ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

***OneStep* qMethyl™ Panel**: A method to indicate pluripotency and characterize human embryonic stem cells

Lam Nguyen, Jill Petrisko, Manuel Krispin, Xi-Yu Jia, Zymo Research Corporation, Irvine, CA

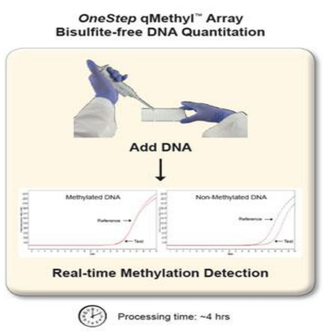
Abstract

Pluripotency is the ability of embryonic stem cells to differentiate into multiple cell types.[1] Pluripotent cells have epigenetic signatures that reflect their ability to generate multiple cell types.[2] Different DNA methylation patterns in gene regions vary between pluripotent and differentiated cells as a result of processes such as development, carcinogenesis, genomic imprinting disorders, and cell reprogramming[3,4,5.] In human pluripotent cells, gene promoter regions in the ***NANOG***, ***RAB25***, and ***PTPN6*** genes have been shown to maintain low levels of DNA methylation compared to differentiated cell types.[3,6] Conversely, gene promoter regions of ***MGMT, GBP3***, and ***LYST*** have been shown to maintain high levels of methylation in pluripotent cells compared to differentiated cell types.[3,4] Here we present a simple, straightforward, and bisulfite-free procedure for rapid, DNA methylation assessment for the above mentioned genes.

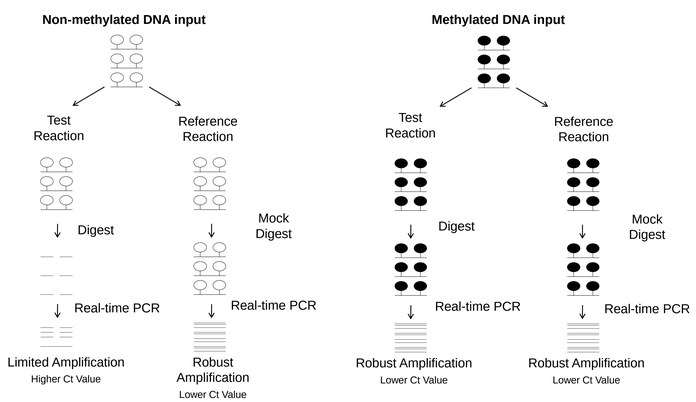
Introduction

The ***OneStep* qMethyl™ Panel (Human Pluripotent Stem Cell Panel I)** from Zymo Research provides a simple, straightforward, and bisulfite-free procedure for rapid, DNA methylation assessment of: *NANOG, RAB25, PTPN6, MGMT, GBP3, and LYST* in any cell type. The reagents in the plate are already premixed and optimized for robust amplification and detection. Simply add DNA into the appropriate well and then quantitate via real-time PCR… *OneStep!*

Workflow



The whole process is achieved in a streamlined, single, premixed reaction on a real-time, 96-well PCR plate and can be performed in < 5 hours. These specific regions of interests include *NANOG, RAB25, PTPN6, MGMT, GPB3, and LYST.* The performance of the panel has been validated using rigorous standards to ensure repeatability, accuracy and sensitivity.
An overview of the technology is shown below:



The sample DNA is divided in two parts; a **Test Reaction** and a **Reference Reaction**. **Test Reaction** samples are digested with **M**ethylation **S**ensitive **R**estriction **E**nzymes (MSREs) while the **Reference Reaction** samples are not (mock digest). Following digestion, DNA from both samples is used for real-time PCR.

Assay Performance

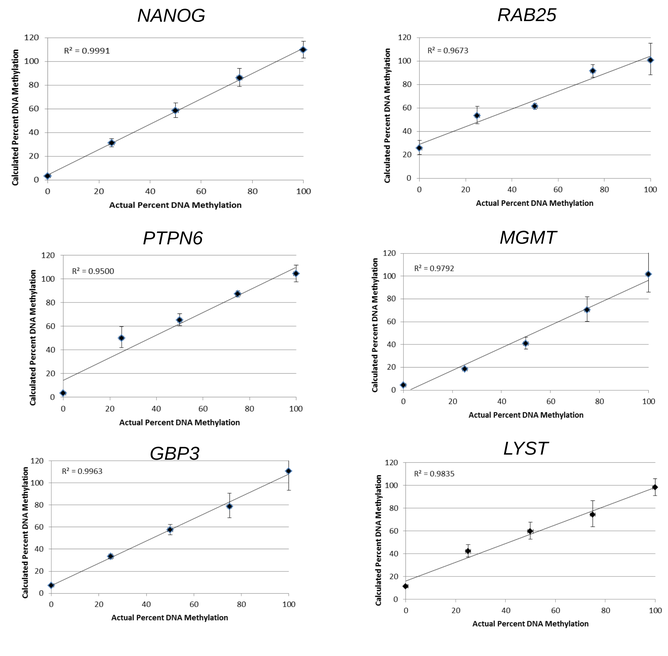


Figure 1. Results of *OneStep* qMethyl™ Panel closely correlate with actual DNA methylation percent values. A standard curve using *NANOG*, *RAB25, PTPN6, MGMT, GBP3,* and *LYST* primer sets from the ***OneStep* qMethyl™ Panel** was performed with 0%, 25%, 50%, 75%, and 100% methylated DNA from mixtures of human non-methylated and methylated DNA. DNA samples were assayed in triplicates using the ABI™ 7500 series.

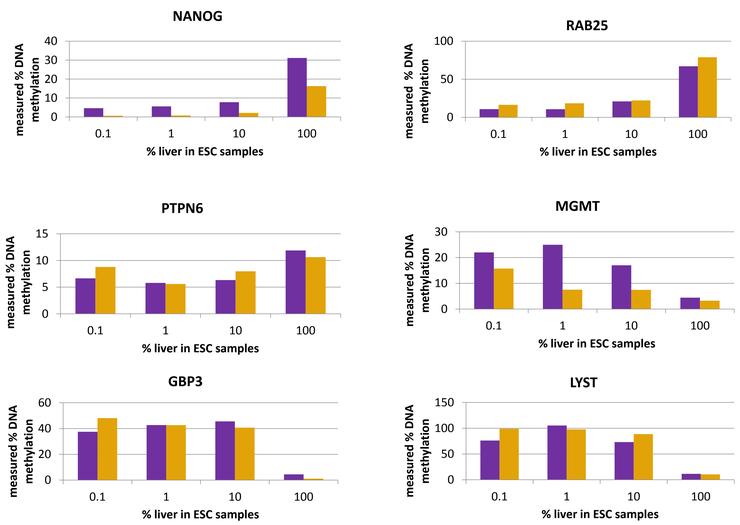
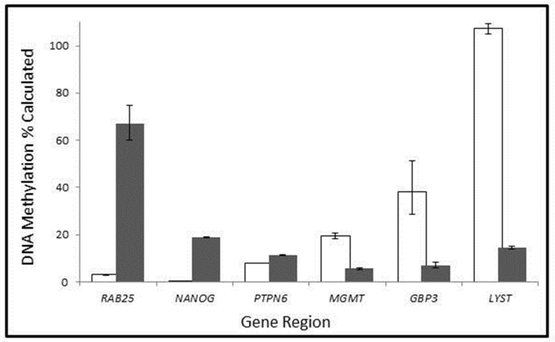


Figure 2. Differentiated (liver cell) DNA can be distinguished from embryonic stem cell DNA using the *OneStep* qMethyl™ Panel. Differentiated DNA can be detected from two DNA stem cell populations (shown in purple and orange bars) using the ***OneStep* qMethyl™ Panel**. Investigating the six regions of interest, liver DNA was spiked into human embryonic stem cells at 10%, 1% and 0.1%. As little as 1% (~330 cells) of liver DNA can be detected within the heterogeneous DNA population. Assay performed in duplicates on the ABI™ 7500 series.

Biological Applications



Cell Population	*RAB25*	*NANOG*	*PTPN6*	*MGMT*	*GBP3*	*LYST*
Differentiated	+	+	+	-	-	-
Pluripotent	-	-	-	+	+	+

Figure 3. A pluripotent stem cell line can be distinguished from a differentiated cell line using the *OneStep* qMethyl™ Panel. The graph above illustrates average DNA methylation percentages for the human differentiated cells (shown as grey bars) and human stem cell line (shown as white bars) for gene regions *RAB25*, *NANOG*, *PTPN6*, *MGMT*, *GBP3*, and *LYST*. The human pluripotent stem cell line shows a distinctly different pattern than the human differentiated cells.

Conclusions

- The ***OneStep* qMethyl™ Panel** can be used to efficiently quantify percent DNA methylation in regions of *RAB25, NANOG, PTPN6, MGMT, GBP3,* and *LYST*.
- The ***OneStep* qMethyl™ Panel** can be used to indicate pluripotency in stem cells lines by comparison with a differentiated cell line.
- The ***OneStep* qMethyl™ Panel** is a cost effective way to quantify DNA methylation in specific gene regions for large numbers of samples.

References

1. Tavakoli *et al.* (2009). *BMC Cell Biology* 10 (44) doi:1186/1471-2121-10-44.
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4. Calvanese *et al.* (2008). *PLOS One* 3:9: e3294. doi:10.1371/journal.pone.0003294.
5. Chamberlain *et al.* (2010). *PNAS* 107 (41):17668-17673.
6. Deb-Rinker *et al.* (2005). *J. Biol. Chem*. 280 (8):6257-6260.

For more information on the ***OneStep* qMethyl™ Panel**, ***OneStep* qMethyl ™Arrays**, ***OneStep* qMethyl™Kit**, and ***OneStep* qMethyl™ *Lite*** go to www.zymoresearch.com.