

1 SUPPLEMENTARY MATERIAL

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3 TABLES

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Table S1. Oligonucleotide Primers used in this Study

<i>Purpose</i>	<i>Primer</i>	<i>Sequence^a</i>
Cloning of internal 937bp fragment of <i>gosD</i> in pORI19	IM199F IM199R	tgcggaagctt cgatgattacgagcccacgtac ctatg ctctaga gttgcccttcgagtcgtac
Amplification of tetW	tetWFw tetWRv	tcagctgctgacatgctcatgtacggtaaggaagca gcgacggtcgaccataactctgattgtgccg
Confirmation of site specific homologous recombination	pORI19 for 0199-FW	Attgtgagcggataacaatttcac caagagtcagaaccgatcg
Amplification of internal fragment of chromosomal encoded gene from <i>B. breve</i> JCM 7017	7017_1848f 7017_1848r	cagaagtcaccaacgtcaag gattccggcaagatccacctg
Amplification of internal fragment pMP7017 encoded gene 7017pl_0024	7017pl_0024f 7017pl_0024r	gaaccaccaagctgcctgacgac gtgaagctttatcctgggcacg
Amplification of internal fragment of UCC2003 <i>rbsA</i>	rbsAF rbsAR	caagaggcgtgggtatggtatc actcgcgaccaacatcaacacc

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^a Restriction sites incorporated into oligonucleotide primer sequences are indicated in bold

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1 **Table S2. Genes displaying a deviating codon usage in pMP7017.**

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<i>ORF</i>	<i>Product</i>
pMP7017_0002	hypothetical protein
pMP7017_0013	hypothetical protein
pMP7017_0014	Conserved hypothetical protein with a helix-turn-helix motif
pMP7017_0023	Cell surface protein precursor with Cna protein B-type domain and Gram-positive cocci surface proteins LPxTG motif profile
pMP7017_0041	hypothetical protein
pMP7017_0043	hypothetical protein
pMP7017_0044	hypothetical protein
pMP7017_0045	hypothetical protein
pMP7017_0048	hypothetical protein
pMP7017_0050	transposase
pMP7017_0058	hypothetical protein
pMP7017_0068	hypothetical protein
pMP7017_0071	hypothetical protein
pMP7017_0075	hypothetical protein
pMP7017_0081	hypothetical protein
pMP7017_0091	hypothetical protein
pMP7017_0099	transposase
pMP7017_0101	Acetyltransferase
pMP7017_0107	hypothetical protein
pMP7017_0110	hypothetical protein
pMP7017_0111	hypothetical protein
pMP7017_0115	hypothetical protein
pMP7017_0119	hypothetical protein
pMP7017_0120	HipA domain-containing protein
pMP7017_0121	hypothetical protein
pMP7017_0127	hypothetical protein with putative flagellar basal body-associated domain protein
pMP7017_0132	hypothetical protein
pMP7017_0136	Protein translocase subunit secE
pMP7017_0139	hypothetical protein
pMP7017_0144	methyltransferase domain protein
pMP7017_0151	hypothetical protein
pMP7017_0155	hypothetical protein
pMP7017_0157	hypothetical protein
pMP7017_0158	hypothetical protein
pMP7017_0159	hypothetical protein
pMP7017_0162	hypothetical protein
pMP7017_0166	hypothetical protein
pMP7017_0169	hypothetical protein
pMP7017_0172	hypothetical protein
pMP7017_0173	hypothetical protein
pMP7017_0175	hypothetical protein
pMP7017_0177	transposase
pMP7017_0182	hypothetical protein
pMP7017_0186	Conserved hypothetical secreted protein with NlpC/P60 family domain
pMP7017_0198	Single-strand DNA binding protein

pMP7017_0200	Conserved hypothetical secreted protein with prokaryotic membrane lipoprotein lipid attachment site and Gram-positive cocci surface proteins LPxTG motif profile
pMP7017_0202	Conserved hypothetical protein
pMP7017_0204	Hypothetical secreted protein
pMP7017_0205	hypothetical protein
pMP7017_0206	Hypothetical protein
pMP7017_0208	hypothetical protein
pMP7017_0210	hypothetical protein
pMP7017_0211	hypothetical protein
pMP7017_0212	hypothetical protein
pMP7017_0213	hypothetical protein
pMP7017_0215	hypothetical protein
pMP7017_0216	hypothetical protein
pMP7017_0219	hypothetical protein
pMP7017_0226	hypothetical protein
pMP7017_0229	hypothetical protein
pMP7017_0230	hypothetical protein

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1 **Table S3. CRISPR/Cas repeats in pMP7017 and *B. longum* subsp. *longum*.**

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<i>CRISPR system</i>	<i>Direct repeat</i>	<i>Repeat variant</i>	<i>Terminal repeat</i>	<i>Locus length (bp)</i>	<i>Spacers</i>
pMP7017_CRISPR1	ACCTACCCCGCAGGCGCGGGGATAAACC	ACCTACCCCGCAGGCGCGGGGATAAACC	ATCTACCCCGCAGGCGCGGGGATAAACC	636	10
p1-6B CRISPR1	TACCTACCCCGCACACGCGGGGATAAACCTT	N/A	AACCTACCCCGCACATGCGGGGATAAACCTG	152	2
p44B CRISPR1	TACCTACCCCGCACACGCGGGGATAAACCTT	N/A	AACCTACCCCGCACATGCGGGGATAAACCTG	152	2
p2-2B CRISPR1	ACCTACCCCGCAGGCGCGGGGATAAAA	ACCTACCCCGCAGGCGCGGGGATAAACCTACCCCGCACACGCGGGGATAAACCTACCCCGCACACGCGGGGATAAAATGCCTACCCCGCACACGAGGGGATAAAA	ACCTACCTCGTACACGCGGGGATAAA	642	10
pMP017_CRISPR2	ATCTACCCCGCACATGCGGGGATAAACC	ATCTACCCCGCACACGCGGGGATAAACC ATCTACCCCGCACGCGGGGATAAACC ATCTACCCCGCATGCGGGGATAAACC	ATCTACCCCGCACACGCGGGGATAAACC	942	15
p1-6B CRISPR2	ATCTACCCCGCACACGCGGGGATAAACC	N/A	ATCTACCCCGCACATGCGGGGATAAACC	1240	20
p44B CRISPR2	ATCTACCCCGCACACGCGGGGATAAACC	CATCTACCCCGCACACGCGGGGATAAACC AATCTACCCCGCACACGCGGGGATAAACC	N/A	751 ++	12 ++
P2-2B CRISPR2	ATCTACCCCGCACACGCGGGGATAAACC	N/A	N/A	88 ++	1 ++

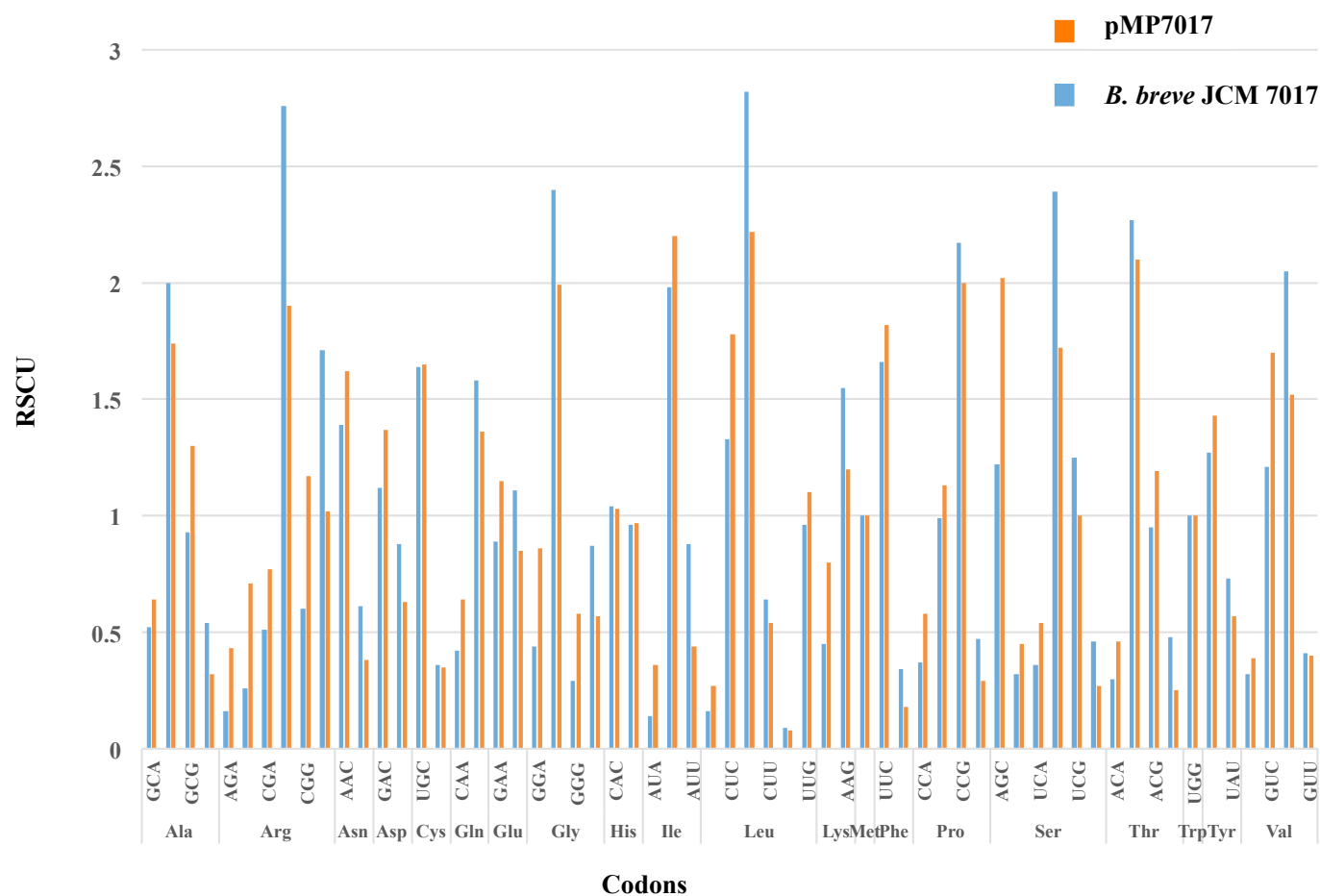
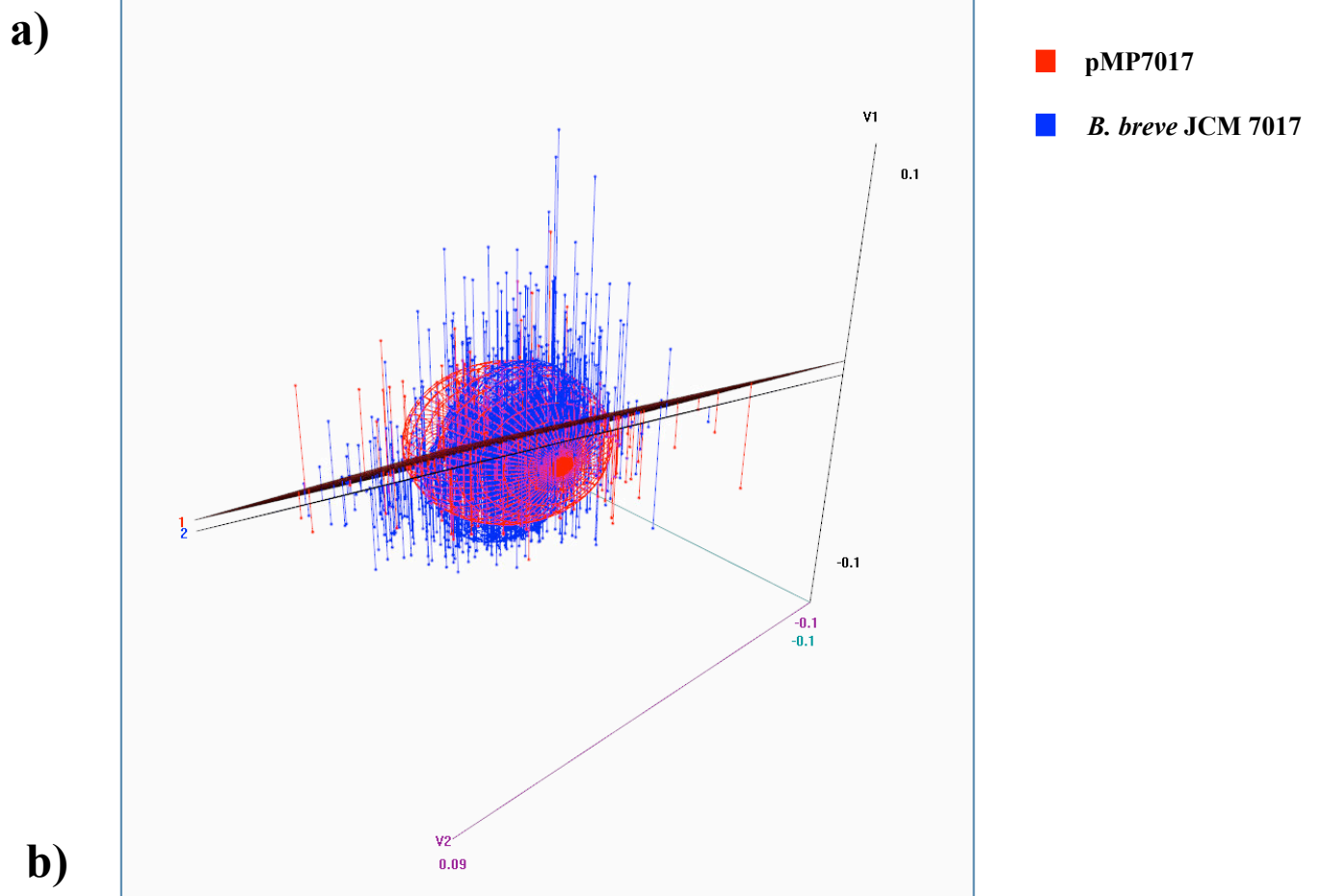


Figure S2

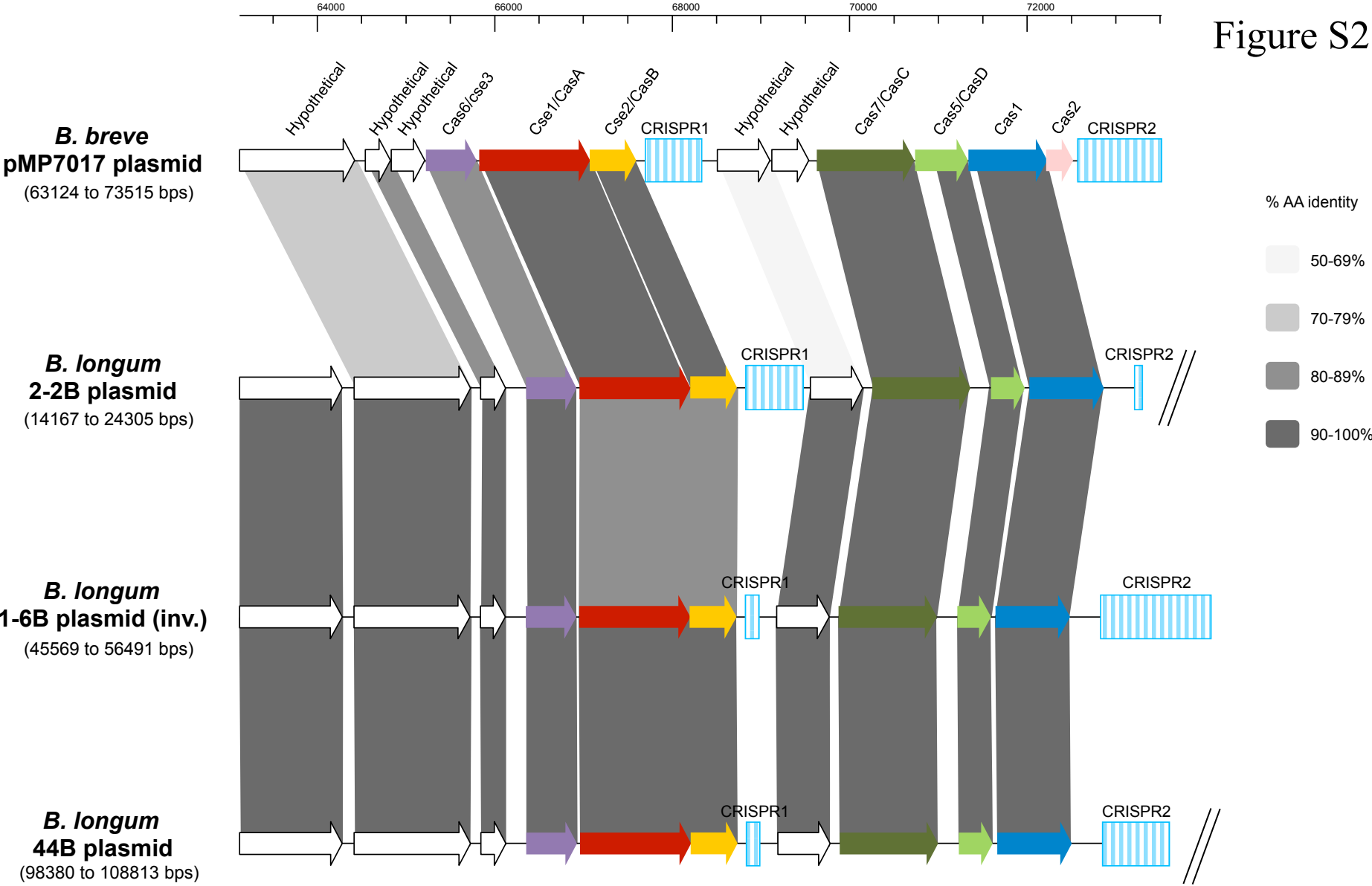
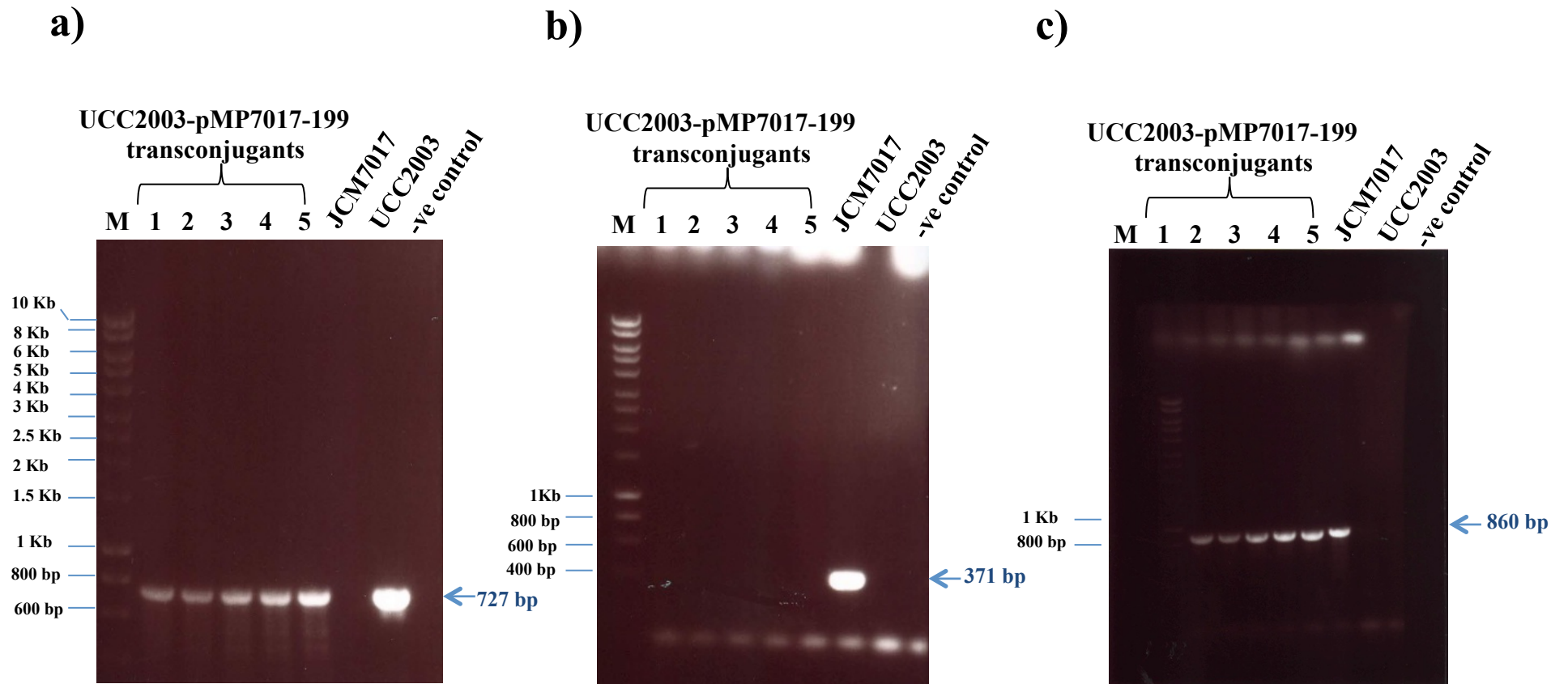


Figure S3



1 **FIGURE LEGENDS**

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3 **Figure S1. Codon usage comparison of pMP7017 and the host.**

4 a) 3D scatterplot representing principal component analysis (PCA) conducted on the
5 predicted ORFs of plasmid (red) and host (blue) and computed using scatter3D
6 module implemented in the statistical package R. As indicated by the overlap of
7 the relative regression surfaces (red and blue spheres), codon usage of host fits
8 with that of the plasmid.

9 b) Bidimensional barplot indicating the relative synonymous codon usage value
10 (RSCU) computed for of all the codons encountered in the plasmid (orange) and
11 the host (pale blue).

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13 **Figure S2. Presence of CRISPR/Cas systems in pMP7017 and *B. longum* subsp.** 14 ***longum*.**

15 Locus map showing the comparison of the CRISPR/Cas system of pMP7017
16 megaplasmid and the draft sequence online available of *B. longum* subsp *longum* 1-
17 6B, *B. longum* subsp. *longum* 2-2B, *B. longum* subsp. *longum* 44B.

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19 **Figure S3. PCR confirmation of UCC2003 pMP7017 transconjugants.** Colony

20 PCRs on five *B. breve* UCC2003 pMP7017-199 transconjugants was performed using
21 primer pairs that amplify an internal fragment of (a) *B. breve* UCC2003 rbsA, (b) *B.*
22 *breve* JCM7017 7017_1848 and (c) pMP7017_0024. Molecular weight marker (M) is
23 included in each gel. Control PCRs were performed incorporating DNA of either *B.*
24 *breve* UCC2003, *B. breve* JCM7017 or no DNA.