m⁶A RNA Modifications of Reprogramming Genes in Mouse and Human Embryonic Stem Cells

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ABSTRACT

N6-methyladenosine (m⁶A) RNA modification is a recently emerging topic regarding the control of cell fate transitions in mammalian embryonic stem cells. m⁶A is one of the most prevalent modifications on both mRNAs and noncoding RNAs in eukaryotes with an estimated 12,000 m⁶A sites in over 7,000 genes. The mark is deposited by a heterodimer of methyltransferase-like 3 and 14 (Mettl3 and Mettl14) and can be removed by the RNA demethylase enzymes FTO and ALKBH5. Obesity risk gene FTO encodes the first identified m⁶A demethylase. Mutations in FTO have been associated with increased risk for obesity and type II diabetes. Recently, impairment of the status of m⁶A regulation has led to prolonged expression of Nanog in ES cells and the inability to exit from self-renewal stages toward differentiation. m⁶A RNA immunoprecipitation (MeRIP) is a method to monitor the status of m⁶A and can map the locations of m⁶A modifications transcriptome-wide. We have optimized the method by employing a monoclonal antibody against m⁶A modifications and the use of magnetic A/G beads. The streamlined protocol is easy to process and can produce results with high signal to noise (SN) ratio. With the protocol we analyzed the m⁶A RNA levels of different reprograming genes in mouse and human ES cells.

MAGNA MeRIP™ m6A WORKFLOW

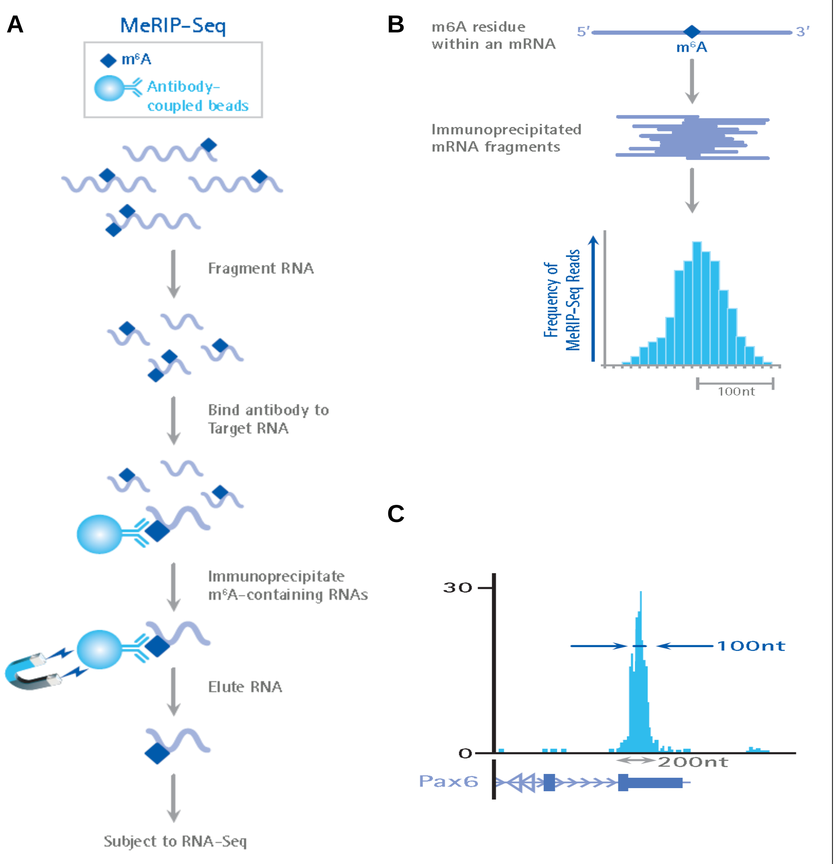


Figure 1. Overview of the Magna MeRIP™ m6A Kit

The Magna MeRIP™ m⁶A Kit (Cat. # 17-10499) uses the MeRIP method to enable identification and transcriptome-wide profiling of m⁶A. In the MeRIP assay, RNA is chemically fragmented into 100 nucleotides or smaller fragments followed by magnetic immunoprecipitation with a monoclonal antibody toward m⁶A. After immunoprecipitation, isolated RNA fragments can be subjected with qRT-PCR or RNA sequencing (RNA-seq).

RESULTS

Examples of Magna MeRIP™ m⁶A Data Using EEF1A1 Control Assay

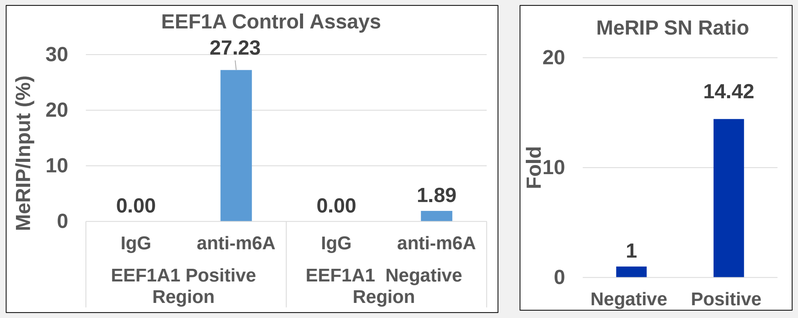


Figure 2. Successful retrieval of methylated RNA by Magna MeRIP™ m⁶A Assay (mRNA) MeRIP was performed using mRNA from HEK293 cells. Purified RNA was then analyzed by RT-qPCR using Positive Control Primers (MeRIP Primers Human EEF1A1 Positive, Part # CS220017) and Negative Control Primers (MeRIP Primers Human EEF1A1 Negative, Part # CS220018).

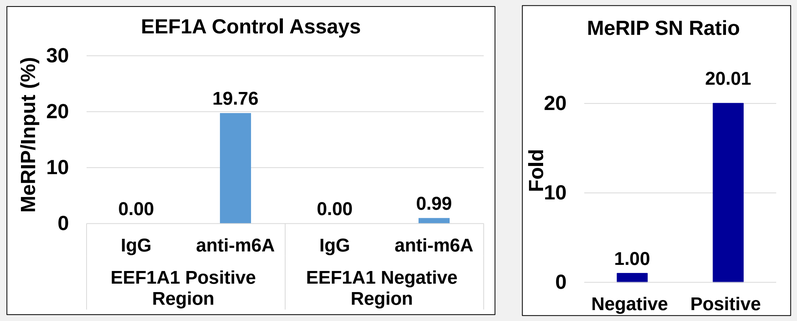


Figure 3. Successful retrieval of methylated RNA by Magna MeRIP m6A Assay (total RNA) MeRIP was performed using total RNA from HEK293 cells. Purified RNA was then analyzed by RT-qPCR using Positive Control Primers (MeRIP Primers Human EEF1A1 Positive, Part # CS220017) and Negative Control Primers (MeRIP Primers Human EEF1A1 Negative, Part # CS220018).

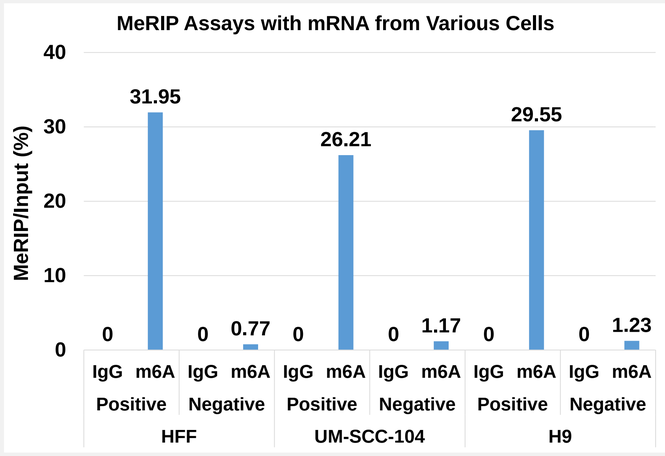
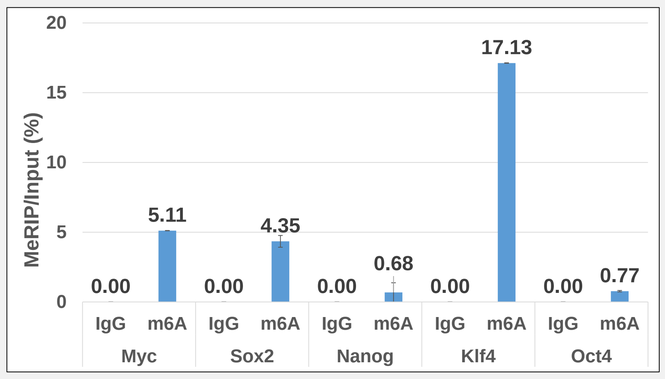


Figure 4. Successful retrieval of methylated RNA by MeRIP™ m6A Assay with mRNA from various cells
MeRIP was performed using mRNA from xeno-free human foreskin fibroblast cells (HFF, Cat. # SCC058), UM-SCC-104 human head & neck squamous carcinoma cells (Cat. # SCC072), and human embryonic stem cells (H9). Purified RNA was then analyzed by RT-qPCR using Positive Control Primers (MeRIP Primers Human EEF1A1 Positive, Part # CS220017) and Negative Control Primers (MeRIP Primers Human EEF1A1 Negative, Part # CS220018).

Analysis of the m⁶A RNA Levels of the Different Reprograming Genes in Mouse and Human ES cells

A. Murine ESC Core Pluripotency Factors



B. Human ESC Core Pluripotency Factors

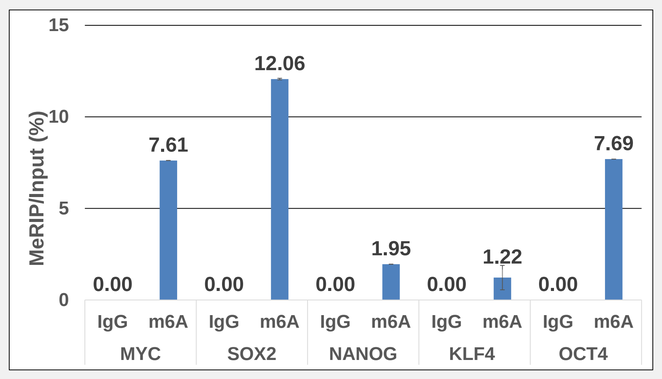


Figure 5. Analysis of m⁶A RNA Levels of Reprograming Genes in Mouse and Human ES cells
MeRIP was performed using mRNA from PluriStem 129/S6 murine ES cells (EMD Millipore, Cat. # SCR012) and H9 human ES cells. Purified RNA was then analyzed by RT-qPCR using MeRIP m⁶A peak primers for Myc, Sox2, Nanog, Klf4, and Oct4. PCR primer sequences for mouse reprograming genes were provided by Howard Chang (Stanford University). Human PCR primers were designed from published MeRIP-Seq data. One step RT-qPCR was performed with Bio-Rad qPCR machine and iTaq ™ Universal One-Step Kits (Bio-Rad). Percent input recovery was calculated by standard curve drawn with 0.1% input sample or delta delta Ct methods.

CONCLUSIONS

- Magna MeRIP m6A Kit (Cat. # 17-10499) allows:
  - Reliable recovery of m6A modified RNAs.
  - Simplifies performance of MeRIP using integrated set of key buffers, enzymes, and reagents
  - Streamlined protocol is easy to process and can produce results with high SN ratio
- RT-qPCR results with mouse ES core pluripotency factors were mostly consistent with published reports (Batista, J. et al. (2014) Cell Stem Cells) except for the mouse Nanog m6A signal which was lower than published results.
- In Human ES cells, clear m6A signals was observed in Myc and Sox2 and to lesser extent Nanog and Klf4.
- Significantly higher m6A expression in human ES cells relative to 129 ES/S6 mouse ES cells may be explained by PCR primer design in region of high mRNA expression levels.

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