

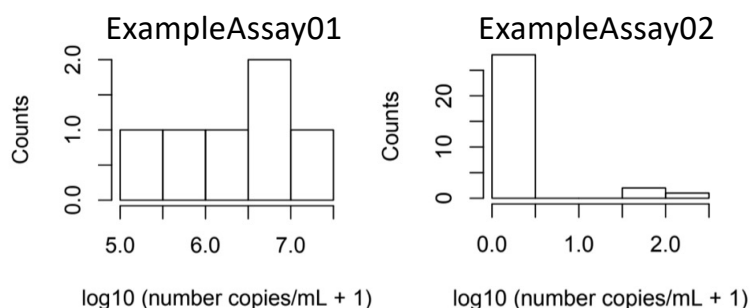


# Jonah Ventures qPCR Report Files Explained

## Histograms

These histograms show the distribution of the data for each assay tested.

Y-axis: Represents count of samples in each category.



X-axis: Log transformed copies per 100mL (if data on water volume is provided) after adding 1 to show zeros.

## Table 1

An internal identification number.

Jonah Sample ID provided by client.

Name of the assay.

RunID	SampleID	Bullfrog01
JVQ0001	0010	27648
JVQ0001	0011	0
JVQ0001	0012	0
JVQ0001	0013	126
JVQ0001	0014	5279

The number of copies per filtered 100mL of water (if data provided) for each assay averaged across internal replicates.

## Table 2

An internal identification number.

Jonah Sample ID provided by client.

Name of the assay.

RunID	SampleID	Bullfrog01
JVQ0001	100.0	100.0
JVQ0001	0011	0
JVQ0001	0012	0
JVQ0001	0013	33.3
JVQ0001	0014	100.0

Shows the percent of all replicates that were above the detection limit for each assay.

## Table 3

Each client gets an individual ID number.

An internal identification number, used to look up calibration curves for each sample.

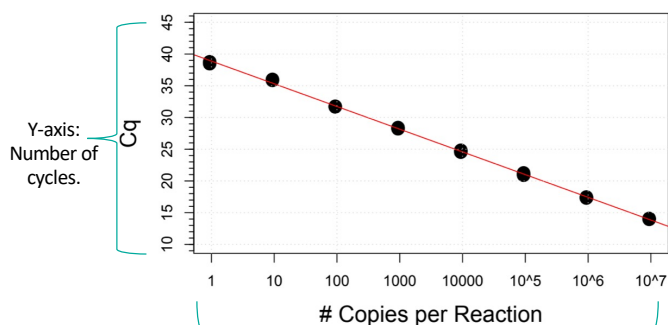
The qPCR assay that was used in the sample. If multiple primers are used on one sample, they will be listed separately by assay.

Jonah Sample ID provided by the client.

The number of DNA copies detected per filtered 100mL of water. Each sample is run three times using the exact same protocol to evaluate the results.

ClientID	RunID	Assay	SampleID	Rep1	Rep2	Rep3
0001	JVQ1234	BigheadCarp01	0001	567	678	789
0001	JVQ1234	Silver Carp01	0002	2345	3456	1234
0001	JVQ1234	NorthernPike01	0003	0	0	0
0001	JVQ4567	NorthernPike01	0004	0	95	0

## Calibration Curves



X-axis: Log10 of the number of copies in the standard.

"RunID = JVQ0055" → An internal identification number, which can be matched to samples.

"Assay = Bullfrog01" → The name of the assay used in the calibration curve.

"r2 = 0.999" → The goodness of fit for the linear relationship.

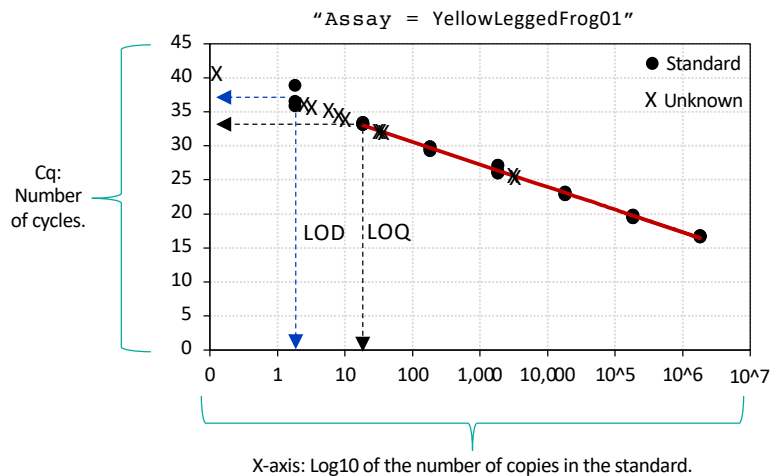
"efficiency = 95.3%" → How close to a doubling of product with each PCR cycle. 100% efficiency is the ideal.



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## Limit of detection and Limit of Quantification

Samples with Cq values that exceed 35 are difficult to interpret because they exhibit higher variability and prone to false positives due to low copy number. These samples should be interpreted with caution



**Limit of Detection (LOD):** lowest concentration at which 95% replicates are positive but are highly variable and therefore cannot be accurately quantified. The MIQE guideline is  $3 c/r_{xn}$  ( $Cq \sim 36$ )

**Limit of Quantification (LOQ):** lowest concentration at which replicates are quantified with precision and accuracy. The MIQE guidelines recommend replicate Cq's be within 0.5 Cq of each other with a standard deviation of  $< 0.3$  among replicates.

Cq value. Each sample is run three times using the exact same protocol to evaluate the results.

SampleID	Rep1	Rep2	Rep3	
0001	41.11	No Cq	No Cq	?
0002	36.26	No Cq	36.98	✓
0003	35.67	34.86	34.94	✓
0004	32.81	32.72	32.78	✓

**SampleID 0001:** One out of three replicates positive with a high Cq. Does not meet criteria for a true positive, without re-testing consider negative

**SampleID 0002:** Two out of three replicates positive with Cqs between the LOD and LOQ. Likely a true positive but at concentrations that can not be reliably quantified.

**SampleID 0003:** All three replicates positive with Cqs higher than the LOQ. Positive but at concentrations that can not be reliably quantified.

**SampleID 0004:** All three replicates positive Cqs lower than the LOQ with an  $SD < 0.3$ . Positive and quantifiable