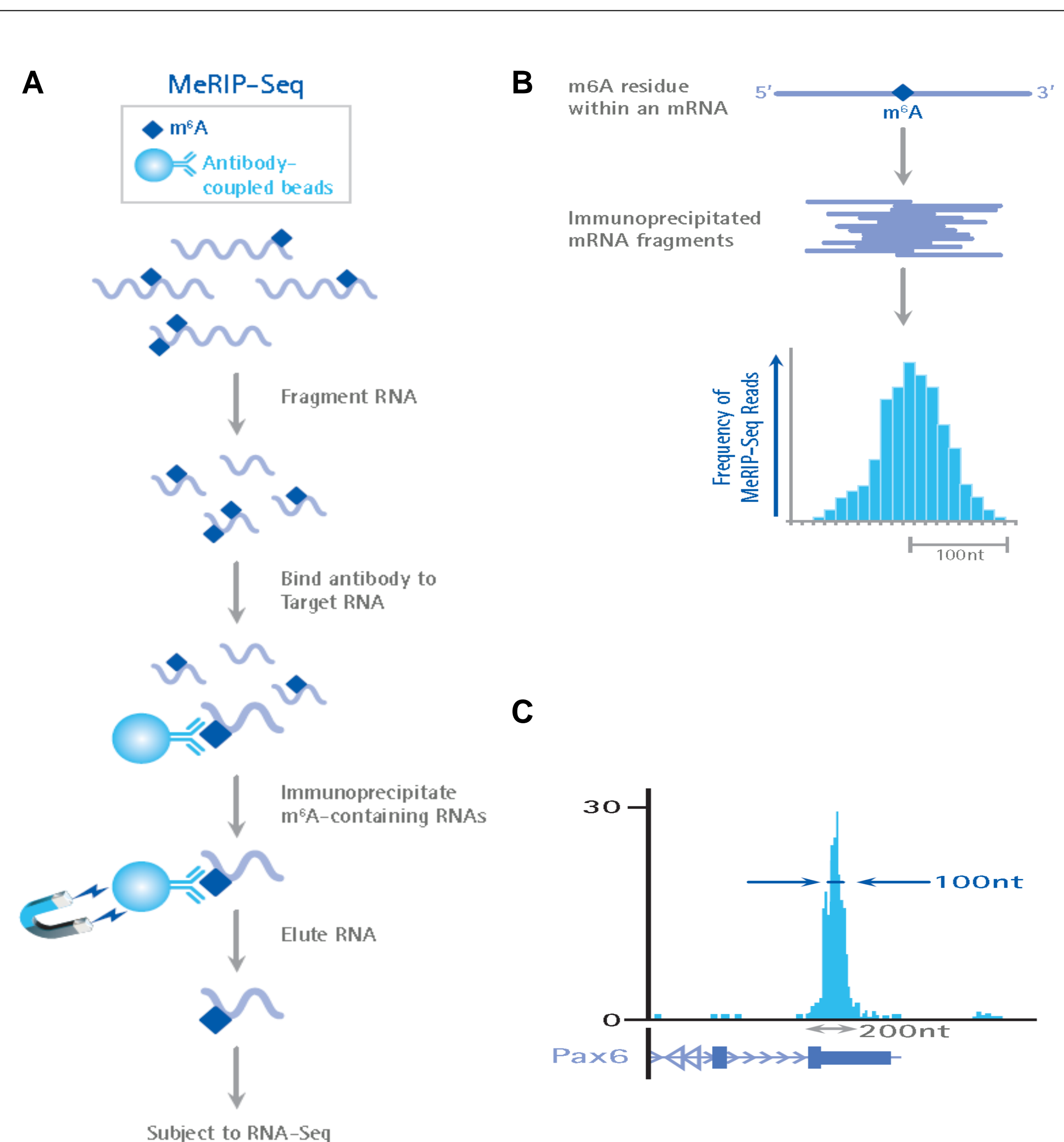


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## ABSTRACT

N<sup>6</sup>-methyladenosine (m<sup>6</sup>A) RNA modification is a recently emerging topic regarding the control of cell fate transitions in mammalian embryonic stem cells. m<sup>6</sup>A is one of the most prevalent modifications on both mRNAs and noncoding RNAs in eukaryotes with an estimated 12,000 m<sup>6</sup>A 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 m<sup>6</sup>A demethylase. Mutations in FTO have been associated with increased risk for obesity and type II diabetes. Recently, impairment of the status of m<sup>6</sup>A regulation has led to prolonged expression of Nanog in ES cells and the inability to exit from self-renewal stages toward differentiation. m<sup>6</sup>A RNA immunoprecipitation (MeRIP) is a method to monitor the status of m<sup>6</sup>A and can map the locations of m<sup>6</sup>A modifications transcriptome-wide. We have optimized the method by employing a monoclonal antibody against m<sup>6</sup>A 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 m<sup>6</sup>A RNA levels of different reprogramming genes in mouse and human ES cells.

## MAGNA MeRIP™ m<sup>6</sup>A WORKFLOW

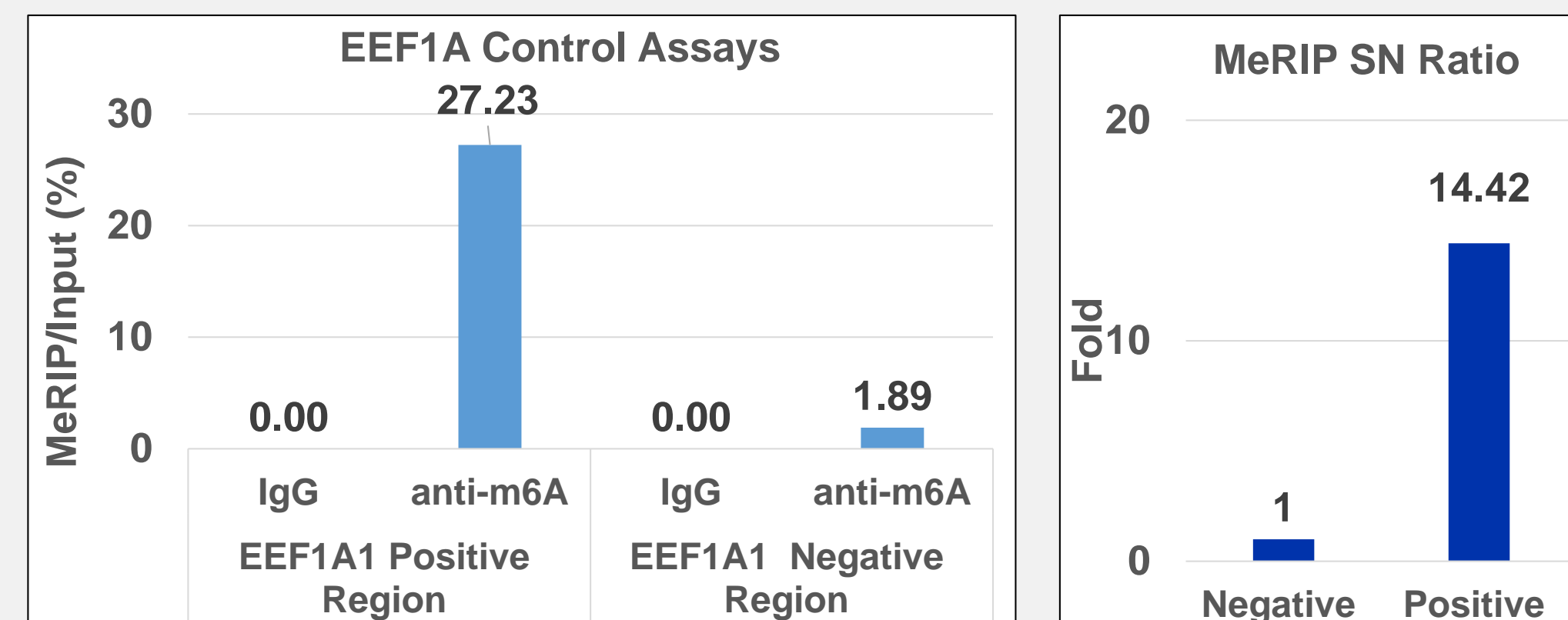


**Figure 1. Overview of the Magna MeRIP™ m<sup>6</sup>A Kit**

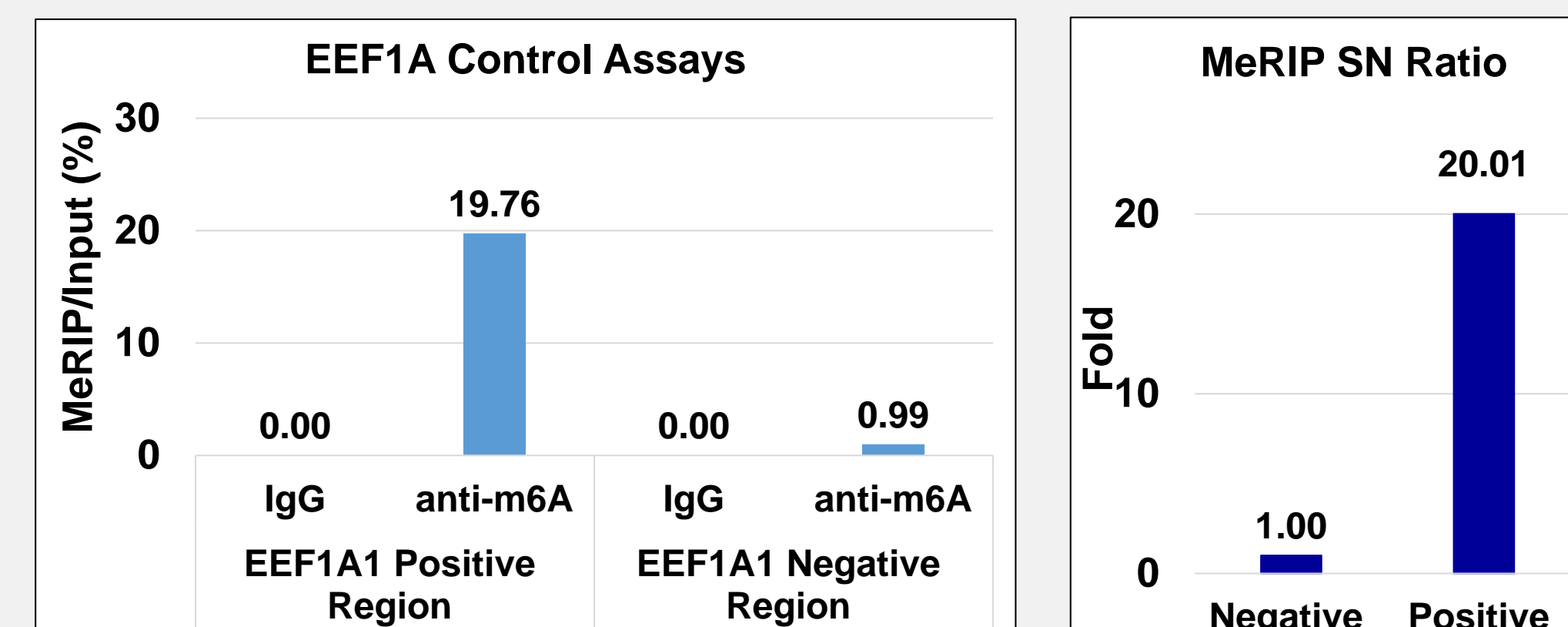
The Magna MeRIP™ m<sup>6</sup>A Kit (Cat. # 17-10499) uses the MeRIP method to enable identification and transcriptome-wide profiling of m<sup>6</sup>A. In the MeRIP assay, RNA is chemically fragmented into 100 nucleotides or smaller fragments followed by magnetic immunoprecipitation with a monoclonal antibody toward m<sup>6</sup>A. After immunoprecipitation, isolated RNA fragments can be subjected with qRT-PCR or RNA sequencing (RNA-seq).

## RESULTS

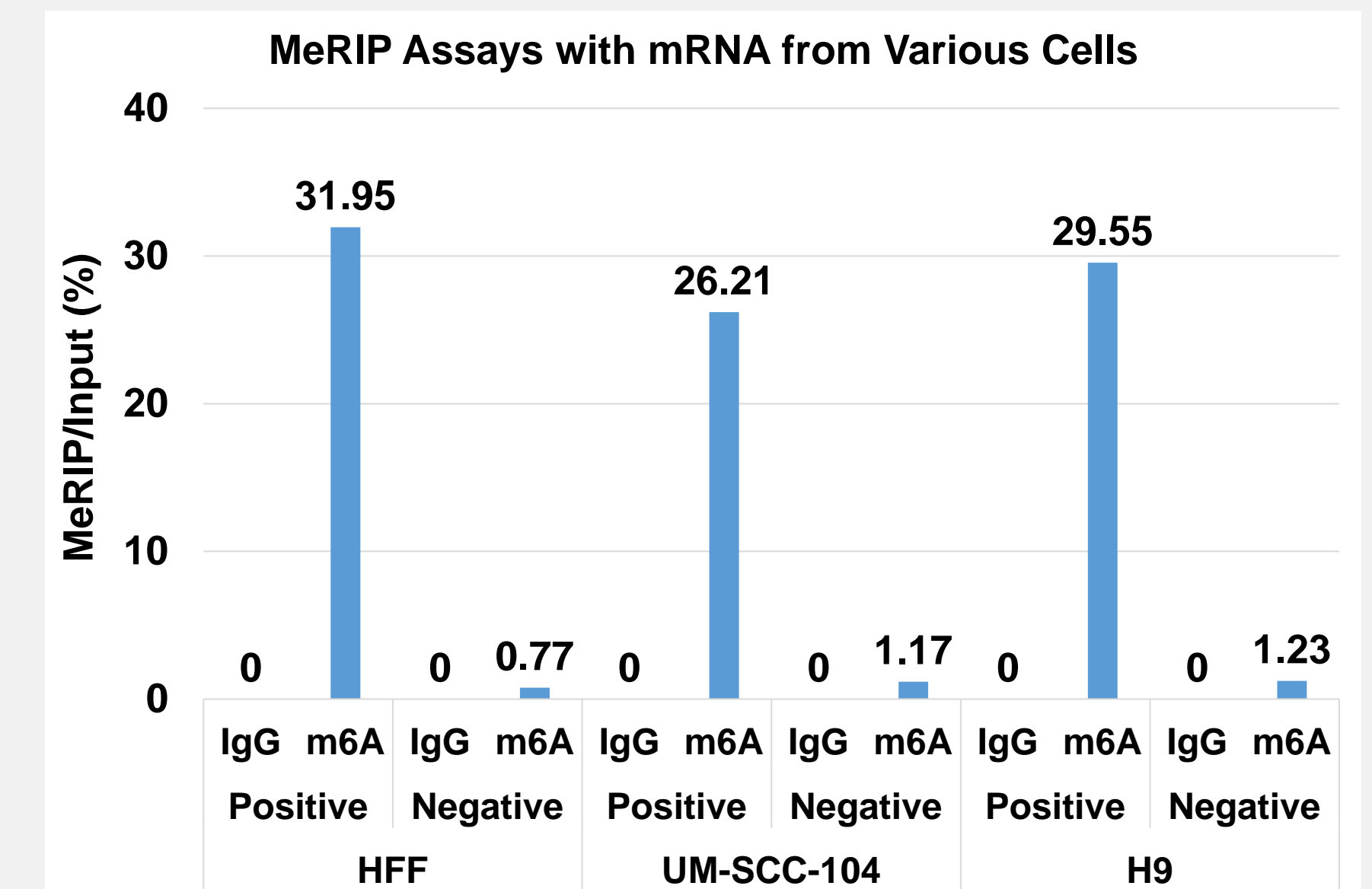
### Examples of Magna MeRIP™ m<sup>6</sup>A Data Using EEF1A1 Control Assay



**Figure 2. Successful retrieval of methylated RNA by Magna MeRIP™ m<sup>6</sup>A 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).



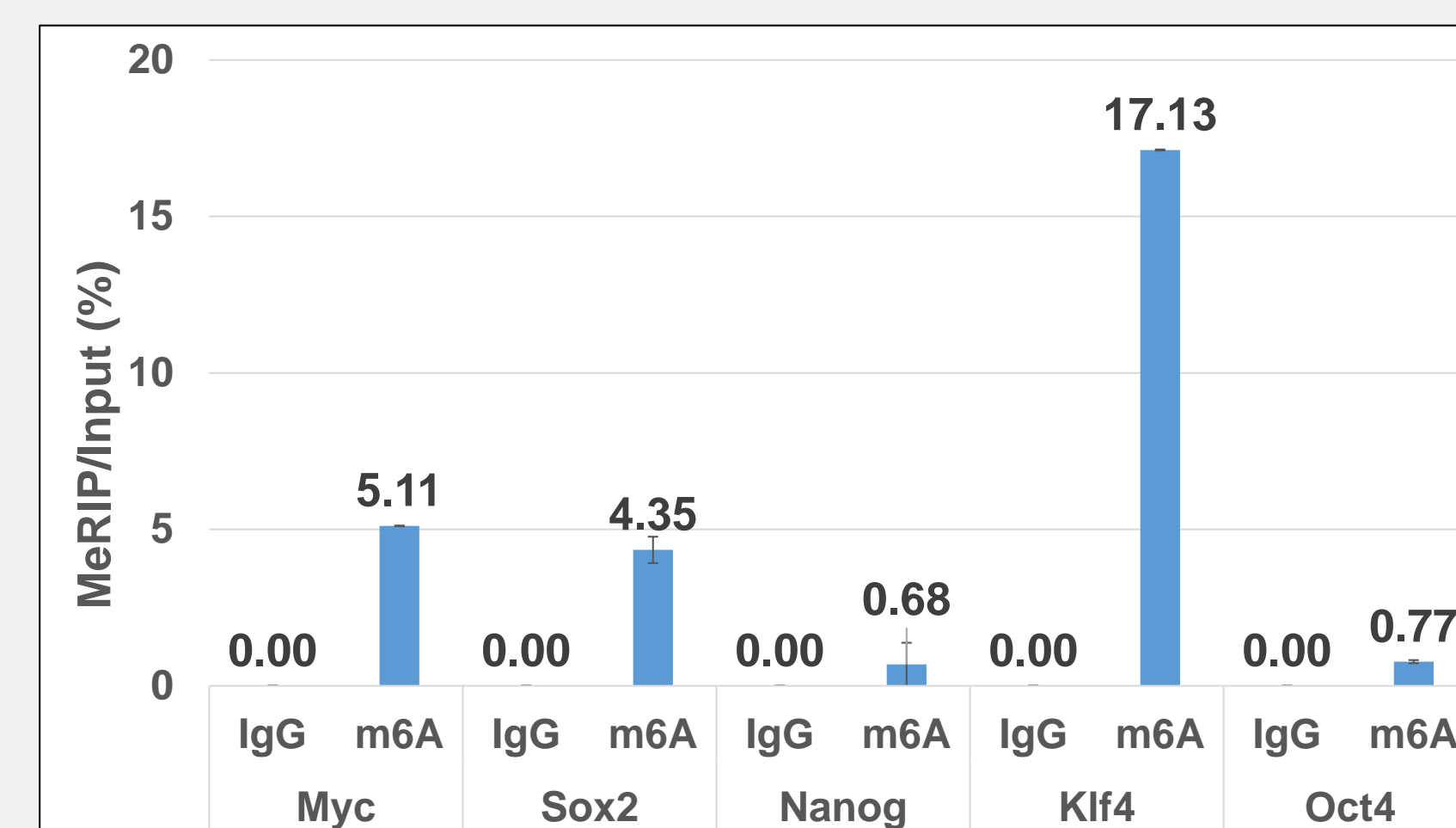
**Figure 3. Successful retrieval of methylated RNA by Magna MeRIP™ m<sup>6</sup>A Assay (total RNA)** MeRIP was performed using total RNA from HEK293 cells. Purified RNA was then analyzed by RT-qPCR using Positive Control Primers (MeRIP Primers Human EEF1A1 Positive, Part # CS220017) and Negative Control Primers (MeRIP Primers Human EEF1A1 Negative, Part # CS220018).



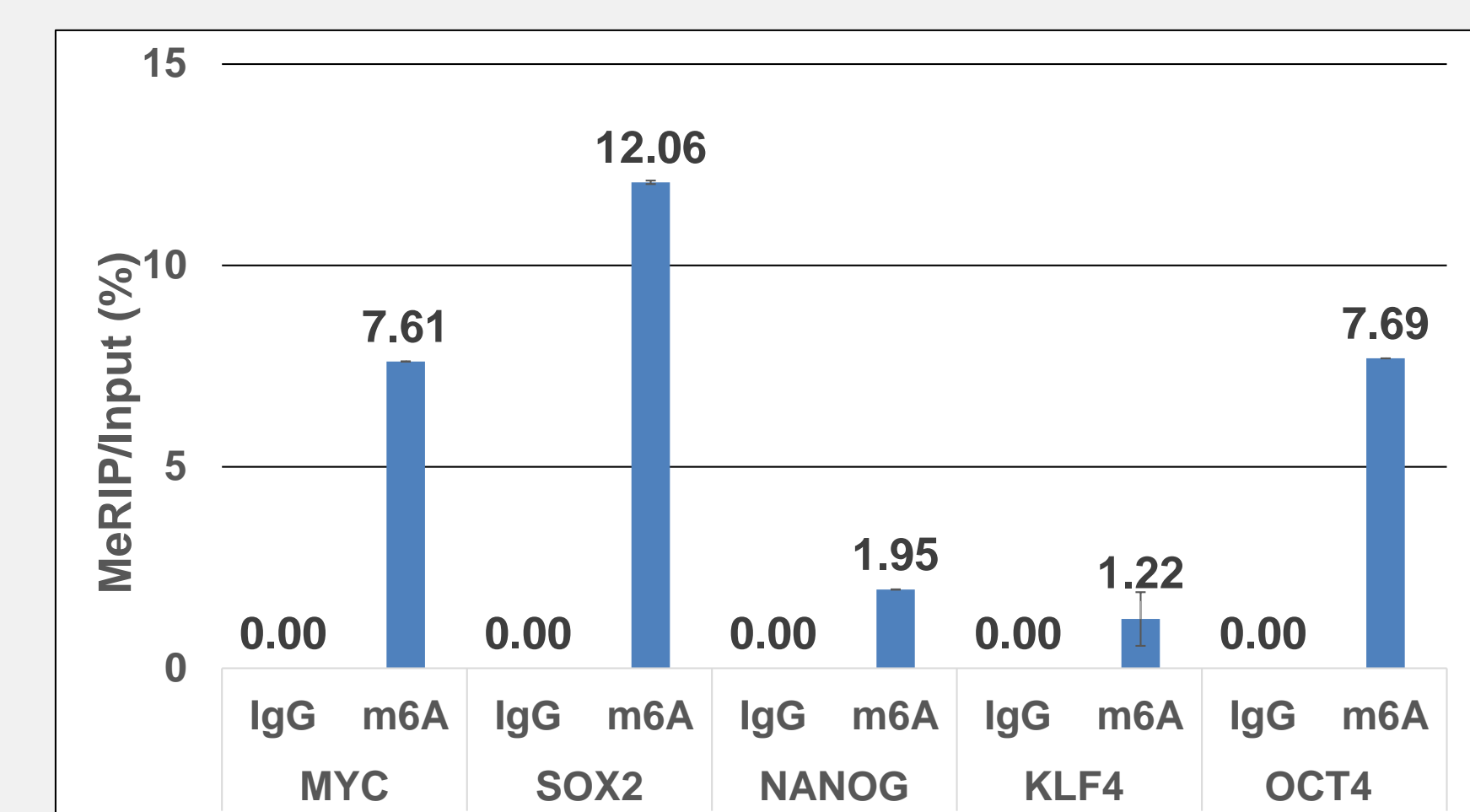
**Figure 4. Successful retrieval of methylated RNA by MeRIP™ m<sup>6</sup>A Assay with mRNA from various cells** MeRIP was performed using mRNA from xeno-free human foreskin fibroblast cells (HFF, Cat. # SCC058), UM-SCC-104 human head & neck squamous carcinoma cells (Cat. # SCC072), and human embryonic 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 m<sup>6</sup>A RNA Levels of the Different Reprogramming Genes in Mouse and Human ES cells

#### A. Murine ESC Core Pluripotency Factors



#### B. Human ESC Core Pluripotency Factors



**Figure 5. Analysis of m<sup>6</sup>A RNA Levels of Reprogramming 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 m<sup>6</sup>A peak primers for Myc, Sox2, Nanog, Klf4, and Oct4. PCR primer sequences for mouse reprogramming 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 m<sup>6</sup>A Kit (Cat. # 17-10499) allows:
  - Reliable recovery of m<sup>6</sup>A 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 m<sup>6</sup>A signal which was lower than published results.
- In Human ES cells, clear m<sup>6</sup>A signals was observed in Myc and Sox2 and to lesser extent Nanog and Klf4.
- Significantly higher m<sup>6</sup>A 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.