Rapid and accurate identification of human induced pluripotent stem cells with a novel multiplex gene expression assay

Kathryn Sciabica[1], Knut Woltjen[2], Akitsu Hotta[2], Handy Yowanto[1], Jeff Chapman[1] and Hans Dewald[1]

[1]Beckman Coulter, Inc., P.O. Box 8000, Brea, CA, USA

[2]Ontario Human Induced Pluripotency Stem Cell Facility, The Hospital for Sick Children (SickKids®), Toronto, Ontario, Canada

Introduction

Recent methods to reprogram human somatic cells, in order to create induced pluripotent stem cells (iPSCs), intend to provide important tools for drug discovery and models for the study of disease. The ultimate goal is to create an alternative to controversial embryonic stem cells (ESCs) and generate patient-specific pluripotent cells for autologous regeneration. Reprogramming methods include the transfer of genetic material, protein transduction and/or application of chemicals to promote epigenetic modification and the expression of key pluripotency markers. Stable reprogramming results from the upregulation of endogenous pluripotency-associated genes and repression of differentiation-associated genes. However, the mechanistic progression and timing of complete reprogramming are generally accepted as being highly stochastic. Somatic cell reprogramming often results in a heterogeneous population in which some cells remain in a partially reprogrammed state, while others are fully competent iPS cells. A technical challenge faced by researchers is the ability to rapidly and accurately predict the pluripotent capacity of these cells. A novel, multiplex RT-PCR§ method in combination with capillary electrophoresis laser induced fluorescence (CE-LIF) surmounts this challenge by offering simultaneous, quantitative detection of gene expression for 2 to 40 genes with a minimal requirement for template RNA input. In this study, a multiplex panel of 27 genes was used to generate an expression profile that, when compared to human ESCs, characterizes and defines fully reprogrammed human iPSCs derived from adult fibroblasts. Similar types of gene panels can be used to efficiently monitor the quality of iPSC maintenance cultures and subsequent differentiation into specific cell lineages. Additionally, this method can be effectively applied to all studies that involve gene expression research and biomarker detection.

Induced Pluripotent Stem Cell Multiplex

	Gene Name	Accession #	GeXP Fragment Size
Housekeeping genes for normalization →	TBP	NM_003194	145
	MYBL2	NM_002466	152
	DPPA4	NM_018189	157
→	ZFP42	NM_174900	169
	PHC1	NM_004426	181
	SALL4	NM_020436	188
	SOX2	NM_003106	192
→	GUSB	NM_000181	200
	Oct4	NM_002701	204
	cMyc	NM_002467	210
	DPPA2	NM_138815	214
	DNMT3B	NM_006892	218
→	GAPDH	NM_002046	250
	Lin28	NM_024674	253
→	MXs-LIN28	MXs-hLIN28	257
	GDF3	NM_020634	268
→	CCNG1	NM_004060	275
→	PHB	NM_002634	280
→ Viral reprogramming factors	MXs-Oct4	MXs-hOct4	283
→	MXs-Nanog	MXs-hNanog	295
	STELLA	NM_199286	301
	Kan(r)	Kan(r)	325
	TERTv1	NM_198253	331
	Klf4	NM_004235	334
	Nanog	NM_024865	343
→	MXs-Sox2	MXs-hSox2	349
→	MXs-Klf4	MXs-hKlf4	354
→	MXs-cMyc	MXs-hcMyc	358

Table 1. The induced pluripotent stem cell (iPSC) multiplex gene panel contains 16 genes of interest, six viral reprogramming factors (blue arrows) and five reference (housekeeping) genes (black arrows), plus one internal control gene Kan(r).

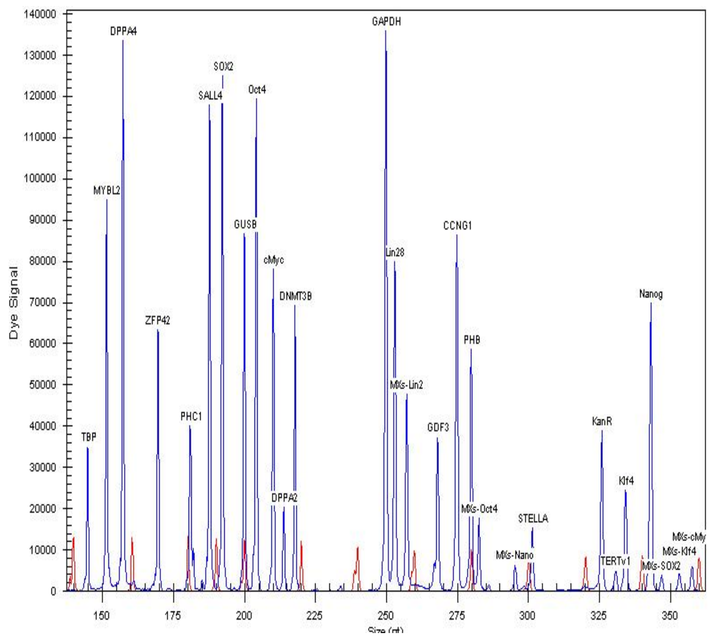


Figure 2. Electropherogram of a multiplex XP-PCR reaction with the iPSC multiplex gene panel. A 1:1:1 mixture of RNA extracted from iPSC (4YA), hESC (CA-1) and 4 day transfected human fibroblast (BJ-MXs) was assayed via XP-PCR with the iPSC multiplex primers to demonstrate the detection of each gene in the multiplex as an discrete fragment.

Gene Expression Profiles

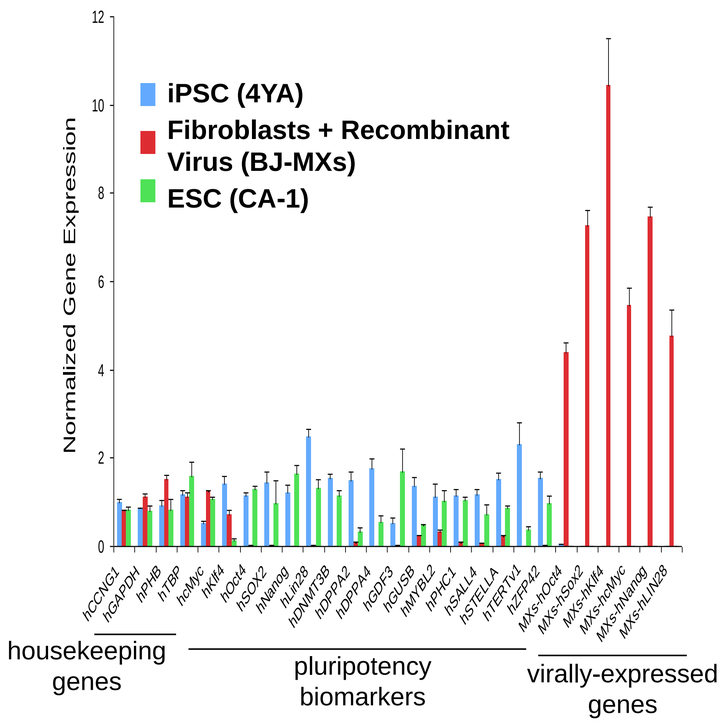
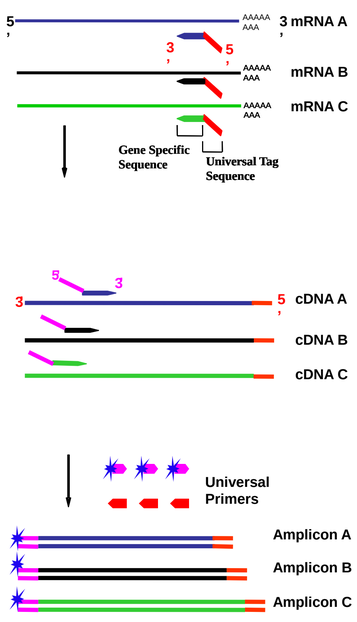


Figure 3. Results from the iPSC multiplex gene expression assay. Similar expression patterns are observed for both human iPSCs (4YA) and human ESCs (CA-1), whereas this pattern clearly differs from a fibroblast (differentiated) cell line soon after transduction with recombinant virus (BJ-MXs).

eXpress Profiling* Technology (XP-PCR): Multiplex Universal Priming Strategy

A. RT-PCR of multiple target transcripts by chimeric and universal primers



Each target mRNA is detected by the gene-specific sequence of a chimeric reverse primer in the reverse transcription reaction.

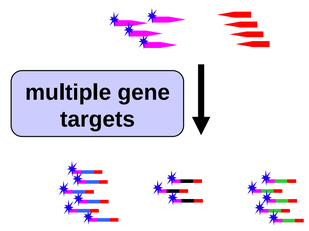
↓

PCR§ starts with priming by chimeric forward primers to produce a double stranded template.

↓

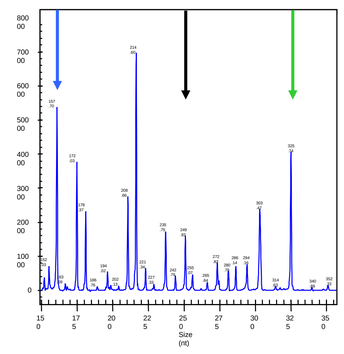
In later cycles of PCR§, amplification is predominantly carried out by universal forward and universal reverse primers due to favorable ratios.

B. Universal Amplification of Multiplex Targets



All gene targets in the multiplex panel are uniformly amplified by the single pair of universal primers. The universal forward primer is dye-labeled.

C. Separation of PCR§ products with CE



The PCR§ amplified, fluorescent-dye-labeled fragments can be detected and quantified by the GeXP system. They are separated based on size by capillary electrophoresis (CE) on the GeXP system.

Figure 1. Schematic of the eXpress Profiling Technology which utilizes chimeric and universal primers. The eXpress Profiling technology (XP-PCR) uses a combined gene-specific, universal-priming strategy that converts multiplexed PCR§ to a two-primer amplification process with universal primers. As a result, the gene ratio of RNA samples is maintained during the PCR§ process. This strategy overcomes the variations in amplification efficiency of different genes during the conventional amplification process without compromising the detection sensitivity.

CONCLUSIONS

GeXP with XP-PCR technology is an important tool for stem cell research because it can facilitate and expedite:

- Detection of the expression of 2 - 30+ genes, therefore limiting the need for large sample quantities
- Comparison of delivery methods for reprogramming factors
- Characterization of a biomarker signature for each step of somatic cell reprogramming
- Identification and assessment of the pluripotent capacity of individual colonies
- Monitoring the maintenance of stemness
- Characterization of biomarker signatures for each step of differentiation of toward specific lineages