Supplementary Information

Terpene Synthase Genes Originated from Bacteria through Horizontal Gene Transfer Contribute to Terpene Diversity in Fungi

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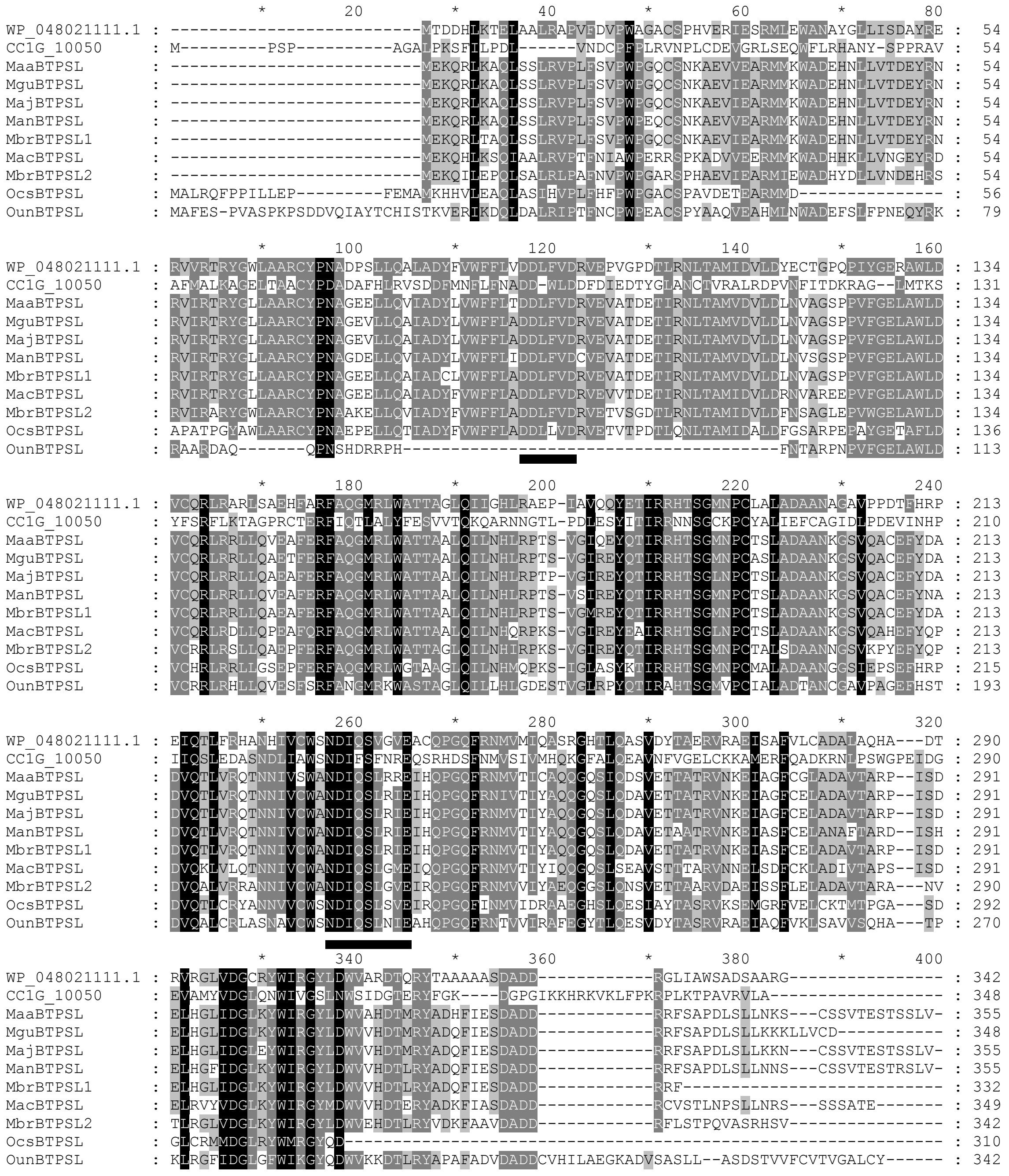

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                           *        20         *        40         *        60         *        80
WP_048021111.1 : --------------------------MTDDHLKTELAALRAPVFDVPWAGACSPHVERIESRMLEWANAYGLLISDAYRE :  54
CC1G_10050     : M---------PSP----------AGALPKSFILPDL------VNDCPFPLRVNPLCDEVGRLSEQWFLRHANY-SPPRAV :  54
MaaBTPSL       : --------------------------MEKQRLKAQLSSLRVPLFSVPWPGQCSNKAEVVEARMMKWADEHNLLVTDEYRN :  54
MguBTPSL       : --------------------------MEKQRLKAQLSSLRVPLFSVPWPGQCSNKAEVIEARMMKWADEHNLLVTDEYRN :  54
MajBTPSL       : --------------------------MEKQRLKAQLSSLRVPLFSVPWPGQCSNKAEVIEARMMKWADEHNLLVTDEYRN :  54
ManBTPSL       : --------------------------MEKQRLKAQLSSLRVPLFSVPWPEQCSNKAEVVEARMMKWADEHNLLVTDEYRN :  54
MbrBTPSL1      : --------------------------MEKQRLTAQLSSLRVPLFSVPWPGQCSNKAEVIEARMMKWADEHNLLVTDEYRN :  54
MacBTPSL       : --------------------------MEKQHLKSQIAALRVPTFNIAWPERRSPKADVVEERMMKWADHHKLLVNGEYRD :  54
MbrBTPSL2      : --------------------------MEKQILEPQLSALRLPAFNVPWPGARSPHAEVIEARMIEWADHYDLLVNDEHRS :  54
OcsBTPSL       : MALRQFPPILLEP---------FEMAMKHHVLEAQLASIHVPLFHFPWPGACSPAVDETEARMMD--------------- :  56
OunBTPSL       : MAFES-PVASPKPSDDVQIAYTCHISTKVERIKDQLDALRIPTFNCPWPEACSPYAAQVEAHMLNWADEFSLFPNEQYRK :  79

                           *       100         *       120         *       140         *       160
WP_048021111.1 : RVVRTRYGWLAARCYPNADPSLLQALADYFVWFFLVDDLFVDRVEPVGPDTLRNLTAMIDVLDYECTGPQPIYGERAWLD : 134
CC1G_10050     : AFMALKAGELTAACYPDADAFHLRVSDDFMNFLFNADD-WLDDFDIEDTYGLANCTVRALRDPVNFITDKRAG--LMTKS : 131
MaaBTPSL       : RVIRTRYGLLAARCYPNAGEELLQVIADYLVWFFLTDDLFVDRVEVATDETIRNLTAMVDVLDLNVAGSPPVFGELAWLD : 134
MguBTPSL       : RVIRTRYGLLAARCYPNAGEVLLQAIADYLVWFFLADDLFVDRVEVATDETIRNLTAMVDVLDLNVAGSPPVFGELAWLD : 134
MajBTPSL       : RVIRTRYGLLAARCYPNAGEVLLQAIADYLVWFFLADDLFVDRVEVATDETIRNLTAMVDVLDLNVAGSPPVFGELAWLD : 134
ManBTPSL       : RVIRTRYGLLAARCYPNAGDELLQVIADYLVWFFLIDDLFVDCVEVATDETIRNLTAMVDVLDLNVSGSPPVFGELAWLD : 134
MbrBTPSL1      : RVIRTRYGLLAARCYPNAGEELLQAIADCLVWFFLADDLFVDRVEVATDETIRNLTAMVDVLDLNVAGSPPVFGELAWLD : 134
MacBTPSL       : RVIRTRYGLLAARCYPNAGEELLQAIADYFVWFFLADDLFVDRVEVVTDETIRNLTAMIDVLDRNVAREEPVFGELAWLD : 134
MbrBTPSL2      : RVIRARYGWLAARCYPNAAKELLQVIADYFVWFFLADDLFVDRVETVSGDTLRNLTAMIDVLDFNSAGLEPVWGELAWLD : 134
OcsBTPSL       : APATPGYAWLAARCYPNAEPELLQTIADYFVWFFLADDLLVDRVETVTPDTLQNLTAMIDALDFGSARPEPAYGETAFLD : 136
OunBTPSL       : RAARDAQ-------QPNSHDRRPH---------------------------------------FNTARPNPVFGELAWLD : 113

                           *       180         *       200         *       220         *       240
WP_048021111.1 : VCQRLRARLSAEHFARFAQGMRLWATTAGLQIIGHLRAEP-IAVQQYETIRRHTSGMNPCLALADAANAGAVPPDTFHRP : 213
CC1G_10050     : YFSRFLKTAGPRCTERFIQTLALYFESVVTQKQARNNGTL-PDLESYITIRRNNSGCKPCYALIEFCAGIDLPDEVINHP : 210
MaaBTPSL       : VCQRLRRLLQVEAFERFAQGMRLWATTAALQILNHLRPTS-VGIQEYQTIRRHTSGMNPCTSLADAANKGSVQACEFYDA : 213
MguBTPSL       : VCQRLRRLLQAETFERFAQGMRLWATTAALQILNHLRPTS-VGIREYQTIRRHTSGMNPCASLADAANKGSVQACEFYDA : 213
MajBTPSL       : VCQRLRRLLQAEAFERFAQGMRLWATTAALQILNHLRPTP-VGIREYQTIRRHTSGLNPCTSLADAANKGSVQACEFYDA : 213
ManBTPSL       : VCQRLRRLLQVEAFERFAQGMRLWATTAALQILNHLRPTS-VSIREYQTIRRHTSGMNPCTSLADAANKGSVQACEFYNA : 213
MbrBTPSL1      : VCQRLRRLLQAEAFERFAQGMRLWATTAALQILNHLRPTS-VGMREYQTIRRHTSGMNPCTSLADAANKGSVQACEFYDA : 213
MacBTPSL       : VCQRLRDLLQPEAFQRFAQGMRLWATTAALQILNHQRPKS-VGIREYEAIRRHTSGLNPCTSLADAANKGSVQAHEFYQP : 213
MbrBTPSL2      : VCRRLRSLLQAEPFERFAQGMRLWATTAGLQILNHIRPKS-VGIREYQTIRRHTSGMNPCTALSDAANNGSVKPYEFYQP : 213
OcsBTPSL       : VCHRLRRLLGSEPFERFAQGMRLWGTAAGLQILNHMQPKS-IGLASYKTIRRHTSGMNPCMALADAANGGSIEPSEFHRP : 215
OunBTPSL       : VCRRLRHLLQVESFSRFANGMRKWASTAGLQILLHLGDESTVGLRPYQTIRAHTSGMVPCIALADTANCGAVPAGEFHST : 193

                           *       260         *       280         *       300         *       320
WP_048021111.1 : EIQTLFRHANHIVCWSNDIQSVGVEACQPGQFRNMVMIQASRGHTLQASVDYTAERVRAEISAFVLCADALAQHA---DT : 290
CC1G_10050     : IIQSLEDASNDLIAWSNDIFSFNREQSRHDSFNMVSIVMHQKGFALQEAVNFVGELCKKAMERFQADKRNLPSWGPEIDG : 290
MaaBTPSL       : DVQTLVRQTNNIVSWANDIQSLRREIHQPGQFRNMVTICAQQGQSIQDSVETTATRVNKEIAGFCGLADAVTARP--ISD : 291
MguBTPSL       : DVQTLVRQTNNIVCWANDIQSLRIEIHQPGQFRNIVTIYAQQGQSLQDAVETTATRVNKEIAGFCELADAVTARP--ISD : 291
MajBTPSL       : DVQTLVRQTNNIVCWANDIQSLRIEIHQPGQFRNMVTIYAQQGQSLQDAVETTATRVNKEIAGFCELADAVTARP--ISD : 291
ManBTPSL       : DVQTLVRQTNNIVCWANDIQSLRIEIHQPGQFRNMVTIYAQQGQSLQDAVETAATRVNKEIASFCELANAFTARD--ISH : 291
MbrBTPSL1      : DVQTLVRQTNNIVCWANDIQSLRIEIHQPGQFRNMVTIYAQQGQSLQDAVETTATRVNKEIASFCELADAVTARP--ISD : 291
MacBTPSL       : DVQKLVLQTNNIVCWANDIQSLGMEIQQPGQFRNMVTIYIQQGQSLSEAVSTTTARVNNELSDFCKLADIVTAPS--ISD : 291
MbrBTPSL2      : DVQALVRRANNIVCWANDIQSLGVEIRQPGQFRNMVVIYAEQGGSLQNSVETTAARVDAEISSFLELADAVTARA---NV : 290
OcsBTPSL       : DVQTLCRYANNVVCWSNDIQSLSVEIRQPGQFINMVIDRAAEGHSLQESIAYTASRVKSEMGRFVELCKTMTPGA---SD : 292
OunBTPSL       : DVQALCRLASNAVCWSNDIQSLNIEAHQPGQFRNTVVIRAFEGYTLQESVDYTASRVRAEIAQFVKLSAVVSQHA---TP : 270

                           *       340         *       360         *       380         *       400
WP_048021111.1 : RVRGLVDGCRYWIRGYLDWVARDTQRYTAAAAASDADD------------RGLIAWSADSAARG---------------- : 342
CC1G_10050     : EVAMYVDGLQNWIVGSLNWSIDGTERYFGK----DGPGIKKHRKVKLFPKRPLKTPAVRVLA------------------ : 348
MaaBTPSL       : ELHGLIDGLKYWIRGYLDWVAHDTMRYADHFIESDADD------------RRFSAPDLSLLNKS---CSSVTESTSSLV- : 355
MguBTPSL       : ELHGLIDGLKYWIRGYLDWVVHDTMRYADQFIESDADD------------RRFSAPDLSLLKKKLLVCD----------- : 348
MajBTPSL       : ELHGLIDGLEYWIRGYLDWVVHDTMRYADQFIESDADD------------RRFSAPDLSLLKKN---CSSVTESTSSLV- : 355
ManBTPSL       : ELHGFIDGLKYWIRGYLDWVVHDTLRYADQFIESDADD------------RRFSAPDLSLLNNS---CSSVTESTRSLV- : 355
MbrBTPSL1      : ELHGLIDGLKYWIRGYLDWVVHDTLRYADQFIESDADD------------RRF--------------------------- : 332
MacBTPSL       : ELRVYVDGLKYWIRGYMDWVVHDTERYADKFIASDADD------------RCVSTLNPSLLNRS---SSSATE------- : 349
MbrBTPSL2      : TLRGLVDGLKYWIRGYLDWVEHDTLRYVDKFAAVDADD------------RFLSTPQVASRHSV---------------- : 342
OcsBTPSL       : GLCRMMDGLRYWMRGYQD-------------------------------------------------------------- : 310
OunBTPSL       : KLRGFIDGLGFWIKGYQDWVKKDTLRYAPAFADVDADDCVHILAEGKADVSASLL--ASDSTVVFCVTVGALCY------ : 342
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Fig S1. Multiple sequence alignment of selected terpene synthases. These includes 9 bacterial terpene synthase-like (BTPSL) proteins and representative terpene synthase WP_048021111.1 and CC1G_10050 from bacteria (Burkholderia) and fungi (*Coprinopsis cinerea* okayama7#130), respectively. The positions of DDxx(x)D and NDxxSxxxE motifs are indicated by black bars.

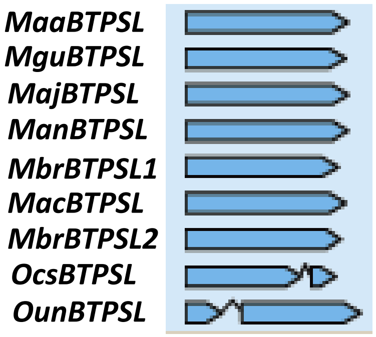

Fig S2. Intron-exon organization of bacterial terpene synthase-like (BTPSL) genes. These include seven *BTPSL* genes from *Metarhizium* (intronless) and two *BTPSL* genes from *Ophiocordyceps* (one intron)

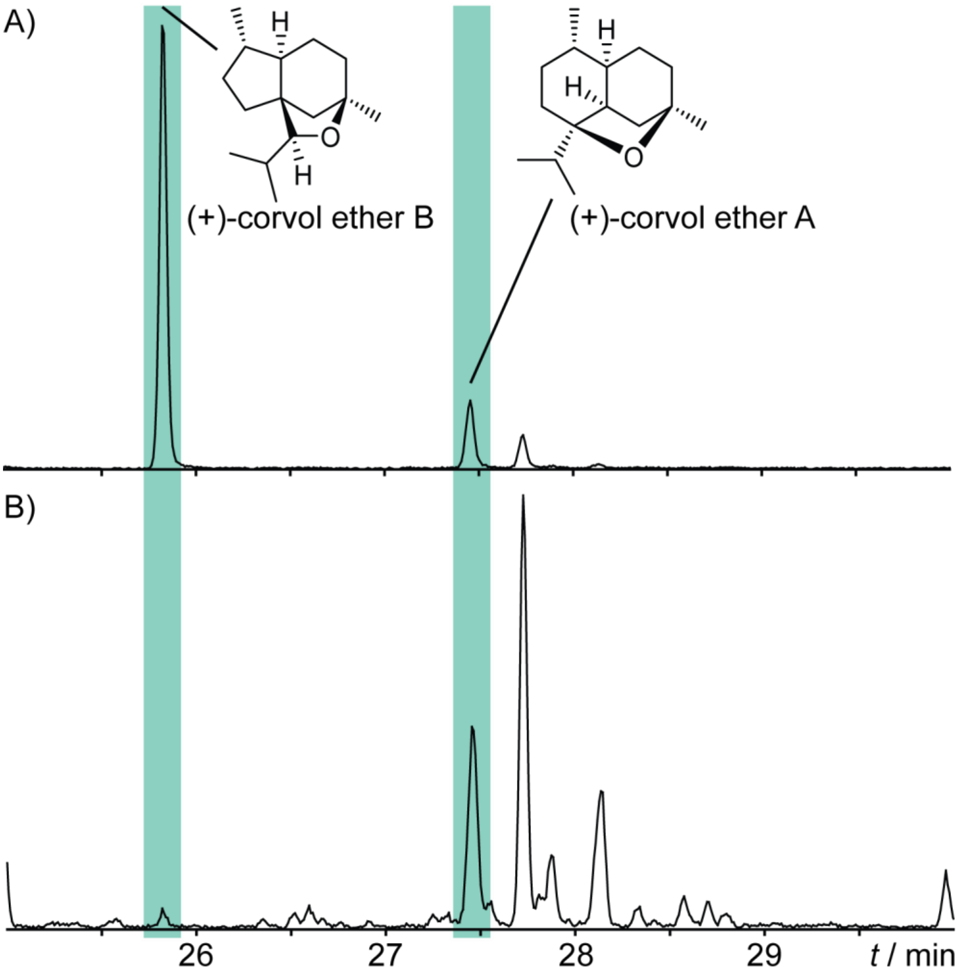

Fig S3. Terpene products identification. Total ion chromatograms of extracted incubations of farnesyl diphosphate with A) recombinant corvol ether synthase (BAJ27126) from *Kitasatospora setae* and B) recombinant MajBTPSL under the same conditions. The peaks highlighted feature the same mass spectra.

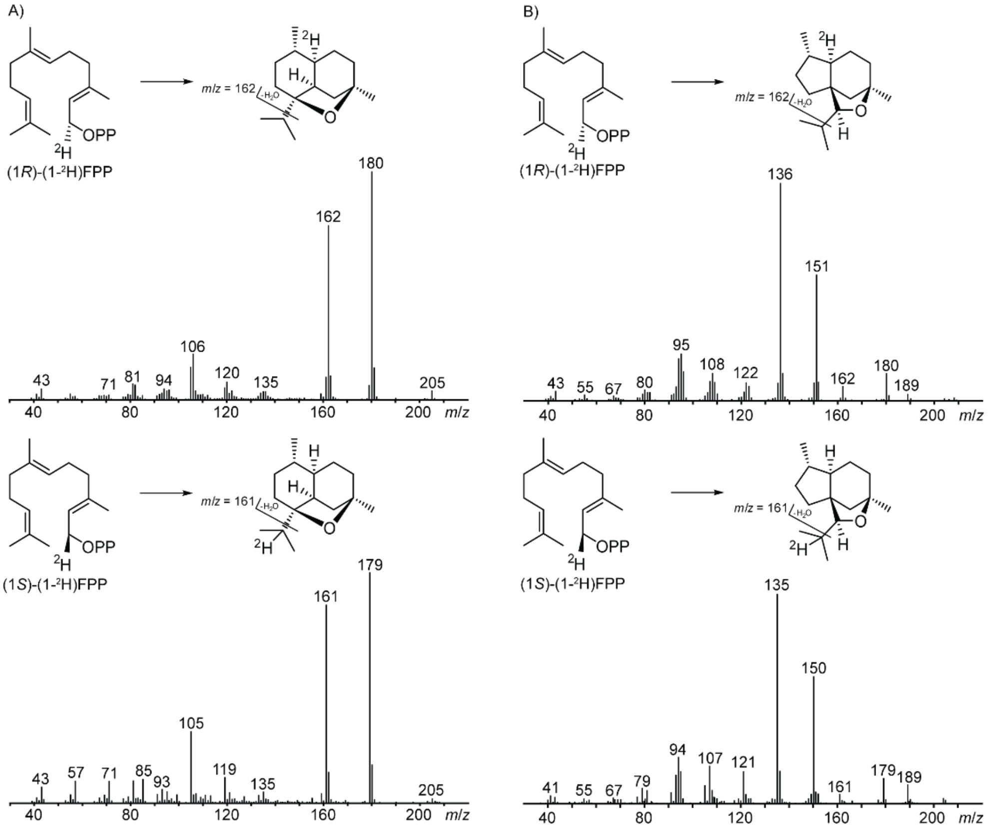

Fig S4. Mass spectra of terpenes. EI-MS spectra of deuterated A) corvol ether A and B) corvol ether B, both generated by incubating recombinant MajBTPSL and (1*R*)-(1-^{2}H)farnesyl diphosphate (top) as well as (1*S*)-(1-^{2}H)farnesyl diphosphate (bottom). The 1,3-hydride shift into the isopropyl group shows the same stereochemical course as for the bacterial corvol ether synthase from *Kitasatospora setae* indicating the same absolute configuration of the obtained products.

Table S1 BTPSL genes from eight species of entomopathogenic fungi.

Organism	*BTPSL*s	New_names	Length (aa)
Metarhizium acridum (MAC)	*MAC_05714*	*MacBTPSL*	349
Metarhizium majus (MAJ)	*MAJ_08936*	*MajBTPSL*	355
Metarhizium guizhouense (MGU)	*MGU_11447*	*MguBTPSL*	348
Metarhizium brunneum (MBR)	*MBR_10393*	*MbrBTPSL1*	332
	MBR_09977	*MbrBTPSL2*	342
Metarhizium anisopliae (MAN)	*MAN_10655*	*ManBTPSL*	355
Metarhizium robertsii (MAA)	*MAA_08668*	*MaaBTPSL*	355
Ophiocordyceps sinensis (OCS)	*OCS_03958*	*OcsBTPSL*	310
Ophiocordyceps unilateralis (XA68)	*XA68_4644*	*OunBTPSL*	342

Table S2 Primers used for gene cloning and neighbor gene confirmation

Gene name	Primers	sequence
MacBTPSL	Forward	5'-ATGGAAAAACAACACTTGAAATCTC-3'
	Reverse	5'-TCACTCAGTCGCGGACGAGGAACTTC-3'
MaaBTPSL	Forward	5'-ATGGAAAAACAAAGACTGAAAGC-3'
	Reverse	5'-CTAGACCAAGCTGCTCGTTGACTC-3'
MajBTPSL	Forward	the same as MaaBTPSL Forward
	Reverse	the same as MaaBTPSL Reverse
ManBTPSL	Forward	5'-ATGGAAAAACAAAGATTGAAAGCTC-3'
	Reverse	5'-CTAAACCAAGCTTCTCGTTGACTCAG-3'
MbrBTPSL1	Forward	5'-ATGGAAAAACAAAGATTGACAGCTC-3'
	Reverse	5'-TCAAAAGCGACGATCATCGGCATC-3'
MbrBTPSL2	Forward	5'-ATGGAAAAACAAATCTTGGAGCCTC-3'
	Reverse	5'-TTACACACTATGTCTAGAAGCAACTTG-3'
MguBTPSL	Forward	the same as MaaBTPSL Forward
	Reverse	5'-TCAGTCACAGACGAGCAACTTTTTTTTTAG-3'
BcTPS	Forward	5'-ATGACAGACGACCATCTGAAAACCGAAC-3'
	Reverse	5'-TCAGCCGCGCGCCGCCGAGTCCGCCGAC-3'
MbrBTPSL2NBF	Forward	5'-GAATGGGTCCTCTTCGTGACTTTG-3'
MbrBTPSL2NBR	Reverse	5'-CAATCATAGCCGTCAAGTTGCGCAG-3'
MAA_11029/MaaBTPSLNB	Forward	5'-ATGCCGCTGGCAGACTTGCGGTAC-3'
MaaBTPSNB1	Reverse	5'-GACTCAAGTACTGGATACGAGGTTAC-3'
MaaBTPSNB2	Forward	5'-GTCAGTCACCAGCAGATTATGCTC-3'
MaaBTPLS/MAA_08869NB	Reverse	5'-TACACAAGGCCGGCAAGAACAGTG-3'