

Appendix A

	Platform	Normalisation	Method of Cq determination	Quantification method	Plots summary statistics
QuantGenius	Web	To ref genes	N/A	Absolute (Standard Curve)	No
ELIMU-MDx	Web	To ref genes	N/A	Absolute (Standard Curve) and Relative	No
shinyCurves	Web/R	N/A	N/A	Absolute (Standard Curve)	No
PIPE-T	Web	Numerous methods (from HTqPCR)	N/A	Relative	No
SATqPCR	Web	To ref genes	N/A	Relative	Yes
Auto-qPCR	Web/Python	To ref genes	N/A	Absolute (Standard Curve) and Relative	Yes
Chainy	Web/R	To ref genes	first derivative maximum of the curve, second derivative maximum of the curve, Guescini et al (2002)'s tangent to the point of inflection or Corbett Research's take-off method	Relative	Yes
LEMming	R	Linear Error Model	N/A	Relative	No
pcr	R	To ref genes	N/A	Relative (delta Cq or standard curve)	Yes
HTqPCR	R	Ref genes, quartile mean, rank-invariant normalising feature	N/A	Relative	Yes
ReadqPCR/ NormqPCR	R	To ref genes	N/A	Relative	No
qpcR	R		Fitting multi parameter logistic curve.		Yes
qpcr	Python	To ref genes	N/A	Relative	Yes
Spreadsheet	Misc	To ref genes	N/A	Relative	No

	Scalability	Summary statistics	Outlier identification	Calculate Primer calibration	Use primer efficiency
QuantGenius	Requires copy and pasting input	No	Yes	No	No (but filters based on it)
ELIMU-MDx	If you can create RDML files	No	No	No	No
shinyCurves	If in correct excel format and sample NT/control positions	No	Manual	No	No
PIPE-T	If files in tab separated format	two condition tests	Yes	No	No
SATqPCR	If files in tab separated txt format	t-test or ANOVA	No	No	Yes
Auto-qPCR	If input files in right format	t-test or ANOVA	Yes	No	No
Chainy	Manual upload of files	permutation approach equivalent to the REST software	Yes	Yes	Yes
LEMming					
	If imported into R	t-test and Wilcoxon signed-rank test.	No	No	No
pcr	If imported into R	t-test, ANOVA and signed-rank test.	No	Yes	No
HTqPCR	Yes	t-test, Mann-Whitney Test and limma package linear modals	Yes	No	No
ReadqPCR/ NormqPCR	Yes	No	No	No	No
qpcR	Yes	F-test for model selection	Yes	Yes	
qpcr	If input files in right format	No	Yes	Yes	Yes
Spreadsheet	No	t-test	No	No	Yes

	QC	Reproducible	Copy number	Input
QuantGenius	No melt/amp curve. Highlights outliers, outside LOD and efficiency	No	Yes	Copy and paste each target and reference gene separately
ELIMU-MDx	No melt/amp curves. Checks LOD, efficiency and control Cq.	If you can host it?	No	RDML, excel
shinyCurves	Both melt/amp curve with qpcR	Yes (If you identify the same outliers)	Yes	csv, (custom) xlsx, xls
PIPE-T	No melt/amp curve. Highlights outliers, outside LOD and efficiency	Yes	No	tsv
SATqPCR	No	Yes	No	txt
Auto-qPCR	Filters out samples with SD cutoff. No melt/amp curve	Yes	Yes	csv, txt
Chainy	Plots amp curve and highlight outliers that dont fit sigmoidal	Yes	No	RDML, csv, raw qPCR machine excel, plate plan
LEMming	No	Yes	No	R data.frame
pcr	No	Yes	No	R data.frame
HTqPCR	No melt/amp curve. Functions to plot wells, conduct PCA, calc variation. Can flag High Cq value and high variable samples	Yes	No	raw qPCR machine excel
ReadqPCR/ NormqPCR	No melt/amp curve. Functions to pairwise results across replicates/plates. Can impute missing values. flag High Cq value and high variable samples	Yes	No	raw qPCR machine excel
qpcR		Yes	No	R data.frame
qpcr	Filters out samples with SD cutoff. No melt/amp curve	Yes	No	csv, (custom) excel
Spreadsheet	Filters out samples with SD cutoff. No melt/amp curve	No	No	(custom) excel

	Output	GUI	Last update	Release date	Number of well	Normalising gene selection
QuantGenius	txt, xls	Yes	2017 Feb	2017 Feb	Unlimited	No
ELIMU-MDx	RDML, excel	Yes	2020 Dec	2019 Oct	Unlimited	No
shinyCurves	csv, png	Yes	2021 Oct	2021 Oct	96 or 364	No
PIPE-T	tsv, PNGs	Yes	2019 Nov	2019 Nov	Unlimited	No
SATqPCR	txt, png	Yes	2019 Aug	2019 Aug	Unlimited	stability parameter and coefficient of variation
Auto-qPCR	csv, png	Yes	2021 Oct	2021 Oct	Unlimited	No
Chainy	csv, png	Yes	2020 Aug	2017 May	Unlimited	Yes (geNorm method from NormqPCR)
LEMming	R data.frame	No	2015 Sept	2015 Sept	Unlimited	No
pcr	R data.frame and plots	No	2020 April	2018 May	Unlimited	No
HTqPCR	R S4 object and plots	No	??	2009 Dec	Unlimited	No
ReadqPCR/ NormqPCR	R S4 object and plots	No	2018 July	2012 Jul	Unlimited	Yes (geNorm or NormFinder)
qpcR	R S3 object and plots	No	2018 June	2008 July	Unlimited	
qpcr	txt, jpg	No	2022 Feb	2021 Aug	Unlimited	No
Spreadsheet	excel	No	2021 Dec	2021 Dec	Unlimited	No