

Additional file 1

PCR reaction conditions

For all PCR reactions, the following reaction mixture was used:

- 1 µl or 0.5 µl genome prep
- 2.5 µl forward primer (10 pM)
- 2.5 µl reverse primer (10 pM)
- 1 µl dNTP mix (10 mM each)
- 1 µl DyNAzyme II DNA Polymerase (Finnzymes)
- 5 µl 10× Reaction buffer
- 37 µl dH₂O

The reactions were subjected to thermal cycling using the following program: Initial denaturing at 95 °C for 3 minutes, then 30 cycles of 95 °C for 1 minute, 45-55 °C (depending on the primers) for 1 minute and 72 °C for 1 minute, followed by an elongation step of 7 minutes at 72 °C.

Initial PCR detection of *nhe* genes in strains NVH 391/98, NVH 883/00, and INRA AF2

The primer pairs based on earlier published sequences of *nhe* that were initially positive for the three strains NVH 391/98, NVH 883/00, and INRA AF2 were the following three forward primers: cggttcatctgttgcgacagc, gcgaagcaatggtagatgta, and acgttacaacgttgaagtac, and the two reverse primers: tgaattaattttgcgtatgaatccattgc and gcatatgaatccattgcaa. All three forward primers gave positive PCR results in reactions with both reverse primers.

Details of the PCR experiments that confirm that INRA AF2 has the same gene variants as strains NVH 391/98 and NVH 883/00 for 13 genes:

The PCR reactions listed below were positive and of the correct size for strain INRA AF2 using primers designed from the corresponding sequences in NVH 391/98 and NVH 883/00. The targeted genes were *yvrG*, *yvrH*, *orf2* upstream of *cytK*, *cytK*, the gene encoding an ABC transporter permease protein (*ABC*), *yvfT*, *yvfU*, *plcR*, *papR*, *nheA*, *nheB*, *nheC*, and the gene encoding a deoxyribonucleotide regulator (*DeoR*).

Forward primer	Reverse primer	Targeted gene	Size
yvfF6	yvfR6	<i>ABC</i>	1640 bp
yvfF11	yvfR7	<i>ABC, yvfT</i>	1711 bp
yvfF6	yvfR5	<i>ABC, yvfT</i>	1814 bp
yvfF11	yvfR5	<i>ABC, yvfT</i>	972 bp
yvfF11	yvfR4	<i>ABC, yvfT</i>	1199 bp
yvfF11	yvfR3	<i>ABC, yvfT</i>	1341 bp
yvfF4	yvfR8	<i>yvfT, yvfU</i>	1677 bp
yvfF4	yvfR2	<i>yvfT, yvfU</i>	1388 bp
yvfF2	yvfR7	<i>yvfT</i>	933 bp
yvfF13	plcR391R2	<i>yvfU, plcR</i>	806 bp
plcR391F4	plcR391R2	<i>plcR</i> promoter	537 bp
plcR391F2	plcR391R4	<i>plcR, papR</i>	1367 bp
plcR391F4	plcR391R4	<i>plcR, papR</i>	1840 bp
plcR391F1	plcR391R4	<i>plcR, papR</i>	1321 bp
RRf3	RegulatorR2	<i>yvrH</i>	671 bp
RRf3	HKr6	<i>yvrH, yvrG</i>	1743 bp
RRf6	HKr6	<i>yvrH, yvrG</i>	1118 bp
RRf5	HKr6	<i>yvrH, yvrG</i>	957 bp
RRf3	HKr6	<i>yvrH, yvrG</i>	1743 bp
HKf6	HKR1	<i>yvrG</i>	895 bp
HKf6	R8	<i>yvrG</i>	1451 bp
HK1	R6	<i>yvrG</i>	660 bp
HK2	R5	<i>yvrG, orf2</i>	645 bp
HK1	orf2R4	<i>yvrG, orf2</i>	1256 bp
HKf6	R4FR	<i>yvrG, orf2</i>	2009 bp
orf2F1	R3fr	<i>orf2, cytK</i>	948 bp
orf2F3	Rent3	<i>orf2, cytK</i>	1734 bp
F3fr	Rent1	<i>cytK</i>	627 bp
F6FR	R2cytK	<i>cytK</i>	674 bp
Fent1	Rent2	<i>cytK</i>	651 bp
Fent1	Rent3	<i>cytK</i>	748 bp
Fent2	Rent3	<i>cytK</i>	554 bp
nhe391F12	nhe391R13	<i>nheA</i> promoter	832 bp
nhe391F12	nhe391R9	<i>nheA</i>	1152 bp
nhe391F12	nhe391R8	<i>nheA</i>	1864 bp
nhe391F11	nhe391R9	<i>nheA</i>	538 bp
nhe391F11	nhe391R8	<i>nheA</i>	1246 bp
nhe391F12	nhe391R6	<i>nheA, nheB</i>	2596 bp
nhe391F11	nhe391R6	<i>nheA, nheB</i>	1978 bp
nhe391F7	nhe391R7	<i>nheA, nheB</i>	814 bp
nhe391F9	nhe391R7	<i>nheA, nheB</i>	1626 bp
nhe391F7	nhe391R4	<i>nheA, nheB</i>	1050 bp
nhe391F8	nhe391R2	<i>nheB</i>	861 bp
nhe391F1	nhe391R14	<i>nheB, nheC</i>	1267 bp
nhe391F8	nhe391R5	<i>nheB, nheC</i>	1487 bp
nhe391F2	nhe391R10	<i>nheC, deoR</i>	1530 bp
nhe391F10	nhe391R11	<i>deoR</i>	863 bp

Primer sequences:

F3fr:	aacagatcgggtcaaaatgc	RRf6:	ccgctcagctatttcagctc
F6FR:	gcattatctgctgtgtggc	yvfF11:	gcagtggaaacgtggaagtct
Fent1:	agtaacttggctctgattccg	yvfF13:	ggcactgtatctttctctgt
Fent2:	atgaaacagatgcacgagge	yvfF2:	aagaaaaagacaggaagcggta
HK1:	gaacaggacttggctctgc	yvfF4:	gcattgcaccaacaaaaag
HK2:	caattgaaagtgaacttggg	yvfF6:	ggggtggagagatggaaaag
HKf6:	gatgaggatgtaatcgcgaaa	yvfR2:	cgcataagcttcgtaataactt
HKR1:	cgagaccaagtctctgttc	yvfR3:	gcgctcttcgcgttttacta
HKr6:	tgagccaacgcattgtatgt	yvfR4:	atgcgattgcatttgcgtga
nhe391F1:	tgacaggcagaaattcaaa	yvfR5:	ttgccggtatgctagaacaa
nhe391F10:	tgaaatcgctcgaaaaatgg	yvfR6:	ccgcttctgtctttttctt
nhe391F11:	cgtaataaggcgcgtgtgtt	yvfR7:	cgcatagatgcgccttacta
nhe391F12:	tcagcttcatggtggaattg	yvfR8:	gaaatgaagtacgccttatacaaca
nhe391F2:	gcaatggtgcaaacacatc		
nhe391F7:	aaacgaagcagagtaacagaaa		
nhe391F8:	aagatgcaatggaacgcact		
nhe391F9:	gcttactacgaaggggagca		
nhe391R10:	aagcaatcgtctcatttgc		
nhe391R11:	tgcaatgttatatctttcactcc		
nhe391R13:	acattcagcccttgcactct		
nhe391R14:	cggcatcaattgtttctgtc		
nhe391R2:	ctcccattgtatgccactga		
nhe391R4:	gctgtggctacagaggaacc		
nhe391R5:	tgttgattgcgcttgaaaaa		
nhe391R6:	tcccttgcagagttgctt		
nhe391R7:	caacctgttaatccctttgtca		
nhe391R8:	atcaacgagctccgtaaagc		
nhe391R9:	actactcatcgcgctcacct		
orf2F1:	gcaacgaaaggttctctcca		
orf2F3:	gtctgttatgagcttaggttgg		
orf2R4:	cgaaggaaatgaaagaggaga		
plcR391F1:	gatgaggggttaacgcaaa		
plcR391F2:	gcgtgcaagcagaaaagctagg		
plcR391F4:	ggtgattttaccgcaacag		
plcR391R1:	ttgctcaccatcccattaca		
plcR391R2:	tcggttaaaccctcatcactc		
plcR391R4:	ttgctggggacgatattagc		
R2cytK:	gaatacataaataattggtttcc		
R3fr:	gtgttttgcatttgaccg		
R4FR:	caccagcgatgactaagcc		
R5:	tatgttcttagggaagagc		
R6:	gttactacaatcactttcc		
R8:	ggagagaaccttctgtgc		
RegulatorR2:	ttcggattctcgcaaaagaagat		
Rent1:	cgtgcatctgtttcatgagg		
Rent2:	tattgttcgttcaccttgc		
Rent3:	aatcgtaataggtgttccgg		
RRf3:	tcacactatctctccccta		
RRf5:	aaccaagcaaccacagta		