

Titration Validation - Spike-in qPCR

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To validate the volumetric mixing of the two-sample titration ERCC plasmids were spiked into the unmixed pre- and post-exposure samples. The plasmids were quantified using qPCR. The qPCR assay standard curves had a high level of precision with R^2 values close to 1 and amplification efficiencies between 0.84 and 0.9 for all standard curves (Table 1). The qPCR assays targeting the ERCCs spiked into the post-exposure samples had R^2 values and slope estimates close to 1 (Table 1). The expected slope is 1, for a doubling every cycle. Slope estimates less than 1 were attributed to the assay standard curve amplification factors being less than 2 (Table 1). The 1-4 titration factor samples had Ct values consistently above the regression line (Figure 1). For the pre-exposure ERCCs a regression line was fit to the log2 pre-exposure sample proportion for titrations 1-4 and the unmixed pre-exposure sample. The change in pre-exposure sample proportion between titrations 5, 10, and 15 ($0.03 - 3.05e^{-5}$) is too small for qPCR to accurately detect changes in ERCC spike-in concentration. For the ERCCs spiked into the pre-exposure samples the R^2 values were low, less than 0.6, with slope estimates between -1.5 and -2.1 (Table 1) when a regression line was fit to the Ct values and log2 Pre-exposure sample proportion 1, with a -1 expected slope. These results indicate that the unmixed pre- and post-exposure samples were volumetrically mixed according to the mixture design.

Table 1: ERCC Spike-in qPCR summary statistics. R^2 , Efficiency (E), and amplification factor (AF) for standard curves. R^2 and slope for titration qPCR results for the titration series.

Individual	Treatment	Std. R^2	E	AF	R^2	Slope
E01JH0004	Post	0.9996	86.19	1.86	0.98	0.92
E01JH0011	Post	0.9995	87.46	1.87	0.95	0.90
E01JH0016	Post	0.9991	87.33	1.87	0.95	0.84
E01JH0017	Post	0.9968	85.80	1.86	0.89	0.93
E01JH0038	Post	0.9984	86.69	1.87	0.95	0.94
E01JH0004	Pre	0.9972	84.36	1.84	0.53	-2.09
E01JH0011	Pre	0.9999	87.93	1.88	0.52	-1.56
E01JH0016	Pre	0.9990	84.22	1.84	0.60	-1.95
E01JH0017	Pre	0.9979	89.78	1.90	0.32	-1.66
E01JH0038	Pre	0.9994	84.30	1.84	0.21	-1.86

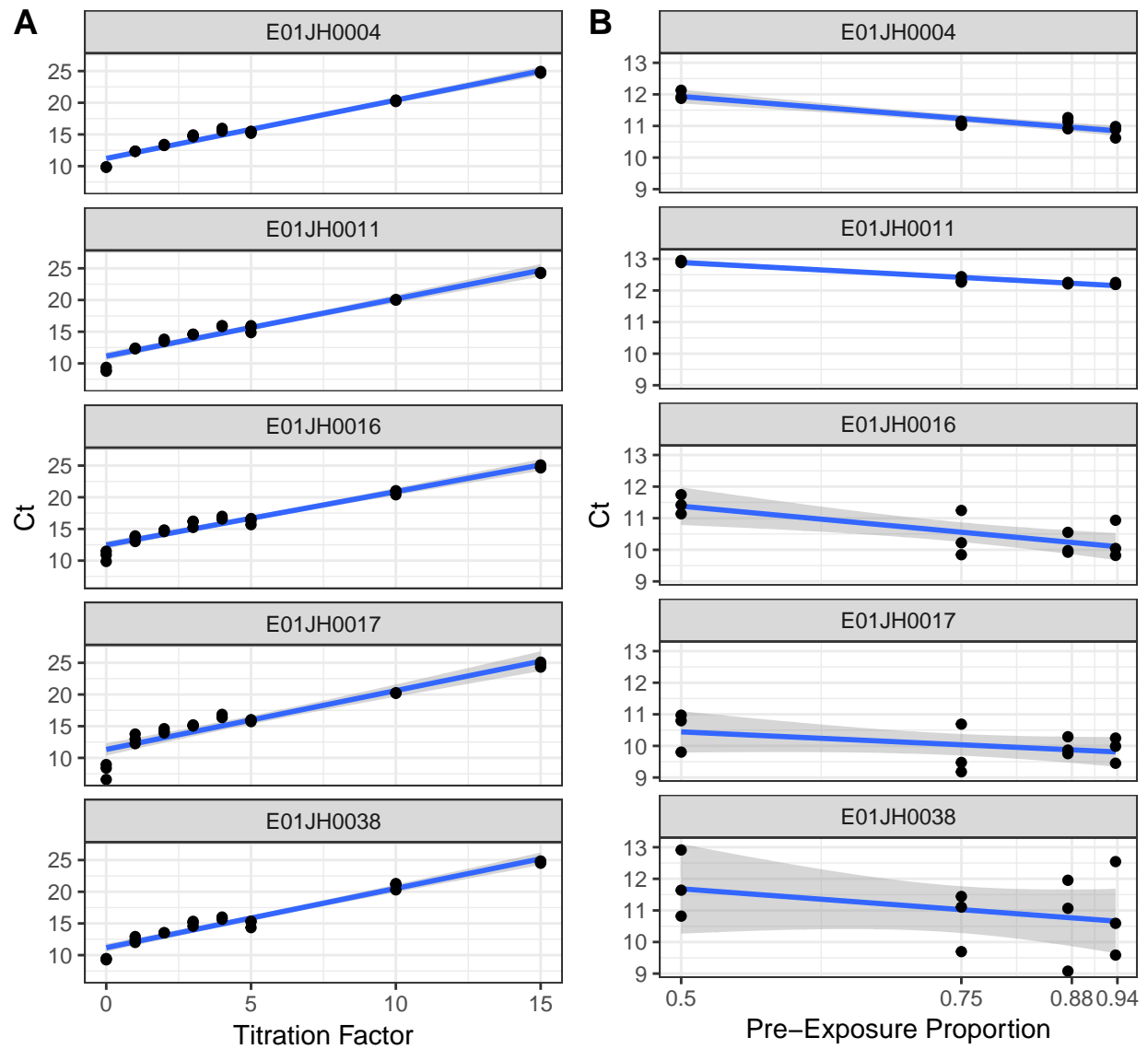


Figure 1: qPCR ERCC spike-in results for ERCC spiked into unmixed (A) Post-exposure samples and (B) Pre-exposure samples (titrations 1-4 only). X-axis is on a log2 scale with expected slope of 1 and -1 for Post-exposure and Pre-exposure spike-ins respectively.