POSTER PRESENTATION



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Amplification-free whole-genome shotgun bisulfite sequencing of mouse primordial germ cells

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From Epigenetics & Chromatin: Interactions and processes Boston, MA, USA. 11-13 March 2013

Background

Dynamic epigenetic reprogramming occurs during mammalian germ cell development, whereas the targets of this process including DNA demethylation and de novo methylation remain poorly understood. Here, we examined genome-wide methylation profiles in developing primordial germ cells (PGCs) of mice using high-throughput shotgun sequencing of bisulfite-treated DNA (whole-genome shotgun bisulfite sequencing; WGSBS), which accurately quantifies whole-genome methylation levels at single-base resolution.

Materials and methods

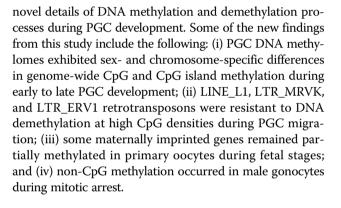
Using Illumina sequencing libraries, we scaled down the construction and analysis to nanogram quantities of DNA by generating a new WGSBS library, termed the postbisulfite adapter tagging (PBAT) method. PBAT libraries were generated from 2,000-5,000 PGCs and WGSBS analysis was performed using Illumina HiSeq 2000. Thus, we could provide complete maps of cytosine methylation in developing male and female PGCs during gonadal sex determination (at E10.5, E13.5, and E16.5).

Results

This DNA methylome study demonstrated genome-wide DNA demethylation, with erasure of genomic imprinting and X-inactivation during gonadal sex determination and gender-specific differences in genome-wide and genespecific (a part of CpG islands) DNA methylation levels in developing PGCs. Some of these global/local changes in DNA methylation during PGC progression were consistent with previous as well as more recent studies. However, our complete DNA methylome maps revealed important and

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Conclusions

In this study, we performed WGSBS mapping with thousands of mammalian cells (equal to approximately 20-50 ng genomic DNA) using the PBAT method. Our data and techniques can therefore serve as a platform for future studies to elucidate the role of epigenetic modifications in germline development and other biological processes.

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Published: 8 April 2013

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doi:10.1186/1756-8935-6-S1-P116

Cite this article as: Kobayashi *et al.*: **Amplification-free whole-genome shotgun bisulfite sequencing of mouse primordial germ cells.** *Epigentics & Chromatin* 2013 **6**(Suppl 1):P116.

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