Chordoma Foundation Cell Line Validation

UM-Chor5

Cell Line Phenotype and Expression Analysis Report

April 20, 2018

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Cell Line Receiving

Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability
Live Cells	March 13, 2018	N/A	2	17	n/a	n/a

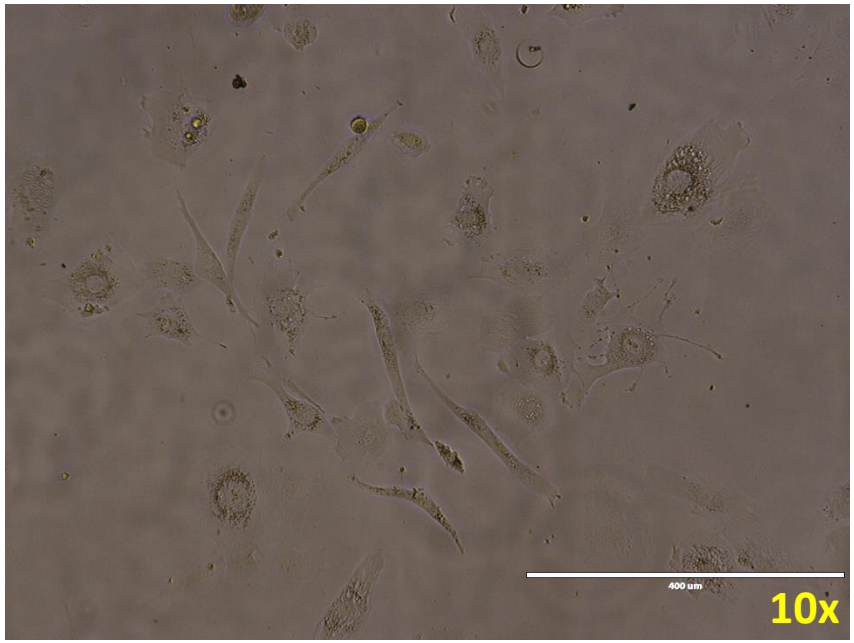
Growth Conditions

Media:

4:1 IMDM/RPMI + 20% FBS + 1X Non Essential Amino Acids + Pen/Strep + 1X Anti-anti

→Passage when ~80-90% confluent

→Change media every 2-3 days



UM-Chor5 arrival, live in T25.

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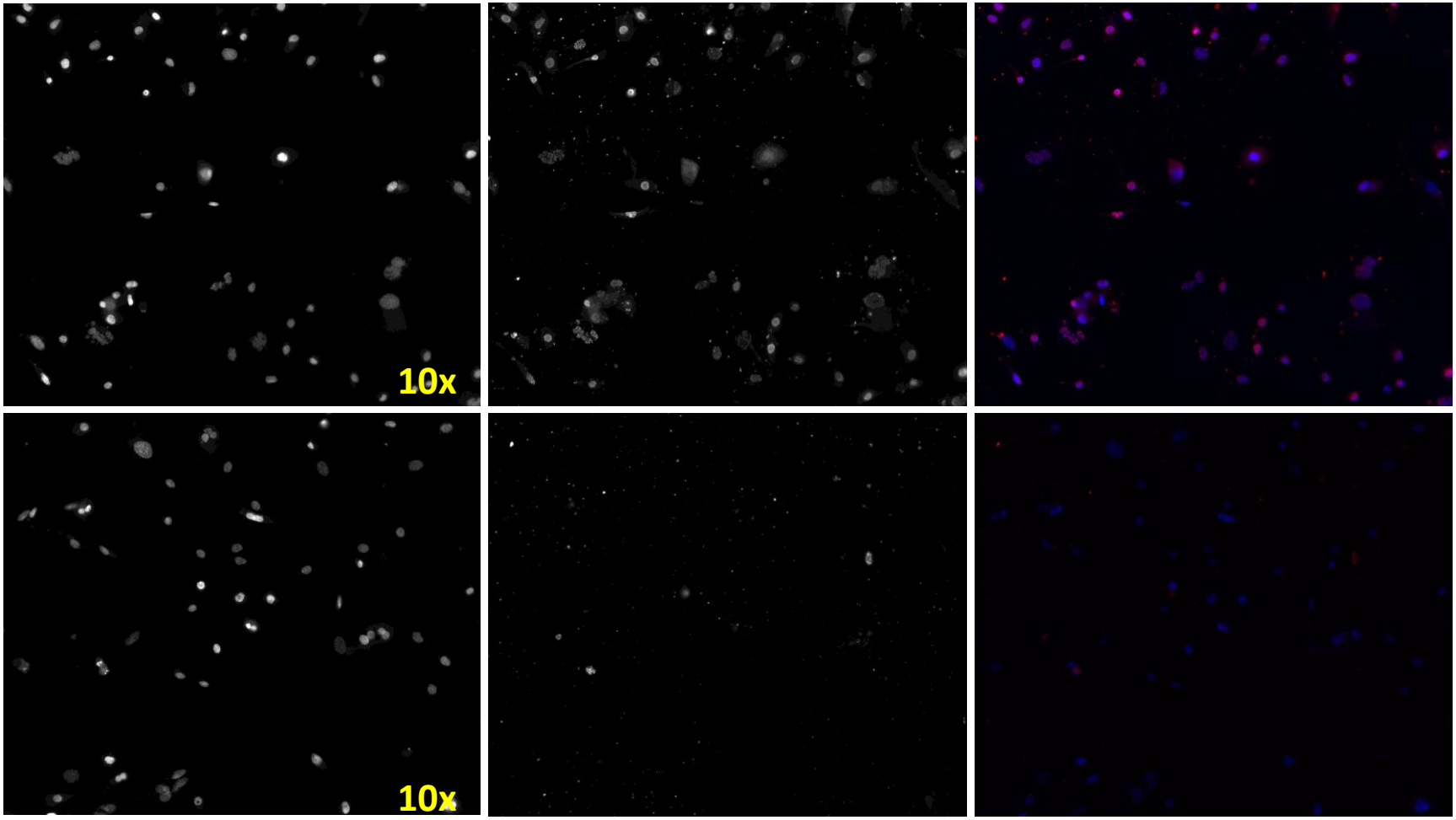
Cell Line Immunofluorescence Validation

UM-Chor5 Anti-Brachyury versus Secondary-only Negative Control



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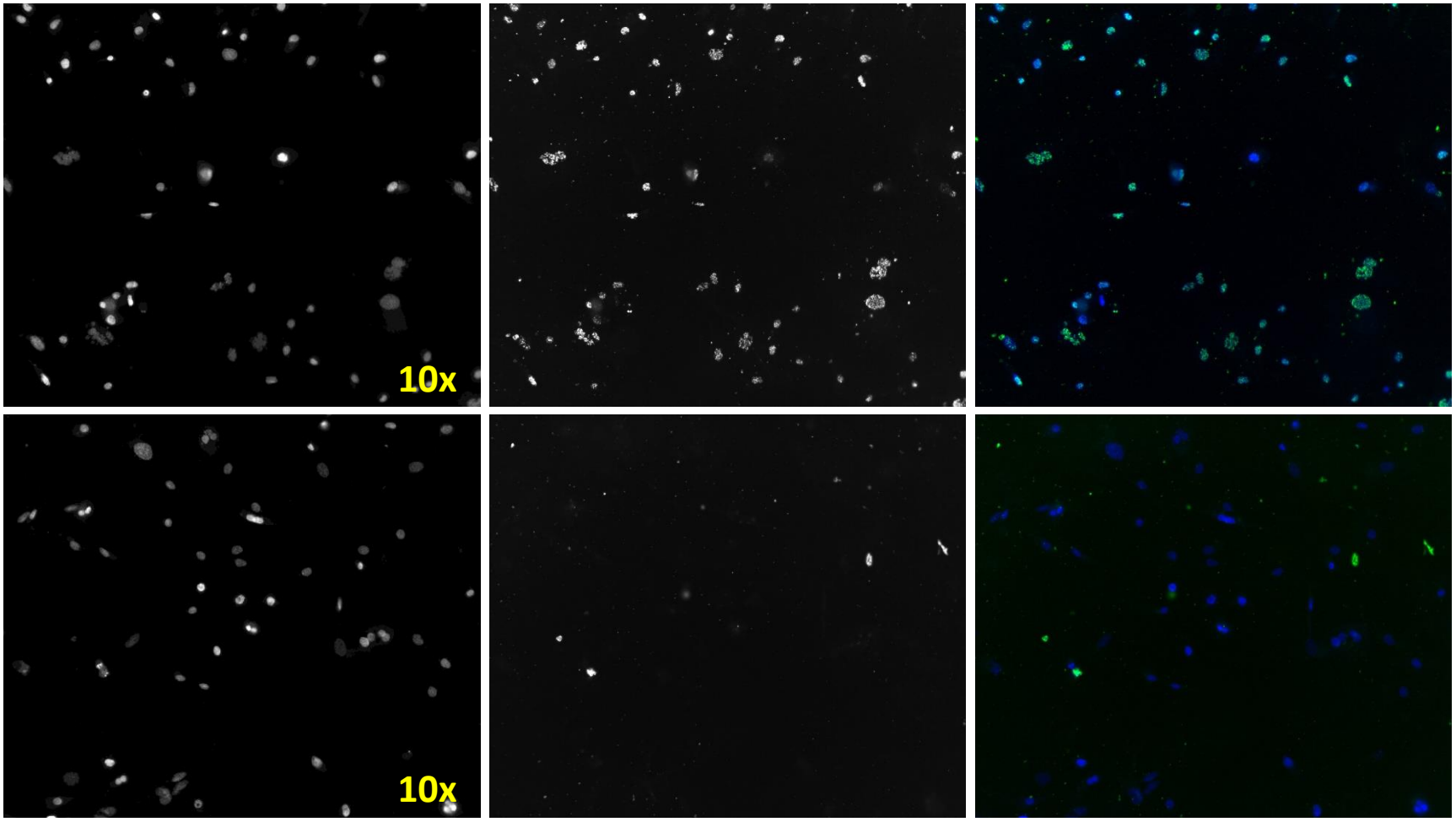
Cell Line Immunofluorescence Validation

UM-Chor5 Anti-CD24 versus Secondary-only Negative Control



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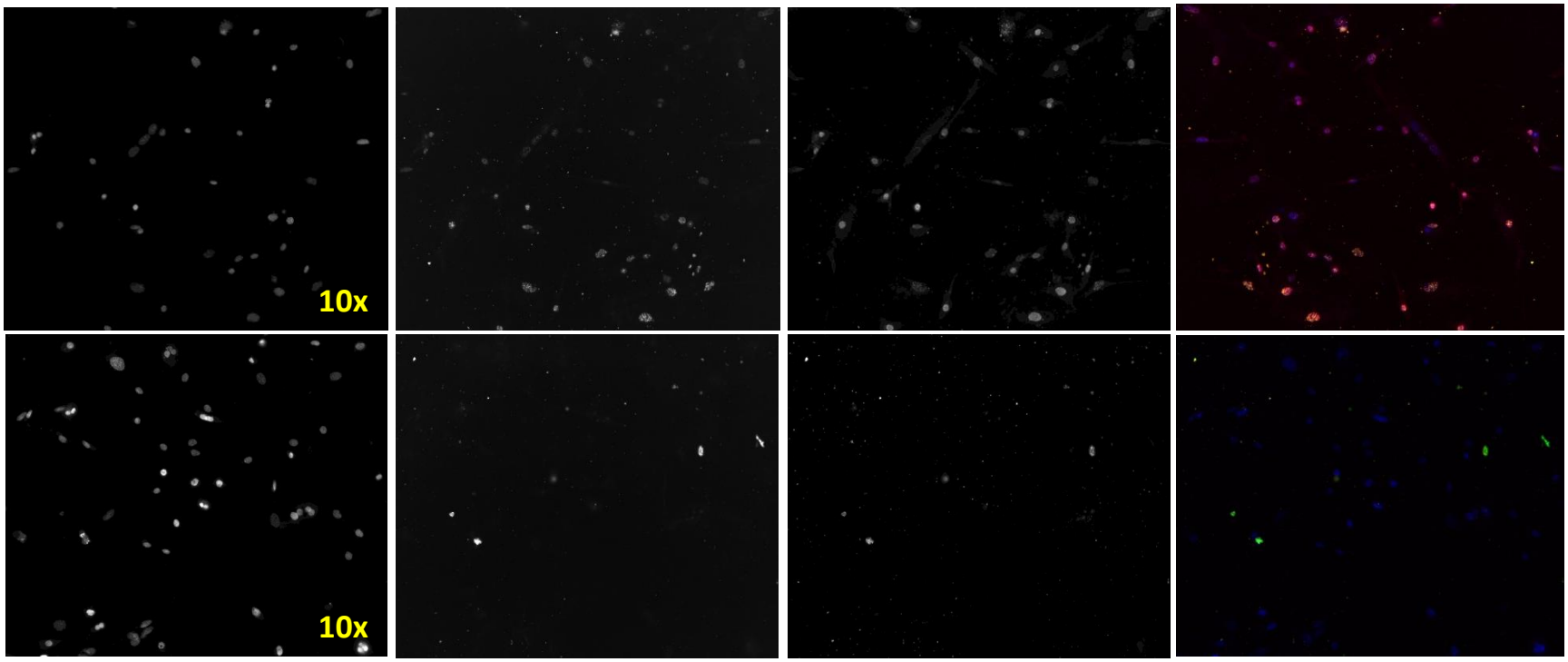
Cell Line Immunofluorescence Validation

UM-Chor5 Anti-Ini1 versus Secondary-only Negative Control



Comments:

Ini1 signal is positive and nuclear.

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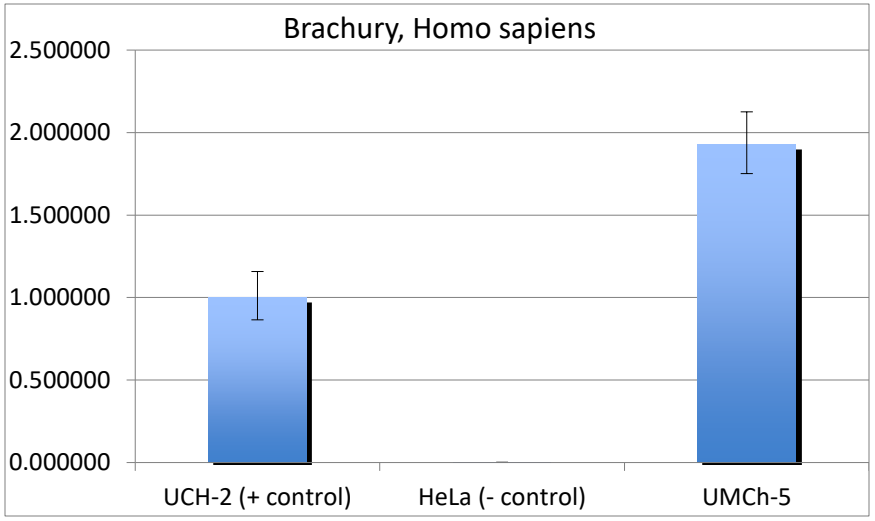
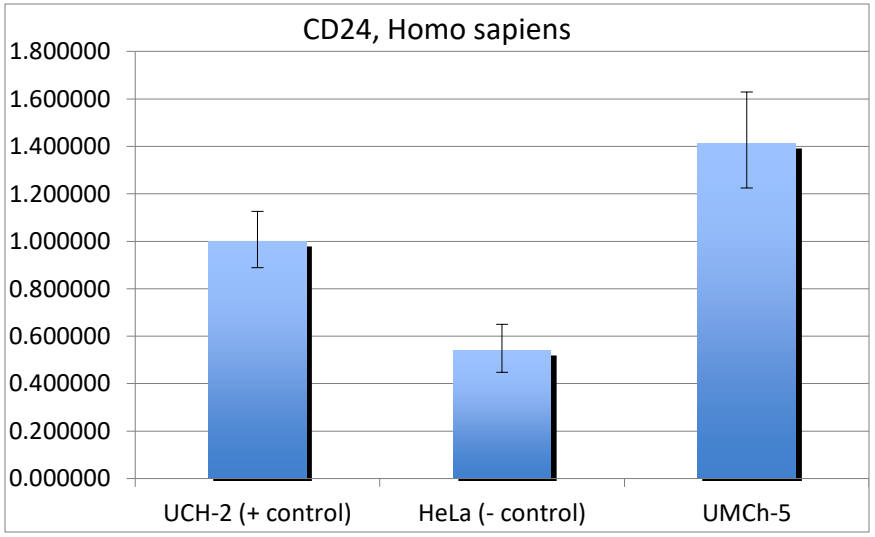
Cell Line PCR Validation

Relative quantification of Brachyury and CD24 gene in UM-Chor5 cell line

Sample	Brachury, Homo sapiens	Neg. Error	Pos. Error
UCH-2 (+ control)	1.000000	0.135689	0.156991
HeLa (- control)	0.001345	0.000168	0.000192
UMCh-5	1.929321	0.177805	0.195855



Sample	CD24, Homo sapiens	Neg. Error	Pos. Error
UCH-2 (+ control)	1.000000	0.111536	0.125538
HeLa (- control)	0.539107	0.091686	0.110475
UMCh-5	1.412872	0.187716	0.216477



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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

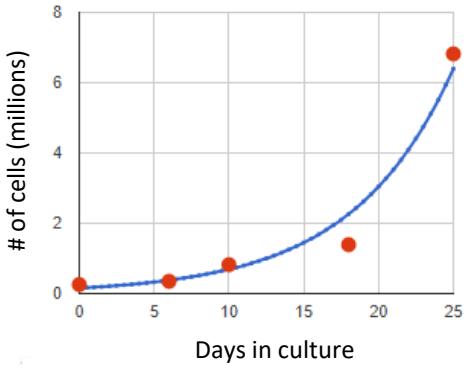
Results summary report of UM-Chor5

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

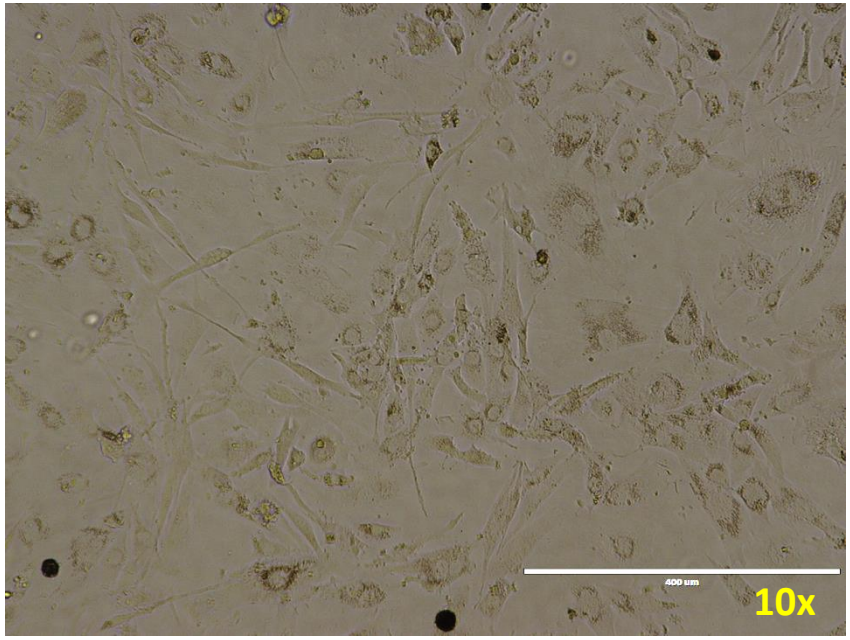
Cell lot generated

Stock Lot#	1027-0131
Cells per vial	300K
Lot Viability	96%
Passages	21

Cell Line Growth: Cell doubling time= 96 hours



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.



UM-Chor5, lot#1027-131

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