

# TaqMan<sup>®</sup> OpenArray<sup>®</sup> Human Stem Cell Panel

A set of validated gene expression markers to characterize human stem cells

OpenArray® technology is a nanofluidics platform for lowvolume, solution-phase reactions that streamlines real-time PCR studies using large numbers of samples and assays. A single TaqMan® Human Stem Cell Panel holds over 2,600 realtime PCR assays. The panel combines the efficiency of high-throughput platforms with the robust gene expression performance of TaqMan® Assays.

The TaqMan® OpenArray® Human Stem Cell Panel is available for use on both the QuantStudio™ 12K Flex Real-Time PCR System and the OpenArray® Real-Time PCR System. Users can be confident of high-quality results on any OpenArray® platform.



# Validated gene expression markers

The TaqMan® OpenArray® Human Stem Cell Panel contains characterization markers of undifferentiated human stem cells or their differentiated derivatives. The panel contains a well-defined set of 631 validated gene expression markers to characterize human embryonic stem (hES) cell identity as well as assessment of phenotypic variations between embryonic stem cell isolates [1].



Genes were selected for inclusion in the panel based on the following criteria:

- 1. Expression in human embryonal carcinoma cells, primordial germ cells, or hES cells
- 2. Known role in maintenance of pluripotency in hES cells
- 3. Identification in microarray-based experiments as being correlated with the stem cell state
- 4. Expression known to be characteristic of specific differentiation lineages in the post-implantation embryo and mature tissues.

The format of the TaqMan<sup>®</sup> OpenArray<sup>®</sup> panel allows scientists to run 1–4 replicates in parallel.

# Panel arranged in gene group formats

NANOG, POU5F1 (OCT4), and SOX2 represent gene products that are functionally associated with maintenance of the undifferentiated embryonic stem cell state as studied in mouse embryonic stem cells [2–7]. SOX2 is a pluripotentency marker. The human array also includes developmentally regulated transcripts used to identify hES cells such as TDGF1, DNMT3B, GABRB3, and GDF3, which are also useful in the identification of pluripotent stem cells, although their role in maintaining the undifferentiated state is less clear. Together with NANOG and POU5F1, TDGF1, DNMT3B, GABRB3, and GDF3 constitute a set of six markers to define undifferentiated hES cells. Pluripotent stem cells can give rise to differentiated cells and tissues for all three embryonic germ layers, and 50 genes are included in these arrays to mark differentiation. For example, *AFP* and *NEUROD1* have been observed to be upregulated upon differentiation into extraembryonic endoderm and neuroectoderm; *CDX2, GCM1, KRT1*, and *EOMES* are markers for trophoblast; and *MYF5, ACTC, HBB, COL1A1, COL2A1*, and *DDX4* are examples of mesodermal markers for muscle, cardiac muscle, blood, bone, cartilage and germ cells, respectively (Table 1).

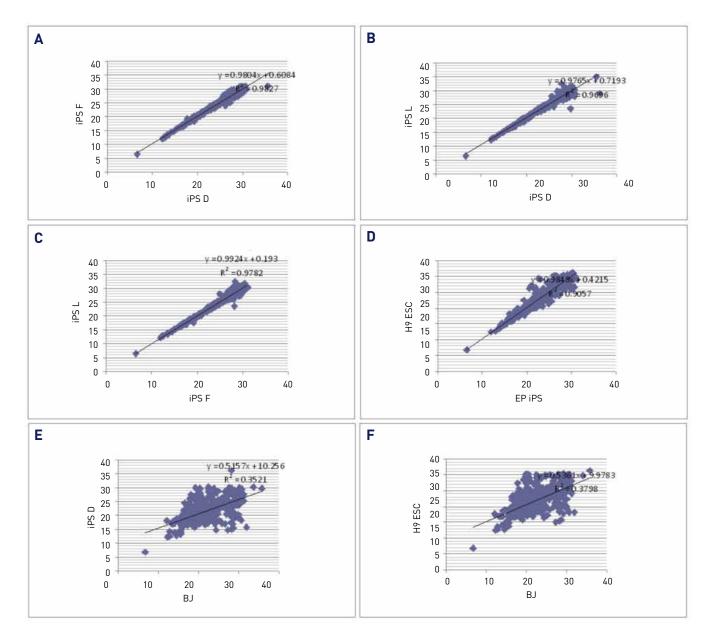
### Generates over 10,000 data points per run

Figure 1 depicts the breadth of data that can be generated with pluripotent and differentiating cells using OpenArray<sup>®</sup> plates on the QuantStudio<sup>™</sup> 12K Flex system.

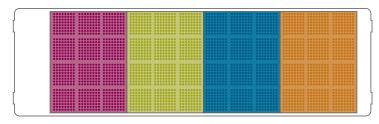
The configuration for the TaqMan® OpenArray® Human Stem Cell Panel Plate is shown in Figure 2. The four colors represent four different samples that can be loaded onto the plate. Alternatively, four replicate samples can be loaded onto the plate. The QuantStudio<sup>™</sup> 12K Flex system can run four plates at once, generating over 10,000 data points.

Contact your local Life Technologies support representative for more information on the panel as well as sample preparation details.

Group	Genes	Human gene symbols
Expression in undifferentiated cells	6	NANOG, POU5F1, TDGF1, DNMT3B, GABRB3, and GDF3
Maintenance of pluripotency	3	NANOG, POU5F1, and SOX2
Correlation to stemness	33	BRIX, CD9, COMMD3, CRABP2, EBAF, FGF4, FGF5, FOXD3, GAL, GBX2, GRB7, IFITM1, IFITM2, IL6ST, IMP2, KIT, LEFTB, LIFR, LIN28, NODAL, NOG, NR5A2, NR6A1, PODXL, PTEN, REST, SEMA3A, SFRP2, TERT, TFCP2L1, UTF1, Xist, and ZFP42
Differentiation markers	50	ACTC, AFP, CD34, CDH5, CDX2, CGB, COL1A1, COL2A1, DDX4, DES, EOMES, FLT1, FN1, FOX A2, GATA4, GATA6, GCG, GCM1, GFAP, HBB, HBZ, HLXB9, IAPP, INS, IPF1, ISL1, KRT1, LAMA1, LAMB1, LAMC1, MYF5, MYOD1, NES, NEUROD1, NPPA, OLIG-2, PA X4, PA X6, PECAM1, PTF1A, RUNX2, SERPINA1, SOX17, SST, SYCP3, SYP, T, TAT, TH, and WT1
Controls	6	ACTB, RAF1, CTNNB1, GAPD, EEF1A1, and 18S



**Figure 1.** Pairwise correlation of gene expression levels from 631 transcripts. Induced pluripotent stem (iPS) cells vs. other iPS cell replicates of the same cell culture (iPS D, F, L clones in panels A, B, and C), H9 embryonic stem cells (ESC) vs. episomal (EP) iPS cells (Panel D), BJ fibroblasts vs. iPS cell clone D (panel E), and BJ fibroblasts vs H9 embryonic stem cells (panel F). Average Ct values are plotted for the 631 transcripts, and correlation coefficients ( $r^2$ ) are shown. The greatest difference in expression was observed for fibroblasts vs. iPS cells or embryonic stem cells ( $r^2 < 0.38$ ) across the 631 transcripts. Embryonic stem cells show greater similarity to iPS cells ( $r^2 > 0.86$ ), while iPSC clones show nearly identical transcript expression ( $r^2 > 0.96$ ).



**Figure 2. TaqMan® OpenArray® Human Stem Cell Panel Plate.** Each through-hole contains a primer pair specific for a cancer gene or a control. Each assay is repeated four times on the TaqMan® OpenArray® plate. Run a single sample to generate four data points per assay, or run four different samples on the plate, as depicted here by the color groupings.

#### **Ordering information**

Product	No. of Targets/ Controls	Format	Pack size	Cat. No.
TaqMan® OpenArray® Human Stem Cell Panel*	4	OpenArray®	1 plate	4475370
TaqMan® OpenArray® Human Stem Cell Panel QuantStudio® 12K Flex system*	4	OpenArray®	1 plate	4475390
Related products required for use with panels				
TaqMan® OpenArray® Real-Time PCR Master Mix			5 mL	4462164
TaqMan® OpenArray® Real-Time PCR Accessories Kit (for use with OpenArray® Real-Time PCR System)			Enough for 10 plates	4453993
QuantStudio" 12K Flex OpenArray® Accessories Kit	Enough for 10 plates	4469576		

\*Each TaqMan® Array is suitable to run 1–4 replicates in parallel.

#### References

- 1. Muller FJ, Schuldt BM, Williams R, et al. (2011) A bioinformatic assay for pluripotency in human cells. *Nat Methods* 8(4):315–317
- 2. Nichols J, Zevnik B, Anastassiadis K et al. (1998) Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* 95(3):379–391.
- 3. Matin MM, Walsh JR, Gokhale PJ et al. (2004) Specific knockdown of Oct4 and beta2-microglobulin expression by RNA interference in human embryonic stem cells and embryonic carcinoma cells. *Stem Cells* 22(5):659–668.
- 4. Scholer HR, Balling R, Hatzopoulos AK et al. (1989) Octamer binding proteins confer transcriptional activity in early mouse embryogenesis. *EMBO J* 8(9): 2551–2557.
- 5. Mitsui K, Tokuzawa Y, Itoh H et al. (2003) The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell* 113(5):631–642.
- 6. Chambers I, Colby D, Robertson M et al. (2003) Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* 113(5):643–655.
- 7. Avilion AA, Nicolis SK, Pevny LH et al. (2003) Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev* 17(1):126–140.



#### Learn more at lifetechnologies.com

For Research Use Only. Not for use in diagnostic procedures. ©2012 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners. TaqMan\* is a registered trademark of Roche Molecular Systems, Inc., used under permission and license. C026964 1012