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Electronic Supporting Information

International ring-trial for the validation of an event-specific Golden Rice 2 quantitative real-time PCR method

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Supplementary Materials and Methods

Materials. Frozen leaves of non-GM rice (*Oryza sativa* L. ssp. Japonica, cultivar Kaybonnet) and GM rice (homozygous for GR2-R transformation event, cv. Kaybonnet) were provided by IRRI as individual packages each containing leaves from a single rice plant. Genomic DNA was extracted from each plant independently and DNA extracted from plants with the same genetic background was pooled and homogenised only after it was checked for quality and for absence of contamination.

DNA extraction. Genomic DNA was extracted from rice leaves ground with liquid nitrogen with either a CTAB DNA extraction method (modified from ISO 21571 [a]) or a phenol-chloroform method optimised for rice DNA extraction (modified from Sambrook and colleagues[b]). The integrity of the extracted DNA was checked with a 1 % agarose gel electrophoresis and the absence of DNA inhibition was assessed as described by Zel and colleagues.[c] The extracted DNA was quantified fluorimetrically using the Quant-iT™ PicoGreen dsDNA Assay Kit (Molecular Probes, Eugene, U.S.), on the basis of a five point standard curve ranging from 1 to 500 ng/mL, with a Biorad (Hercules, U.S.) VersaFluor fluorometer. Before sample preparation the concentration of each DNA extract was measured ten times, and the ten values were averaged. Only non-degraded DNA free from inhibitors was used for qPCR experiments.

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[a] International Standard (ISO) 21571:2005 *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction*. International Organization for Standardization, Geneva, 2005.

[b] Sambrook, J., Fritsch, E.F., Maniatis, T. 1989 *Molecular Cloning: A Laboratory Manual*, 2nd Edition; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989; Vol. 3, pp. E3-E4.

[c] Žel, J., Mazzara, M., Savini, C., Cordeil, S., Camloh, M., Štebih, D., Cankar, K., Gruden, K., Morisset, D., Van den Eede, G. Method Validation and Quality Management in the Flexible Scope of Accreditation: An Example of Laboratories Testing for Genetically Modified Organisms. *Food Anal. Methods* **2008**, *1*, 61-72.

Table S1. Primer and probe sequences for taxon-specific PLD-GR assay and GR2 event-specific assay, and amplicon size

Name	Sequence (5'–3')	Amplicon size (bp)
PLD-GR For	GGCAAGAACAACACCATTGACA	
PLD-GR Rev	CGGCTTTCCATGCAAATGA	124
PLD-GR Probe	FAM-ATTCGCCGCGCCAAGAACTTCA-TAMRA	
GR2 For	TGGCCGTATCCGCAATGTG	
GR2 Rev	GCTGCGCCTCTAACCAGGTAT	121
GR2 Probe	FAM-TCGATATGATTCCTTCATCGAGCGAGC-TAMRA	

Table S2. PLD standard curve samples DNA content per reaction, expressed in hge and in weight

Sample code	S1 PLD	S2 PLD	S3 PLD	S4 PLD	S5 PLD
PLD taxon-specific hge per reaction	250 000	25 000	2 500	250	40
Total amount of DNA per reaction (ng/5 µl)	125	12.5	1.25	0.125	0.02

Table S3. PLD blind samples DNA content per reaction, expressed in hge and in weight

Sample code	B1 PLD	B2 PLD	B3 PLD	B4 PLD	B5 PLD
PLD taxon-specific hge per reaction	200 000	20 000	2 000	200	50
Total amount of DNA per reaction (ng/5 µl)	100	10	1.0	0.10	0.025

Table S4. GR2 standard curve samples DNA content per reaction, expressed in hge and in weight

Sample code	S1 GR2	S2 GR2	S3 GR2	S4 GR2	S5 GR2
GR2 hge per reaction	25 000	5 000	1 000	200	40
Total amount of DNA per reaction (ng/5 µl)	12.5	2.5	0.5	0.10	0.02

Table S5. GR2 blind samples GM and non-GM DNA content per reaction, expressed in hge, in percentage and in weight

Sample code	**B1 GR2**	**B2 GR2**	**B3 GR2**	**B4 GR2**	**B5 GR2**
GR2 hge per reaction (GM hge/reaction)	20 000	6 000	1 800	200	50
PLD taxon-specific hge per reaction (rice hge/reaction)	200 000	200 000	200 000	200 000	200 000
GR2 GM % (GM hge/rice hge x 100)	10.0	3.0	0.9	0.1	0.025
Total amount of DNA per reaction (ng/5 µl)	100	100	100	100	100

Table S6. Laboratories participating to the international validation study

Laboratory	Country
Agro-Product Safety Research Center, Chinese Academy of Inspection and Quarantine No A3	CN
ANSES Laboratoire de la Santé des Végétaux, unité bactériologie, virologie et OGM	FR
Austrian Agency for Health and Food Safety, Competence Centre for Biochemistry	AT
Department of Food Science & Biotechnology, Kyung Hee University	KR
Food Safety & Quality Microbial & Molecular Analytics, Nestec Ltd, Nestlé Research Center	CH
GMO Analytical Evaluation Laboratory, National Food Research Institute	JP
GMO Unit, Environmental Health Division, Department of Chemistry Malaysia	MY
Joint Center for Life Sciences, School of life science and Biotechnology, Shanghai Jiao Tong University	CN
Landesamt für Verbraucherchutz des Landes Sachsen-Anhalt, Fachbereich Lebensmittelsicherheit	DE
National Centre for Food, Spanish Food Safety Agency and Nutrition, Biotechnology Unit	ES
National Institute of Biology	SI
Post Entry Quarantine Station Molecular and Biotechnology Laboratory, Bureau of Plant Industry	PH
Referral Centre for Molecular Diagnosis of Transgenic Planting Material, National Bureau of Plant Genetic Resources	IN
Veterinary Public Health Institute for Lazio and Toscana Regions, National Reference Centre for GMO Analysis	IT
Veterinary Public Health Laboratory, Agri-Food & Veterinary Authority of Singapore	SG
Walloon Agricultural Research Centre — Department Valorization of Agricultural Products	BE

Table S7. Hge content values for PLD blind samples obtained by averaging the hge content determined by participating laboratories (LAB) with the PLD-GR taxon-specific assay for the four replicated samples of each level; outlying values are marked with an asterisk

Haploid genome equivalents content					
LEVEL / **LAB**	**50**	**200**	**2 000**	**20 000**	**200 000**
1	34	150	1 869	20 736	182 609
2	21	97	1 580	24 739	182 674
3	36	140	1 885	22 058	198 527
4	33	124	1 703	21 121	185 232
5	42	149	1 872	19 532	196 362
6	34	146	1 874	21 229	202 428
7	33	155	1 989	23 071	195 423
8	33	136	1 942	23 754	210 409
9	27	104	1 726	23 022	202 514
10	40	169	2 092	21 078	182 481
11	33	135	1 362*	21 210	201 611
12	35	140	1 885	20 472	183 702
13	35	143	1 815	16 602*	200 787
14	41	183	2 388	28 869	267 765*
15	44	164	2 164	25 333	175 993
16	35	120	1 789	18 797	155 825

Table S8. Hge content values determined for PLD blind samples by participating laboratories (LAB) with the PLD-GR taxon-specific assay; outlying series are highlighted in bold. Hge values highlighted in italic were extrapolated beyond the range of the standard curve

Haploid genome equivalents content																				
SAMPLE	**50**				**200**				**2 000**				**20 000**				**200 000**			
LAB	**REP 1**	**REP 2**	**REP 3**	**REP 4**	**REP 1**	**REP 2**	**REP 3**	**REP 4**	**REP 1**	**REP 2**	**REP 3**	**REP 4**	**REP 1**	**REP 2**	**REP 3**	**REP 4**	**REP 1**	**REP 2**	**REP 3**	**REP 4**
1	*28*	*26*	43	39	142	164	144	150	1 923	2 176	1 821	1 556	19 246	19 528	20 730	23 441	169 560	179 681	188 567	192 630
2	*17*	*23*	*24*	*22*	130	86	78	93	1 578	1 922	1 378	1 443	24 872	25 275	24 875	23 936	167 344	182 336	201 149	179 866
3	35	40	35	34	150	131	149	130	1 937	1 638	1 947	2 018	22 121	21 615	22 836	21 659	203 187	202 774	199 358	188 789
4	*29*	40	*33*	*32*	168	118	96	113	1 537	1 690	2 069	1 516	19 396	22 871	21 044	21 174	209 398	205 219	163 489	162 822
5	42	56	40	31	148	163	148	138	1 774	1 934	2 077	1 702	17 092	19 935	17 561	23 539	208 690	199 127	174 263	203 369
6	39	*31*	*29*	39	190	121	148	124	1 872	1 868	1 733	2 024	21 425	23 398	20 336	19 757	207 139	196 544	202 302	203 727
7	*30*	35	38	*30*	161	163	147	147	2 084	1 939	1 902	2 033	23 635	22 397	23 173	23 081	189 737	199 253	196 885	195 818
8	*31*	47	*33*	*22*	146	148	113	139	1 895	1 953	1 958	1 961	22 788	24 371	21 695	26 161	185 366	208 812	205 502	241 956
9	*25*	*24*	*25*	*32*	123	91	97	106	1 856	1 467	1 845	1 736	21 024	25 202	21 819	24 043	203 462	208 765	199 821	198 009
10	51	*30*	35	46	158	177	160	180	2 171	2 043	2 058	2 098	23 539	19 919	20 142	20 710	185 920	176 129	184 631	183 242
11	*31*	*26*	*34*	41	117	146	136	141	**1 708**	**1 881**	**0**	**1 859**	21 868	21 010	20 190	21 772	175 941	184 812	215 662	230 028
12	*27*	38	38	36	145	157	124	133	1 799	1 864	1 928	1 950	19 912	18 921	21 124	21 932	176 548	176 411	190 224	191 624
13	34	37	*30*	40	133	154	162	123	1 842	1 894	1 563	1 960	**21 352**	**0**	**21 395**	**23 659**	205 830	184 341	189 595	223 383
14	42	46	42	35	174	198	166	192	2 392	2 411	2 632	2 116	27 857	30 513	26 301	30 802	**272 358**	**288 117**	**221 537**	**289 050**
15	44	53	34	44	168	190	184	116	1 897	2 391	2 282	2 084	25 228	26 414	23 770	25 919	179 100	174 686	154 747	195 440
16	30	46	42	*24*	137	143	98	101	1 763	1 710	1 851	1 831	17 624	18 419	20 190	18 955	176 200	170 869	137 349	138 881

Table S9. Values of slope, PCR efficiency and R^2 obtained in the validation study with PLD-GR taxon-specific assay in plate A and plate C; n.a.: not assessed

Lab	Plate	Slope	PCR Efficiency (%)	R^2
1	A	-3.43	95.7	0.999
	C	-3.39	97.2	0.999
2	A	-3.36	98.3	0.997
	C	-3.37	97.9	0.997
3	A	-3.47	94.1	0.999
	C	-3.46	94.7	0.998
4	A	-3.47	94.2	0.999
	C	-3.46	94.5	0.999
5	A	-3.52	92.4	0.997
	C	-3.49	93.4	0.999
6	A	-3.46	94.5	0.997
	C	-3.40	96.9	0.999
7	A	-3.38	97.8	0.998
	C	-3.45	94.9	0.996
8	A	-3.41	96.5	0.999
	C	-3.42	96.1	0.998
9	A	-3.51	92.9	0.999
	C	n.a.	n.a.	n.a.
10	A	-3.48	93.6	0.998
	C	-3.46	94.6	0.999
11	A	-3.45	94.9	0.999
	C	-3.44	95.4	0.999
12	A	-3.49	93.6	0.999
	C	-3.50	93.0	0.999
13	A	-3.43	95.6	0.999
	C	-3.40	96.7	0.998
14	A	-3.40	96.9	0.996
	C	-3.36	98.4	0.996
15	A	-3.33	99.7	0.997
	C	-3.28	101.8	0.995
16	A	-3.44	95.3	0.997
	C	-3.40	96.8	0.997
Mean		-3.43	95.7	0.998

Table S10. Hge content values for GR2 blind samples obtained by averaging the hge content determined by participating laboratories (LAB) with the GR2 event-specific assay for the four replicated samples of each level; outlying values are marked with an asterisk; outlying laboratories are marked with two asterisks

Haploid genome equivalents content					
LEVEL / **LAB**	**50**	**200**	**1 800**	**6 000**	**20 000**
1	55	232	2 125	6 720	22 208
2**	5 340*	505*	3 280*	2 439*	4 885*
3	53	211	1 959	6 546	20 829
4	63	247	1 912	5 913	18 465
5	61	232	1 992	7 068	21 409
6	62	220	2 111	6 492	20 400
7	49	205	1 910	5 872	18 465
8	56	217	2 237	7 432	24 511
9	81	295	2 262	6 592	20 072
10	68	266	2 166	6 790	21 742
11	55	223	1 821	6 064	19 123
12	59	231	2 076	6 656	20 864
13	63	234	2 019	6 497	19 279
14	60	221	1 938	6 268	12 280
15	452*	218	1 794*	5 033*	16 569
16	45	174	1 640	5 939	18 939

Table S11. Hge content values determined for GR2 blind samples by participating laboratories (LAB) with the GR2 event-specific assay; outlying series are highlighted in bold

Haploid genome equivalents content																				
SAMPLE	**50**				**200**				**1 800**				**6 000**				**20 000**			
LAB	**REP 1**	**REP 2**	**REP 3**	**REP 4**	**REP 1**	**REP 2**	**REP 3**	**REP 4**	**REP 1**	**REP 2**	**REP 3**	**REP 4**	**REP 1**	**REP 2**	**REP 3**	**REP 4**	**REP 1**	**REP 2**	**REP 3**	**REP 4**
1	55	53	66	45	237	246	212	231	2 125	2 181	2 114	2 079	6 282	6 956	6 646	6 995	21 384	23 085	22 145	22 220
2	**19 494**	**1 778**	**46**	**43**	**43**	**1 781**	**31**	**164**	**1 778**	**5 477**	**4 085**	**1 781**	**31**	**164**	**5 477**	**4 085**	**46**	**0**	**19 494**	**0**
3	53	54	58	48	220	198	212	213	1 997	1 828	2 031	1 981	6 525	6 555	6 774	6 329	20 669	20 777	21 270	20 602
4	60	67	68	58	239	247	254	248	1 898	1 843	2 098	1 810	5 739	6 074	5 923	5 915	18 630	19 529	17 873	17 829
5	55	66	66	57	250	226	228	222	1 963	2 004	2 077	1 926	7 018	6 914	7 242	7 098	21 385	21 950	20 171	22 132
6	58	72	59	60	241	225	213	202	2 037	2 136	2 042	2 231	6 557	6 517	5 774	7 120	21 164	18 388	22 106	19 942
7	50	49	58	41	220	204	197	197	1 984	1 855	1 862	1 940	5 946	5 780	6 021	5 742	17 622	18 813	18 839	18 584
8	52	58	59	54	228	217	218	205	2 285	2 174	2 272	2 219	7 264	7 411	7 806	7 245	23 809	24 996	25 358	23 882
9	75	77	94	77	299	305	293	284	2 280	2 251	2 281	2 235	6 676	6 562	6 392	6 740	19 845	20 066	20 003	20 373
10	63	67	74	67	274	244	268	276	2 137	2 064	2 375	2 086	6 840	6 627	7 201	6 495	21 288	22 205	22 104	21 371
11	49	54	62	55	233	223	231	204	1 791	1 906	1 791	1 798	5 867	6 016	6 090	6 283	18 211	18 707	19 621	19 954
12	57	60	65	53	243	232	221	226	2 073	2 103	2 064	2 066	6 575	6 054	6 873	7 123	19 380	19 642	22 393	22 040
13	61	61	67	62	239	248	221	230	2 005	2 090	1 987	1 994	6 467	6 469	6 411	6 643	18 566	20 359	19 072	19 119
14	64	63	56	57	233	213	211	226	1 962	2 064	1 987	1 740	6 627	6 481	5 830	6 136	23 074	22 166	19 709	20 171
15	**50**	**51**	**58**	**1 647**	218	235	199	220	**1 984**	**1 938**	**1 801**	**1 453**	**4 408**	**5 592**	**3 738**	**6 394**	17 061	15 982	18 180	15 054
16	60	40	46	35	192	163	164	179	1 614	1 586	1 617	1 744	5 368	6 288	5 897	6 202	20 757	19 459	18 107	17 434

Table S12. Values of slope, PCR efficiency and R^2 obtained in the validation study with GR2 event-specific assay (plate B)

Lab	Slope	PCR Efficiency (%)	R^2
1	-3.57	90.5	0.999
2	-3.55	91.4	0.999
3	-3.57	90.5	1.000
4	-3.81	83.1	0.996
5	-3.63	88.6	0.999
6	-3.71	86.0	0.998
7	-3.66	87.5	0.998
8	-3.50	93.0	0.997
9	-3.93	79.8	0.993
10	-3.71	86.2	0.998
11	-3.70	86.3	0.998
12	-3.67	87.4	0.997
13	-3.71	86.0	0.996
14	-3.66	87.6	0.996
15	-3.54	91.8	0.994
16	-3.50	93.1	0.996
Mean	-3.65	88.0	0.997

Table S13. GM % values for GR2 blind samples obtained by averaging the GM % content determined by participating laboratories (LAB) with the combined method for the four replicated samples of each level; outlying values are marked with an asterisk; outlying laboratories are marked with two asterisks

GM content (%) [a]					
LEVEL / **LAB**	**0.025**	**0.1**	**0.9**	**3.0**	**10**
1	0.026	0.11	1.0	3.2	10
2**	3.0*	0.28*	1.6*	1.2*	2.3*
3	0.028	0.11	1.0	3.3	11
4	0.033	0.13	1.1	3.1	9.7
5	0.034	0.13	1.1	4.0	12
6	0.030	0.11	1.0	3.2	9.8
7	0.028	0.12	1.0	3.1	10
8	0.029	0.12	1.2	3.7	12
9	-	-	-	-	-
10	0.034	0.13	1.0	3.2	11
11	0.030	0.12	1.0	2.9	10
12	0.033	0.13	1.2	3.6	11
13	0.030	0.12	1.0	3.2	9.3
14	0.024	0.09	0.80	2.7	8.3
15	0.27*	0.12	1.2*	2.8*	9.1
16	0.026	0.11	1.1	3.7	12

[a] GM % = (GM hge /rice hge) x 100, equivalent to GM % expressed as mass/mass

Table S14. GM % values determined for GR2 blind samples by participating laboratories (LAB) with the combined method; outlying series are highlighted in bold

GM content (%) [a]																				
SAMPLE	**0.025**				**0.1**				**0.9**				**3.0**				**10**			
LAB	**REP 1**	**REP 2**	**REP 3**	**REP 4**	**REP 1**	**REP 2**	**REP 3**	**REP 4**	**REP 1**	**REP 2**	**REP 3**	**REP 4**	**REP 1**	**REP 2**	**REP 3**	**REP 4**	**REP 1**	**REP 2**	**REP 3**	**REP 4**
1	0.026	0.025	0.032	0.022	0.12	0.12	0.10	0.11	1.0	0.97	1.0	1.0	3.1	3.5	3.2	3.0	9.8	11	11	9.7
2	**11**	**0.87**	**0.022**	**0.023**	**0.021**	**0.86**	**0.13**	**0.091**	**0.87**	**2.8**	**2.0**	**0.85**	**0.014**	**0.091**	**2.6**	**1.92**	**0.026**	**2015**	**9.1**	**2015**
3	0.028	0.027	0.029	0.027	0.12	0.11	0.11	0.12	1.1	1.0	1.0	1.0	3.4	3.3	3.4	3.2	10	10	11	12
4	0.028	0.033	0.040	0.031	0.12	0.13	0.13	0.13	1.1	1.3	1.1	1.1	3.3	2.9	3.2	3.0	8.7	9.2	10	10
5	0.027	0.033	0.040	0.034	0.16	0.11	0.13	0.13	1.2	1.0	1.2	1.2	4.3	3.8	4.4	3.4	10	12	12	12
6	0.028	0.034	0.029	0.029	0.11	0.12	0.11	0.10	0.97	1.1	0.96	1.0	3.3	3.0	3.1	3.4	10	8.9	10	9.9
7	0.029	0.027	0.031	0.025	0.12	0.11	0.12	0.12	1.1	1.0	1.0	1.1	3.2	3.1	3.3	3.0	9.9	10	10	10
8	0.027	0.027	0.030	0.031	0.12	0.10	0.12	0.12	1.3	1.1	1.2	1.2	3.7	3.6	4.2	3.2	13	13	13	11
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	0.032	0.032	0.037	0.035	0.13	0.12	0.13	0.15	1.0	0.97	1.1	1.0	3.2	3.3	3.3	3.2	11	11	11	10
11	0.030	0.026	0.029	0.033	0.11	0.11	0.12	0.12	0.99	0.92	0.98	0.92	2.9	2.9	3.0	2.9	11	10	9.2	9.5
12	0.033	0.035	0.035	0.030	0.14	0.13	0.12	0.13	1.2	1.1	1.2	1.1	3.6	3.5	3.6	3.6	11	11	12	11
13	0.028	0.028	0.035	0.030	0.13	0.12	0.10	0.11	1.1	0.97	1.1	1.1	3.2	3.3	3.2	3.0	8.8	10	9.8	8.5
14	0.024	0.024	0.026	0.021	0.095	0.080	0.090	0.084	0.83	0.81	0.76	0.80	2.7	2.6	2.8	2.5	8.2	8.3	8.8	7.9
15	**0.029**	**0.027**	**0.036**	**0.97**	0.13	0.12	0.11	0.12	**1.3**	**0.95**	**1.2**	**1.3**	**2.6**	**3.1**	**2.3**	**3.2**	9.0	8.8	11	7.9
16	0.033	0.023	0.029	0.020	0.12	0.10	0.10	0.11	1.0	1.1	1.1	1.1	3.9	3.8	3.4	3.6	11	12	12	12

[a] GM % = (GM hge /rice hge) x 100, equivalent to GM % expressed as mass/mass

Figure S1. Plate set-up and loading order for plate A, performed with **PLD** taxon-specific assay reaction mix and **PLD** standard curve samples and blind samples. Light blue, no template control (NTC); light violet (S1 PLD-S5 PLD), PLD standard curve samples quantified with PLD assay; green (U1 PLD-U20 PLD), PLD blind samples quantified with PLD assay.

	1	2	3	4	5	6	7	8	9	10	11	12
a	NTC	NTC	NTC	S1 PLD	S1 PLD	S1 PLD	S2 PLD	S2 PLD	S2 PLD	S3 PLD	S3 PLD	S3 PLD
b				S4 PLD	S4 PLD	S4 PLD	S5 PLD	S5 PLD	S5 PLD			
c	U1 PLD	U1 PLD	U1 PLD	U2 PLD	U2 PLD	U2 PLD	U3 PLD	U3 PLD	U3 PLD	U4 PLD	U4 PLD	U4 PLD
d	U5 PLD	U5 PLD	U5 PLD	U6 PLD	U6 PLD	U6 PLD	U7 PLD	U7 PLD	U7 PLD	U8 PLD	U8 PLD	U8 PLD
e	U9 PLD	U9 PLD	U9 PLD	U10 PLD	U10 PLD	U10 PLD	U11 PLD	U11 PLD	U11 PLD	U12 PLD	U12 PLD	U12 PLD
f	U13 PLD	U13 PLD	U13 PLD	U14 PLD	U14 PLD	U14 PLD	U15 PLD	U15 PLD	U15 PLD	U16 PLD	U16 PLD	U16 PLD
g	U17 PLD	U17 PLD	U17 PLD	U18 PLD	U18 PLD	U18 PLD	U19 PLD	U19 PLD	U19 PLD	U20 PLD	U20 PLD	U20 PLD
h												

Figure S2. Plate set-up and loading order for plate B, performed with **GR2** event-specific assay reaction mix and **GR2** standard curve samples and blind samples. Light blue, no template control (NTC); orange (S1 GR2-S5 GR2), GR2 standard curve samples quantified with GR2 assay; yellow (U1 GR2-U20 GR2), GR2 blind samples quantified with GR2 assay.

	1	2	3	4	5	6	7	8	9	10	11	12
a	NTC	NTC	NTC	S1 GR2	S1 GR2	S1 GR2	S2 GR2	S2 GR2	S2 GR2	S3 GR2	S3 GR2	S3 GR2
b				S4 GR2	S4 GR2	S4 GR2	S5 GR2	S5 GR2	S5 GR2			
c	U1 GR2	U1 GR2	U1 GR2	U2 GR2	U2 GR2	U2 GR2	U3 GR2	U3 GR2	U3 GR2	U4 GR2	U4 GR2	U4 GR2
d	U5 GR2	U5 GR2	U5 GR2	U6 GR2	U6 GR2	U6 GR2	U7 GR2	U7 GR2	U7 GR2	U8 GR2	U8 GR2	U8 GR2
e	U9 GR2	U9 GR2	U9 GR2	U10 GR2	U10 GR2	U10 GR2	U11 GR2	U11 GR2	U11 GR2	U12 GR2	U12 GR2	U12 GR2
f	U13 GR2	U13 GR2	U13 GR2	U14 GR2	U14 GR2	U14 GR2	U15 GR2	U15 GR2	U15 GR2	U16 GR2	U16 GR2	U16 GR2
g	U17 GR2	U17 GR2	U17 GR2	U18 GR2	U18 GR2	U18 GR2	U19 GR2	U19 GR2	U19 GR2	U20 GR2	U20 GR2	U20 GR2
h												

Figure S3. Plate set-up and loading order for plate C, performed with **PLD** taxon-specific assay reaction mix, **PLD** standard curve samples and **GR2** blind samples. Light blue, no template control (NTC); light violet (S1 PLD-S5 PLD), PLD standard curve samples quantified with PLD assay; light green (U1 GR2-U20 GR2), GR2 blind samples quantified with PLD assay.

	1	2	3	4	5	6	7	8	9	10	11	12
a	NTC	NTC	NTC	S1 PLD	S1 PLD	S1 PLD	S2 PLD	S2 PLD	S2 PLD	S3 PLD	S3 PLD	S3 PLD
b				S4 PLD	S4 PLD	S4 PLD	S5 PLD	S5 PLD	S5 PLD			
c	U1 GR2	U1 GR2	U1 GR2	U2 GR2	U2 GR2	U2 GR2	U3 GR2	U3 GR2	U3 GR2	U4 GR2	U4 GR2	U4 GR2
d	U5 GR2	U5 GR2	U5 GR2	U6 GR2	U6 GR2	U6 GR2	U7 GR2	U7 GR2	U7 GR2	U8 GR2	U8 GR2	U8 GR2
e	U9 GR2	U9 GR2	U9 GR2	U10 GR2	U10 GR2	U10 GR2	U11 GR2	U11 GR2	U11 GR2	U12 GR2	U12 GR2	U12 GR2
f	U13 GR2	U13 GR2	U13 GR2	U14 GR2	U14 GR2	U14 GR2	U15 GR2	U15 GR2	U15 GR2	U16 GR2	U16 GR2	U16 GR2
g	U17 GR2	U17 GR2	U17 GR2	U18 GR2	U18 GR2	U18 GR2	U19 GR2	U19 GR2	U19 GR2	U20 GR2	U20 GR2	U20 GR2
h												
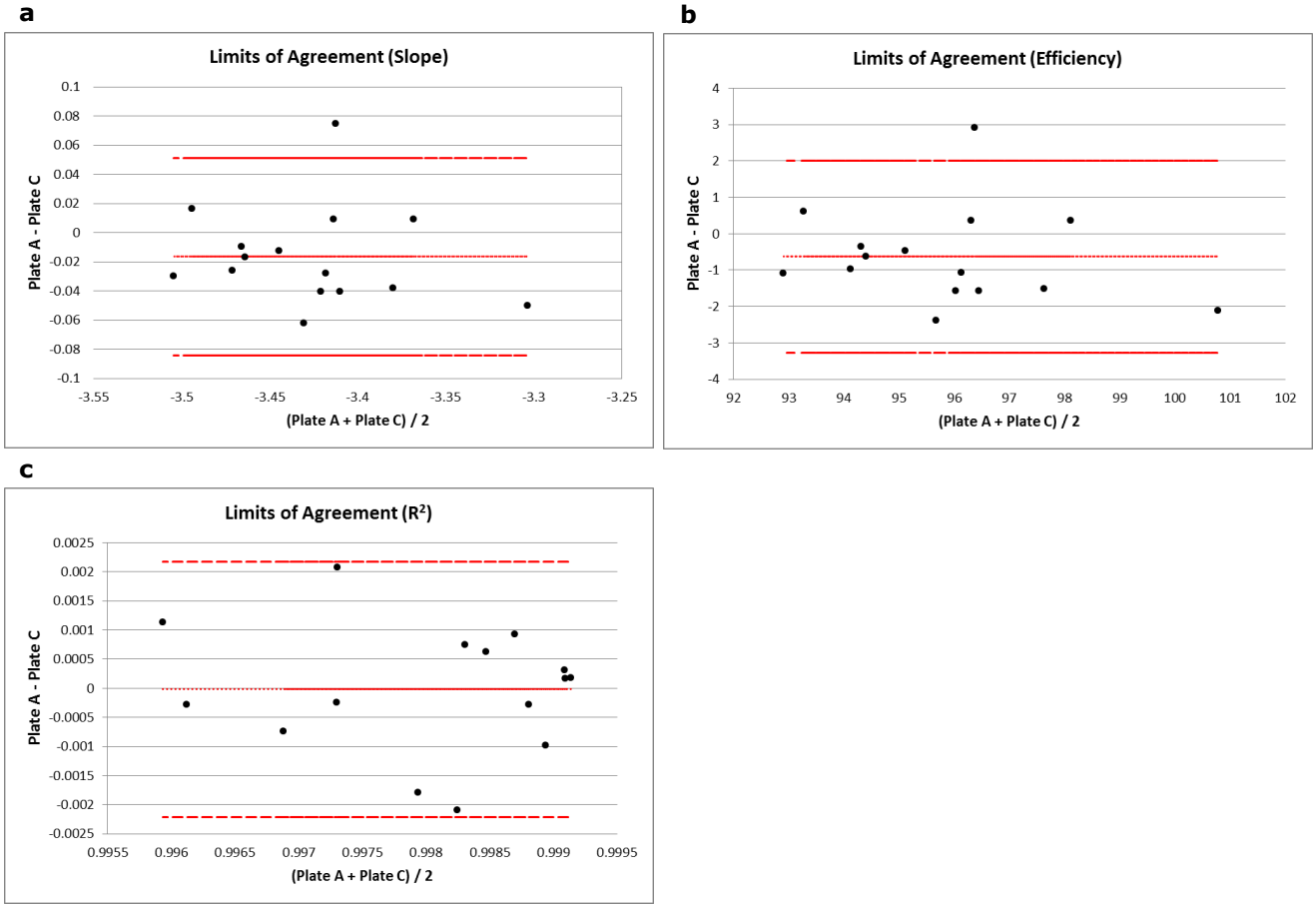
Figure S4. Scatterplots of the comparison of the measured slope (a), efficiency (b) and linearity (c) values obtained by participating laboratories for plate A and plate C with PLD-GR taxon-specific assay.