Towards an accurate analysis of pluripotency: Analysis of marker gene expression by digital droplet PCR

stemgenomics
cell integrity

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BACKGROUND

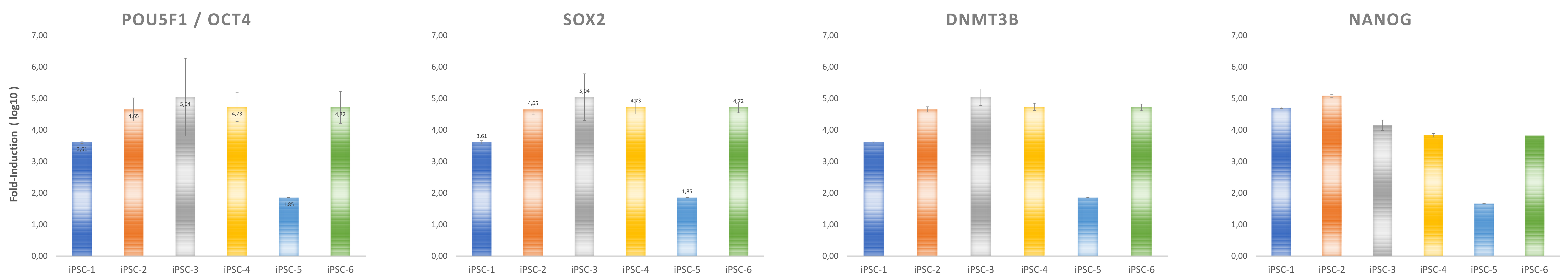
- Pluripotency is a critical characteristic of stem cells, allowing them to **differentiate** into various cell types in the body.
- Understanding pluripotency is essential for harnessing stem cells in **regenerative medicine** and **disease treatment**.
- **Molecular tools** play a crucial role in studying **pluripotency and differentiation stages** by providing precise insights into gene expression profiles.
- Study question: **How accurate is digital PCR for studying gene marker expression in pluripotent cells?**

CONCLUSIONS

Our innovative digital PCR test is

- **Targeted:** this digital PCR assay was developed to evaluate the expression of 16 marker genes representing Undifferentiated stage and the three germ layers.
- **Highly sensitive:** it provides accurate insights into undifferentiated status of cells and trilineage differentiation potential.
- **Easy to use:** it accepts three different input sample types - RNA, cell pellet, and cells in RLT buffer.
- **A fast service:** the results are delivered within 3 working days after sample reception.
- **Easy to understand:** the results are clear and straightforward, facilitating interpretation and decision-making in pluripotency and differentiation studies.

RESULTS



Six pluripotent cell lines (iPSC-1 to iPSC-6) were evaluated using optimized protocols, and the relative expression of 4 genes was analyzed by digital droplet PCR.

- Expression levels observed for each gene **were previously normalized** against a **differentiated cell line.**
- There was significant **overexpression** of undifferentiated genes (DNMT3B, NANOG, POU5F1, SOX2) in all cell lines tested.

METHOD

GENE SELECTION

This selection was meticulously based on recommendations from the **International Society for Stem Cell Research (ISSCR)** and **relevant scientific publications.**

Housekeeping gene: *B2M*

Undifferentiated genes: *NANOG, DNMT3B, SOX2, POU5F1/OCT4*

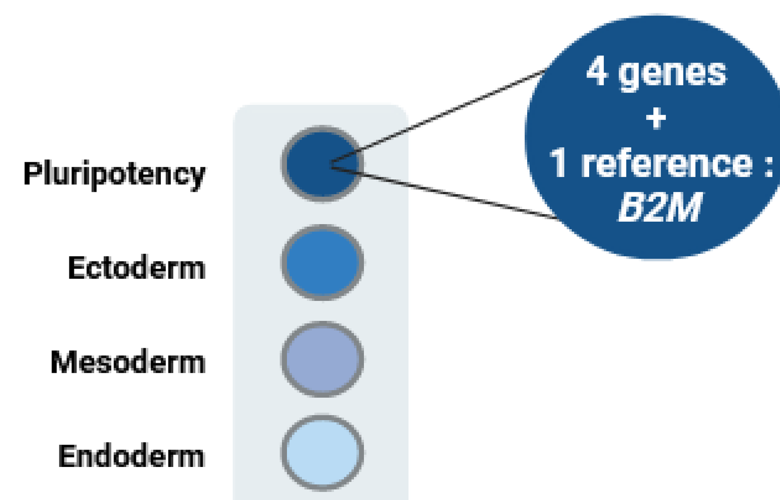
Ectoderm genes: *MAP2, PAX6, EN1, NEFL*

Mesoderm genes: *HAND1, CDH5, MYOG, TBXT*

Endoderm genes: *AFP, FOXA2, SOX17, SST*

MULTIPLEXING TEST DESIGN

The proposed design for this test highlights the **distribution** and **multiplexing** of each of the four genes of interest specific to each differentiation stage along with the reference gene.



EXTRACTION

In this test, gene expression is the key focus, requiring **RNA** as the starting material. Since the provided sample consisted of live cells, an **RNA extraction** step was necessary.

REVERSE TRANSCRIPTION

The extracted RNA was **reverse-transcribed into cDNA**, enabling its use for quantification by digital PCR.

DATA ANALYSIS

For normalization, gene expression levels were adjusted relative to the housekeeping gene B2M. The R1 ratio was determined for pluripotent lines.

$$\textbf{Ratio 1 (undifferentiated cell line)} = \frac{\text{Gene of interest}}{\text{Housekeeping gene (B2M)}}$$

The same calculation was performed on the control cell line. A fully differentiated fibroblast line was tested under the same conditions to determine the R2 ratio.

$$\textbf{Ratio 2 (control cell line)} = \frac{\text{Gene of interest}}{\text{Housekeeping gene (B2M)}}$$

By dividing the two previously obtained ratios, the expression level of each gene was calculated.

$$\textbf{Expression level} = \frac{\text{Ratio 1 (pluripotent cell line)}}{\text{Ratio 2 (control cell line)}}$$