Pluripotency Prediction Using a Key Set of Six Epigenetic Biomarkers

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Abstract

The ability to accurately characterize the pluripotent state of human embryonic stem (ES) cells or induced pluripotent stem (iPS) cells is paramount to the field of human stem cell research. Pluripotent stem cells display cell morphology, cell surface markers, a gene expression profile, and an epigenetic signature distinct from that of partially or fully differentiated cells. Routine measures employed to characterize the pluripotent state of an ES or iPS cell line include embryoid body formation, karyotyping, expression of transcription factors OCT4, SOX2, and NANOG, expression of specific cell surface antigen markers such as SSEA and TRA, and more recently, characterization of the cells' epigenetic profile.

Genome-wide DNA methylation signatures for human ES and iPS cell lines have been studied using both reduced-representation bisulfite sequencing (RRBS) (Bock *et al*. 2010) and bead array platforms (Bibikova *et al*. 2006). While these genome-wide methods are the most accurate means for characterizing the epigenetic variability among ES and iPS cells lines, they are expensive and cumbersome for high-throughput research laboratories wanting to routinely monitor the quality of their pluripotent cell lines.

Introduction

The *OneStep* qMethyl™ Human Pluripotent Stem Cell Panel I uses Methylation-Sensitive Restriction Enzyme (MSRE) digestion and real-time PCR to predict the pluripotent state of an ES cell or an iPS cell line based upon a specific epigenetic profile of six key genes: RAB25, NANOG, PTPN6, MGMT, GBP3, and LYST. These gene regions have been established in the literature (Nishino *et al*. 2011, Calvanese *et al*. 2008, and Deb-Rinker *et al*. 2005) to show differential DNA methylation in their promoter regions between human ES and iPS cells, and differentiated cells based upon bisulfite sequencing.

OneStep qMethyl Panel Workflow

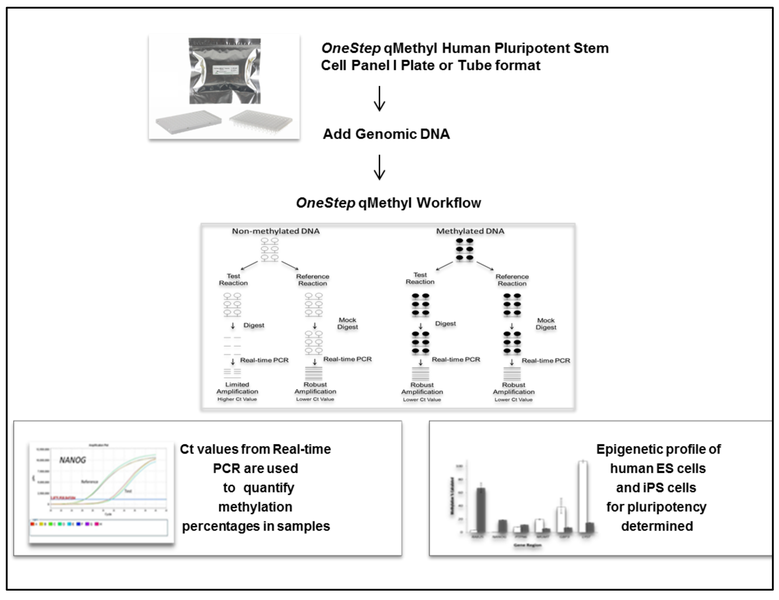

Figure 1. The *OneStep* qMethyl™ Human Pluripotent Stem Cell Panel contains master mixes containing either active or inactive Methylation Sensitive Restriction Enzymes (MSREs), Taq polymerase, dNTPs, buffers, primers, and the fluorescent dye SYTO 9® for real-time PCR. The genomic DNA sample is divided into two wells; a Test Reaction, and a Reference Reaction. Test Reaction samples are digested with the MSREs while the Reference Reaction is not (mock digest). Following digestion, the DNA in each well is amplified using real-time PCR. Methylation percentages for each sample are determined using the Ct values of both the Test and Reference Reaction using a derivation of the $2^{-\Delta CT}$ method.

Methods

In order to test the effectiveness of promoter regions of RAB25, NANOG, PTPN6, MGMT, GBP3, and LYST as epigenetic biomarkers for pluripotency prediction in ES cells, genomic DNA from five human ES cell lines ES-017, ES-035, ES-049, ES-051, ES-053, (BioTime), one mesenchymal stem cell (MSC) line (PromoCell), one neural progenitor stem cell (NPC) line (Millipore), and fully differentiated liver cells were run in triplicates for each of the six promoter regions using the *OneStep* qMethyl™ Human Pluripotent Stem Cell Panel I protocol. In a separate experiment conducted at Rutgers University, the *OneStep* qMethyl™ Human Pluripotent Stem Cell Panel I protocol was tested on iPS cell lines (transformed using Yamanka factors) reprogrammed from fibroblasts and cryopreserved lymphocytes.

All samples were run in a 96 well plate on the Applied BioSystem 7500 real-time thermocycler using a two hour digestion at 37°C, followed by enzyme activation at 95°C for ten minutes, and 40-45 cycles of 95°C for 30 seconds, 65°C for 1 minute, and 72°C for 1 minute. Reads of fluorescence were taken in the extension phase. Ct values were converted to DNA methylation percentages based upon the $2^{-\Delta CT}$ method (Livak and Schmittgen, 2001).

Results

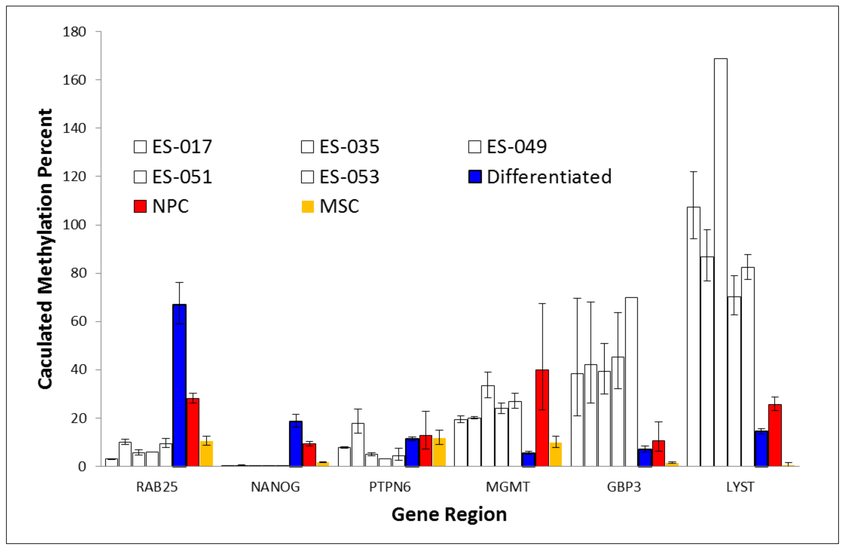

Figure 2. The methylation patterns of DNA from five ES cell lines ES-017, ES-035, ES-049, ES-051, ES-053, (BioTime) (shown as white bars) were consistent with the patterns of DNA methylation percentages in the promoter regions of RAB25, NANOG, PTPN6, MGMT, GBP3, and LYST shown in the reported literature, while differentiated liver cell DNA (shown in blue) had a distinctly opposite pattern of DNA methylation. The MSC line (shown in yellow) and the NPC line (shown in red) showed patterns unlike that of either the ES cell lines or the fully differentiated liver cell DNA.

References

1. Bock , C. *et al*., 2011. Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell* 144,439-452.
2. Bibikova, M. *et al.,* 2006 . Human embryonic stem cells have a unique epigenetic signature. *Genome Res.* 16:1075-1083 .
3. Nishino, K. *et al.,* 2011. DNA methylation dynamics in human induced pluripotent stem cells over time. PLoS Genetics 7, (5):e1002085. doi 10.1371/journal.pgen. 1002085
4. Calvanese, V. *et al*., 2008. Cancer genes hypermethylated in human embryonic stem cells. PLoS ONE 3, (9):e3294. doi 10.1371/journal.pone.0003294
5. Deb-Rinker, P. *et al*., 2005. Sequential DNA methylation of the Nanog and Oct-4 upstream regions in human NT2 cells during neuronal differentiation. *JBC* 280(8) 6257-6260.
6. Livak and Schmittgen, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25, 402-408.

Results

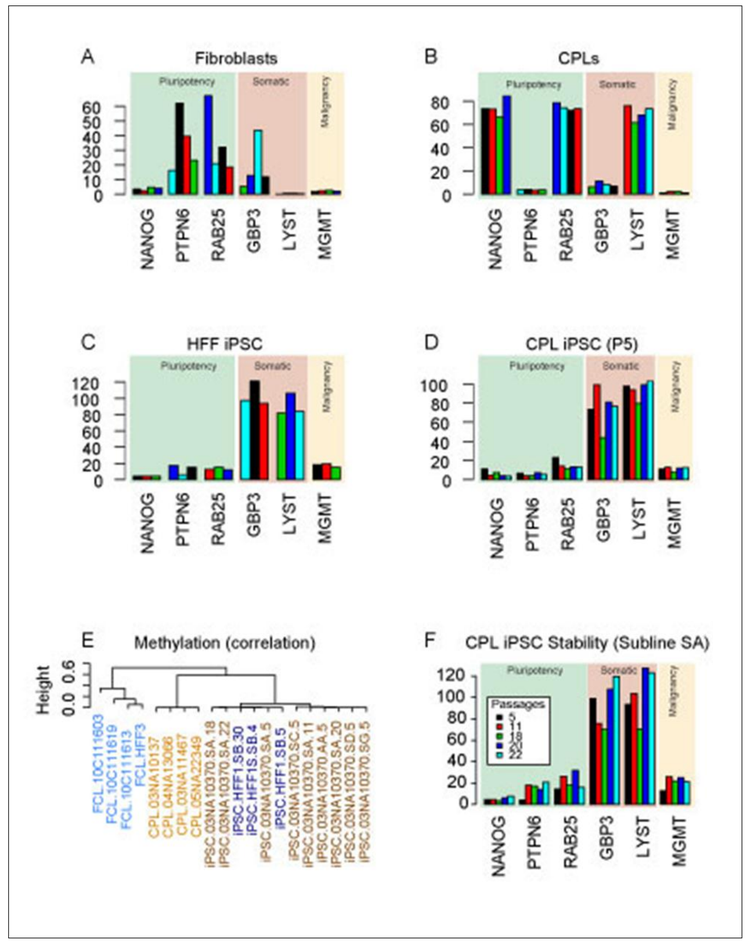

Figure 3. The DNA methylation patterns of fibroblasts (A) and cryopreserved lymphocytes (B) were distinctly different from that of the iPS cells derived from fibroblasts (C), and cryopreserved lymphocytes (D) when tested with the *OneStep* qMethyl™ Panel. iPS cells displayed methylation patterns of pluripotency similar to human ES cells. Hierarchical clustering confirmed that iPS cells were more similar to each other than the fibroblasts or cryopreserved lymphocytes from which they were derived (E). Multiple passaging of an iPS cell line for 5 to 22 passages showed that the DNA methylation pattern was stable in each of the six gene regions (F). Data courtesy of Dr. Ronald Hart, Rutgers University

Conclusions

•The ***OneStep* qMethyl™ Panel** can be used to indicate pluripotency in iPS cells lines by comparison with a known ES cell line This is achieved by quantifying the percent of DNA methylation in regions of RAB25, NANOG, PTPN6, MGMT, GBP3, and LYST and comparing the overall pattern of the six genes.

• The epigenetic profile of an ES or iPS cell line can be characterized using the ***OneStep* qMethyl™ Panel** to indicate if the cell line is in a pluripotent state or an intermediate state of differentiation such as a neural progenitor cell or a mesenchymal stem cell.

•The ***OneStep* qMethyl™ Panel** utilizes MSRE digestion and real-time PCR to easily quantify DNA methylation in specific gene regions for large numbers of samples. Compared to targeted sequencing the cost of running the panel is much lower.

• The six gene regions of the ***OneStep* qMethyl™ Panel** can be multiplexed to increase the sample throughput and further reduce the cost per sample. Please inquire about multiplexing options for this panel.