Technology from Kyoto University

Sample preparation method for DNA methylation analysis

We are looking to out-license the technology for its commercialization.

Nobel method for increasing a yield of PCR amplifiable DNA sample after bisulfite treatment for DNA methylation analysis

◆Background

Methylation of DNA is one of epigenetic mechanisms and is deeply associated with embryo generation, cell differentiation, phenotype differences, various diseases, and the like. Further, methylation analysis of DNA is also used for sorting iPSC. In recent years, attempts are made to perform methylation analysis of single cell DNA using RRBS (Reduced Representation Bisulfite Sequencing) method capable of examining the methylation of DNA at a single base level. However, the DNA sample is fragmented because the bisulfite treatment is performed under a severe condition, and sufficient amount of DNA for PCR amplification is not supplied for further amplification for the next step.

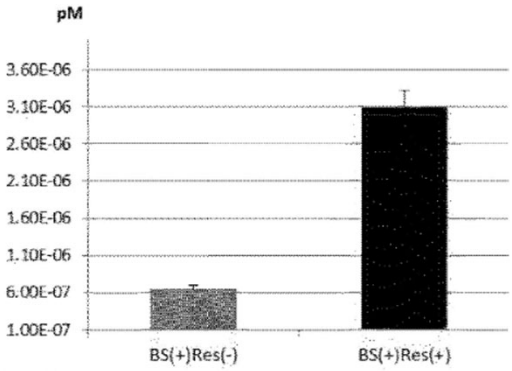
◆Description

Performing rescue including a step of treating a DNA sample after bisulfite treatment with single-stranded DNA ligase solves such problems, and can perform PCR amplification after bisulfite.

Rescue reaction The rescue reaction included following four steps:

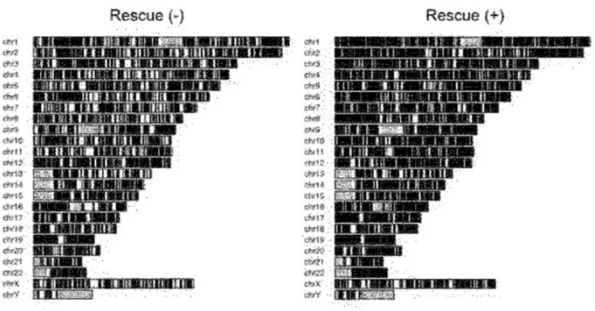
1) Immobilization on beads
2) Phosphatase reaction
3) Kinase reaction
4) Single-stranded DNA ligase reaction

[Fig 1]



The figure showing the effect of treatment with (Right) and without (Left) single-stranded DNA ligase.
The vertical axis represents the DNA amount (ng/μl) and shows 4.8 times difference.

[Fig 2]



The figure shows the result of analyzing the sequence obtained from the DNA obtained from the human ES cell line by the next generation sequence with (Right) and without (Left) rescue treatment.
The dark part (black) indicates the site of the CpG site that can be obtained in each chromosome. Rescue increased the number of CpG sites for approximately 1.6 times more.

◆Development Stage

This rescue method increases the yield of PCR-amplifiable DNA samples after bisulfite treatment

◆Applications

- Diagnosis of cancer and genetic diseases
- Discovery of drug discovery targets
- Understanding gene expression control at the single cell level improvement and livestock productivity improvement
- Crop improvement and livestock productivity improvement

◆Intellectual Property

- JP6976567
- WO2017/104675

Applicant:Kyoto University

◆Offers

- Patent License
- Option agreement

◆Contact

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