

Chordoma Foundation Cell Line Validation

U-CH11

Cell Line Phenotype and Expression
Analysis Report

June 16, 2015

Cell Line Receiving

Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability
Frozen Vials	October 15, 2014	N/A	10	p. 10	7.6×10^5	90.4%

Growth Conditions

Media:

4:1 IMDM/RPMI + 10% FBS

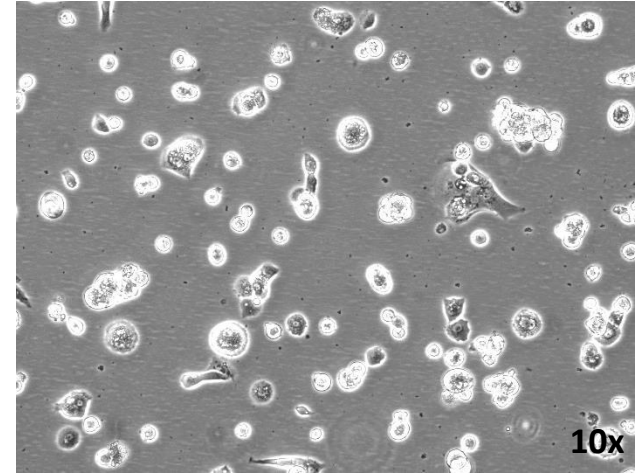
+ l-glutamine + Pen/Strep

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

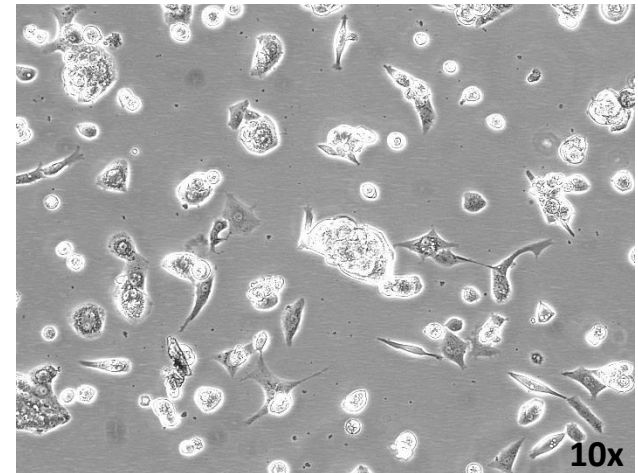
→ Change media every 5-7 days

Phase Contrast Image Review

Ten vials of various passages of cells arrived. One was thawed on 10/20/2014. Culture was visibly clear of contamination but only a few cells sat down despite good viability. Eventually recovered.



24 Hours Post Thaw (10/21/2014)

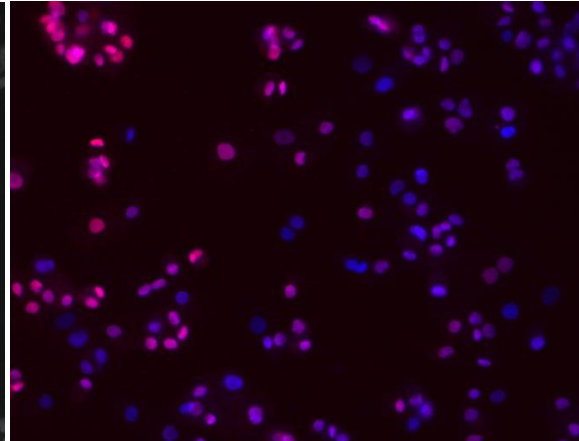
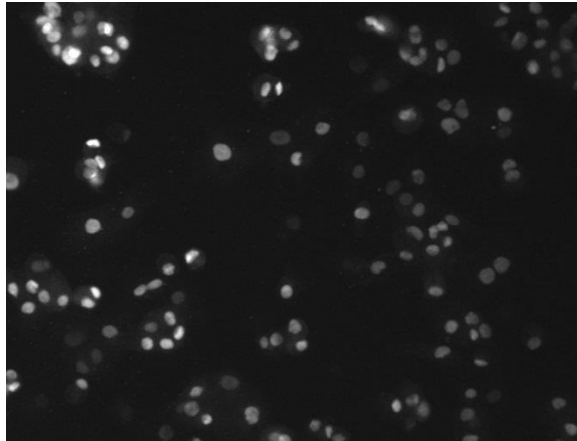
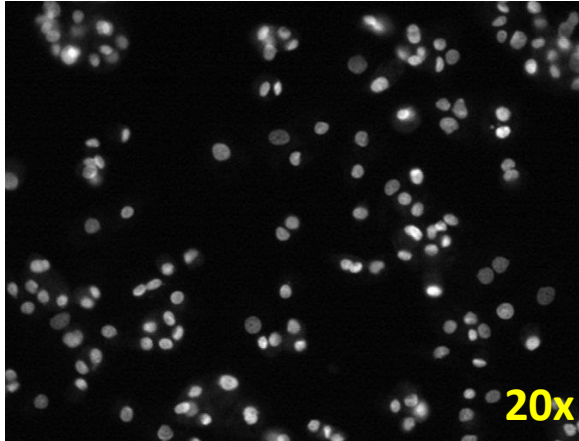


48 Hours Post Thaw (10/22/2014)

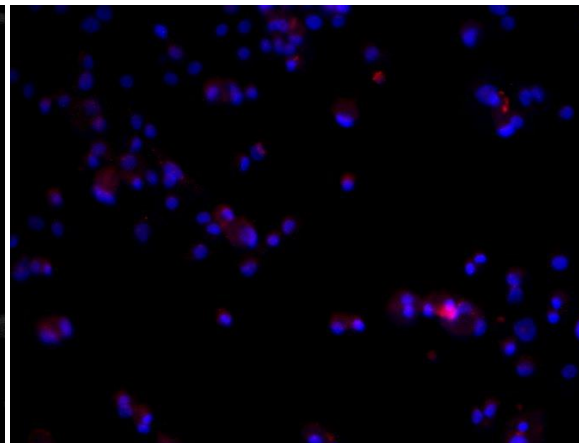
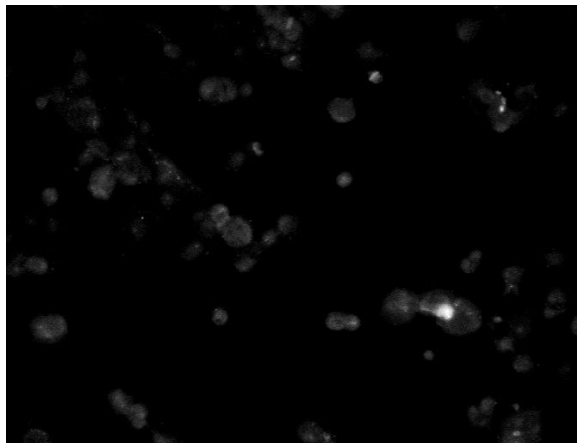
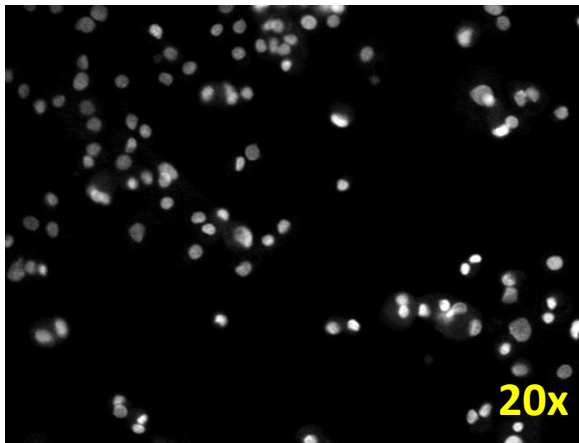
Cell Line Immunofluorescence Validation

U-CH11 p.12 Anti-Brachyury versus Secondary-only Negative Control

U-CH11



U-CH11
2°
Only
Control



Hoechst Nuclear Stain

Anti-Brachyury Channel

Color Composite

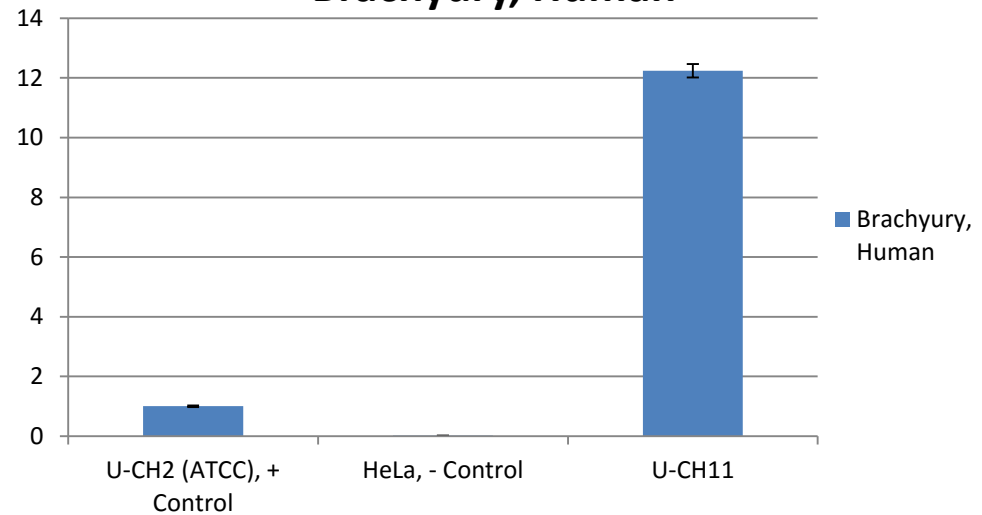
Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in U-CH11 cell line

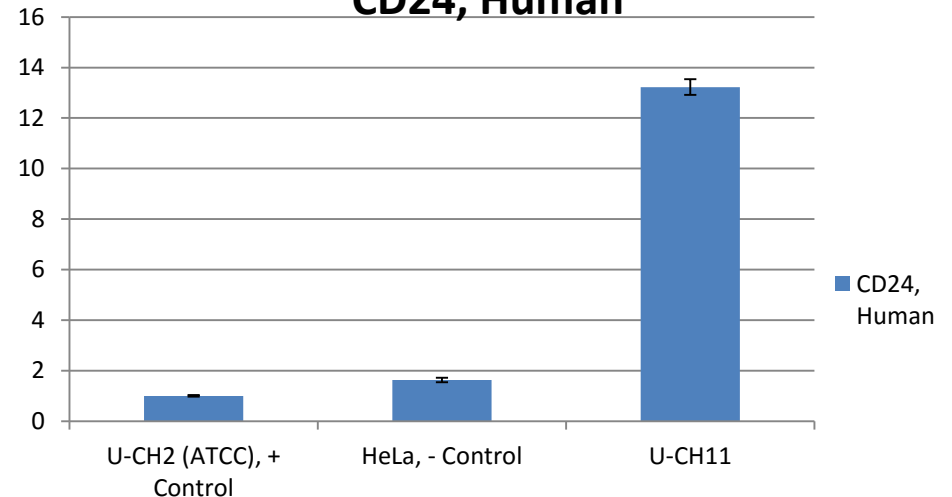
Sample Name	Brachyury, Human	Neg Error	Pos Error
U-CH2 (ATCC), + Control	1	0.02543	0.02610
HeLa, - Control	0.01222	0.00084	0.00091
U-CH11	12.23594	0.22113	0.22520

Sample	CD24, Human	Neg. Error	Pos. Error
U-CH2 (ATCC), + Control	1	0.0315	0.0326
HeLa, - Control	1.6318	0.0861	0.0908
U-CH11	13.2262	0.3030	0.3101

Brachyury, Human



CD24, Human



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.

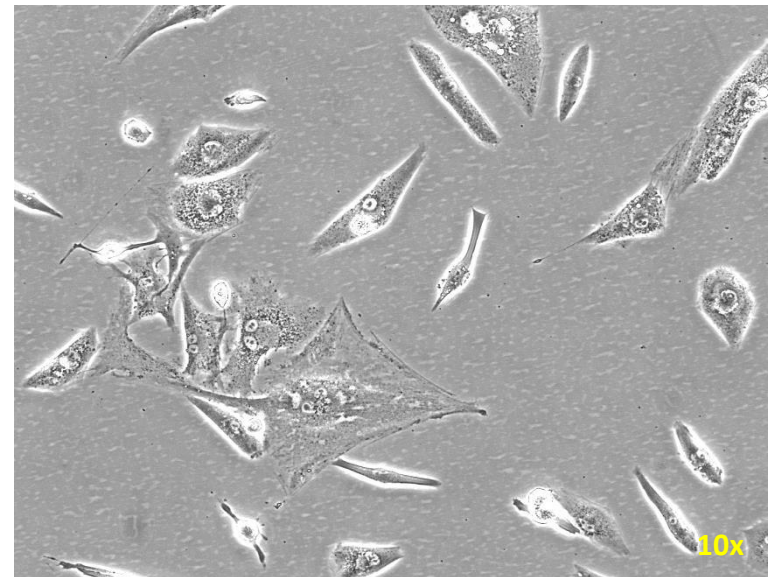
Cell Line Validation Results

Results summary report of U-CH11

TEST	SPECIFICATION	RESULTS
Cell Growth	Immortalized	Doubling time = 15 days
STR Analysis	Human, unique	Pass
IF Validation	Signal in nucleus	Pass
PCR Validation	Expressing Brachyury and CD24	Pass

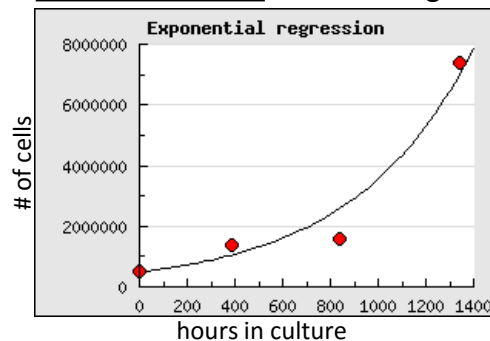
Cell lot generated

Stock Lot#	EB1018-062
Cells per vial	5.0x10 ⁵
Lot Viability	77.3%
Passages	p. 19



U-CH11 Thaw of lot EB1018-062

Cell Line Growth: Cell doubling time= 15 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.