Chordoma Foundation Cell Line Validation

U-CH7

Cell Line Phenotype and Expression Analysis Report

May 7, 2015

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Cell Line Receiving

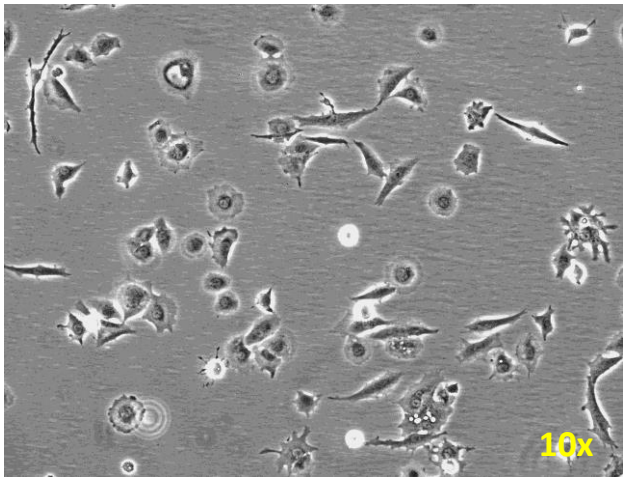
Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability
frozen vials	October 8, 2014	frozen	2	p. 47	$1.2x10^6$ cells	97.5%

Growth Conditions

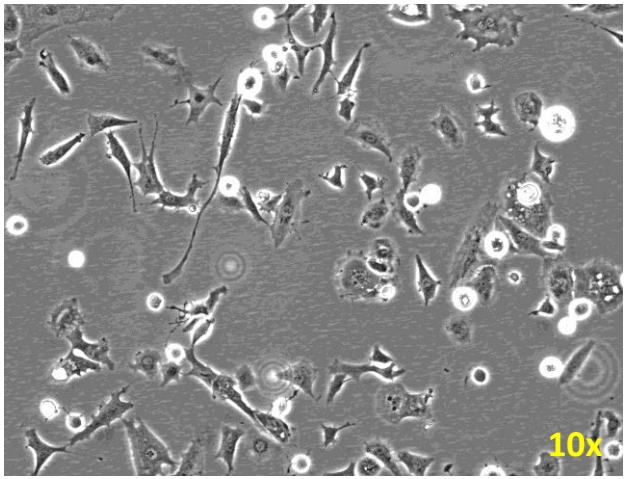
Media:
4:1 IMDM/RPMI + 10% FBS
+ Pen/strep
→Passage when ~80-90% confluent (1:3)
→Change media every 3 days

Phase Contrast Image Review

Cells were thawed into a T25 flask. Were still slightly sparse after 24 hours. They are visibly clear of contamination and grow well. Were ready to expand in 6 days.



24 Hours Post Thaw (10/08/2014)



48 Hours After Thaw (10/09/2014)

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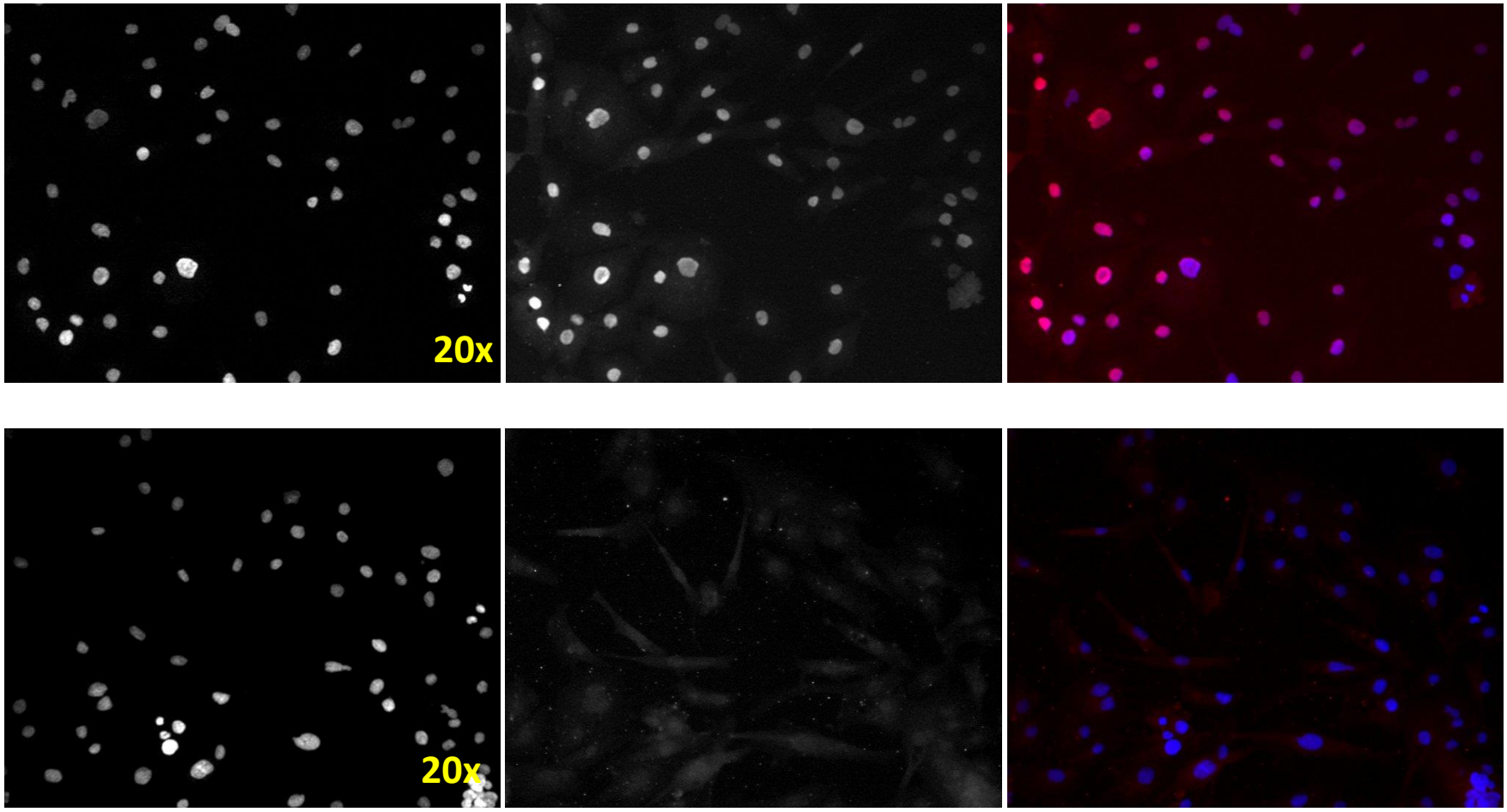
Cell Line Immunofluorescence Validation

U-CH7 p.49 versus Secondary Only Control



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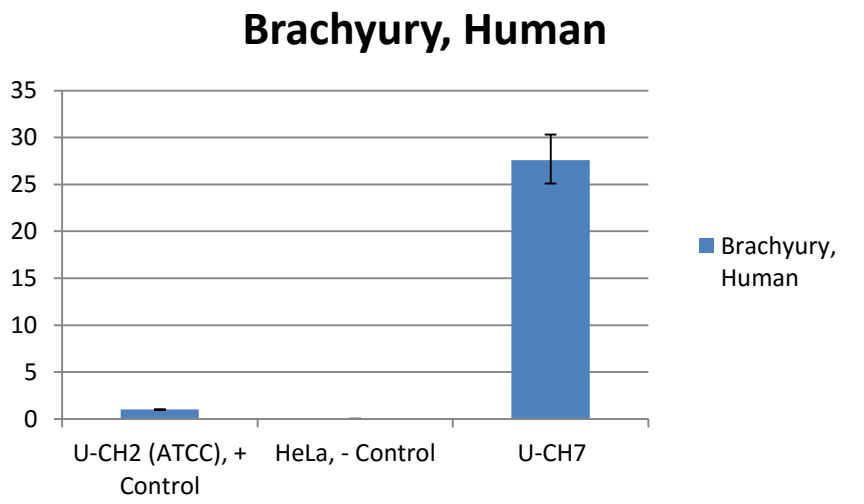
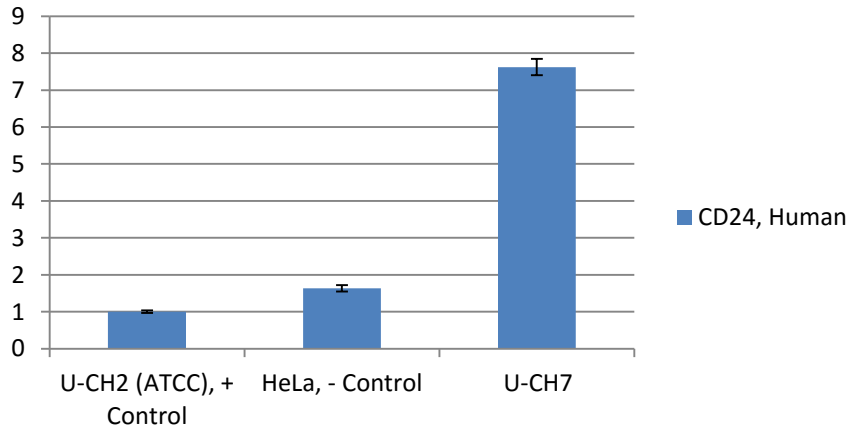
Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in U-CH7 cell line

Sample Name	Brachyury, Human	Neg Error	Pos Error
U-CH2 (ATCC), + Control	1	0.0254	0.0261
HeLa, - Control	0.0122	0.0008	0.0009
U-CH7	27.5778	2.4957	2.7440



Sample	CD24, Homo sapiens	Neg. Error	Pos. Error
U-CH2 (ATCC), + Control	1	0.0315	0.0326
HeLa, - Control	1.6318	0.0861	0.0908
U-CH7	7.6177	0.2172	0.2236



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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

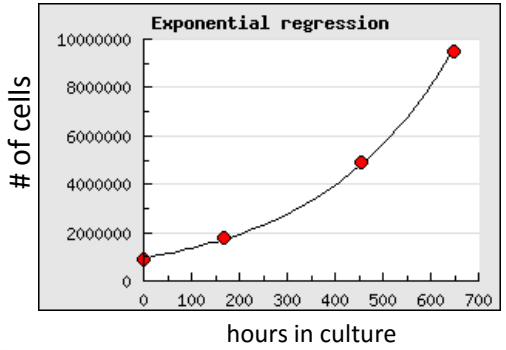
Results summary report of U-CH7

TEST	SPECIFICATION	RESULTS
Cell Growth	Immortilized	Doubling time = 8 days
STR Analysis	Human, unique	Pass
IF Validation	Signal in nucleus	Pass
PCR Validation	Expressing Brachyury and CD24	Pass

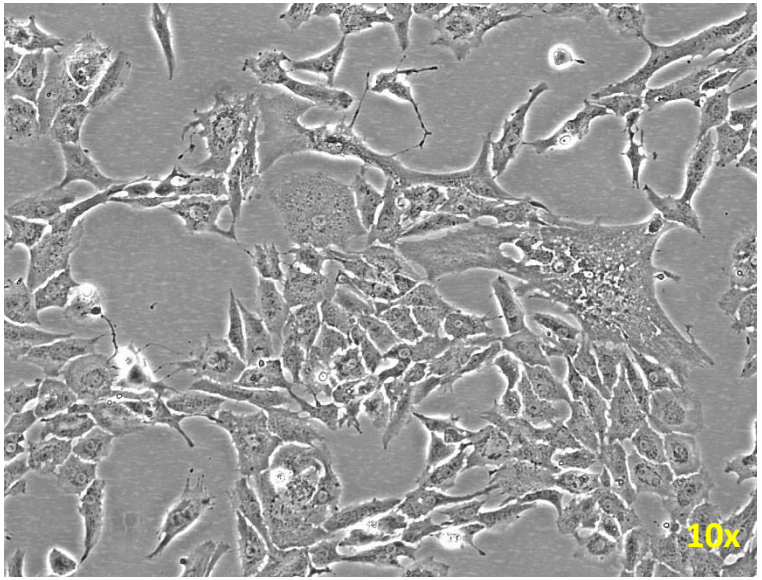
Cell lot generated

Stock Lot#	EB1018-003
Cells per vial	$5.0x10^5$
Lot Viability	98%
Passages	p. 56

Cell Line Growth: Cell doubling time= 8 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-CH7 Vala cell lot EB1018-003 7 days after viability thaw

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