

Table S1. Strains of soybean bradyrhizobia used in this study

Strain	Relevant characteristics	Source or reference
USDA110	Non-HRS strain	USDA ^a
NK5	HRS field isolate from Nagakura, Niigata, Japan	1
NK6	HRS field isolate from Nagakura, Niigata, Japan	1
USDA123	HRS field isolate from USA	2
T2	HRS field isolate from Tokachi, Hokkaido, Japan	3
USDA135	HRS field isolate from USA	2
2281	HRS field isolate from Heilongjiang Province, China (USDA3622)	4
NC6	Non-HRS strain	1
Kas1-4	Non-HRS strain	6
Kas2-2	Non-HRS strain	6
Tsu4-125	Non-HRS strain	6
Yam3-2	Non-HRS strain	6
Hok1-31	Non-HRS strain	6
Kag1-5	Non-HRS strain	6
Fuk1-11	Non-HRS strain	6
USDA122	Non-HRS strain	6
NC4	Non-HRS strain	6
NK2	Non-HRS strain	6
Kas1-7	Non-HRS strain	6
Yam3-7	Non-HRS strain	6
Hok10-2	Non-HRS strain	6
Fuk1-6	Non-HRS strain	6
Kag1-3	Non-HRS strain	6
S23321	Non-symbiotic strain that does not nodulate soybean plants	7

^a USDA, U.S. Department of Agriculture, Beltsville, MD.

Table S2. Genome size of HRS strains of soybean bradyrhizobia estimated by pulsed-field gel electrophoresis^a

Strain	RE	Fragment size (kb)	Total fragment size (Mb)
Non-HRS			
USDA110	<i>Smi</i> I	2600, 2000, 1500, 1200, 1100, 330, 150, 120, 110	9.1
HRS			
NK5	<i>Smi</i> I	4500, 2100, 1100, 880, 440, 400, 380, 130	9.9
	<i>Pme</i> I	5000, 2000, 1000, 900, 500	9.4
NK6	<i>Smi</i> I	3500, 2100, 1800, 1200, 450, 430	9.5
	<i>Pme</i> I	5000, 2500, 1400, 1100	10.0
USDA135	<i>Smi</i> I	4600, 3300, 1400, 740, 640	10.7
2281	<i>Smi</i> I	4600, 3300, 1300, 950	10.2
USDA123	<i>Smi</i> I	4200, 1600, 1100, 650, 580, 500, 400, 380, 340, 270, 240, 200, 160	10.6
	<i>Pme</i> I	4200, 3100, 2000, 1400	10.7
T2	<i>Smi</i> I	2700, 1700, 1100, 1050, 950, 620, 490, 440, 390, 260, 240, 170, 140, 120	10.4
	<i>Pme</i> I	4900, 2300, 1600, 1200	10.0

^a USDA110 was used as a control strain. RE: Restriction enzyme. The averages of total sizes of chromosome fragments were 9.65 Mb (NK5), 9.75 Mb (NK6), 10.65 Mb (USDA123), and 10.20 Mb (T2).

Table S3. Parameters of genome sequences of HRS strains and coverage statistics of their mapping to the USDA110 genome^a

Strain	HRS or non-HRS strains	Average length (bp)	Total reads	GC content (%)	Mapped reads (%)	Not mapped reads (%)	Average coverage	Maximum coverage
NK5	HRS group A	225	2,170,102	62.3	80	20	42	13,947
NK6	HRS group A	218	2,496,052	63.4	81	19	47	10,097
USDA135	HRS group B	214	1,752,256	62.6	63	37	25	10,197
2281	HRS group B	220	2,436,194	62.1	63	37	35	16,642
T2	HRS group C	229	1,553,580	63.4	58	42	21	3,375
USDA123	HRS group C	218	2,030,892	63.4	58	42	27	4,659
USDA110	non-HRS	221	1,545,842	63.8	99	1	37	98
NC6	non-HRS	226	2,173,812	63.3	90	11	48	497
Kas1-4	non-HRS	232	1,611,664	61.8	98	2	38	99
Kas2-2	non-HRS	233	1,404,884	62.4	93	7	32	253
Tsu4-125	non-HRS	223	1,816,360	62.2	93	7	40	381
Yam3-2	non-HRS	210	1,349,040	62.6	95	5	28	140
Hok1-31	non-HRS	224	2,174,490	61.9	95	5	49	385
Kag1-5	non-HRS	216	2,963,944	61.4	92	8	62	626
Fuk1-11	non-HRS	217	2,473,576	62.0	92	8	52	570
USDA122	non-HRS	227	2,192,500	62.2	92	8	48	652
NC4	non-HRS	212	3,391,300	61.6	91	9	69	676
NK2	non-HRS	229	2,347,168	61.9	92	8	52	523
Kas1-7	non-HRS	228	2,368,846	61.9	91	9	52	565
Yam3-7	non-HRS	231	2,225,346	61.9	92	8	50	483
Hok10-2	non-HRS	230	1,462,946	62.4	91	9	32	332
Fuk1-6	non-HRS	224	1,958,166	62.4	93	8	43	477
Kag1-3	non-HRS	228	960,644	63.1	92	8	21	200

^aThe MiSeq reads of the non-HRS strain USDA110 were also mapped to the genomes of USDA110 (5) and those of the six HRS strains.

Table S4. Copy numbers of insertion sequences (ISs) in non-HRS strains by genome mapping

IS			Non-HRS strain																	Standard deviation	
Name in this study	ISFinder	Synonyms ^a	USDA110	NC6	Kas1-4	Kas2-2	Tsu4-125	Yam3-2	Hok1-31	Kag1-5	USDA122	NC4	NK2	Kas1-7	Yam3-7	Hok10-2	Fuk1-6	Kag1-3	Fuk1-11		Average
ISRj1	ISRj1	RS α	18	15	18	14	12	19	12	14	11	11	10	11	12	11	10	10	12	12.9	2.9
ISRj2	ISRj2	RS β	18	16	13	16	15	23	13	14	15	17	15	15	15	13	14	16	18	15.6	2.4
ISFK1	ISFK1	FK1	8	7	7	7	5	7	6	6	6	6	6	6	6	7	6	6	7	6.4	0.7
IS1632	IS1632	IS1632	3	2	2	4	4	3	4	11	2	2	3	2	2	2	4	2	3	3.2	2.2
ISBj6_B	ISBj6_B	ISB20	3	3	3	6	12	3	10	8	4	4	3	4	4	3	3	4	4	4.8	2.7
ISB27		ISB27	4	4	3	3	2	4	3	4	3	3	3	3	4	3	3	3	3	3.2	0.6
ISBj2	ISBj2	ISBj2	12	12	11	23	25	17	17	20	14	15	14	16	15	14	14	14	14	15.7	3.8
ISBj3	ISBj3	ISBj3	5	5	4	0	1	2	0	2	2	2	2	2	2	2	2	2	2	2.2	1.4
ISBj4	ISBj4	ISBj4	7	7	6	6	5	5	5	7	4	3	3	4	4	5	3	3	4	4.8	1.4
ISBj5	ISBj5	ISBj5	6	5	5	5	5	8	5	8	4	5	6	5	5	4	5	5	4	5.3	1.2
ISBj6		ISBj6	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0.4	0.5
ISBj7	ISBj7	ISBj7	13	13	12	16	14	15	15	18	12	14	13	13	14	12	13	10	14	13.6	1.8
ISBj8	ISBdi2	ISBj8	4	5	4	7	6	4	5	5	15	5	9	7	6	5	5	6	5	6.1	2.6
ISBj9	ISBj9	ISBj9	2	2	2	4	3	2	3	3	0	0	0	0	0	0	0	0	0	1.2	1.4
ISBj10		ISBj10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
ISBj11	ISBj11	ISBj11	7	6	5	0	0	11	0	0	2	2	2	2	2	2	2	2	2	2.8	2.9
ISBj12	ISBj12	ISBj12	1	1	1	2	1	0	1	4	3	4	3	3	3	3	3	3	3	2.3	1.2
ISBj7_B	ISBj7_B	ISBj13	3	3	3	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0.8	1.4
ISBj5_B	ISBj5_B	ISBj14	8	5	7	2	4	4	5	4	2	2	2	3	2	2	2	4	3	3.6	1.8
ISBj2_B	ISBj2_B	ISBj15	3	3	3	3	3	3	3	10	2	3	3	3	3	3	3	3	3	3.4	1.7
IS1631	IS1631		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total			126	115	111	120	119	135	109	140	102	99	98	100	100	92	93	94	102	109.1	14.7

^a Copy numbers of ISs were estimated by BWA mapping to USDA110 (see text). ISFinder indicates approved IS name in the ISFinder database (<https://www-is.biotoul.fr>).

^b Synonyms used in genome paper of *B. diazoefficiens* USDA110 (5).

Table S5. Replication initiator and partitioning genes

BLASTP results											
Replicon	Start location	End location	Direction	Length (aa)	Gene	Similarity	E-Value	Subject length	Gene	Bacteria	Accession no.
chromosome	703219	703881	-1	220	<i>parA</i>	100	4.55E-139	220	chromosome partitioning protein ParA	<i>Bradyrhizobium diazoefficiens</i> USDA 110	WP_011088682
chromosome	2218858	2219526	1	222	<i>parA</i>	100	4.69E-156	222	chromosome partitioning protein ParA	<i>Bradyrhizobium japonicum</i>	WP_028182039
chromosome	3560215	3561627	1	470	<i>dnaA</i>	100	0	470	chromosomal replication initiation protein	<i>Bradyrhizobium diazoefficiens</i>	WP_011083652
chromosome	3637588	3638331	1	247	<i>parA</i>	99	2.46E-150	247	chromosome partitioning protein ParA	<i>Bradyrhizobium diazoefficiens</i> USDA 110	WP_011083586
chromosome	3773140	3773994	1	284	<i>parA</i>	100	0	284	chromosome partitioning protein ParA	<i>Bradyrhizobium diazoefficiens</i> USDA 110	WP_011083458
chromosome	3774132	3775019	1	295	<i>parB</i>	99	0	302	chromosome partitioning protein ParB	<i>Bradyrhizobium diazoefficiens</i> USDA 110	WP_011083457
chromosome	5279373	5280461	1	362	<i>repA</i>	96	0	365	replication protein A	<i>Bradyrhizobium elkantii</i>	WP_028343842
chromosome	5618807	5619931	1	374	<i>repA</i>	89	0	425	replication protein A	<i>Bradyrhizobium diazoefficiens</i> USDA 110	WP_011082883
pNK6a	72066	73331	-1	421	<i>repC</i>	95	0	439	replication initiation protein RepC	<i>Bradyrhizobium japonicum</i>	WP_028153003
pNK6a	73555	74574	-1	339	<i>repB</i>	76	1.47E-133	330	chromosome partitioning protein ParB	<i>Methylosinus</i> sp. LW3	WP_024881649
pNK6a	74647	75834	-1	395	<i>repA</i>	91	0	393	chromosome partitioning protein ParA	<i>Methylocystaceae</i>	WP_024881650
pNK6a	178827	179813	-1	328	<i>parB</i>	92	0	333	chromosome partitioning protein ParB	<i>Bradyrhizobium japonicum</i>	WP_028133144
pNK6a	179803	180567	-1	254	<i>parA</i>	97	1.48E-178	254	chromosome partitioning protein ParA	<i>Bradyrhizobium japonicum</i>	WP_028133143
pNK6b	45368	46498	-1	376	<i>repC</i>	99	0	410	replication initiation protein RepC	<i>Bradyrhizobium japonicum</i>	WP_028153806
pNK6b	46815	47834	-1	339	<i>repB</i>	100	0	339	plasmid partitioning protein	<i>Bradyrhizobium japonicum</i>	WP_028153807
pNK6b	47837	49057	-1	406	<i>repA</i>	100	0	394	chromosome partitioning protein ParA	<i>Bradyrhizobium japonicum</i>	WP_028153808
pNK6b	181819	182709	-1	296	<i>repC</i>	82	5.42E-138	410	replication initiation protein RepC	<i>Bradyrhizobium</i> sp. OHSU_III	WP_024584971
pNK6b	182935	183948	-1	337	<i>repB</i>	65	3.87E-60	332	replication protein B	<i>Bradyrhizobium</i> sp. OHSU_III	WP_029880243
pNK6b	183945	185138	-1	397	<i>repA</i>	100	0	397	chromosome partitioning protein ParA	<i>Bradyrhizobium japonicum</i>	WP_028152592
pNK6c	115359	116573	1	404	<i>repA</i>	100	0	404	chromosome partitioning protein ParA	<i>Bradyrhizobium japonicum</i>	WP_028152782
pNK6c	116570	117589	1	339	<i>repB</i>	100	0	339	chromosome partitioning protein ParB	<i>Bradyrhizobium japonicum</i>	WP_028152783
pNK6c	117786	119018	1	410	<i>repC</i>	99	0	410	replication initiation protein RepC	<i>Bradyrhizobium japonicum</i>	WP_028152784
pNK6c	130199	130960	1	253	<i>parA</i>	100	0	253	chromosome partitioning protein ParA	<i>Bradyrhizobium japonicum</i>	WP_028152789
pNK6c	130950	131930	1	326	<i>parB</i>	100	0	326	chromosome partitioning protein ParB	<i>Bradyrhizobium japonicum</i>	WP_028152790
pNK6d	13470	14345	-1	291	<i>repC</i>	91	1.33E-133	441	replication initiation protein RepC	<i>Bradyrhizobium japonicum</i>	WP_028181486
pNK6d	14678	15706	-1	342	<i>repB</i>	99	0	342	replication protein B	<i>Bradyrhizobium japonicum</i>	WP_028153002
pNK6d	15703	16773	-1	356	<i>repA</i>	100	0	404	chromosome partitioning protein ParA	<i>Bradyrhizobium japonicum</i>	WP_028153001
pNK6d	43476	45155	-1	559	<i>parB</i>	100	0	559	plasmid partitioning protein	<i>Bradyrhizobium japonicum</i>	WP_028152389

Table S6. Copy numbers of insertion sequences (ISs) in the genomes of NK6, USDA6, and USDA110 strains.

IS					HRS strain NK6								non-HRS strain ^d		
					Chromosome ^b			Plasmid					NK6 mapping ^c	USDA110	USDA6
Name in this study	ISFinder	Synonyms ^a	Family	Group	V1+V2 region	Core	Total	pNK6a	pNK6b	pNK6c	pNK6d	Plasmid total			
ISRj1	ISRj1	RS α	IS630	-	38	76	114	8	1	1	1	11	203	15	9
ISRj2	ISRj2	RS β	IS3	IS150	8	8	16	1	1	0	0	2	54	12	7
ISFK1	ISFK1	FK1	IS21	-	7	0	7	3	4	0	2	9	7	6	5
IS1632	IS1632	IS1632	IS256	-	6	0	6	1	1	0	1	3	13	2	2
ISBj6_B	ISBj6_B	ISB20	IS701	-	3	0	3	2	0	0	0	2	4	3	2
ISB27		ISB27	IS66		27	22	49	8	17	5	3	33	64	3	3
ISBj2	ISBj2	ISBj2	IS5	IS427	14	8	22	2	0	0	2	4	27	10	10
ISBj3	ISBj3	ISBj3	IS3	IS150	0	0	0	0	0	0	0	0	1	4	1
ISBj4	ISBj4	ISBj4	IS110	IS1111	1	2	3	0	0	0	0	0	6	6	1
ISBj5	ISBj5	ISBj5	IS630	-	0	0	0	0	0	0	0	0	9	5	5
ISBj6		ISBj6	IS110	IS1111	0	0	0	0	0	0	0	0	0	1	0
ISBj7	ISBj7	ISBj7	IS6	-	4	0	4	1	0	0	1	2	17	11	10
ISBj8	ISBdi2	ISBj8	IS1380		27	25	52	5	3	2	2	12	90	4	5
ISBj9	ISBj9	ISBj9	IS701	-	0	0	0	0	0	0	0	0	0	2	0
ISBj10		ISBj10	IS21		0	0	0	0	0	0	0	0	0	1	2
ISBj11	ISBj11	ISBj11	IS21	-	0	0	0	0	0	0	0	0	0	5	1
ISBj12	ISBj12	ISBj12	ISNCY	ISLbi1	0	0	0	2	0	0	2	4	0	1	2
ISBj7_B	ISBj7_B	ISBj13	IS66	-	0	0	0	2	0	0	2	4	0	3	1
ISBj5_B	ISBj5_B	ISBj14	IS5	IS5	0	0	0	2	0	0	2	4	1	7	1
ISBj2_B	ISBj2_B	ISBj15	IS5	IS5	2	1	3	2	0	0	2	4	5	3	2
IS1631	IS1631		IS21	-	11	16	27	2	0	0	2	4	34	0	0
Total					148	158	306	41	27	8	22	98	535	104	69

^a Synonyms used in genome paper of *B. diazoefficiens* USDA110 (5).

^b IS copy numbers were counted separately within and outside the variable regions V1 and V2 of NK6 chromosome.

^c IS copy number was estimated by mapping MiSeq sequences of NK6 (Table 3).

^d IS copy numbers are shown according to refs (5) for USDA110 and (8) for USDA6.

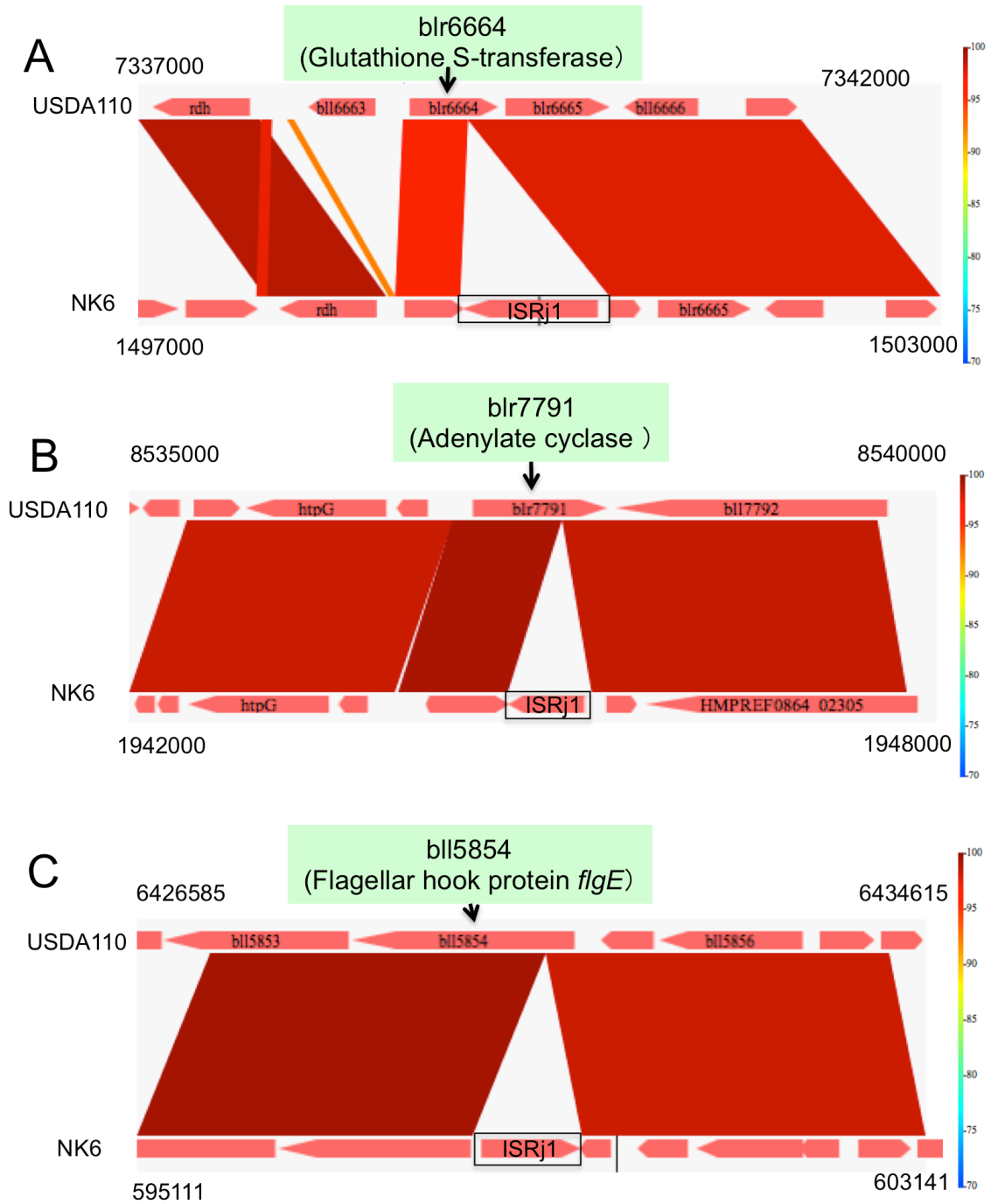


Fig. S1. Gene disruption by ISRj1 insertion in the chromosome of the HRS strain NK6. The disrupted genes, *blr6664* (A), *blr7791* (B), and *bli5854* (C), are important for survival or environmental responses.

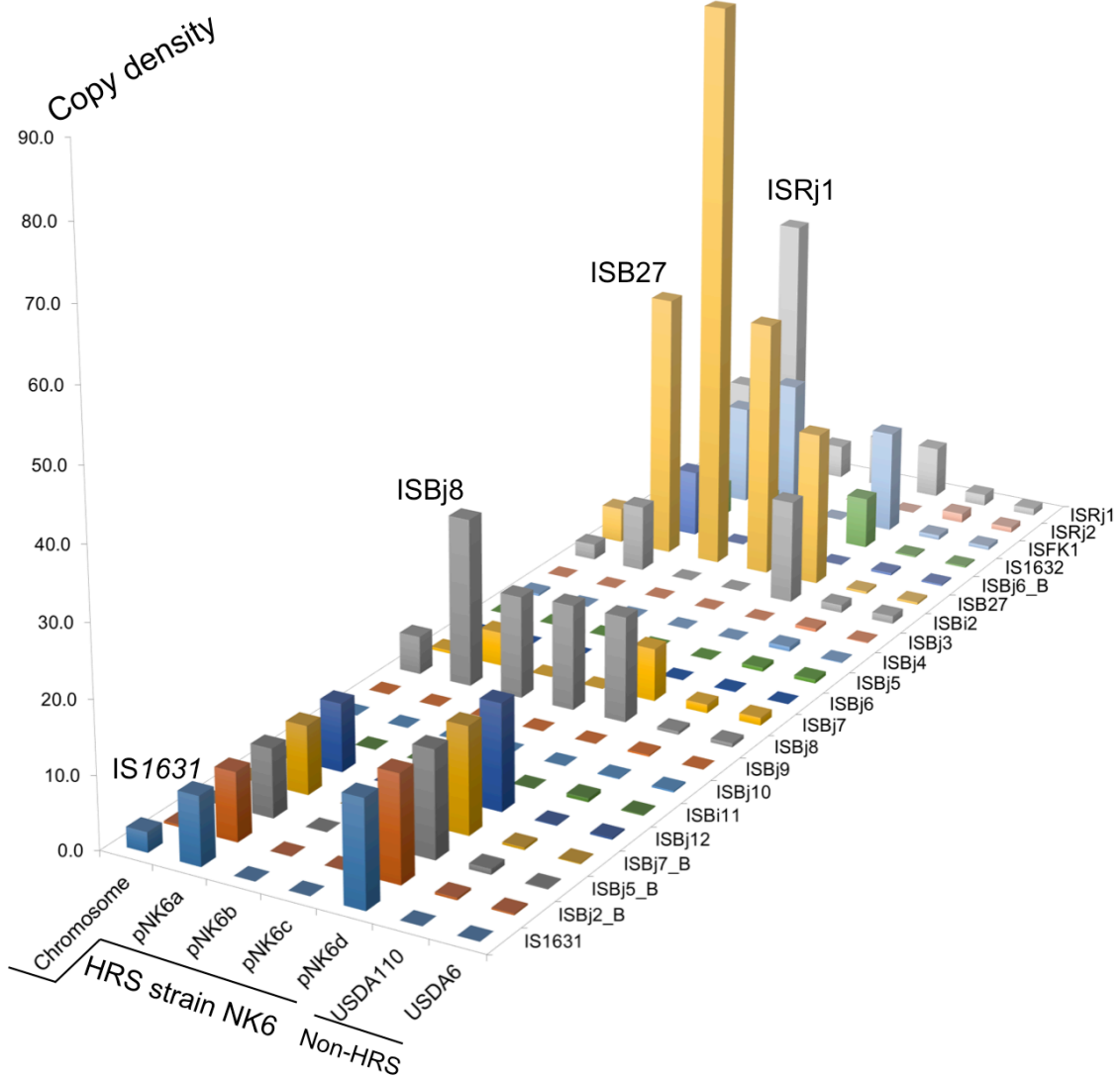


Fig. S2. Density of 21 insertion sequences (ISs) in the genomes of NK6, USDA110, and USDA6. The IS density is expressed as the copy number–based length (Mb). Note that plasmids pNK6a and pNK6d had higher density of ISs, including *IS1631*.

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