Certificate of Analysis

Thaw and Culture Details

Cell Line Name	**WIZ03e-H9CAGhM3Dq**	
WiCell Lot Number	**WB67889**	
Parent Material	WIZ03e-H9CAGhM3Dq-WB67299	
Provider/Client	University of Wisconsin – Dr. Su-Chun Zhang	
Banked By	WiCell	
Thaw and Culture Recommendations	WiCell recommends thawing 1 vial into 3 wells of a 6 well plate using mTeSR™Plus and Matrigel®.	
Protocol	WiCell Feeder Independent Pluripotent Stem Cell Protocol	
Culture Platform Prior to Freeze	Medium: mTeSR™Plus	Matrix: Matrigel®
Passage Number	p43 Cells were cultured for 42 passages prior to freeze. Plated cells at thaw should be labeled passage 43.	
Date Vialed	07-JUNE-2022	
Vial Label	WIZ03e-H9CAGhM3Dq p43 WB67889	
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

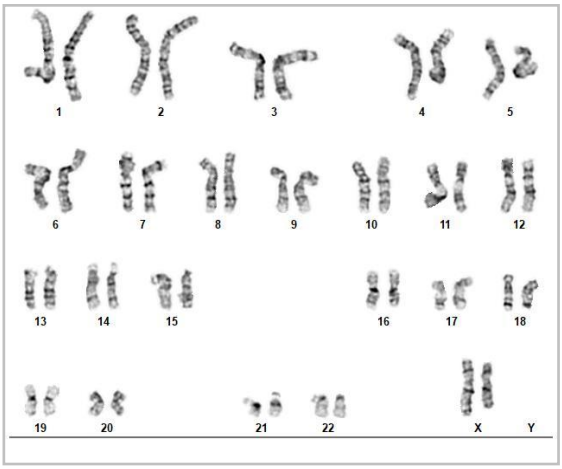
Test Description	Test Provider	Test Method	Test Specification	Result
Karyotype	WiCell	G-T-L Banding performed on 20 metaphase cells	Expected karyotype	See Report
	Results: 46,XX ***Interpretation:*** This is a normal karyotype; no clonal abnormalities were detected at the stated band level of resolution.			
Post-Thaw Viable Cell Recovery	WiCell	Thaw using specified Thaw & Culture Recommendations	≥ 15 Undifferentiated Colonies prior to passage, ≤ 30% Differentiation prior to passage, and recoverable attachment after passage	Pass
Identity by STR	WiCell	PowerPlex 16 HS System by Promega™	Consistent with 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
Expression of Reporter Proteins	WiCell	Fluorescence microscopy	Expression of reporter proteins in undifferentiated state	Pass

Approval Date	WiCell Quality Assurance Approval
04-August-2022	8/4/2022 X DLG JKG WiCell Quality Assurance Signed by: Graham, Dawn

Chromosome Analysis Report: 092553

Date Reported: Friday, June 24, 2022
Cell Line: WIZ03e-H9CAGhM3Dq-WB67889
Submitted Passage #: 43
Date of Sample: 6/16/2022
Specimen: Human Modified ESC
Results: 46,XX

Cell Line Sex: Female
Reason for Testing: LOT_RELEASE
Investigator: WiCell Stem Cell Bank, WiCell



Cell: 1
Slide: G01
Slide Type: Karyotype

Total Counted: 20
Total Analyzed: 8
Total Karyogrammed: 4
Band Resolution: 375 - 400

Interpretation:
This is a normal karyotype; no clonal abnormalities were detected at the stated band level of resolution.

Completed by: Pam Mill
Reviewed and Interpreted by: Kaitlin C. Lenhart, PhD, FACMG

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".

Short Tandem Repeat

Requestor: WiCell Stem Cell Bank, WiCell
Samples Received: 16Jun22, 15Jun22, 14Jun22, 13Jun22
STR Amplification Date: 22Jun22

Form SOP-89.01
Version 8.0

Sample Name	STAN220i-504C2-DB35478 p15	STAN256i-649C2-DB44439 p15	WIZ03e-H9CAGhM3Dq-WB67889 p43	STAN223i-509C3-DB44168 p14	STAN222i-509C2-DB44165 p14	STAN255i-649C1-DB44436 p15	PENN003i-661-4-DB36301 p15
Label on tube	92557	92556	92553	92545	92544	92501	92500
FGA	[redacted]						
TPOX							
D8S1179							
vWA							
Amelogenin							
Penta_D							
CSF1PO							
D16S539							
D7S820							
D13S317							
D5S818							
Penta_E							
D18S51							
D21S11							
TH01							
D3S1358							
Allelic Polymorphisms	28	26	24	28	28	26	25
Matches*		92501	See Matches Comment	92544	92545	92556	
Comments		[1]Allelic Imbalance		[2]Allelic Imbalance		[1]Allelic Imbalance	

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: 16Jun22, 15Jun22, 14Jun22, 13Jun22
STR Amplification Date: 22Jun22

Sample Name	WIC-WA09-MB-002 p27
Label on tube	92481
FGA	[redacted]
TPOX	
D8S1179	
vWA	
Amelogenin	
Penta_D	
CSF1PO	
D16S539	
D7S820	
D13S317	
D5S818	
Penta_E	
D18S51	
D21S11	
TH01	
D3S1358	
Allelic Polymorphisms	24
Matches*	See Matches Comments
Comments	

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: 16Jun22, 15Jun22, 14Jun22, 13Jun22
STR Amplification Date: 22Jun22

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 24-28 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%.

Matches: Samples 92553 and 92481 are 100% match to each other and to 84552, 84553, 84656, 84930, 84931, 84932, 86113, 89607, 90917, 90918 and additional profiles. Additional matches can be provided upon request.

[1]**Allelic Imbalance:** Allelic imbalance was observed in sample 92556 and 92501 at the Amelogenin loci. This could be the result of chromosomal gains, losses, and/or amplifications in the cell line.

[2]**Allelic Imbalance:** Allelic imbalance was observed in sample 92545 at the vWA loci. This could be the result of chromosomal gains, losses, and/or amplifications in the cell line.

6/28/2022 X Molly Miles Tech #1 Characterization Signed by: Miles, Molly	6/28/2022 X Anna Lisa Larson Tech #2 Characterization Signed by: Larson, Anna Lisa	6/28/2022 X Dawn Graham QA Review Quality Assurance Signed by: Graham, Dawn

Raw data is available upon request.

Mycoplasma Assay Report

PCR-based assay performed by WiCell
WiCell Stem Cell Bank, WiCell
17Jun22

Form SOP-83.01
Version 5.0

Sample Name	Result	Interpretation
WIZ03e-H9CAGhM3Dq-WB67889 p43 (92553)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
STAN223i-509C3-DB44168 p14 (92545)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
STAN222i-509C2-DB44165 p14 (92544)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
WC-24-02-DS-M-WB67887 p14 (92525)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
STAN255i-649C1-DB44436 p15 (92501)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
PENN003i-661-4-DB36301 p15 (92500)	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).

6/17/2022 X Julia Graham Tech #1 Characterization Signed by: Graham, Julia	6/20/2022 X Amber Kuhn Tech #2 Characterization Signed by: Kuhn, Amber	6/20/2022 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 #: 22061406
DATE RECEIVED: 23-Jun-22
TEST INITIATED: 01-Jul-22
TEST COMPLETED: 15-Jul-22

SAMPLE NAME / DESCRIPTION:
WC-24-02-DS-M-WB67887
STAN220i-504C2-DB35478
STAN222i-509C2-DB44165
STAN223i-509C3-DB44168
WC007i-FX13-2-WB67902
WIZ03e-H9CAGhM3Dq-WB67889
WC026i-5807-3-WB67904
WIZ04e-H9CAGmChry-WB67905
SCRP6904i-WB67890
PENN078i-SV10-DB36423

UNIQUE IDENTIFIER: N/A

TEST RESULTS:

# Tested	# Positives (Growth)	- Control
10	0	2 Negatives

TEST SUMMARY:

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

REFERENCE: Processed according to LAB-003: Sterility Test Procedure
PD #: 000053
TEST METHODOLOGY: USP - Direct Transfer

COMMENTS: NA

REVIEWED BY _____ DATE 18 JUL 2022

Verification of mCherry Reporter Protein

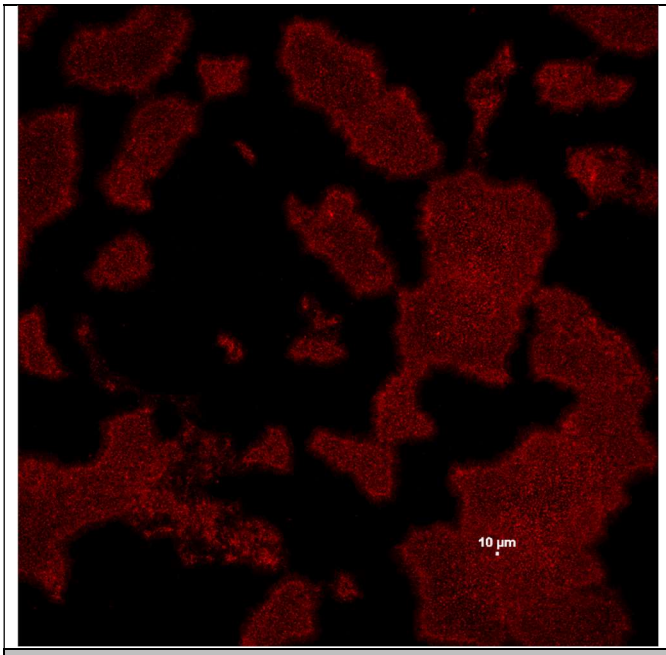
Cell Line-Lot Number	WIZ03e-H9CAGhM3Dq -WB67889
Sample ID	15575
Passage Number	43
Assay Date	15Jul22
Reported By/Date	KLP 19Jul22
Reviewed By/Date	JB 21JUL22
QA Review & Processed By/Date	AA 25Jul22
Notes	☒ N/A

Data Acquisition

- Culture imaged by the University of Wisconsin Optical Imaging Core using a Leica DMi8 Fluorescent Microscope (Filter sets for 4 color widefield fluorescence acquisition using a Hg Arc Lamp).

Results

- Does this lot express the mCherry reporter protein?
  - Yes ☒
  - No ☐



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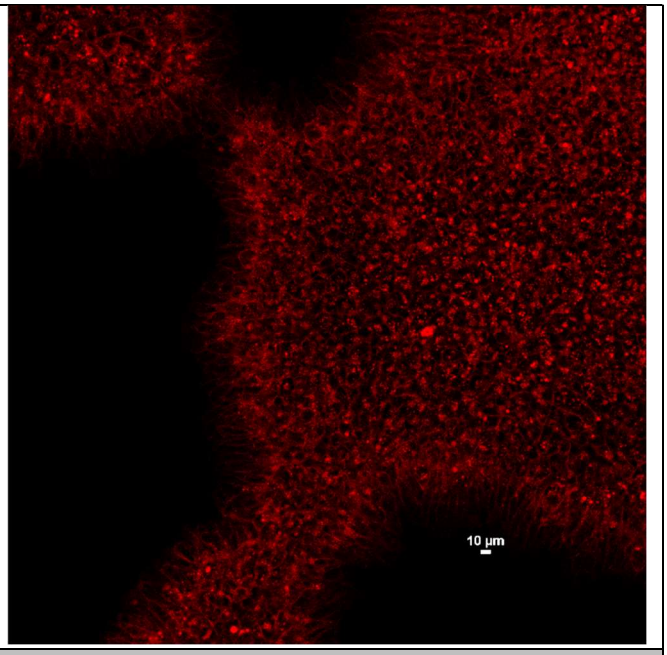

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