Chordoma Foundation Cell Line Validation

U-CH17S

Cell Line Phenotype and Expression Analysis Report

April 6, 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),	8.47×10^5	60%

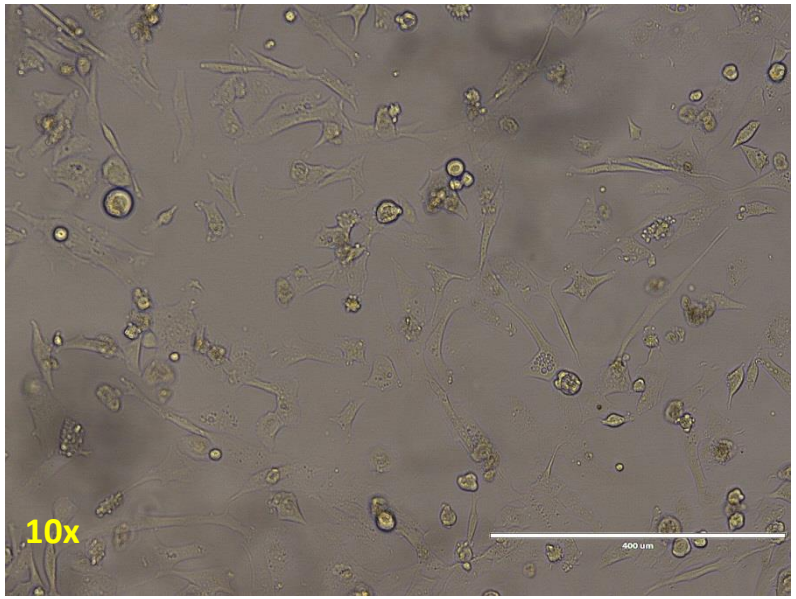
Growth Conditions

Media:

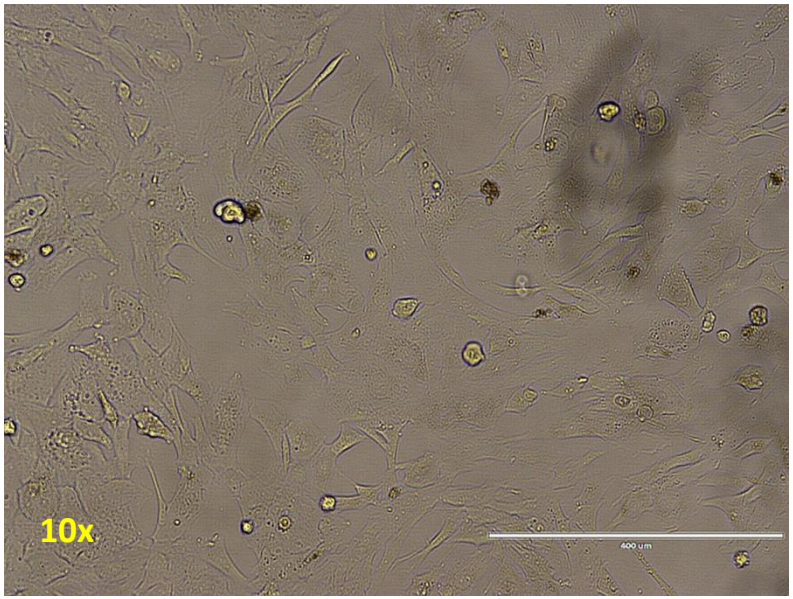
4:1 IMDM/RPMI + 10% FBS + l-Glutamine + Pen/Strep

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

→Change media every 5-7 days



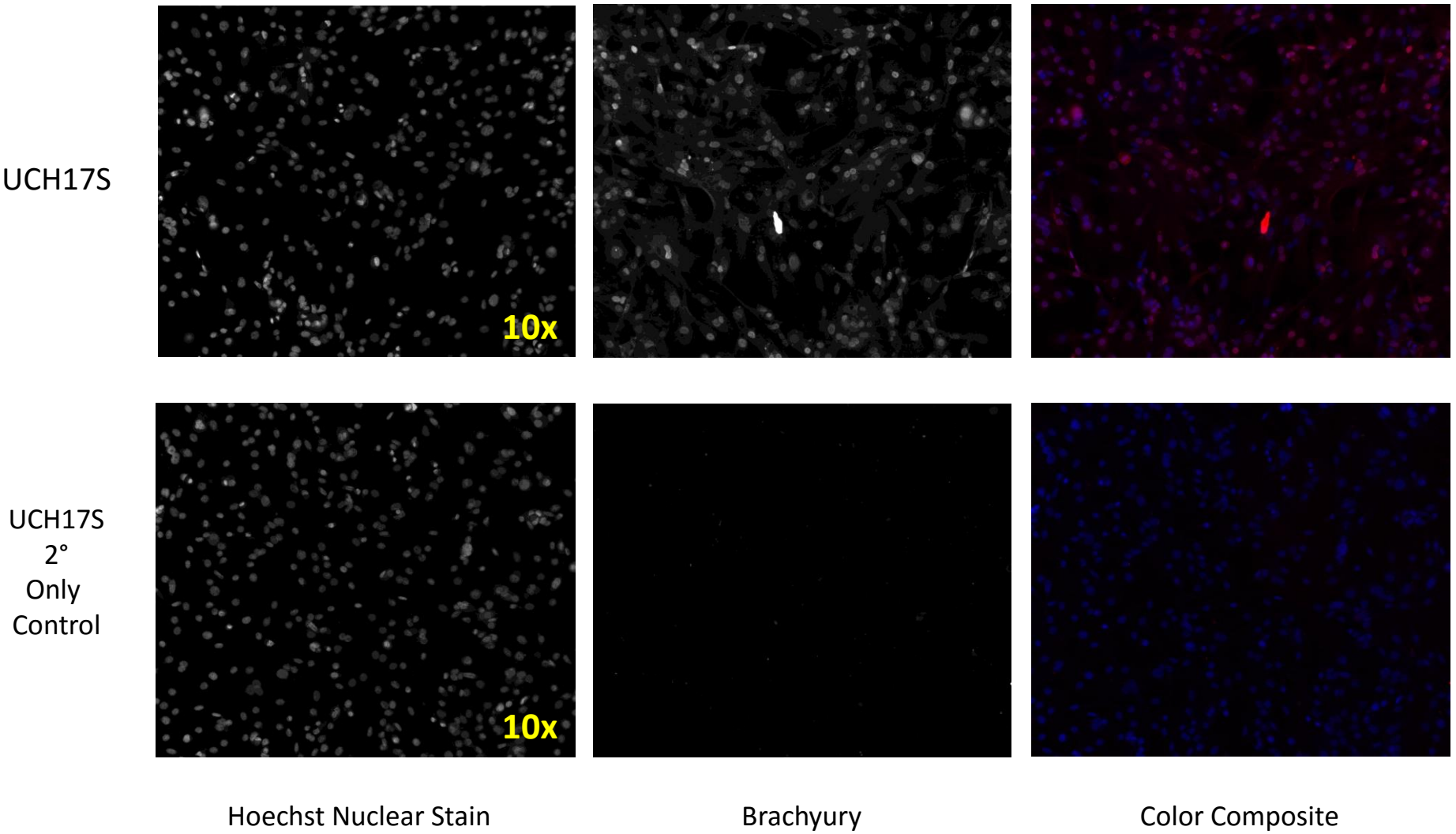
UCH17S 24 hours post thaw into a tissue culture treated T25 flask.



UCH17S 72 hours post thaw into a tissue culture treated T25 flask.

Cell Line Immunofluorescence Validation

UCH17S p.(1) Anti-Brachyury versus Secondary-only Negative Control



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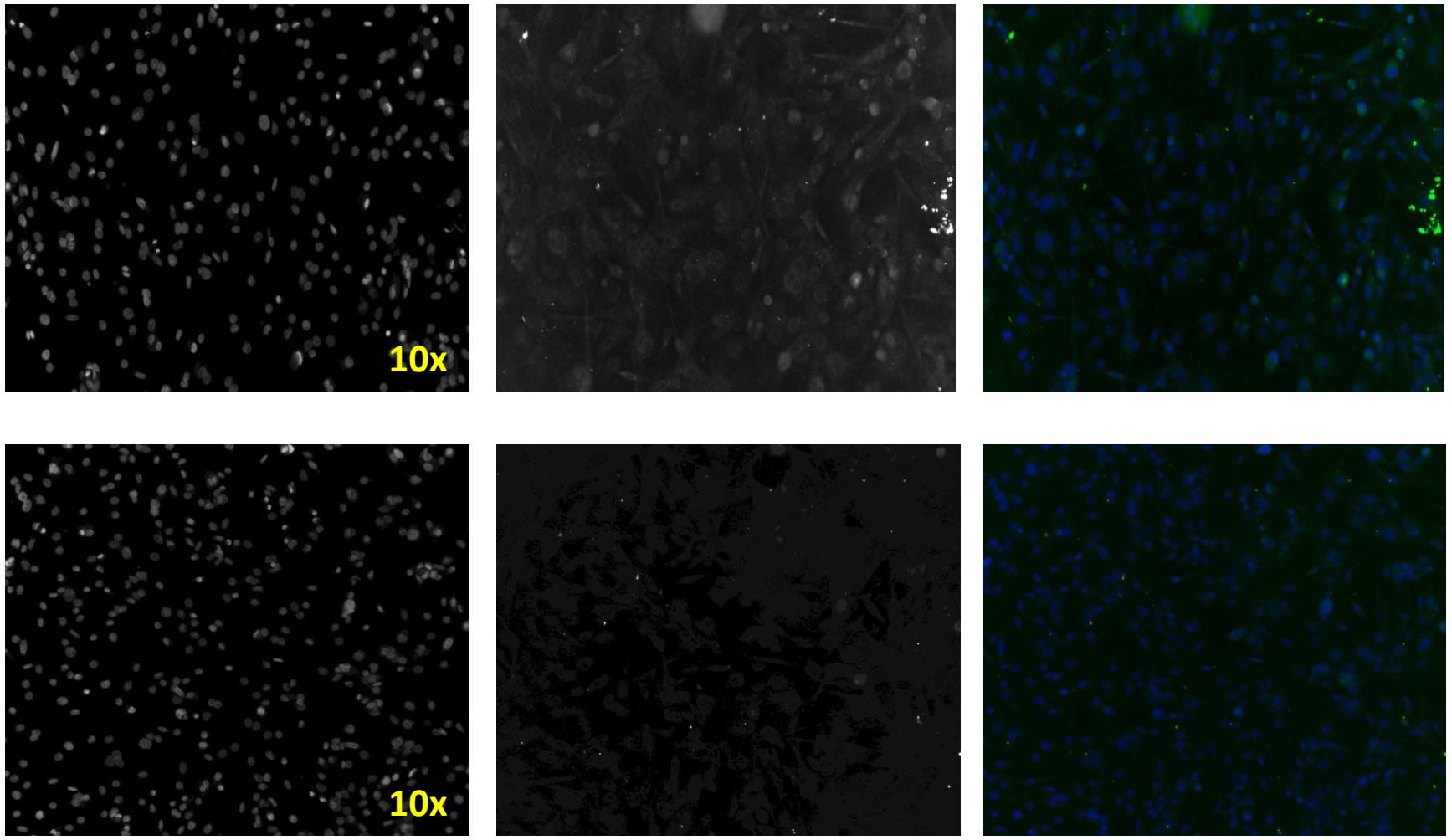
Cell Line Immunofluorescence Validation

UCH17S p.(1) Anti-CD24 versus Secondary-only Negative Control



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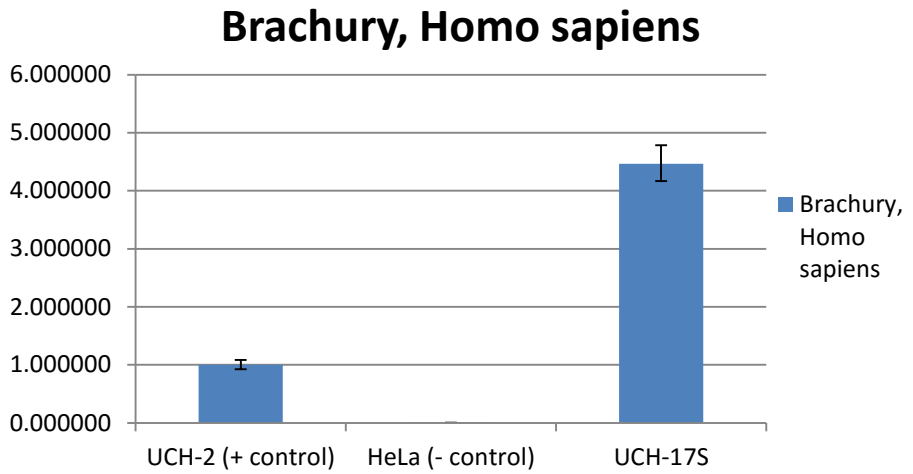
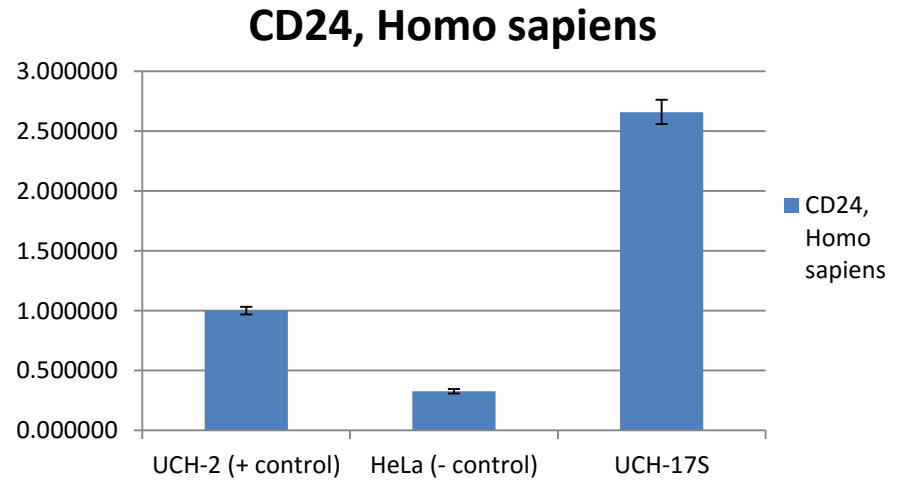
Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in UCH17S 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-17S	4.464586	0.297671	0.318936



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-17S	2.658127	0.100607	0.104565



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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

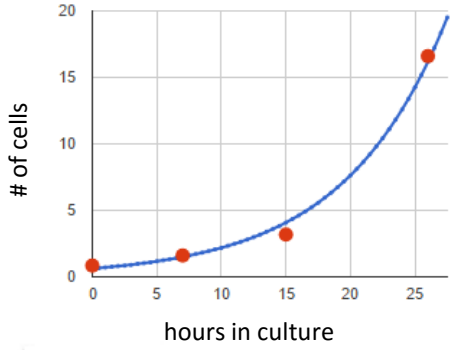
Results summary report of UCH17S

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

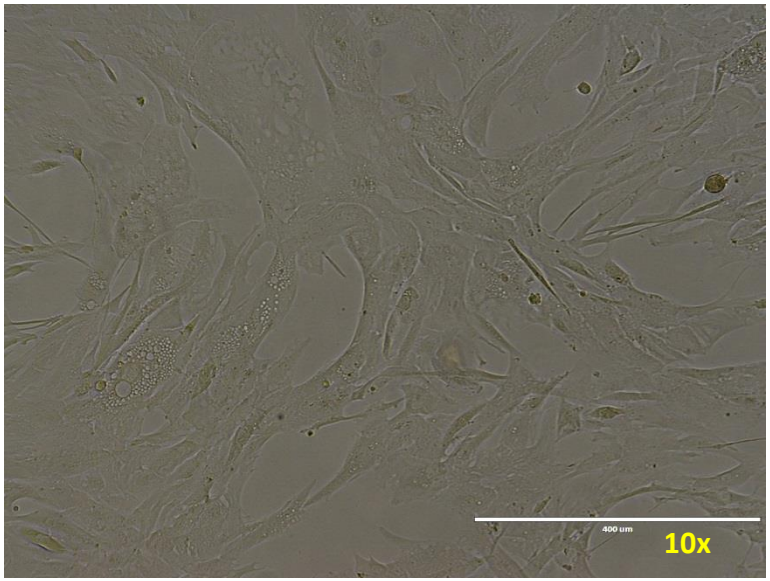
Cell lot generated

Stock Lot#	1022-185
Cells per vial	650k
Lot Viability	92%
Passages	p.5

Cell Line Growth: Cell doubling time= 5-7 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.



UCH17S Thaw of lot Vala 1022-185

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