Dynamic-array based method for high throughout and flexible assessment of pluripotency in PSCs

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Background and Objectives: The mechanisms responsible for maintaining pluripotency during expansion of pluripotent stem cells (PSCs) to date remain unclear. Continuous propagation of PSCs is associated with varying levels of spontaneous differentiation as well as genomic aberrations. Any changes to the quality of the cells have to be detected in order to pass or fail produced batches of cells (e.g. cell banks). Differentiation of pluripotent cells can be detected by gene expression analysis, where reduction of expression of self-renewal genes and increased levels of expression of differentiation markers is observed. Current methods such as hPSC Scorecards™, can detect changes by comparing samples to reference material, however, scorecards also require high quantity of RNA for analysis. We therefore aimed to develop a method which would allow more flexible, high throughput analysis for pluripotent gene expression analysis by applying dynamic array methodology.

Comparison of a dynamic array gene expression panel against Scorecards™ in PSCs directed differentiation assay

Methods

Design: Cells from a CGT-RCiB10 Master Cell Bank (MCB) were profiled by gene expression using hPSC Scorecard™ (Life Technology; Table 1) and by dynamic array using a CGT panel of Taqman™ gene expression assays (Figure 1) . Cell potency was assessed by differentiation into the 3-germ layers of embryoid bodies (EBs) cultured in serum-containing medium. Cells were cultured for 3 passages, then divided into 6 well plates. Non-differentiated cells were harvested on day 0. Ectoderm, endoderm and mesoderm differentiated cells were harvested after 6 days of directed differentiation (Figure 1) RNA was extracted using Qiagen mini Rneasy kits, and reverse transcriptase was performed using Quantitect RT (Qiagen). RNA (8µg) was used for each scorecard sample and the equivalent of 0.5µg RNA was used for dynamic array samples. Scorecards were run on Quantstudio 7 (life technologies) and 48.48 dynamic arrays were run on Biomark HD (Fluidigm). Statistical analysis was performed using Genex 6 (MultiD)

Table 1: Scorecard™ panel of genes

Control	Ectoderm	Endoderm	Mesoderm
ACTB	CDH9	AFP	FGF4
ACTB	COL2A1	CABP7	ABCA4
ACTB	DMBX1	CDH20	ALOX15
ACTB	DRD4	CLDN1	BMP10
CTCF	EN1	CPLX2	CDH5
EP300	LMX1A	ELAVL3	CDX2
SMAD1	MAP2	EOMES	COLEC10
	MYO3B	FOXA1	ESM1
Self renewal	NOS2	FOXA2	FCN3
CXCL5	NR2F1/NR2F2	FOXP2	FOXF1
DNMT3B	NR2F2	GATA4	GDF3
HESX1	OLFM3	GATA6	HAND1
IDO1	PAPLN	HHEX	HAND2
LCK	PAX3	HMP19	HEY1
NANOG	PAX6	HNF1B	HOPX
POU5F1	POU4F1	HNF4A	IL6ST
SOX2	PRKCA	KLF5	NKX2-5
TRIM22	SDC2	LEFTY1	NPPB
	SOX1	LEFTY2	NR5A2
	TRPM8	NODAL	ODAM
	WNT1	PHOX2B	PDGFRA
	ZBTB16	POU3F3	PLVAP
		PRDM1	PTHLH
		RXRG	RGS4
		SOX17	SNAI2
		SST	T
			TBX3
			TM4SF1

Figure 1: Differentiation analysis by Scorecard™ and dynamic arrays

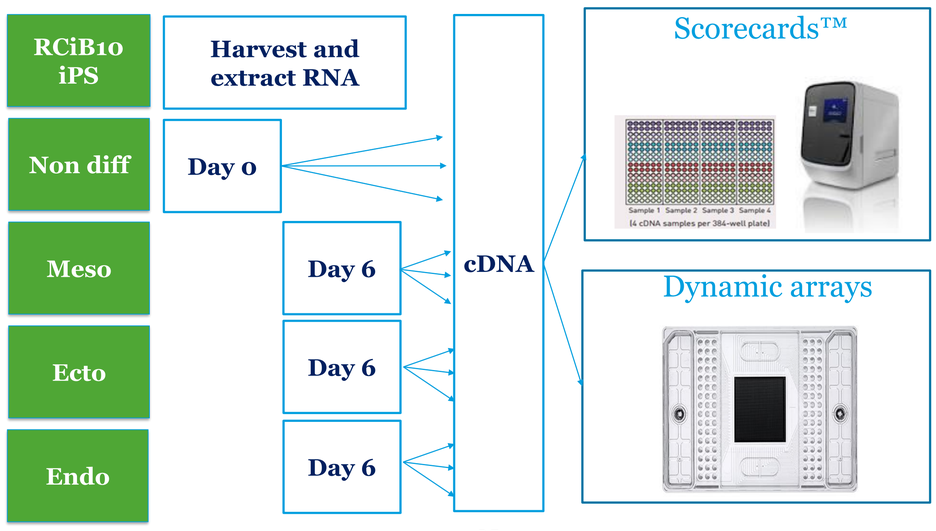


Figure 2: Genes shared by the two platforms

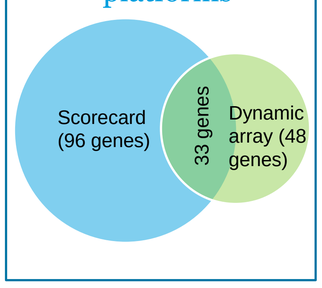
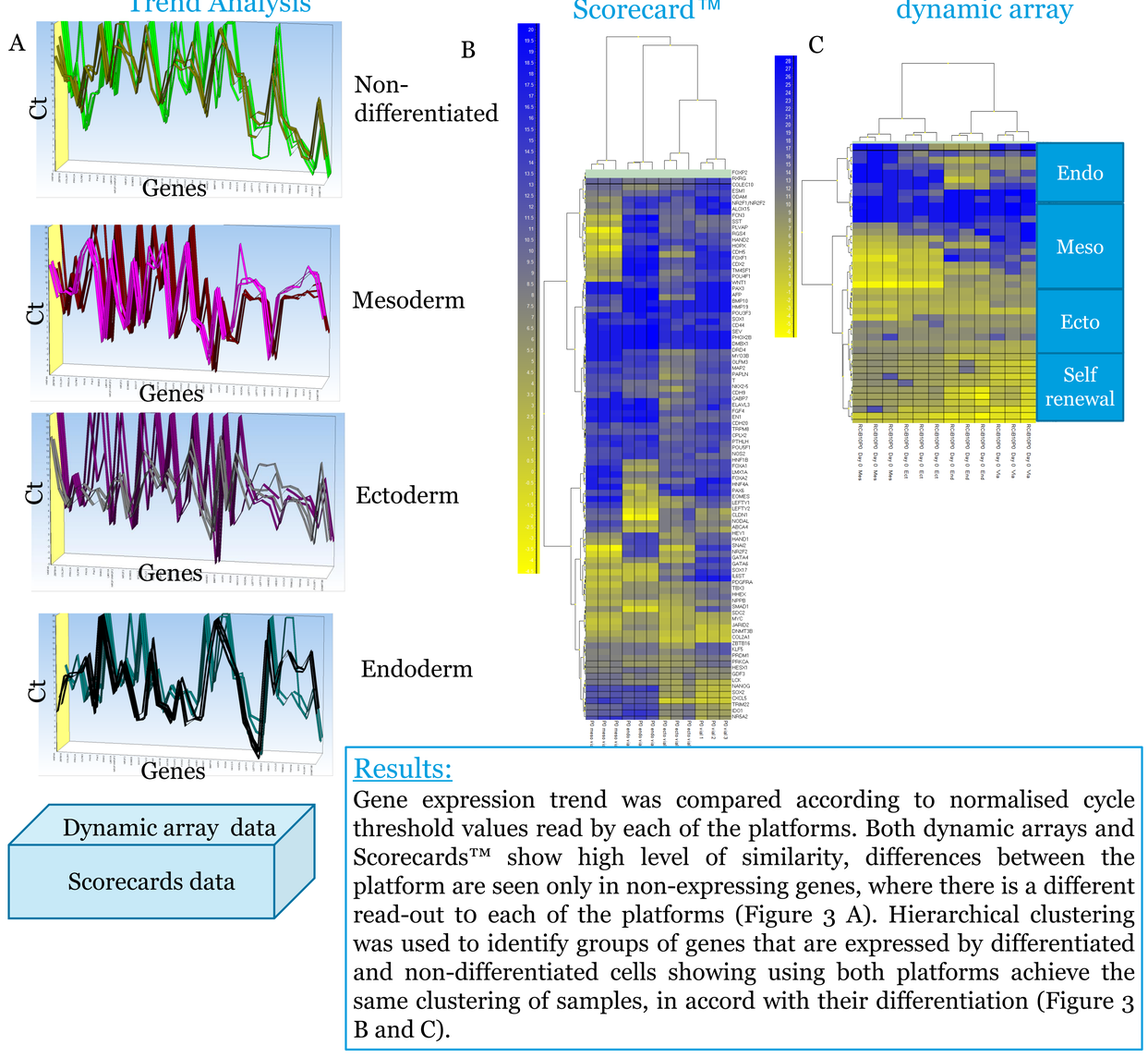


Figure 3: A comparison of differentiation analysis by Scorecard™ and dynamic arrays

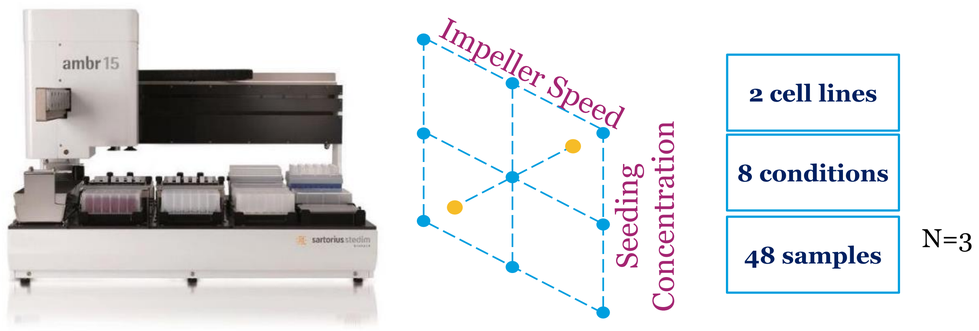


Results:
Gene expression trend was compared according to normalised cycle threshold values read by each of the platforms. Both dynamic arrays and Scorecards™ show high level of similarity, differences between the platform are seen only in non-expressing genes, where there is a different read-out to each of the platforms (Figure 3 A). Hierarchical clustering was used to identify groups of genes that are expressed by differentiated and non-differentiated cells showing using both platforms achieve the same clustering of samples, in accord with their differentiation (Figure 3 B and C).

Comparison of a dynamic array gene expression panel against Scorecards™ during PSCs culture optimisation

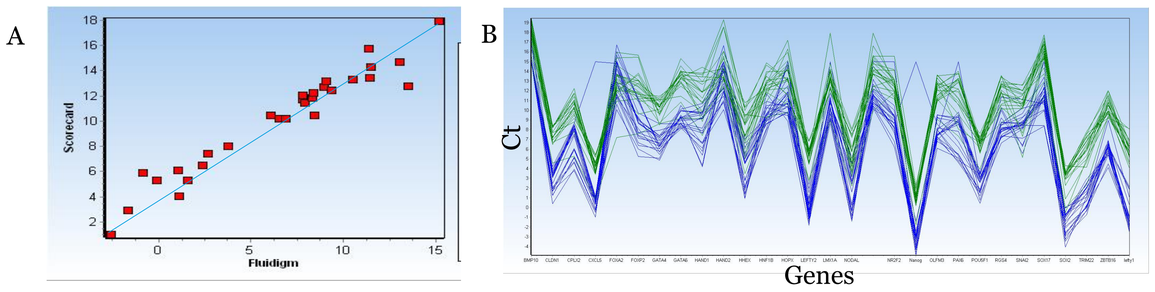
Methods

Figure 4: Multi-component bioreactor optimisation



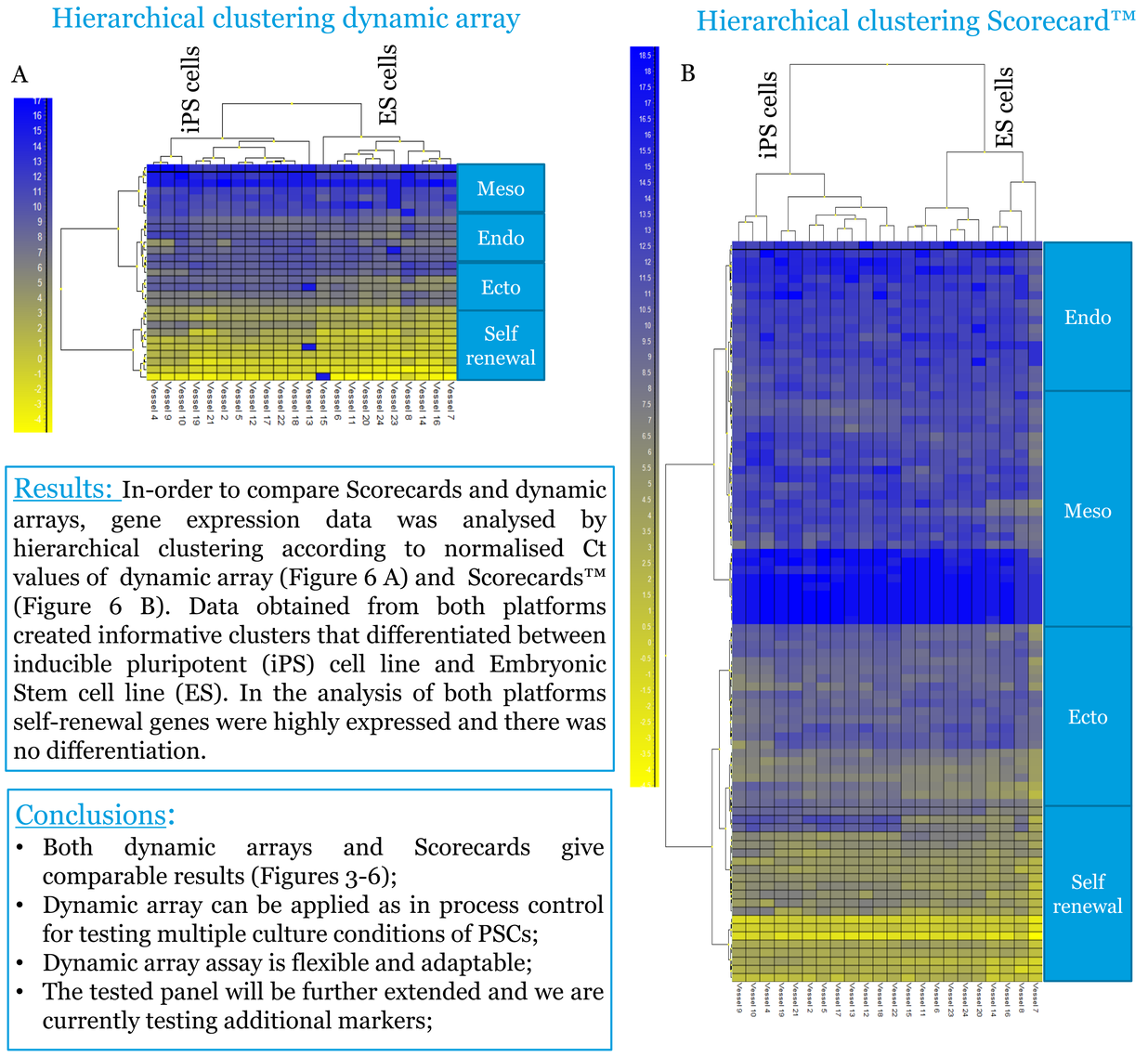
Design: Culture conditions were optimised on AMBR 15 bioreactor (Figure 4). The effect of culture conditions such as impeller speed, media type and seeding concentration on gene expression was analysed by hPSC Scorecards™ (Life Technologies) and dynamic arrays and the two platforms were compared (Figures 5 and 6).

Figure 5: CGT dynamic array panel vs Scorecard™ correlation analysis



Results: Gene expression trend was compared according to normalised cycle threshold values read by each of the platforms. The trend of gene expression shows correlation (Figure 5 A). Both dynamic arrays and Scorecards™ show high level of similarity in gene expression, however the raw data reads on dynamic arrays show overall lower Ct values compared to Scorecards due to differences in the methods (Figure 5 B) however the trend between different genes remains similar.

Figure 6: CGT dynamic array panel vs Scorecard™ comparison



Results: In-order to compare Scorecards and dynamic arrays, gene expression data was analysed by hierarchical clustering according to normalised Ct values of dynamic array (Figure 6 A) and Scorecards™ (Figure 6 B). Data obtained from both platforms created informative clusters that differentiated between inducible pluripotent (iPS) cell line and Embryonic Stem cell line (ES). In the analysis of both platforms self-renewal genes were highly expressed and there was no differentiation.

Conclusions:

- Both dynamic arrays and Scorecards give comparable results (Figures 3-6);
- Dynamic array can be applied as in process control for testing multiple culture conditions of PSCs;
- Dynamic array assay is flexible and adaptable;
- The tested panel will be further extended and we are currently testing additional markers;

We work with Innovate UK

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