Certificate of Analysis

Thaw and Culture Details

Cell Line Name	**JHU055i**	
WiCell Lot Number	**DB41083**	
Provider/Client	Johns Hopkins University – Laboratory of Dr. Lewis Becker	
Banked By	Johns Hopkins University – Laboratory of Dr. Lewis Becker	
Thaw and Culture Recommendations	WiCell recommends thawing 1 vial into 3 wells of a 6 well plate using TeSR™-E8™ and Recombinant Human Vitronectin. WiCell recommends thawing using ROCK Inhibitor for best results.	
Protocol	WiCell Feeder Independent Pluripotent Stem Cell Protocol	
Culture Platform Prior to Freeze	Medium: E8	Matrix: Vitronectin
Passage Number	p6 Cells were cultured for 5 passages prior to freeze and post reprogramming. Plated cells at thaw should be labeled passage 6.	
Date Vialed	06-June-2016	
Vial Label	P055 P6 6/6/16 0.7M	
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.	

Certificate of Analysis

Results

Test Description	Test Provider	Test Method	Test Specification	Result
Karyotype	WiCell	G-T-L Banding performed on 20 metaphase cells	Expected karyotype	See Report
	Results: 47,XX,+X[2]/47,XX,+X,t(9;10)(p24;q24)[2]/46,XX[15] Nonclonal findings: 46,XX,del(4)(q31.1) ***Interpretation:*** This is an abnormal karyotype. There are two related abnormal clones. The cells in the primary clone (two of twenty cells examined; representative image on the left) contain an additional copy of chromosome X. Gain of chromosome X is recurrently acquired in pluripotent stem cell cultures. The cells in the secondary clone (two of twenty cells examined; representative image on the right) contain the gain of chromosome X and an apparently balanced translocation between the short (p) arm of chromosome 9 and the long (q) arm of chromosome 10. No other clonal abnormalities were detected at the stated band level of resolution. There is a nonclonal finding, listed above. Nonclonal findings may result from technical artifact, but may be due to a developing clonal abnormality or to low-level mosaicism.		
Post-Thaw Viable Cell Recovery	WiCell	Thaw using specified Thaw & Culture Recommendations	Recoverable attachment after passage	Pass
Identity by STR	WiCell	PowerPlex 16 HS System by Promega™	Defines STR profile of deposited cell line	See Report
Mycoplasma	WiCell	PCR	Amplification of mycoplasma specific DNA detected with negative result	Pass
Sterility	Steris	Native Product Direct Transfer using FTM and TSB (ST/07)	Negative for growth following 14 days of culture	Pass

Testing Reported by Provider

The Provider stated that some or all of the additional analyses listed below may have been performed for this cell line. For more information, publication and dbGaP links, where available, are provided on the cell line specific web page on the WiCell website.

- Embryoid bodies
- Infinium® Expanded Multi-Ethnic Genotyping Array (MEGA^{EX})

Certificate of Analysis

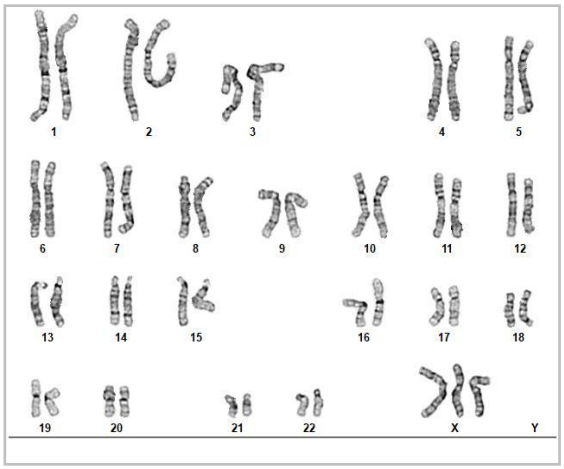
Approval Date	WiCell Quality Assurance Approval
02-June-2022	6/2/2022 X JKG JKG WiCell Quality Assurance Signed by: Gay, Jenna

Chromosome Analysis Report: 091687

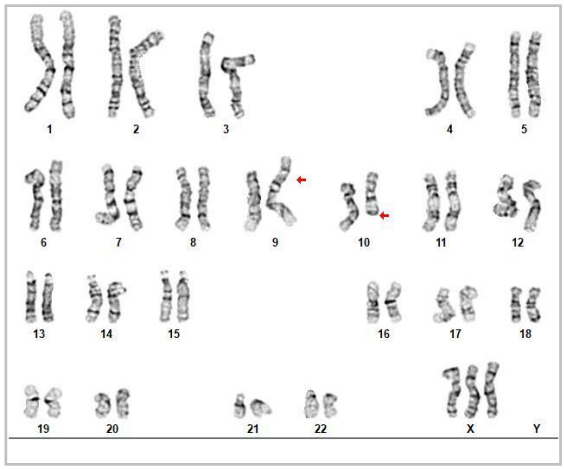
Date Reported: Friday, April 29, 2022
Cell Line Sex: Female
Cell Line: JHU055i-DB41083
Reason for Testing: LOT_RELEASE
Submitted Passage #: 8
Date of Sample: 4/21/2022
Investigator: WiCell Stem Cell Bank, WiCell
Specimen: Human IPSC
Results: 47,XX,+X[2]/47,XX,+X,t(9;10)(p24;q24)[2]/46,XX[15]

Nonclonal findings: 46,XX,del(4)(q31.1)

Cell: 4 **Slide: G03** **Slide Type: Karyotyping**



Cell: 4 **Slide: G02** **Slide Type: Karyotyping**



Total Counted: 20
Total Analyzed: 10
Total Karyogrammed: 6
Band Resolution: 400 - 475

Interpretation:

This is an abnormal karyotype. There are two related abnormal clones.

The cells in the primary clone (two of twenty cells examined; representative image on the left) contain an additional copy of chromosome X. Gain of chromosome X is recurrently acquired in pluripotent stem cell cultures.

The cells in the secondary clone (two of twenty cells examined; representative image on the right) contain the gain of chromosome X and an apparently balanced translocation between the short (p) arm of chromosome 9 and the long (q) arm of chromosome 10.

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

There is a nonclonal finding, listed above. Nonclonal findings may result from technical artifact, but may be due to a developing clonal abnormality or to low-level mosaicism.

Completed by: Jennifer Pecos, CG(ASCP)
Reviewed and Interpreted by: Kaitlin C. Lenhart, PhD, DABMGG

Case #: 091687 ***Cell Line: JHU055i-DB41083***

For internal use only

Date:_____ Sent By:____ Sent To:_____ QC Review By: ____

Limitations: This assay allows for microscopic visualization of numerical and structural chromosome abnormalities. The size of structural abnormality that can be detected is >3-10Mb, dependent upon the G-band resolution obtained from this specimen. For the purposes of this report, band level is defined as the number of G-bands per haploid genome. It is documented here as "band level", i.e., the range of bands determined from the four karyograms in this assay. Detection of heterogeneity of clonal cell populations in this specimen (i.e.,mosaicism) is limited by the number of metaphase cells examined, documented here as "# of cells counted".

This assay was conducted solely for listed investigator/institution. The results of this assay are for research use only. Unless otherwise mutually agreed in writing, the services provided to you hereunder by WiCell Research Institute, Inc. ("WiCell") are governed solely by WiCell's Terms and Conditions of Service, found at www.wicell.org/privacyandterms. Any terms you may attach to a purchase order or other document that are inconsistent, add to, or conflict with WiCell's Terms and Conditions of Service are null and void and of no legal force or effect.

Short Tandem Repeat

Requestor: WiCell Stem Cell Bank, WiCell
Samples Received: 20Apr22, 18Apr22
STR Amplification Date: 23Apr22

Sample Name	JHU055i-DB41083 p7	JHU158i-DB36358 p6	JHU052i-DB41077 p9	[redacted]
Label on tube	91639	91601	91600	
FGA	[redacted]			
TPOX				
D8S1179				
vWA				
Amelogenin				
Penta_D				
CSF1PO				
D16S539				
D7S820				
D13S317				
D5S818				
Penta_E				
D18S51				
D21S11				
TH01				
D3S1358				
Allelic Polymorphisms	28	28	28	28
Matches*				90435, 90771, 90291
Comments			Minor Contamination	

Identifying information has been redacted to protect donor confidentiality. If more information is required, please contact info@wicell.org

**Note: The STR profile of the following sample is an exact match for the given sample/samples.*

Short Tandem Repeat

Requestor: WiCell Stem Cell Bank, WiCell
Samples Received: 20Apr22, 18Apr22
STR Amplification Date: 23Apr22

Form SOP-89.01
Version 8.0

Assay Description: STR analysis is performed using the PowerPlex 16 HS System by Promega™. Results are reported as 13 CODIS STR markers, Amelogenin for gender determination and two low-stutter, highly discriminating pentanucleotide STR markers.

Results: The genotypic profiles comprise a range of 28 allelic polymorphisms across the 15 STR loci analyzed.

Interpretation: 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 suggests that the cells submitted correspond to the cell lines as named and were not contaminated with any other human cells or a significant amount of mouse feeder layer cells.

Sensitivity: Sensitivity limits for detection of STR polymorphisms unique to either this or other human cell lines is ~2-5%.

Minor Contamination: Sample 91600 shows signs of possible contamination. The most likely explanation for this result is that two cultures have been mixed.

4/30/2022 X Molly Miles Tech #1 Characterization Signed by: Miles, Molly	5/2/2022 X Anna Lisa Larson Tech #2 Characterization Signed by: Larson, Anna Lisa	5/2/2022 X Andy Arntz QA Review Quality Assurance Signed by: Arntz, Andy

Raw data is available upon request.

Mycoplasma Assay Report

PCR-based assay performed by WiCell

WiCell Stem Cell Bank, WiCell

23Apr22

Form SOP-83.01
Version 5.0

Sample Name	Result	Interpretation
JHU055i-DB41083 p7 (91639)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
Positive (+) Control	Positive	
Negative (-) Control	Negative	

Assay Description
Sample is tested for presence of mycoplasma using EZ-PCR™ Mycoplasma Detection Kit (Sartorius).

4/23/2022 X Molly Miles Tech #1 Characterization Signed by: Miles, Molly	4/26/2022 X Amber Kuhn Tech #2 Characterization Signed by: Kuhn, Amber	4/26/2022 X Dawn Graham QA Review Quality Assurance Signed by: Graham, Dawn

A gel image is available upon request.

Native Product Sterility Report

STERIS

WiCell
504 S Rosa Road, Rm 101
Madison, WI 53719

SAMPLE #: 22040783
DATE RECEIVED: 14-Apr-22
TEST INITIATED: 28-Apr-22
TEST COMPLETED: 12-May-22

SAMPLE NAME / DESCRIPTION:

JHU038i-DB40987
JHU039i-DB40991
JHU040i-DB41044
JHU043i-DB41052
JHU048i-DB41068
JHU055i-DB41083
JHU158i-DB36358
JHU171i-DB36374
JHU197i-DB41411
JHU235i-DB37044
[redacted]
JHU185i-DB41395
JHU052i-DB41077
iPS(IMR90)-4-WB67850
iPS(IMR90)-4-WB67851
iPS(IMR90)-4-WB67852
iPS(IMR90)-4-WB67853
PENN102i-96-1-DB36580
PENN104i-321-6-DB34693

UNIQUE IDENTIFIER: N/A

TEST RESULTS:

# Tested	# Positives (Growth)	- Control
19	0	2 Negatives

TEST SUMMARY:

# Samples	Media Type	Volume (mL)	Incubation Temperature (° C)	Incubation Duration (Days)
19	TSB	40	20-25	14
19	FTG	40	30-35	14

Native Product Sterility Report

STERIS

REFERENCE: Processed according to LAB-003: Sterility Test Procedure

PD #: 000053

TEST METHODOLOGY: USP - Direct Transfer

COMMENTS: NA

REVIEWED BY Lonnie Buckhard DATE 23May2022