DAkkS
Deutsche
Akkreditierungsstelle
D-PL-19128-01-00

GeneCon
International GmbH

Administration & Laboratory
Grengracht 39
D-52499 Baesweiler
Germany
Fon: +49 (0) 2401 8960182
Fax: +49 (0) 2401 8960183
Mobil: +49 (0) 173 5341373
service@genecon-int.de
www.genecon-int.de

Testreport / Analysis certificate

Customer address

Company:	Pfeifer und Langen GmbH&Co KG Werk Elsdorf
Street/PO box:	Dürener Strasse 40
Country/Town:	D 50189 Elsdorf

Sample to be analysed:

Sample:	F0149-6
Sample arrival:	07.04.2021
Processing period:	07.04.2021 to 21.05.2021
Samplecode GeneCon GmbH:	22105003-10
Sample description:	viscous, transparent, homogenous, 11,1g,

Analysis

- ***Isolation-method:***

°Macherey & Nagel Nucleo Spin Food Kit

Isolation according to standard work instruction GeneCon International GmbH

- ***Qualitative GMO(1) - Screening***

checked on	result		method
SD16	**not detected**	**0,01ppm**	**GCI PCR 90 (2020-01)**

The following controls are included: a) total DNA from the production strain, b) inhibition control via positive-material spiked sample (one per charge), c) negative control

°DNA was isolated of 1g sample material.

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Detection limits are influenced by matrix and processing effects. All test methods are being performed in accordance with the standard operating procedures by GeneCon International GmbH.

[redacted]

A. Wambach
(general manager)

R. Matyjaszczyk
(laboratory manager)

(1) GVO = gentechnisch veränderte Organismen; (2) CaMV = Cauliflower Mosaic Virus; (3) LOD = Limit of Detection; (4) LOQ = Limit of Quantitation

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Sample to be analysed:

Sample:	F0149-7
Sample arrival:	07.04.2021
Processing period:	07.04.2021 to 21.05.2021
Samplecode GeneCon GmbH:	22105003-20
Sample description:	viscous, transparent, homogenous, 11,1g,

Analysis

- ***Isolation-method:***

°Macherey & Nagel Nucleo Spin Food Kit

Isolation according to standard work instruction GeneCon International GmbH

- ***Qualitative GMO(1) - Screening***

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(general manager) (laboratory manager)

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Sample to be analysed:

Sample:	F0149-10
Sample arrival:	07.04.2021
Processing period:	07.04.2021 to 21.05.2021
Samplecode GeneCon GmbH:	22105003-30
Sample description:	viscous, transparent, homogenous, 11,1g,

Analysis

- ***Isolation-method:***

°Macherey & Nagel Nucleo Spin Food Kit

Isolation according to standard work instruction GeneCon International GmbH

- ***Qualitative GMO(1) - Screening***

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Sample to be analysed:

Sample:	F0152-3
Sample arrival:	07.04.2021
Processing period:	07.04.2021 to 21.05.2021
Samplecode GeneCon GmbH:	22105003-40
Sample description:	viscous, homogenous, transparent, 11,1g,

Analysis

- ***Isolation-method:***

°Macherey & Nagel Nucleo Spin Food Kit

Isolation according to standard work instruction GeneCon International GmbH

- ***Qualitative GMO(1) - Screening***

checked on	result		method
SD16	**not detected**	**0,01ppm**	**GCI PCR 90 (2020-01)**

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[redacted]

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Sample to be analysed:

Sample:	F0152-5
Sample arrival:	07.04.2021
Processing period:	07.04.2021 to 21.05.2021
Samplecode GeneCon GmbH:	22105003-50
Sample description:	viscous, homogenous, transparent, 11,1g,

Analysis

- ***Isolation-method:***

°Macherey & Nagel Nucleo Spin Food Kit

Isolation according to standard work instruction GeneCon International GmbH

- ***Qualitative GMO(1) - Screening***

checked on	result		method
SD16	**not detected**	**0,01ppm**	**GCI PCR 90 (2020-01)**

The following controls are included: a) total DNA from the production strain, b) inhibition control via positive-material spiked sample (one per charge), c) negative control

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Sample to be analysed:

Sample:	F0152-7
Sample arrival:	07.04.2021
Processing period:	07.04.2021 to 21.05.2021
Samplecode GeneCon GmbH:	22105003-60
Sample description:	viscous, homogenous, transparent, 11,1g,

Analysis

- ***Isolation-method:***

°Macherey & Nagel Nucleo Spin Food Kit

Isolation according to standard work instruction GeneCon International GmbH

- ***Qualitative GMO(1) - Screening***

checked on	result		method
SD16	**not detected**	**0,01ppm**	**GCI PCR 90 (2020-01)**

The following controls are included: a) total DNA from the production strain, b) inhibition control via positive-material spiked sample (one per charge), c) negative control

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Sample to be analysed:

Sample:	F0155-5
Sample arrival:	07.04.2021
Processing period:	07.04.2021 to 21.05.2021
Samplecode GeneCon GmbH:	22105003-70
Sample description:	viscous, homogenous, transparent, 11,2g,

Analysis

- ***Isolation-method:***

°Macherey & Nagel Nucleo Spin Food Kit

Isolation according to standard work instruction GeneCon International GmbH

- ***Qualitative GMO(1) - Screening***

checked on	result		method
SD16	**not detected**	**0,01ppm**	**GCI PCR 90 (2020-01)**

The following controls are included: a) total DNA from the production strain, b) inhibition control via positive-material spiked sample (one per charge), c) negative control

°DNA was isolated of 1g sample material.

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Sample to be analysed:

Sample:	F0155-15
Sample arrival:	07.04.2021
Processing period:	07.04.2021 to 21.05.2021
Samplecode GeneCon GmbH:	22105003-80
Sample description:	viscous, homogenous, transparent, 11g,

Analysis

- ***Isolation-method:***

°Macherey & Nagel Nucleo Spin Food Kit

Isolation according to standard work instruction GeneCon International GmbH

- ***Qualitative GMO(1) - Screening***

checked on	result		method
SD16	**not detected**	**0,01ppm**	**GCI PCR 90 (2020-01)**

The following controls are included: a) total DNA from the production strain, b) inhibition control via positive-material spiked sample (one per charge), c) negative control

°DNA was isolated of 1g sample material.

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Sample to be analysed:

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Processing period:	07.04.2021 to 21.05.2021
Samplecode GeneCon GmbH:	22105003-90
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Isolation according to standard work instruction GeneCon International GmbH

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Validation and verification report for PCR-detection system (GCI 90 2020-01) for the *SD16*-sequence of *Pseudomonas cichorii*

incl. extension of validation for the matrix enzymesolution

Gene of interest *SD16*:

[redacted]

Primer sequences:

The primer sequences were provided by “Pfeifer und Langen GmbH & Co. KG”. and verified via blasting with “*Primer 3 Output*” against the specific *SD16*-gene as well as against the production strain *E.coli BL21(DE3)* and further *E.coli*-strains.

Primer:	
PL0013 F	[redacted]
PL0014 R	[redacted]

Probe-sequence:

The probe sequence was provided by “Pfeifer und Langen GmbH & Co.KG” as well. This sequence was verified via blasting with “*Primer 3 Output*” against the specific *SD16*-gene as well as against the production strain *E.coli BL21(DE3)* and further *E.coli*-strains, also.

Probesequenz P&L	[redacted]

The 3’-quencher “[redacted]” was exchanged against the 3’-quencher “[redacted]”.

Probe: [redacted]

Positive DNA:

Positive DNA was isolated from "*E.coli* BL21 (DE3) ΔarsB::SD16/ ΔglvBC::SD16, Klon B3/12" and provided by "Pfeifer und Langen GmbH & Co.KG" subsequently.

PCR conditions:

Several PCR conditions were tested like 2- or 3-step PCR or the annealing temperature which was defined in first place by the melting point of the primer system and was specified in following steps:

Mpt(Melting point temperature): X+4°C
Mpt: X+2°C
Mpt X
Mpt X-2°C
Mpt X-4°C

Primer and Probe concentration were determined via several dilution series, whereas concentrations of the other reagents were based on the information provided by the manufacturer.

Limit of detection:

Serial dilutions of the positive DNA provided were carried out in multiple determinations. The dilution level at which reproducible results were obtained is used as limit of detection (LOD). As shown in fig.1, the samples with a concentration up to 1ng/mL *SD16*-DNA (SD16^4, see table 1; see fig.1) could be detected stable and reproducibly. Samples with a concentration of 0,0001ng/µL *SD16* were not detected reproducibly. The negative control H_2O showed no amplification.

Therefore the LOD of the PCR-detection system (GCI 90 2020-01) for the *SD16*-sequence of *Pseudomonas cichorii* is **1 ng/ml *SD16*-DNA.**

Table 1: Series of dilutions of positive DNA extracted from "in *E.coli* BL21 (DE3) ΔarsB::SD16/ ΔglvBC::SD16, Klon B3/12"

Positive DNA	DNA [ng/µL]	DNA [pg/µL]	Dilution factor
SD16	117	measured	
SD16^1	1	calculated	:11,7
SD16^2	0,1	calculated	:117
SD16^3	0,01	calculated	:1170
SD16^4	0,001	calculated	:11700
SD16^5	0,0001	calculated	:23400

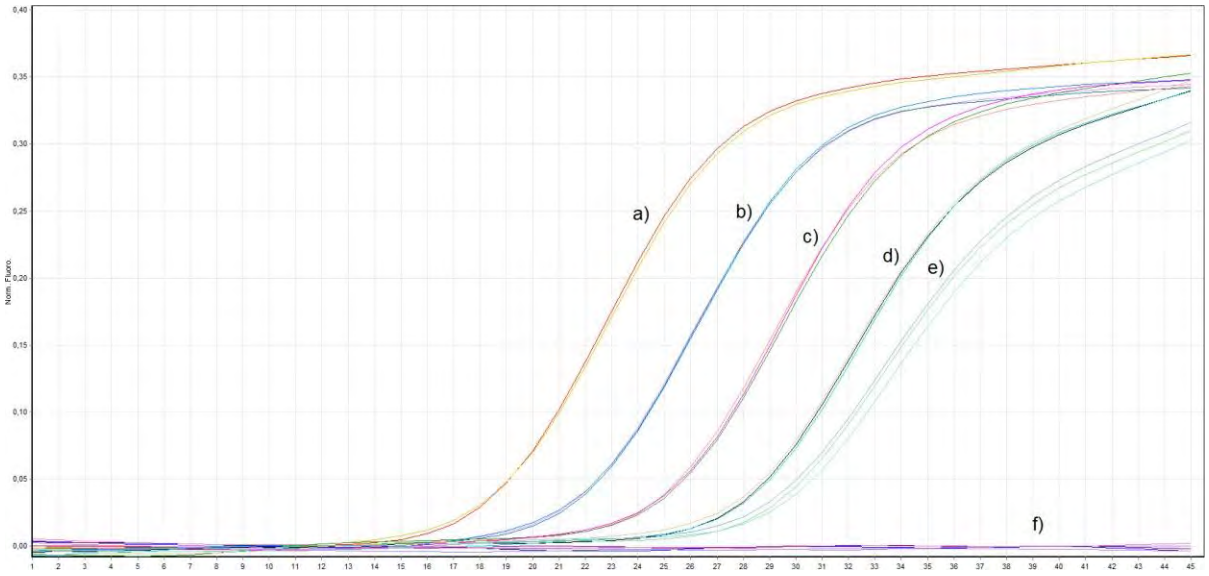

Figure 1: fluorescence curves during PCR amplification of *SD16*-gene in *E.coli* BL21 (DE3) ΔarsB::SD16/ ΔglvBC::SD16, Klon B3/12" in several dilutions (see table1): a) positive control *SD16* [DNA:117ng/µL]; b) *SD16*^1 [DNA:1ng/µL]; c) *SD16*^2 [0,1ng/µL]; d) *SD16*^3 [0,01ng/µL]; e) *SD16*^4 [0,001ng / mL]; f) *SD16*^5 [0,0001ng/µL] and negative control H_2O; coordinate: number of PCR cycles; y-coordinate: normalized fluorescence

LOD *SD16* final: 1ng/ml

LOD matrix syrup (enzymesolution):

For validation of the PCR-detection system (GCI 90 2020-01) for the *SD16*-sequence of *Pseudomonas cichorii* in the enzymesolution a real sample (F0155_5), provided by "Pfeifer & Langen GmbH & Co.KG", was spiked with positive DNA from "*E.coli* BL21 (DE3) ΔarsB::SD16/ ΔglvBC::SD16, Klon B3/12" in several dilutions before the DNA-extraction of the matrix in a way that the extracted sample had defined concentrations in 1mL spiked sample (see table 2). The DNA of those spiked samples were extracted and tested afterwards to determine the LOD of the target gene in the matrix of the extracted enzymesolution.

Table 2: Series of *SD16*-spiked samples with final concentrations before DNA-extraction

Spiked Samples	Final concentration SD16 spiked enzymsolutionsample [ng/µL]	
SD16_spike	1	calculated
SD16_spike ^1	0,1	calculated
SD16_spike ^2	0,01	calculated
SD16_spike ^3	0,001	calculated
SD16_spike ^4	0,0001	calculated

As control DNA positive DNA from "*E.coli* BL21 (DE3) ΔarsB::SD16/ ΔglvBC::SD16, Klon B3/12" was used. Negative control was nuclease-free H_2O, as well as unspiked extracted DNA of the provided enzymesolution.
As shown in fig.1, the samples with a spiked concentration up to 1ng/mL *SD16*-DNA (SD16_spike ^3, see table 2; see fig.2) before DNA-extraction could be detected stable and reproducibly. The negative samples H_2O and unspiked sample-material show no amplification. Samples spiked to a concentration of 0,0001ng/µL were not detected reproducibly.
The detected samples spiked with DNA from the enzymesolution show that inhibition control works with the positive DNA-spiked enyzmesolution-matrix as well.

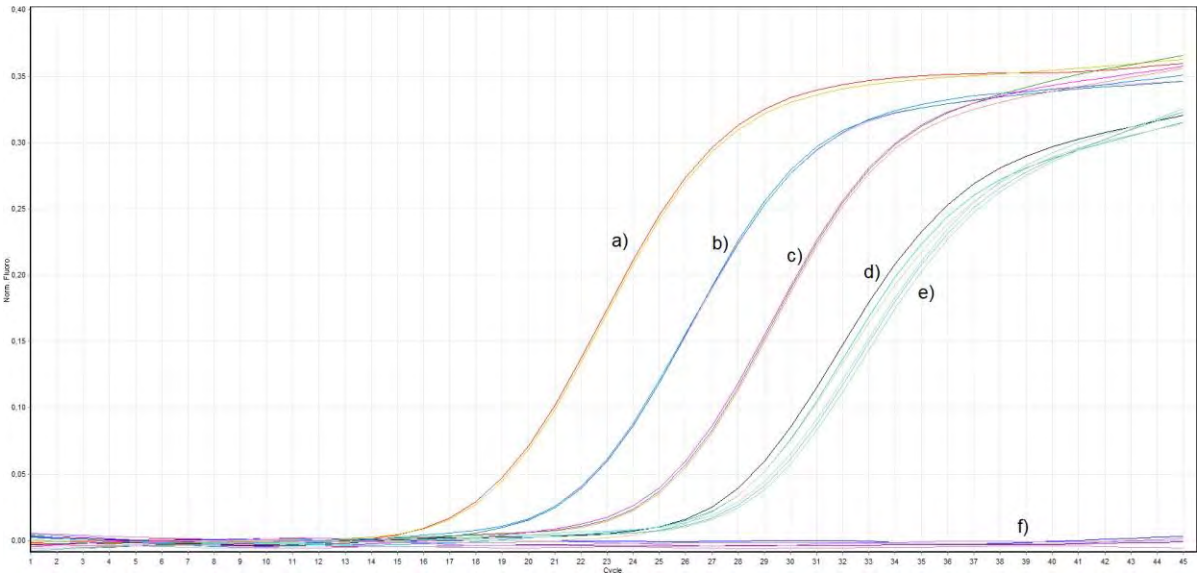

Figure 2: fluorescence curves during PCR amplification of SD16-gene in several samples: a) positive control E.coli BL21 (DE3) ΔarsB::SD16/ ΔglvBC::SD16, Klon B3/12 [DNA: 117ng/µL]; b) SD16_spike; c) SD16_spike^1; d) SD16_spike^2; e) SD16_spike^3; f) SD16_spike^4 and negative controls: unspiked

enzymesolution-sample and H2O; coordinate: number of PCR cycles; y-coordinate: normalized fluorescence; DNA concentration: see table 2

The results of the validation of of the PCR-detection system (GCI 90 2020-01) for the SD16-sequence of Pseudomonas cichorii in the spiked enzymesolution (sample matrix) confirm the previous results of the detection of *SD16* in extracted DNA from "*E.coli* BL21 (DE3) ΔarsB::SD16/ ΔglvBC::SD16, Klon B3/12". Therefore it is shown, that the used method of DNA-extraction has no influence on the detection limit and performance of the detection-system.
Due to the fact, that the desired LOD of this PCR-detection system is 0,01ng/µL SD16, it is possible to use a sample *SD16* [0,01ng/µL] as reference to exclude false positive results with a lower concentration.