

Certificate of Analysis - Amended

Product Description	iPS IMR90-4				
Cell Line Provider	University of Wisconsin – Laboratory of Dr. Ja	University of Wisconsin – Laboratory of Dr. James Thomson			
Lot Number	iPS(IMR90)-4-MCB-01	iPS(IMR90)-4-MCB-01			
Date Vialed	23-July-2008				
Passage Number	p29 ¹				
Culture Platform	Feeder Independent				
	Media: TeSR	Matrix: Matrigel			

The following testing specifications have been met for the specified product lot:

Test Description	Test Provider	Test Method	Test Specification	Result
Post-Thaw Viable Cell Recovery	WiCell	SOP-CH-305	≥ 15 Undifferentiated Colonies, ≤ 30% Differentiation	Pass
Identity by STR	UW Molecular Diagnostics Laboratory	PowerPlex 1.2 System by Promega	Consistent with known profile	Pass
Sterility - Direct transfer method	Apptec	30744	Negative	Pass
Mycoplasma	Apptec	30055	No contamination detected	Pass
Karyotype by G-banding	WiCell	SOP-CH-003	Normal karyotype	Pass

¹These cells were cultured for 28 passages post reprogramming, at least 3 of them in mTeSR™1/ Matrigel®. WiCell adds +1 to the passage number to best represent the overall passage number of the cells at thaw. Fibroblasts were reprogrammed at p18.

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. WiCell is not responsible for damages or injuries that may result from the use of these cells.

Date of Lot Release	Quality Assurance Approval		
	2/26/2018		
10-September-2008	HEB Quality Assurance Signed by Bruner, Haley		



Short Tandem Repeat Analysis*

Sample Report: 3330-STR

UW HLA#: 59440

Sample Date: 09/04/08

iPS(IRM90)-4-UCB-1

Received Date: 09/04/08

Requestor: WiCell Research Institute

Test Date: 09/10/08, 09/17/08

File Name: 080911,080918

Report Date: 09/19/08

Sample Name: (label on tube) 3330-STR

Description: DNA Extracted by WiCell

257 ug/mL; 260/280 = 1.97

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	Identifying information
D7S820	6-14	has been redacted to
D13S317	7-15	protect donor
D5S818	7-15	confidentiality. If
CSF1PO	6-15	more information is
TPOX	6-13	required, please, contact WiCell's
Amelogenin	NA	Technical Support.
TH01	5-11	
vWA	11, 13-21	

Comments: Based on the DNA 3330-STR submitted dated and received on 09/04/08 from WiCell, this sample (UW HLA# 59440) matches exactly the STR profile of the human stem cell line iPS(IMR90) comprising 16 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human iPS(IMR90) stem cell line were detected and the concentration of DNA required to achieve an acceptable STR genotype (signal/ noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. These results suggest that the 3330-STR DNA sample submitted corresponds to the iPS(IMR90) stem cell line and it was not contaminated with any other human stem cells or a significant amount of mouse feeder layer cells. Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is ~5%. A preliminary copy of this report was issued via electronic mail to the WI Cell Research Institute on Monday, September 22, 2008.

HLA/Molecular Diagnostics Laboratory

HLA/Molecular Diagnostics Laboratory

* Testing to assess engraftment following bone marrow transplantation was accomplished by analysis of human genetic polymorphisms at STR loci. This methodology has not yet been approved by the FDA and is for investigational use only.

File: Final STR Report

Test Facility:

This report is confidential. No part may be used for advertising or public announcement without written permission. Results apply only to the sample(s) tested.



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WiCell Research Institute

September 15, 2008 P.O. #:

STERILITY TEST REPORT

Sample Information:

hES Cells

iPs (IMR90)-1-DL-1

ES02-DL-2

iPs (Foreskin)-1-DL-1

4: WA14-DL-2

ES06-DL-3 5:

TE04-FTDL-1

7: WA13.C-DL-2

iPS(IMR90)-4-MCB-1

9: BG01-DL-1

Date Received:

Date in Test:

Date Completed:

August 26, 2008

August 28, 2008

September 11, 2008

Test Information:

Test Codes: 30744, 30744A

Immersion, USP / 21 CFR 610.12

Procedure #: BS210WCR.201

QA	R	e	۷i	e	W	е	d	1
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Reviewed:

Testing conducted in accordance with current Good Manufacturing Practices.

Test Facility:

This report is confidential. No part may be used for advertising or public announcement without written permission. Results apply only to the sample(s) tested.



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September 15, 2008 P.O. #:

STERILITY TEST REPORT

Sample Information:

hES Cells

8: iPS(IMR90)-4-MCB-1

Date Received:

August 26, 2008

Date in Test: Date Completed:

August 28, 2008 September 11, 2008

Test Information:

Test Codes: 30744, 30744A Immersion, USP / 21 CFR 610.12 Procedure #: BS210WCR.201

TEST PARAMETERS	PRODUCT			
Approximate Volume Tested	0.5 mL	0.5 mL		
Number Tested	2	2		
Type of Media	SCD	FTM		
Media Volume	400 mL	400 mL		
Incubation Period	14 Days	14 Days		
ncubation Temperature	20 °C to 25 °C	30 °C to 35 °C		
RESULTS	2 NEGATIVE	2 NEGATIVE		

QA Reviewed:	Page 1 Signed	Reviewed:	Page 1 Signed	





FINAL STUDY REPORT

STUDY T	T	LE	:
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MYCOPLASMA DETECTION:

"Points to Consider"

PROTOCOL NUMBER:

30055F

TEST ARTICLE IDENTIFICATION:

iPS (IMR90)-4-MCB-1

SPONSOR:

WiCell Research Institute

PERFORMING LABORATORY:

WuXi AppTec, Inc.

STUDY NUMBER:

110537

RESULT SUMMARY:

Considered negative for mycoplasma

contamination

Reference PO # RP2108

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QUALITY ASSURANCE UNIT SUMMARY

STUDY: Mycoplasma Detection: "Points to Consider"

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA,

standard operating proc of study protocols and s	edures and a standar tandard operating pro	rd protocol. The Quality Assi	urance Unit maintains copies this study on the dates listed tegrity of the study.
<u>Critical Phase</u> Dilution of Controls Final Report	<u>Date</u> 08/29/08 09/26/08	Study Director 09/04/08 09/29/08	<u>Management</u> 09/29/08 09/29/08
The findings of these ins	spections have been	reported to management and	the Study Director.
Quality Assurance Audit	or:_		Date: 9/29/08
	GOOD LABORATO	RY PRACTICES STATEME	NT
The study referenced in Good Laboratory Practic	this report was conducted (GLP) regulations se	cted in compliance with U.S. F et forth in 21 CFR part 58.	Food and Drug Administration
The studies not perform Laboratory Practice Sta article.	ed by or under the di tement and include cl	rection of WuXi AppTec, Inc., haracterization and stability o	are exempt from this Good f the test compound(s)/test
Study Director:			Date: 9/29/00
Professional Personne	el Involved:		
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1.0 PURPOSE

To demonstrate that a test article consisting of a cell bank, production or seed lots, or raw materials is free of mycoplasmal contamination, according to "Points to Consider" criteria.

2.0 SPONSOR:

WiCell Research Institute

3.0 TEST FACILITY:

WuXi AppTec, Inc.

4.0 SCHEDULING

5.0

DATE SAMPLE RECEIVED:

8/26/08

STUDY INITIATION DATE:

8/28/08

STUDY COMPLETION DATE:

09/29/08

TEST ARTICLE IDENTIFICATION: WiCell Research Institute iPS (IMR90)-4-MCB-1

6.0 SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing. Unused test samples remained in the storage area until the study was completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

7.0 TEST ARTICLE CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

The Vero cells were maintained by WuXi AppTec's Cell Production Laboratory.

8.0 EXPERIMENTAL DESIGN

8.1 Overview

Whereas no single test is capable of detecting all mycoplasmal strains, freedom from mycoplasmal contamination may be demonstrated by the use of both an indirect and direct procedure.

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8.2 Justification for Selection of the Test System

Contamination of cell cultures by mycoplasma is a common occurrence and is capable of altering normal cell structure and function. Among other things, mycoplasma may affect cell antigenicity, interfere with virus replication, and mimic viral actions. Testing for the presence of mycoplasma for cell lines used to produce biologicals is recommended by the FDA, Center for Biologics Evaluation and Research (CBER) under "Points to Consider."

9.0 EXPERIMENTAL SUMMARY

Study Number: 110537

Protocol Number: 30055F

The indirect method of detection allows visualization of mycoplasma, particularly non-cultivable strains, by growing the mycoplasma on an indicator cell line and then staining using a DNA-binding fluorochrome stain. The indicator cell line should be easy to grow, have a large cytoplasmic to nuclear area ratio and support the growth of a broad spectrum of mycoplasma species. The African green monkey kidney cell line, Vero, fits this description and was used in this assay. The assay was performed with negative and positive controls. Both a strongly cyto-adsorbing (*M. hyorhinis*) and a poorly cyto-adsorbing (*M. orale*) mycoplasma species were used as positive controls. Poor cyto-adsorbing mycoplasma species may not give reliable positive results when inoculated in low numbers. A second dilution of *M. orale* was used to ensure cyto-adsorption. Staining the cultures with DNA binding fluorochrome allows for the detection of mycoplasma based on the staining pattern observed. Only the cell nuclei demonstrate fluorescence in the negative cultures but nuclear and extra-nuclear fluorescence is observed in positive cultures.

Direct cultivation is a sensitive and specific method for the detection of mycoplasma. The agar and broth media employed supply nutrients necessary for the growth of cultivable mycoplasmas. These media also supply a source of carbon and energy, and favorable growth conditions. The direct assay was performed with both negative and positive controls. A fermentative mycoplasma (*M. pneumoniae*) and a non-fermentative mycoplasma (*M. orale*) were used as positive controls. The procedure employed in this study is based on the protocol described in the 1993 Attachment # 2 to the "Points To Consider" document, as recommended by the FDA, Center for Biologics Evaluation and Research (CBER).

10.0 TEST MATERIAL PREPARATION

10.1 Test Article Identification:

Test Article Name: iPS (IMR90)-4-MCB-1

General Description: iPS Cells
Number of Aliquots used: 1 x 15 mL
Stability (Expiration): Not Given

Storage Conditions: Ultracold (< -60°C)

Safety Precautions: BSL-1
Intended Use/Application: Not Given

Study Number: 110537 Protocol Number: 30055F

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10.2 Test Sample Preparation

The test article was thawed in a water bath at $37 \pm 2^{\circ}\text{C}$ and 1:5 and 1:10 dilutions of the test article were prepared in sterile phosphate buffered saline (PBS). 1.0 mL of the undiluted sample, the 1:5 and 1:10 dilutions were then inoculated onto each of two (2) coverslips (per sample/dilution) containing Vero cells. The coverslips were incubated in incubator E959 for 1-2 hours at $37 \pm 1^{\circ}\text{C}$ / $5 \pm 2^{\circ}\text{CO}_2$ and then 2.0 mL of EMEM, 8% Fetal Bovine Serum (FBS) was added to each coverslip. The coverslips were returned to incubator E959 at $37 \pm 1^{\circ}\text{C}$ / $5 \pm 2^{\circ}\text{CO}_2$. After three days of incubation, the coverslips were fixed, stained, and then read using an epifluorescent microscope.

0.2 mL of the undiluted test article was then inoculated onto each of two SP-4 agar plates, and 10.0 mL was inoculated into a 75 cm² flask containing 50 mL of SP-4 broth. The plates were placed in an anaerobic GasPak system and incubated at $36 \pm 1^{\circ}\text{C}$ for a minimum of 14 days.

The broth flask was incubated aerobically at $36\pm1^{\circ}\text{C}$, and subcultured onto each of two SP-4 agar plates (0.2 mL/plate) on Days 3, 7, and 14. These subculture plates were placed in an anaerobic GasPak system and incubated at $36\pm1^{\circ}\text{C}$ for a minimum of 14 days. The broth flask was read each working day for 14 days. The SP-4 agar plates (Day 0) were read after 14 days of incubation. The SP-4 broth subculture plates (Days 3, 7, and 14) were read after 14 days incubation.

10.3 Controls and Reference Materials

10.3.1 Sterile SP-4 broth served as the negative control for both the direct and indirect assays.

10.3.2 Positive Controls

a. Indirect Assay

- **a.1** Strongly cyto-adsorbing species *M. hyorhinis* GDL (ATCC #23839) at 100 or fewer colony forming units (CFU) per inoculum.
- **a.2** Poorly cyto-adsorbing species *M. orale* (ATCC #23714) at 100 or fewer CFU and at approximately 100 ID₅₀ per inoculum

b. Direct Assay

- **b.1** Nonfermentative mycoplasma species *M. orale* (ATCC #23714) at 100 or fewer CFU per inoculum.
- **b.2** Fermentative mycoplasma species *M. pneumoniae* FH (ATCC #15531) at 100 or fewer CFU per inoculum.

10.3.3 Control Preparation

a. Negative Controls

a.1 1.0 mL of sterile SP-4 broth was inoculated onto each of two (2) coverslips containing Vero cells to serve as the negative control in the indirect assay.

Study Number: 110537 Protocol Number: 30055F WuXi AppTec

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a.2 0.2 mL of SP-4 broth was inoculated onto each of two (2) SP-4 agar plates to serve as the negative control in the direct assay. 10.0 mL of SP-4 broth was inoculated into a 75 cm² flask containing 50 mL of SP-4 broth to serve as the negative control in the direct assay.

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b. Positive Controls

- **b.1** *M. hyorhinis, M. orale,* and *M. pneumoniae* were diluted to less than 100 CFU per inoculum in sterile SP-4 broth. 1.0 mL of *M. hyorhinis* and *M. orale* at less than 100 CFU/mL was inoculated onto each of two (2) coverslips containing Vero cells. 1.0 mL of *M. orale* at 100 ID $_{50}$ CFU per inoculum was also inoculated onto each of two (2) coverslips containing Vero cells. These coverslips served as the positive controls in the indirect assay.
- b.2 The coverslips were incubated in incubator E950 for 1-2 hours at $37 \pm 1^{\circ}\text{C}$ / $5 \pm 2\%$ CO₂ and then 2.0 mL of EMEM, 8% Fetal Bovine Serum (FBS) was added to each coverslip. The coverslips were returned to incubator E950 at $37 \pm 1^{\circ}\text{C}$ / $5 \pm 2\%$ CO₂. After three days of incubation, the cell cultures were fixed, stained, and then read using an epifluorescent microscope.
- b.3 0.2 mL of *M. orale* and *M. pneumoniae* at less than 100 CFU/plate was inoculated onto each of two (2) SP-4 agar plates. 10.0 mL of *M. orale* and *M. pneumoniae* at less than 10 CFU/mL (≤100 CFU/inoculum) were each inoculated into a 75 cm² flask containing 50 mL of sterile SP-4 broth.
- b.4 The agar plates were placed in an anaerobic GasPak system and incubated at $36 \pm 1^{\circ}$ C for 14 days. The broth cultures were incubated aerobically at $36 \pm 1^{\circ}$ C for a minimum of 14 days and were read each working day for 14 days. On Days 3, 7, and 14, 0.2 mL from each broth culture flask was subcultured onto each of two (2) SP-4 agar plates. These subculture plates were placed in an anaerobic GasPak system and incubated at $36 \pm 1^{\circ}$ C. The subculture plates were observed microscopically for the presence of mycoplasma colonies after a minimum of 14 days incubation.
- **c.** See Section 15.0, Results, for the results of these controls.

11.0 DATA ANALYSIS

The results of this study were based on visual observations, therefore, no data analysis was required.

12.0 STATISTICAL METHODS

The results of this study were qualitative, therefore, no statistical analysis was required.

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EVALUATION CRITERIA 13.0

Final evaluation of the validity of the assay and test article results was based upon the criteria listed below and scientific judgment.

13.1 **Indirect Assay**

DETECTION OF MYCOPLASMA CONTAMINATION BY INDIRECT ASSAY

	MYCOPLASMA FLUORESCENCE OBSERVED
Controls	(AT LEAST ONE COVERSLIP REQUIRED FOR THE EVALUATION)
Negative Control	-
M. hyorhinis	+
M. orale (≤100 CFU)	+/-*
M. orale (100 ID ₅₀)	+

^{*}Mycoplasma must be observed for at least one dilution of the poorly cyto-adsorbing mycoplasma species M. orale.

13.2 **Direct Assay**

DETECTION OF MYCOPLASMA CONTAMINATION BY DIRECT ASSAY

	NEGATIVE CONTROL	M. PNEUMONIAE	M. ORALE
Broth (Color change or turbidity change)	-	+/-	+/-
Agar Day 0 (at least one plate)	-	+	+
Agar Day 3, 7, 14 (at least one plate on one day)	-	+	+
Results	-	+	+

14.0 **TEST EVALUATION**

Indirect Assay 14.1

Hoechst stain will bind to most DNA containing organisms and organelles present in the culture; this includes indicator cell nuclei, prokaryotes including mycoplasma and cell debris. The source of DNA is determined by the staining pattern. The indicator cell nuclei fluoresce brightly and are generally 10-20 μm in diameter. Mycoplasma fluorescence is less intense, is extra-nuclear and typically appears as small round bodies approximately 0.3 µm in diameter.

14.2 **Direct Assay**

Change in color or turbidity of broth culture can be an indicator of the presence of Fermentative mycoplasma produce acid from the mycoplasma growth. carbohydrates in the medium causing the pH of the medium to drop and the broth to turn yellow in color. Nonfermentative mycoplasma produce ammonia by arginine hydrolysis causing the pH to rise and the broth to turn red. In general, growth of mycoplasma can cause the broth to become turbid. These changes must be confirmed by agar plate subculture or DNA-staining since changes in the appearance of the broth culture can also be caused by the properties of the inoculum.

Mycoplasma colonies grow down into the agar causing the center of the colony to appear opaque and the peripheral surface growth to appear translucent. These "fried-egg" colonies vary in size, 10-500 μm, and can be readily observed unstained using a light microscope.

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14.3 Indirect Assay and Direct Assay Results Interpretation

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Mycoplasmal fluorescence	-	+	+/-	+/-	_
Broth (Color change or turbidity change)	-	+/-	+/-	+/-	+*
Agar - Day 0 (at least one plate)	***	+/-	+/-	+	-
Agar - Day 3, 7, 14 (at least one plate on one day)	-	+/-	+	+/-	_
THEN: OVERALL FINAL RESULT	-	+	+	+	-

^{*}A change in the appearance of a broth culture must be confirmed by positive subculture plate(s).

14.4 Positive Results

The test article is considered positive if the direct assay (agar or broth media procedure) or the indirect assay (indicator cell culture procedure) show evidence of mycoplasma contamination and resemble the positive controls for the procedure.

14.5 Negative Results

The test article is considered as negative if both the direct assay (agar and broth media procedure) and the indirect assay (indicator cell culture procedure) show no evidence of mycoplasma contamination and resemble the negative control for each procedure.

15.0 RESULTS

Indirect Assay and Direct Assay Results

		DIRECT		
	INDIRECT	DAY 0 AGAR PLATES	BROTH FLASKS AND ASSOCIATED SUBCULTURE PLATES	Overall
Test Article: iPS (IMR90)-4-MCB-1	Negative	Negative	Negative	Negative
Negative Control	Negative	Negative	Negative	Negative
M. hyorhinis	Positive		All of the processing constraints in Fig. 9 (2), and the process of the process o	Positive
M. orale	Positive	Positive	Positive	Positive
M. pneumoniae		Positive	Positive	Positive

For the indirect assay, the coverslips for the undiluted test article were read and determined negative.

16.0 ANALYSIS AND CONCLUSION

- 16.1 The results of the negative and positive controls indicated the validity of this test.
- These findings indicated that the test article, iPS (IMR90)-4-MCB-1, is considered negative for the presence of mycoplasma contamination.

17.0 **DEVIATIONS:** None.

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18.0 AMENDMENTS: None.

19.0 RECORD RETENTION

An exact copy of the original final report and all raw data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

20.0 TECHNICAL REFERENCES

- 20.1 Barile, Michael F. and McGarrity, Gerard J. (1983). "Isolation of Mycoplasmas from Cell Culture by Agar and Broth Techniques." Methods in Mycoplasmology, Vol II, ed. J.G. Tully and S. Razin. (New York: Academic Press) pp. 159-165.
- 20.2 Del Giudice, Richard A. and Joseph G. Tully. 1996. "Isolation of Mycoplasma from Cell Cultures by Axenic Cultivation Techniques," ed. J.G. Tully and S. Razin, Molecular and Diagnostic Procedures in Mycoplasmology, Vol. II (New York: Academic Press).
- 20.3 McGarrity, Gerard J. and Barile, Michael F. 1983. "Use of Indicator Cell Lines for Recovery and Identification of Cell Culture Mycoplasmas," ed. J.G. Tully and S. Razin, Methods in Mycoplasmology, Vol. II (New York: Academic Press).
- 20.4 Masover, Gerald and Frances Becker. 1996. "Detection of Mycoplasma by DNA Staining and Fluorescent Antibody Methodology," ed. J.G. Tully and S. Razin, Molecular and Diagnostic Procedures in Mycoplasmology, Vol. II (New York: Academic Press).
- 20.5 Schmidt, Nathalie J. and Emmons, Richard W. 1989. "Cell Culture Procedures for Diagnostic Virology," ed. Nathalie J. Schmidt and Richard W. Emmons, 6th ed., Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections (Washington: American Public Health Association).
- 20.6 U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER). 1993. "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals."



WiCell Cytogenetics Report: 000709-082708 3330-KAR

Report Date: August 26, 2010

Case Details:

Cell Line: iPS (IMR90)-4-MCB-1 (3330)

Passage #: 18+32(7)

Date Completed: 9/3/2008
Cell Line Gender: Female

Investigator: National Stem Cell Bank

Specimen: iPS cells on Matrigel

Date of Sample: 8/27/2008

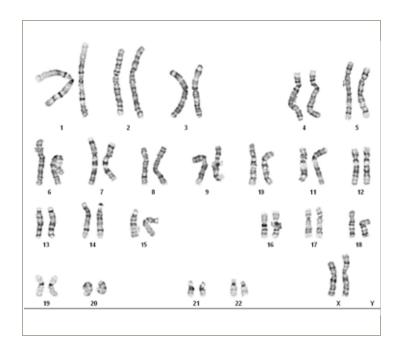
Tests, Reason for: WiCell Bank - Lot Release

Results: 46,XX

Completed by KL, CLSp(CG), on 9/2/2008

Reviewed and interpreted by KDM, PhD, FACMG, on 9/3/2008

Interpretation: No abnormalities were detected at the stated band level of resolution.



Cell: S01-02

Slide: A

Slide Type: Karyotyping

Cell Results: Karyotype: 46,XX

of Cells Counted: 20

of Cells Karyotyped: 4

of Cells Analyzed: 8

Band Level: 425-550

Results Transmitted by Fax / Email / Post

Sent By:_____

Date:_____Sent To: