Supplementary Material

Title: High resolution estimates of relative gene abundance with quantitative ratiometric regression PCR (qRR-PCR)

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Table S1. Residual sum squared (RSS) for univariate and bivariate fits

Values along the diagonal represent RSS values for univariate logistic fits while all other values represent RSS values for bivariate fits modeled from the logistic fits for the corresponding cycle “*x*” and cycle “*y*”.

	Cycle *y*										
Cycle *x*	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
0	34341										
1	9442	14646									
2	5671	5556	8308								
3	5309	5560	6075	6813							
4	5644	5938	6222	6311	6690						
5	5967	6197	6410	6439	6417	6762					
6	6812	6850	6975	7040	7049	7063	7680				
7	9046	8745	8729	8880	9036	9154	9107	10611			
8	273183	272874	274885	278612	283649	287826	289828	289380	299040		
9	600758	622958	620285	627573	642616	653847	657567	657551	658368	689742	
10	817713	828996	914228	895377	912971	935689	956006	980079	1002207	1028898	1103609

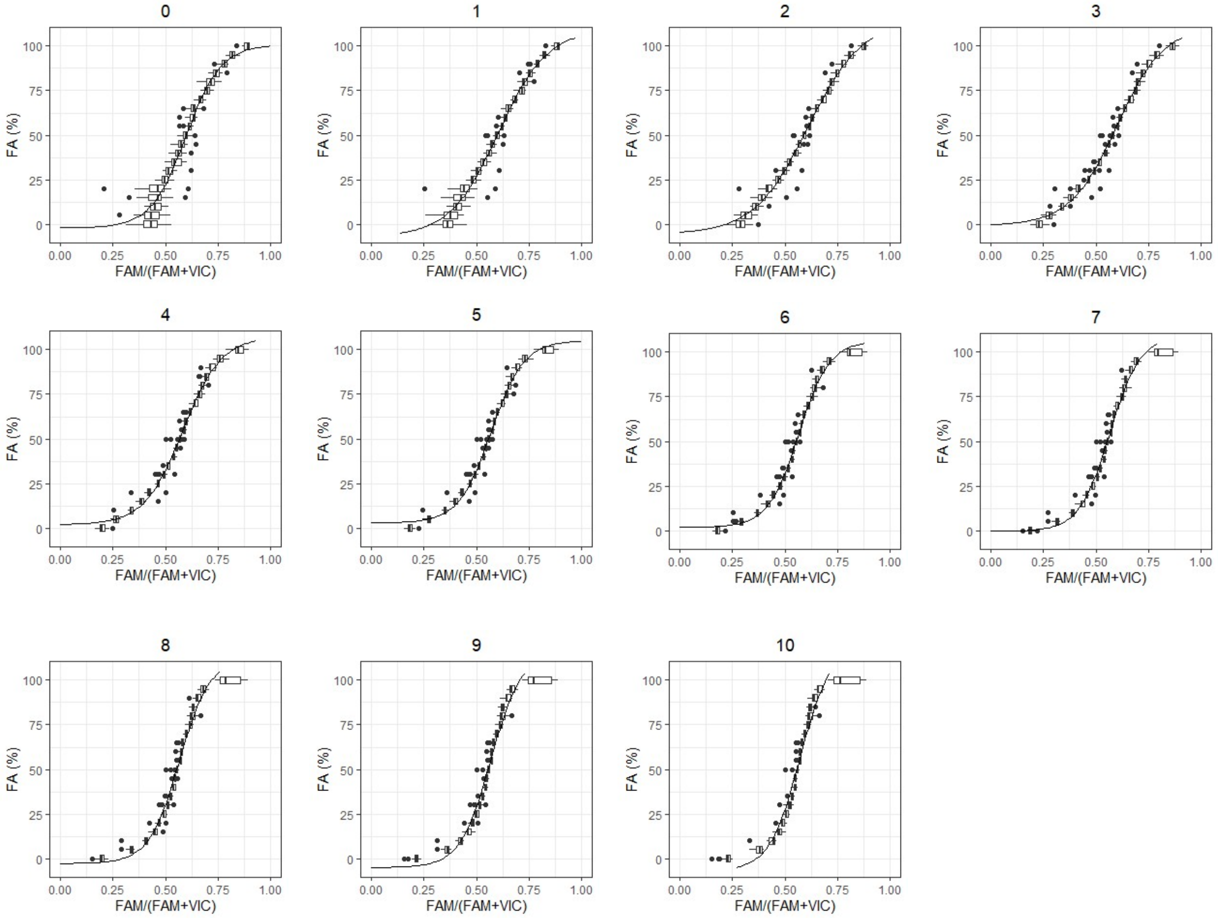

Figure S1. Univariate four parameter logistic fits. The number at the top of each plot represents the cycle after the VIC Ct from which the fluorescence ratio data was extracted for the fits.

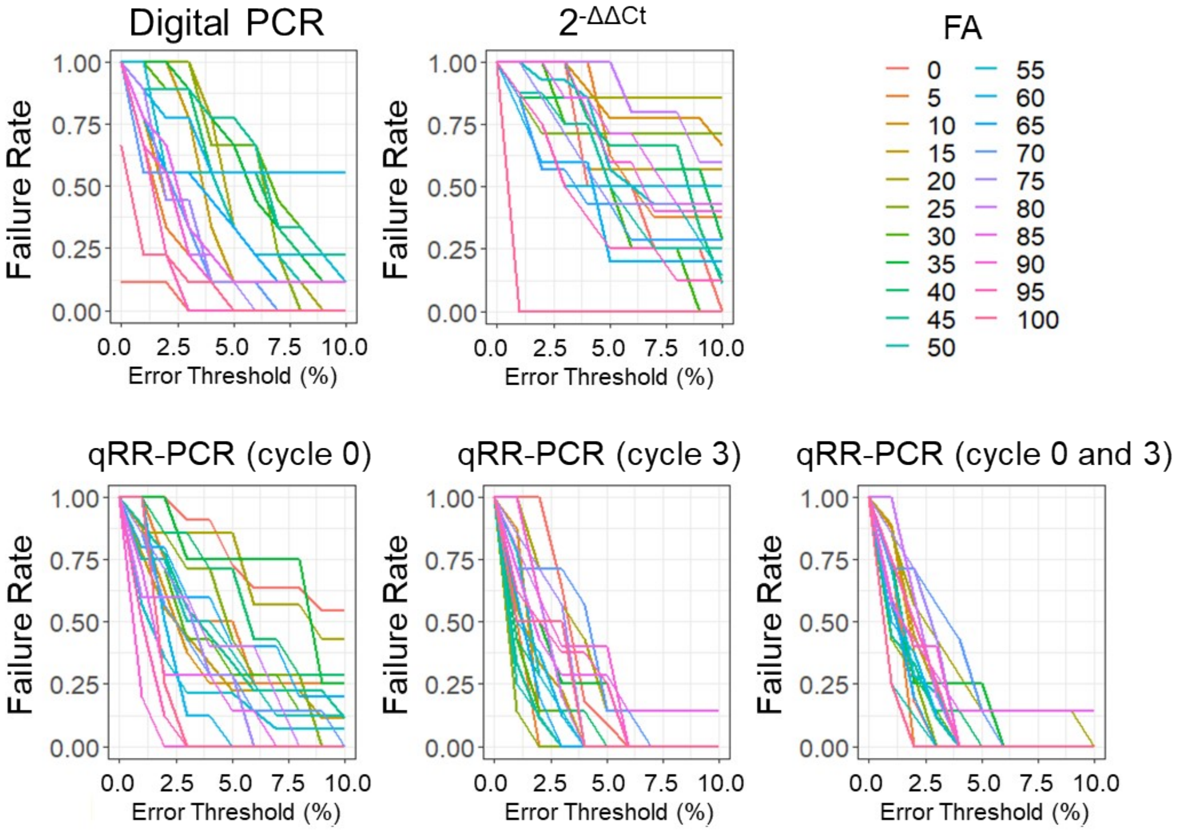

Figure S2. Standard curve failure rate at varying thresholds. Each line tracks the rate at which points in each FA data set that failed to fall within the given thresholds

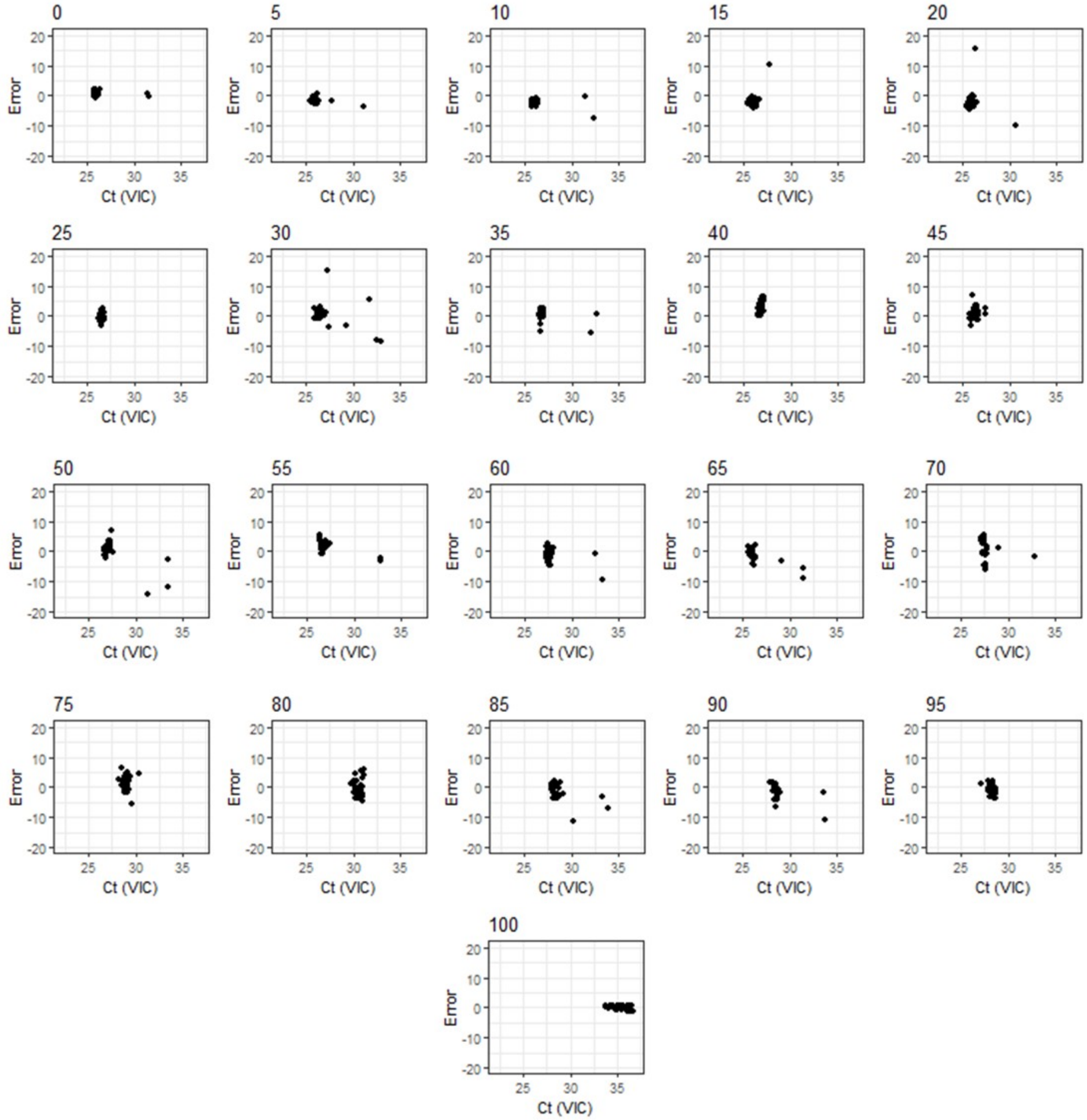

Figure S3. Prediction error percentage versus VIC Ct for each replicate with a given fractional abundance value shows clustering with approximately 2 cycles for Ct values without a clear correlation between Ct and error.

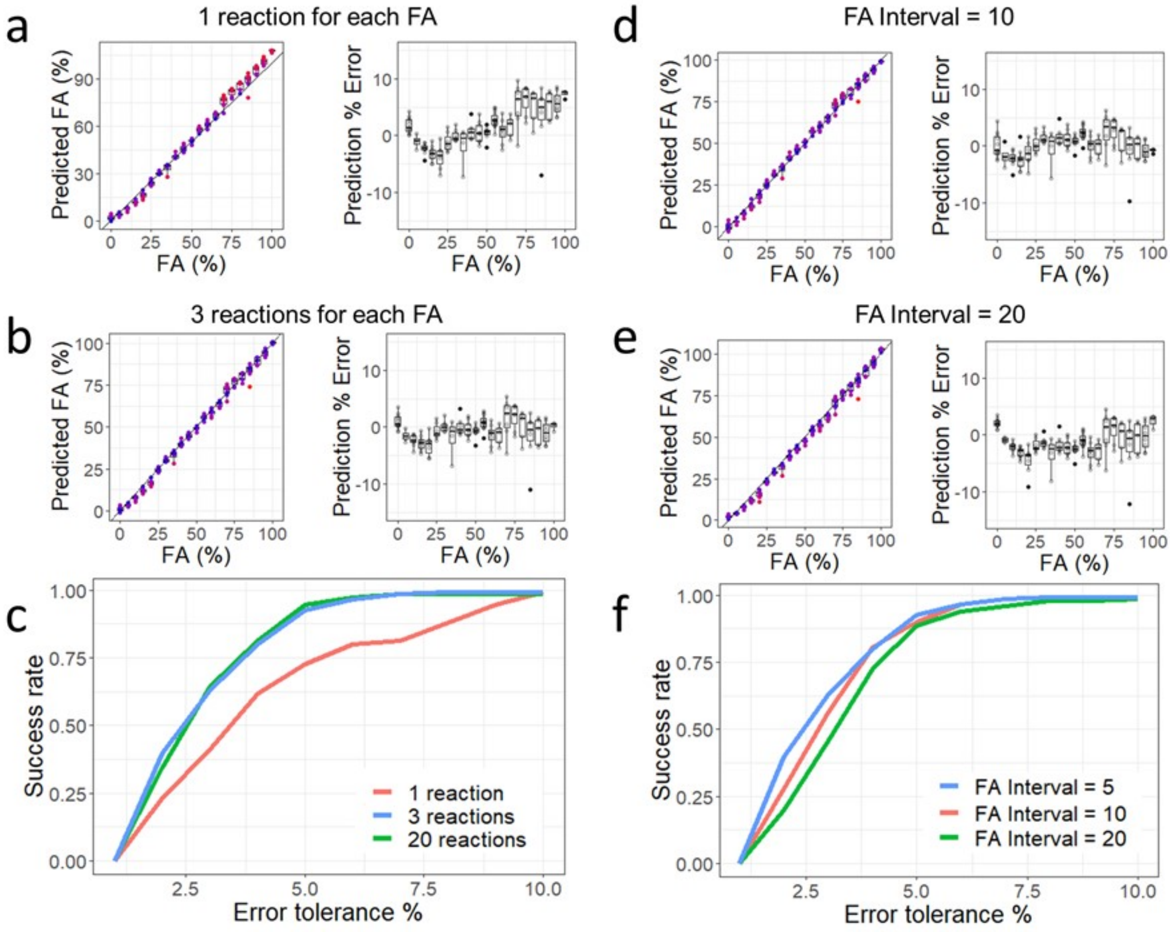

Figure S4. qRR-PCR model performance using smaller training sets. Prediction of FA was compared between using (a) only single point at each FA, and (b) 3 replicates at each FA as training set. (c) Success rate of the model trained with 3 replicates is comparable to the prediction model trained with ~20 replicates no matter the error tolerance is.

Instead of training the model with reactions containing varying FA in 5% increments from 0% to 100%, prediction of FA was compared between (d) reactions containing FA in 10% increments, and (e) reactions containing FA in 20% increments. (f) The model trained with FA in 5% increments shows higher success rates (92.7%) at 5% error tolerance, while the success rate of 10% increments is 90.1% and the success rate of 20% increments is 88.7%.