

Chordoma Foundation Cell Line Validation

UM-Chor1

Cell Line Phenotype and Expression
Analysis Report

April 17, 2015

Cell Line Receiving

Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability
Flasks (T25)	October 08, 2013	confluent	2	p. 36	N/A	N/A

Growth Conditions

Media:

1:4 RPMI/IMDM + 10% FBS

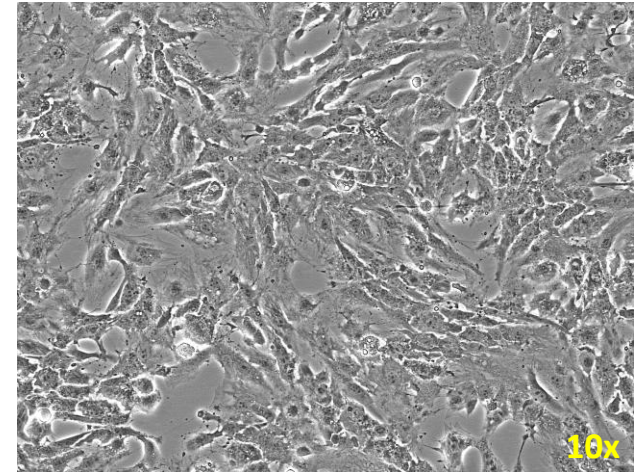
+ NEAA + Antibiotic/Antifungal

→ Passage when ~80-90% confluent (1:2, 1:3)

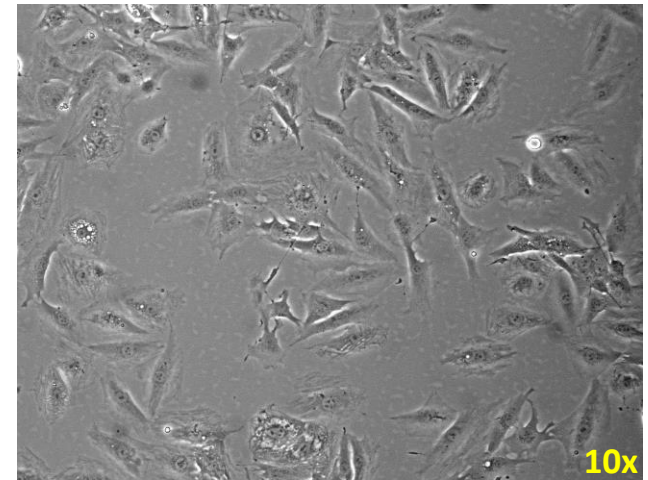
→ Change media every 2-3 days

Phase Contrast Image Review

Cells arrived live, in 2 T25 flasks. Were confluent and split after 24 hours. They are visibly clear of contamination and grow well.



Date Received (10/08/2013)

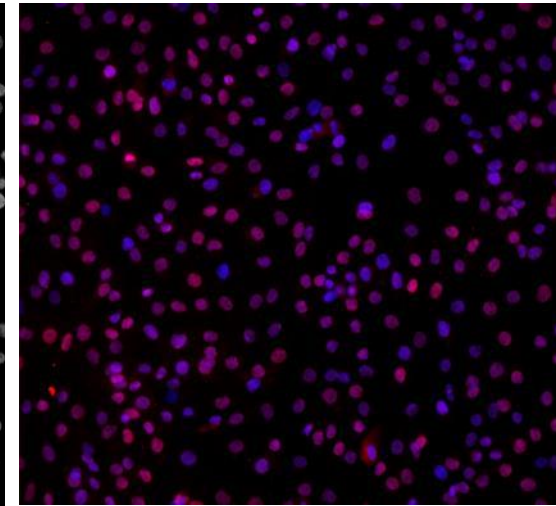
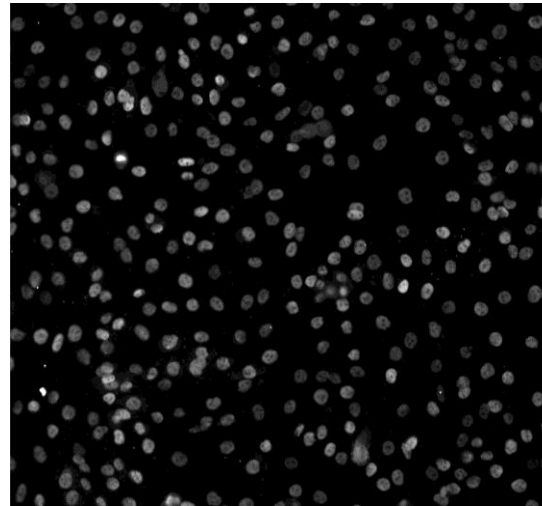
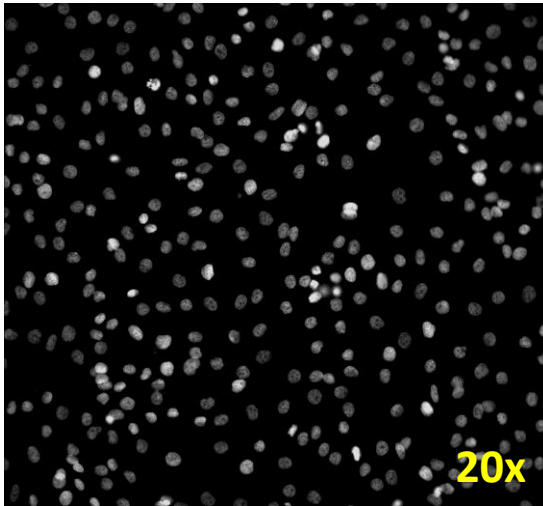


24 hours post initial in-house passage
(10/10/2013)

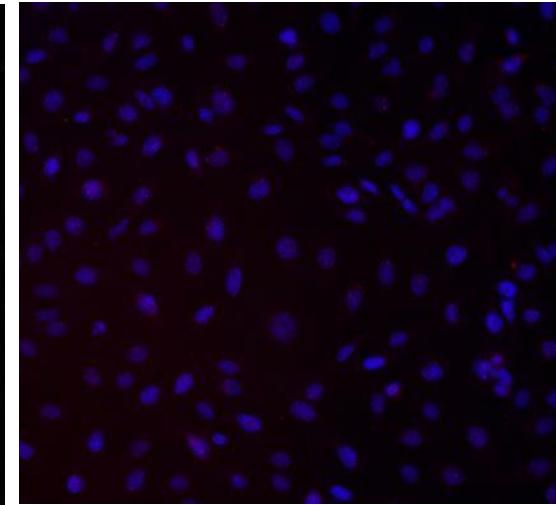
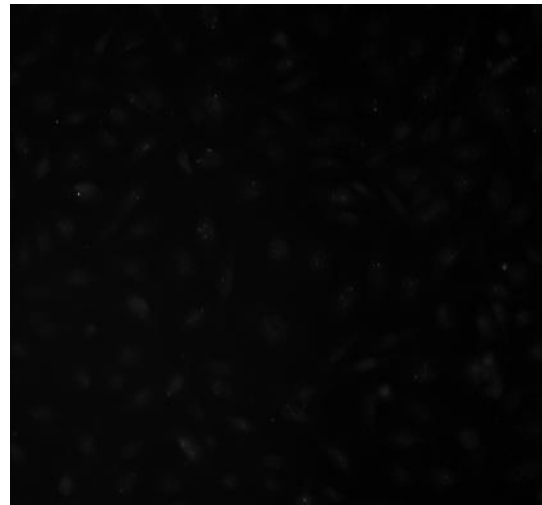
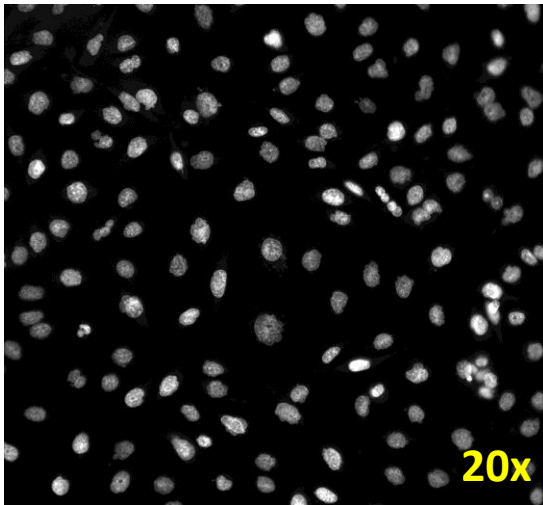
Cell Line Immunofluorescence Validation

UM-Chor1 (supplied by U. of Michigan) p.63 versus Non-Chordoma Negative Control

UM-Chor1
Cells



MDA-MB-231
Negative
Control



Hoechst Nuclear Stain

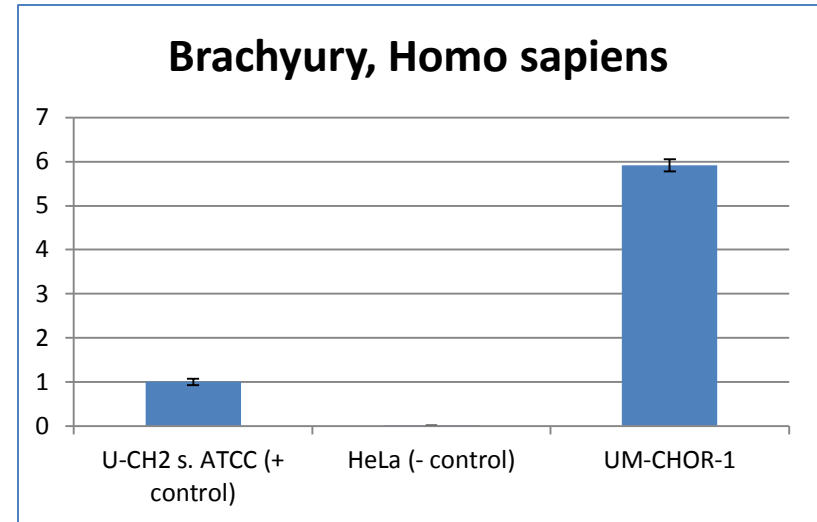
Anti-Brachyury Antibody

Color Composite

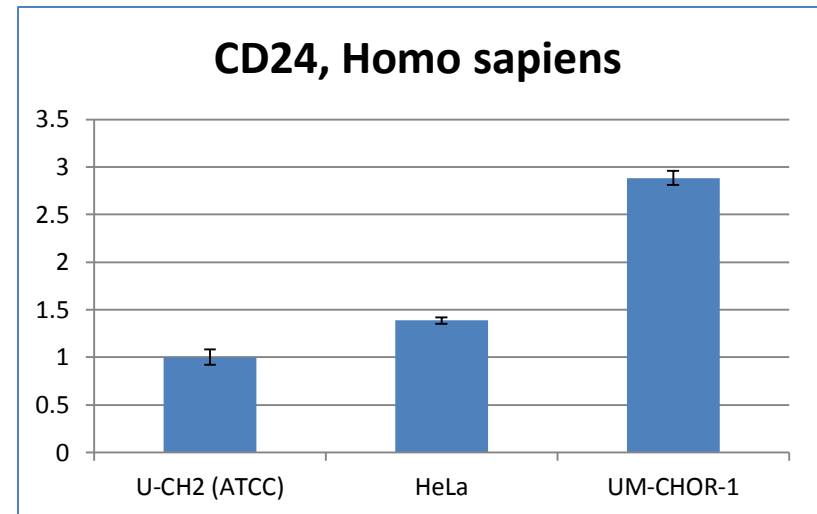
Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in UM-Chor1 cell line

Sample	<u>BRACHYURY</u> , Homo sapiens	<u>Neg. Error</u>	<u>Pos. Error</u>
U-CH2 s. ATCC (+ control)	1	0.071225	0.076687
HeLa (- control)	0.009183	0.00052	0.00055
UM-CHOR-1	5.912447	0.135544	0.138725



Sample	<u>CD24, Homo 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
UM-CHOR-1	2.884621	0.07226	0.074117



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.

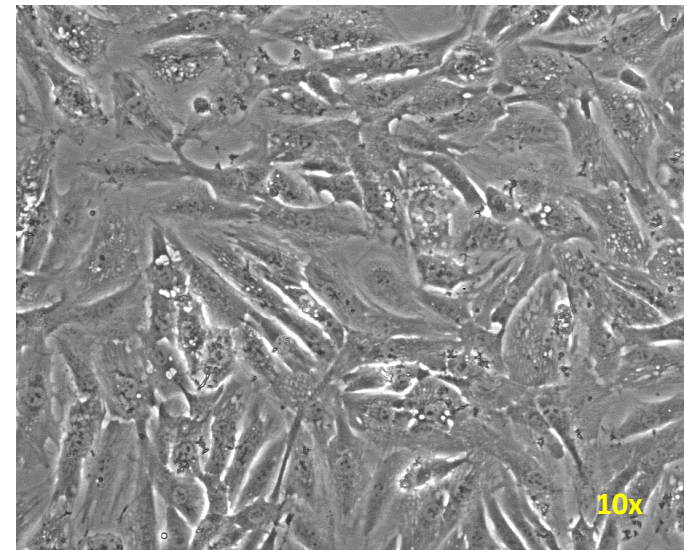
Cell Line Validation Results

Results summary report of UM-Chor1

TEST	SPECIFICATION	RESULTS
Cell Growth	Immortalized	Doubling time = 5 days
STR Analysis	Human, unique	Pass
IF Validation	Signal in nucleus	Pass
PCR Validation	Expressing Brachyury and CD24	Pass

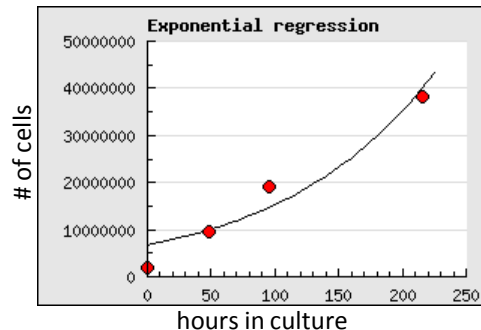
Cell lot generated

Stock Lot#	EB1013-012
Cells per vial	1.0x10 ⁶
Lot Viability	92.7%
Passages	p. 42



UM-Chor1 Vala cell lot EB1013-012
viability thaw

Cell Line Growth: Cell doubling time= 5 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.