Certificate of Analysis

Thaw and Culture Details

Cell Line Name	**WIZ04e-H9CAGmChry**	
WiCell Lot Number	**WB67905**	
Parent Material	WIZ04e-H9CAGmChry-WB67287	
Provider/Client	University of Wisconsin – Dr. Su-Chun Zhang	
Banked By	WiCell	
Thaw and Culture Recommendations	WiCell recommends thawing 1 vial into 4 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	p38 Cells were cultured for 37 passages prior to freeze. Plated cells at thaw should be labeled passage 38.	
Date Vialed	17-JUNE-2022	
Vial Label	WIZ04e-H9CAGmChry p38 WB67905	
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

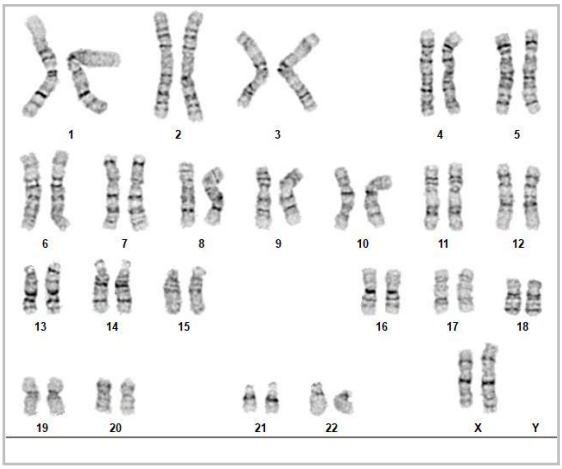
Test Description	Test Provider	Test Method	Test Specification	Result
Karyotype	WiCell	G-T-L Banding performed on 20 metaphase cells	Expected karyotype	See Report
	Results: 46,XX ***Interpretation:*** This is a normal karyotype; no clonal abnormalities were detected at the stated band level of resolution.			
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: 092908

Date Reported: Tuesday, July 26, 2022
Cell Line: WIZ04e-H9CAGmChry-WB67905
Submitted Passage #: 38
Date of Sample: 7/15/2022
Specimen: Human Modified ESC
Results: 46,XX

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



Cell: 22
Slide: G02
Slide Type: Karyotype

Total Counted: 20
Total Analyzed: 8
Total Karyogrammed: 4
Band Resolution: 425 - 475

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

Completed by: Jennifer Pecos, CG(ASCP)
Reviewed and Interpreted by: Vanessa Horner, 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: 14Jul22, 15Jul22
STR Amplification Date: 16Jul22

Sample Name	WIZ04e-H9CAGmChry-WB67905 p38	SCRP6904i-WB67890 p15	STAN164i-352C1-WB67917 p19	STAN095i-102C4-WB67915 p14
WiCell CTR No.[1]	92908	92907	92906	92893
FGA	[redacted]			
TPOX				
D8S1179				
vWA				
Amelogenin				
Penta_D				
CSF1PO				
D16S539				
D7S820				
D13S317				
D5S818				
Penta_E				
D18S51				
D21S11				
TH01				
D3S1358				
Allelic Polymorphisms	24	26	28	26
Matches*	See Matches Comment		80615, 80762	73739, 73725
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 a 100% match for the given sample/samples unless otherwise specified.*

[1] CTR No.: Characterization Test Request Number; also known as a laboratory accessioning number.

Short Tandem Repeat

Requestor: WiCell Stem Cell Bank, WiCell
Samples Received: 14Jul22, 15Jul22
STR Amplification Date: 16Jul22

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 ~2-4%.

Matches: 92908 is a match to 92553, 92481, 90918, 90917, 89607, 86113, 84932, 84931, 84930, 84656, and to additional profiles. Additional matches provided upon request.

7/20/2022 X Molly Miles Tech #1 Characterization Signed by: Miles, Molly	7/21/2022 X Amber Kuhn Tech #2 Characterization Signed by: Kuhn, Amber	7/21/2022 X Hunter Hefti QA Review Quality Assurance Signed by: Hefti, Hunter

Mycoplasma Assay Report

PCR-based assay performed by WiCell

WiCell Stem Cell Bank, WiCell

19Jul22

Form SOP-83.01
Version 5.0

Sample Name	Result	Interpretation
PENN004i-277-1-WB67929 p19 (92918)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
PENN165i-M2-21-WB67928 p27 (92917)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
STAN248i-617C1-WB67923 p17 (92916)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
INC170 16Jul22 KLP 2/2 (92915)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
INC170 16Jul22 KLP 1/2 (92914)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
INC169 16Jul22 JG (92913)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
INC123 16Jul22 JH (92912)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
INC149 16Jul22 MMM (92911)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
WIZ04e-H9CAGmChry-WB67905 p38 (92908)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
SCRP6904i-WB67890 p15 (92907)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
STAN164i-352C1-WB67917 p19 (92906)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
STAN095i-102C4-WB67915 p14 (92893)	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).

7/19/2022 X Justin Hobson Tech #1 Characterization Signed by: Hobson, Justin	7/20/2022 X Amber Kuhn Tech #2 Characterization Signed by: Kuhn, Amber	7/20/2022 X Hunter Hefti QA Review Quality Assurance Signed by: Hefti, Hunter

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

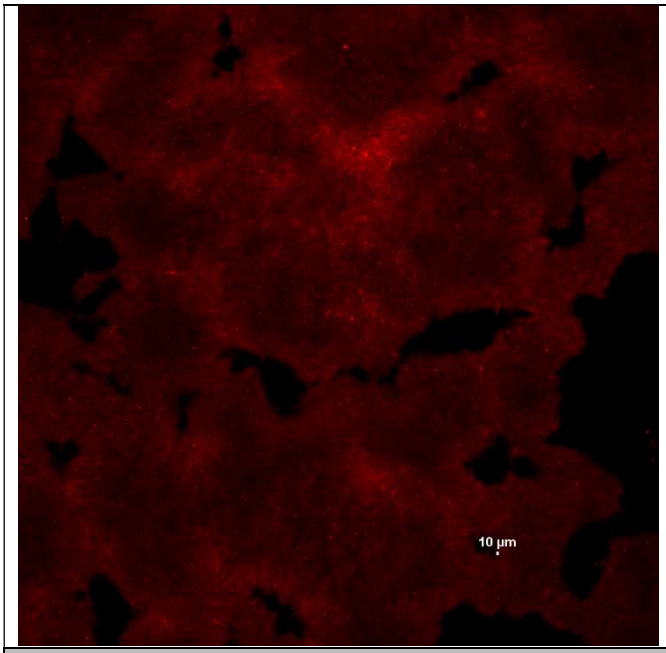
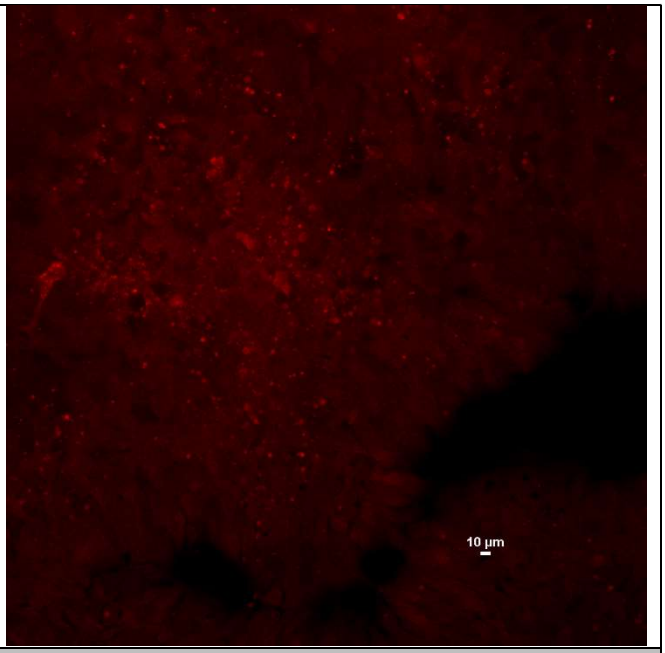
Cell Line-Lot Number	WIZ04e-H9CAGmChry-WB67905
Sample ID	15574
Passage Number	38
Assay Date	15Jul22
Reported By/Date	KLP 19Jul22
Reviewed By/Date	JB 25JUL22
QA Review & Processed By/Date	AA 25Jul22
Notes	☒ N/A

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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