

# Total mRNA Quantification in Single Cells: Sarcoma Cell Heterogeneity

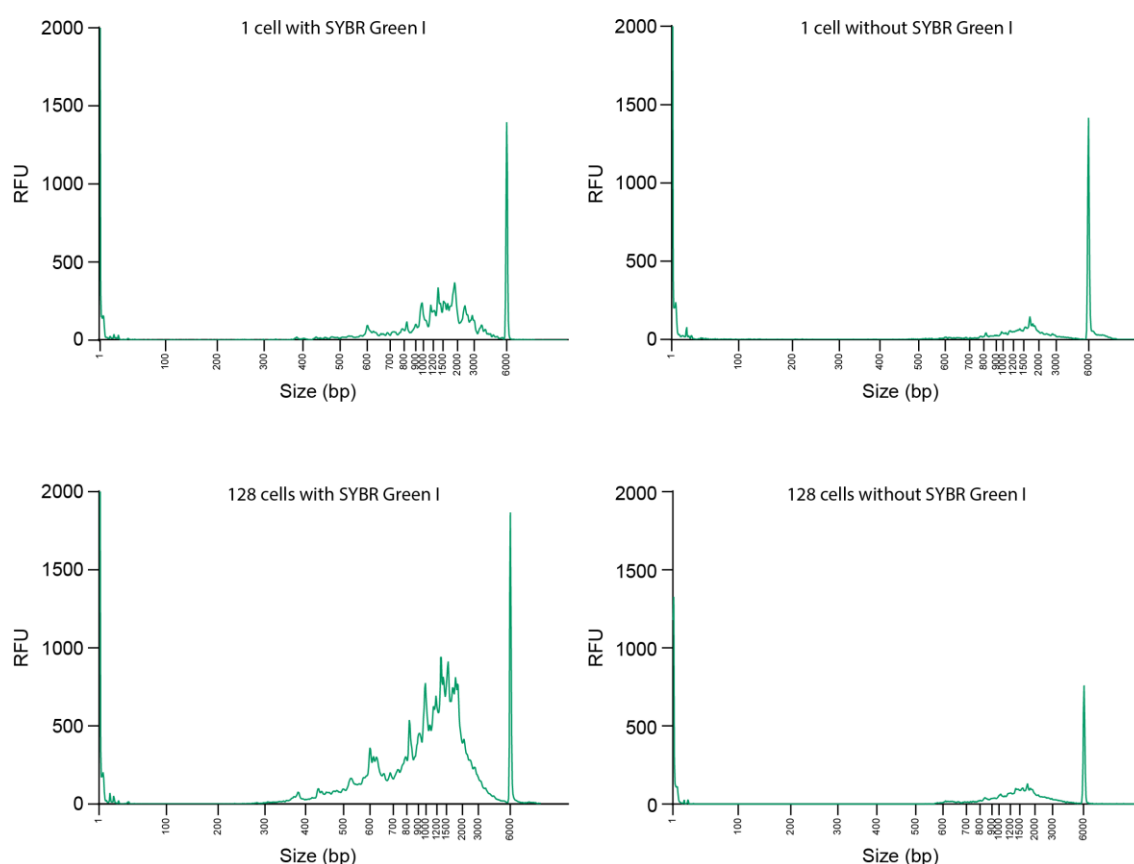
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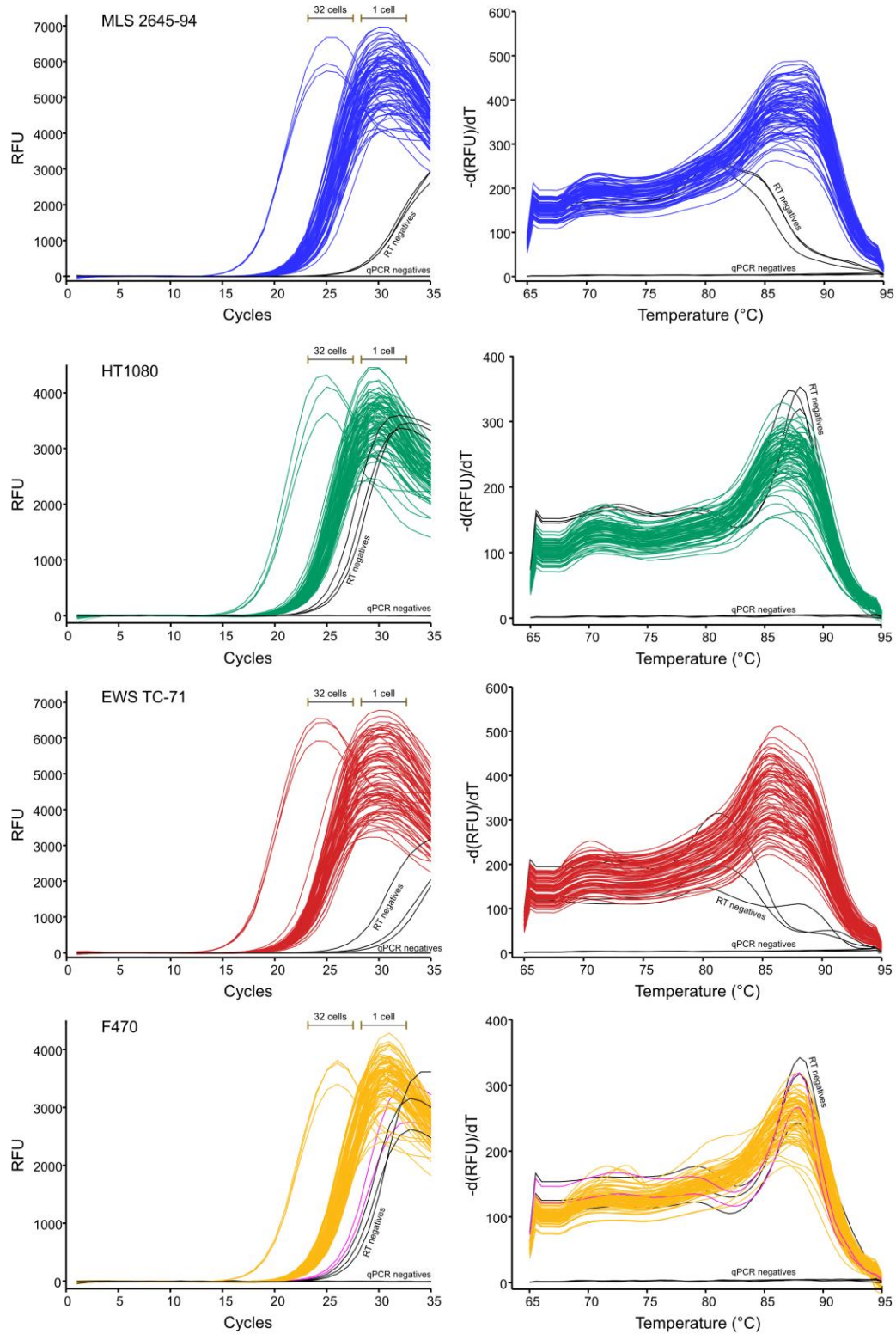
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**Figure S1.** Size distribution assessment of preamplified cDNA. Size distribution analysis using Fragment Analyzer analysis of purified cDNA, preamplified for a limited number of cycles with and without SYBR Green I added to the reaction. Representative electropherograms for 128 cells and single cells are shown. RFU, Relative fluorescence unit.



**Figure S2.** Preamplification and melting curves. **(A)** Preamplification curves of cDNA generated from myxoid liposarcoma (MLS) 2645-94, HT1080, Ewing sarcoma (EWS) TC-71 and F470. Single cells ( $n = 79-81$ ), 32 cells ( $n = 3$ ), cell-free negatives, *i.e.* reverse transcription negatives and qPCR negatives ( $n = 3$  each). The two samples in magenta were interpreted as empty wells or apoptotic/necrotic cells. **(B)** Melting curves of preamplified cDNA from MLS 2645-94, HT1080, EWS TC-71 and F470. Single cells ( $n = 79-81$ ), 32 cells ( $n = 3$ ), cell-free negatives, *i.e.* reverse transcription negatives and qPCR negatives ( $n = 3$  each). The two samples in magenta were interpreted as empty wells or apoptotic/necrotic cells. RFU, Relative fluorescence unit; T, Temperature.

**Table S1.** Pairwise statistical comparisons of relative expression levels between cell lines. Statistical tests were performed with Tukey's multiple comparison test.

<b>Single cells</b>	<b>Mean difference</b>	<b>Adjusted P value</b>
MLS 2645-94 vs. HT1080	0.09	0.67
MLS 2645-94 vs. EWS TC-71	-0.49	<0.01
MLS 2645-94 vs. F470	0.73	<0.01
HT1080 vs. EWS TC-71	-0.58	<0.01
HT1080 vs. F470	0.64	<0.01
EWS TC-71 vs. F470	1.22	<0.01
<b>32 cells</b>	<b>Mean difference</b>	<b>Adjusted P value</b>
MLS 2645-94 vs. HT1080	0.85	<0.01
MLS 2645-94 vs. EWS TC-71	-0.55	<0.01
MLS 2645-94 vs. F470	1.40	<0.01
HT1080 vs. EWS TC-71	-1.40	<0.01
HT1080 vs. F470	0.54	<0.01
EWS TC-71 vs. F470	1.95	<0.01