

Supplementary Material

Evaluation of *Ogataea (Hansenula) polymorpha* for hyaluronic acid production

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Table S1. List of primers used in this work

Primer name	Sequence to amplify	Primer orientation	Restriction enzyme	'Primer sequence 5'→3'
attB_hasB_F	<i>attL</i> (<i>hasB</i>)	Forward	NA	CCAGTTCCCTGAAATTATTCCCCCT
attP_hasB_R	<i>attL</i> (<i>hasB</i>)	Reverse	NA	CAGCTTCTTGAATTGCACCATCAAT
attB_hasAp_F	<i>attL</i> (<i>hasAp</i>)	Forward	NA	CTCTTCTTAGGCATCCTTCTATC
attP_hasAp_R	<i>attL</i> (<i>hasAp</i>)	Reverse	NA	GCAGCACTAGCTGGAAACC
hasB_F	<i>hasB</i>	Forward	HindIII	AAAAAG CTTAATGTT CAGATCAAGAAGATTGTTGA A
hasB_R	<i>hasB</i>	Reverse	Sall	AAAGTCGACTTACACACGTTGCTTC
hasAp_F	<i>hasAp</i>	Forward	HindIII	AAGCTT AGCTT TATGAATACTTATCTC
hasAp_R	<i>hasAp</i>	Reverse	XhoI	AAAGTCGACGC TTACAATGT GATTGA
AOX- Integration_F ¹	AOX promoter	Forward	NA	CAGTTTGCC CTACTTG ATC
AOX- Integration_R ₁	AMO ³ terminator	Reverse	NA	GTAGGAAGG CTGGATGTC

NA: Not applied

¹These primers were designed to confirm the insert integration when the plasmid pHIPH4 is used for cloning.

²In bold, the recognizing sequence for the respective enzyme.

³AMO: Amino oxidase

Table S2. List of plasmids used in this work

Name	Description	Reference
pBSK_hasB	Cloning vector carrying the synthetic <i>hasB</i> gene encoding the UDP-glucose dehydrogenase from <i>Xenopus laevis</i>	This work
pBSK_hasAp	Cloning vector carrying the synthetic <i>hasAp</i> gene encoding the hyaluronic acid synthase from <i>Pasteurella multocida</i>	This work
pBSK_hasAs	Cloning vector carrying the synthetic <i>hasAs</i> gene encoding the hyaluronic acid synthase from <i>Streptococcus zooepidemicus</i>	This work
pGEM-T Easy	Commercial plasmid used in the cloning steps	Promega
pGEM_hasB	pGEM-T Easy plasmid carrying the <i>hasB</i> gene	This work
pHIPH4	<i>O. polymorpha</i> integrative plasmid containing the native promoter pAOX and the terminator TamO	[1]
pHIPZ7	<i>O. polymorpha</i> integrative plasmid containing the native promoter pTEF1 and the terminator TamO	[1]
pHIPZ18_eGFP_SKL	<i>O. polymorpha</i> integrative plasmid containing the native promoter pAHD1 and the terminator TamO and carrying the gene encoding eGFP	[1]
pHIPH4_hasB	pHIPH4-derived plasmid carrying the <i>hasB</i> gene under control of the pAOX promoter	This work
pHIPH4_hasAs	pHIPH4-derived plasmid carrying the <i>hasAs</i> gene under control of the pAOX promoter	This work
pHIPH4_ScSInt13	Synthetic plasmid containing a genetic switch controlling the expression of <i>hasAp</i> and <i>hasB</i> and carrying a gene of a serine-type phage integrase-13, codon-optimized for <i>S. cerevisiae</i>	This work
pHIPZ7_hasAp	pHIPZ7-derived plasmid carrying the <i>hasAp</i> gene under control of the pTEF1 promoter	This work
pHIPZ18_hasAp	pHIPZ18-derived plasmid carrying the <i>hasAp</i> gene under control of the pADH1 promoter	This work
pHIPZ18_hasB	pHIPZ18-derived plasmid carrying the <i>hasB</i> gene under control of the pADH1 promoter	This work

Table S3. List of strains used in this work

Name	Description	Reference
<i>E. coli</i> DH10B	Bacteria strain for plasmid cloning	Invitrogen
<i>O. polymorpha</i> NCYC 495 <i>yku80</i>	Methylotrophic yeast with a NHEJ-deficient phenotype; referred to as Wild Type (WT) in this work	[1]
EMB100.1	WT strain transformed with the linearized pHIPH4_hasB plasmid. The hasB gene was integrated into the genome under control of the pAOX promoter. This strain was used as an intermediate for the construction of the EMB101 and EMB102 strains.	This work
EMB101	EMB100.1 transformed with the linearized pHIPZ18_hasAp plasmid. The hasAp gene was integrated into the genome under control of the pADH1 promoter. The hasB gene is regulated by the pAOX promoter.	This work
EMB102	EMB100.1 transformed with the linearized pHIPZ7_hasAp plasmid. The hasAp gene was integrated into the genome under control of the pTEF1 promoter. The hasB gene is regulated by the pAOX promoter.	This work
EMB103	WT strain transformed with the linearized pHIPH4_ScsInt13 containing a genetic switch controlling the expression of hasAp and hasB and carrying a gene for a serine-type phage integrase-13, codon-optimized for <i>S. cerevisiae</i> and under control of the pAOX promoter. The genes hasB and hasAp are regulated by the pGDP promoter from <i>S. cerevisiae</i> and the pADH1 promoter from <i>O. polymorpha</i> , respectively.	This work
EMB100.2	WT strain transformed with the linearized pHIPZ18_hasB plasmid. The hasB gene was integrated into the genome under control of the pAHD1 promoter. This strain was used as an intermediate for the construction of EMB104.	This work
EMB104	EMB100.2 transformed with the linearized pHIPH4_hasAs plasmid. The hasAs gene was integrated into the genome under control of the pAOX promoter. The hasB gene is regulated by the pADH1 promoter.	This work

Sequence S1. Nucleotide sequence of the *hasB* gene from *Xenopus laevis* (Genbank ID: MH728986). The start and stop codons of the ORF are shown in bold.

ATGTTTCAGATCAAGAAGATTGTTATTGGTGCCTACGTCGGTGGTCCAACCTGT
TCTGTCATTGCCACAGATGCTGCCCTGACATTAAGCTCACTGTTGGAATGTGAACCAAGC
CAGGATCAATGCTTGAATAGTGACACTTGCCTATCTACGAACCAGGTTGAAGGAAG
TCGTAGAGTCATGCAGGGAAAGAATTGTTCTACTCAACTGACATTGATGGTCAATT
CAAGAACGCTGATTGGTGTTCATCTCACTAACACTCCAACAAAAACTACCGTATGGG
TAAGGGAAAGGGCAGCCGACTGAAATACATTGAGGCTGCCTAGAAGAATAGTACAG
AATAGTAACGGATAACAAGATTGTTACAGAGAAATCTACTGTGCCAGTTAGAGCTGCTG
AATCAATAAGACGTACTCGATGCAAATACTAAACCAGATTGAACITGCAGGTATTG
AGTAACCCAGAGTTTGGCAGAGGGTACAGCCATTAAGGATTGAAGAACCCCTGATA
GAGTTTGATAGGTGGTACGAAACCCCTGAAGGTAGAAAGCTGTTAGAGCTTGT
GACGTATCGAACACTGGTACCATCTGAGAAAATCATAACCACAAACACCTGGTCTT
CTGAGTTGAGTAAGTTAGCAGCCAACGCATTCTAGCTCAAAGAATTCTTCATCAAC
TCAATTAGTGCCTATGTGAAGCCACAGGAGCTGACGTGAAGAGGTTGCCAGAGCTAT
TGGTATGGATCAAAGAATTGGTAACAAGTTGAAGGCTTCAGTGGATTGGAGGTT
CATGTTTCAAGAAGGACGTTGAACCTGGTTACTTGTGAGGTGTTAAACTGACAG
AAGTGGCCAAGTACTGGCAACAAGTGATTGATATGAATGATTATCAAAGGAGACGTT
TACAACTAGGATAATCGATTGTTGTTAACACCGTGACCGATAAGAAAATCGCATTGT
TAGGTTCGCTTCAAGAAGGATACTGGTACAGGAGTACTAGAGAGAGTAGTTCAATCTATAC
TCTAAGTATTGATGGATGAAGGTGCTAAGTTACATATCTACGATCCAAAGGTCCCACG
TGAGCAGATCATCACTGACTTGAGTCAACCTGGTGTGACGACAGGGTTCTC
AATTGGTCCACATAAGTACAGATTGTTACGAAGCCTGTGAGAATGCACACGCTATGGTC
ATTGTTACTGAATGGATATGTTCAAGGAATTAGATTCAATAGAATCCATAGGATGAT
GTTAAAGCCTGCTTCATATTGATGGTAGACGTGTTAGATGAATTGCATGGAGAATT
GCAAAACATTGGATTTCAGGTGGAAACCATCGGAAAGAAGGTAGCTCAAAAGAAT
ACCATTCACTCCAAGTGTGATATCCCTAAGTTGGTTACAGGACTTGCCACACAAGA
AGCAACGTGTGAA

Sequence S2. Nucleotide sequence of the *hasAp* gene from *Pasteurella multocida* (Genbank ID: MH728990). The start and stop codons of the ORF are shown in bold.

ATGAATACCTTATCTCAAGCCATCAAGGCATACAATTCAAATGACTATCAATTGGCTT
GAAATTGTCGAAAAGTCAGCAGAAATCTACGGACGTAAGATAGTAGAGTTTCAGATT
ACTAAGTGCAAGGAGAAATTGTCATGCCCATCCAAGTGTCAATTCTACGCTCATCCTTCAGT
CAACTCAGCACATTGTCAGTAAACAAAGAGGAAAGGTTAATGTGCGATTCTCCAT
TGGATATTGCTACCCATTGTTGTCAGTAAAGAAATTAGTGTGTTACTGAAAGAAAAGTGAATG
AGAAGAACACATTGAAGAATAAGTGGAAAGTTGTTACTGAAAGAAAAGTGAATG
CTGAAGTTCGTGTGACTGGTACCAAGGATTCCCAAAGGATTGGTGTGGCA
CCATTACCTGACCATGTGAATGACTTACTGGTACAAGAAGAGAAAGAACGTTAG
GTATCAAACCAAGAACATCAACACGTGGTTGTCAATAATTGTCACCACTTCAATCGT
CCTGCAATCTTAAGTATAACTTGGCATGCTTAGTTAATCAAAGACTCACTATCCATT
GAGGTGATTGTCACAGATGATGGATCACAAGAAGATTGTCAGACAAAAGGACAATGGTTCCAAGCTAG
TGCTGCTAGGAATATGGTTGAGATTAGCAAAGTATGATTTCATTGGTTGTGGATTG

CGATATGGCACCTAACCCATTATGGTGCATTCATATGTCGCTGAATTGTTAGAAGATG
 ATGATTGACAATCATTGGACCAAGAAAGTACATTGATAACACAACATATCGACCCAAA
 GGACTTCTAAACAATGCATCTTGTGGAATCATTGCCAGAAGTTAAGACCAATAACT
 CAGTGGCCGAAAAGGTGAAGGTACCGTTCATGGATTGGAGGTGGAGCAATTGCA
 AAAGACTGAAAACCTTAAGATTGTCAGACTCTCCTTTAGATTCTCGCAGCTGGTAATGT
 TGCTTCGCCAAGAAGTGGTGAACAAATCTGGATTCTTGATGAAGAGTTCAACCATT
 GGGGTGGTGAAGATGTTGAGTTGGATATAGATTGTTAGGTATGGTCATTCTCAAGA
 CTATTGACGGTATCATGGCCTACCATCAAGAGCCACCTGGTAAGGAAAACGAAACAGA
 TAGGGAAGCTGAAAGAACATCACATTGGATATTATGAGGGAGAAGGTACCATATATT
 TACAGGAAGTTGTCATCGAAGATTCACACATCAATAGAGTCCCTTGGTTCTATC
 TATATCCCAGCTTACAACGTGCCAATTATATTCAACCGTGTGATTCTGCCTGAAAC
 CAGACAGTTGAGATTGGAAGTCTGTATTGCAATGATGGTCTACAGATAATACTTG
 GAAAGTTATCAACAAAGTTGACGGTAACAATCCAAGAGTCAGAATCATGAGTAAACCAA
 ATGGTGGTATTGCTAGTGCCTAATGCAGCAGTGAGTTGCCAAGGGATATTACATA
 GGTCAATTAGATTCAAGATGACTATTGGAGGCCAGATGCCGTAGAGTTATGTTGAAAGA
 GTTCTGAAAGACAAAACTTGGCTGTGTATATACAACAAACAGAAATGTCATCCTG
 ATGGTTCTTGTAGCAAATGGTACAACACTGCCAGAGTTAGTAGGGAGAAGTTGACT
 ACTGCAATGATTGCTCATCACTCCGTATGTTCACTATCAGGGCATGGCATTGACCGAT
 GGTTTAATGAGAAGATTGAGAATGCTGTGACTACGATATGTTCTGAAGTTGAGTGA
 AGTGGTAAGTTCAAGCACTTAAACAAAATCTGCTATAACAGGGTATTGCATGGTATA
 ATACAAGTATTAAGAAGTTGGTATCCAAAAGAAGAACCATTCGTGGTGTCAACCA
 GACTTGAACAGGAACGGAACTACTACAATTACGACGAGTCGATGACTTAGAT
 GAGTCTAGGAATACATCTTAACAAAACAGCTGAGTACCGAGGAAGAAATTGACATCT
 TAAAGGACATTAAGATCATAACAAAACAAGGACGCTAAATAGCAGTATCTATCTCTA
 CCCAAATACTTGAATGGTTGGTCAAGAAATTGAATAACATCATCGAGTACAACAAG
 AACATATTGTTATTGCTTGCATGTGGACAAGAACCATTGACCCAGATATCAAGAA
 AGAGATATTGGCTTCTACCACAAGCATCAAGTGAATATTGTTGATGAATAACGATATCT
 CATACTACACATCAAACCGTTAATCAAGACCGAGGCACATTATCAAACATTAATAA
 GTTGTACAGTTGAACCTGAATTGTAATATCATATTGACAATCATGACTCTTGT
 CGTGAAGAATGATTCTATGCCTATATGAAGAAGTACGATGTTGATGAATTCTCAG
 CCTTAACTCATGATTGGATTGAAAAGATTAACGCACATCCACCATTCAAGAAGTTGATT
 AAGACATACTTAAAGATAATGACTGAAATCTATGAACGTTAAAGGAGCTAGTCAAG
 GAATGTTATGACATATGCATTGGCTCACGAATTGTTGACTATTATCAAAGAGGTTATCA
 CTTCTGCCAATCTATCGATTCTGTACCGAAATACACACTGAGGACATATGGTTCAAT
 TTGCATTGTTGATCTGGAAAAGAAAAACTGGTACATTGTTAACAGACAAGTACCTG
 ACATACATGCCCTGGGAGAGGAAGTGCAATGGACCAATGAACAAATTGAATCAGCTA
 AACGTGGAGAAAACATTCCAGTGAACAAAGTTCATATCAATTCAATCACATTGTAA

Sequence S3. Nucleotide sequence of the *hasAs* gene from *Streptococcus zooepidemicus* (Genbank ID: AF414053.1). The start and stop codons of the ORF are shown in bold.

ATGAGAACTTGAAAAATTAAATTACAGTTGTCATTCTCAATTCTGGTTTATTAA
 ATTATGTTAATGTTATTGTTGCTCGGTGCTAAAGGTTATTGTCATTCTCAATTATGTTCTTATT
 AATTGCATATTGTTAGTTAAAGTCTTGTCAATTCTTATAAACCAAGGTTAG
 AGCAGGTCAATATAAGTTGCTGCAATTATTCCATCATATAATGAAGATGCTGAATCTT

TATTGGAAACTTGAATCAGTCACAAACAAACATATCCATTGCCAGAAATTATGTT
 GTTGATGATGGTCAGCCGATGAAACAGGTATTAAAAGAATTGAAGATTATGTTAGAG
 ATACTGGTGTATTATCATCTAACATGTTATTGTCATCGTAGCGAAAAAAATCAAGGTAAA
 AGACATGCACAAGCATGGGCTTCGAAAGAAGCGATGCAGATGTTCTGACAGTGA
 TTCAGATACATATTTATCCAGATGCTTCCAAGAATTATTGAAACTTCAATGATCC
 TACAGTTTCGAGCTACAGGTCTTGAATGTTAGAAATAGACAAACTAATTGTTAA
 CAAGATTGACTGATATTAGATATGATAATGCATTGGTGTGAAAGAGCAGCACAAATCA
 GTTACTGGTAATATTAGTTGTTCTGGCCTTGTCAAGTTATAGAAGAGAAGTTGTTG
 TTCTTAATATTGATAGATATTAATCAAACATTCTAGGTATTCCAGTTCAATTGGTGA
 TGATAGATGTTAACTAATTATGCTACTGATTGGTAAACAGTTATCAATCTACAGC
 TAAATGTATTACTGATGTTCCAGATAAAATGTCTACATATTGAAACAACAAATAGAT
 GGAATAAAATCATTCTCAGAGAATCTATTTCAGTTAAAAAAATTATGAATAATCCA
 TTCGGTCTTGTGGACTATTGGAAAGTTCTATGTTCATGATGTTAGTTATTCAAGTGT
 TGATTCTCGTTGATAATGTTAGAGAGTCGATTGTTAAGAGTTGGCATTCTGGTT
 ATTATTTCAATTGTTGCTTGTGAGAAATATTCAATTATGTTGAAACATCCTTGTCTT
 CTTGTTATCTCTTCTATGGTGTTCATTTATTCAAGTGTGAAATTGTT
 CATTGTTCACTATTAGAAATGCTGATTGGGTACTAGAAAAAAATTGTTGTA**A**

Sequence S4. Nucleotide sequence of the gene of serine integrase-13 (Int13), codon-optimized for *S. cerevisiae*. The start and stop codons of the ORF are shown in bold.

ATGGCGGTGGATTACATCAGACTCAACCCAAGAGCAGCGAGTGAAGGGCACA
 GTATTGAAAGCCAAAAAAAGAAACTGGCTTATTGTGAAATCCAAGGATGGATGA
 CTACAGGTTCTACATCGAAGAGGGCATATCCGGAAAAACACAAATAGACCGAAGCTT
 AAGCTATTAACTGAAACATATCGAAAAGGGAAAATTAACATTATTGTTCTACAGGCT
 GGATAGGTTGACTAGGTCTGTGATCGATTACATAAGCTATTAAACTTTACAGGAAC
 ATGGGTGCGGTTAAATCTGCTACAGAAACTTACGACACAACACTGCAAACCGAAG
 GATGAGTATGGGTATAGTGAGTCTCTAGCCCAGGGAGACAGAAAATATGAGTGAG
 CGTATTAAACTAAACCTGAAACACAAAGCTTGGTGAAGGGAAAGAGTAGGGCGA
 TTCCCTATGGATTGACTTGTCAAGATGATGAAAGCTTGTGAAGAATGAAAAGCTGCA
 ATTTATTGGACATGGCGAAAGGGTGAGAACGGCTGGTCCGTCAATAGGATCGTCAA
 CTATCTTAATTAACTAACATGATCGTAACGGTACCTAACGGTGCTACGTTGTT
 AAGGAACCCCTGCACTATATGGCGTACAAGGTGGAATGATAAAATCGCAGAGAACAC
 ACACGAGGGTATAATTAGCAAGGAACGTTCAACCGTCTGCAGCAAACACTGCAGAC
 CGTAGCATCCATCACAGACGTGATGTGAAAGGAACATACATATTCAAGGAGTTGA
 GATGTCGGTTGTGATCAGACGCTGTCCGTTAATAGGTTATTAGAAGCGTAAGGAT
 GGAACAGAGTACTGTGGTGTCTTATAGGTGTCAGCCATGTATTAGCAAAACAGTA
 CAATTAGCTATCGCGAAGCTAGGTTCTGAAGGCCCTAACGAGTACATGTCACGG
 TGGAATTCCAGACAGTGAAGACGAGGTGATACCCAGAAAAGTGAAGAGAGAAATGT
 TGGAATCTCAGCTGCAACAGATCGCAAGAAAGAGGGAGAAATACCAAAAGGCATGG
 CGAGCGATTAAATGTCGATGATGAAATTGAGAAACTTATGGTCGAGACCCGTGAAACT
 TATGACGAATGCAAGCAAAACTGGAGAGTTGCCAGGACCTATTAGATCGACGAG
 ACATATTGAAGGAAATAGTTACATGTTCATCAAACATTCAATGATTAGACTCCGA
 GAAGCAAAAGGAGTTATCAAAATTATAAGGACTATCCGTTACACCGTCAAAGAG
 CAGCAACCTATCAGACCTGATAAGTCTAACAGAGTAAGGGTAAACAGAAAGTGATA
 ATTACGGAAGTGGAGTTTACCAAGT**AA**

Sequence S5. The nucleotide sequence of the pGEM_*hasB* plasmid. The sequence of the *hasB* gene is shown in yellow.

TATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTACAACGTCGTGACTGGGAAAA
CCCTGGCGTTACCCAACITAATCGCCTTGCAGCACATCCCCCTTCGCCAGCTGCCGA
ATAGCGAAGAGGCCCGCACCGATGCCCTCCAAACAGTGCAGCAGCTGAATGGCGA
ATGGACGCCCTGTAGCGCGCATTAAGCGCGGGGTGTTACGCGCAGCG
TGACCGCTACACTGCCAGCGCCCTAGCGCCGCTCCTTCGCTTCTCCCTCCTTCT
CGCCACGTTGCCGGCTTCCCCGTCAAGCTCAAATCGGGGCTCCCTTAGGGTCCG
ATTAGTGCCTTACGGCACCTGACCCCCAAAAACTGATTAGGGTGTGGTTACGTA
GTGGGCCATGCCCTGATAGCGGTTTCGCCCTTGACGTTGGAGTCCACGTTCTTA
ATAGTGGACTCTTGTCCAAACTGGAACAACACTCAACCCTATCTCGTCTATTCTTTG
ATTATAAGGGATTTGCCGATTGCCCTATTGGTAAAAATGAGCTGATTAAACAA
AAATTAAACCGAATTAAACAAATATTACGCTTACAATTCTGATGCCGTATTTC
TCCTTACGCATCTGTGCCGTATTACACCCGATCAGTGGCATTTCGGGAAATGTG
CGCGAACCCCTATTGTTATTCTAAACATTCAAATATGTATCCGCTCATGAGA
CAATAACCTGATAAAATGCTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAAC
ATTCCGTGTCGCCCTATTCCCTTTGCCGATTTGCCTCCTGTTTGCTCACCCA
GAAACGCTGGTAAAGTAAAAGATGCTGAAGATCAGTGGGTGCACGAGTGGTTACA
TCGAACTGGATCTCAACAGCGTAAGATCCTGAGAGTTGCCCGAAGAACGTTT
CCAATGATGAGCACTTAAAGTCTGCTATGCGCGGTATTATCCGTATTGACGCC
GGCGAACAGCAACTCGTCGCCGCATAACTATTCTCAGAATGACTGGTTGAGTACTC
ACCAACTCACAGAAAAGCATCTACGGATGGCATGACAGTAAGAGAATTATGAGTGT
GCCATAACCAGTGAACACTGCGGCCACTTACTCTGACAACGATCGGAGGAC
CGAAGGAGCTAACCGCTTTTGACAAACATGGGGATCATGTAACTGCCCTGATCGT
TGGGAACCGGAGCTGAATGAAGCCATACAAACGACGAGCGTGACACCACGATGCC
GTAGCAATGCAACACGTTGCCAAACTATTAACTGGCGAAACTACTACTCTAGCTC
CCGGCAACAATTAAAGACTGGATGGAGGCGATAAGTTGCAGGACCACTCTGCGC
TCGGCCCTCCGGCTGGCTGGTTATTGCTGATAAAATCTGGAGCCG TGAGCGTGGTCT
CGCGGTATCATGCACTGGGCCAGATGTTAGCGTAAGCCCTCCGTATCGTAGTTATCTA
CACGACGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGG
TGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTACTCATATATACTTGT
TGATTTAAACTTCATTAAATTAAAAGGATCTAGGTGAAGATCCTTTGATAATCT
CATGACCAAAATCCCTAACGTGAGTTCTGTTCCACTGAGCGTCAGACCCGTAGAAA
AGATCAAAGGATCTTGTGAGATCCTTTCTGCGCGTAATCTGCTGCTGCAAACAA
AAAAACCAACGCTACCGAGCGGGTTGTTGCCGATCAAGAGCTACCAACTCTTTT
CCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAAACTGTTCTAGTGTAGCC
GTAGTTAGGCCACCACTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTGCTAA

TCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTCTTACCGGGTGGACTCA
AGACGATAGTTACCGGATAAGGCAGCAGCGCTGGCTGAACGGGGGTTCTGCACAC
AGCCCAGCTGGAGCGAACGACCTACACCGAACTGAGATAACCTACAGCGTGAGCTATG
AGAAAGGCCACGCCCTCCGAAGGGAGAAAGGCCAGACAGGTATCCGTAAGCGCAG
GGTCGAACAGGAGAGCGCACGGAGCTCCAGGGAAACGCCCTGGTATCTTAT
AGTCCTGTCGGTTGCCACCTCTGACTTGAGCGTCGATTGTGATGCTCGTCAGGG
GGCGGAGCCTATGAAAAACGCCAGCAACGCCCTTTACGGTCTGCCCTTTG
CTGGCCTTTGCTCACATGTTCTCTGCGTTATCCCTGATTCTGTGGATAACCGTATT
ACGCCCTTGAGTGAGCTGATAACCGCTGCCAGCCGAACGACCGAGCGCAGCGAGT
CAGTGAGCGAGGAAGCGGAAGAGCGCCAATACGCAAACGCCCTCCCCGCGCGTTG
GCCGATTCAATTGAGCTGGCACGACAGGTTCCGACTGGAAAGCGGGAGTGAG
CGCAACGCAATTGAGTTAGCTCACTCATTAGGCACCCAGGCTTACACTTAT
GCTTCCGGCTCGTATGTTGTGGAATTGTGAGCGGATAACAATTACACAGGAAACA
GCTATGACCATGATTACGCCAAGCTATTAGGTGACACTATAGAATACTCAAGCTATGC
ATCCAACGCCGTGGAGCTCTCCATATGGTCGACCTGCAGGCCCGAATTCACTA
GTGATTAAAAGCTTAATGTTAGATCAAGAAGATTGTTGATTGGTGCCTGTTACGT
CGGTGGTCCAACCTGTTCTGTCATTGCACAGATGTGCCCTGACATTAAGGTCACTGTTG
GGATGTGAACCAAGCCAGGATCAATGCTTGAATAGTGACACTTGCCTATCTACGAAC
CAGGTTGAAGGAAGTCGTAGAGTCATGCAGGGAAAGAAATTGTTACTCAACTGA
CATTGATGGTCAATTCAAGAAGCTGATTGGTGTTCATCTCAGTCAACACTCCAACAA
AAACTTACGGTATGGTAAGGGAAAGGGCAGCCGACTGAAATACATTGAGGCTTGC
TAGAAGAATAGTACAGAATAGTAACGGATAACAGATTGTTACAGAGAAATCTACTGTG
CCAGTTAGAGCTGCTGAATCAATAAGACGTATCTCGATGCAAATACTAAACCAGATT
GAACCTGCAGGTATTGAGTAACCCAGAGTTTGCAGAGGGTACAGCCATTAAGGATT
TGAAGAACCTGATAGAGTTGATAGGTGGTACGAAACCCCTGAAGGTCAAGAAC
TGTAGAGCTTGTGACGTACGAACACTGGTACCATCTGAGAAAATCATAACCA
CAAACACCTGGCTTCTGAGTTGAGTAAGTTAGCAGCCAACGCATTCTAGCTCAAAGA
ATTCTCAATCAACTCAATTAGTGCCTATGTGAAGCCACAGGAGCTGACGTTGAAGA
GGTGGCCAGAGCTATTGGTATGGATCAAAGAATTGTAACAAGTTTGAAGGCTTCAG
TGGGATTGGAGGTTCATGTTTCAAGAAGGACGTTGAACCTGGTTACTGTGAGG
TGTAAACTGCACGAAGTGGCCAAGTACTGGCAACAAGTGATTGATATGAATGATT
CAAAGGAGACGTTTACAACTAGGATAATCGATTGTTGTTAACACCGTGACCGATAA
GAAAATCGCATTGTTAGGTTGCTTCAAGAAGGATAACAGGTGATACTAGAGAGT
AGTCAATCTATCTCAAGTATTGATGGATGAAGGTGCTAAGTTACATATCTACGAT
CCAAAGGTCCCACGTGAGCAGATCATCACTGACTTGAGTCAACCTGGTGTGAGCTGA
CGACAGGGTTCTCAATTGGTCCACATAACTACAGATTGACGAAGCCTGTGAGAATG
CACACGCTATGGTACTGAATGGGATATGTTCAAGGAATTAGATTCAATAGA

ATCCATAGGATGATGTTAAAGCCTGCTTCATATTGATGGTAGACGTGTTAGATGAA
TTGCATGGAGAATTGCAAAACATTGGATTCAGTGGAAACCATCGAAAGAAGGTAG
CTTCAAAAAGAATACCATTCACTCCAACGTGCTGATATCCCTAACGTTACAGGAC
TTGCCACACAAGAACGAAACGTGTAAAGTCGACTTAATCGAATTCCCGGGCCCAT
GGCGGCCGGGAGCATGCGACGTCGGGCCATTGCCCC

Sequence S6. The nucleotide sequence of the pHIPZ7_*hasAp* plasmid. The sequence of the *hasAp* gene is shown in green.

AGGGGATATCCTCGAGACTGCCTTGAGGCTTGTGCGGTAAATAAGTATATAGG
ACACGACAATCTAGTAATCTCCACTATTGACGAGCTCGTGAACGCGAAATAGTTT
TCCATCTGGTCTGTAGGCATCAGCCCGCGTCATCCTCTGGCAGGAGCAGCGGCTC
AGGGCCGGCTGGCGGGCTGATCCAGAAAGTCGAGGTTAGATCCCCCACACACCAT
AGCTTCAAAATGTTCTACTCCTTTTACTCTTCAGATTTCAGTCTCGACTCCGCGATCG
CCGTACCACTCAAAACACCAAGCACAGCATACTAAATTCCCTTTCTCTCTCTAG
GGTGTGTTAATTACCGTACTAAAGGTTGGAAAAGAAAAAGAGACCGCCTGTTTC
TTTTCTCGAAGGAATAAAATTATCACGTTCTTTCTTGAAATT
TTTTAGTTTTCTCTTCAGTGCACCTCATTGATATTAAAGTTAATAAACGGTCTCAA
TTTCTCAAGTTTCAGTTCACTTTCTGTTCTATTACAACCTTTTACTCTGTTCA
GAAAGAAAGCATAGCAATCTAATCTAAGGGCGGTGTTGACAATTAAATCGGCATA
GTATATCGGCATAGTATAATACGACAAGGTGAGGAACCTAACCATGCCAAGTTGACC
AGTGCCTCCGGTGCCTACCGCGCGACGTGCCGGAGCGGTGAGTCTGGACCG
ACCGGCTGGGTTCTCCGGACTTCGTGGAGGACGACTTCGCCGGTGGTCCGGAC
GACGTGACCTGTTCATCAGCGGGTCCAGGACCAAGGTGGTGGCCGGACAACACCG
CCTGGGTGGTGGTGCCTGGACGAGCTGTACGCCAGTGGTGGCCGGAGGTGCGTCC
ACGAACCTCCGGGACGCCCTCCGGCGGCCATGACCGAGATCGCGAGCAGCCGTGG
GGCGGAGTCGCCCTCGCGACCCGGCGCAACTGCGTGCACCTCGTGGCCGGAGGA
GCAGGACTGACACGTCCGACGGCGGCCACGGTCCCAGGCCTGGAGATCCGTCCCC
CTTTCTTGTGATATCATGTAATTAGTTATGTCACGTTACATTACGCCCTCCCC
ACATCCGCTTAACGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATT
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TAATCATGGTCAAGCTGTTCTGTGAAATTGTTATCCGCTCACAAATTCCACACAAC
ATACGAGCCGAAGCATAAAGTGTAAAGCCTGGGTGCCTAATGAGTGAGCTAACTCA
CATTAATTGCGTGCCTCACTGCCGCTTCCAGTCGGAAACCTGCGTGCAGCTGC
ATTAATGAATCGGCCAACGCGCGGGAGAGGCGGTTGCGTATTGGCGCTTCCGCT
TCCTCGCTCACTGACTCGCTGCCCTGGTCGGCTGCCAGCGGTATCAGCTCA
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TGCCTCTCTGTTCCGACCCCTGCCCTACCGGATACCTGTCGCCTTCTCCCTCGGG
AAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTGGTAGGTCGTTCG
CTCCAAGCTGGCTGTGCACGAACCCCCGTTCAGCCCACCGCTGCGCTTATCCG
GTAACATCGTCTTGAGTCCAACCCGTAAGACACGACTTATGCCACTGGCAGCAGCC
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GGTGGCCTAACTACGGCTACACTAGAACAGACTATTGGTATCTGGCTCTGCTGAAG
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AGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACGCTGCGCGTAACCACACACCC
GCCCGCCTTAATGCGCCGCTACAGGGCGCGTCGCCATTGCCATTAGGCTGCGCAA
CTGTTGGGAAGGGCGATCGGTGCAGGGCCTTCGCTATTACGCCAGCTGGCGAAAGGG
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TAAAACGACGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTGGAGCTCCAC
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CGTCTCTACGTTCCAGCTACATGGCAGTTCTAAGACGGGAAGTAAGATGACACTAGTAG
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TAGCCCACCAAGGTATTGTCCTGTGCGTAATTGGCACCGAGACGACTCGAATAAG
TTGGCAATAAAATTCTTCACTATATAAGAGGAGACATTCCCACATGAGATT
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GTCAGACTCTCCTTTAGATTCTCGCAGCTGTAATGTTGCTTCGCCAAGAAGTGGTT
GAACAAATCTGGATTCTTGATGAAGAGTTCAACCATTGGGGTGGTAAGATGTTGAGT
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ACCATCAAGAGCCACCTGTAAGGAAAACGAAACAGATAAGGAAGCTGAAAGAAC
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TGCCAATTATATTCAACGTTGTTGATTCTGCCTGAACCAGACAGTTGAGATTGGA
AGTCTGTATTGCAATGATGGTTCTACAGATAATACTTGGAAAGTTATCAACAAAGTTGA
CGGTAAACAATCCAAGAGTCAGAATCATGACTAAACCAAATGGTGGTATTGCTAGTGCT
TCTAATGCAGCAGTGAGTTGCCAAAGGATATTACATAGGTCAATTAGATTAGATGA
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TGTGGACAAGAACCATTTGACCCAGATATCAAGAAAGAGATATTGGCTTCTACCAC
AAGCATCAAGTGAATATTGTTGAATAACGATATCTCATACTACACATCAAACCGTT
AATCAAGACCGAGGCACATTATCAAACATTAATAAGTTGTCACAGTTGAACATTGAATT
GTGAATATATCATATTGACAATCATGACTCTTGGTGTGAAGAATGATTCTTATGCCT
ATATGAAGAAGTACGATGTTGGTATGAATTCTCAGCCTTAACTCATGATTGGATTGAA

AAGATTAACGCACATCCACCATTCAAGAAGTTGATTAAGACATACTTAACGATAATG
ACTTGAAATCTATGAACGTTAAAGGAGCTAGTCAAGGAATGTTATGACATATGCATTG
GCTCACGAATTGTTGACTATTATCAAAGAGGGTATCACTTCTGCCAATCTATCGATTCT
GTACCAGAACATACAACACTGAGGACATATGGTTCAATTGCATTGTTGATCTTGGAAAAA
GAAAAGCTGGTCATGTTAACAGACAAGTACCTGACATACATGCCTGGGAGAGG
AAGTTGCAATGGACCAATGAACAAATTGAATCAGCTAACACGTGGAGAAAACATTCCAG
TGAACAAAGTTCATATAATTCAATCACATTGTAAAGGCCTAA

Sequence S7. The nucleotide sequence of the pHIPZ18_*hasAp* plasmid. The sequence of the *hasAp* gene is shown in green.

TCGAGACTTGCCTTGAAGGCTTGTGCGGTAAATAAGTATATAGGACACGACAATC
TAGTAATCTCCACTATTGACGAGCTCGACTCGAAAATAGGTTTCCATCTGGTCT
GTAGGCATCAGCCCAGCGTCATCCTCTGCGCAGGAGCAGCGGGCTCAGGCCGGCCT
GGCGGGCTGATCCAGAAAGTCGAGGTTCAGATCCCCACACACCAGCTTCAAAT
GTTTCTACTCCTTTTACTCTTCCAGATTTCTGGACTCCGCGATGCCGTACCACTT
CAAAACACCCAAGCACAGCATACTAAATTTCCTCTTCTCCTCTAGGGTGTGTTAA
TTACCCGTACTAAAGGTTGGAAAAGAAAAAGAGACGCCCTCGTTCTTTCTCGTC
GAAAAAGGCAATAAAATTTCACGTTCTTTCTGAAATTTTTTAGTTTT
TTCTCTTCAGTGACCTCATTGATATTAAAGTTAAATAACGGTCTCAATTCTCAAGTT
TCAGTTTCATTTCTGTTCTTACAACTTTTACTCTGTTCTAGAAAGAAAGC
ATAGCAATCTAATCTAAGGGCGGTGTTGACAATTAAATCGGCATAGTATATCGGCA
TAGTATAATACGACAAGGTGAGGAACCTAAACCATGGCAAGTTGACCAGTGCCGTCC
GGTGTCAACCGCGCGACGTCGCCGGAGCGGTGAGTTCTGGACCGACCGGGCTCGGG
TTCTCCGGACTTCGAGGACACTCGCCGGTGTGGTCCGGACGACGTGACCT
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TTGCCGGAAAGCTAGAGTAAGTAGTCGGCTGCAACTTATCCGCCTCCATCCAGTCTATTAAATTG
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ACCATTCAAGAAGTTGATTAAGACATACTTAAACGATAATGACTTGAAATCTATGAACG
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AACAAAGACAAGTACCTTGACATACATGCCCTGGGAGAGGAAGTTGCAATGGACCAATG
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TTCAATCACATTGAAAGGCCTAAAGGGGATATCC

Sequence S8. The nucleotide sequence of the pHIPZ18_*hasB* plasmid. The sequence of the *hasB* gene is shown in yellow.

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CGTCATCCTCCTGCGCAGGAGCAGCAGGGCTCAGGGCCGCTGGCCGGCTGATCCAG
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CCAGGACCAGGTGGTCCGGACAACACCCCTGGCTGGGTGCGCGCCCTGGAC
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CCCACGGTCCCAGGCCCTCGGAGATCCGCCCCCTTTCCTTGTGATATCATGTAATT
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TCCCTTAGTGAGGGTAATTCCGAGCTTGGCGTAATCATGGTCAAGCTGTTCTGTG
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AAGCCTGGGTGCTAATGAGTGAGCTA ACTCACATTAAATTGCGT GCGCTACTGCC
GCTTCCAGTCGGAAACCTGTCGTGCCAGCTGCTTAATGAATGCCAACCGCG
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CAGAATCAGGGATAACCGCAGGAAAGAACATGTGAGCAAAGGCCAGCAAAGGCC
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CGGTATGGTAAGGGAAAGGGCAGCCGACTTGAAATACATTGAGGCTGCGCTAGAAGA
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ACCTGGTCTCTGAGTTGAGTAAGTTAGCAGCCAACGCATTCTAGCTCAAAGAATTCT
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TCGCATTGTTAGGTTGCTTCAAGAAGGATAACAGGTGATACTAGAGAGACTAGTTCA
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GGGTTCTCAATTGGTCCACATAAGTACAGATTGATGAGCCTGTGAGAATGCACAC
GCTATGGTCAATTGTTACTGAATGGGATATGTTCAAGGAATTAGATTCAATAGAATCCA

TAGGATGATGTTAAAGCCTGCTTCATATCGATGGTAGACGTGTTAGATGAATTGCA
TGGAGAATTGCAAACATTGGATTCAGGTGAAACCATCGAAAGAAGGTAGCTTC
AAAAGAATACCATTCACTCCAATGCTGATATCCCTAACAGTCGGTTACAGGACTGCC
ACACAAGAACGAAACGTGTAACTCGACT

Sequence S9. The nucleotide sequence of the pHIPH4_*hasB* plasmid. The sequence of the *hasB* gene is shown in yellow.

AAGCTTAATGTTCAGATCAAGAAGATTGTTGATTGGTGCCGGTACGTCGGTGGTCC
AACCTGTTCTGTCATTGCACAGATGTGCCCTGACATTAAGGTCACTGTTGTGGATGTGAA
CCAAGCCAGGATCAATGCTTGAATAGTACACTTGCCTATCTACGAACCAGGTTGA
AGGAAGTCGTAGACTCATGCAGGGAAAGAATTGTTCACTCAACTGACATTGATGGT
GCAATTCAAGAAGCTGATTGGTGTTCATCTCAGTCAACACTCCAACAAAAACTTACGG
TATGGTAAGGGAAAGGGCAGCCGACTTGAAATACATTGAGGCTTGCCTAGAAGAATA
GTACAGAATAGTAACGGATACAAGATTGTTACAGAGAAATCTACTGTGCCAGTTAGAG
CTGCTGAATCAATAAGACGTATCTCGATGCAAATACTAAACCAGATTGAACCTGCAG
GTATTGACTAACCCAGAGTTTGGCAGAGGTACAGCCATTAAGGATTGAAGAACCC
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TGTGTGACGTACGAACACTGGTACCATCTGAGAAAATCATAACCACAAACACCTG
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GCACGAAGTGGCCAAGTACTGGCAACAAGTGATTGATATGAATGATTATCAAAGGAGA
CGTTTACAACACTAGGATAATCGATTGTTAACACCGTGACCGATAAGAAAATCGC
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GGCCGACGCGACGCTCCTGCGGACCACGGTGGCTGGCGAGGCCAGTTGTGAACGAG
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CATGAGGTGTCGACTGCAGAGATGCCGTTGCTCTCACCGCTACAGGACGAACGGC
GTGGCCAGCAGGCCCTGATCCATTCTATGAGGCCATCTGACGGTGTCCCTGAGTGC
GTACTCCACTCTGTAGCGACTGGACATCTGAGACTGGCTTGCTGTGGATGCACC
AATTAATTGTCGCCGATGCATCCTGCACCGCAAGTTAAAACCCACTCGCTTAGC
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CAACGTGACCTTGCCTAACCGGACGGCGTACCCACTGCTGTGCGCTGCTACCAGA
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TTCTCACAGTCAAATGCGGGTAACCGGCCAGAAAGTAAATTCTATGCTACCGTGC
AGTGAATCCGACATCCCCAGTTTGCCTACTTGATCACAGATGGGTCAGCGCTGCC
GCTAAAGTGTACCCAAACCGTCCCCACACGGTCCATCTATAAAACTGCTGCCAGTGCACG
GTGGTGACATCAAATCTAAAGTACAAAAAC

Sequence S10. The nucleotide sequence of the pHIPH4_*hasAs* plasmid. The sequence of the *hasAs* gene is shown in blue.

AAGCTTAAAAATGAGAACTTGAAGAAATTAAATTACAGTTGTCATTCTCAATTCTG
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 CAACTGGTGGGGTGTGGACAGGCTGTTCTCCACAGTGCAAATGCGGGTAACCGGC
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 TTGATCACAGATGGGTCAGCGCTGCCATACTGACCTAACCGTCCCCACACGGTCC
 ATCTATAAAACTGCTGCCAGTGCACGGTGGTACATCAATCTAAAGTACAAAAAC

Sequence S11. The nucleotide sequence of the pHIPH4_ScSInt13 plasmid. The sequence of the *hasB* gene is shown in yellow. The reverse sequence of the *hasAp* gene is shown in green. The sequence of the Int13 encoding gene is shown in red.

GGCGCCCCCTGCATTAAATCACCAACCCGCTACGATGACAGGCTCGCAGTGCAG
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TCATCAAACATTCAATGATTAGAGTCCGAGAACGAAAGGAGTTATATCAAATTAA
TAAGGACTATCCGTTACACCGTCAAAGAGCAGCAACCTATCAGACCTGATAAGTCTAA

GACAGGTAAGGGTAAACAGAAAGTGATAATTACGGAAGTGGAGTTTACCAAGTAAAA
 GCTTGCATGCCTGCAGGTGACTCTAGAGGATCGATCCCCGGGCCTGGACATCCAGCCT
 TCCTACGCCATGACCACCTCCGAGGCTAAGAGGGCCGTGCACAAGGAGACCAAGGAC
 AAAACCTCGAGACTTGCCTTGAAGGCTTGTGCGGTAAATAAGTATATAGGACACG
 ACAATCTAGTAATCTCCACTATTGACGAGCTCGTCACTGCGAAAATAGGTTTCCAT
 CTGGTCTGTAGGCATCAGCCCCGGCGTCATCCTCCTGCGCAGGAGCAGCGGGCTCAGGG
 CCGGCCTGGCGGGCTGATCCAGAAAGTCGAGGTTCA

Figure S1. Confirmation of integration of both genes into the EMB101 strain by colony PCR of 12 colonies selected after transformation and plating on selective medium. The upper gel shows amplification of the *hasB* gene (1505 bp) using the primer pair hasB_F and hasB_R. The bottom gel shows amplification of the *hasAp* gene (2966 bp) using the primer pair hasAp_F and hasAp_R. The agarose content was 1% and the ladders used were 1kB Ladder Plus M1191/M1192 (M1; Sinapse Inc) 1 kb Ladder K9 (M2; Kasvi). The *O. polymorpha* NCYC495 *yku80* was used as a negative control (C-) for the PCR reactions. The pHIPH4_*hasB* and pHIPZ18_*hasAp* plasmids were used as positive controls (C+) for the PCR reactions of *hasB* and *hasAp*, respectively.

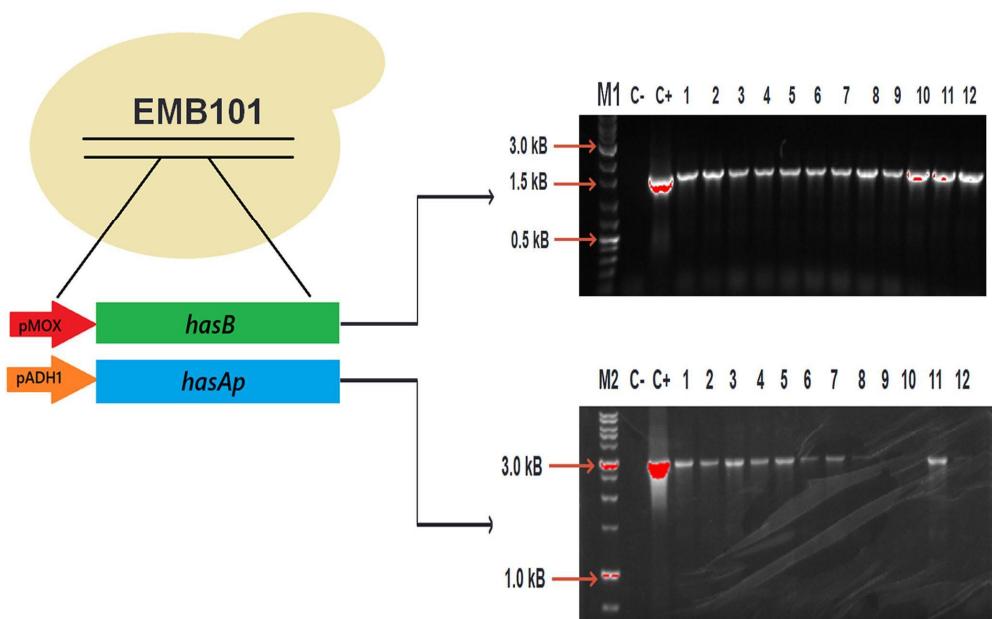


Figure S2. Colony PCRs to verify the *hasAp* (2966 bp) and *hasB* (1505 bp) genes stability in the genome of EMB101 strain after three successive passages on YPD supplemented with zeocin and hygromycin. Five colonies were selected after the transformation and plating on YPD plate containing both antibiotics for the verification of *hasB* and *hasAp* stability on the genome of *O. polymorpha* NCYC495 *yku80*. All PCR reactions for *hasB* gene were performed using the primer pair hasB_F and hasB_R while the PCRs reactions for *hasAp* gene using the primer pair hasAp_F and hasAp_R. In all PCRs reactions performed, the *O. polymorpha* NCYC495 *yku80* was used as a negative control (C-) and the pHIPH4_*hasB* and pHIPZ18_*hasAp* plasmids were used as positive controls (C+) for the of *hasB* and *hasAp*, respectively. The fragments amplified at the height of 1.5 kb in the upper gels correspond to the *hasB* gene and the fragments in the height of 3.0 kb at the bottom gels correspond to the *hasAp* gene. The black arrows indicate a passaging to another YPD plate supplemented with zeocin and hygromycin. The agarose content was 1% and the ladder used was 1 kb Plus DNA Ladder (M1; Invitrogen). **(A)** Colony PCR for the *hasAp* gene of the first passaging on selective medium, **(B)** Colony PCR for the *hasB* (upper gel) and *hasP* (bottom gel) genes of the second passaging on YPD plate with both antibiotics. **(C)** Colony PCR for the *hasB* (upper gel) and *hasAp* (bottom gel) genes of the third passaging on a YPD plate with both antibiotics.

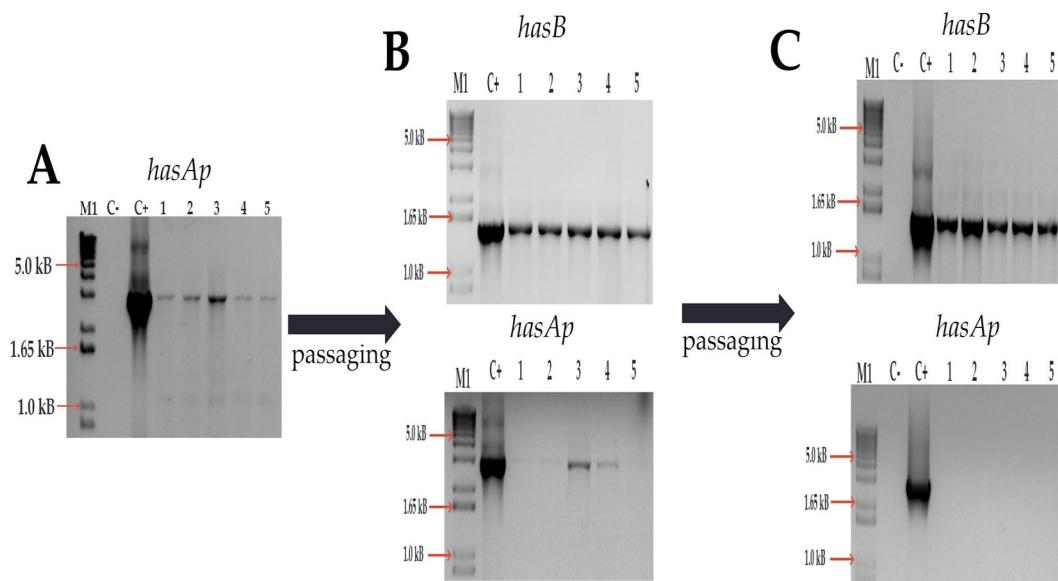


Figure S3. Confirmation of integration of both genes into the EMB102 strain by colony PCR of 12 colonies selected after the transformation and plating on selective medium. The upper gel shows amplification of the *hasB* gene (1.6 kB) using the primer pair hasB_F and hasB_R. The bottom gel shows amplification of the *hasAp* gene (2.9 kB) using the primer pair hasAp_F and hasAp_R. The agarose content was 1% and the ladders used were 1kB Ladder Plus M1191/M1192 (M1; Sinapse Inc) 1 kb Ladder K9 (M2; Kasvi). The *O. polymorpha* NCYC495 *yku80* was used as a negative control (C-) for the PCR reactions. The pHIPH4_*hasB* and pHIPZ7_*hasAp* plasmids were utilized as positive controls (C+) for the PCR reactions of *hasB* and *hasAp*, respectively.

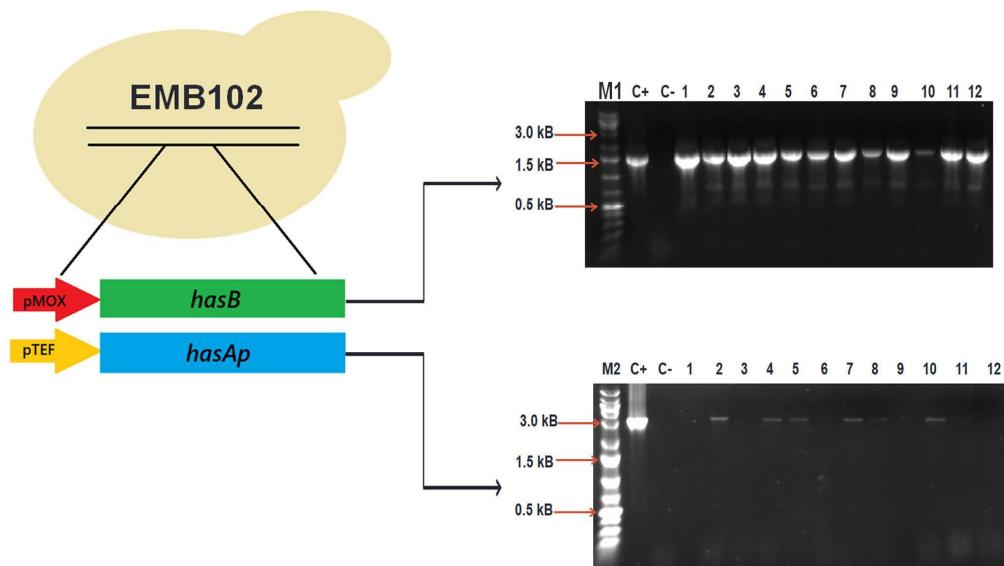


Figure S4. Scheme representing the genetic switch constructed to control the expression of both *hasB* and *hasAp* by a serine integrase. (A) The gene encoding the Int13 codon-optimized for *S. cerevisiae* is regulated by the promoter pAOX which is inducible by methanol (details in Figure S14). Thus, the addition of methanol leads to the production of Int13 that recognizes the sites *attB* and *attP* flanking both *has* genes synthesized in reverse complement orientation. The action of Int13 causes a rotation of 180° in both genes resulting in two different flanking sequences named *attL* and *attR*. In the final, both genes are in ORF with promoter and terminator and can be properly transcribed. The correct gene orientation as well the formation of *attL* sequence could be evaluated by PCR using the pair of primer *attB_hasB_F* and *attP_hasB_R* for *hasB* (resulting fragment of 493 bp) and *attB_hasAp_F* and *attP_hasAp_R* for *hasAp* (resulting fragment 827 bp). When the genes are in the initial orientation, the primer annealing fails and no amplification occurs. The black arrows represent the primer orientation. (B) Confirmation by PCR of the both genes rotation in the EMB103 strain after methanol induction. For all PCRs reactions, the genomic DNA was utilized as the template and for the extraction the phenol/chloroform method was applied according to [2]. The gel in the left shows the fragment (827 bp) containing the *attL* sequence and the *hasAp* flipped and in the right the fragment for *hasB* (493 bp). The *O. polymorpha* NCYC495 *yku80* was used as a negative control (C-) for the PCR reactions. The pHIPZ18_*hasAp* plasmid which contains the *hasAp* in ORF was utilized as positive controls (C+) for the PCR reactions of *hasAp*. The plasmid PKLAC2-BP constructed previously for our group (REF) was utilized as the control positive for the PCR reaction of *hasB* once all plasmids constructed in this work has the *hasB* gene controlled by endogenous promoters of *O. polymorpha* instead of pGDP promoter from *S. cerevisiae*.

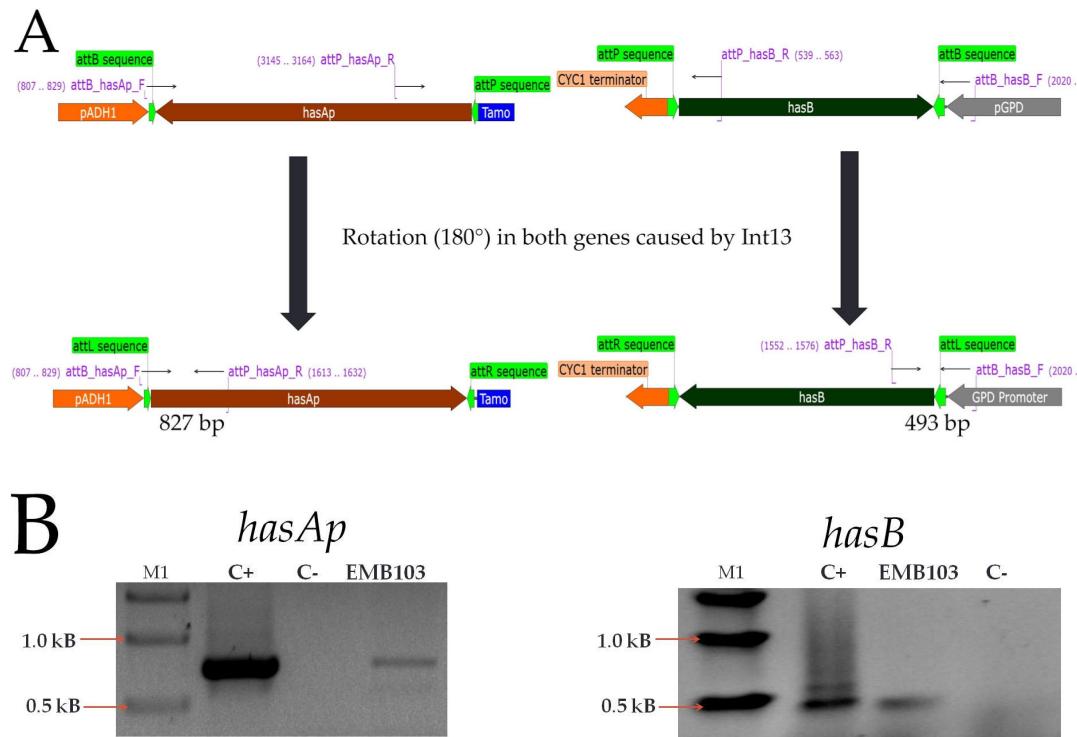


Figure S5. Confirmation of integration of both genes into the EMB104 strain by colony PCR of 12 colonies selected after the transformation and plating on selective medium. The upper gel shows amplification of the *hasB* gene (1.6 kB) using the pair of primers hasB_F and hasB_R. The bottom gel shows amplification of the *hasAs* gene between the pAOX (pMOX) promoter and the AMO terminator (1.5 kB) using the pair of primers AOX-Integration_F and AOX-Integration_R. The agarose content was 1% and the ladder used was 1kB Ladder M1181/M1182 (M1; Sinapse Inc). The *O. polymorpha* NCYC495 *yku80* was used as a negative control (C-) for the PCR reactions. The pHIPZ18_*hasB* and pHIPH4_*hasAs* plasmids were used as positive controls (C+) for the PCR reactions of *hasB* and *hasAs*, respectively.

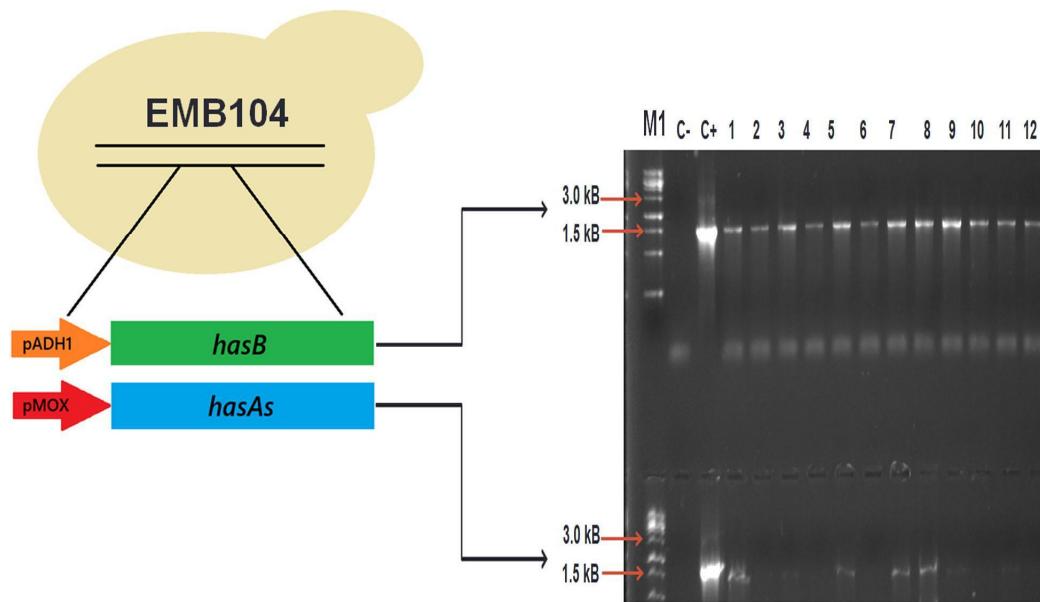


Figure S6. Map of the synthetic plasmid pBSK_hasB harbouring the *hasB* gene from *Xenopus laevis*. Oligonucleotide primers for amplification of *hasB* (HasB_F and HasB_R) are shown on their annealing sites.

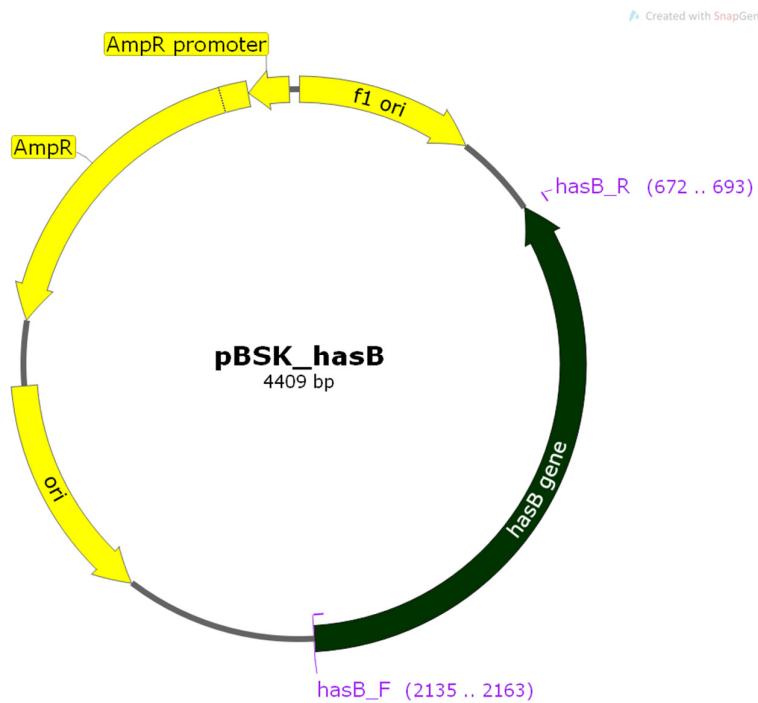


Figure S7. Map of the synthetic plasmid pBSK_hasAp harbouring the *hasA* gene from *Pasteurella multocida*. Primers for amplification of *hasAp* (HasAp_F and HasAp_R) are shown on their annealing sites.

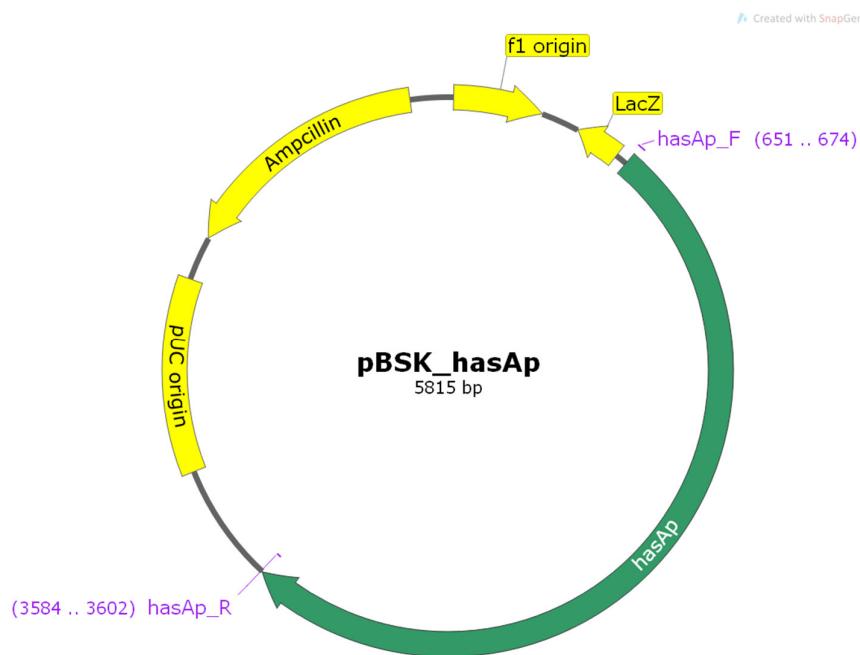


Figure S8. Map of the synthetic plasmid pBSK_hasAs harbouring the *hasA* gene from *Streptococcus zooepidemicus*. Restriction sites for HindIII and XbaI are shown.

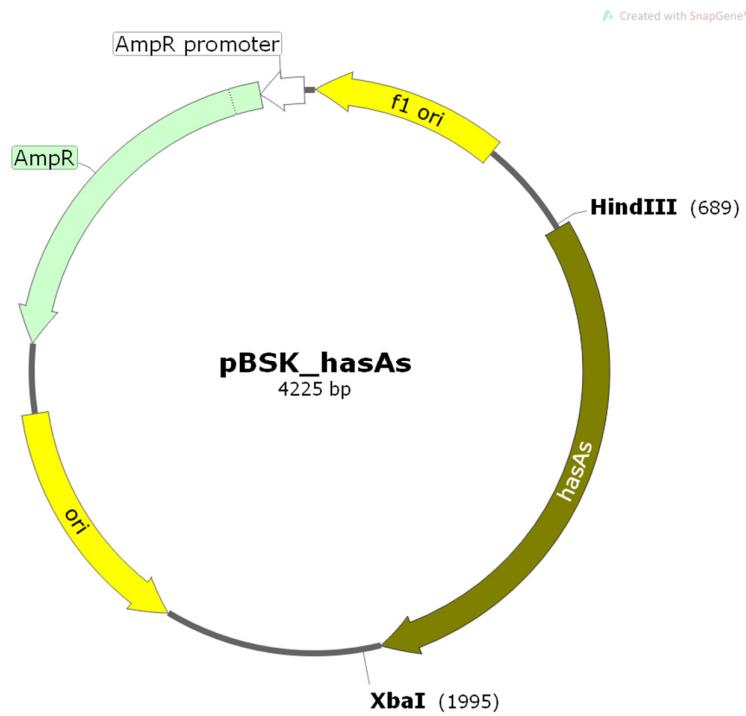


Figure S9. Map of the pGEM_hasB plasmid harbouring the *hasB* gene from *X. laevis*. Restriction sites for HindIII and SalI are shown.

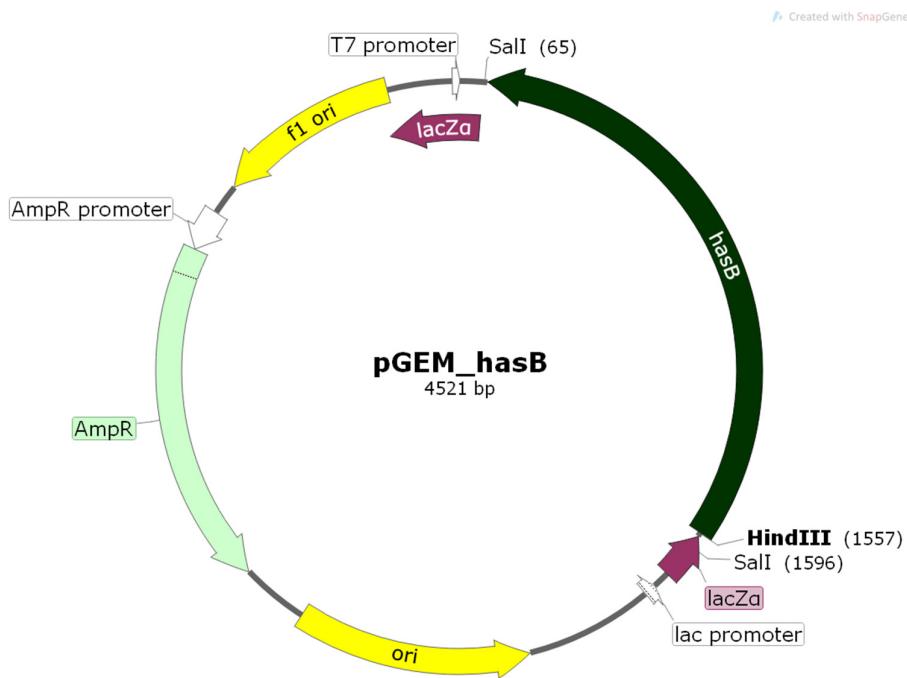


Figure S10. Map of the pHIPZ7_hasAp plasmid bearing the *hasAp* gene from *P. multocida*. Primers for amplification of *hasAp* (HasAp_F and HasAp_R) are shown on their annealing sites. Restriction sites for HindIII and XhoI are shown.

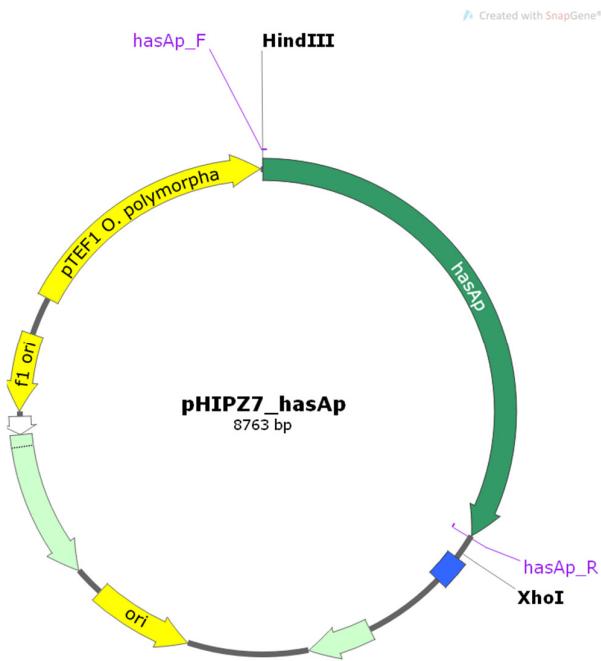


Figure S11. Map of the pHIPZ18_hasAp plasmid harbouring the *hasA* gene from *P. multocida*. Primers for amplification of *hasAp* (HasAp_F and HasAp_R) are shown on their annealing sites. Restriction sites for HindIII and XhoI are shown.

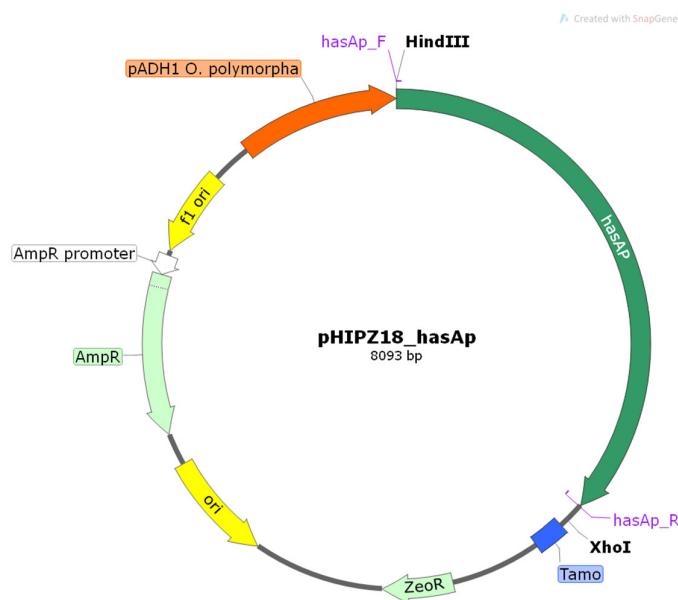


Figure S12. Map of the pHIPZ18_hasB plasmid bearing the *hasB* gene from *X. laevis*. Primers for amplification of *hasB* are shown on their annealing sites. Restriction sites for HindIII and SalI are shown.

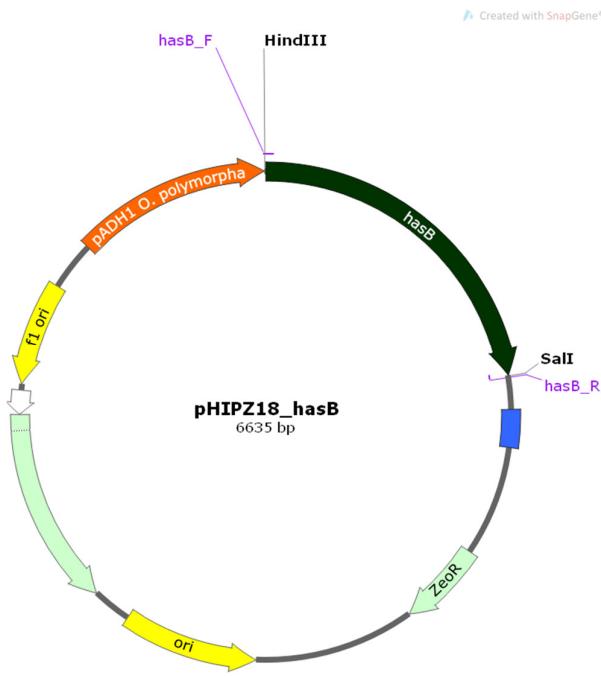


Figure S13. Map of the pHIPH4_hasB plasmid harbouring the *hasB* gene from *X. laevis*. Primers for amplification of *hasB* (HasB_F and HasB_R) are shown on their annealing sites. Restriction sites for HindIII and SalI are shown.

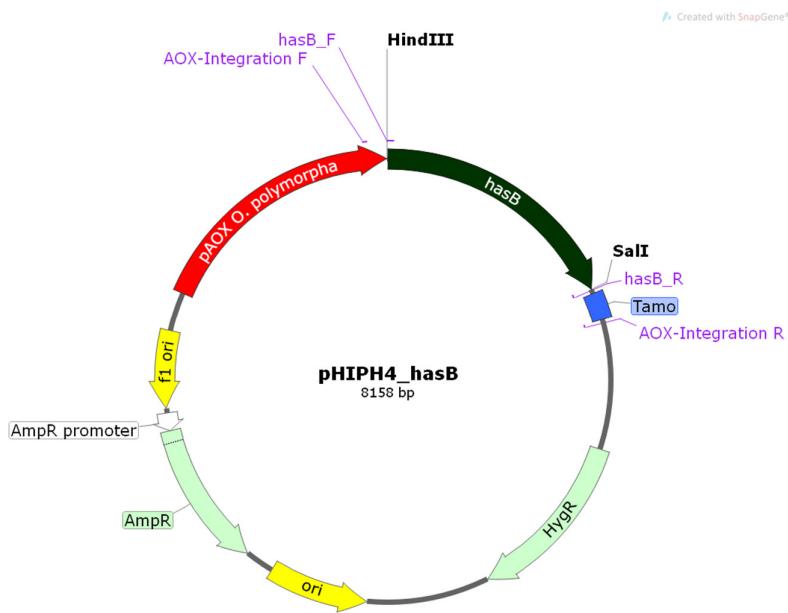


Figure S14. Map of the pHIPH4_hasAs plasmid harbouring the *hasA* gene from *S. zooepidemicus*. Primers for amplification of *hasA* (AOX-Integration F and AOX-Integration R) are shown on their annealing sites. Restriction sites for HindIII and XbaI are shown.

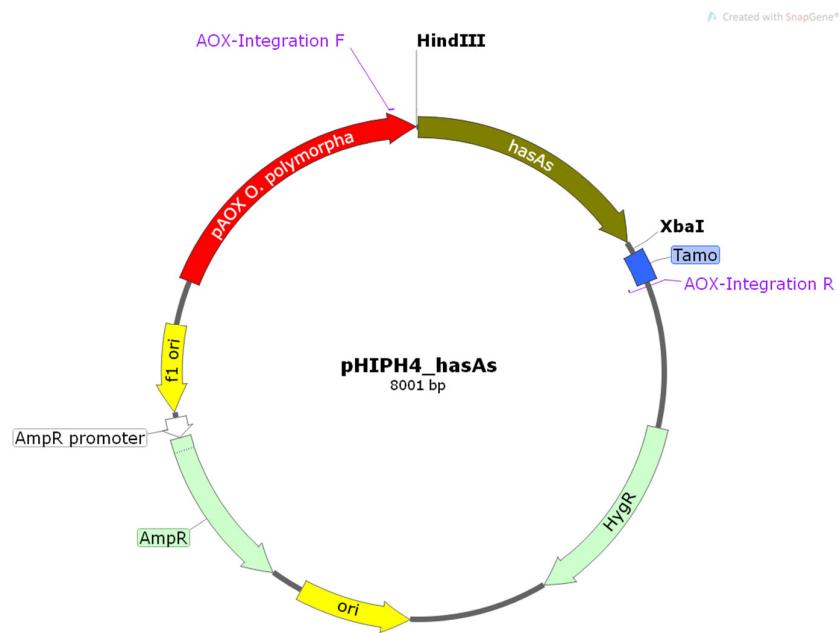


Figure S15. Map of the pHIPH4_ScSInt13 plasmid harbouring the *hasB* gene from *X. laevis* and the *hasA* gene from *P. multocida*. Primers for amplification of *hasB* (HasB Forward and HasB Reverse) and *hasAp* (HasAp Forward and HasAp Reverse) are shown on their annealing sites.

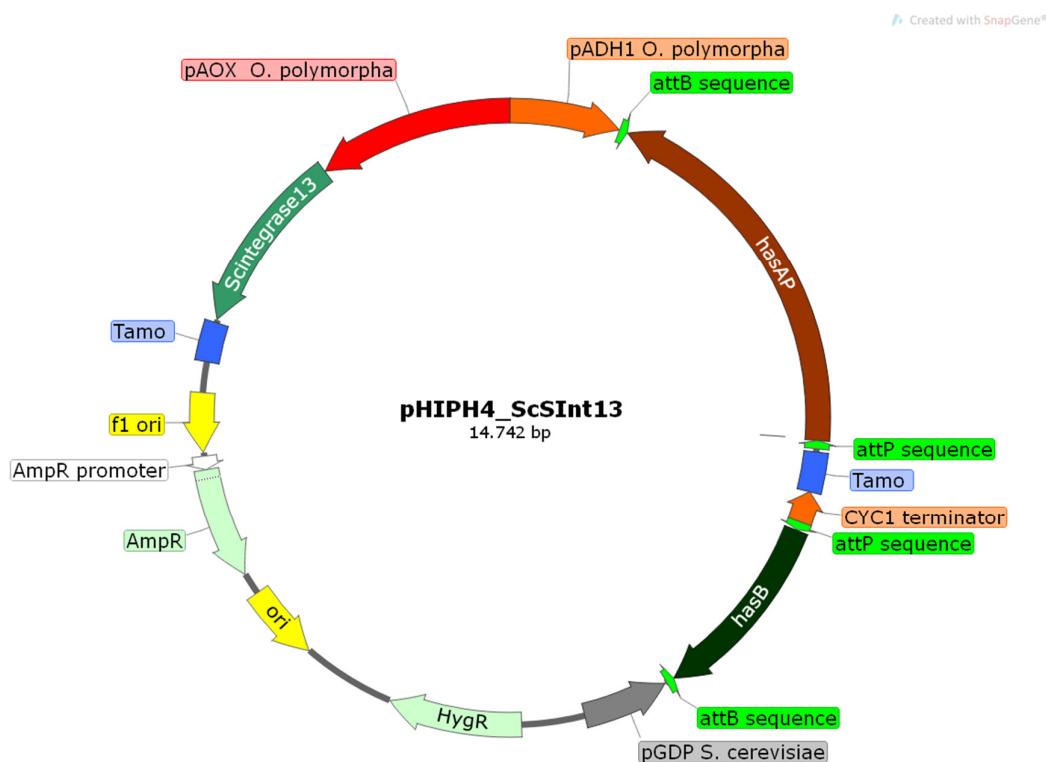


Figure S16. Standard curve obtained by the carbazole method for quantifying HA.

$$y = 0.0015x - 0.0023$$

$$R^2 = 0.9873$$

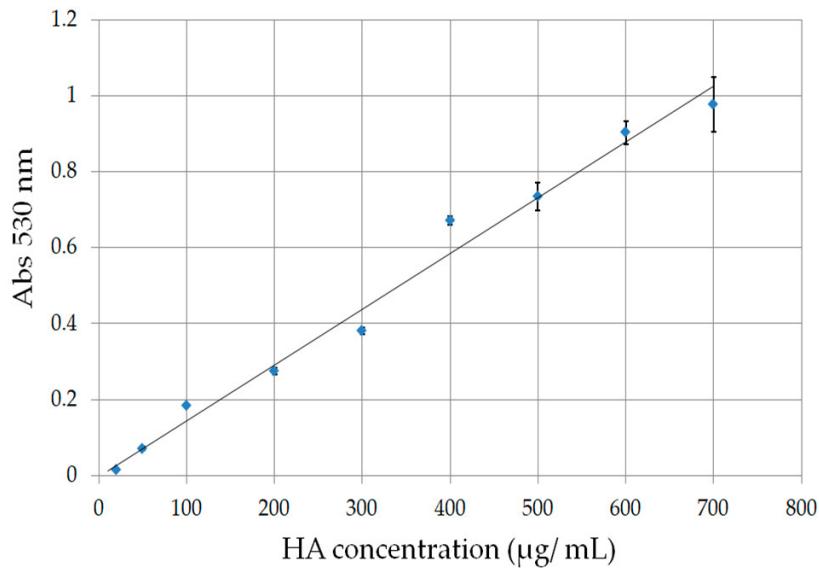
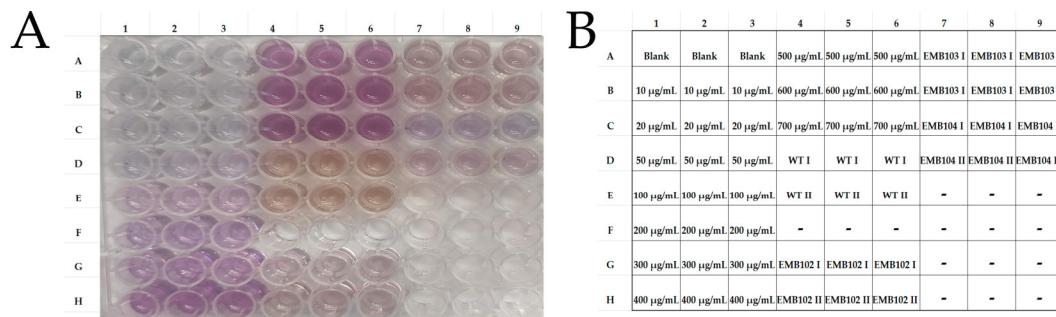


Figure S17. Carbazole assay plate picture. (A) The 96-well plate of the carbazole assay performed to quantify the HA in the supernatants after the 48 h of cultivation. (B) The plate design indicating the position of each sample in (A). I and II indicated the biological replicate. All quantifications were performed in technical replicate. The empty wells are represented with a line."



References

1. van der Klei, I.J. The Hansenula polymorpha expression system Available online: <https://www.rug.nl/research/molecular-cell-biology/research/the-hansenula-polymorpha-expression-system> (accessed on May 19, 2020).
2. Sambrook, J.; Russell, D.W. *Molecular Cloning - A Laboratory Manual*; 3rd ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor: New York, 2001;
3. V. Gomes, A.M.; C. M. Netto, J.H.; Carvalho, L.S.; Parachin, N.S. Heterologous Hyaluronic Acid Production in *Kluyveromyces lactis*. *Microorganisms* 2019, 7, 294, doi:10.3390/microorganisms7090294.