Supplementary materials

Table S1 gBlock gene fragments used in this study

gBlock No.	Gene of interest	sequence length	DNA content (in ng/ µl)	copy No. of stock solution
I	PPV-1 PCMV PLHV-3 HEV PCV3	499 bp	1,69	3,3 x 10^9 copies/ µl
II	PLHV-1 PLHV-2 PCV4 SARS-CoV-2 TTSuV-1 TTSuV-2	653 bp	2,62	3,9 x 10^9 copies/ µl
III	pGAPDH huGAPDH PERVpol PCV2	690 bp	1,13	1,6 x 10^9 copies/ µl
V	PCV1 PCV2 PCV3 PCV4 PLHV-3	623 bp	4,72	7,4 x 10^9 copies/ µl

Table S2: Experimental details concerning quality control criteria when applying a $\log_{10}$ dilutional series of the gBlock in the respective qPCR approaches

PCR assay	reference	internal control	PCR efficiency[1]
HEV	[4]	Flu (NA)	0.90
PCMV	[2]	pGAPDH	1.07
PLHV-1	[3]	pGAPDH	1.01
PLHV-2	[3]	pGAPDH	1.01
PLHV-3	[13]	pGAPDH	1.00
PCV1	[12]	pGAPDH	0.90
PCV2	[12]	pGAPDH	1.09
PCV3	[12]	pGAPDH	0.95
PCV4	[12]	pGAPDH	0.85
PPV-1	[1]	pGAPDH	0.94
PERV pol	[11]	pGAPDH	0.95

[1]PCR efficiency curve was calculated with the qPCRsoft software from Analytik Jena (Jena, Germany) and the PCR efficiency was determined as the slope R^2

Table S3 Virus-specific oligosequences integrated in gBlocks I, II, III and IV

GOI*	reference	Oligosequence of the amplicon (5' → 3')
gBlock I		
PPV-1	[1]	CAGAATCAGCAACCTCACCACCAACCAAAATATATAATAATGATCTAACT GCAAGCTTAATGGTCGCACTAGACACCAATAACACACTTCCATACACACC AGCAGC
PCMV	[2]	ACTTCGTCGCAGCTCATCTGAGAGAGCTCGACCGCCGCCCTGGCAACCTC GGAATCCCAGAAC
PLHV-3	[3]	AAGGACCCCAAAGAGGAAAATCAATTTTATGGTTCACCTTCTACCTTTCC TTACAGAGTATGCAGTGCCTCAG
HEV	[4]	GGTGGTTTCTGGGGTGACCGGGCTGATTCTCAGCCCTTCGCAATCCCCTA TATTCATCCAACCAACCCCT
PCV3	[5]	AGTGCTCCCCATTGAACGGTGGGGTCATATGTGTTGAGCCATGGGGTGG GTCTGGAGAAAAAGAAGAGGCTTTGTCCTGGGTGAGCGCTGGTAGTTCCC GCCAGAAGTGGTTTGGGGGTGAAGTAACGGCTGTGT
gBlock II		
PLHV-1	[3]	CTCACCTCCAAATACAGCGACCTGGTCTACTGAATCGCCGCTAACAGGTC ACTATGGAACACACGATTCAAGC
PLHV-2	[3]	GTCACCTGCAAATACACAGGCCTGGTCTACTGAAGCGCTGCCAATAGGTC AATATGGAACATACGATTCAAGCC
PCV4	[6]	CAGCGACCTTAAAGCGGCTGTGGCCGCCCTGAATGCCGGCAGCTCAATGA GTGAAGTGGCCCGTGAGTTCCCGTCTGTATTTATAAGGTATGGGCGTGGC CTCCGGGACTACGTCATTACTGC
SARS-CoV-2	[7]	ACAGGTACGTTAATAGTTAATAGCGTACTTCTTTTTCTTGCTTTCGTGGT ATTCTTGCTAGTTACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGT ACTGCTGCAATAT

TTSuV-1	[8]	CGAATGGCTGAGTTTATGCCGCCAGCGGTAGACAGAACTGTCTAGCGAC TGGGCGGGTGCCGGAGGATCCCTGATCCGGAGTCAAGGGGCCTATC
TTSuV-2	[8]	CGAATGGCTGAGTTTATGCCGCTGGTGGTAGACACGAACAGAGCTGAGT GTCTAACCGCCTGGGCGGGTGCCGGAGCTCCTGAGAGCGGAGTCAAGGG GCTTATC
gBlock III		
pGAPDH	[9]	ACATGGCCTCCAAGGAGTAAGAGCCCCTGGACCACCAACCCCAGCAAGAG CACGCGAGGAGGAGAGAGGCCCTCAGCTGCTGGGGAGTCACAGCCCCAAC TCGATC
huGAPDH	[10]	GGCGATGCTGGCGCTGAGTACGTCGTGGAGTCCACTGGCGTCTTCACCAC CATGGAGAAGGCTGGGGCTCATTTGCAGGGGGGAGCCAAAAGGGTCATCA TCTCTGCCCCCTCTGCTGATGCCCCCATGTTCGTCATGGGTGTGGACCA
PERV pol	[11]	CGACTGCCCCAAGGGTTCAAGAACTCCCCGACCATCTTTGACGAAGCCCT ACACAGGGACCTGGCCAACTTCAGGATCCAACACCCTCAGGTGACCCTCC TCCAGTACGTGGATGACCTGCTTCTGGCGGGAGCCACCAAACAGGACTGC TTAGAAGGTACGAAGGCACTACTGCTGGAATTGTCTGACCTAGGCTACAG AGCCTCTGCTAAGAAGGCCCAGATTTGCAGGAGAGA
PCV2	[12]	CGGATATTGTATTCCTGGTCGTATATACTGTTTTCGAACGCAGTGCCTAG GCCTACGTGGTCTACATTTCCAGCAGTTTGTAGTCTCAGCCATAGCTGAT TTCTTTTGTTGTTTGGTTGGAAGTAATCAATAGTGGAATCTAGGACAGG

Table S2 (Continuation)

GOI	reference	Oligosequence of the amplicon (5’ → 3’)
gBlock IV		
PCV1	[12]	AACCCCATAAGAGGTGGGTGTTCACCCTTAATAATCCTTCCGAGGAGGAG AAAAACAAAATACGGGAGCTTCCAATCTCCCTTTTTGATTATTTTGTTTG CGGAGAGGAAGGTTTGGAAGAGGGTAGAA
PCV2	[12]	CTGAGTCTTTTTTATCACTTCGTAATGGTTTTTATTATTCACTTAGGGTT AAGTGGGGGGTCTTTAAGATTAAATTCTCTGAATTGTACATACATGGTTA TACGGATATTGTAATCCTGGTCGTATATACTGTTTTCGAACGCAGT
PCV3	[12]	CATAAATGCTCCAAAGCAGTGCTCCCCATTGAACGGTGGGGTCATATGTG TTGAGCCATGGGGTGGGTCTGGAGAAAAAGAAGAGGCTTTGTCCTGGGTGA
PCV4	[12]	ATTATTAAACAGACTTTATTTGTGTCATCACTTCGGATACTACACTTGAT CTTAGCCAAAAGGCTCGTTGATTGAGTGATCACTACGCATTATCCCTGT
PLHV-3	[13]	AACAGCGCCAGAAAAAAAGGACCCCAAAGAGGAAAATCAATTTTATGGTT CACCTTCTACCTTTCC

*GOI = gene of interest

Figure S1 Workflow of using gBlock gene fragments as standard for the determination of the copy number using gBlock I as example

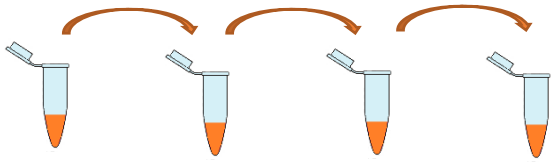
(1) Determination of the genes of interest

(2) Design of a gBlock

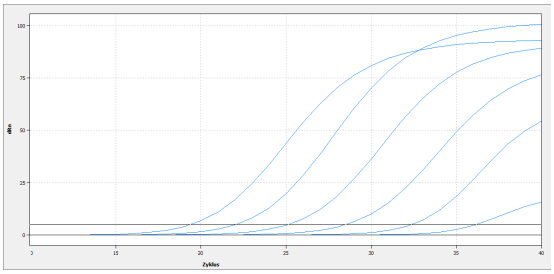
(3) Calculation of the copy number

$$copy\ number\ per\ \mu l = c \times M \times 1 \times 10^{-15}\ \frac{mol}{fmol} \times Avogadro's\ number$$

(4) Preparation of a log_{10} dilutional series



(5) Comparative analysis of the qPCR assays using the log_{10} dilutional series of the gBlock



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