Is Linear Regression Curve Fitting the Best Model for Stem-Loop RT-qPCR Data Analysis?

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PURPOSE

- Small interfering RNAs (siRNAs) have become a new class of therapeutics in gene therapy.
- Stem-loop RT-qPCR is the most commonly used method to quantify the small RNAs in various biological matrices, which may exert significant matrix effect on the method sensitivity and accuracy.
- To reduce the matrix effect and increase assay sensitivity, we investigated the potential of optimizing regression models during data analysis, other than the use of extensive sample purification procedures.

METHOD(S)

- Analyte X was spiked into 1 mg/mL liver tissue lysate of cynomolgus monkey to prepare a 7-point STD curve (*64,000 to 15.625 pg/mL*) and 2 sets of QCs (*32,000 to 31.25 pg/mL*).
- The precision and accuracy assay of Stem-loop RT-qPCR was performed with QuantStudio 7 Flex real-time PCR system.
- Two regression models, a linear and a non-linear 4-parameter logistic (4-PL) regression, were used to assess the precision and accuracy.

RESULT(S)

- Significant matrix effect for analyte X was observed with Cycle Threshold (Ct) values of ~ 26 for bank liver lysates.
- Non-linear regression with 4-PL was found to be superior to linear regression in fitting the generated Ct values over nominal standard curve concentrations.
  - ✓ For linear regression fitting, the bias% of standards and QC samples spans a wide range, with 2 out of 7 standards and 3 out of 12 QC samples over 20%.
  - ✓ In contrast, for nonlinear regression fitting, the bias% values of standards and QC samples showed to be in a narrow range, with none above 20%.

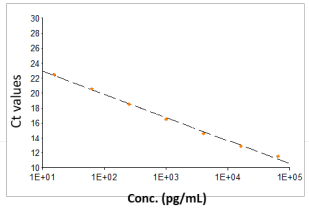
A

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1: 64,000		QC1: 32,000								QC1: 32,000	
C	STD2: 16,000		QC2: 8,000								QC2: 8,000	
E	STD3: 4,000		QC3: 2,000								QC3: 2,000	
G	STD4: 1,000		QC4: 500								QC4: 500	
I	STD5: 250		QC5: 125								QC5: 125	
K	STD6: 62.5		QC6: 31.25								QC6: 31.25	
M	STD7: 15.625											
O	STD8: Blank											

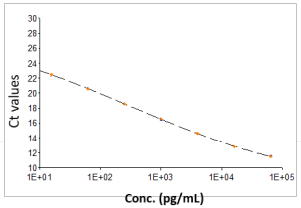
B

	1	2	3	4	5	6	7	8	9	10	11	12
A	11.537	11.477	12.059	12.049							12.020	11.993
C	12.827	12.827	13.694	13.712							13.638	13.614
E	14.638	14.613	15.559	15.586							15.486	15.453
G	16.592	16.476	17.499	17.533							17.474	17.431
I	18.578	18.538	19.480	19.489							19.407	19.411
K	20.605	20.545	21.507	21.551							21.490	21.509
M	22.505	22.435										
O	25.953	25.934										

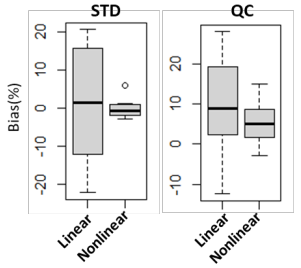
C



D



E



Comparison of linear and nonlinear curve fittings in precision and accuracy assessment of the siRNA.

- The assessment was performed with the plate layout as shown in ***A***.
- Raw Ct values were listed in ***B***.
- Linear (C) and nonlinear (D) 4-PL regression curve fittings were performed.
- The accuracy of standards and QC samples calculated with linear and nonlinear regression models was depicted in ***E***.

CONCLUSION(S)

We hereby propose that non-linear regression should be an option as curve fitting model, instead of linear regression, in analyzing stem-loop qPCR data, especially when obvious matrix effect is observed.

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