Development of a Real-Time Polymerase Chain Reaction Method to Measure Ligation Efficiency

Jessica Qiu, Priscilla Chen, and Serena Lin
Department of Microbiology and Immunology, University of British Columbia

SUPPLEMENTAL MATERIAL

SUPPLEMENTARY TABLE 1 Raw Data from replicates used in the initial qPCR with oligonucleotide cohesive ends of 0%, 50%, and 100% CG content and controls.

Sample	Replicate Number	Quantification Cycle (Cq) Value	Mean Cq Value for Sample	Standard Error for Mean Cq Value	Melt Temperature (°C)
Control A1	1	*NS	**N/A	N/A	None
	2	NS			None
	3	NS			None
Control B1	1	NS	N/A	N/A	None
	2	NS			None
	3	NS			None
Control C1	1	NS	N/A	N/A	None
	2	NS			None
	3	NS			None
Control D	1	18.5	19.4	1.0	76.0
	2	18.2			76.0
	3	21.4			75.5
0% GC Content	1	NS	N/A	N/A	None
	2	NS			None
	3	NS			None
50% GC Content	1	NS	N/A	N/A	None
	2	NS			None
	3	NS			None
100% GC Content	1	NS	N/A	N/A	None
	2	NS			None
	3	NS			None

*NS: No Signal
**N/A: Not Applicable as some replicates contained no signal

SUPPLEMENTARY TABLE 2 Raw Data from replicates used in qPCR on modified oligonucleotide with controls.

Sample	Replicate Number	Quantification Cycle (Cq) Value	Mean Cq Value for Sample	Standard Error for Mean Cq Value	Melt Temperature (°C)
Control A2	1	*NS	**N/A	N/A	None
	2	NS			None
	3	NS			None
Control B2	1	17.6	N/A	N/A	81.0
	2	NS			None
	3	NS			None
Control B3	1	23.8	N/A	N/A	80.5
	2	29.1			None
	3	NS			None
Control C2	1	23.0	N/A	N/A	80.5
	2	NS			None
	3	28.3			80.0
Control C3	1	NS	N/A	N/A	None
	2	NS			None
	3	26.5			80.0
Control D	1	21.8	22.0	1.0	76.0
	2	23.8			75.5
	3	20.4			75.5
1 Hour Ligated DNA Sample	1	14.2	14.3	0.0	80.5
	2	14.4			80.5
	3	14.4			80.5

*NS: No Signal

**N/A: Not Applicable as some replicates contained no signal