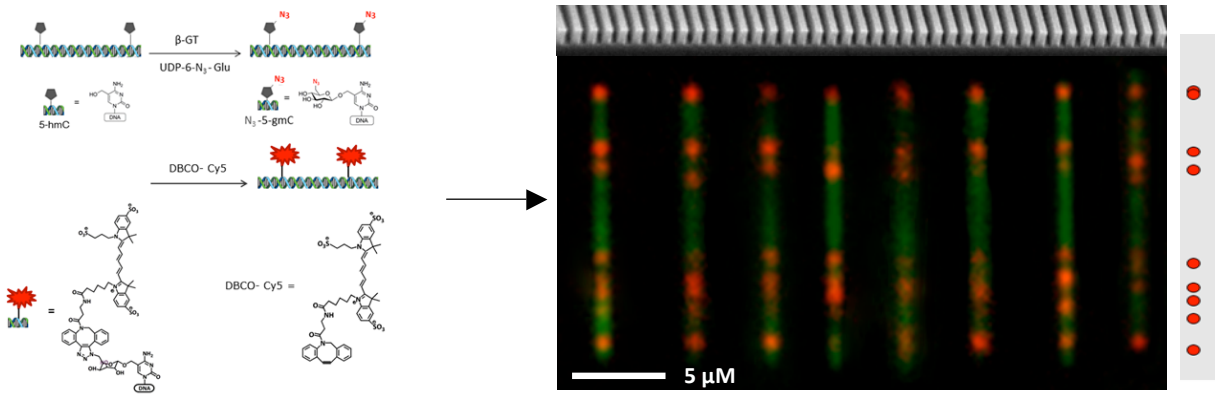

Figure 1: Left: schematic representation of the 5hmC labeling procedure. Right: Covalent labeling of 5hmC sites with a fluorescent dye for single molecule mapping in nanochannels. 5hmC sites were incorporated into the Lambda genome at known sites using nick translation. A glucosyltransferase enzyme was used to transfer an azido modified sugar onto each 5hmC residue and further labeled with an alkyne modified fluorescent dye by a click chemistry.

To demonstrate the viability of such an approach for epigenetic mapping, we have engineered 10 specific 5hmC sites within the 48.5-kb genome of lambda bacteriophage. The hydroxymethylated sites along the DNA were then labeled using the above procedure with Alexa Fluor 555. The DNA was stained with YoYo-1 intercalating dye and extended in nanofluidic channels. Channel cross-section was 50nm X50nm, resulting in extension of 85% of the b-form contour length, sufficient extension to generate distinct patterns along the DNA.

For mapping human genomic DNA we used dual color labeling of DNA extracted from peripheral blood cells. First, we generated a sequence specific barcode along the genome using nick translation with fluorescent nucleotides (Atto532-dUTP). In a second step we labeled 5hmC with Cy5 in order to generate the epigenetic pattern. The sample was extended in the nanochannels and imaged in three colors on an EM-CCD camera. Laser illumination at 473nm, 532nm & 635nm was used for excitation.

RESULTS AND DISCUSSION

Dual-color fluorescence images of the lambda phage sample revealed that the DNA was decorated with fluorescent spots. The theoretical pattern and several examples of individual genomes decorated with multiple fluorescent spots indicating 5hmC sites are shown in Figure 1. The expected 5hmC pattern is clearly reconstructed by the fluorescent labels, indicating that labeling was highly specific.

The human genomic DNA was labeled with an additional color at specific sequence motifs (GCTCTTC) to generate a locus specific pattern of dots that can be used in order to map the molecule onto the reference genome[3]. DNA molecules typically spanning several hundred Kbp (and up to 2Mbp) were visualized in the channels. The green dots represent 5hmC sites and red spots representing sequence-specific labels are clearly seen along the stretched molecules

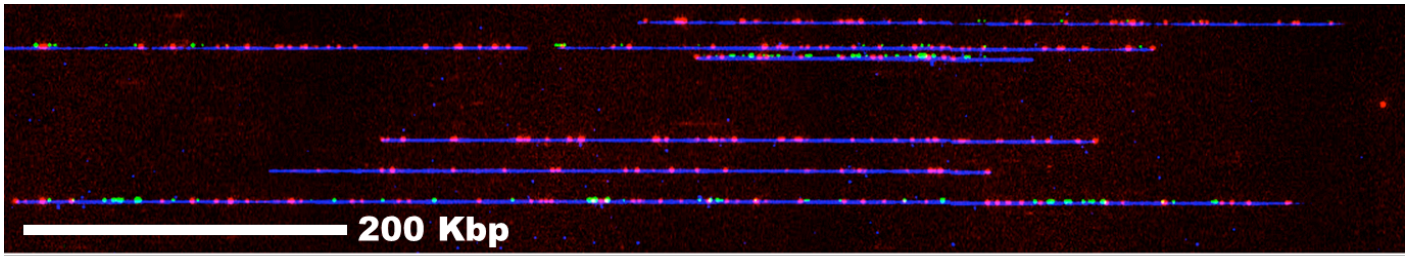

Figure 2: Genetic and epigenetic mapping of single DNA molecules. Three colors were used to label DNA extracted from human peripheral blood cells. Blue: DNA (YOYO-1), green: 5hmC (Cy5), Red: sequence-specific labels (Atto532).

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