




# Certificate of Analysis

## Thaw and Culture Details

Cell Line Name	<b>AI11e-OTX2YFP</b>	
WiCell Lot Number	<b>WB68605</b>	
Parent Material	AI11e-OTX2YFP- DB66696	
Provider/Client	Allen Institute - Dr. Boaz Levi	
Banked By	WiCell	
Thaw and Culture Recommendations	WiCell recommends thawing 1 vial into 3 wells of a 6 well plate using mTeSR™ 1 and Matrigel®.	
Protocol	WiCell Feeder Independent Pluripotent Stem Cell Protocol	
Culture Platform Prior to Freeze	Medium: mTeSR™ 1	Matrix: Matrigel®
Passage Number	p59 Cells were cultured for 58 passages prior to freeze. Cells were modified at passage 40. Plated cells at thaw should be labeled passage 59.	
Date Viald	27-September-2024	
Vial Label	AI11e-OTX2YFP  p59 WB68605  Store at -135C or colder Made in United States Research Use Only 	
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.	



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## Results

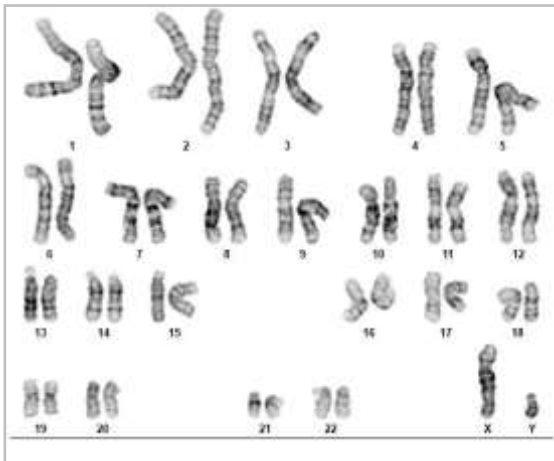
Test Description	Test Provider	Test Method	Test Specification	Result
Karyotype	WiCell	G-T-L Banding performed on 20 metaphase cells	Expected karyotype	See Report
	<p><b>Results:</b> 46,XY Nonclonal findings: 46,XY,del(6)(p21.3)</p> <p><b>Interpretation:</b> This is a normal karyotype; no clonal abnormalities were detected at the stated band level of resolution.</p> <p>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.</p>			
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

Approval Date	WiCell Quality Assurance Approval
18-December-2024	<div><div>X</div><div>HEB</div><div>WiCell Quality Assurance</div><div>Signed by: Bruner, Haley</div></div> <div>12/18/2024</div>

**Date Reported:** October 23, 2024  
**Cell Line:** AI11e-OTX2YFP-WB68605  
**Submitted Passage #:** 59  
**Date of Sample:** 10/14/2024  
**Specimen:** Human Modified ESC  
**Results:** 46,XY

**Cell Line Sex:** Male  
**Reason for Testing:** LOT\_RELEASE  
**Investigator:** WiCell Stem Cell Bank, WiCell

**Nonclonal findings:** 46,XY,del(6)(p21.3)



**Cell:** 44  
**Slide:** G01  
**Slide Type:** Karyotype  
**Total Counted:** 20  
**Total Analyzed:** 9  
**Total Karyogrammed:** 4  
**Band Resolution:** 350 - 425

## Interpretation:

**This is a normal karyotype; no 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:** Pam Mill  
**Reviewed and Interpreted by:** Justin Schleede, 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".*

*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](http://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

Sample Receipt Date: 15Oct24

STR Amplification Date: 28Oct24

Form SOP-89.01

Version 14.0

Sample Name	UCSD225i-NDC1-3-WB68598 p13	AI11e-OTX2YFP-WB68605 p59
WiCell CTR No. <sup>1</sup>	104380	104361
FGA	Identifying information has been redacted to protect donor confidentiality. If more information is required, please contact <a href="mailto:info@wicell.org">info@wicell.org</a>	20, 24
TPOX		8, 11
D8S1179		12, 13
vWA		15, 17
Amelogenin		X, Y
Penta_D		10, 13
CSF1PO		12, 13
D16S539		9, 13
D7S820		8, 12
D13S317		8, 11
D5S818		9, 11
Penta_E		10, 12
D18S51		17, 18
D21S11		28, 32.2
TH01		9.3, 9.3
D3S1358		15, 15
Allelic Polymorphisms	27	28
Matches <sup>2</sup>	90752, 90751, 61476, 66801, 90753, 66943	See Results
Comments		

<sup>1</sup> CTR No.: Characterization Test Request Number; also known as a laboratory accessioning number.

<sup>2</sup> The STR profile of the sample(s) listed are a 100% match for the given sample unless otherwise specified.



# Short Tandem Repeat

Requestor: WiCell Stem Cell Bank, WiCell

Sample Receipt Date: 15Oct24

STR Amplification Date: 28Oct24

Form SOP-89.01

Version 14.0

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

**Results:** The genotypic profiles comprise a range of 27-28 allelic polymorphisms across the 15 STR loci analyzed. Sample 104361 is a 100% match to 103887, 103763, 101599, 99632, 98996, 98995, 97828, 97757 and additional profiles. Additional matches can be provided upon request.

**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 suggest 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%.

10/28/2024	10/29/2024	10/31/2024
<div>X Amber Kuhn</div>	<div>X John Raff</div>	<div>X Jennifer Leny</div>
Tech #1 Characterization Signed by: Kuhn, Amber	Tech #2 Characterization Signed by: Raff, John	QA Review Quality Assurance Signed by: Leny, Jennifer

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# Mycoplasma Assay Report

PCR-based assay performed by WiCell  
WiCell Stem Cell Bank, WiCell  
17Oct24

Form SOP-83.01  
Version 6.0

Sample Name	Result	Interpretation
WA01-WB68078 p37 (104357)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
AI11e-OTX2YFP-WB68605 p59 (104361)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
UCSD225i-NDC1-3-WB68598 p13 (104380)	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).

10/17/2024	10/17/2024	10/18/2024
<b>X</b> John Raff	<b>X</b> Kaylie Petersen	<b>X</b> Dawn Graham
Tech #1 Characterization Signed by: Raff, John	Tech #2 Characterization Signed by: Petersen, Kaylie	QA Review Quality Assurance Signed by: Graham, Dawn

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A gel image is available upon request.

Native Product Sterility Report



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

SAMPLE #: 24110413  
DATE RECEIVED: 14-Nov-24  
TEST INITIATED: 18-Nov-24  
TEST COMPLETED: 02-Dec-24

SAMPLE NAME / DESCRIPTION: UCSD225i-NDC1-3-WB68598  
AI11e-OTX2YFP-WB68605  
AI06e-SOX2YFP-WB68606  
AI03e-DCXYFP-WB68639  
STAN346i-59C2-DB35814  
STAN348i-851C1-DB35826  
STAN347i-66C3-DB35817  
STAN150i-337C1-DB35823  
STAN149i-298C1-DB35820  
H1 AAVS-Runx+23V-WB68661

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

AUTHORIZED BY 

DATE 03DEC2024

Specific test results may not be indicative of the characteristics of any other samples from the same lot or similar lots. This test report shall not be reproduced, except in full, without prior written approval. Liability is limited to the costs of the tests. Results applied to samples as received.