Chordoma Foundation Cell Line Validation

U-CH17P

Cell Line Phenotype and Expression Analysis Report

June 19, 2017

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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)	$1.60x10^6$	80%

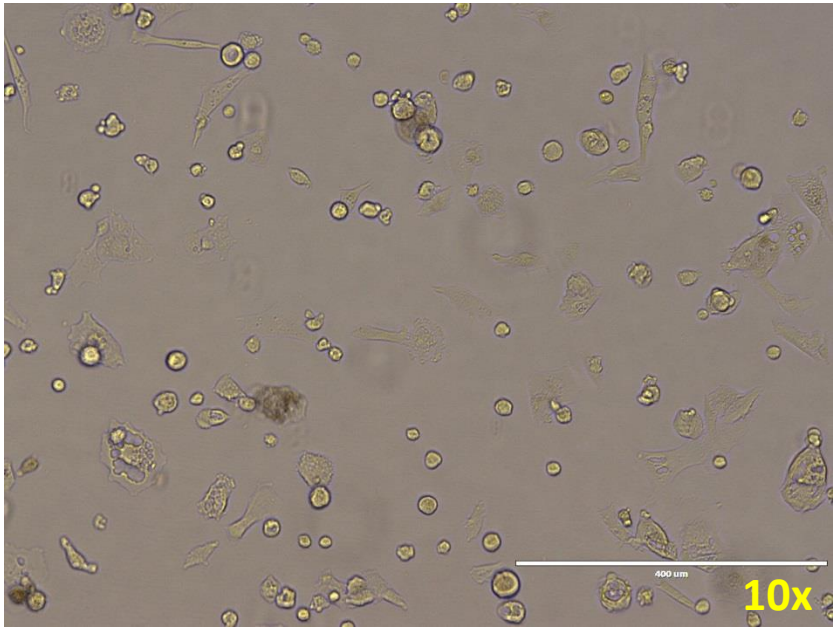
Growth Conditions

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-CH17P 24hrs post thaw.

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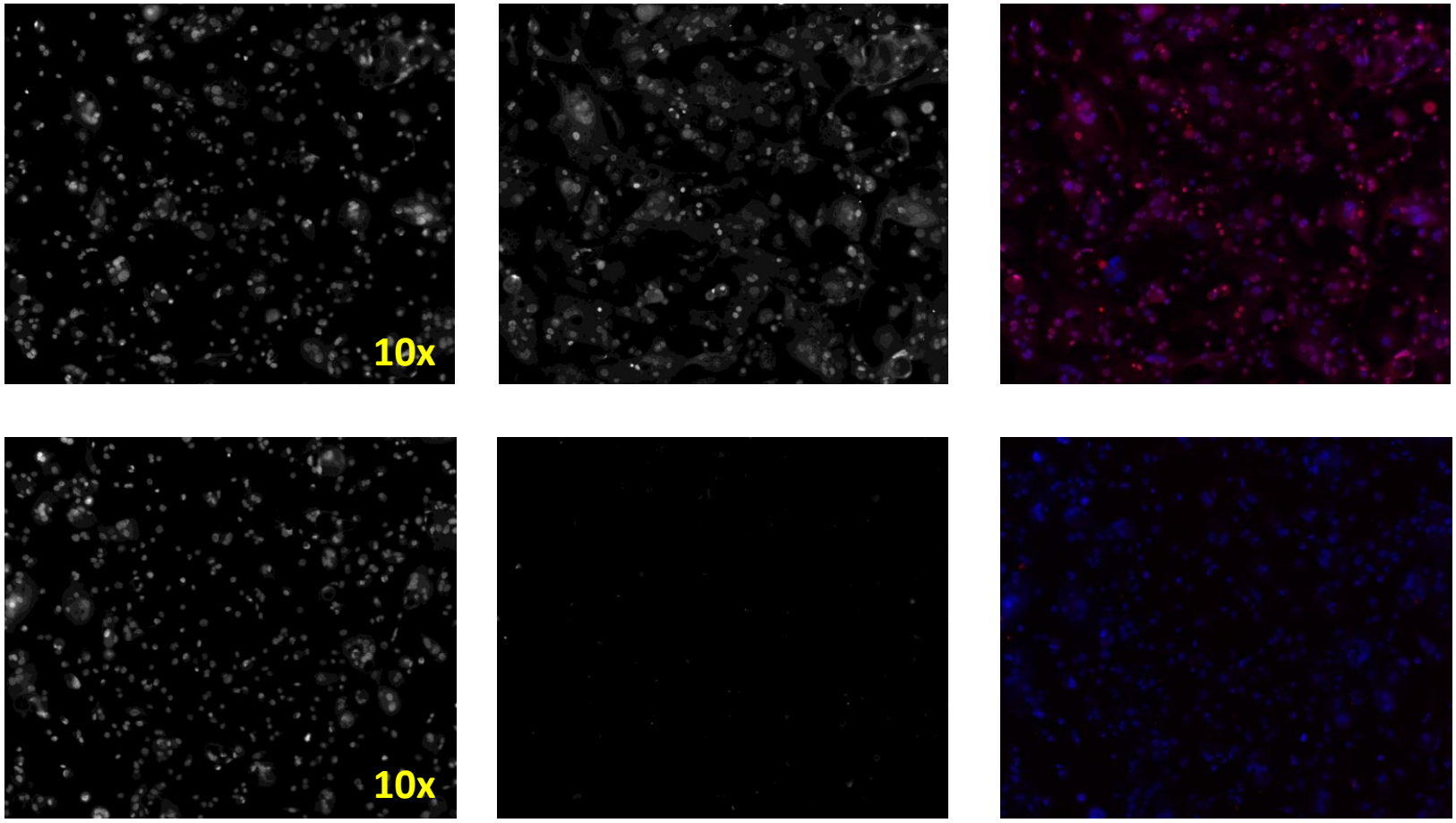
Cell Line Immunofluorescence Validation

U-CH17P Anti-Brachyury versus Secondary-only Negative Control



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Cell Line Immunofluorescence Validation

U-CH17P Anti-CD24 versus Secondary-only Negative Control

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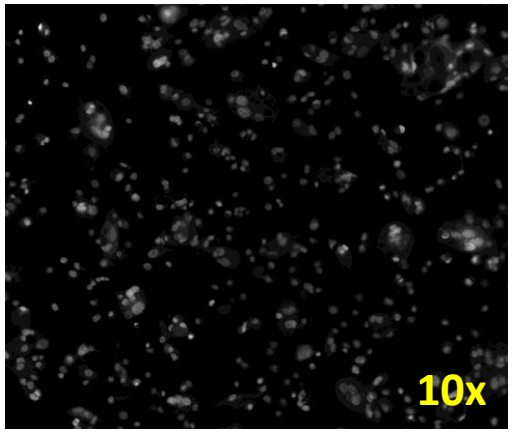
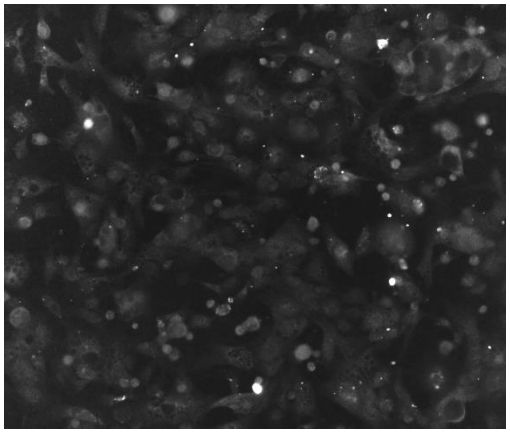
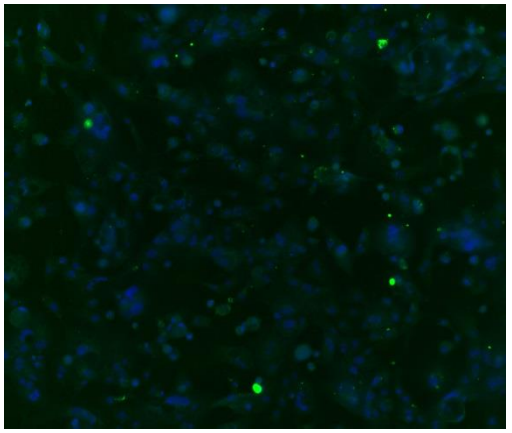
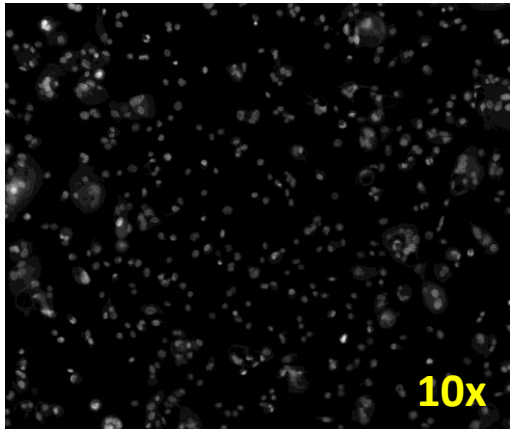
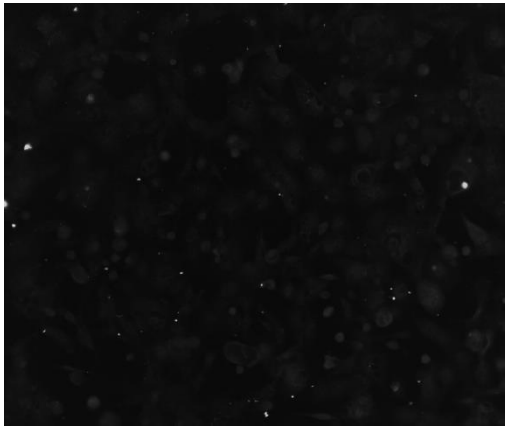


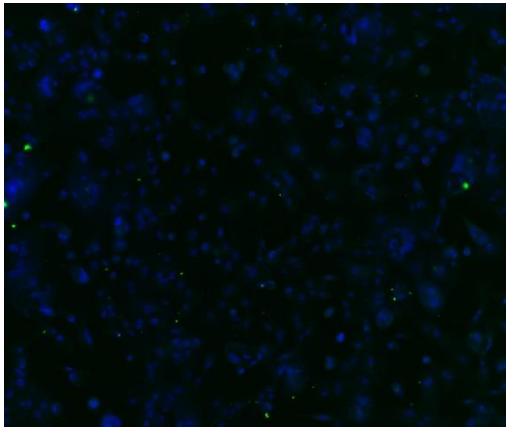




U-CH17P 2° Only Control







Hoechst Nuclear Stain | CD24 | Color Composite

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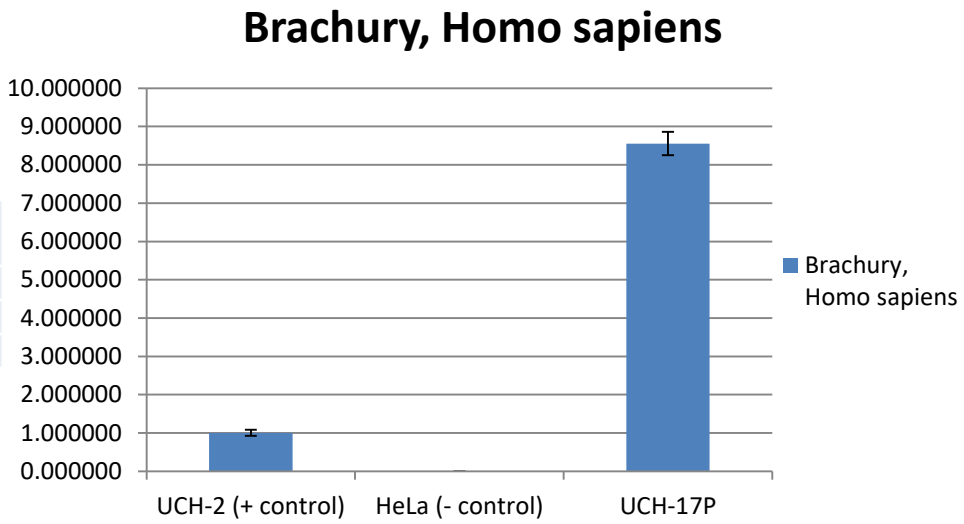
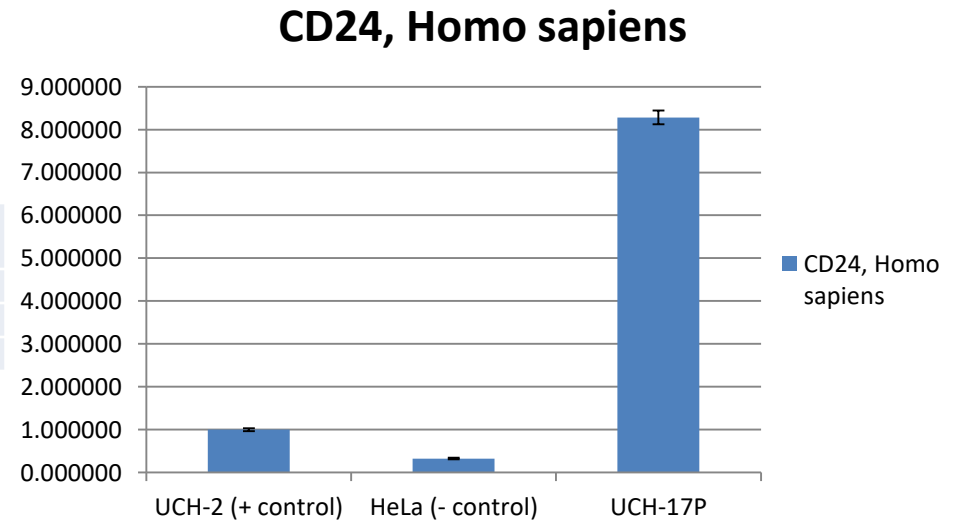
Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in U-CH17P cell line

Sample	Brachury, Homo sapiens	Neg. Error	Pos. Error
UCH-2 (+ control)	1.000000	0.076062	0.082324
HeLa (- control)	0.000820	0.000052	0.000055
UCH-17P	8.550729	0.303171	0.314315



Sample	CD24, Homo sapiens	Neg. Error	Pos. Error
UCH-2 (+ control)	1.000000	0.032074	0.033137
HeLa (- control)	0.326105	0.017748	0.018769
UCH-17P	8.282120	0.160858	0.164044



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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.

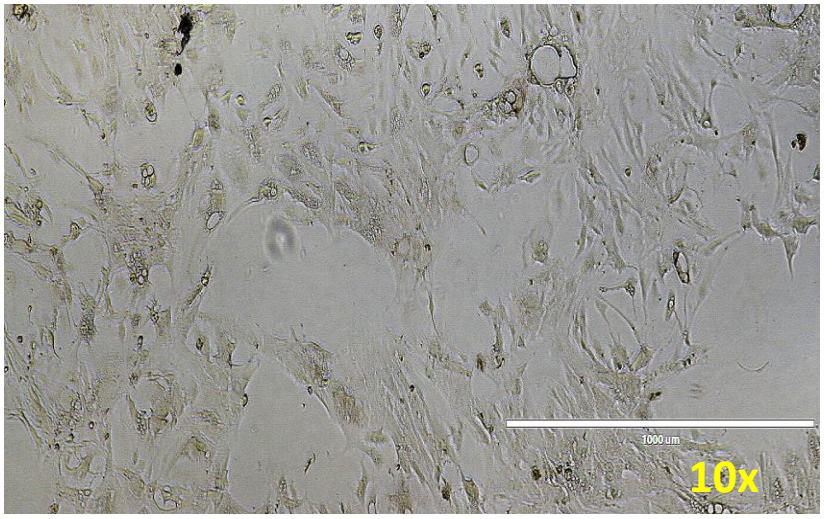
Cell Line Validation Results

Results summary report of U-CH17P

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

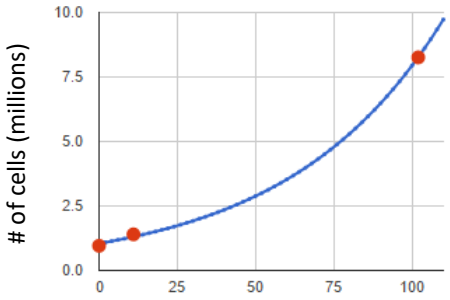
Cell lot generated

Stock Lot#	1027-037
Cells per vial	500K
Lot Viability	94%
Passages	7



U-CH17P, lot#1027-037

Cell Line Growth: Cell doubling time= 34 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.

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