



Cedars-Sinai Biomanufacturing Center
Certificate of Analysis (COA)

Cell Line Name	CS21iHD-60n5
CS Vial ID #(s)	VIAL ID
Date Viald	8/02/2024
Passage Number	14

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		
<i>PrimeView Global Gene Expression Profile Assay (PluriTest)</i>	Pluripotency score ≥ 20 and novelty score ≤ 1.6	Pass
<i>Immunocytochemistry (IF-IC)</i>	OCT3/4, NANOG, SOX2, TRA-1-60, TRA-1-81, SSEA4	Pass
TaqMan® hPSC Scorecard™ Assay	Confirm appropriate expression of self-renewal factors	Pass
Differentiation		
<i>EB Formation</i>	Successful Embryoid Body (EB) formation after 14 days	Pass
TaqMan® hPSC Scorecard™ Assay or Differentiation PCR	Tri-lineage differentiation potential <i>Endoderm, Ectoderm and Mesoderm</i>	Pass
Reprogramming Plasmid Integration		
<i>Genomic DNA PCR</i>	Confirm the presence or absence of exogenous reprogramming plasmids	Absent
Parent Cell Line Lineage Determination		
TCRB + TCRG T-Cell Clonality Assay <i>(Blood derived cell lines only)</i>	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%	N/A

Dhruv Sareen, Ph.D; CS Biomanufacturing Ctr.

Dhruv Sareen, Ph.D

Executive Director, Cedars-Sinai Biomanufacturing Center



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PARENT LINE IDENTIFICATION AND INFORMATION:

Parent Cell Line: GM03621

Age at Tissue Sampling: 29

Phenotypic Sex: ☐ Male ☒ Female

Clinical Diagnosis (if known): Clinically Affected

Specific Mutations (if known): HTT 18 and 60. CAG repeats

Additional Information: Onset at age 18 years; similarly affected grandmother and father; 46,XX.

REPROGRAMMING INFORMATION:

iPSC Line Name: CS21iHD-60n5

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



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
7 days	7 days	7 days
	1:18	
☐	☒	☐

Passaging Frequency: 7 days

Average Split Ratio: 1:18

Cell Line Preferred Method: ☐ ☒ ☐

Rate of Differentiation: ☐ High ($\geq 50\%$) ☐ Moderate (30-40%) ☒ Low ($\leq 20\%$)

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: 13

Karyotyping Analysis & Results: 46,XX

Interpretation: Normal Female Karyotype

Comments:

Cytogenetic analysis using G-banding at or below the 425 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 : 35.811

Novelty Score, Pass <1.6 : 1.234



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 #:

26

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:

iPSC (Day 0):

Score:

EBs (Day 14):

Score:

Self-Renewal	Ectoderm	Mesoderm	Endoderm
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A

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, EOMES

PARENT CELL LINE LINEAGE DETERMINATION:

(Blood derived cell lines only)

T-Cell Clonality Assay:

TCR- $\alpha\beta$		TCR- $\gamma\delta$	
☐ Positive	☐ Negative	☐ Positive	☐ Negative

Final Result:

☐ T-Cell Derived ☐ Non T-Cell Derived ☐ TBD ☒ N/A



CELL LINE AUTHENTICATION:

Parent Cell Line: GM03621

AMEL	CSF1PO	D13S317	D16S539	D5S818	D7S820	TH01	TPOX	vWA

iPSC Line: CS21iHD-60n5

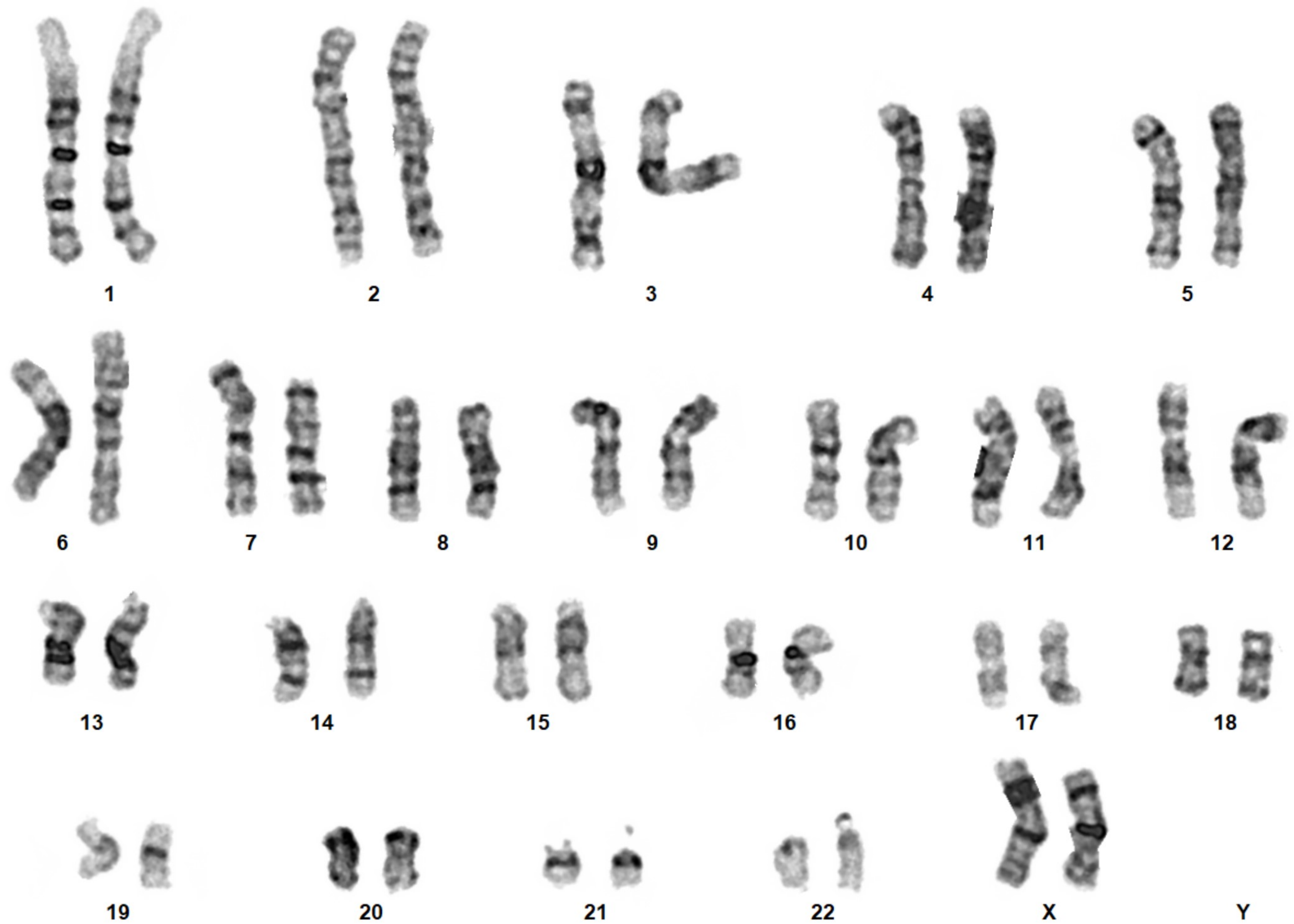
AMEL	CSF1PO	D13S317	D16S539	D5S818	D7S820	TH01	TPOX	vWA

% Identity Match: TBD

IDEXX IBR #(s): TBD

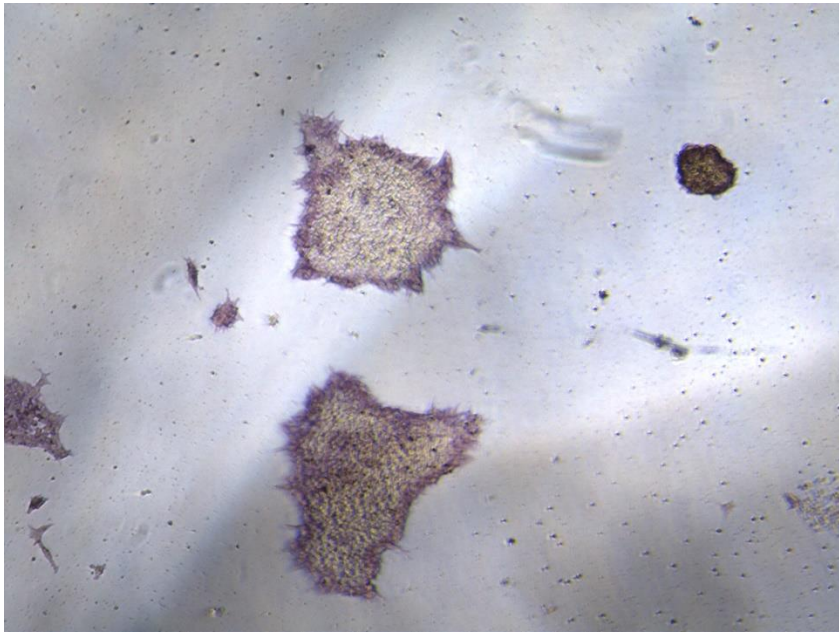
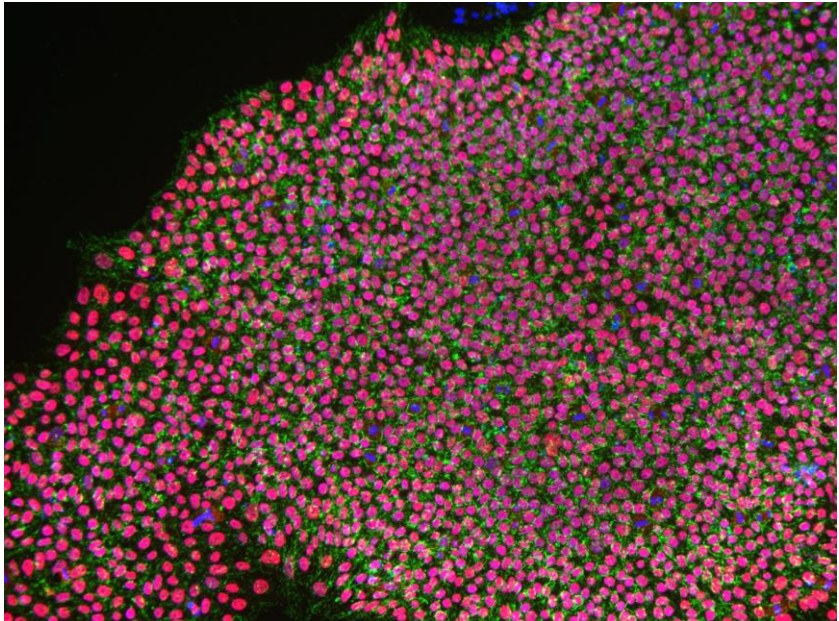
ADDITIONAL INFORMATION:

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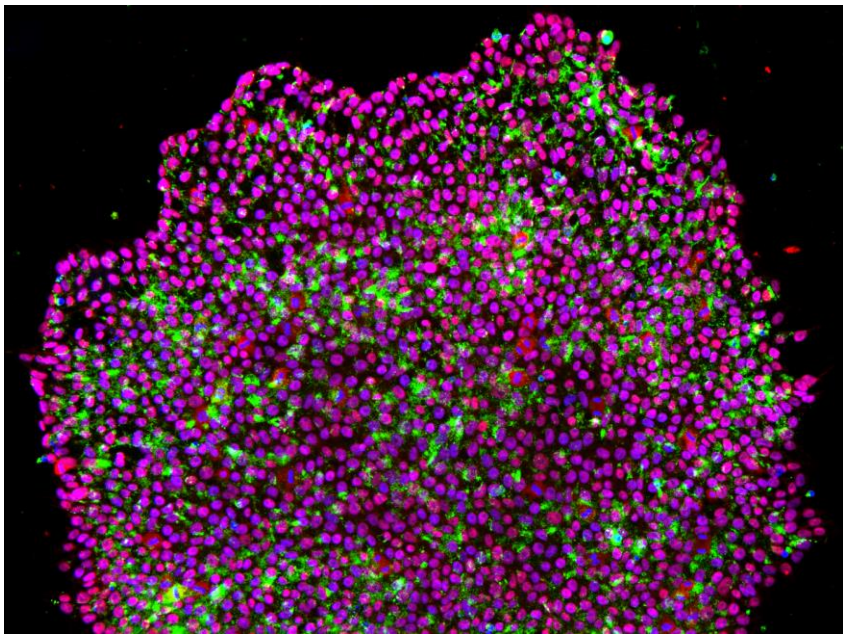


21iHD-60n5 P13



CS21iHD-60n5	
Alkaline Phosphatase Staining	 A brightfield micrograph showing several clusters of cells stained for Alkaline Phosphatase activity. The clusters are dark purple, indicating positive staining, and are surrounded by a lighter, less stained area.
Oct4/SSEA4/DAPI	 A fluorescence micrograph showing a dense cluster of cells stained for Oct4 (red), SSEA4 (green), and DAPI (blue). The cells are densely packed, and the staining is uniform across the cluster.



CS21iHD-60n5	
SOX2/TRA-1-81/DAPI	
NANOG/TRA-1-60/DAPI	