

Chordoma Foundation Cell Line Validation

U-CH14

Cell Line Phenotype and Expression
Analysis Report

May 16, 2017

Cell Line Receiving

Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability
Frozen Vials	January 12, 2017	N/A	3	p (0)	2.85×10^5	66%

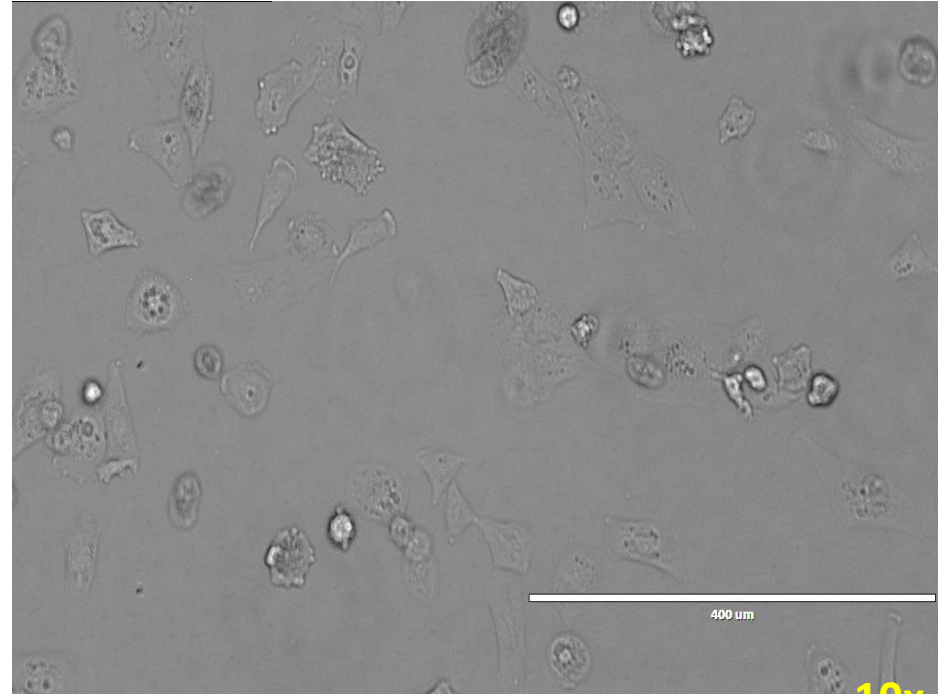
Growth Conditions

Media:

4:1 IMDM/RPMI + 10% FBS + l-Glutamine +
Pen/Strep

→ Passage when ~80-90% confluent (no more
than 1:2)

→ Change media every 5-7 days

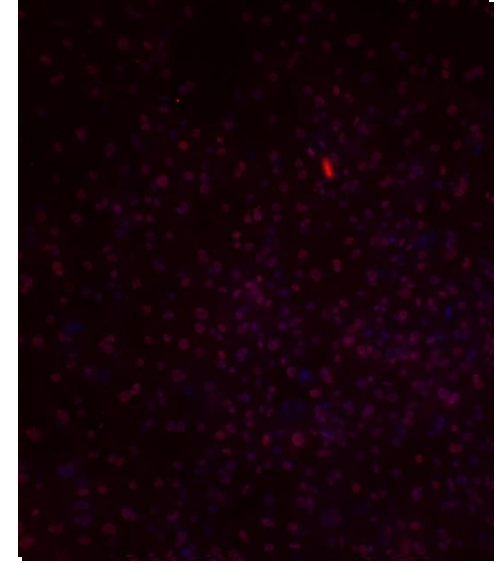
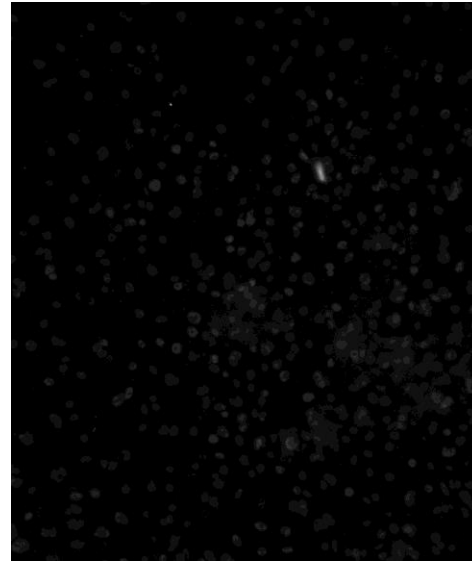
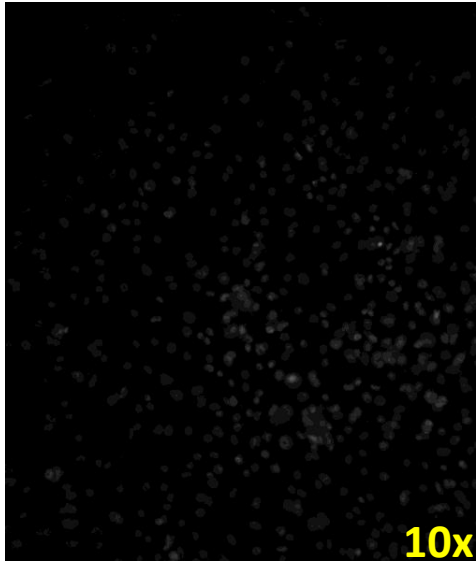


U-CH14 24hrs post thaw.

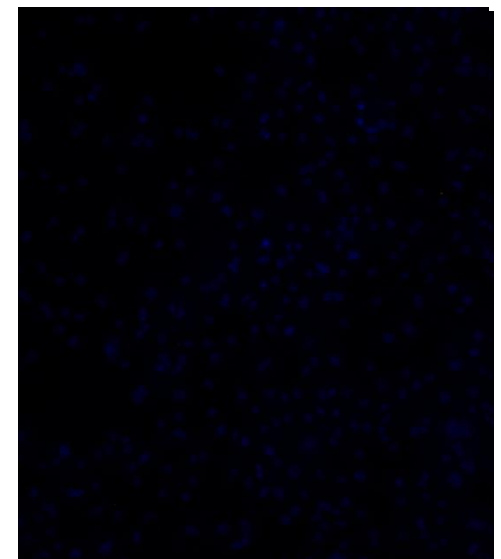
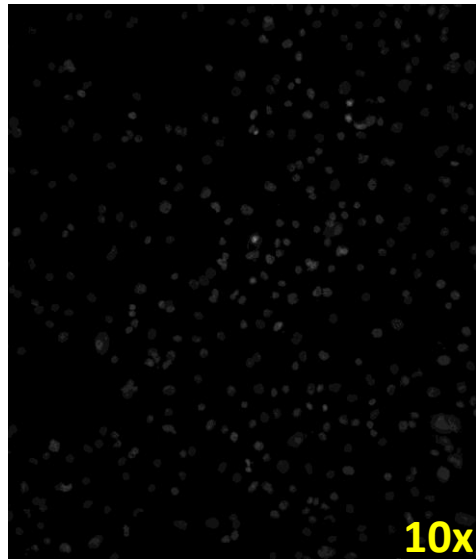
Cell Line Immunofluorescence Validation

U-CH14 Anti-Brachyury versus Secondary-only Negative Control

U-CH14



U-CH14
2°
Only
Control



Hoechst Nuclear Stain

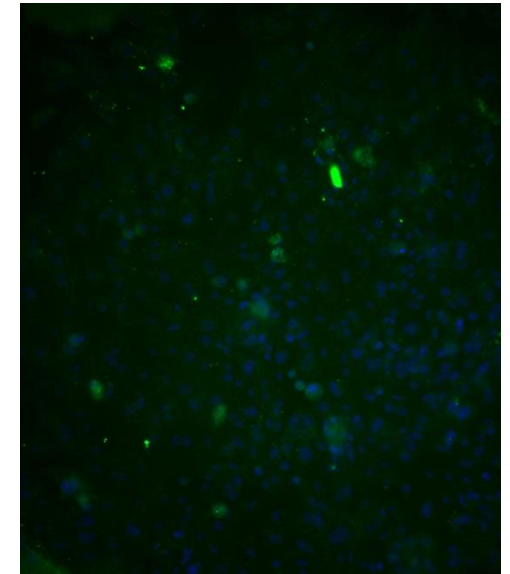
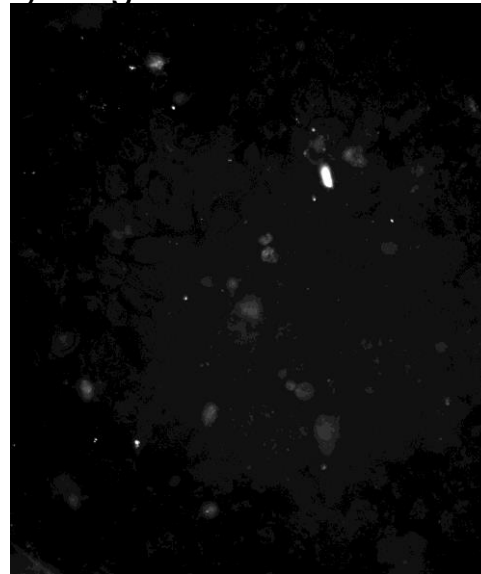
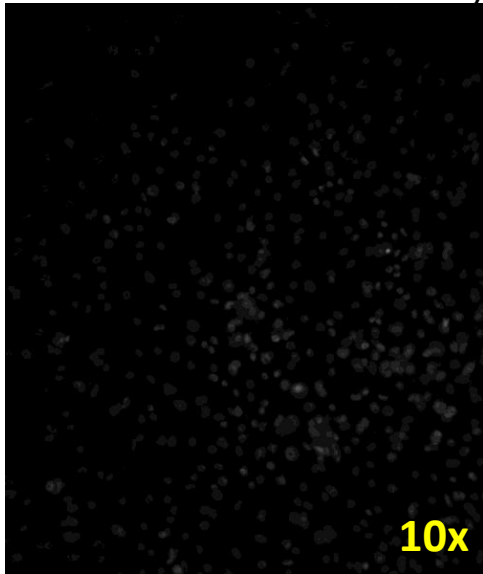
Brachyury

Color Composite

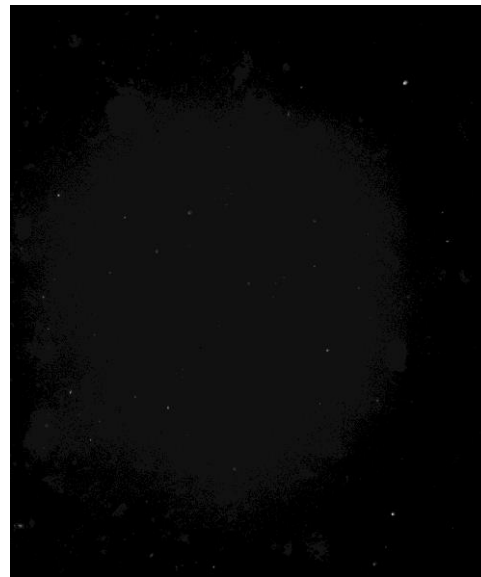
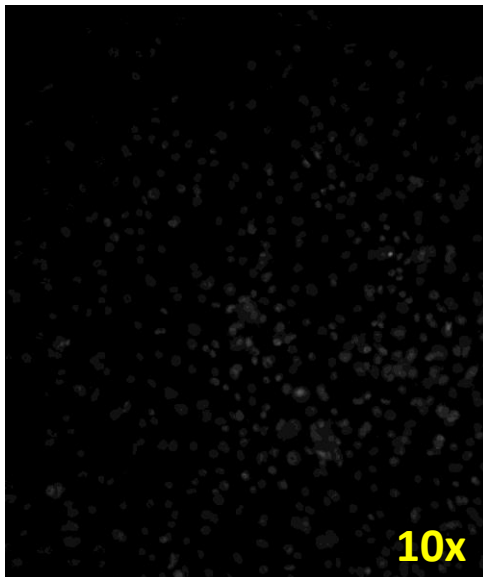
Cell Line Immunofluorescence Validation

U-CH14 Anti-CD24 versus Secondary-only Negative Control

U-CH14



U-CH14
2°
Only
Control



Hoechst Nuclear Stain

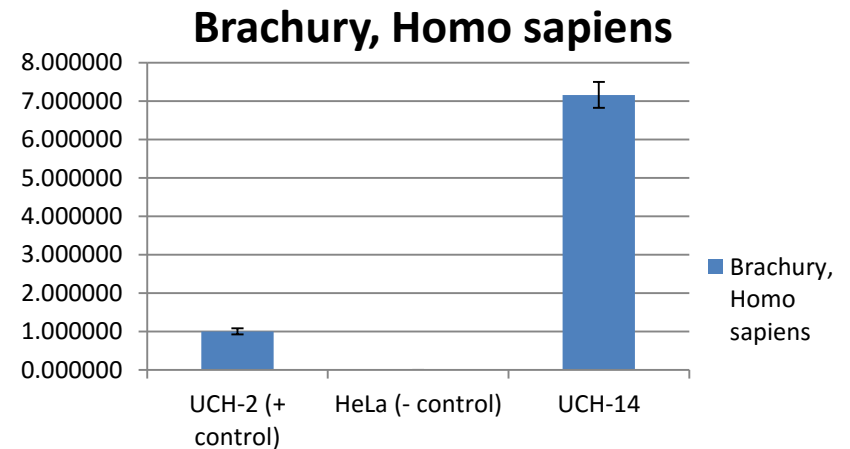
CD24

Color Composite

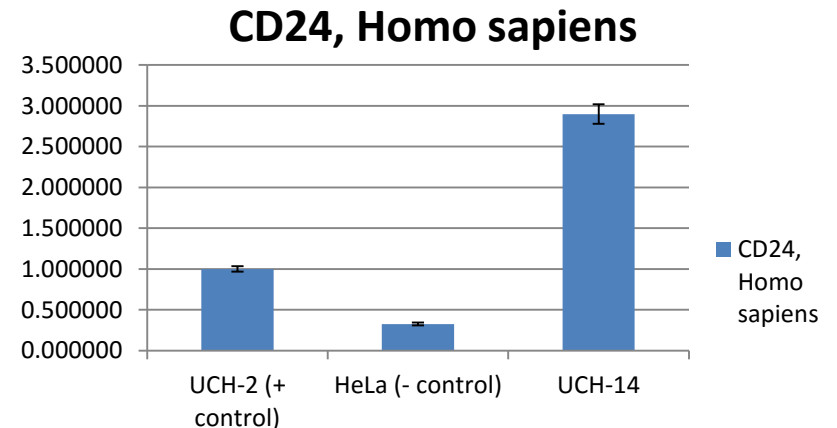
Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in U-CH14 cell line

Sample	Brachyury, Homo sapiens	Neg. Error	Pos. Error
UCH-2 (+ control)	1	0.076061975	0.08232368
HeLa (- control)	0.00081977	5.18264E-05	5.5324E-05
UCH-14	7.15881518	0.328702959	0.34452197



Sample	CD24, Homo sapiens	Neg. Error	Pos. Error
UCH-2 (+ control)	1	0.032074043	0.03313688
HeLa (- control)	0.3261052	0.01774769	0.01876917
UCH-14	2.89650235	0.118301913	0.12333947



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.

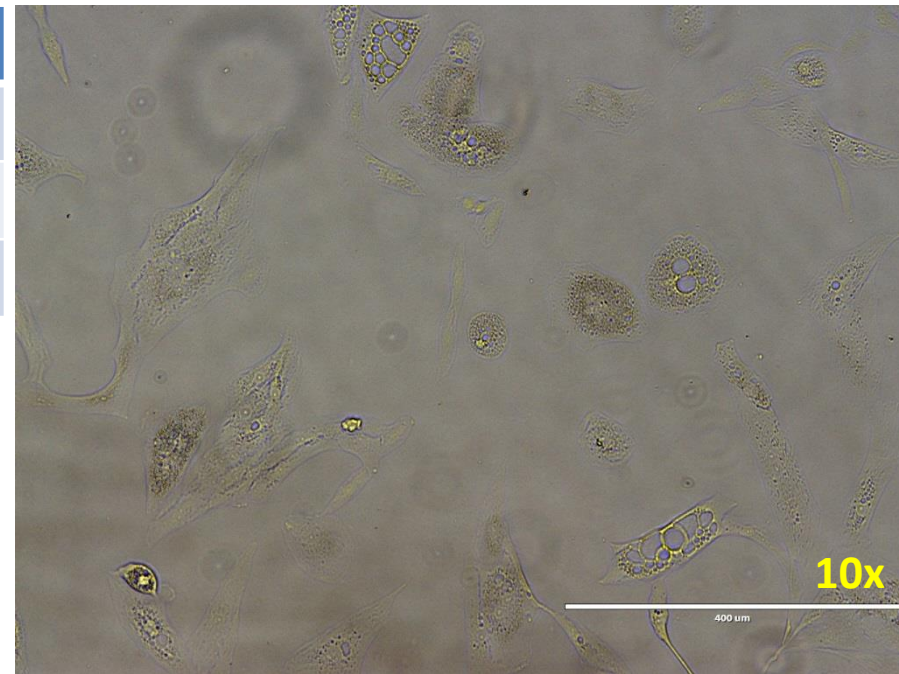
Cell Line Validation Results

Results summary report of U-CH14

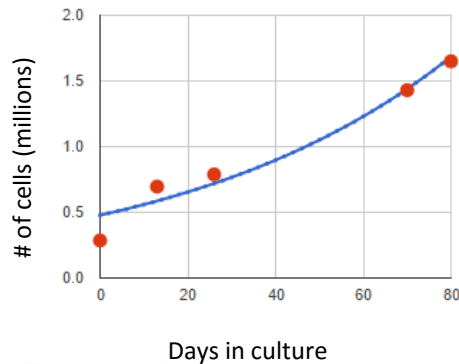
TEST	SPECIFICATION	RESULTS
Cell Growth	Immortalized	Pass
STR Analysis	Human, unique	Pass
IF Validation	Signal in nucleus	Pass
PCR Validation	Expressing Brachyury and CD24	Pass

Cell lot generated

Stock Lot#	1027-026
Cells per vial	500k
Lot Viability	96%
Passages	7



Cell Line Growth: Cell doubling time= 44 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.

U-CH14, lot 1027-002.