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	Bovine01	Dog01	E.coli01	Human01	Poultry01	Sheep01	Swine01
CALR6YCD	0	28	108	159	29	0	458
LYVJG6FY	0	0	0	266	0	0	2664
IU73H24R	432	0	1483	9466	0	0	0
FE3S98PB	36	0	811	380	43	0	617
WH8O0AFK	91	17	1118	2404	194	0	237
RIOC3AU6	75	0	322	1019	55	0	215
2IKE2YXD	68	0	295	935	50	0	197
HK84IP64	132	0	258	1721	0	0	1800
EA0E3V6U	97	25	4	87	0	0	0
565VKE6V	210	10	15	143	36	0	0

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	Bovine01	Dog01	E.coli01	Human01	Poultry01	Sheep01	Swine01
CALR6YCD	0	33.3	100	100	100	0	66.6
LYVJG6FY	0	0	0	100	0	0	66.6
IU73H24R	33.3	0	100	100	0	0	0
FE3S98PB	66.6	0	100	100	66.6	0	66.6
WH8O0AFK	100	33.3	100	100	100	0	33.3
RIOC3AU6	100	0	100	100	100	0	33.3
2IKE2YXD	100	0	100	100	100	0	33.3
HK84IP64	100	0	100	100	0	0	100
EA0E3V6U	100	33.3	100	100	0	0	0
565VKE6V	100	33.3	100	100	66.6	0	0

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
Swine01			
CALR6YCD	665	708	0
LYVJG6FY	3160	4833	0
IU73H24R	0	0	0
FE3S98PB	0	1019	833
WH8O0AFK	710	0	0
RIOC3AU6	0	0	644
2IKE2YXD	0	0	591
HK84IP64	2645	1791	963
EA0E3V6U	0	0	0
565VKE6V	0	0	0
Bovine01			
CALR6YCD	0	0	0
LYVJG6FY	0	0	0
IU73H24R	0	0	1295
FE3S98PB	49	0	58
WH8O0AFK	141	82	50
RIOC3AU6	48	99	77
2IKE2YXD	44	91	70
HK84IP64	17	148	230
EA0E3V6U	86	69	135
565VKE6V	286	311	33
Human01			
CALR6YCD	102	253	123
LYVJG6FY	102	160	236
IU73H24R	11690	10880	5828
FE3S98PB	509	343	287
WH8O0AFK	2847	2003	2363
RIOC3AU6	1028	708	1322
2IKE2YXD	943	649	1212

HK84IP64	1353	1994	1816
EA0E3V6U	42	67	151
565VKE6V	203	122	103
Sheep01			
CALR6YCD	0	0	0
LYVJG6FY	0	0	0
IU73H24R	0	0	0
FE3S98PB	0	0	0
WH8O0AFK	0	0	0
RIOC3AU6	0	0	0
2IKE2YXD	0	0	0
HK84IP64	0	0	0
EA0E3V6U	0	1	0
565VKE6V	1	0	0
Dog01			
CALR6YCD	54	29	0
LYVJG6FY	0	0	0
IU73H24R	0	0	0
FE3S98PB	0	0	0
WH8O0AFK	0	0	50
RIOC3AU6	0	0	0
2IKE2YXD	0	0	0
HK84IP64	0	0	0
EA0E3V6U	0	75	0
565VKE6V	29	0	0
Poultry01			
CALR6YCD	36	42	8
LYVJG6FY	0	0	0
IU73H24R	0	0	0
FE3S98PB	0	46	84
WH8O0AFK	257	43	282
RIOC3AU6	32	2	130
2IKE2YXD	29	2	119
HK84IP64	0	0	0
EA0E3V6U	0	0	0

565VKE6V	40	68	0
E.coli01			
CALR6YCD	116	84	125
LYVJG6FY	0	0	0
IU73H24R	1162	972	2315
FE3S98PB	902	782	749
WH8O0AFK	1198	1062	1093
RIOC3AU6	317	275	373
2IKE2YXD	290	252	342
HK84IP64	251	266	258
EA0E3V6U	9	0	4
565VKE6V	36	0	9

Sample metadata

SampleId	ClientSampleID	Date	Time	Volume Filtered (mL)	SiteName	Notes
CALR6YCD	ST_Sample1	6/7/2022	07:43:09UTC	120	JonahVentures1	NA
LYVJG6FY	ST_Sample2	6/7/2022	07:51:22UTC	40	JonahVentures2	NA
IU73H24R	ST_Sample3	6/7/2022	08:15:56UTC	5	JonahVentures3	NA
FE3S98PB	ST_Sample4	6/7/2022	08:49:38UTC	90	JonahVentures4	NA
WH8O0AFK	ST_Sample5	6/7/2022	09:11:29UTC	85	JonahVentures5	NA
RIOC3AU6	ST_Sample6	6/7/2022	09:37:54UTC	110	JonahVentures6	NA
2IKE2YXD	ST_Sample7	6/7/2022	09:55:45UTC	120	JonahVentures7	NA
HK84IP64	ST_Sample8	6/7/2022	10:39:21UTC	100	JonahVentures8	NA
EA0E3V6U	ST_Sample9	6/7/2022	10:56:33UTC	105	JonahVentures9	NA
565VKE6V	ST_Sample10	6/7/2022	11:20:02UTC	71	JonahVentures10	NA

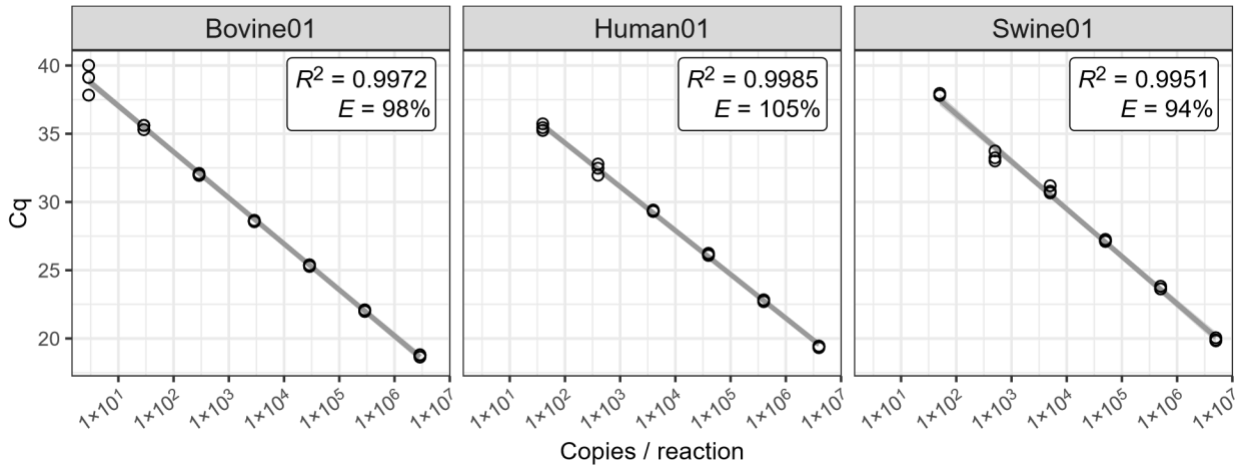
Methods and calibration curves

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

Warning: The closing backticks on line 4 ("‘‘‘") in template_qPCRreport.Rmd do
not match the opening backticks " ‘‘‘" on line 2. You are recommended to fix
either the opening or closing delimiter of the code chunk to use exactly the ## same numbers of backticks and same level of indentation (or blockquote).



Human Forward primer: 5’ CAGCAGCCATTCAAGCAATCC 3

Human Reverse primer: 5’ GGTGGAGACCTAATTGGGCTGATTAG 3

Human Probe: 5’ /5Cy5/TATCGGCGA/TAO/TATCGGTTTCATCCTCG/3IAbRQSp/ 3

Bovine Forward primer: 5’ CAGCAGCCCTACAAGACCTGT 3

Bovine Reverse primer: 5’ GAGGCCAAATTGGGCGGATTAT 3

Bovine Probe: 5’ /5HEX/CATCGGCGACATTGGTTTCATTTTAG/BHQ1/ 3

Swine Forward primer: 5’ ACAGCTGCACTACAAGCAATGC 3

Swine Reverse primer: 5’ GGATGTAGTCCGAATTGAGCTGATTAT 3

Swine Probe: 5’ /56-FAM/CATCGGAGA/ZEN/CATTGGATTTGTCCTAT/3IABkFQ/ 3

Primer/probe reference: Cadwell et al., 200

Amplicons from the NADH dehydrogenase, subunit 5 (ND5) gene were amplified via qPCR from genomic DNA samples using the ND5 FWD and ND5 REV primers, and ND5 Probes corresponding to human, bovine, and swine. A standard curve was generated for each run to correspond to targeted regions of each of the ND5 genes. qPCR reactions contain 4.0 uL of QuantaBio PerfeCTa Multiplex qPCR ToughMix Low ROX (Catalog Number 89497-290), 500 nM of each primer, 300 nM of each probe, 4.0 uL of gDNA (or 1.33 uL of each gBlock for standard curve wells), and 2.4 uL of Nuclease-free H2O for a total reaction volume of 20 uL. qPCR amplification was carried out on the Agilent AriaMx 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

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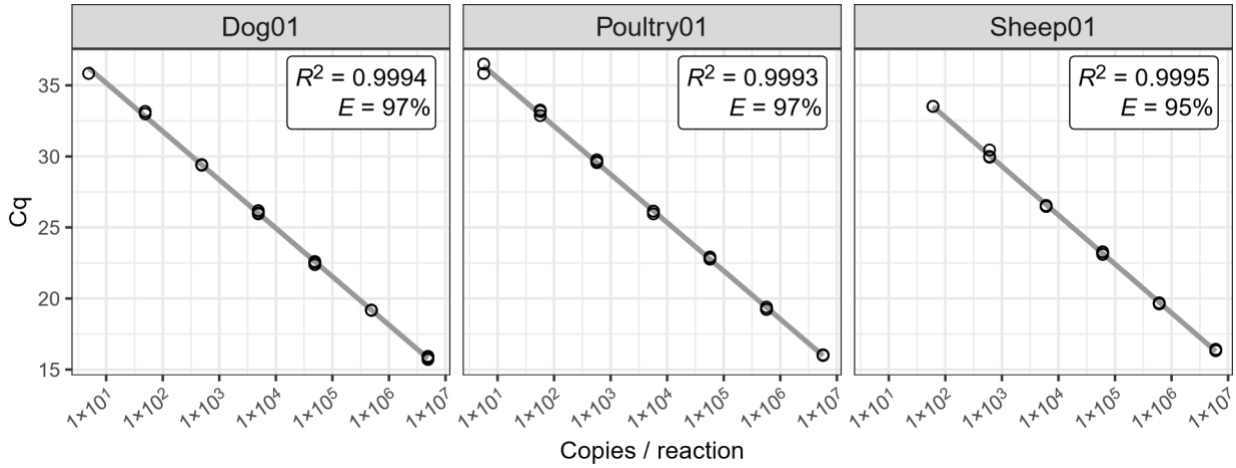

Dog Forward primer: 5’ CACATCTAAGCAACGCAGCATAA 3’

Dog Reverse primer: 5’ AGATCGGCGACTAAAAGTCAGAA 3’

Dog Probe: 5’ /5HEX/TCCGGCCCC/ZEN/TTAGCCAATGCC/3IABkFQ/ 3’

Poultry Forward primer: 5’ CGTYATCACAAACCTATTCTCAGCAAT 3’

Poultry Reverse primer: 5’ TTGGGTTGTCGACTGAAAATCC 3’

Poultry Probe: 5’ /5Cy5/CCCTACATY/TAO/GGACAMACCCTAGTAGAGTGAGCC/3IAbRQSp/ 3’

Sheep Forward primer: 5’ GCAATACACTATACACCTGACACAACAA 3’

Sheep Reverse primer: 5’ CAGATAAAAAATATTGATGCCCCGTTTG 3’

Sheep Probe: 5’ /56-FAM/CTCCTCTGT/ZEN/AACCCACATTTGCCGAGA/3IABkFQ/ 3’

Primer/probe reference: Dancer et al., 2014

Amplicons from the Cytochrome b (Cytb) gene were amplified via qPCR from genomic DNA samples using the Cytb FWD and Cytb REV primers, and Cytb Probes corresponding to dog, poultry, and sheep. A standard curve was generated for each run to correspond to targeted regions of each of the Cytb genes. qPCR reactions contain 4.0 uL of QuantaBio PerfeCTa Multiplex qPCR ToughMix Low ROX (Catalog Number 89497-290), 500 nM of each primer, 300 nM of each probe, 4.0 uL of gDNA (or 1.33 uL of each gBlock for standard curve wells), and 2.4 uL of Nuclease-free H2O for a total reaction volume of 20 uL. qPCR amplification was carried out on the Agilent AriaMx 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.

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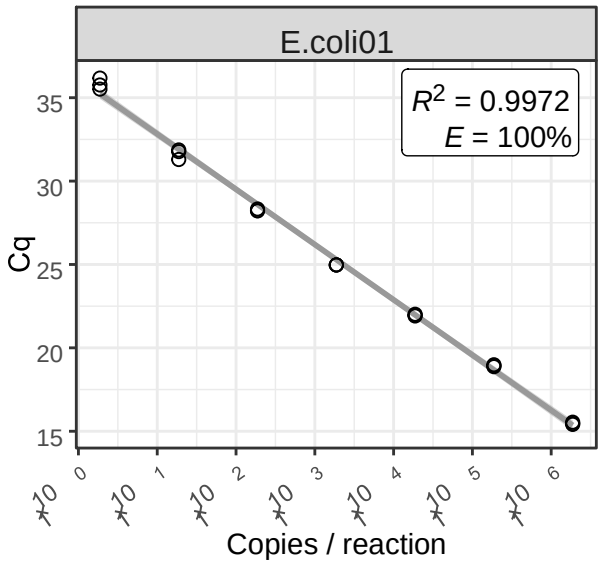

Forward primer: 5’ CAATGGTGATGTCAGCGTT 3

Reverse primer: 5’ ACACTCTGTCCGGCTTTTG 3

Probe: 5’ /56-FAM/TTGCAACTG/ZEN/GACAAGGCACCAGC/3IABkFQ/ 3

Primer/probe reference: Srinivasan et al., 201

An amplicon from the uidA gene was amplified via qPCR from genomic DNA samples using E. coli FWD and REV primers and probe. A standard curve was generated for each run to correspond to targeted region of the E. coli, uidA 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 Agilent AriaMx 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.