Cedars-Sinai Biomanufacturing Center
Certificate of Analysis (COA)

Cell Line Name	CS83iCTR-33n1
CS Vial ID #(s)	VIAL ID
Date Vialed	2/17/2023
Passage Number	15

The following testing specifications have been met for the specified cell line:

Test Description	Test Specification	Result
Mycoplasma	No contamination detected	Pass
Alkaline Phosphatase Staining	Positive AP staining	Pass
Karyotype by G-Banding	Normal Karyotype	Pass
Pluripotency		
PrimeView Global Gene Expression Profile Assay (PluriTest)	Pluripotency score ≥ 20 and novelty score ≤ 1.6	Pass
Immunocytochemistry (IF-IC)	OCT3/4, NANOG, SOX2, TRA-1-60, TRA-1-81, SSEA4	Pass
TaqMan® hPSC Scorecard™ Assay	Confirm appropriate expression of self-renewal factors	N/A
Differentiation		
EB Formation	Successful Embryoid Body (EB) formation after 14 days	Pass
TaqMan® hPSC Scorecard™ Assay or Differentiation PCR	Tri-lineage differentiation potential *Endoderm, Ectoderm and Mesoderm*	Pass
Reprogramming Plasmid Integration		
Genomic DNA PCR	Confirm the presence or absence of exogenous reprogramming plasmids	Absent
Parent Cell Line Lineage Determination		
TCRB + TCRG T-Cell Clonality Assay (Blood derived cell lines only)	Confirm presence or absence of clonal T-cell receptor beta chain and gamma chain gene rearrangements in iPSCs	N/A
Cell Line Authentication		
STR Analysis	Confirm identity matching score is above 80%	Confirmed

Dhruv Sareen, PhD; CS Biomanufacturing Ctr.

Dhruv Sareen, Ph.D
Executive Director, Cedars-Sinai Biomanufacturing Center

CEDARS-SINAI BIOMANUFACTURING CENTER

iPSC Line: CS83iCTR-33n1

CONTACT INFORMATION:

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USA

PARENT LINE IDENTIFICATION AND INFORMATION:

Parent Cell Line:	GM02183
Age at Tissue Sampling:	21
Phenotypic Sex:	☐ Male ☒ Female
Clinical Diagnosis (if known):	Clinically Normal
Specific Mutations (if known):	33 CAG repeats
Additional Information:	At risk (50%) for Huntington's Disease

REPROGRAMMING INFORMATION:

iPSC Line Name:	CS83iCTR-33n1
Vial ID(s):	VIAL ID
Starting Cell Type:	☐ PBMC ☒ Fibroblast ☐ Other:
Reprogramming Method:	☒ Episomal ☐ Sendai Virus ☐ Other:
Reprogramming Factors:	☒ Oct3/4 ☒ Sox2 ☒ KLF4 ☒ L-Myc ☒ shp53 ☒ Lin28
☐ Other:	

CULTURING INFORMATION:

MEDIUM:

Growth Medium:	mTeSR Plus
Company:	StemCell Technologies
Catalog #:	100-0276

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SUBSTRATE:

Substrate Specification:	Cultrex UltiMatrix RGF Basement Membrane Extract
Company:	R&D Systems
Catalog #:	BME001-10
Coating Concentration:	1.0mg per 6-well plate or 0.16mg per well

PASSAGING METHOD:

Method:	STEMPRO EZPassage Tool	Versene (EDTA)	ReLeSR
Passaging Frequency:	7 days	7 days	7 days
Average Split Ratio:			1:12
Cell Line Preferred Method:	☐	☐	☒

Rate of Differentiation: ☐ High (≥50%) ☐ Moderate (30-40%) ☒ Low (≤20%)

Freezing Media:	Cryostor CS10
Recovery Media:	mTeSR Plus

CHARACTERIZATION OF UNDIFFERENTIATED PLURIPOTENT CELL LINE:

G-BAND KARYOTYPE:

Performed By:	Cedars-Sinai Department of Pathology and Laboratory Medicine
Passage Number:	25
Karyotyping Analysis & Results:	46,XX
Interpretation:	Normal Female Karyotype
Comments:	Cytogenetic analysis using G-banding at or below the 400 band level of resolution on slides of these cultured cells revealed a 46,XX normal female karyotype. No consistent numerical or structural abnormality was observed, and there was no demonstrable clinically significant abnormality.

PLURITEST:

Final Result: ☒ Pass ☐ Fail ☐ Further Evaluate ☐ TBD ☐ N/A

Pluripotency Score, Pass >20:	30.17
Novelty Score, Pass <1.6:	1.342

IMMUNOCYTOCHEMISTRY:

Pluripotency Marker:

AP	SSEA-4	Tra-1-60	Tra-1-81	Nanog	Oct4	Sox2
☒	☒	☒	☒	☒	☒	☒

PLASMID INTEGRATION ANALYSIS:

Absence of plasmid integration confirmed by gDNA PCR:

Result:

EBNA Negative	EBNA Positive	TBD	N/A
☒	☐	☐	☐

Passage #: Unknown

CHARACTERIZATION OF DIFFERENTIATION POTENTIAL:

This cell line has been assessed for differentiation potential by:

☒ 14 Day Embryoid Body Formation ☐ TaqMan® hPSC Scorecard™ Assay ☒ PCR ☐ N/A

hPSC SCORECARD DATA ANALYSIS:

	Self-Renewal	Ectoderm	Mesoderm	Endoderm
iPSC (Day 0):	N/A	N/A	N/A	N/A
Score:				
EBs (Day 14):	N/A	N/A	N/A	N/A
Score:				

Comments:

In lieu of the hPSC Scorecard, embryoid body differentiation was assessed by PCR. PCR analysis confirmed gene expression of all three germ layers for Day 14 EBs as follows:
Endoderm: AFP
Ectoderm: NCAM
Mesoderm: MSX, HAND

PARENT CELL LINE LINEAGE DETERMINATION:

(Blood derived cell lines only)

T-Cell Clonality Assay:

TCR-αβ		TCR-γδ	
☐ Positive	☐ Negative	☐ Positive	☐ Negative

Final Result: ☐ T-Cell Derived ☐ Non T-Cell Derived ☐ TBD ☒ N/A

CEDARS-SINAI BIOMANUFACTURING CENTER

iPSC Line: CS83iCTR-33n1

CELL LINE AUTHENTICATION:

Parent Cell Line: GM02183

AMEL	CSF1PO	D13S317	D16S539	D5S818	D7S820	TH01	TPOX	vWA
X	12	14	11	11, 12	8, 12	9, 9.3	8	16, 19

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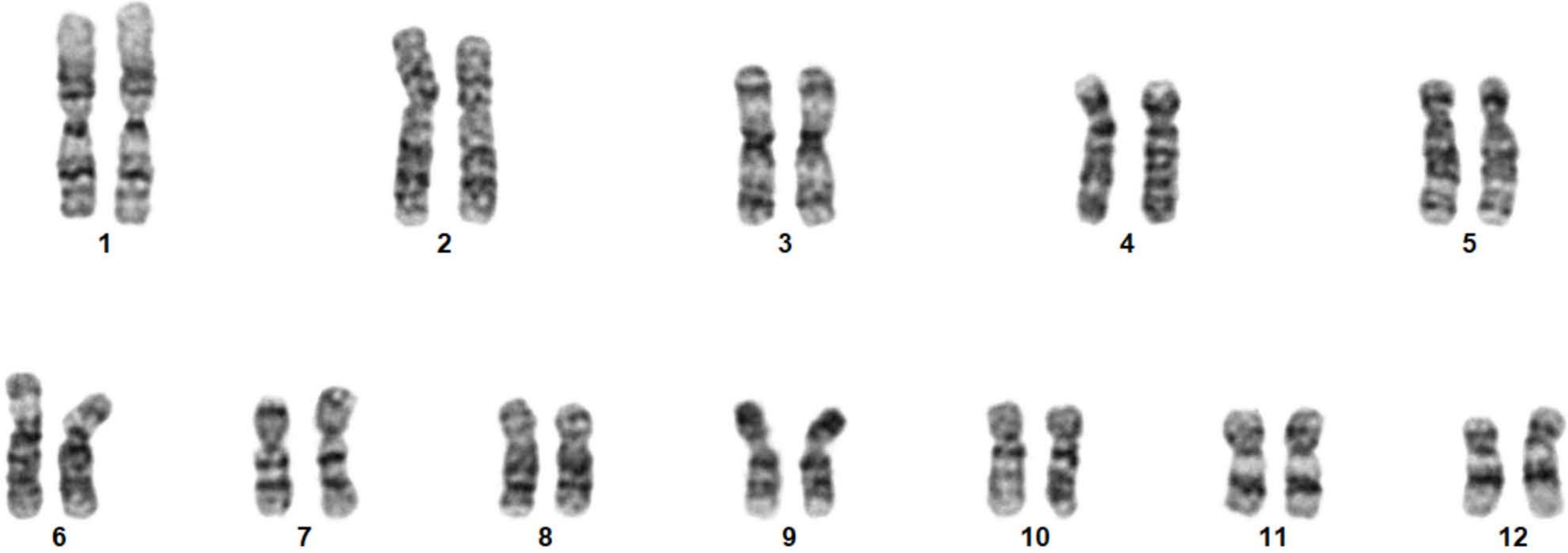
% Identity Match: 100%

IDEXX IBR #(s): 3020-2020 #19

ADDITIONAL INFORMATION:

FACS Analysis:
% OCT4 Positive: 96.8%
% SSEA4 Positive: 99.5%
% Double Positive: 97.9%

qRT-PCR analysis confirmed the endogenous pluripotency gene expression for OCT3/4 (POU5F1), SOX2, LIN28, KLF4, L-Myc.
qRT-PCR analysis confirmed lack of exogenous gene expression.



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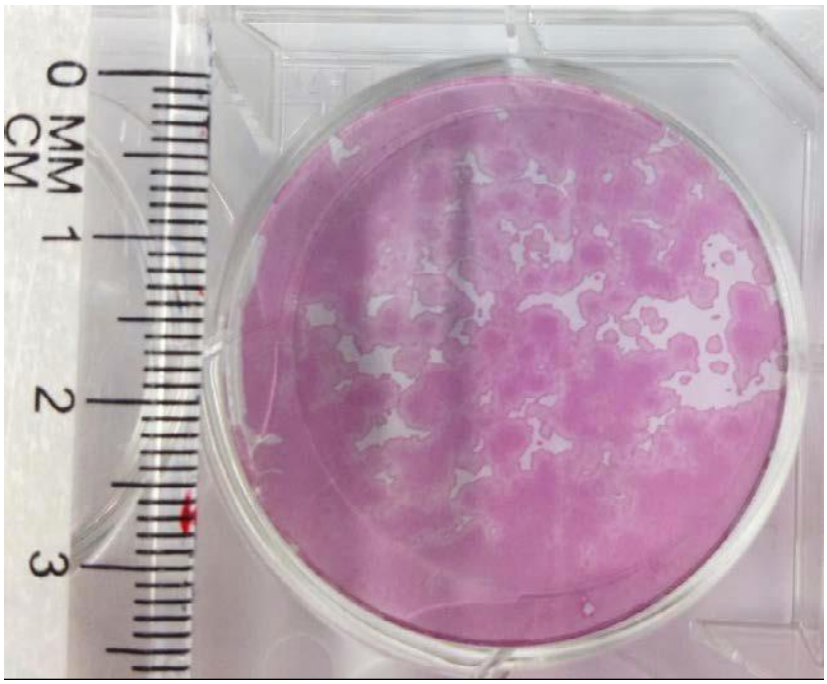
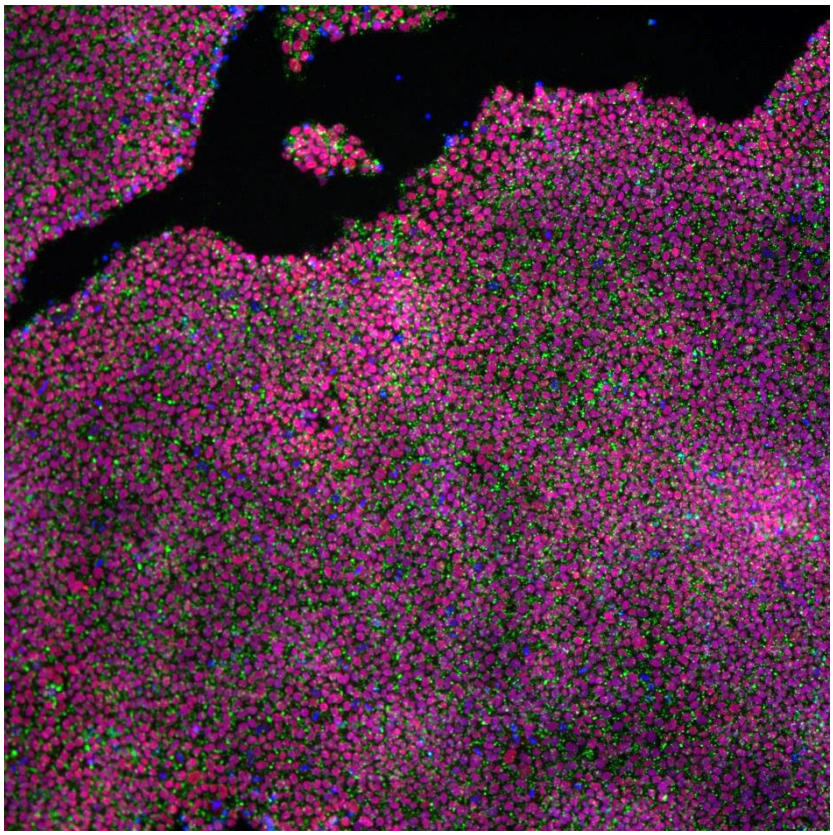
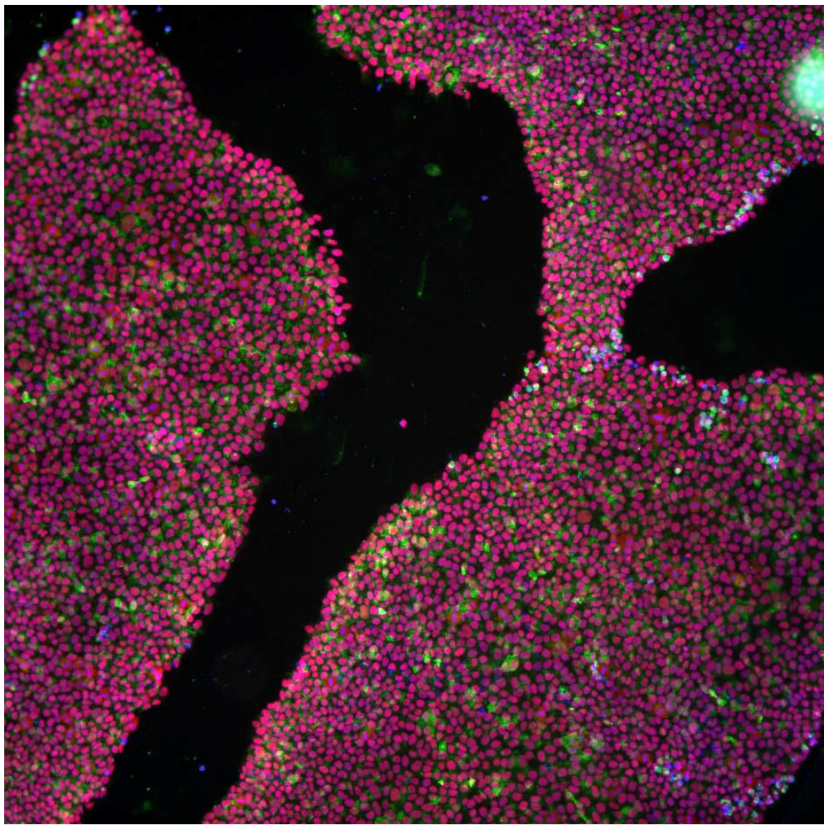
CS83iCTR-33n1	
Alkaline Phosphatase Staining	
Oct4/SSEA4/DAPI	

CS83iCTR-33n1	
SOX2/TRA-1-81/DAPI	
NANOG/TRA-1-60/DAPI