



Supplementary Figure S4. Comparison of unmodified and 5'-o-TINA modified primer concentrations at three different annealing times and two different annealing temperatures (Ta). Unmodified and 5'-o-TINA modified primers were compared on the same plate. *A single standard with 14 copies per well was excluded from the data analysis, as the Cq was 44.1 compared to a mean Cq of 42.1 with a SD of 0.1. **Both triplicate measurements were excluded for efficiency calculation, as the Cq's were approximately 1.5 cycle lower than expected (highlighted in red).

Eff. summary	Unmodified		5' o-TINA modified	
	Ta (°C); ann. time (seconds)	Efficiency (%)	R ²	Efficiency (%)
66; 5	91.3	0.990	102.4	0.995
66; 10	106.9	0.994	107.0	0.998
66; 30	110.2	0.996	111.5	0.997
70; 5	53.2	0.973	103.5	0.992
70; 10	70.7	0.994	106.1	0.995
70; 30	95.8	0.996	110.9	0.996