Cell Line Information Sheet

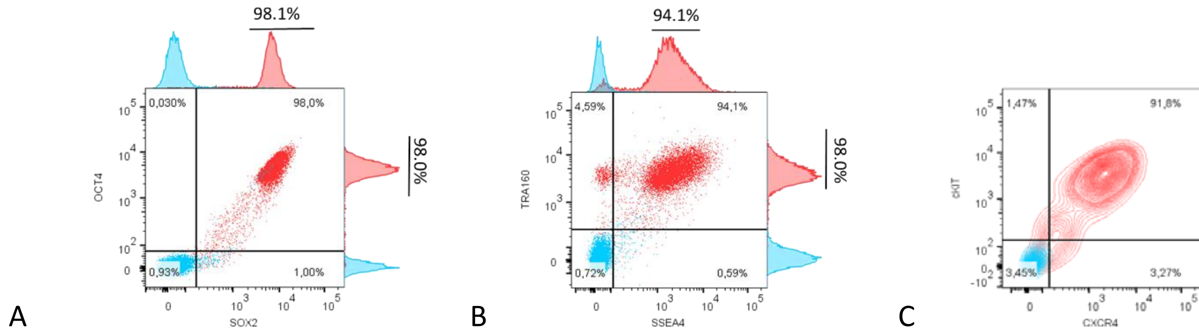
Cell Line Name and Description: Example; Human Induced Pluripotent Stem Cells
Consent status: Research only; Not consented for distribution
Depositor Name and Institution: Dr. Christine Bear; The Hospital for Sick Children
Parental Name and Description: Example; Peripheral Blood
Disease: Cystic Fibrosis; F508del-CFTR
Donor Information: Female; XX yrs
Reprogramming Method: Sendai viral expression of Oct4, Sox2, Klf4, and Myc genes
Culture Format: Culture medium: E8 (Life Technologies #A1517001); Subst rate: Matrigel (Corning #354277)
Incubation Conditions: 37°C, 5% CO_2, >95% RH, Subculture: single cell passaging u sing GCDR (StemCell Technologies #07174)
Passage No: P3+7
Thaw Recommendations: 1 vial should be thawed into 2 wells of a 6 well plate

Test Description	Method	Expected Result	Result
Expression of pluripotency-associated proteins	Flow cytometry	≥ 80% of population is positive for expression of surface markers (SSEA4, Tra-1-60), and intracellular marker (OCT4).	**Antigen** / **% Expressing-cells** SSEA4 94.1% Tra-1-60 98.0% OCT4 98.0% SOX2 98.1% (Histograms shown in Figure 1)
Gene expression of pluripotency markers	qRT-PCR	≥ 80% expression measured in hESC reference standard (HES2 hESCs on Matrigel).	**Gene** / **Relative Expression** OCT4 203% NANOG 159% DNMT3B 157%
Germ layer differentiation	Directed Differentiation Followed by qPCR	Increased expression of germ lineage-specific marker relative to starting pluripotent cell population	**Germ Layer** / **Gene** / **Fold Induction** Endoderm SOX17 4,300 Mesoderm HAND1 91,800 Ectoderm SOX1 67
Definitive endoderm differentiation – gene expression	Directed Differentiation Followed by qPCR	Increased expression of additional endoderm lineage-specific marker relative to starting pluripotent cell population	**Gene** / **Fold Induction** GATA 6 12,000 GATA 4 7,000 FOXA2 2,700
Definitive endoderm differentiation – protein expression	Directed Differentiation Followed by Flow cytometry	≥ 80% of population is double positive for expression of DE markers (cKIT and CXCR4)	**% Expressing-cells** 91.8% (Histograms shown in Figure 2)
Mycoplasma	Lonza MycoAlert Plus kit	None detected	None detected
Identity	STR: PCR profiling of 9 STR regions plus Amelogenin for gender determination.	Consistent with expected[1]	Consistent with parental - Amel: XX CSF1PO:10,10 D21S11:32.2,33.2 THOI:9,9.3 D13S317:8,12 D5S818:11,12 TPOX: 8,9 D16S539:8,12 D7S820:9,13 vWA:15,17
Karyotype	G-banding analysis detecting structural abnormality of size >3-10Mb	Normal karyotype, 46 XX or 46 XY 19/20 cells normal[2]	Normal karyotype, 46 XX at passage 3+5
Post-Thaw Viability	Cell count and viability using Nucleocounter	Viable cell count and viability within 7 days post thaw	Viable cell count 1.1E+06 Viability 87.0%
Residual Sendai	RT-PCR against Sendai viral elements	None detected in PCR amplification	None detected

1 STR results are compared to the STR profile of the parental cells.
2 ISCI standards (Stem Cell Rev and Rep (2009) 5:301-314)

Cells distributed by CCRM are intended for research purposes only and are not intended for use in humans.
Appropriate biosafety precautions should be followed when working with these cells. The end user is responsible for ensuring that the cells are handled and stored in an appropriate manner. CCRM is not responsible for damages or injuries that may result from the use of these cells.

Supplemental Figure 1: Expression proteins by flow cytometry. A. Pluripotency associated proteins OCT4 and SOX2. B. Pluripotency associated proteins TRA160 and SSEA4. C. DE associated proteins after DE differentiation.



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Karyotype on fixed cells of P3+5 iSv.PB.CF.G

Laboratory No: **O16/0324**

Date of Receipt:	19/08/2016	Analysed By:	Frankie Shaw
Date of Report:	06/09/2016	Checked By:	Rachel Newby

Clinical details: Stem cells for karyotyping

Karyotype: 46,XX

Chromosome analysis of the fixed cell suspension from this stem cell line, P3+5 iSv.PB.CF3.G, has shown an apparently normal female karyotype in 20 cells examined.

The preparations obtained from this sample were of sufficient quality to detect numerical and large structural abnormalities.

Authorised by: A J Clarkson

Amanda Clarkson, Lead Clinical Scientist

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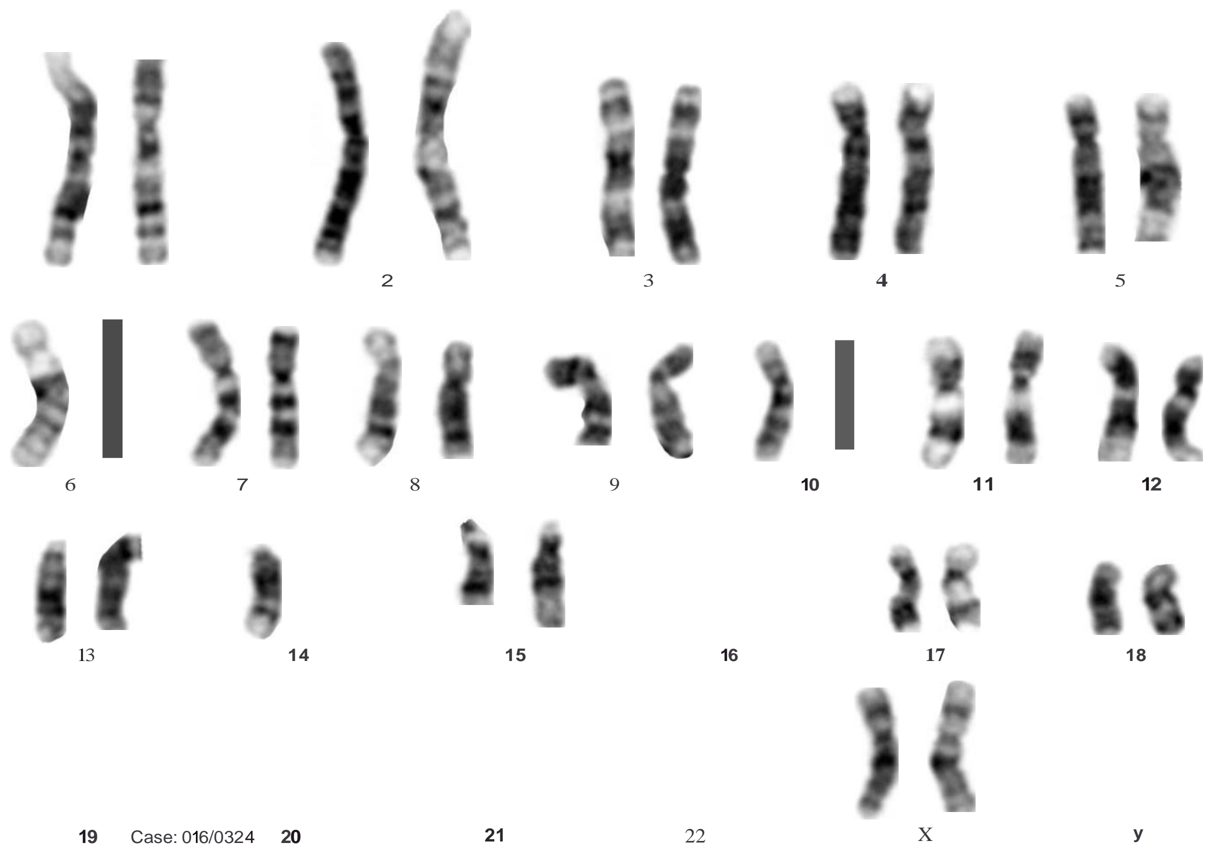

Case: 016/0324
Name: iSv.PB.CF3.G,P3+5
Dute: 06/09/2016
Result: 46,XX