

NHCDR ID - ND50021	Source cell type - Fibroblast
Cell line ID - CR0000011	Reprogramming method - Episome
Lot numbers - R139036381	Growth conditions - mTeSR and Matrigel (SOP# G103-0179)
R139036386	Passage method - Dispase (SOP# G103-0179)
Depositor - CRM	Total Passage Number - 35
	Number of passages at RUCDR - 6
	Number of cells /vial - $2 \times 10^6$

Property	Test Name	Test Method	Criteria for Passing	Test Result
Sterility	Mycoplasma Contamination	qPCR	Negative	Pass
Identity	SNP Trace	96 SNP assay	Matches source cells, if available	Pass
Pluripotency	Live colony image	Visual inspection	Majority of colonies have smooth edges, are mostly circular and consist of small, tightly compacted cells with large nuclear to cytoplasm ratios	Pass
	AP Stain	Live cell fluorescent stain for Alkaline Phosphatase	Positive	Pass
	Immunofluorescence	Fixed cell staining for Oct4 and Tra-1-60	Expression of Oct4 and Tra-1-60	N/A
	FACS	Flow cytometry for the expression of Oct4 and Tra-1-60	Minimum 90% Oct4/Tra-1-60 double positive	99%
	RUCDR Pluritest	HT12 array for gene expression	>18 Pluripotency	N/A
			<2.1 Novelty	N/A
	iPSC Scorecard	96 well qPCR assay on undifferentiated cells for the expression of pluripotency markers	Positive score for pluripotency and negative score for differentiation	N/A
	Differentiation Scorecard	96 well qPCR assay on differentiated cells for the expression of markers of all 3 germ layers	Negative score for pluripotency and positive score for differentiation	N/A
Exogenous reprogramming factor expression	Sendai Persistence	qPCR assay to detect sendai viral sequences	< 1.0 RNA molecules per cell	N/A
			$R^2$ value $\geq 0.95$	N/A
	Episome Persistence	qPCR assay to detect episome sequences	< 0.5 DNA molecules per cell	N/A
			$R^2$ value $\geq 0.95$	N/A
Genetic Stability	Karyotyping	G-band analysis	Expected chromosome complement (Dependant on subject)	N/A
Viability	Test Thaw	Live cell dye followed by confluency quantitation	At least 10 pluripotent colonies at 72hr post-thaw	Pass

# Cryopreservation

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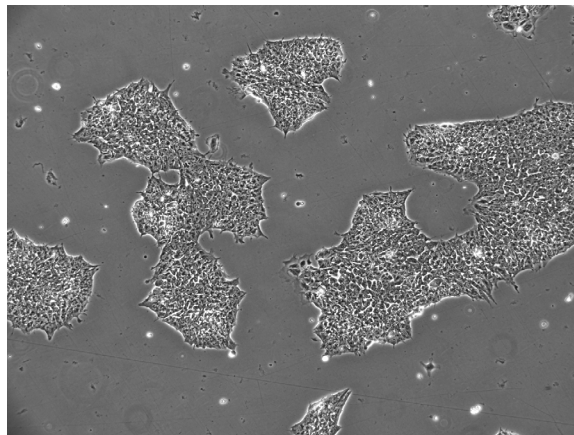
Cryopreservation date - 08/19/2013

Cryopreservation method - accutase and mFreSR (SOP# G103-0179)

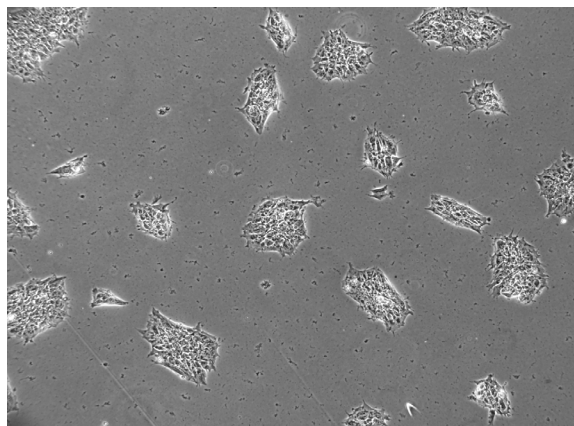
Cell density -  $2 \times 10^6$  /vial

	R139036381	R139036386
Recomended thaw density	1 vial in 2 well(s) of a 6 well plate (9.6cm <sup>2</sup> )	1 vial in 2 well(s) of a 6 well plate (9.6cm <sup>2</sup> )
# Colonies after 24 hours	>10	>10
# Colonies after 72 hours	>30	>30

## Post thaw viability



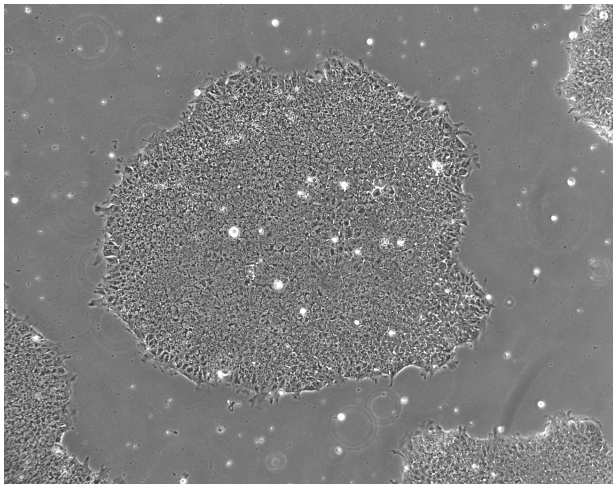
10x magnification 3 days after thaw (R139036381)



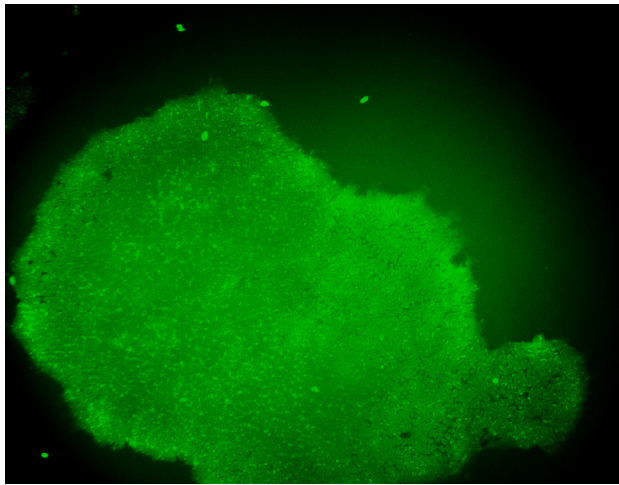
10x magnification 3 days after thaw (R139036386)

# Pluripotency

Live colony image (10X)



AP stain (10X)



## FACS Analysis

