

DETECTION OF QUARANTINE AND BLACKLEG DISEASE-CAUSING BACTERIA IN POTATO SEEDS BY PCR

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September 2, 2019.*



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BIOREBA AG



- Family-owned company (12 collaborators)
- Partner in Agro-diagnostics (plant pathogens) since 1980
- Export in > 100 countries
- ISO 9001 certified
- ISO/IEC 17025 accredited lab
- R+D, production, logistic and sales in Switzerland



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Deutschland

Polen

Belgien

Tschechische Republik

Luxemburg

Slowakei

Schweiz

Österreich

Frankreich

Ungarn

Liechtenstein

Slowenien

Kroatien

Bosnien und Herzegowina

Monaco

San Marino

Italien

Serbien

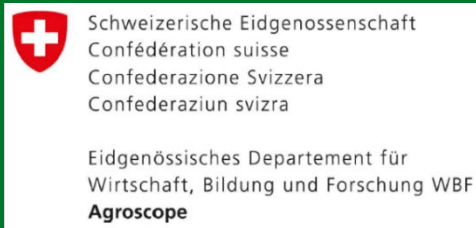
Montenegro

Google

Blackleg disease testing on seed potatoes

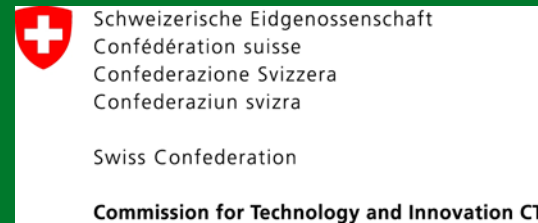


Partners in Switzerland



Contractor

-Development of PCR-based diagnostic tests (CTI project)



-Sample preparation

-DNA extraction and analysis

Blackleg disease in Switzerland

- Testing is done mainly on imported seed potato lots
- Mostly classes SE, S (A).
- We test the following pathogens:
 - *Dickeya* sp.
 - *Pectobacterium atrosepticum*
 - *Pectobacterium wasabiae* (*P. parmentieri* on potato)
 - *Pectobacterium carotovorum* subsp. *brasiliense*

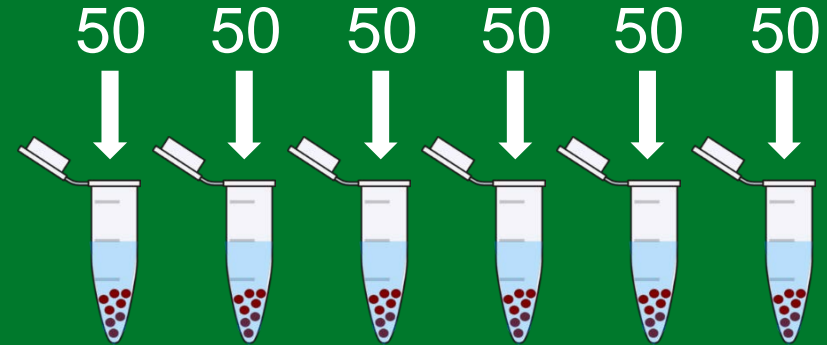
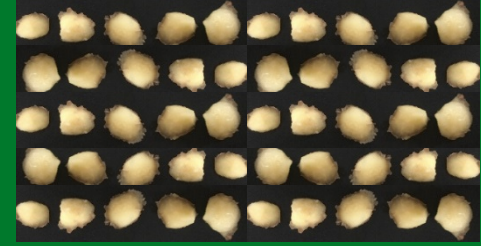
Step 1: Sample preparation



300 tubers = 1 sample



Subsamples 6 x 50

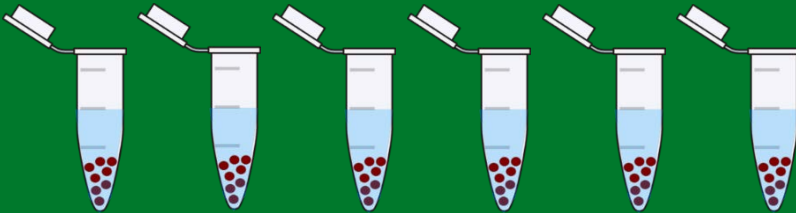


Step 2: DNA Extraction

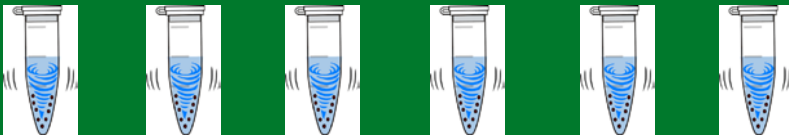


Enrichment:

6 x 50: 48h bei 26° C

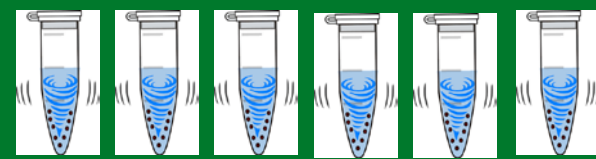
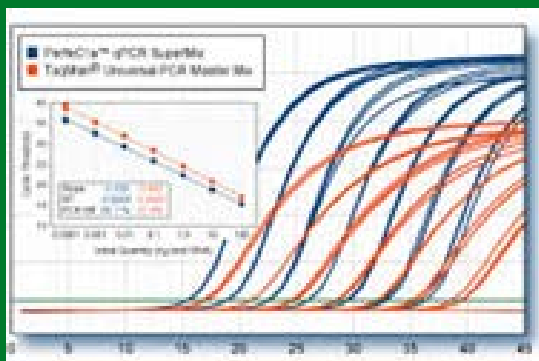


6 x 50: DNA Extraktion





Step 3: PCR Analysis

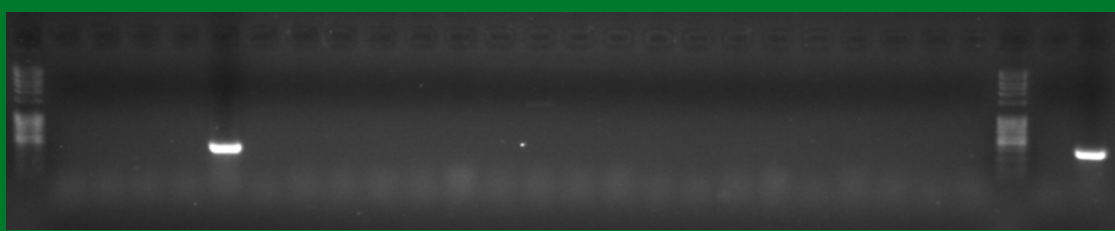


6 x qPCR:

- *Dickeya* sp. (Dsp)
- *Pectobacterium atrosepticum* (Patr)

6 x PCR: *Pectobacterium wasabiae* (*parmentieri*) (Pwas)

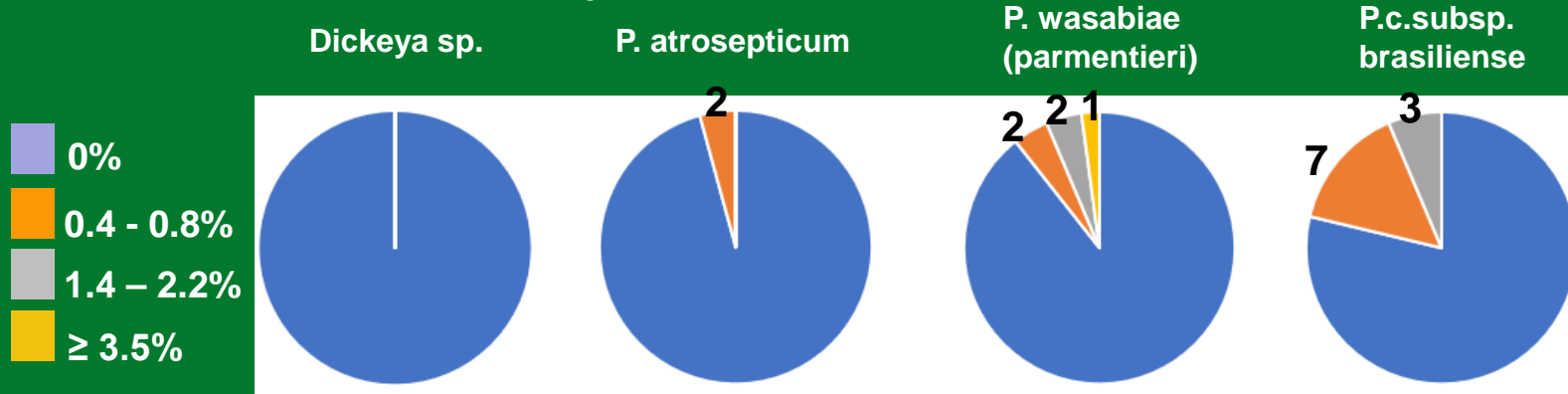
6 x PCR: *Pectobacterium carotovorum* ssp. *brasiliense* (Pcbr)



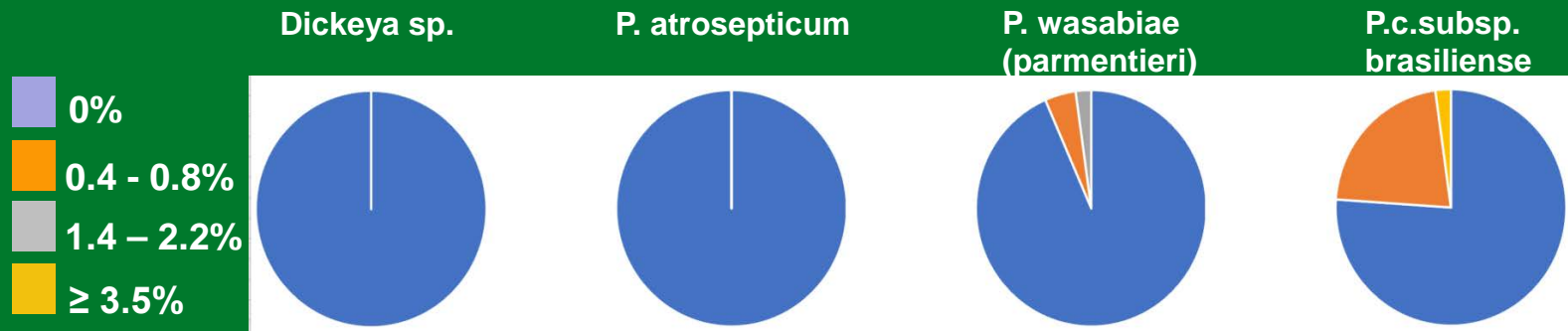
Total: 18 PCR
Reactions per seed
potato lot

Results in 2017 and 2018

2018: 47 imported seed lots



2017: 46 imported seed lots



Quarantine bacteria testing

Clavibacter michiganensis subsp. sepedonicus (Cms)
(causal agent of bacterial ring rot)

Ralstonia solanacearum (Rs)
(causal agent of bacterial brown rot)



Quarantine bacteria: workflow

Customers

Collection of the seed lots (200 tubers each)

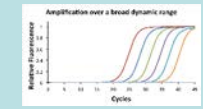
Sample preparation

DNA extraction and qPCR

Seed potato company

Lab in Germany

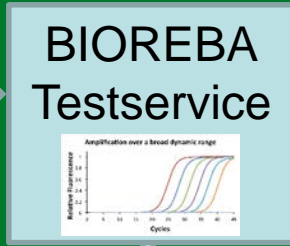
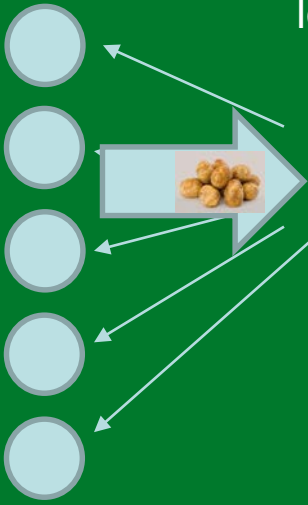
BIOREBA Testservice



Lab-bill to seed company

BIOREBA-bill to seed company

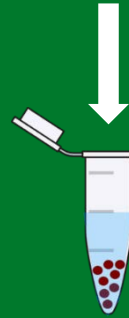
BIOREBA – reporting direct to customers



Quarantine bacteria: sampling



200 tubers = 1 sample



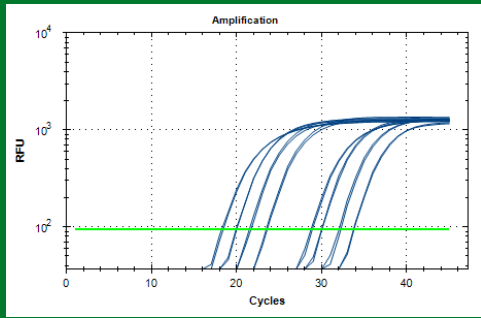
DNA extraction

qPCR analysis

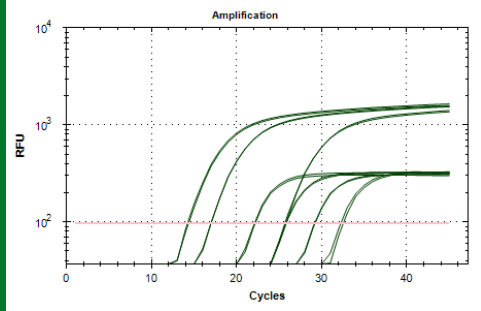


Quarantine bacteria: qPCR

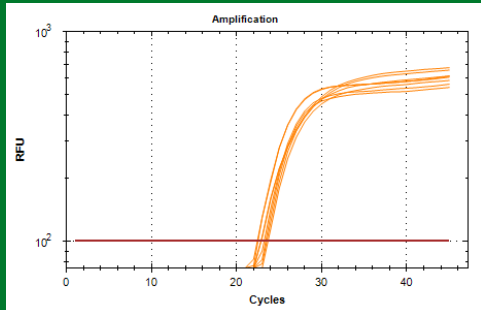
Triplex qPCR:



Clavibacter michiganensis subsp. sepedonicus (Cms)



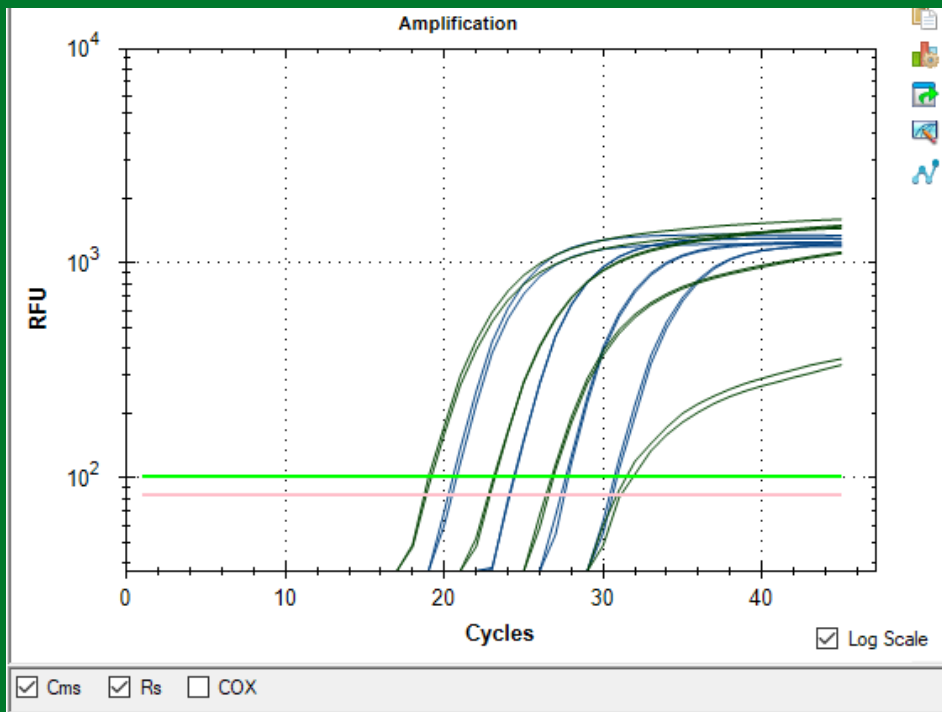
Ralstonia solanacearum (Rs)



Internal control gene (Cox)



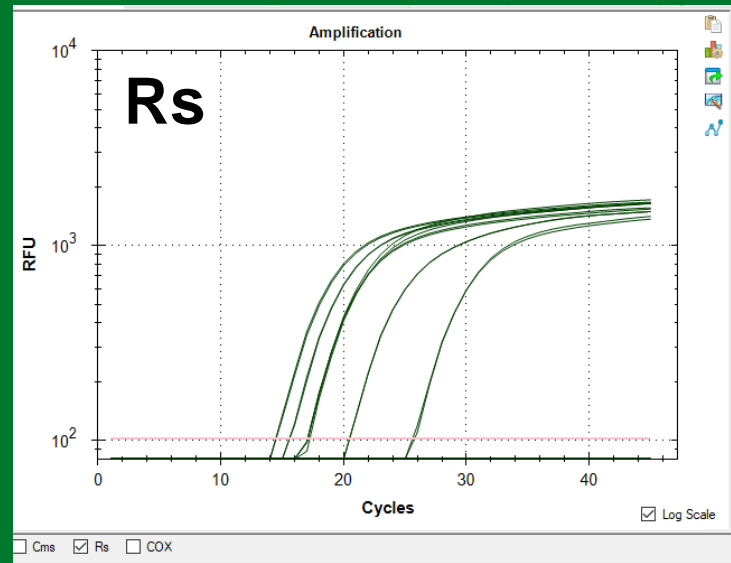
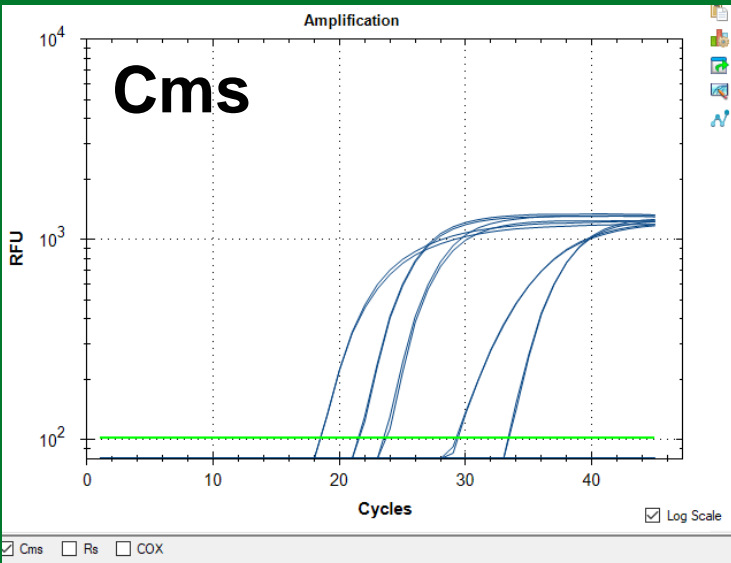
Quarantine bacteria: standards



Well	Fluor	Target	Content	Sample	Cq
A11	FAM	Cms	Unkn-41	CR10e5	30.66
A12	FAM	Cms	Unkn-41	CR10e5	30.85
F09	FAM	Cms	Unkn-38	CR10e8	20.47
F10	FAM	Cms	Unkn-38	CR10e8	20.73
G09	FAM	Cms	Unkn-39	CR10e7	24.35
G10	FAM	Cms	Unkn-39	CR10e7	24.31
H09	FAM	Cms	Unkn-40	CR10e6	27.59
H10	FAM	Cms	Unkn-40	CR10e6	27.75
A11	HEX	Rs	Unkn-41	CR10e5	30.84
A12	HEX	Rs	Unkn-41	CR10e5	31.09
F09	HEX	Rs	Unkn-38	CR10e8	18.65
F10	HEX	Rs	Unkn-38	CR10e8	18.81
G09	HEX	Rs	Unkn-39	CR10e7	22.82
G10	HEX	Rs	Unkn-39	CR10e7	22.71
H09	HEX	Rs	Unkn-40	CR10e6	26.33
H10	HEX	Rs	Unkn-40	CR10e6	26.48

Cms/Rs double-infected samples: dilution serie from bacteria spiked into healthy tuber sap.

Quarantine bacteria: example



Well	Fluor	Target	Content	Sample	Cq
C09	FAM	Cms	Unkn-35	Cms #2016-5-3-7 1:10	23.56
C10	FAM	Cms	Unkn-35	Cms #2016-5-3-7 1:10	23.80
D07	FAM	Cms	Unkn-28	Rs Stamm D2 10e8	N/A
D08	FAM	Cms	Unkn-28	Rs Stamm D2 10e8	N/A
E07	FAM	Cms	Unkn-29	Rs #50137 (25.01.07)	N/A
E08	FAM	Cms	Unkn-29	Rs #50137 (25.01.07)	N/A
E09	FAM	Cms	Unkn-37	Cms #2017-5-3-19 1:10	33.40
E10	FAM	Cms	Unkn-37	Cms #2017-5-3-19 1:10	33.33
F05	FAM	Cms	Unkn-22	Rs Race 1 10e6	N/A
F06	FAM	Cms	Unkn-22	Rs Race 1 10e6	N/A
F07	FAM	Cms	Unkn-30	Cms #2016-5-3-4	18.50
F08	FAM	Cms	Unkn-30	Cms #2016-5-3-4	18.43
G07	FAM	Cms	Unkn-31	Cms #2016-5-3-4 1:10	21.64
G08	FAM	Cms	Unkn-31	Cms #2016-5-3-4 1:10	21.55
H05	FAM	Cms	Unkn-24	Rs Stamm R2 10e8	N/A
H06	FAM	Cms	Unkn-24	Rs Stamm R2 10e8	N/A
H07	FAM	Cms	Unkn-32	Cms #2016-5-3-5	29.21
H08	FAM	Cms	Unkn-32	Cms #2016-5-3-5	29.33

Well	Fluor	Target	Content	Sample	Cq
B07	HEX	Rs	Unkn-26	Rs Stamm 97 10e8	17.18
B08	HEX	Rs	Unkn-26	Rs Stamm 97 10e8	17.06
B11	HEX	Rs	Unkn-42	NTC	N/A
B12	HEX	Rs	Unkn-42	NTC	N/A
C07	HEX	Rs	Unkn-27	Rs Stamm D1 10e8	15.69
C08	HEX	Rs	Unkn-27	Rs Stamm D1 10e8	15.63
C09	HEX	Rs	Unkn-35	Cms #2016-5-3-7 1:10	N/A
C10	HEX	Rs	Unkn-35	Cms #2016-5-3-7 1:10	N/A
D07	HEX	Rs	Unkn-28	Rs Stamm D2 10e8	14.54
D08	HEX	Rs	Unkn-28	Rs Stamm D2 10e8	14.46
E07	HEX	Rs	Unkn-29	Rs #50137 (25.01.07)	20.45
E08	HEX	Rs	Unkn-29	Rs #50137 (25.01.07)	20.46
E09	HEX	Rs	Unkn-37	Cms #2017-5-3-19 1:10	N/A
E10	HEX	Rs	Unkn-37	Cms #2017-5-3-19 1:10	N/A
F05	HEX	Rs	Unkn-22	Rs Race 1 10e6	25.68
F06	HEX	Rs	Unkn-22	Rs Race 1 10e6	25.83
F07	HEX	Rs	Unkn-30	Cms #2016-5-3-4	N/A
F08	HEX	Rs	Unkn-30	Cms #2016-5-3-4	N/A
G07	HEX	Rs	Unkn-31	Cms #2016-5-3-4 1:10	N/A

Rs phylotypes I, II, and III are detected

Quarantine bacteria: reporting

TES.004.2.PRO, Version: 7 - 03.04.2018

Analysenbericht

Seite 1 von 1

Reinach, 13.06.2019

Vador Dark
Schattenseite 5
31724 Schwarzersterne

Sehr geehrter Herr Vador

Wir haben Ihre Probe auf das Vorkommen der Erreger der Quarantänekrankheiten Ringfäule, *Clavibacter michiganensis* subsp. *sepedonicus* (Cms), und der Schleimkrankheit, *Ralstonia solanacearum* (Rs), mit folgendem Ergebnis untersucht:

Probenbezeichnung:		Ergebnis:	
Testnr.:	2019 - 6 - 5 - 5	Ringfäule (Cms):	negativ
Probennr.:	OGK-151-19-707	Schleimkrankheit (Rs):	negativ
Sorte:	Solist		
OGK Nr. oder Probenehmer:	OGK151000000195		

Wir möchten uns sehr für das Vertrauen bedanken, welches Sie unserer Dienstleistung entgegenbringen. Falls Sie Fragen zu den angewandten Methoden und Messunsicherheiten, oder andere Fragen zum Ergebnis haben, zögern Sie nicht uns per Email (admin@bioreba.ch) oder telefonisch zu kontaktieren.

Dieser Bericht darf ohne schriftliche Genehmigung der BIOREBA AG nicht auszugsweise kopiert werden.

Mit freundlichen Grüßen



Dr. Denise Altenbach,
Leiterin Testservice



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Thank you



Your BIOREBA Team

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