Product Information and Testing - Amended

Product Information

Product Name	WA21
Lot Number	WB0096
Parent Material	WA21-WB0051
Depositor	WiCell
Banked by	WiCell
Thaw Recommendation	Thaw 1 vial into 5 wells of a 6 well plate.
Culture Platform	Feeder Independent Medium: mTeSR1 Matrix: Matrigel
Protocol	WiCell Feeder Independent Protocol
Passage Number	p26 These cells were cultured for 25 passages prior to freeze, 6 of them in mTeSR1/Matrigel. Cells were derived in MEF Conditioned Medium on Matrigel. WiCell adds +1 to the passage number at freeze so that the number on the vial best represents the overall passage number of the cells at thaw.
Date Vialed	27-June-2011
Vial Label	WB0096 WA21 p26 LK 27JUN11
Biosafety and Use Information	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. Cells distributed by WiCell are intended for research purposes only and are not intended for use in humans.

Lot Specific Testing Performed by WiCell

The following tests were performed on this specific 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	Bionique	M250	No contamination detected	Pass
Karyotype by G-banding	WiCell	SOP-CH-003	Normal karyotype	Pass

Product Information and Testing - Amended

General Cell Line Testing Performed by WiCell

The following tests were performed on the cell line. The tests do not apply to any particular lot.

Test Description	Test Provider	Test Method
Differentiation Potential by Teratoma	WiCell	SOP-CH-213 SOP-CH-214
HLA	UW Molecular Diagnostics Laboratory	PowerPlex 1.2 System by Promega
ABO	American Red Cross	For ABO: Olsson ML, Chester MA. A rapid and simple ABO genotype screening method using a novel B/O2 versus A/O2 discriminating nucleotide substitution at the ABO locus. Vox Sang 1995; 69(3):242-7. For RHD: Singleton BK, Green CA, Avent ND, Martin PG, Smart E, Daka A, Narter-Olaga EG, Hawthorne LM, Daniels G. The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group phenotype. Blood 2000; 95(1): 12-8.
Growth Curve (Doubling Time)	WiCell	Varies by culture platform
Flow Cytometry for ESC Marker Expression	UW Flow Cytometry Laboratory	SOP-CH-101 SOP-CH-102 SOP-CH-103 SOP-CH-105
Array Comparative Genomic Hybridization (aCGH)	WiCell	SOP-CH-308 SOP-CH-309 SOP-CH-310
Comprehensive Human Virus Panel	Charles River	ID 91/0

Amendment(s):

Reason for Amendment	Date
CoA updated to include copyright information.	See Signature
CoA updated for format changes, including adding fields of thaw recommendation, vial label, protocol, and banked by, and removal of footnotes. General Cell Line Testing CoA added to lot CoA.	24-JUN-2013
Original CoA	19-SEP -2011

Date of Lot Release	Quality Assurance Approval
19-September-2011	1/3/2014 X AMC AMC Quality Assurance Signed by: [redacted]

UW Health
University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory
[redacted]

Short Tandem Repeat Analysis*

Sample Report: 10160-STR | UW HLA#: 65663 | Sample Date: 07/21/11, Lab Received 07/21/11

Requestor: WiCell Research Institute

Test Date: 07/29/11, 08/04/11 | File Name: 110729, 110804 | Report Date: 08/05/11

Sample Name: (label on tube) 10160-STR

Description: WI Cell Research Institute provided genomic DNA
256.00 ug/mL 260/280=1.99

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	11,11
D7S820	6-14	9,9
D13S317	7-15	11,12
D5S818	7-15	11,11
CSF1PO	6-15	12,13
TPOX	6-13	8,12
Amelogenin	NA	X,Y
TH01	5-11	8,9
vWA	11, 13-21	18,18

Comments: Based on the 10160-STR DNA dated and received on 07/21/11 from WiCell, this sample (UW HLA# 65663) exactly matches the STR profile of the human stem cell line WA21, comprising 12 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human WA21 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 10160-STR DNA sample submitted corresponds to the WA21 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%.

[redacted] 8/5/11
Date
Molecular Diagnostics Laboratory

[redacted] 08/05/11
Date
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:
[redacted]

WuXi AppTec

WiCell Research Institute
[redacted]

August 23, 2011
P.O. #: [redacted]

STERILITY TEST REPORT

Sample Information: hES Cells
1: MEF SOP-CC-006D34 10179 (2)
2: WA21-WB0089 10180 (2)
3: WA21-WB0096 10181 (2)
4: WA01-WB0097 10182 (2)
5: WA24-WB0068 10185 (2)

Date Received: August 03, 2011
Date in Test: August 08, 2011
Date Completed: August 22, 2011

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	10	10
Type of Media	SCD	FTM
Media Volume	400 mL	400 mL
Incubation Period	14 Days	14 Days
Incubation Temperature	20 °C to 25 °C	30 °C to 35 °C
RESULTS	8 NEGATIVE 2 POSITIVE	9 NEGATIVE 1 POSITIVE

Note: The SCD samples for MEF SOP-CC-006D34 10179 and WA01-WB0097 10182 were positive.

The FTM sample for MEF SOP-CC-006D34 10179 was positive.

[redacted] 08-24-11
QA Reviewer **Date**

[redacted] 08.24.11
Technical Reviewer **Date**

Testing conducted in accordance with current Good Manufacturing Practices.

[redacted]

bionique Testing Laboratories

MYCOPLASMA TESTING SERVICES

BIONIQUE® TESTING LABORATORIES, INC.

[redacted]

APPENDIX

Document ID #:	DCF9002F
Title:	QUALITY ASSURANCE REPORT - GMP
Effective Date:	03/12/10
Edition #:	01

QUALITY ASSURANCE REPORT – GMP

TEST PERFORMED	PROCEDURAL REFERENCE	TEST PERFORMED	PROCEDURAL REFERENCE
☒ M-250	SOP's 3008, 3011, 3013	☐ M-700	SOP's 3008, 3009, 3010
☐ M-300	SOP's 3008, 3014	☐ M-800	SOP's 3008, 3011, 3016
☐ M-350	SOP's 3008, 3014, 3015		

Bionique Sample ID #(s) 101065

This testing procedure was performed in compliance with the FDA's Current Good Manufacturing Practice (cGMP) standards (to the extent that the regulations pertain to the procedures performed) as specified in the Code of Federal Regulations, Title 21 Parts 210 and 211 [21 CFR 210 & 211]. All related records derived from the test procedures have been reviewed by the Quality Assurance Department. The individual's signature below verifies that the methods and procedures referenced above have been followed and that the Final Report accurately reflects the raw data generated during the course of the procedures. All records, including raw data and final reports are archived on site for a minimum of seven years.

The specified test's procedures determine the intervals at which samples are inspected. The medium used for testing must pass quality control mycoplasmal growth promotion testing and sterility testing. Traceability of all of the components used is assured and supporting documentation can be supplied upon request.

Quality Assurance Review Date: 8/11/11

Reviewed By [redacted] QA Assistant: [redacted]

NOTE:

1. Prior to receipt at Bionique® Testing Laboratories, Inc., the stability of the test article is the responsibility of the company submitting the sample. Bionique Testing Laboratories Inc. will assume responsibility for sample stability following receipt and prior to being placed on test.
2. This test is for the detection of microbiological growth and does not require statistical validation.

REFERENCES

Regulatory:

1. Department of Health and Human Services, Food and Drug Administration (USA) [FDA]. Code of Federal Regulations [CFR], Title 21 CFR Part 210, Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs; General. FDA. Office of the Federal Register, National Archives and Records Department.
2. Department of Health and Human Services, Food and Drug Administration (USA) [FDA]. Code of Federal Regulations [CFR], Title 21 CFR Part 211, Current Good Manufacturing Practice for Finished Pharmaceuticals. FDA. Office of the Federal Register, National Archives and Records Department.
3. Department of Health and Human Services, Food and Drug Administration (USA) [FDA]. Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, Director, Center for Biologics Evaluation and Research, FDA. May, 1993. Docket No. 84N-0154.
4. Department of Health and Human Services, Food and Drug Administration (USA) [FDA]. Code of Federal Regulations [CFR], Title 21 CFR Part 610.30, General Biological Products Standards; Subpart D, Test for Mycoplasma. FDA. Office of the Federal Register, National Archives and Records Department.

General:

1. Barile MF, Kern J. Isolation of Mycoplasma arginini from commercial bovine sera and its implication in contaminated cell cultures. Proceedings of the Society for Experimental Biology and Medicine, Volume 138, Number 2, November 1971.
2. Chen, T.R. In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Experimental Cell Research, 104: 255-262, 1977.
3. Carolyn K. Lincoln and Daniel J. Lundin. Mycoplasma Detection and Control. U. S. Fed. for Culture Collections Newsletter, Vol. 20, Number 4, 1990.
4. Fetal Bovine Serum; Proposed Guideline. National Committee For Clinical Laboratory Standards (NCCLS), Vol. 10, Number 6, 1990. (NCCLS publication M25-P).
5. McGarrity GJ, Sarama J, Vanaman V. Cell Culture Techniques. ASM News, Vol. 51, No. 4, 1985.
6. Tully JG, Razin S. Methods in Mycoplasmology, Volumes I and II. Academic Press, N.Y., 1983.
7. Barile MF, Razin S, Tully JG, Whitcomb RF. The Mycoplasmas, Volumes 1-4. Academic Press, N.Y., 1979.
8. http://www.bionique.com/ - Safe Cells Insights

bionique Testing Laboratories

MYCOPLASMA TESTING SERVICES

BIONIQUE TESTING LABORATORIES, INC [redacted]

Document#: DCF3013D
Edition#: 10
Effective Date: 07/15/2003
Title: **M-250 FINAL REPORT SHEET**

M-250 FINAL REPORT

Direct Specimen Culture
Procedure 3008, 3011, 3013

TO: **WiCell QA**
WiCell Research Institute
[redacted]

BTL SAMPLE ID#: **66065** P.O.#: [redacted] DATE REC'D: **07/14/2011**

TEST/CONTROL ARTICLE:

WA21-WB0096 10160

LOT#: **NA**

DIRECT CULTURE SET-UP (DAY 0) DATE: **07/14/2011**

INDICATOR CELL LINE (VERO) SEE DNA FLUOROCHROME RECORD SHEET

				DATE
THIOGLYCOLLATE BROTH	DAY 7	+	⊖	07/21/2011
	DAY 28	+	⊖	08/11/2011
BROTH-FORTIFIED COMMERCIAL				
0.5 mL SAMPLE	DAY 7	+	⊖	07/21/2011
6.0 mL BROTH	DAY 28	+	⊖	08/11/2011
BROTH-MODIFIED HAYFLICK				
0.5 mL SAMPLE	DAY 7	+	⊖	07/21/2011
6.0 mL BROTH	DAY 28	+	⊖	08/11/2011
BROTH-HEART INFUSION				
0.5 mL SAMPLE	DAY 7	+	⊖	07/21/2011
6.0 mL BROTH	DAY 28	+	⊖	08/11/2011

(See Reverse)

Document#: DCF3013D
Edition#: 10
Effective Date: 07/15/2003
Title: M-250 FINAL REPORT SHEET

SAMPLE ID#: **66065**		AEROBIC	MICROAEROPHILIC	DATE
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	07/21/2011
	DAY 14	+ ⊖	+ ⊖	07/28/2011
	DAY 21	+ ⊖	+ ⊖	08/04/2011
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	07/21/2011
	DAY 14	+ ⊖	+ ⊖	07/28/2011
	DAY 21	+ ⊖	+ ⊖	08/04/2011
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	07/21/2011
	DAY 14	+ ⊖	+ ⊖	07/28/2011
	DAY 21	+ ⊖	+ ⊖	08/04/2011
BROTH SUBCULTURES (DAY 7)		DATE: **07/21/2011**		
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	07/28/2011
	DAY 14	+ ⊖	+ ⊖	08/04/2011
	DAY 21	+ ⊖	+ ⊖	08/11/2011
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	07/28/2011
	DAY 14	+ ⊖	+ ⊖	08/04/2011
	DAY 21	+ ⊖	+ ⊖	08/11/2011
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	07/28/2011
	DAY 14	+ ⊖	+ ⊖	08/04/2011
	DAY 21	+ ⊖	+ ⊖	08/11/2011

RESULTS: No detectable mycoplasmal contamination

[redacted]

8/11/11
Date

Laboratory Director
[redacted] Ph.D.

M-250 Procedural Summary: The objective of this test is to ascertain whether or not detectable mycoplasmas are present in an in vitro cell culture sample, be it a primary culture, hybridoma, master seed stock or cell line. This procedure combines an indirect DNA staining approach to detect non-cultivable mycoplasmas with a direct culture methodology utilizing three different mycoplasmal media formulations. The indirect approach involves the inoculation of the sample into a mycoplasma-free VERO (ATCC) indicator cell line and performing a DNA fluorochrome assay after 72-120 hours of incubation. The direct culture aspect of the test utilizes three different mycoplasmal media including both broth and agar formulations. The sample is inoculated into each of the 3 broth formulations and also onto duplicate plates (0.1 mL/plate) for each of the 3 agar formulations. Subculture from broth to fresh agar plates is carried out after 7 days incubation. Agar plates are incubated aerobically and microaerophillically in order to detect any colony forming units morphologically indicative of mycoplasmal contamination. Issuance of the final report with signature of the Laboratory Director signifies that the required controls were performed concurrently with the test sample(s) as detailed in the referenced SOPs and that all test conditions have been found to meet the required acceptance criteria for a valid test, including the appropriate results for the positive and negative controls.

bionique Testing Laboratories

MYCOPLASMA TESTING SERVICES

BIONIQUE® TESTING LABORATORIES, INC.

[redacted]

Document ID #:	DCF3008A
Title:	**DNA FLUOROCHROME ASSAY RESULTS**
Effective Date:	3/24/10
Edition #:	07

DNA-FLUOROCHROME ASSAY RESULTS

Procedures 3008, 3009, 3011

Sample ID # **66065** **M-250** Date Rec'd: **07/14/2011** P.O. # [redacted]

Indicator Cells Inoculated: Date/Initials: 7/14/11 / KG

Fixation: Date/Initials: 7/18/11 / MK

Staining: Date/Initials: 7/18/11 / MK

TEST/CONTROL ARTICLE:

WA21-WB0096 10160

LOT# **NA**

WiCell QA
WiCell Research Institute

[redacted]

Phone: [redacted]

Fax #:

DNA FLUOROCHROME ASSAY RESULTS:

X **NEGATIVE:** A reaction with staining limited to the nuclear region, which indicates no mycoplasmal contamination.

_____ **POSITIVE:** A significant amount of extranuclear staining which strongly suggests mycoplasmal contamination.

_____ **INCONCLUSIVE:**

_____ A significant amount of extranuclear staining consistent with low - level mycoplasmal contamination or nuclear degeneration.

_____ A significant amount of extranuclear staining consistent with bacterial, fungal or other microbial contaminant or viral CPE. Morphology not consistent for mycoplasmal contamination.

COMMENTS:

Date: 7/18/11 Results Read by: MK Date of Review: 7/18/11 Reviewed by: SG

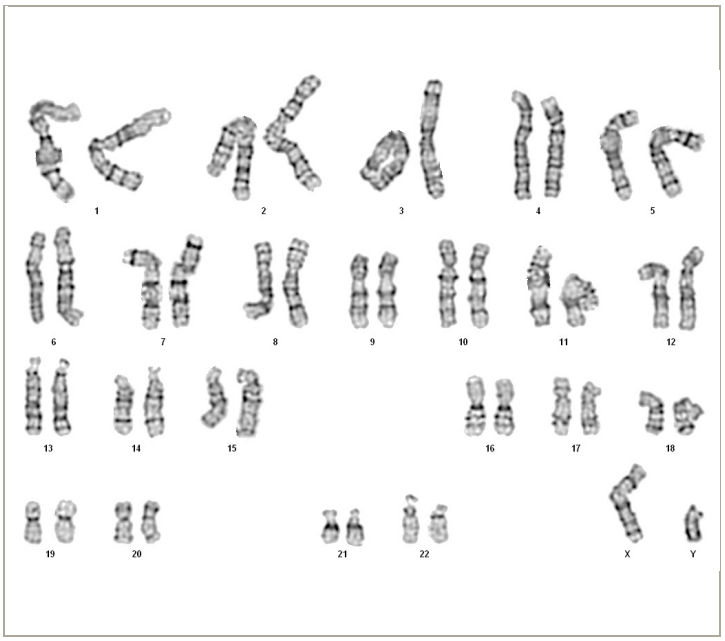
WiCell Cytogenetics Report: 006177

Report Date: July 19, 2011

Cell Line: WA21-WB0096 10160
Passage #: 27
Date of Sample: 7/13/2011
Date Completed: 7/19/2011

Specimen: hESC on Matrigel
Cell Line Gender: Male
Reason for Testing: Lot Release Testing
Investigator: [redacted] Wisconsin International Stem Cell Bank

Results: 46,XY



Cell: S01-47
Slide: 3(6)KARYOTYPE
Slide Type: Karyotyping

# of Cells Counted: 20
# of Cells Karyotyped: 4
# of Cells Analyzed: 8
Band Level: 450-550

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

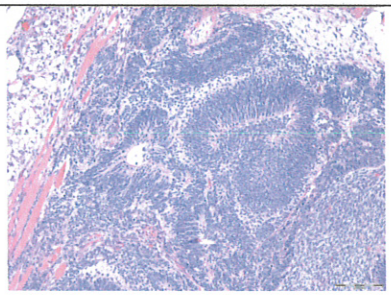
Completed by [redacted]**, CG(ASCP), on 7/19/2011**
Reviewed and interpreted by [redacted]**, PhD, FACMG, on 7/19/2011**

Results Transmitted by Fax / Email / Post
Sent By:_____
QC Review By: _____
Date:_____
Sent To:_____
Results Recorded: _____

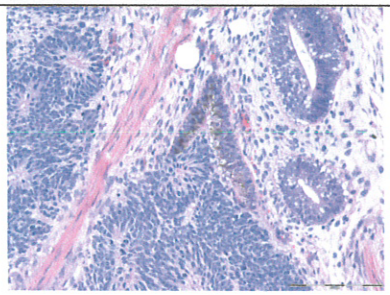
Cell Line: WA21 **Cell Lot Number:** NA (1) **Sample Number:** 3773

ECTODERM

Structure Name: Neuroectoderm **Magnification:** 100X **Slide ID:** A

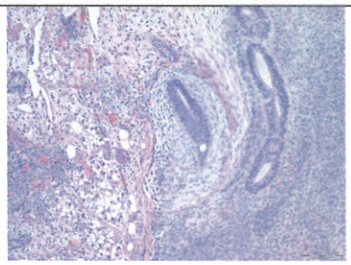


Structure Name: Pigmented Neural **Magnification:** 200X **Slide ID:** A

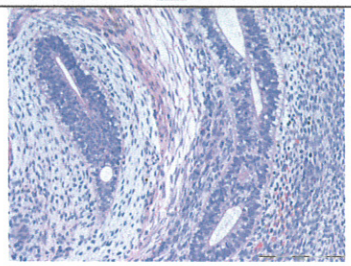


ENDODERM

Structure Name: Bronchial Mucosa **Magnification:** 100X **Slide ID:** A

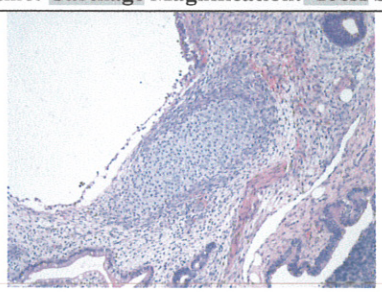


Structure Name: Bronchial mucosa **Magnification:** 200x **Slide ID:** A

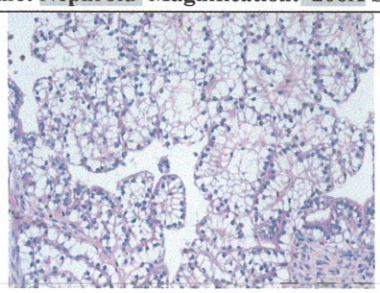


MESODERM

Structure Name: Cartilage **Magnification:** 100X **Slide ID:** A



Structure Name: Nephroid **Magnification:** 200X **Slide ID:** A



Comments: Structures identified include Ectoderm (2), Mesoderm (2) and Endoderm (1)

Sample(s) were assessed for the presence of differentiation into cell types characteristic of the three embryonic germ layers, which, if present in the sample(s) examined, are represented in the photographs above. The individual's signature below verifies that this report accurately reflects the pathology observed.

Pathologist (By/Date): [redacted]

QA Review (By/Date):

(1) Cell lot is WB00006. Added by JME on 15MAR2021 for clarification.

UW Health
University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory
[redacted]

Date: 07/01/2010 17:06:27

To: WiCell Research Institute
Cytogenetics Lab
510 Charmany Dr., Suite 59
Madison, WI 53719

Re: High-resolution HLA results

Patient

Name / HLA / MR# / received	Dates		HLA DNA-based typing*							
			Method: PCR-SSP			Direct Sequencing				PCR-SSP
			A*	B*	C*	DRB1*	DRB3*	DRB4*	DRB5*	DQB1*
WICELL, 1018-HLA	DQB SSP		03:01	44:03	06:02	07:01				
63371 /	A,B,C SSP	06/30/2010	29:02	50:01	16:01	14:01/14:54				
06/30/2010	DRB Seq	06/30/2010								

[redacted]
HLA/Molecular Diagnostics Laboratory
7-1-10 TW
Date

[redacted]
HLA/Molecular Diagnostics Laboratory
7/6/10
Date

This test was developed and its performance characteristics determined by the UWHC Clinical Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. However, the FDA does not require licensure of analyte specific reagents since the laboratory is approved, under CLIA, for high complexity testing.

[I:\db\tx\ttmaster\vttmreport.frx]

American Red Cross

National Molecular Blood Group and Platelet Testing Laboratory
[redacted]

07/09/10

SAMPLE: **DNA on 1018-ABO** (ML10-0988)

Date received: 06/29/10
Sample date: not provided

INSTITUTION: WiCell Research Institute/National Stem Cell Bank (WICELL)

HISTORY: DNA sample from cell line.

TESTING REQUESTED: Genotype for *ABO* and *RH*

DNA TESTING PERFORMED: *ABO*: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) testing for nucleotide positions 261 (O^1), 467 (A^2), 703 (B), and 1096 (B and O^2). *RH*: PCR-multiplex analysis for *RHD* exons 4, 7, the inactivating *RHD* pseudogene and C/c genotyping. Zygosity determination by hybrid box detection. AS-PCR for exon 3 (455A>C). *RHCE:* PCR-RFLP for e/E in exon 5 (676G>C).

DNA MOLECULAR RESULTS:

	Genotype	**Predicted Phenotype**
1018-ABO:	*ABO*AO^1; RHD, RHC, RHc, RHe*	**Group A; RhD+, C+ c+ E- e+**

RH COMMENTS: The sample was negative for the *RHD*-inactivating pseudogene and the D-, *RHD-CE-D* hybrid.

[redacted]
Scientific Director

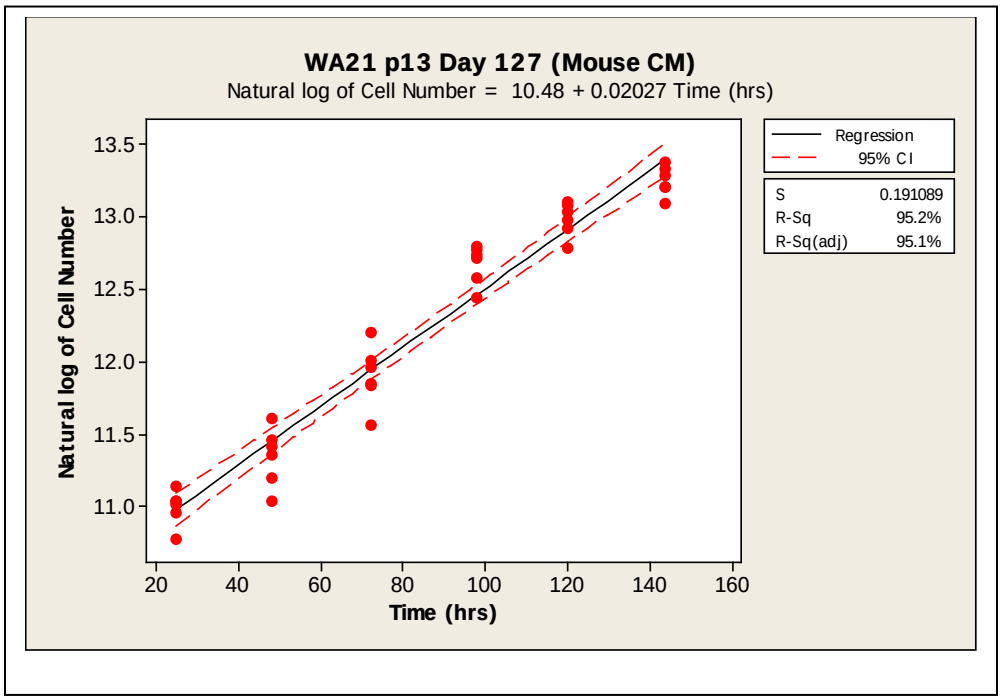
[redacted]
Molecular Biologist

THE MOLECULAR TEST METHODS WERE DEVELOPED, AND THEIR PERFORMANCE CHARACTERISTICS DETERMINED BY THE MOLECULAR RED CELL AND PLATELET TESTING LABORATORY AT THE AMERICAN RED CROSS PENN-JERSEY REGION. THE FDA HAS NOT REVIEWED OR APPROVED THE REAGENTS USED. THESE RESULTS ARE NOT INTENDED AS THE SOLE MEANS FOR CLINICAL DIAGNOSIS OR PATIENT MANAGEMENT DECISIONS. **LIMITATIONS:** The genotype may not always reflect the red cell phenotype. New mutations that inactivate gene expression or rare new variant alleles may not be identified in these assays.

Please Give Blood.

Characterization Report- Growth Characteristics

Sample ID: 4835	Cell lot #: N/A	Cell Line: WA21 Culture Conditions: mouse CM/ Matrigel	Passage: p13 (Day 127)
Notebook # and page(s) where growth curve procedure is detailed: Flowchart – Notebook #109 p. 138		Notebook & page(s) where data is captured for this growth curve assay is written: Notebook # 109 pp. 138-142	Date of Day 0: 10-JUL-2010
Growth Curved performed by: LB/NG/JJ		Report prepared by/date: JMJ – 21-JAN-2011	QA review by/date: JKT 25Jan11



Regression Analysis: Natural log of Cell Number versus Time (hrs)

The regression equation is
Natural log of Cell Number = 10.5 + 0.0203 Time (hrs)

Predictor	Coef	SE Coef	T	P
Constant	10.4753	0.0732	143.18	0.000
Time (hrs)	0.0202741	0.0007789	26.03	0.000

S = 0.191089 R-Sq = 95.2% R-Sq(adj) = 95.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	24.742	24.742	677.60	0.000
Residual Error	34	1.242	0.037		
Total	35	25.984			

Slope ± 95% C.I.

0.020 ± 0.002

Apparent Doubling Time (hrs) ± 95% C.I.

34.2 ± 2.0

Apparent Doubling Time (95% C.I.)

31.7 hrs – 37.1 hrs

Characterization Report- Growth Characteristics

Sample ID: 4835	Cell lot #: N/A	Cell Line: WA21 Culture Conditions: mouse CM/ Matrigel	Passage: p13 (Day 127)
Notebook # and page(s) where growth curve procedure is detailed: Flowchart – Notebook #109 p. 138		Notebook & page(s) where data is captured for this growth curve assay is written: Notebook # 109 pp. 138-142	Date of Day 0: 10-JUL-2010
Growth Curved performed by: LB/NG/JJ		Report prepared by/date: JMJ – 21-JAN-2011	QA review by/date: JKT 25Jan11

Photo Day 0- Colonies before splitting	Photo Day 1
NOT DONE	NOT DONE
Photo Day 2	Photo Day 3
NOT DONE	NOT DONE
Photo Day 4	Photo Day 5
NOT DONE	NOT DONE

WiCell Cytogenetics Report: 003803 WISC5489

Report Date: *6/8/2011*
Date of Sample: *10/29/2010*
Investigator: [redacted]
Reason for Testing: *Lot release testing*
Specimen: *hESC on Matrigel, CM*
Karyotype Results: *46,XY*

Test: *WA21-WB0051-p18 (Male)*
Reference: *WA09-MCB-01-E.3-p19(2) (Female)*
Project: [redacted]
Funding: [redacted]
CGH Accession #: *000388*
GEO Accession #:

Microarray Results:

☐ ***arr(1-22,X)x2 – Female***

☒ ***arr(1-22)x2,(XY)x1 – Male***

☐ ***Consistent with a Balanced Karyotype (Karyotype Unavailable)***

☒ ***Consistent with the Karyotype Results***

☐ ***Inconsistent with the Karyotype Results***

☐ ***Additional Findings***

Interpretation:

CNV gains/losses

- *There were **57** copy number gains and losses identified, including **2** pseudoautosomal regions and **12** copy number changes due to the reference DNA*
- *There is a >1Mb gain at 10q11.22. This CNV is likely cell line specific, is in a region of known copy number variation, and likely a benign finding.*
- *Select CNVs are detailed in the table below*

Chr	Band (Genomic Position)	Width	Aberration Type	Classification	Genes
1	arr 1p36.33(750,231-1,489,997)x1	739,766	Loss	Uncertain Significance – Likely Benign	AGRN, ATAD3A, ATAD3B, ATAD3C, AURKAIP1, B3GALT6, C1orf159, CCNL2, CENTB5, CPSF3L, DVL1, FAM132A, GCUD2, GLTPD1, HES4, ISG15, KLHL17, MRPL20, MXRA8, NOC2L, PLEKHN1, PUSL1, SAMD11, SCNN1D, SDF4, SSU72, TAS1R3, TNFRSF18, TNFRSF4, TTLL10, UBE2J2, VWA1
1	arr 1p12p11.2(120,577,445-120,874,481)x1	297,035	Loss	Uncertain Significance – Likely Benign	FAM72B, FCGR1B
*3	arr 3p14.1(65,160,247-65,209,465)x3	49,217	Gain	Uncertain Significance – Likely Benign	
7	arr 7p13(44,038,257-44,051,445)x3	13,187	Gain	Uncertain Significance – Likely Benign	DBNL
7	arr 7q22.1(101,931,083-102,096,488)x3	165,405	Gain	Uncertain Significance – Likely Benign	MGC119295, POLR2J2, POLR2J3, RASA4
7	arr 7q31.1(109,814,376-110,938,507)x3	1,124,130	Gain	Uncertain Significance – Likely Benign	IMMP2L, LRRN3
10	arr 10p11.21(37,490,459-37,524,112)x3	33,653	Gain	Uncertain Significance – Likely Benign	ANKRD30A
10	arr 10q11.22(46,186,744-47,228,866)x3	1,042,121	Gain	Uncertain Significance – Likely Benign	ANXA8, ANXA8L1, ANXA8L2, GPRIN2, PPYR1, SYT15
10	arr 10q11.22(47,746,246-47,901,718)x3	155,471	Gain	Uncertain Significance – Likely Benign	ANXA8, ANXA8L1
10	arr 10q11.22(48,555,198-48,977,828)x3	422,630	Gain	Uncertain Significance – Likely Benign	
10	arr 10q23.2(88,831,886-88,955,268)x3	123,382	Gain	Uncertain Significance – Likely Benign	FAM35A, GLUD1
15	arr 15q11.2(20,920,976-21,194,540)x1	273,563	Loss	Uncertain Significance – Likely Benign	GOLGA8E
16	arr 16p13.11(14,799,715-14,885,721)x1	86,005	Loss	Uncertain Significance – Likely Benign	NOMO1
17	arr 17q21.31q21.32(41,788,253-42,121,257)x3	333,003	Gain	Uncertain Significance – Likely Benign	ARL17, ARL17P1, LRRC37A2, NSF
X	arr Xq28(152,034,225-152,735,977)x3	701,751	Gain	Uncertain Significance – Likely Benign	ABCD1, ATP2B3, BCAP31, BGN, DUSP9, FAM58A, IDH3G, MAGEA1, PDZD4, PLXNB3, PNCK, SLC6A8, SRPK3, SSR4, TREX2, UCHL5IP, ZNF275
X	arr Xq28(153,064,834-153,165,618)x1	100,784	Loss	Uncertain Significance – Likely Benign	OPN1LW, OPN1MW, OPN1MW2, TEX28

*Select differentially expressed genes are in bold and underlined; classifications are based on ACMG draft guidelines *Aberration marked manually and included in report*

Notes:

- *Karyotype Information – no abnormalities were detected at the stated band level of resolution*
- *Published CNVs (1) – Narva et al: arr 10q11.22(46,186,744-47,228,866)x3*

References: *Werbowetski-Ogilvie, T, Bosse, M, Stewart, M, et al. (2008). Characterization of human embryonic stem cells with features of neoplastic progression. Nature Biotechnology 27, 91-97; Wu, H, Kim, K, Mehta, K, et al. (2008). Copy number variant analysis of human embryonic stem cells. Stem Cells 26, 1484-1489; Chin, MH, Mason, M, Xie, W, et al. (2009). Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell 5, 111-123; Närvä, E, Autio R, Rahkonen N, et al. (2010). High-resolution DNA analysis of human embryonic stem cell lines reveals culture-induced copy number changes and loss of heterozygosity. Nature Biotechnology 28, 371-377*

Recommendations: *For relevant findings, confirmation and localization is recommended. Contact cytogenetics@wicell.org to request further testing.*

Results Completed By: [redacted] *MS, CG(ASCP)*
Reviewed and Interpreted By: [redacted]*mery, PhD, FACMG*

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aCGH Specifications*:*

- *Platform: NimbleGen 12x135K array (HG18 WG CGH v3.1 HX12)*
- *Relative copy number is determined by competitive differential hybridization of labeled genomic DNA to the 135,000 oligonucleotide whole genome tiling array*
- *Probe length = 60mer, spanning non-repetitive regions of the human genome*
- *Median probe spacing = 21,500*
- *Analysis software: NimbleScan™ , CGH Fusion (RBS v1.0)™*
- *Array design, genomic position, genes and chromosome banding are based on HG18.*
- *Analysis is based on examination of unaveraged and/or 130Kbp (10X) averaged data tracks as noted. Settings for data analysis in Infoquant include an average log-ratio threshold of 0.2, a minimum aberration length of 5 probes, p-value of 0.001. Additional analysis of this data may be performed using different ratio settings and different window averaging to enhance resolution.*
- *Raw data has not yet been deposited in GEO.*
- *Reported gains and losses are based on test to reference ratios within CGHfusion™ and the size of aberration.*
- *Quality assurance monitors: 1) opposite gender reference DNA ratio change in X and Y chromosomes; 2) presence of Xpter and Xq21.3 'pseudoautosomal' (PAR) imbalance; 3) presence of known reference DNA copy number changes. QA measures—PAR (2/2); Reference DNA copy number changes (12); test sample gain or loss of X and Y chromosomes consistent with the opposite gender reference sample.*

Limitations: *This assay will detect aneuploidy, deletions, duplications of represented loci, but will not detect balanced alterations (reciprocal translocations, Robertsonian translocations, inversions, and insertions), point mutations, loss of heterozygosity (LOH), uniparental disomy or imbalances less than 30kb in size. Copy number variants can be attributable to the test or reference samples used. Exact limits of detectable mosaicism have not been determined, but >20% mosaicism is reported to be visualized by aCGH. Actual chromosomal localization of copy number change is not determined by this assay. Other mapping procedures are required for determining chromosomal localization.*

Results Transmitted by ☐ Fax / ☐ Email / ☐ Post **Date:**_____
Sent By:_____ **Sent To:**_____

Charles River Research Animal Diagnostic Services

[redacted]

Sponsor: WiCell Research Institute **Accession #: 2010-034920**

Diagnostic Summary Report

[redacted]

Received: 27 Jul 2010
Approved: 28 Jul 2010, 16:40

Bill Method: [redacted]
Attn: [redacted]
Test Specimen: Human Cells Human
Tel: [redacted]

Sample Set	Service (# Tested)	Profile	Assay	Tested	+	+/-	?
#1	Infectious Disease PCR (2)	All Results Negative					

+ = Positive, +/- = Equivocal, ? = Indeterminate

Service Approvals

Service	Approved By*	Date
Infectious Disease PCR	[redacted]	28 Jul 2010, 16:40

To assure the SPF status of your research animal colonies, it is essential that you understand the sources, pathobiology, diagnosis and control of pathogens and other adventitious infectious agents that may cause research interference. We have summarized this important information in infectious agent **Technical Sheets**, which you can view by visiting http://www.criver.com/info/disease_sheets.

**This report has been electronically signed by laboratory personnel. The name of the individual who approved these results appears in the header of this service report. All services are performed in accordance with and subject to General Terms and Conditions of Sale found in the Charles River Laboratories-Research Models and Services catalogue and on the back of invoices.*

Charles River Research Animal Diagnostic Services

[redacted]

Sponsor: WiCell Research Institute **Accession #: 2010-034920**

Product: Not Indicated **Test Specimen:** Human Cells Human **Received:** 27 Jul 2010

Molecular Diagnostics Infectious Disease PCR Results Report

Department Review: Approved by [redacted] 28 Jul 2010, 16:40*

Human Comprehensive Viral PCR Panel

Sample #: / Code :	1 WA16-WB0029 0988	2 WA21-WB0034 5961
John Cunningham virus	-	-
BK virus	-	-
Herpesvirus type 6	-	-
Herpesvirus type 7	-	-
Herpesvirus type 8	-	-
Parvovirus B19	-	-
Epstein-Barr Virus	-	-
Hepatitis A virus	-	-
Hepatitis B virus	-	-
Hepatitis C virus	-	-
HPV-16	-	-
HPV-18	-	-
Human T-lymphotropic virus	-	-
Human cytomegalovirus	-	-
HIV-1	-	-
HIV-2	-	-
Adeno-associated virus	-	-
Human Foamy Virus	-	-
LCMV PCR	-	-
Hantavirus Hantaan PCR	-	-
Hantavirus Seoul PCR	-	-
Mycoplasma Genus PCR	-	-
DNA Spike	**PASS**	**PASS**
RNA Spike	**PASS**	**PASS**
NRC	**PASS**	**PASS**

Remarks: - = Negative; I = Inhibition, +/- = Equivocal; + = Positive.

Sample Suitability/Detection of PCR Inhibition:
Sample DNA or RNA is spiked with a low-copy number of a exogenous DNA or RNA template respectively. A spike template-specific PCR assay is used to test for the spike template for the purpose of determining the presence of PCR inhibitors. The RNA spike control is also used to evaluate the reverse-transcription of RNA. Amplification of spike template indicates that there is no detectable inhibition and the assay is valid.

NRC:
The nucleic acid recovery control (NRC) is used to evaluate the recovery of DNA/RNA from the nucleic acid isolation process. The test article is spiked with a low-copy number of DNA/RNA template prior to nucleic acid isolation. A template-specific PCR assay is used to detect the DNA/RNA spike.

**This report has been electronically signed by laboratory personnel. The name of the individual who approved these results appears in the header of this service report.*