Thaw and Culture Details

Cell Line Name	**EFNB2-tdTomato/EPHB4-EGFP**
WiCell Lot Number	**DB66613**
Provider	University of Wisconsin – Dr. James Thomson
Banked By	University of Wisconsin – Dr. James Thomson
Thaw and Culture Recommendations	WiCell recommends thawing 1 vial into 8 wells of a 6 well plate.
Culture Platform	Feeder Independent
	Medium: E8 Medium
	Matrix: Matrigel®
Protocol	WiCell Feeder Independent E8 Medium Protocol
Passage Number	p42 These cells were cultured for 41 passages prior to freeze. Cells were targeted for modification individually. B2 was targeted at p25 and then B4 at p30. Plated cells at thaw should be labeled passage 42.
Date Vialed	25-August-2017
Vial Label	25AUG17 H1 B2-B4 C29 p42 JAB
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.

Testing Performed by WiCell

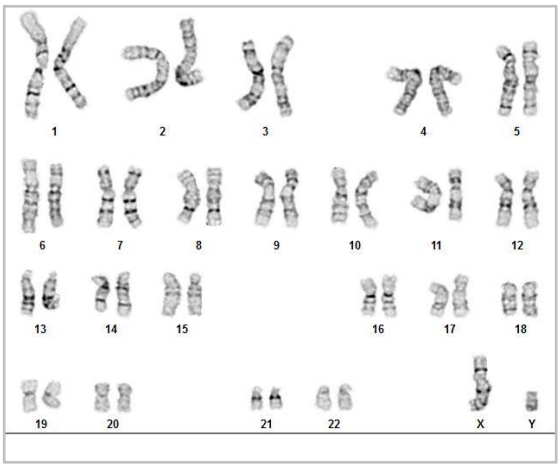
Test Description	Test Provider	Test Method	Test Specification	Result
Karyotype by G-banding	WiCell	SOP-CH-003	Expected karyotype	See Report
Post-Thaw Viable Cell Recovery	WiCell	SOP-CH-305	Recoverable attachment after passage	Pass
Identity by STR	UW Translational Research Initiatives in Pathology Laboratory	PowerPlex 16 HS System by Promega	Defines STR profile of deposited cell line	Pass
Sterility	Steris	ST/07	Negative	Pass
Mycoplasma	WiCell	SOP-QU-004	Negative	Pass

Approval Date	Quality Assurance Approval
04-January-2019	5/27/2020 X HEB HEB Quality Assurance Signed by: Bruner, Haley

Chromosome Analysis Report: 074260

Date Reported: Tuesday, December 18, 2018
Cell Line: EFNB2-tdTomato/EPHB4-EGFP-DB66613 14163
Passage#: 43
Date of Sample: 12/13/2018
Specimen: Human Other
Results: 46,XY

Cell Line Sex: Male
Reason for Testing: Lot release testing
Investigator: [redacted] WiCell



Cell: 7
Slide: G03
Slide Type: Karyotype

Total Counted: 20
Total Analyzed: 8
Total Karyogrammed: 4
Band Resolution: 400 - 525

Interpretation:

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

Completed by: [redacted]
Reviewed and Interpreted by: [redacted] PhD, FACMG

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 Analysis

TRIPath
HISTOLOGY - IHC - MOLECULAR - IMAGING
Department of Pathology and Laboratory Medicine
TRIP Laboratory (Molecular)
https://research.pathology.wisc.edu/trip/
(608) 265-9168

WiCell
Your Lab Partner
characterization@wicell.org
(608) 316-4145

Sample Report:
14163-STR
Sample Name on Tube: 14163-STR
71.2 ng/µL, (A260/280=1.84)
Sample Type: Cells
Cell Count: ~2 million cells

Requestor:
WiCell Research Institute
Quality Assurance Department

Receive Date: 12/17/18
Report Sent: 12/21/18
Assay Date: 12/18/18
File Name: STR 181218 wmr
Report Date: 12/21/18

STR Locus	STR Genotype Repeat #	STR Genotype
FGA	16–18,18.2,19,19.2,20,20.2,21,21.2,22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 43.2, 44.2,45.2, 46.2	20,24
TPOX	6-13	8,11
D8S1179	7-18	12,13
vWA	10-22	15,17
Amelogenin	X,Y	X,Y
Penta_D	2.2, 3.2, 5, 7-17	10,13
CSF1PO	6-15	12,13
D16S539	5, 8-15	9,13
D7S820	6-14	8,12
D13S317	7-15	8,11
D5S818	7-16	9,11
Penta_E	5-24	10,12
D18S51	8-10, 10.2, 11-13, 13.2, 14-27	17,18
D21S11	24,24.2,25,25.2,26-28,28.2,29,29.2, 30, 30.2,31, 31.2,32,32.2,33,33.2, 34,34.2,35,35.2,36-38	28,32.2
TH01	4-9,9.3,10-11,13.3	9.3,9.3
D3S1358	12-20	15,15

<u>Results:</u> Based on the 14163-STR cells submitted by WiCell QA dated and received on 12/17/18, this sample (Label on Tube: 14163-STR) defines the STR profile of the human stem cell line EFNB2-tdTomato/EPHB4-EGFP comprising 28 allelic polymorphisms across the 15 STR loci analyzed.

<u>Interpretation:</u> No STR polymorphisms other than those corresponding to the human EFNB2-tdTomato/EPHB4-EGFP 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. This result suggests that the 14163-STR sample submitted corresponds to the EFNB2-tdTomato/EPHB4-EGFP stem cell line and was not contaminated with any other human stem cells or a significant amount of mouse feeder layer cells.

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

X *RMB* **Digitally Signed on 12/21/18**

[redacted], *BA*
TRIP Laboratory, Molecular

X *WMR* **Digitally Signed on 12/21/18**

[redacted], *PhD, Director / Co-Director*
UWHC Molecular Diagnostics Laboratory / UWSMPH TRIP Laboratory

Native Product Sterility Report

STERIS

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

SAMPLE #: 18111110
DATE RECEIVED: 15-Nov-18
TEST INITIATED: 26-Nov-18
TEST COMPLETED: 10-Dec-18

SAMPLE NAME / DESCRIPTION:
LUEL8357i-3 WB66939 14103
LUEL8679i-4 WB66940 14104
STAN100i-108C4 DB44605 14105
STAN099i-108C2 DB44602 14106
STAN207i-459C2 DB35961 14107
STAN206i-459C1 DB35958 14108
STAN216i-496C1 DB35535 14109
LUEL7159i-7 WB66914 14110
EFNB2-tdTomato/EPHB4-EGFP DB66613 14116
JHU012i-2 DB36196 14117

UNIQUE IDENTIFIER: NA

PRODUCT REGISTRATION: Other: Human iPS cells

TEST RESULTS:

# Tested	# Positives (Growth)	- Control
10	1	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

METHOD VALIDATION / PD #: 000053

TEST METHODOLOGY: USP - Direct Transfer

COMMENTS: Sample labeled LUEL7159i-7 WB66914 14110 is positive in TSB and FTG.

REVIEWED BY _____ DATE 20DEC18

Mycoplasma Detection Assay Report

Testing Performed by WiCell

Lot Release Testing

December 13, 2018

FORM SOP-QU-004.01
Version G Edition 02
Reported by: SM
Reviewed by: JB
Berthold Flash n' Glow 539

#	Sample Name	Reading A		A Ave	Reading B		B Ave	Ratio B/A	Result	Comments/Suggestions
		RLU1	RLU2		RLU1	RLU2				
1	EFBN2-tdTomato/EPHB4-EGFP-DB66613 14163	109	105	107	38	42	40	0.37	Negative	
2	Positive (+) Control	190	185	187.5	10777	10762	10770	57.44	Positive	
3	Negative (-) Control	297	292	294.5	29	26	27.5	0.09	Negative