Jonah Ventures qPCR report

JONAH VENTURES

KNOWLEDGE IN SEQUENCE

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Report prepared for example@jonahventures.com

BatchId = JVB0000

Number of samples analyzed = 10

Average number of copies detected

This table shows the average number of copies detected for each target organism in each sample. Values represent the average number of copies / 100 mL when sample volume was provided. When sample volumes are not known, the values indicate the estimated number of copies in the sample.

SampleId	ZebraMussel01
POJ41N95	1398094
WSU4K9YS	153739
TODYEMST	0
RAIDBBB8	34
XTGOOXPF	77857
BFY675BF	52
QBKTFR9B	182
VPW494K0	0
1E7G8UGU	205
TK3OG1B9	1654

Percent of replicates above detection limit

This table provides data on what percentage of the replicates that were run were above the detection limit. The detection limit is as high as the lowest positive on the calibration curve, but can be up to an order of magnitude lower. For example, a calibration curve might generate a positive at 100 copies and no positive for 10 copies, but the actual detection limit would be 11 copies. See the next section for the range of copy numbers estimated for each assay.

SampleId	ZebraMussel01
POJ41N95	100
WSU4K9YS	100
TODYEMST	0
RAIDBBB8	66.6
XTGOOXPF	100
BFY675BF	100
QBKTFR9B	100
VPW494K0	0
1E7G8UGU	66.6
TK3OG1B9	100

Detailed results

The following table provides the estimated copy number for individual technical replicates for each qPCR assay. Missing values indicate failed reactions or outliers that were removed from the analysis.

SampleID	Replicate1	Replicate2	Replicate3
ZebraMussel01			
POJ41N95	1408613.00	1352231.00	14334378.00
WSU4K9YS	153530.00	152511.00	155176.00
TODYEMST	0	0	0
RAIDBBB8	65.00	38.00	0
XTGOOXPF	73127.00	73631.00	86812.00
BFY675BF	59.00	59.00	39.00
QBKTFR9B	145.00	260.00	140.00
VPW494K0	0	0	0
1E7G8UGU	319.00	0	296.00
TK3OG1B9	1703.00	1435.00	1824.00

Sample metadata

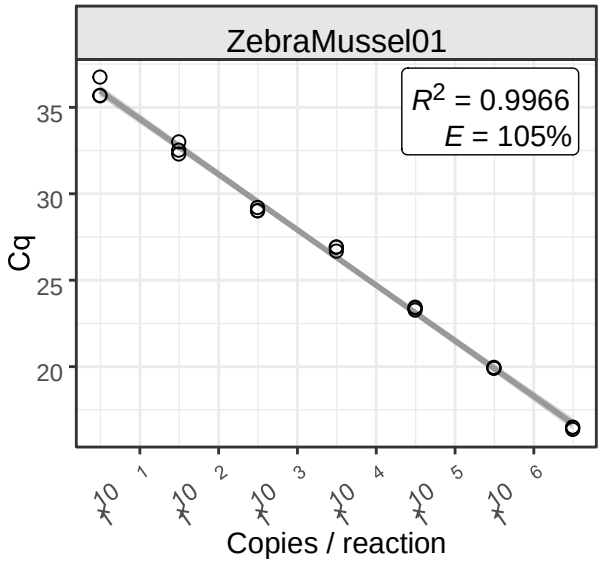
SampleId	ClientSampleID	Date	Time	Volume Water (ml)	SiteName	Notes
POJ41N95	ZM_Sample1	6/7/2022	07:43:09UTC	80	JonahVentures1	NA
WSU4K9YS	ZM_Sample2	6/7/2022	07:51:22UTC	90	JonahVentures2	NA
TODYEMST	ZM_Sample3	6/7/2022	08:15:56UTC	80	JonahVentures3	NA
RAIDBBB8	ZM_Sample4	6/7/2022	08:49:38UTC	87	JonahVentures4	NA
XTGOOXPF	ZM_Sample5	6/7/2022	09:11:29UTC	56	JonahVentures5	NA
BFY675BF	ZM_Sample6	6/7/2022	09:37:54UTC	360	JonahVentures6	NA
QBKTFR9B	ZM_Sample7	6/7/2022	09:55:45UTC	80	JonahVentures7	NA
VPW494K0	ZM_Sample8	6/7/2022	10:39:21UTC	250	JonahVentures8	NA
1E7G8UGU	ZM_Sample9	6/7/2022	10:56:33UTC	250	JonahVentures9	NA
TK3OG1B9	ZM_Sample10	6/7/2022	11:20:02UTC	275	JonahVentures10	NA

Methods and calibration curves

The following pages provide details of the methods used for each qPCR assay and the associated standard curves. Each assay in each run is associated with a calibration curve based typically on a series of 7, 10-fold dilutions of a standard with a known concentration. The calibration curves show the relationship between the $\log_{10}$-transformed standard concentration and the number of PCR cycles at which the detection threshold was reached (Cq). A linear regression is applied to this relationship and the r2 intercept and slope extracted for further analyses.

- RunId = An internal identifier for the standard curve(s) used to calculate copy numbers in the submitted samples. Assays that share a RunId are multiplexed (i.e., multiple targets amplified in a single reaction).
- R^2 = The coefficient of determination, or goodness of fit for the linear relationship (should be > 0.98).
- (E) = The reaction efficiency, or how close to a doubling of product was achieved with each PCR cycle. For a 10-fold dilution, 100% efficiency is for ~3.3 cycles per 10-fold dilution. A range of values is acceptable here, but we try to keep efficiency between 85% - 110%.

RunID: JVQEXMPL



Forward primer: 5’ CCGGCTAGCGGAAGG 3

Reverse primer: 5’ ACCTCGGAGGGCTGTAC 3

Probe: 5’ /56-FAM/GTGATCGGT/ZEN/ATCCGATCGCG/3IABkFQ/ 3

Primer/probe reference: Jonah Venture

An amplicon from the 28S Ribosomal RNA gene was amplified via qPCR from genomic DNA samples using Zebra Mussel FWD and REV primers and probe. A standard curve was generated for each run to correspond to targeted region of the Zebra Mussel, 28S Ribosomal RNA gene. Each qPCR reaction is run in triplicate and contains 8.0 uL of QuantaBio PerfeCTa qPCR ToughMix Low ROX (Catalog Number 97065-966), 500 nM of each primer, 300 nM of probe, 2.0 uL of gDNA, and 6.8 uL of Nuclease-free H2O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 C; followed by 50 cycles of 15 seconds at 95 C and 1 minute at 60 C.