Jonah Ventures qPCR Report

JONAH VENTURES
KNOWLEDGE IN SEQUENCE

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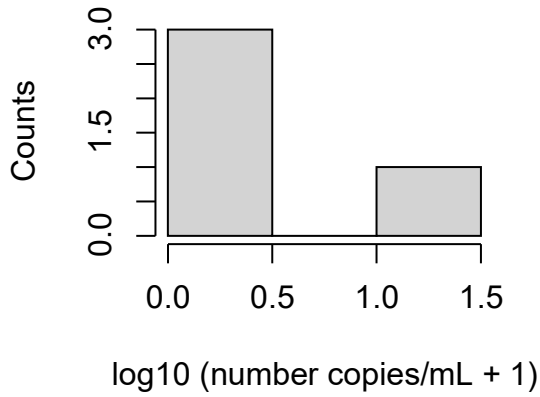info@jonahventures.com

ClientID = 1349

Last Name = CassaniJohn

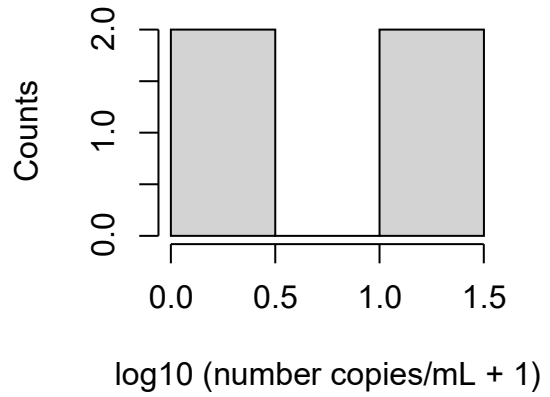BatchID = JVB0055

Number samples analyzed = 4

Histograms

Shown are histograms for the distribution of the data for each assay. x-axis is log transformed copies per 100 mL (if data on water volume provided) after adding 1 to show zeros. y-axis represents count of samples in each category.

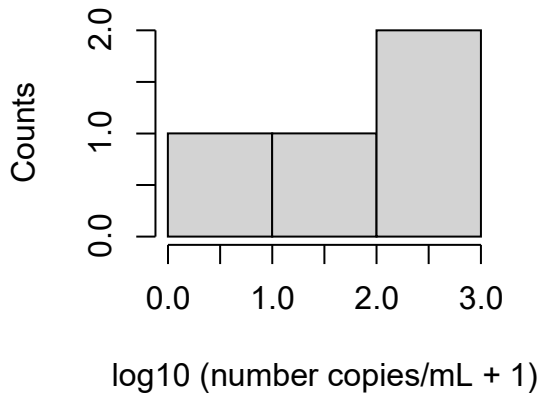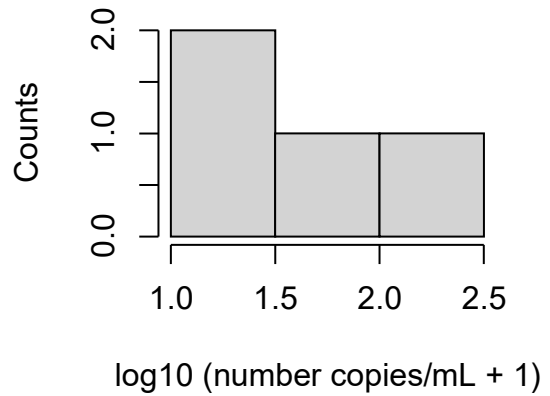## Bovine01

log10 (number copies/mL + 1)

Dog01

log10 (number copies/mL + 1)

E.coli01

log10 (number copies/mL + 1)

Human01

log10 (number copies/mL + 1)

Poultry01

Counts (y-axis): 0.0, 1.5, 3.0
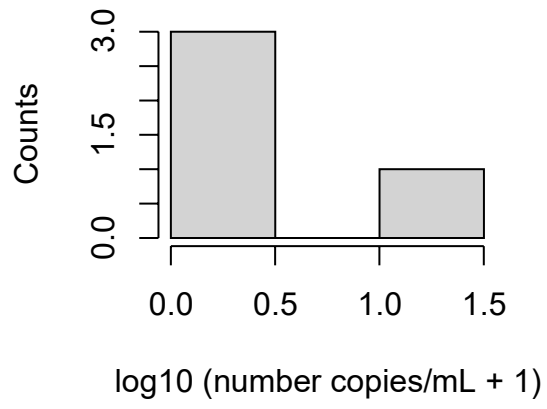
log10 (number copies/mL + 1)

Table ? Average Number of Copies for Each Assay

This table provides data on the average number of copies that were detected for each sample for each assay.

RunID = an internal identification number. Can be used by the client to look up which calibration curve applies to each sample

SampleID = Jonah Sample ID provided by client.

Subsequent columns represent the number of copies per filtered 100 mL of water (if data provided) for each assay averaged across internal replicates. The column name denotes the assay name.

SampleID	Bovine01	Dog01	E.coli01	Human01	Poultry01
1 1HQRV5LL **BC8, Ortiz**	0	0	138	26	0
2 28WTKT65 **BC6 Marsh**	0	18	0	34	0
3 B9RYSB8I **BC4 VS Blvd**.	16	0	16	181	10
4 HZOWVVK6 **BC3 Shady Oaks Pk**	0	12	105	24	0

Table 2. Percent of Replicates Above Detection Limit for Each Assay

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 Table 3 for the range of number of copies for each assay.

RunID = an internal identification number. Can be used by the client to look up which calibration curve applies to each sample

SampleID = Jonah Sample ID provided by client.

Subsequent columns show the percent of all replicates that were above detection limit for each assay. The column name denotes the assay name.

	RunID	SampleID	Bovine01	Dog01	E.coli01	Human01	Poultry01
1	JVQ0119	1HQRV5LL	0.0	0.0	0	100.0	0.0
2	JVQ0119	28WTKT65	0.0	0.0	0	33.3	0.0
3	JVQ0119	B9RYSB8I	33.3	0.0	0	100.0	0.0
4	JVQ0119	HZOWVVK6	0.0	0.0	0	33.3	0.0
5	JVQ0120	1HQRV5LL	0.0	0.0	0	0.0	0.0
6	JVQ0120	28WTKT65	0.0	33.3	0	0.0	0.0
7	JVQ0120	B9RYSB8I	0.0	0.0	0	0.0	33.3
8	JVQ0120	HZOWVVK6	0.0	33.3	0	0.0	0.0
9	JVQ0121	1HQRV5LL	0.0	0.0	100	0.0	0.0
10	JVQ0121	28WTKT65	0.0	0.0	0	0.0	0.0
11	JVQ0121	B9RYSB8I	0.0	0.0	100	0.0	0.0
12	JVQ0121	HZOWVVK6	0.0	0.0	100	0.0	0.0

Table 3. All data (copies/100ml)

	ClientID	RunID	Assay	SampleID	Rep1	Rep2	Rep3
1	1349	JVQ0119	Human01	1HQRV5LL	34	19	24
2	1349	JVQ0119	Human01	28WTKT65	0	0	102
3	1349	JVQ0119	Human01	B9RYSB8I	301	12	229
4	1349	JVQ0119	Human01	HZOWVVK6	73	0	0
5	1349	JVQ0119	Swine01	1HQRV5LL	0	0	0
6	1349	JVQ0119	Swine01	28WTKT65	0	0	0
7	1349	JVQ0119	Swine01	B9RYSB8I	0	0	0
8	1349	JVQ0119	Swine01	HZOWVVK6	0	0	0
9	1349	JVQ0119	Bovine01	1HQRV5LL	0	0	0
10	1349	JVQ0119	Bovine01	28WTKT65	0	0	0
11	1349	JVQ0119	Bovine01	B9RYSB8I	0	0	48
12	1349	JVQ0119	Bovine01	HZOWVVK6	0	0	0
13	1349	JVQ0120	Poultry01	1HQRV5LL	0	0	0
14	1349	JVQ0120	Poultry01	28WTKT65	0	0	0
15	1349	JVQ0120	Poultry01	B9RYSB8I	0	29	0
16	1349	JVQ0120	Poultry01	HZOWVVK6	0	0	0
17	1349	JVQ0120	Sheep01	1HQRV5LL	0	0	0
18	1349	JVQ0120	Sheep01	28WTKT65	0	0	0
19	1349	JVQ0120	Sheep01	B9RYSB8I	0	0	0
20	1349	JVQ0120	Sheep01	HZOWVVK6	0	0	0
21	1349	JVQ0120	Dog01	1HQRV5LL	0	0	0
22	1349	JVQ0120	Dog01	28WTKT65	0	53	0
23	1349	JVQ0120	Dog01	B9RYSB8I	0	0	0
24	1349	JVQ0120	Dog01	HZOWVVK6	0	36	0
25	1349	JVQ0121	E.coli01	1HQRV5LL	113	143	159
26	1349	JVQ0121	E.coli01	28WTKT65	0	0	0
27	1349	JVQ0121	E.coli01	B9RYSB8I	20	6	23
28	1349	JVQ0121	E.coli01	HZOWVVK6	108	127	81

Calibration curves

Each assay in each run is associated with a calibration curve based typically on a series of 7 10-fold dilutions of a known standard.

The calibration curve shown is for the number of cycles vs. the log10 of the number of copies in the standard. A linear regression is applied to this relationship and the r2 intercept and slope extracted for further analyses.

RunID = an internal identification number. Can be used by the client to match the calibration curve applies with each sample for an assay

Assay = the name of the assay for the calibration curve in the particular run

r2 = the coefficient of determination, or goodness of fit for the linear relationship. Typically, r2 should be > 0.98.

efficiency = how close to a doubling of product 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%.
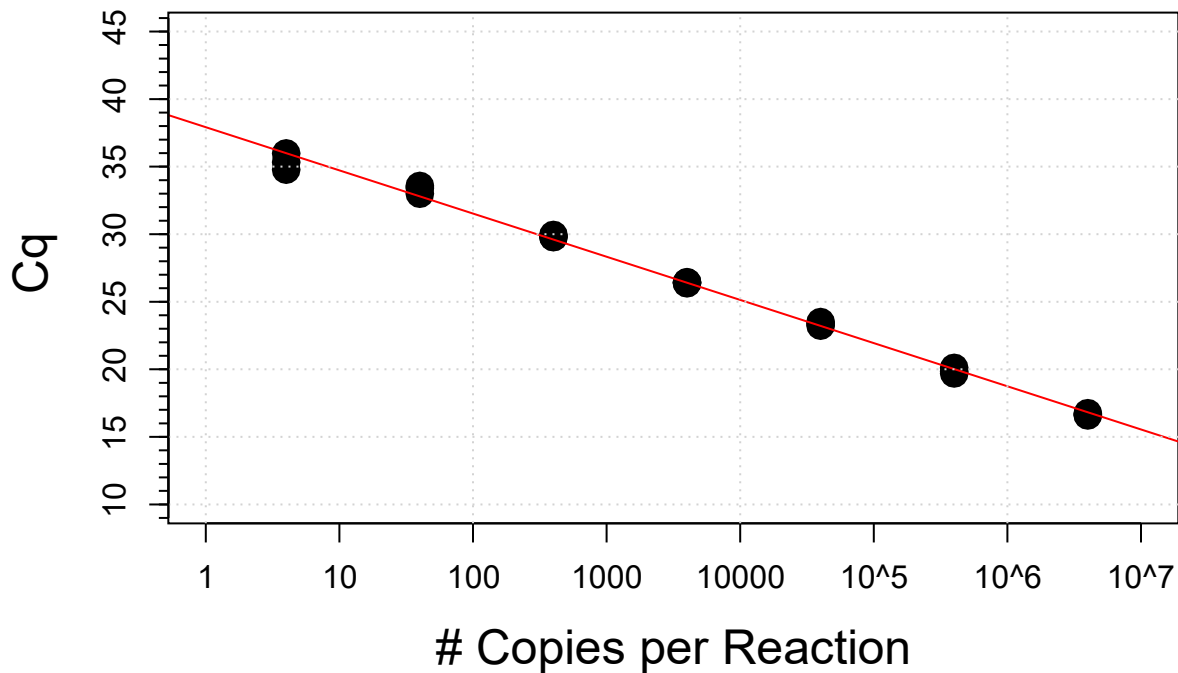
[1] "Calibration Curve"
[1] "RunID = JVQ0119"
[1] "Assay = Human01"
[1] "r2 = 0.996"
[1] "efficiency = 106%"
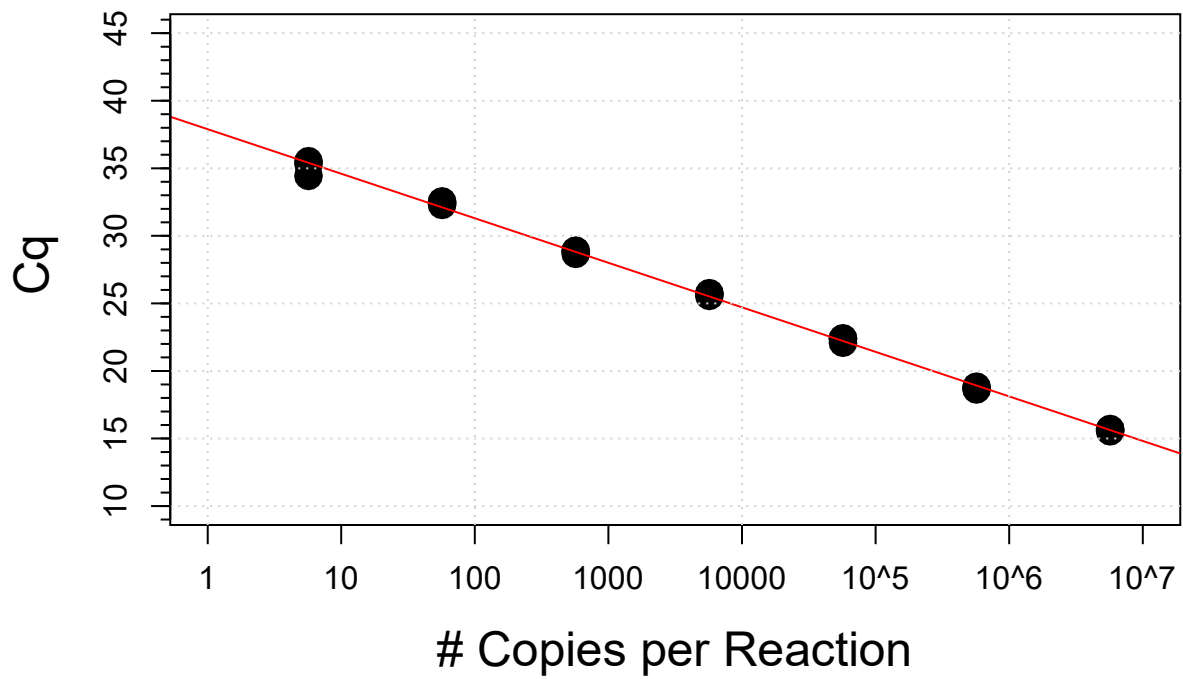
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[1] "RunID = JVQ0120"
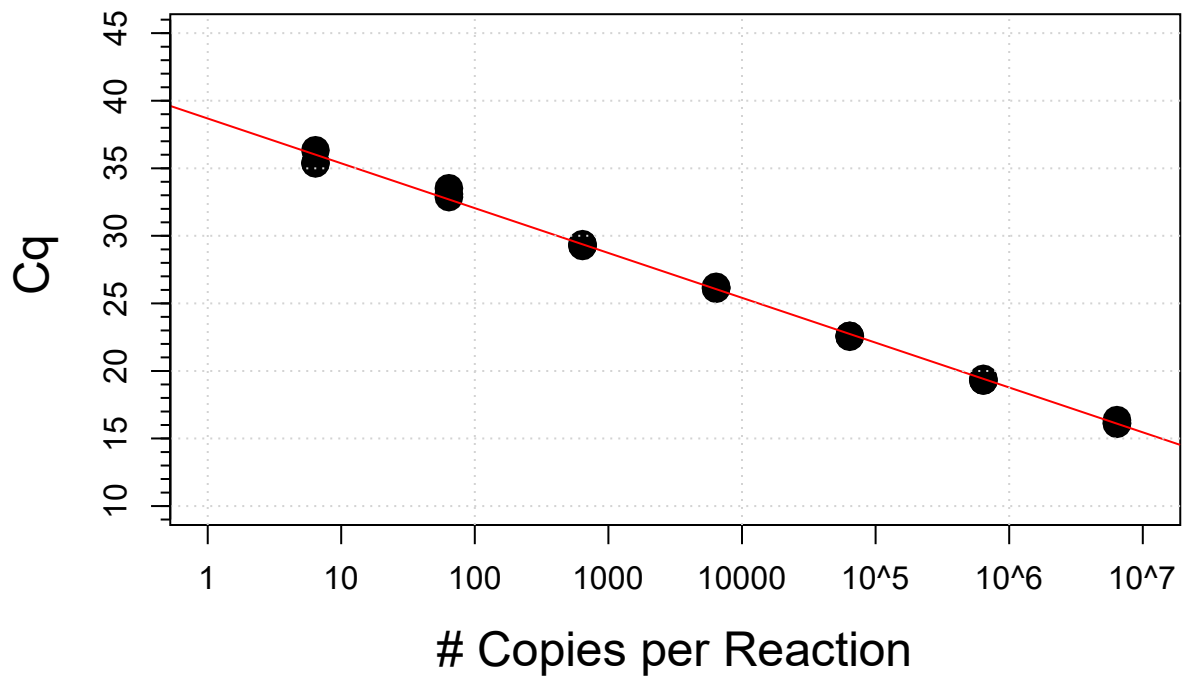[1] "Assay = Poultry01"
[1] "r2 = 0.998"
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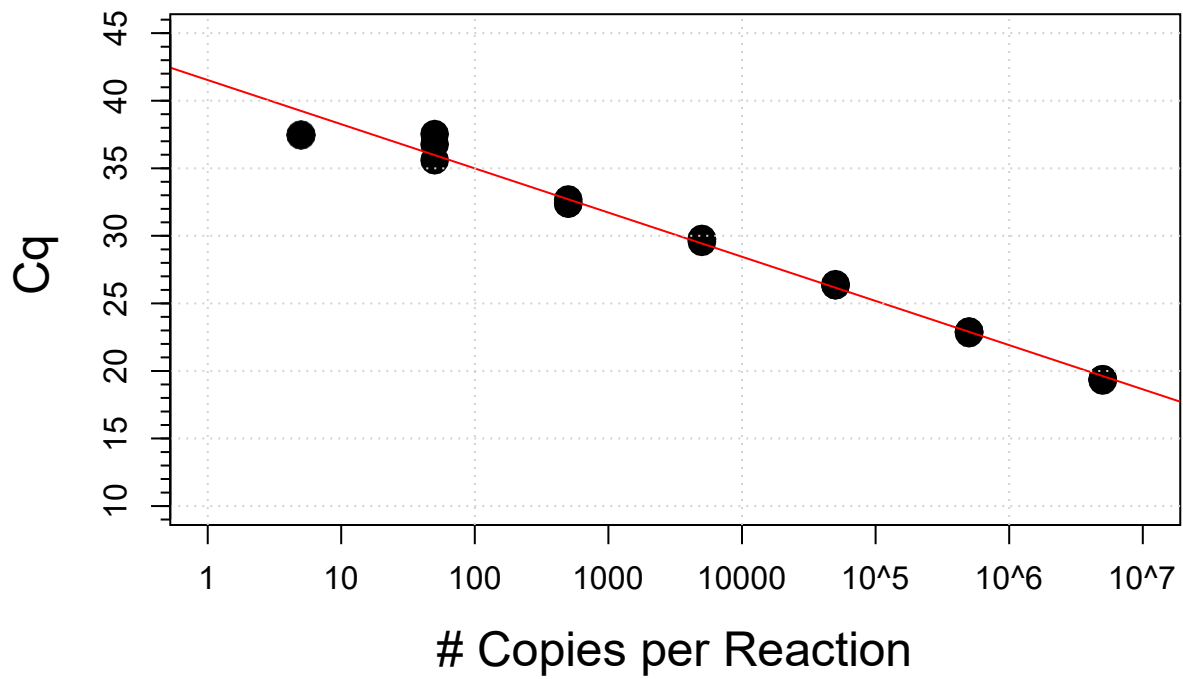
[1] "Calibration Curve"
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[1] "Assay = Swine01"
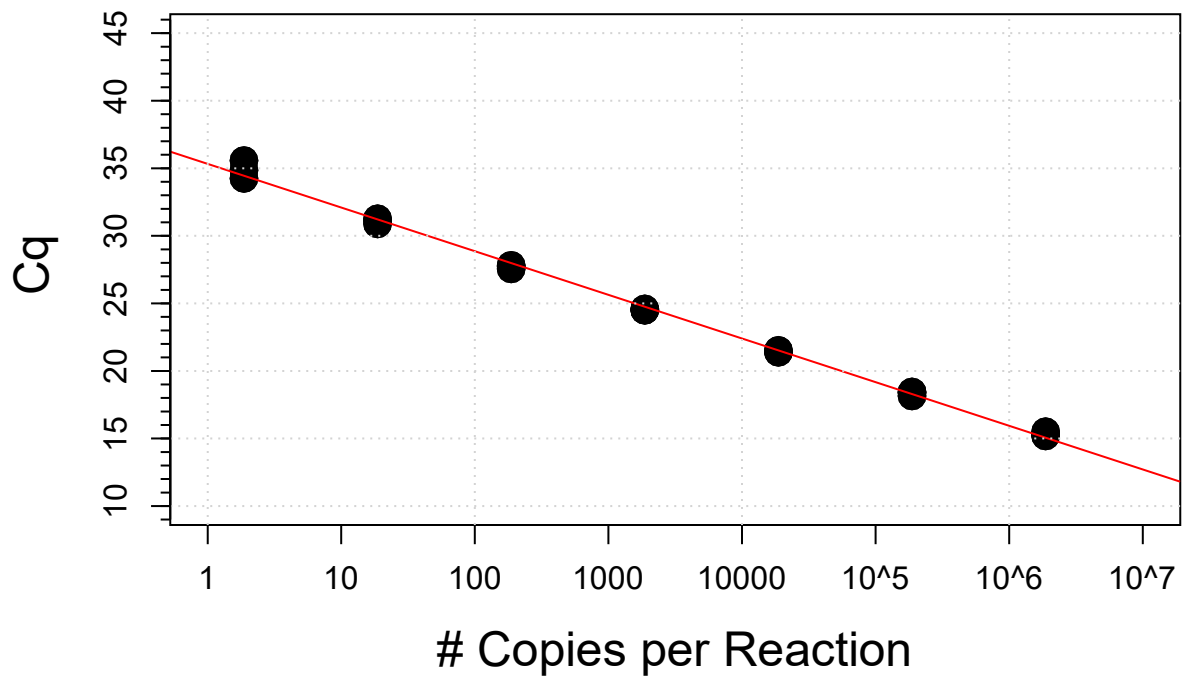[1] "r2 = 0.99"
[1] "efficiency = 102%"

[1] "Calibration Curve"
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[1] "Assay = Sheep01"
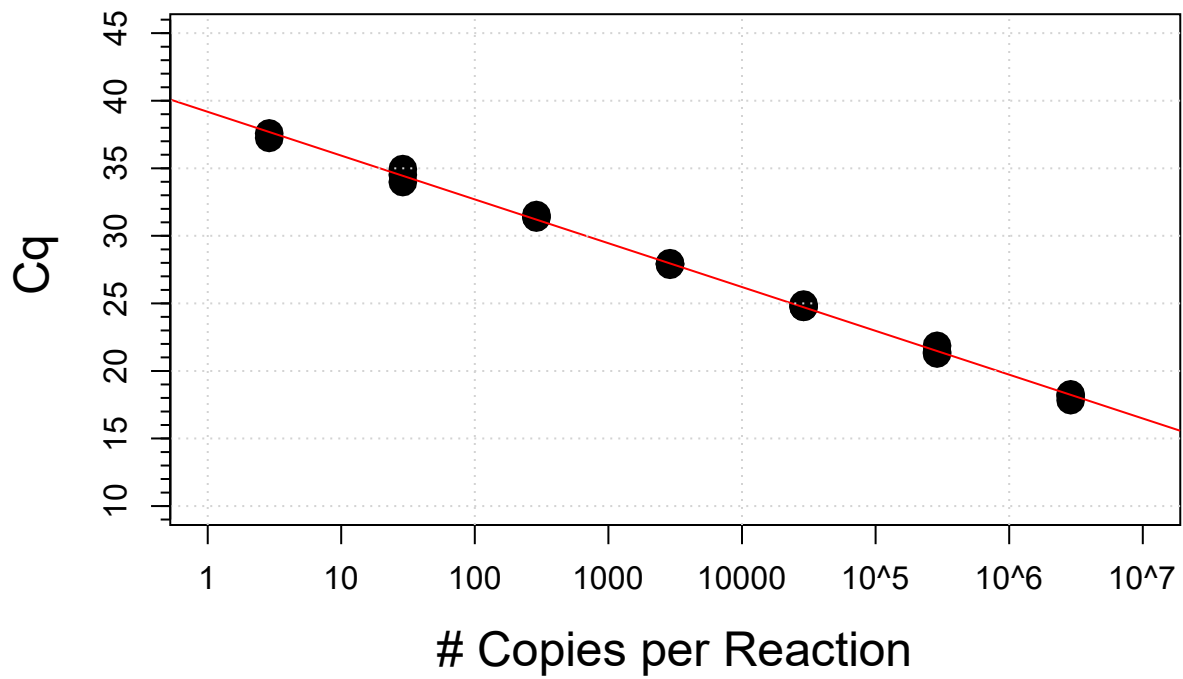[1] "r2 = 0.998"
[1] "efficiency = 100%"

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[1] "RunID = JVQ0121"
[1] "Assay = E.coli01"
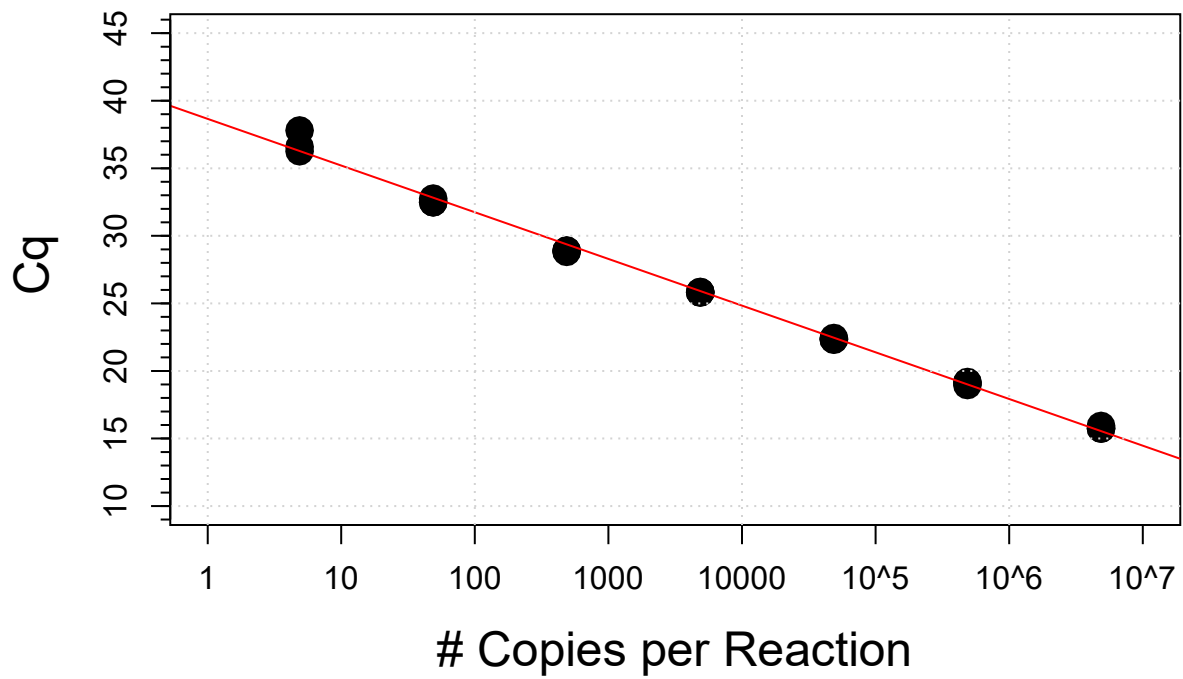[1] "r2 = 0.997"
[1] "efficiency = 104%"

[1] "Calibration Curve"
[1] "RunID = JVQ0119"
[1] "Assay = Bovine01"
[1] "r2 = 0.998"
[1] "efficiency = 103%"

[1] "Calibration Curve"
[1] "RunID = JVQ0120"
[1] "Assay = Dog01"
[1] "r2 = 0.996"
[1] "efficiency = 95%"

Assays

[1] "Assay Name = Bovine01"
[1] "Assay Description = Mitochondrial gene NADH dehydrogenase subunit 5"
[1] "Assay Thermal Profile = 1 cycle at 95 deg C for 5 minutes. 50 cycles at 95 deg"
[2] "C for 15 seconds. 60 deg C for 1 minute"
[1] "Assay Reference = Caldwell, J. M., Raley, M. E., and Levine, J. F. 2007"
[2] "Mitochondrial multiplex real-time PCR as a source tracking method in"
[3] "fecal-contaminated effluents. Environmental Science and Technology"
[4] "41:3277-3283."

[1] "Assay Name = Dog01"
[1] "Assay Description = Mitochondrial Cytochrome b"
[1] "Assay Thermal Profile = 1 cycle at 95 deg C for 5 minutes. 50 cycles at 95 deg"
[2] "C for 15 seconds. 60 deg C for 1 minute"
[1] "Assay Reference = Dancer, D., Baker-Austin, C., Lowther, J. A., Hartnell, R."
[2] "E., Lees, D. N., Roberts, L. O. 2014 Development and Integration of"
[3] "Quantitative Real-Time PCR Methods for Detection of Mitochondrial DNA and"
[4] "Methanobrevibacter smithii nifH Gene as Novel Microbial Source Tracking Tools."
[5] "Environmental Fornesics 15:256-264."

[1] "Assay Name = E.coli01"
[1] "Assay Description = uidA Gene"
[1] "Assay Thermal Profile = 1 cycle at 95 deg C for 5 minutes. 50 cycles at 95 deg"
[2] "C for 15 seconds. 60 deg C for 1 minute"
[1] "Assay Reference = Srinivasan, S., et al., Escherichia coli, enterococci, and"
[2] "Bacteroides thetaiotaomicron qPCR signals through wastewater and septage"
[3] "treatment, Water Research (2011), doi:10.1016/j.watres.2011.02.010"

[1] "Assay Name = Human01"
[1] "Assay Description = Mitochondrial gene NADH dehydrogenase subunit 5"
[1] "Assay Thermal Profile = 1 cycle at 95 deg C for 5 minutes. 50 cycles at 95 deg"
[2] "C for 15 seconds. 60 deg C for 1 minute"
[1] "Assay Reference = Caldwell, J. M., Raley, M. E., and Levine, J. F. 2007"
[2] "Mitochondrial multiplex real-time PCR as a source tracking method in"
[3] "fecal-contaminated effluents. Environmental Science and Technology"
[4] "41:3277-3283."

[1] "Assay Name = Poultry01"
[1] "Assay Description = Mitochondrial Cytochrome b"
[1] "Assay Thermal Profile = 1 cycle at 95 deg C for 5 minutes. 50 cycles at 95 deg"
[2] "C for 15 seconds. 60 deg C for 1 minute"
[1] "Assay Reference = Dancer, D., Baker-Austin, C., Lowther, J. A., Hartnell, R."
[2] "E., Lees, D. N., Roberts, L. O. 2014 Development and Integration of"
[3] "Quantitative Real-Time PCR Methods for Detection of Mitochondrial DNA and"
[4] "Methanobrevibacter smithii nifH Gene as Novel Microbial Source Tracking Tools."
[5] "Environmental Fornesics 15:256-264."