

Supplementary information to

Methanol-based acetoin production by genetically engineered

Bacillus methanolicus

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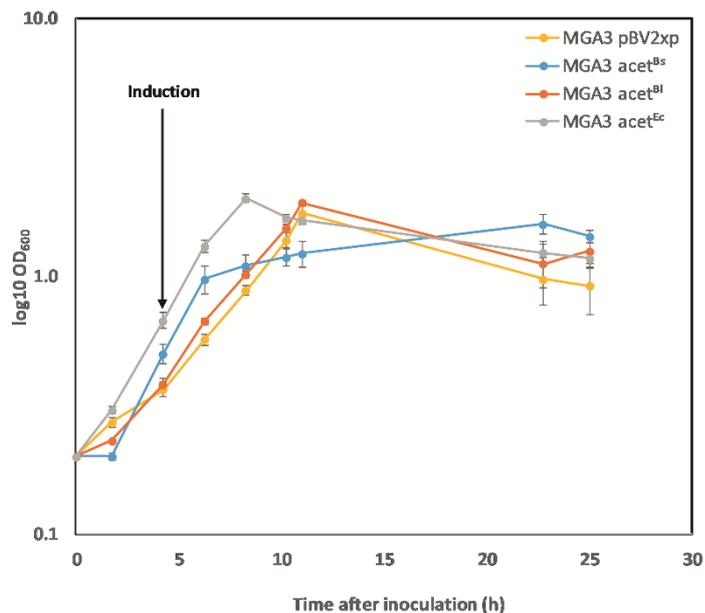


Figure S1 Biomass formation by MGA3 acetoin production strains. The strains were grown in minimal medium with 200 mM methanol and induced with 10 g/L of xylose added to the growth medium 4 hours after inoculation. The OD₆₀₀ was measured every two hours for the first 9 hours, and 25 h after inoculation. The means of triplicates with standard deviations are shown.

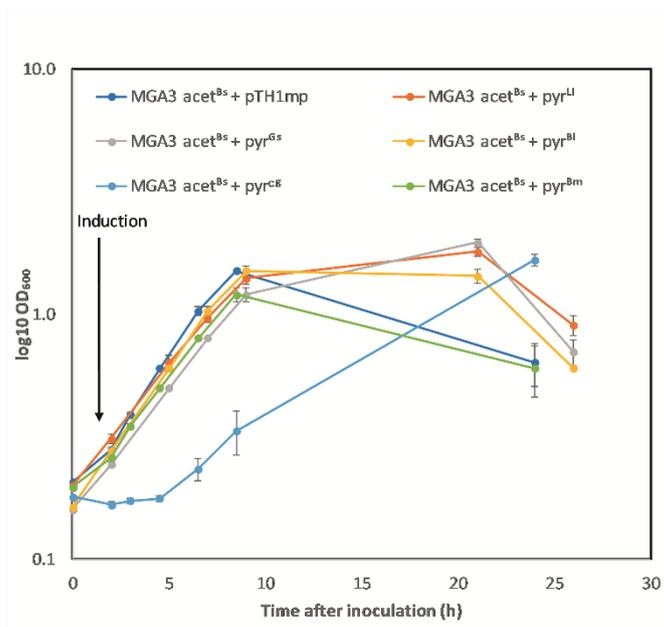


Figure S2 Biomass formation by *B. methanolicus* MGA3 acet^{Bs} strains with a second plasmid overexpressing pyruvate replenishing genes. The strains were grown in minimal medium with 200 mM methanol and induced with 10 g/L of xylose added to the growth medium two hours after inoculation. The OD₆₀₀ was measured every two hours for the first 10 hours, and 25 h after inoculation. The means of triplicates with standard deviations are shown.

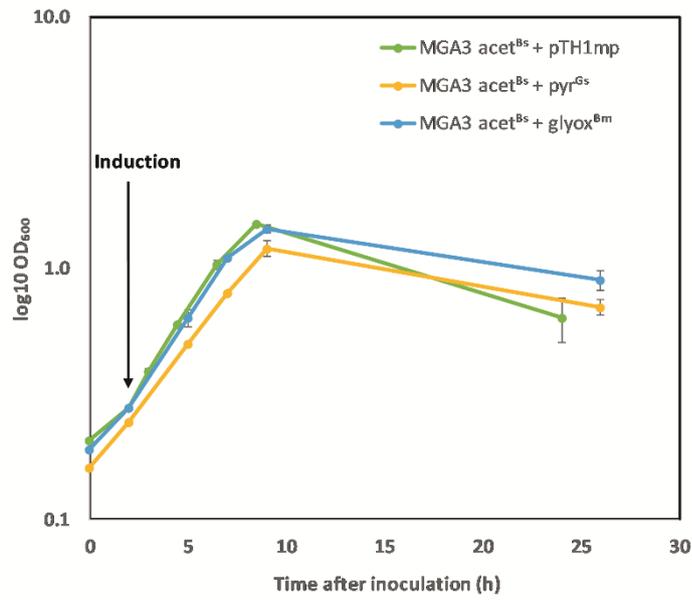


Figure S3 Biomass formation by MGA3 acet^{Bs} strain with overexpressed genes coding for the glyoxylate shunt pathway compared to control strains. The strains were grown in minimal medium with 200 mM methanol and induced with 10 g/L of xylose added to the growth medium two hours after inoculation. The OD₆₀₀ was measured every two hours for the first 10 hours, and 25 h after inoculation. The means of triplicates with standard deviations are shown.

Table S1. Primers used in this study.

Primer name	Sequence 5'→3'	Characteristics
alsSD <i>B. subtilis</i> FW	CTTAAGGGGAAATGGCAAATGACAAAAGCAAAAAGAACAAAATC	Amplification of <i>alsSD</i> from <i>B. subtilis</i>
alsSD <i>B. subtilis</i> RV	ACGGCCAGTGAATTCGAGCTTTATTCAGGGCTTCCTCAGTTGT	Amplification of <i>alsSD</i> from <i>B. subtilis</i>
alsSD <i>B. licheniformis</i> FW	CTTAAGGGGAAATGGCAAATGAATAATGTAGCCGCTAAAAATGAAAC	Amplification of <i>alsSD</i> from <i>B. licheniformis</i>
alsSD <i>B. licheniformis</i> RV	ACGGCCAGTGAATTCGAGCTTTACTCGGGATTGCCTTCGG	Amplification of <i>alsSD</i> from <i>B. licheniformis</i>
budAB <i>E. cloacae</i> FW	CTTAAGGGGAAATGGCAAATGATGCACTCATCTGCCTGCGA	Amplification of <i>budAB</i> from <i>E. cloacae</i>
budAB <i>E. cloacae</i> RV	ACGGCCAGTGAATTCGAGCTTCACAAAATCTGGCTGAGATGGA	Amplification of <i>budAB</i> from <i>E. cloacae</i>
AC01	TAAACAATTACATAAATAGGAGGTAGTACATATGCGTTTTGGACGAATTGCC ACCCAGATGG	Amplification of <i>odx</i> from <i>C. glutamicum</i>
AC02	TAGACCTATGGCGGTACCATATGTTAGGCGTCCACAACCTGGTTGCCAGCT T	Amplification of <i>odx</i> from <i>C. glutamicum</i>
AC03	TAAACAATTACATAAATAGGAGGTAGTACATATGGTTGATTTAATAAAGTT TTAGATTTACAC	Amplification of <i>citM</i> from <i>Lactococcus lactis</i> IL1403
AC04	TAGACCTATGGCGGTACCATATGTTATTTTGTGAATCTTTACCGTTTT	Amplification of <i>citM</i> from <i>Lactococcus lactis</i> IL1403
AC05	TAAACAATTACATAAATAGGAGGTAGTACATATGAACAACATGAGAAGAACG AAGGAAGGACATTT	Amplification of <i>mae</i> from <i>B. licheniformis</i>
AC06	TAGACCTATGGCGGTACCATATGTTAGTCTTGTTTTGCAACAA	Amplification of <i>mae</i> from <i>B. licheniformis</i>
AC07	TAAACAATTACATAAATAGGAGGTAGTACATATGAATTCTGTTACGATTTCA AGCGAGTTG	Amplification of <i>pckA</i> from <i>B. methanolicus</i>
AC08	TTATGCGATAGGACCGCCTTTT	Amplification of <i>pckA</i> from <i>B. methanolicus</i>
AC09	AGGCGTCTATCGCATAAGTAAACAATTACATAAATAGGAGGTAGTAAGAA TGCGCAGAACAAAATCG	Amplification of <i>pyk</i> from <i>B. methanolicus</i>
AC10	TAGACCTATGGCGGTACCATATGTTATAATACGCTCGCATGAC	Amplification of <i>pyk</i> from <i>B. methanolicus</i>
AC11	TAAACAATTACATAAATAGGAGGTAGTACATATGGCATTACCAGCGGGGCA GCCATGAACATTA	Amplification of <i>mae</i> from <i>G. stearothermophilus</i>
AC12	TAGACCTATGGCGGTACCATATGTTACTGCCCTGTATATCCGCCAACCGGGA TATTG	Amplification of <i>mae</i> from <i>G. stearothermophilus</i>
AC13	CGCCATAGGTCTAGAGCTTGTAACAATTACATAAATAGGAGGTAGTAAGAA TGACAGATTTAGAGTACAACAATTA	Amplification of <i>aceA</i> from <i>B. methanolicus</i>
AC14	TGTAACAACGACGCCAGTGAATTCTACTTTTCTTTTAAAAACGTTTTTGA	Amplification of <i>aceA</i> from <i>B. methanolicus</i>
MI09	GATACCAAATACTGTCCTTCTAGTGTAGCCG	Creation of pMI2mp vector
MI10	CGGCTACACTAGAAGGACAGTATTTGGTATC	Creation of pMI2mp vector
aceB_pBx_fw	TGATGGATAAACTTGTTCACAAGGAGGTAGTACATATGTCCACTCAAACGAC AGGC	Amplification of <i>aceBA</i> from <i>B. methanolicus</i> for pBV2xp
aceA_pBx_rv	GTACGGATCCCATTTCCCTTAAGTTATTTTGGCGGATAAATTGTTCTACT TCCG	Amplification of <i>aceBA</i> from <i>B. methanolicus</i> for pBV2xp
aceB_pEC_fw	ATTTCGAGCTCGGTACCCGGGAAAGGAGGCCCTTCAGATGTCCACTCAAACGA CAGGC	Amplification of <i>aceBA</i> from <i>B. methanolicus</i> for pEC-XT99A

aceA_pEC_rv	CCTGCAGGTCGACTCTAGAGTTATTTGCGGGGATAAATTGTTCTACTTCCG	Amplification of <i>aceBA</i> from <i>B. methanolicus</i> for pEC-XT99A
xp-pBV2_fw	CTGCCCCGGGGACGTCGACTCTAACTTATAGGGGTAACACTTAAAAAGAAT CAATAACGA	Creation of pBV2xp vector
xp-pBV2_rv	TGAATTCGAGCTCATGGTACGGATCCATTTCCCCCTTAAGTGAACAAGTTTA TCCATCAACTATCTTAATTGAGTTAGT	Creation of pBV2xp vector

Table S2. Plasmid backbones, promoters and gene sequences used in this study.

Sequence name	Used in plasmid	Nucleotide sequence	Annotation
pBV2xp backbone	pBV2xp pBV2xp- <i>alsSD</i> ^{Bs} pBV2xp- <i>alsSD</i> ^{Bl} pBV2xp- <i>budAB</i> ^{Bc} pBV2xp- <i>aceBA</i> ^{Bm}	<p>GATCTGGAACAAACATCGAGTTGTAAAGTTAAGAGCAGAGTTAAAGAATAAAGAAAAAGGACTGATCCTTGATAAAAAGTCAACTGTCGATAATAGGATAGGTAGATGACGAATATGACTACTTTTTATTCACAAATAGAGAGAGATAGGTAAATGCCCTATTCCACCAAGCTTTAGAAAGTGTCTGTATCGGATATATGCTACATAAAAAATCTTCCACAAAAGATTACGTCGAGAATTTCTGTCTAAAAGATTGGGATTGAAATATGACTGGAAAAACATCTTATGCTTACATTGACTCAGATGTTAAAGTGTGTTTGTAGATAAAAAAGTTGAAAGAAAAAGAGGAACATCAATCCATAAACTTTTTGCTCATACCTTGCTACATGAGGAAAAATCATTAGAGATACCATTGAAGTATATGAAAAACATCAGAAGAAGCAGAAAGGCTAACTGGGTGGAAGCTATACCATATCATATGCTACGTTACATTGACTTTAGCGACCTGAGTTATAAAA CAAGCCTCAGATGTTTATATCTCGAAAGGTTGTGCAAAACAGAAATAACTTTATGATACACAAACAAATTTGAAATGATGAAATTTGATAATGCTCAATGAAAAAGTAAAGAGTTTGGCTTAATCGCAAGGCTCTTTTATTATATCTTTAAAGGTTCTTTAAAGGATGTTTTC TTTGTCAATAAATTTAATCCCTCCCCATTAAAGAAATTAATCAGTGTATAACCCCTAGTAAAAATTTATAGGGTCTTTCTTGTGGATAAACCTCTAATAAATTTAATCCCCCTTGTAAAATTTAATAGGGATTGAAATTAATATTTTGTATCCCTTTATCCCTCAATTAAT TTAATTAGTCTACGTTAATTAATTAATCCAGTCTAAATAAATTAATCAGTACTAATAATTTGATACCTCCCTGATTATATTTTTCACATTTCTCAATAAAAAAACTGCTGAAATCTCAGCAGTTTATGTCACCTTTCTGAGTTCGATTTTTCCGAAAAACATATCCAGCTT CTTCAAGGACTGTTTAAATGTTTCAAAATATCTTTCTTAATCCATCAAATGCAAAATCACTCGGTACAAGATAAACAATTTTTTAACTTTGATCTTAGTGTTTTTTAAACCAGGTACAAAAGAGGCTTAGAGACAGGATTTGCAATTCGCTTTTTAAAGTATGCTGAGCT TTTTCTCTTCAGTTGCTATTGATATACTCTCAAAAGATGTTGAGGATAGGATTTGTTTTGATTTCTTTTTCGAAATTTCTCTTACTGAGTATATCTCTACATATTGTGCATATTGTAAGGCTCTGGAATAATTAATGTTCTGACTCATCCATCATTTTTTAAACAAATTCATCGT GCTCTTTTTCAAAACCTTTGGAAGACCTCTTTTTCTTTTTTAAAGCCTCTTTCATGCTGCTCTCGTCACTGGAATTTCAATATTAGGGTTCCATTTAAAAGTCTCTGAAAGCAAAAGAACCTACGCTTAATCTTAAAAATCGGGGATTCTCTGTGTTATCTCTGTTTTATT ACGGAGCTTACCTTCAAAATAAACCCGACCTTCAAGCAGTTCAAGTAGCCTCTAAGCTGAACAGGAGCTCTGCTCAATTTTTTAGGATGTTTGTAGACTAGGAAAGCAAAAGCTTGTGCTGCGGTATGCATAACGAGTTAGTGAGCATAGAGCGCAATCTCTCAGCAGCTAAAAA ATAGCCCAAAAATCAAAAGATTAATTTAAGATTGTATAATGACCTCTATGTAAGGTATATGCGTACCAGAAATTTGCTAAAGAAAAGTTTTTATTTGCTCTCTGTCATATCTCTCTCCGTTTCAGCAACATAAAACTCTCTTTCCCTTTTTGATCTCTCTGGCGAT TTTATTTCCAGTGGTTGCTGCGATAATACATAATCATCTGTATTTCTCTTTAGGTTTACTCTACTCAATGATCCCTCCGCGATCACTAAAGAACAGTGTAAAAGAGGATTTCACTTGTATGGGTTTTAATAAATGTAAAATGGAGATAGTAAATACAAGTATAAATCCGCC TTTTCCCTTCTCAAAATTAACCTTCTCAGTTTGGCGGTTAGTAAATGAGATTCCGTTAGGTGGATTTTTTTGTTGTTTTTCTCAAAAACCAACAGAGTCTTCATATTCGAAATAATCTATCATATTTTTACTGATTAAAATTTTTTATCCCATGCTCTTCTTAATTC TCTAATATATAATCAATGCTCAATAATTTTTTAAAAAACAGACGCTGTGGAGCTGTCTCAGCCTTTGTTGGTCTTTTTTAAATCATATTCATAAAAAAGTTTTTGGCTCAGGTAATGCTCAACCATCTTATCAATAGCTCTCAATAGTATCTCTAAGTATAAGAAAT CCCTTCGGATGCTGCCAGAATTTGGTGAGCGATTAACTTATTATGATTGAAACATCAACTTTCACTGTTTTCAACAATGCTTTTTTCTTTTTGTTGTTGTTTAACTCTGTGTTTCTTACTTTCAGAAATCTGTAAGATCCATGTAAGGTTCTTAAAGCTGACATTCGCC 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gene: <i>bla</i> (fwd) pUC origin of replication: <i>pUC ori</i> (rev)</p>

<p>pTH1mp backbone</p>	<p>pTH1mp</p> <p>pTH1mp-<i>mae</i>^{Gs}</p> <p>pTH1mp-<i>mae</i>^{Bl}</p> <p>pTH1mp-<i>odx</i>^{Cs}</p> <p>pTH1mp-<i>pckA-pyk</i>^{Bm}</p> <p>pTH1mp-<i>mae</i>^{Gs-aceA}^{Bm}</p>	<pre> GTTCCATTAAGAGCAGCTGATGACTTTAATCCGTTCTTGTCATAAAGTTGAGCAACAGCAACGAGTTTCTCTCGCTATTTCGAAAGATTAGTTAACTAGTAGTAAGCAATAAATCGAAGGAGTTGTTAATGTCACGGATTAAAGCAAAAACACAAAAAAG ACCCCATCATGTTCAAAGAGGCTTTATCAAATAATCTCAATCAATCGGTTGCTGTTCACTAGTTAGTTCGGGTTTAAAAGTAAAAGGAAAGCTGACCGCAATATGATCAATCACTATTCGGCTTCTGTCGAAAGAAAGAAATGATGATCTATAAACACGCTATCAGCCTGATCCGTCAT ACCATTCTGAGTCGGATGATGAATAAAGATGCTATCATTTCTTATAGGAAATATCGGATATAAAATTTAGAAAAATCATATATATAAAAAAGACCTCCGATGATCAACAGCAGCAGCATGCAATAAAGGGTCCCTCAAAGAGGGATCAGTTAACCAAAAGATGAGTAATCCAGACC TTAAGCCTTACCAGCTCAAGGATGGATTACTTTTTTGTGTTAACTTAAAAACGAGTGAACATCAATGCTTAAAAACGGTCAATAACAGCGCTCTCTTCGGGAATGACTACCGTTGAGTTATCTATATCTATTCGCTATCTTATTACTCTTTTATATATGAAAAATAAAAA 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GAGCATTACGAAGCTTAGAGAGTAAGATTGGGGCAGCTTACTATGCAAAAAGCTGAAAAACTAATAGTTGTCAGAGATATCTTCTGTTTAAAGAGACCCGAGAGCGGGCAAGTAAAACTGTATCAAGCCATTTTTGTAAGTGAAGTTATGTCGATGTGGTGGCGAGGTC GTTAAAAATGCTTATCAATAAAGTTGATGCTAGAGGAAGCAATAGACAGTACGGCTCGCGATGGATTTTTCTCACGCTGACGATGCAAAATGTAAGGGAAGCAAGCGCTGAAAGCACAATAATTTCTGCGATGATGGAAGGCTTTAGGAAACTGTTCCAGTACAAAAAGTAAAACTTCGG TTCTGGATTTTTCAGAGCTTAGAGATTACAAAAATCAAGAAAGATACATATCTCTCATTTTCTATGTTGATACCCAGTAAGGAAAAATATTTTGGGAAAACTATATAAGCAGCGGAGTGGACGAGCTTTGGAAAAAGGCGAATAATGGATTACACTCAAATTTGCGAT ATTCGTCAGTGAAGGTAAGCTAAGATTGAGCTGACAGATTTGAAACGATGTCGGGAAGCAATGATGAGCAAAAAAGCTTTCTGAAATCTCAAAATATCCGGTTAAAGGATACCGGATGTTGTCGGGGTAAATGAAGTGAATGAAGTGAATGAAGTGAATGAAGTGAAT TGCGTTGGCAGCTCAAGGTTAATGGATACGGTGGCATTGAAAGGATACATAAAGAGCTGAATCTGTTGATGCGGAGGAGCGGATCTGTTCAAGATTGAGGAAGAAGATGACAGGTTGCAAAATGGTCAATTTGAGGTTATGGCTATGGCTATGAAATTTACATA ATCAAAATAAAAAAGCAGACTTTAGAAGCGCTGCTTTTTAACTAACCCATTTGATTTGTTGAAATGTTTTGATGTTGCACTCTCAGTACAATCTGCTGATGCGCGATAGTTAAGCAGCCGACACCGCCCAACCCGCTGACGCGCTGACGGCTTGTCTGCTCCGGCA TCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGTGCATGTTGAGAGTTTTCCAGCTCATACCAAAAGCGGAGAGCAAAAGCGCTGATGATGCGCTATTTTATAGGTTAATGTCATGATAAATGTTTCTTAGCGATTACAAAAAATAGGCAACGAAAAAGCAAGTAAAGG GATGCGATTTATGATCCCTTAACTTACTTATAAATAATTTATAGCTATTGAAAGAGATAAAGATTTGTTCAAGCTAATATGTTTAAATGTCATCTGATGTTTAAAGGAAATGTTAAATGATTTTTTGTAAATATTTTCTGTATTTCTTTGTAACCCATTTCATAACGAAAT AATATACCTTTGTTATCTTTGTTGATATCTTGATTTTTTCTACTTAAATGATAAGTAGACTATCTACTTTAGGTTTAGGATGAAATATCTCTTGGAACTACTTAAATAGAAAAATCAACTCTGCGCAATAAAGTAATGCAATGAGCGTTTGTATTTAATAATCTTTA GCAACCGGATTTCCAGGATTAATAAATCTCATTAGCTATACTTCAAAAAACAATTTTGGGATATATATCCGCTACTTATGTTAAGGATATATACCAATAATTTAATAGGATTTGTTTAGGAAATTTAACTGCAATATATCTGTTTAAACTTGGAAATATGTTGATCAACAA TTTTATTTCTGAGTTTTCATAAATTTATGCTTATTTCAATGGCAGTTACGAAATACACCTCTTAACTTAAAGGTAAGTAACTTTTCTGAGCCGATTTCTGAGCCGATTTATGATGTTTAACTTATATTTGTCATTTTAACTAATTTTAAAGTAAATAAAGTT TTGACTGTGTTTTATATTTCTCTGTTCAATATAACCTCTTAAATTTGTTTATGAAATTTGCTTATTAACAGTATTATAAACCCTTATTTTTTGTGTTGTAATGAATCTGCTGATTTAGAAAAATACTAAAAATGCCATATTTTTCTCCTTATAAAATAGTATAATATA GCAGAAAAGGATCTAGGTGAAGTCTTTTGTATAATCTCATGACCAAAATCCTTAACTGAGTTTTCGTTCCACTGAGCGTCAGACCCGTCAGAAAAGTCAAAAGGATCTTCTGAGATCTTTTTTCTGCGGTAATCTGCTGTTGCAACAAAAAACCCGCTACCAGCGGTG TTTTGTTGCGGATCAAGAGCTACCACTCTTTTCCGAAGGTAACCTGGCTCAGCAGAGCGCAGATACCAAAATCTGTTCTCTAGTGTAGCCGTAGTTAGGCCAACCTTCAAGAACTCTGTAACCCGCTACATACCCGCTGATGAGTGAAGTAACTTACCGCTTTCGAGT TAAGTCGTGTTACCAGGTTGACTAAGACGATAGTTACCGGATAAGGCGCAGCGTGGGCTGAAACGGGGGTTGTCGACACCGCCAGCTTGGAGCGCAACGACTACACCGAACTGAGATACCTACAGCTGAGCTATGAGAAAGCGCCAGCTTCCGAAAGGAGAAAGCGGACAG GTATCCGTAAGCGCAGGTCGGAACAGGAGCGCAGGAGGAGCTTCCAGGGGAAACGCTGCTTTATAGCTCTGTCGGGTTCCGCACTGACTGAGCGTCGATTTTGTGATGCTGTCAGGGGGGAGCTATGAAAAACCGCAGCAACCGCGCTTTTACGGTT CTGGGCTTTTCTGCGCTTTTCTGACATGATGTTCTTCCGCTTATCCCTGATCTGGATAGCTTACCGCTTTCGAGTGAAGTAACTTACCGCTTTCGAGTGAAGTAACTTACCGCTTTCGAGTGAAGTAACTTACCGCTTTCGAGTGAAGTAACTTACCGCTTTCGAGT GGCGGTTGGCGGATTCATTAATGCACTGGCAGCAGAGGTTCCCGGACTGAAAGCGGGCAGTGAAGCAACGCAATTAATGAGTTAGCTACTTATAGGCCACCCAGGCTTTACACTTATGCTTCGGCTGATGTTGTTGGAATTTGAGGCGATAACAATTTACACAGGAAAC AGCTATGACCATGATTACGCCAAGCTTGGCTGCA </pre>	<p>Methanol dehydrogenase promoter:</p> <p><i>Pmdh/mp</i> (fwd)</p> <p>Chloramphenicol resistance gene:</p> <p><i>Em-R</i> (rev)</p> <p>Origin of replication pTA1060:</p> <p><i>ori pTA1060</i></p> <p>Erythromycin resistance gene:</p> <p><i>Em-R</i> (rev)</p> <p>Origin of replication pUC9:</p> <p><i>ori pUC9</i></p>
<p>pBV2mp backbone</p>	<p>pBV2mp</p>	<pre> GATCTGAAAAACAAATCGAGTTGTTAAGTAAAGAGCACAGTTAAGATAATGAAAAAAGGACTGATCCTTGATAAAAAGTTCAACTGTTGATAATAGGATAGGTAGATGGACGAATATGACTATTTTTATCCAAAAATGAGAGGATAGGTAAAAATGCCCTATTCACCAAG CTTTTGAAGGTGCTGTATCGGATATATGCTACATAAAAAAATCTTTACCAAAAAGATTTAGCTCCAGAATTTCTGCTAAAAGATTTGGGATTGAAATATGACTGAAAAAATCACTTATGCTACATTGACTCAGATGTTAAAGTGTGTTTGTATGAAAAAAGTTGAGAAAAAG AGAGGAACATCAATCCATAAACTTTTCTCATACCTGCTACATGAGGAAAAATCAATTTAGAGATACCCATGGAAGTATATGAAAAAATCTCAGAAAGCAGAACTATGCTGGTGAAGCTATACCATATCATATGCTAGGTTACATTTAGCCTTAGCCCTGAGTTTAAAA CAAGCCTCAGATGTTTATATTTCTGAAAAAGTTGTCGAAAAACAGAAATTAACCTTATGATACACAAACAATTTGAAATCGAGAAATTTGATAATGTCAAATGAAAAAGTAAAGAGTTTGTCTAATGCAAAAGCTTTTTTATTTAATCTTTTAAAGGCTTTTAAAGATTGTTTT TTTGTCAATAAATTTAATCCCTCCCATTAAGAAATTAATCAGTGTATAACCTAGTAAAAATTTTATAGGTTCTTTCTGTTGATAAACCCTCAATTAATTTAATCCCCCTGTTAAAAATTAATAGGATGAAATTAATATTTTGTATCTCTTTATCCCTCAATTAAT TTAATTAGTCTAGGTAAATTAATTAATCCAGTCTAAATAAATTTAATCAGTACTTAAATATTTGATACCTCCCTGATATATTTTTTCACTATTTCTAAATAAAAAAATCTGCTGAAATCTCAGCAGTTTATGCAACATATCCAGCT CTTCAAGGACTGTTAATTTGTTTCAATAATCTTTCTTAATCCATTCAAATGCAAAATCACTCGGTCAGAAATATAAACAATTTTATTTTCAATTTGATCTTAGTGTTTTAAACAGGATCAAAAAGAGGCTTAGAGACAGCATTGTCATTCGCTTTTTAAAGTACTGCTGCACT TTTCTCTCTCAGTTGCTATGATACTCTCAAAAAGTGTGAGGATAGGATTTGTTTGTATTTCTTTGCAATTTCTCCTTACTGAGTATATCTTACATACATTTGTGCATATTTGAAAGGCTTGGAAATTAATTTGTTCTGACTATCCATCATTTTTTAAACAAATTCATCGT GCTCTTTTTTCAAAACCTTTGGAAGACCTCTTTTTCTTTTTTAAAGGCTCTTTACATGCTCTCTGCTCATCTGGAATTTCAATATAGGTTTCCATTTAAAAGTCTCTGAAAGCAAGAACTTACGCTCAATCTTAAAAATCGGGATTCCTCTGTGTTATCTCTGGTTTAT AGCAGGTTTTACTTCAAAATAAAACCGTACCTTTCAAGCAGTTCAAGTAGCCTCAACTGTAACAGGAGTCTTGCCTTTTTTAAAGGCTTTTATGAGCTTTTAAAGGCTTTTAAAGGCTTTTAAAGGCTTTTAAAGGCTTTTAAAGGCTTTTAAAGGCTTTTAAAGGCTTT ATAGCCCCAAAAATCAAAAGATTAATTTAAGATTGTATAATGACCTTATGTAAGGTATATGCTTACCAAAATTTGCTAAAGAAAGTTTTTATTTGCTCTCTGTCATATCTCTCTCGGTTTCAGCAACAATAAATCTCTCTTTCCCTTTTTGATTTCTCTGCGGAT TTTTATTTCCAGTGGTTGCTGTCGATAATACACTAATTCATCTGATTTCTCTTTAGGTTTACTGACTCATTAATGCTCCCGGATCACCTAAAGAACAGTGTAAAGAGGATTTTCACTGATAGGGGTTAATAAATGTTAAATGAGATAGTAAATACAAGTATAAATCCGCC TTTTCCCTTCTCAAAATTAACCTTCTCAGTTTGGCGGCTGTAGTTAAATGAGATTCGGTTAGGTGATTTTTTGTGTTGTTTTTTCATCAAAAACAACAGAGTTCTCTATATCTGAAATAATCTTATCATATTTTACTGGATTTAAATATTTTATCCCATGCTTTCTTAATTC TCTAATATATATCAATGCTCAATATTTTTTTAAAAAACAAGACTGTTTGGAGTCTGCTCAGCCTTTGTTGGTCTTTTTTAAATTCATATTCATAAAAAGTTTTTGGCTCAGGTAATGCTCAACCATCTTATCAATAGCTCTCAATAGTATCTCTAAGTAAATAGTAAT CCCTTCGATGCTGCCAGAAATTCGTGACGGATTAATCTTATATGAAATGAAACATCAACTTCAATGTTTTCAACAATGCTTTTTTCTTTTTGTTGTTCTGTTGTTTAACTCTGATGTTTCTTACTTTCAGAAATCTGTAAGATCCATGTAAGGTTTAAAGTGTACCTTAAAGCT TGTGGCTTTCTAGGTAGATCCCTAAATTTTGTACCTTTTCAAGTAACTTCAAGTAACTTCAAGTAACTTCAAGTAACTTCAAGTAACTTCAAGTAACTTCAAGTAACTTCAAGTAACTTCAAGTAACTTCAAGTAACTTCAAGTAACTTCAAGTAACTTCAAGTAACTTCAAGTAACT ATCTCAAAATCTTCCGAGCTCTGCAAAATATACTGATCACTGATCCACTGATTTTATAGTACAGGACTATCTCAATAAGCCGATATAGCCGCTGATGTTTCAACCATTTGTTCAAGGATAAAGTAACTTTCAGTTGACAGCAAAAGTCCATGAGCAGCTGACGTGAAA AAAGCCGCTCATTAGCCGGCTCCCGGGGACGTCGACTCTAGAGGATCTGACTTTAATCCGCTTCTGTCGATAGTTTGAACAACAGCAACAGGATTTCTCTGCTTATTTCAAAGATTAGTTAACTAGTAGTAAGCAATAAATCGAAGGAGTTGTTTAAATGCTCAGC </pre>	<p>Plasmid replication gene:</p> <p><i>repA</i> (rev)</p> <p>Methanol dehydrogenase promoter:</p> <p><i>Pmdh/mp</i> (fwd)</p> <p>Kanamycin resistance gene:</p> <p><i>kan</i> (rev)</p> <p>Ampicillin resistance gene:</p> <p><i>bla</i> (fwd)</p>

