

Chordoma Foundation Cell Line Validation

JHC7

Cell Line Phenotype and Expression
Analysis Report

May 7, 2015

Cell Line Receiving

Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability
Flasks (T25)	October 15, 2013	slightly sparse	1	p. 42	N/A	N/A

Growth Conditions

Media:

1:1 DMEM/F12+ 10% FBS

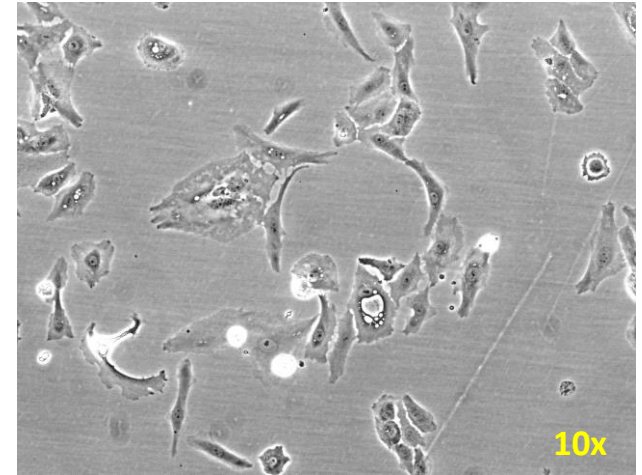
+ l-glutamine + Antibiotic/Antifungal

→ Passage when ~80-90% confluent (1:3, 1:5)

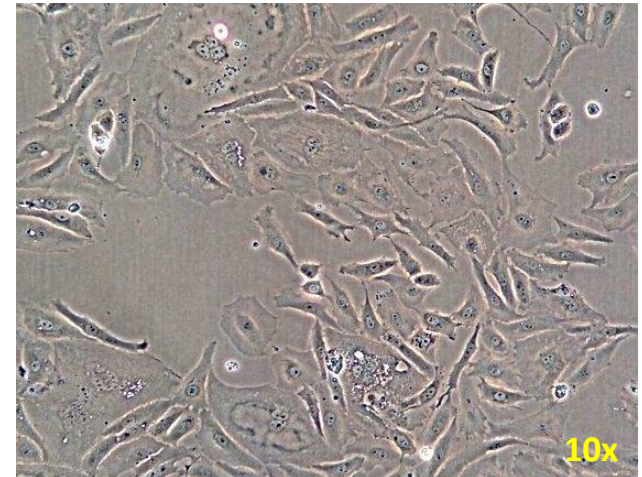
→ Change media every 3-5 days

Phase Contrast Image Review

Cells arrived live, in 1 T25 flask. Were slightly sparse. They are visibly clear of contamination and grow well. Notes on flask indicate they had been seeded on October 12, 2013 thus media was changed to fresh 24 hours after arrival.



Date Received (10/15/2013)

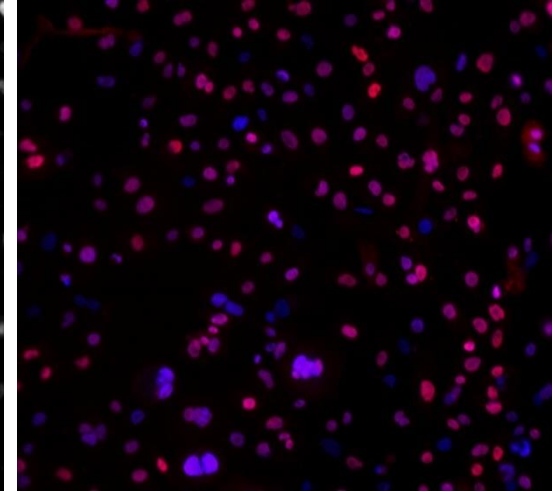
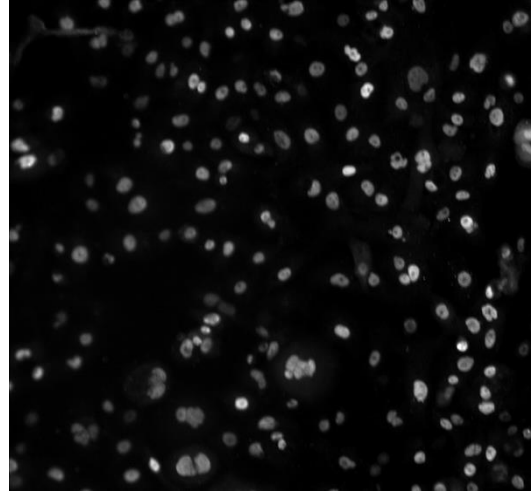
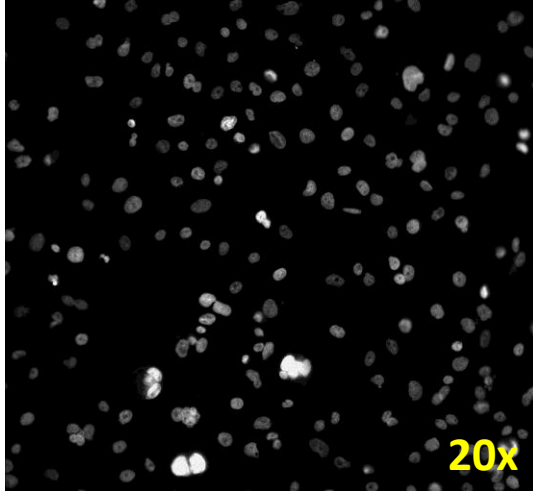


48 Hours After Receiving (10/17/2013)

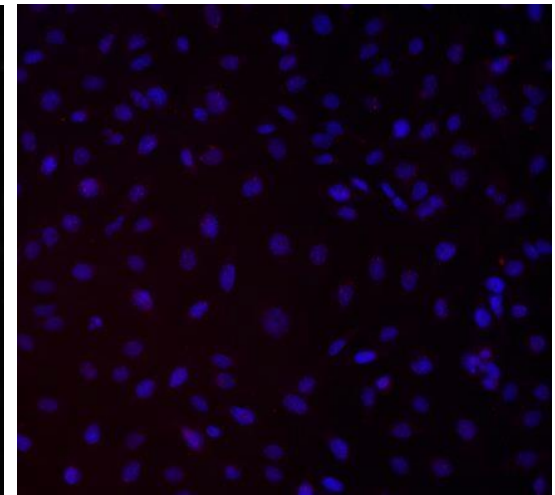
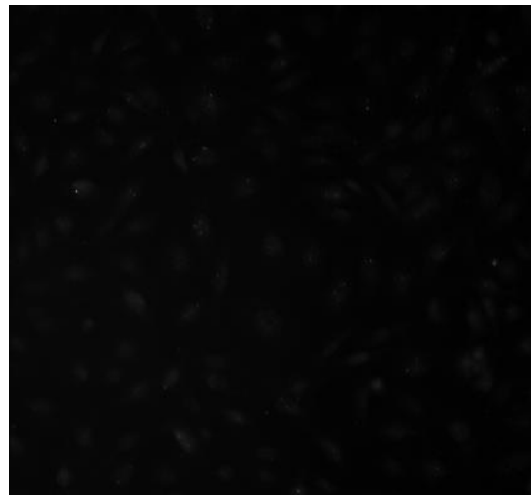
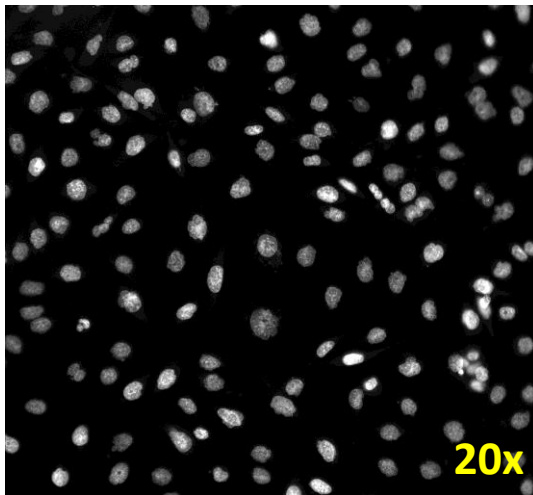
Cell Line Immunofluorescence Validation

JHC7 (supplied by Wake Forest University) p.42 versus Non-Chordoma Negative Control

JHC7
WFBH



MDA-MB-231
Negative
Control



Hoechst Nuclear Stain

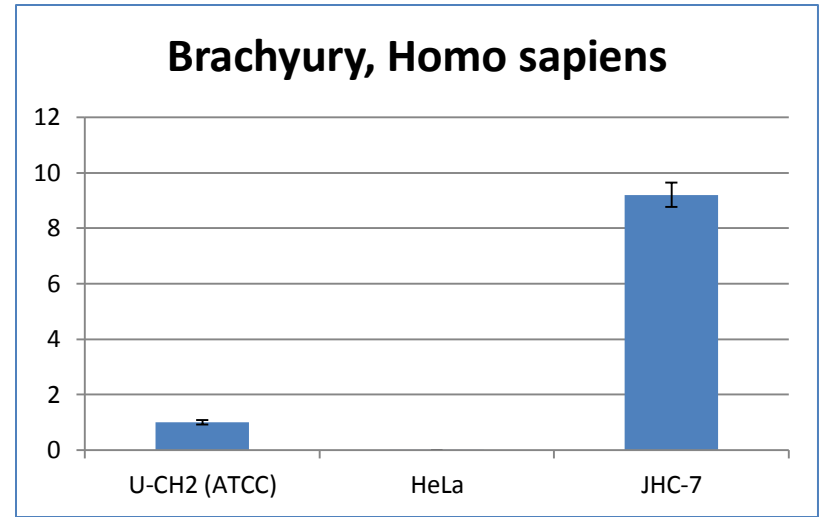
Anti-Brachyury Antibody

Color Composite

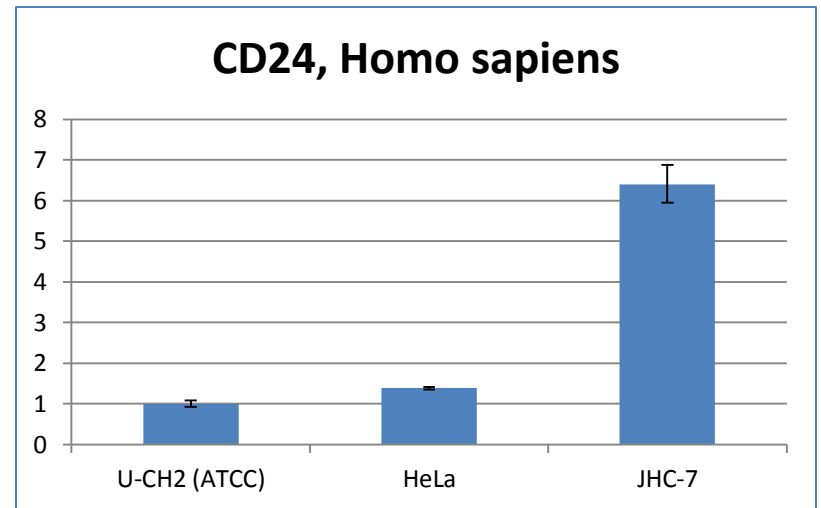
Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in JHC7 cell line

Sample	<u>BRACHYURY,</u> Homo sapiens	<u>Neg. Error</u>	<u>Pos. Error</u>
U-CH2 (ATCC)	1	0.071225	0.076687
HeLa	0.009183	0.00052	0.00055
JHC-7	9.195996	0.43329	0.45471



Sample	<u>CD24, Homo</u> <u>sapiens</u>	<u>Neg. Error</u>	<u>Pos. Error</u>
U-CH2 (ATCC)	1	0.078939	0.085705
HeLa	1.386653	0.03058	0.03127
JHC-7	6.398626	0.4471	0.48069



Tables and associated graphs depict relative quantification of N (top table and graph) and Z (bottom table and graph) gene expression/RNA in TEST cell samples. Gene expression across all assessed lines is set relative to the positive control sample, which is set at 1. The X-axis represents cell lines assessed and the Y-axis represents gene expression relative to positive control.

Cell Line Validation Results

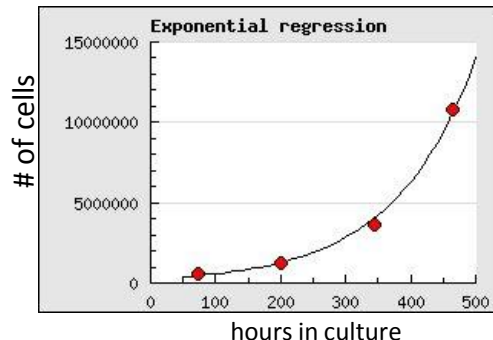
Results summary report of JHC7

TEST	SPECIFICATION	RESULTS
Cell Growth	Immortalized	Doubling time = 3.5-4 days
STR Analysis	Human, unique	Pass
IF Validation	Signal in nucleus	Pass
PCR Validation	Expressing Brachyury and CD24	Pass

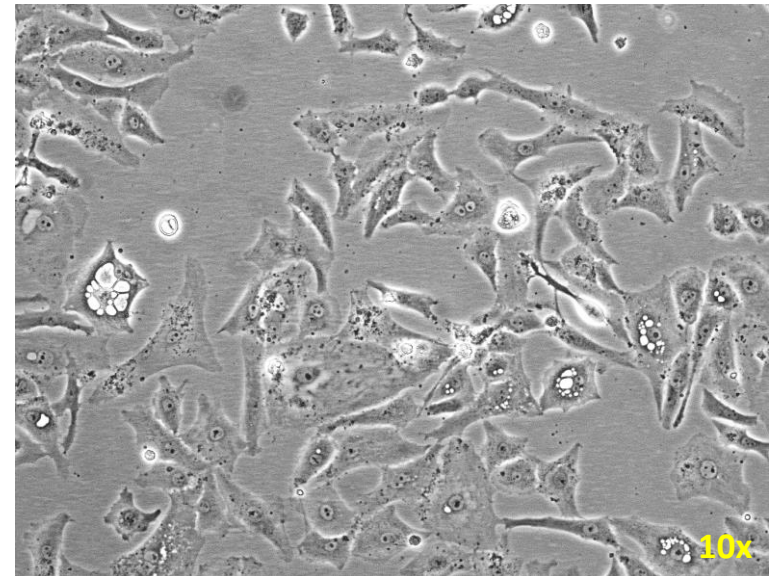
Cell lot generated

Stock Lot#	EB1013-019
Cells per vial	1.0x10 ⁶
Lot Viability	89%
Passages	p. 49

Cell Line Growth: Cell doubling time= 3.5-4 days



Cell growth rates were calculated from an actively growing culture for four passages. Growth rates will likely be slower when calculated from a fresh thaw.



**JHC7 Vala cell lot EB1013-019
viability thaw**