**Supplementary Materials**

**Over-expression of a cyanobacterial gene for 1-deoxy-D-xylulose-5-phosphate synthase in the chloroplast of *Chlamydomonas reinhardtii* perturbs chlorophyll: carotenoid ratios.**

Figure S1: Schematic representation for chloroplast transformation strategy to generate marker-less transformants using the TN72 recipient.Chloroplast transformation plasmid consists of the gene of interest (GOI) under the control of the *psaA* promoter/5’UTR and the *rbcL* 3’UTR. The expression cassette is flanked by homologous sequence that contains wild type *psbH*. The homologous recombination takes place between homologous sequence shared between the plasmid and TN72 plastome, resulting in restoration of *psbH*, loss of *aadA* and the integration of the GOI into the plastome. Thus, the resulting transformants restore their photoautotrophic growth on acetate-free medium but they are sensitive to spectinomycin (SpcS). Homologous recombination is represented by X–X. The diagram is reproduced from (Stoffels et al., 2017).

 MluI

 GOI

 3’*rbcL*

5’*psaA* 1

 *psbH*

*trnE2*

3’*rbcL*

*aadA*

5’*atpA*

*psbH*

*trnE2*

 MluI

 SapI

 SphI

 3’ *rbcL*

 GOI

5’ *psaA* 1

 *psbH*

 *trnE2*

TN72 plastome
(Δ *psbH,* SpcR)

TF plastome
(*psbH*, SpcS)

 Plasmid

SapI

SphI

MluI

Stoffels, L., Taunt, H. N., Charalambous, B., & Purton, S. (2017). Synthesis of bacteriophage lytic proteins against *Streptococcus pneumoniae* in the chloroplast of *Chlamydomonas reinhardtii*. *Plant Biotechnology Journal*, 1–11. https://doi.org/10.1111/pbi.12703

Figure S2: Sequence alignment of the DXS and mDXS proteins.Sequence of the DXS protein

highlighting the amino acids that were deleted to create the mutant DXS protein, abbreviated

as mDXS. The deleted amino acid sequence is highlighted in yellow. The sequence of the

protein from *Synechocystis sp*. PCC 6803 was retrieved from the NCBI database (sequence

identifier: WP-010871718).

DXS 1 MHISELTHPNELKGLSIRELEEVSRQIREKHLQTVATSGGHLGPGLGVVELTVALYSTLD 60
mDXS 1 MHISELTHPNELKGLSIRELEEVSRQIREKHLQTVATSGGHLGPGLGVVELTVALYSTLD 60
 \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

DXS 61 LDKDRVIWDVGHQAYPHKMLTGRYHDFHTLRQKDGVAGYLKRSESRFDHFGAGHASTSIS 120
mDXS 61 LDKDRVIWDVGHQAYPHKMLTGRYHDFHTLRQKDGVAGYLKRSESRFDHFGAGHASTSIS 120
 \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

DXS 121 AGLGMALARDAKGEDFKVVSIIGDGALTGGMALEAINHAGHLPHTRLMVILNDNEMSISP 180
mDXS 121 AGLGMALARDAKGEDFKVVSIIGDGALTGGMALEAINHAGHLPHTRLMVILNDNEMSISP 180
 \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

DXS 181 NVGAISRYLNKVRLSSPMQFLTDNLEEQIKHLPFVGDSLTPEMERVKEGMKRLVVPKVGA 240
mDXS 181 NVGAISRYLNKVRLSSPMQFLTDNLEEQIKHLPFVGDSLTPEMERVKEGMKRLVVPKVGA 240
 \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

DXS 241 VIEELGFKYFGPIDGHSLQELIDTFKQAEKVPGPVFVHVSTTKGKGYDLAEKDQVGYHAQ 300
mDXS 241 VIEELGFKYFGPIDGHSLQELIDTFKQAEKVPGPVFVHVSTTKGKGYDLAEKDQVGYHAQ 300
 \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

DXS 301 SPFNLSTGKAYPSSKPKPPSYSKVFAHTLTTLAKENPNIVGITAAMATGTGLDKLQAKLP 360
mDXS 301 SPFNLSTGKAYPSSKPKPPSYSKVFAHTLTTLAKENPNIVGITAAMATGTGLDKLQAKLP 360
 \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

DXS 361 KQYVDVGIAEQHAVTLAAGMACEGIRPVVAIYSTFLQRGYDQIIHDVCIQKLPVFFCLDR 420
mDXS 361 KQYVDVGIAEQHAVTLAAGMACEGIRPVVAIYSTFLQRGYDQIIHDVCIQKLPVFFCLDR 420
 \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

DXS 421 AGIVGADGPTHQGMYDIAYLRCIPNLVLMAPKDEAELQQMLVTGVNYTGGAIAMRYPRGN 480
mDXS 421 AGIVGADGPTHQGMYDIAYLRCIPNLVLMAPKDEAELQQMLVTGVNYTGGAIAMRYPRGN 480
 \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

DXS 481 GIGVPLMEEGWEPLEIGKAEILRSGDDVLLLGYGSMVYPALQTAELLHEHGIEATVVNAR 540
mDXS 481 GIGVPLMEEGWEPLEIGKAEILRSGDDVLLLGYGS------------------------- 514
 \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

DXS 541 FVKPLDTELILPLAERIGKVVTMEEGCLMGGFGSAVAEALMDNNVLVPLKRLGVPDILVD 600
mDXS 515 ----------------------MEEGCLMGGFGSAVAEALMDNNVLVPLKRLGVPDILVD 553
 \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

DXS 601 HATPEQSTVDLGLTPAQMAQNIMASLFKTETESVVAPGVS 640
mDXS 554 HATPEQSTVDLGLTPAQMAQNIMASLFKTETESVVAPGVS 593
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Figure S3: HPLC profile of terpenoids extracted from freeze-dried transgenic lines; C (black), T1.dxs (pink) and T2.dxs (blue). Peak identifications: (1) β-carotene; (2) chlorophyll α; (3) chlorophyll b; (4) zea; (5) lutein; (6) anteraxanthin and (7) violaxanthin.

Table S1: Primers used in this study

|  |  |  |
| --- | --- | --- |
| **Primer name** |  **Primer sequence (5’🡪3’)** |  **Purpose** |
| Flank 1 | GTCATTGCGAAAATACTGGTGC | Confirm homplasmic integration of transgene into *C. reinhardtii*  |
| RbcL fn | cggatgtaactcaatcggtag | Confirm homplasmic integration of transgene into *C. reinhardtii* |
| RY-psaR | ataggctcttctCATggatttctccttataataac | Sequence the plasmid pRY134a |
| RY-psaSEQ | AACTATTTGTCTAATTTAATAACC | Sequence the plasmid pRY134a |
| RY123a | GGAGGGAAGTAGGCAGTAGC | Sequence the plasmid pRY134a |
| psbH.R | GCAACAGGAACTTCTAAAGC | Confirm homplasmic integration of transgene into C. reinhardtii |
| UH-5’DXS R 1 | AAT ACT TGT GGA AGC GTG CC | Sequence the plasmid pRY134a and pRY134aΔdxs |
| UH-DXS 3’UTR F1 | AAT TAC TCC ACG AAC ACG GC | Sequence the plasmid pRY134a and pRY134aΔdxs |
| UH-DXS 3’UTR R1 | TCC ACT TAA CTC AGA AGC AGG | Sequence the plasmid pRY134a and pRY134aΔdxs |
| UH-mDXS F | GGCACGCTTCCACAAGTATT | Sequence the plasmid pRY134aΔdxs |
| UH-mDXS 1R | CCATAGCAGCCGTGATACCG | Sequence the plasmid pRY134aΔdxs |

**Appendix S1.** Plasmids

**Plasmid pRY134a**

Sequence of pRY134a plasmid, containing *dxs* gene amplified from *Synechocystis* 6803 in pSRSapI backbone. Key: P/5’UTR *psaA* exon 1(red), *dxs* gene (blue), two stop codons (highlighted in turquoise), HA tag (highlighted in red) and 3’UTR *rbcL* (green).

AGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCAATTCGAATCCGCGTTTTCTCCGTGAAAGGGAGGTGTCCTAGGCCTCTAGACGATGGGGGCTTTTTGTTATATTTTACTAAATATATATTATAATTAAAAAAAATTGAATTGTCAATTTTTAATGTACACTTAGTTGAAAGTGCCCCTGTCCCCTTGGCCATATTTAACAGAAGTTATTTATAACGCAGCTGTTTTTTGGAGTCTATAAATTTATAACATCAGTTACTATGGATTTCCCTTTAGTTTTATGGCCTAGGACGTCCCCTTCCCCTTCGATGCTGGAGGCATCCTTTTACGGGACAATAAATAAATTTGTTGCCTCGCCTATCGGCTAACAAGTTCCTTCGGAGTATATAAATATAGGATGTTAATACTGCTATAAACTTTAGTTGCCCAATATTTATATTAGGACGCCAGTGGCAGTGGTACCGCCACTGCCTGCTTCGCAGTATATAAATATAGGCAGTTGGCAGGCAACTGCCACTGACGTCCTATTTTAATACTCCCAAGTTTACTTGCCTAGGCAGTTGGCAGGCAACAAATTTATTTATTGTCCACTAAAATTTATTTGCCCGAAGGGGACGTCCACTAAAATTTATTTACCCGAAGGGGACGTCCTAATATAAATATGGGGATGTCAATGCTCCGTTAGGAAGTAACTAACGTTTTTCAAATAAATTTTATCCCGGAGGGAAGTAGGCAGTAGCCCGCCACTGTCATCCTTTAAGTGGATCTCTCGTCAGGCAATTTGCTTACACCTTTAAATTAAAAATTAAATTTAAAGAAAAGTGAGCTATTAACGCGTAAGCTTTCTTAATTCAACATTTTTAAGTAAATACTGTTTAATGTTATACTTTTACGAATACACATATGGTAAAAAATAAAACAATATCTTTAAAATAAGTAAAAATAATTTGTAAACCAATAAAAAATATATTTATGGTATAATATAACATATGATGTAAAAAAAACTATTTGTCTAATTTAATAACCATGCATTTTTTATGAACACATAATAATTAAAAGCGTTGCTAATGGTGTAAATAATGTATTTATTAAATTAAATAATTGTTATTATAAGGAGAAATCCATGCACATCAGCGAACTGACCCACCCCAATGAGTTAAAGGGTTTATCTATCCGTGAGCTGGAGGAAGTTTCCCGGCAAATCCGAGAAAAACATCTCCAAACTGTGGCCACCAGCGGCGGTCATCTGGGGCCTGGGTTGGGAGTGGTGGAACTGACGGTGGCCCTATATTCCACTCTTGATTTAGACAAAGACCGGGTGATTTGGGATGTGGGACACCAAGCCTATCCCCACAAAATGTTGACGGGACGTTACCATGATTTCCATACCCTGCGGCAAAAAGATGGGGTGGCTGGTTACCTCAAACGCTCTGAAAGTCGTTTTGACCATTTTGGTGCTGGGCACGCTTCCACAAGTATTTCCGCTGGTTTGGGCATGGCTTTAGCTCGAGATGCCAAGGGGGAAGATTTTAAAGTAGTTTCCATCATTGGCGATGGTGCTTTAACCGGGGGCATGGCCTTGGAGGCAATCAACCATGCCGGGCATTTGCCCCATACCAGACTAATGGTGATTCTCAATGACAATGAGATGTCCATTTCCCCCAACGTGGGAGCCATTTCCCGTTATTTAAATAAAGTCCGCCTCAGCAGTCCGATGCAGTTTTTGACGGACAATCTGGAGGAACAGATCAAACACTTGCCCTTTGTGGGGGATTCCCTCACCCCGGAGATGGAAAGGGTCAAGGAGGGAATGAAACGTCTTGTTGTCCCCAAAGTGGGAGCGGTGATTGAAGAACTAGGATTTAAATATTTTGGTCCCATTGACGGTCATAGTTTACAGGAATTAATCGATACCTTTAAACAAGCGGAAAAAGTGCCTGGTCCGGTGTTTGTCCATGTTTCCACCACCAAGGGCAAGGGTTATGACTTAGCCGAAAAGGATCAAGTAGGCTACCACGCCCAAAGTCCTTTCAACCTTTCCACCGGTAAGGCCTATCCTTCTAGTAAGCCGAAACCCCCCAGCTATTCCAAGGTTTTTGCCCATACGTTGACTACCCTGGCCAAGGAAAATCCCAACATTGTCGGTATCACGGCTGCTATGGCGACGGGGACAGGGCTAGATAAACTCCAGGCCAAGCTACCCAAGCAGTATGTGGACGTGGGCATTGCGGAACAACACGCAGTTACTTTGGCGGCGGGCATGGCCTGCGAGGGCATTCGTCCAGTGGTGGCCATCTATTCCACCTTTTTGCAACGGGGTTACGACCAGATTATCCATGACGTTTGTATTCAAAAATTACCCGTATTTTTCTGCCTAGACCGGGCGGGCATTGTGGGGGCGGATGGGCCCACCCACCAAGGGATGTATGACATTGCCTATCTCCGTTGCATTCCCAATCTGGTGCTGATGGCTCCCAAGGATGAAGCAGAGTTACAACAAATGCTGGTTACTGGGGTTAACTACACCGGTGGGGCGATCGCCATGCGTTATCCCCGGGGTAATGGCATCGGGGTGCCCCTGATGGAGGAAGGTTGGGAACCGCTGGAAATTGGTAAAGCTGAAATTCTCCGGTCGGGGGATGATGTGCTTCTGTTGGGCTACGGCTCCATGGTCTATCCCGCTCTGCAAACAGCGGAATTACTCCACGAACACGGCATCGAAGCAACGGTGGTTAATGCCCGCTTTGTTAAACCACTAGATACGGAATTAATTTTGCCCTTGGCCGAGCGCATCGGCAAAGTGGTGACCATGGAAGAAGGTTGCCTCATGGGGGGCTTTGGCTCAGCAGTGGCGGAGGCCCTGATGGATAACAACGTGTTGGTGCCCTTGAAACGTTTAGGGGTGCCCGACATACTTGTGGACCATGCCACTCCGGAACAATCCACCGTTGACCTTGGTTTAACCCCAGCCCAGATGGCGCAGAATATTATGGCTTCACTGTTCAAAACTGAAACTGAATCAGTTGTCGCTCCTGGAGTTAGTTACCCATACGATGTTCCAGATTACGCTTAATAAGCATGCAAGCTTGTACTCAAGCTCGTAACGAAGGTCGTGACCTTGCTCGTGAAGGTGGCGACGTAATTCGTTCAGCTTGTAAATGGTCTCCAGAACTTGCTGCTGCATGTGAAGTTTGGAAAGAAATTAAATTCGAATTTGATACTATTGACAAACTTTAATTTTTATTTTTCATGATGTTTATGTGAATAGCATAAACATCGTTTTTATTTTTATGGTGTTTAGGTTAAATACCTAAACATCATTTTACATTTTTAAAATTAAGTTCTAAAGTTATCTTTTGTTTAAATTTGCCTGTCTTTATAAATTACGATGTGCCAGAAAAATAAAATCTTAGCTTTTTATTATAGAATTTATCTTTATGTATTATATTTTATAAGTTATAATAAAAGAAATAGTAACATACTAAAGCGGATGTAGCGCGTTTATCTTAACGGAAGGCCAGTGGCACGTCCGCCTGCCACTGCCGAATATAAATATGGTTGAGTTGCTTAGTTTACCTTAGCGAAAAGAAGACTTAGCAGCTAGCCTTAACAAACAGTTTTATATTTTATGTTTGTGTTAAATAAAATTAAGAAACTTTAGCTAAAGTTTCCCAACTCATAGAAACGTCATCTAAAATTAAAGAACTGTTGTAAATTTCTAAAATGATTAATAAGAATGCTGCAAATAAAAGGATAAATACAGCCATTAAAACAGTTGTACCCCAGCCTGGTAATACTTTACCTGCTTCTGAGTTAAGTGGACGTAATAAAGTACCTAATGGTGTAACTAAACCAGGTTCTTGGAAGTCTGAATTTACTTTTGATGGTTTAGCTTTAGAAGTTCCTGTTGCCATAATTGATTAAATGAATTAAGCGTTATTAGCGCTATTTTATTTACTTTCTGTAAAAAATAAGGAAAATATTCTTCAGTGCATTCCCTCTCAGGATTATAAATACTCTGAGGATAACGTTCTCTCGTCAAGGGGTTGCTTCTTGTGAGTATAGAAACCTACTAGCACAAGAAATAAATTGCATAAAAATGTATTTACCTAGGACCGCAGTAGGCAGTCCCTTTTCCCCTTCAGAACTGCCTGCTTTAAAAGAATGAAAAAACTGCCTTGTCTGGTAAGTAAAACTCTTTAATTACTCACTAAAGACGATCTTAGAAGTTCTTTGTTCATTTTTTATTTAATATAATATTTGTTATATAAAAATTAAATAATTTTTAATTAATGTTTAACTTTGTAAGGACAGTTTCAAAGTGACATGAATGGCTACTGCAAAAACGAAGTAAGTTATTCTTTCTCAGGGCAAAATTTTGAGTAGATTAATTTTGTTTAAAAATGTGGGACACAGTCGTCAAGTCTTTTGAACTATCTAAGAGATATGTTGAAAAGAGAATAATTTTATTATTAAATGAGCTATGGAAAGTCCAGCTTTTTTCTTTACCTTTTTTTTATGGTTTCTTCTGTTAAGTGTAACTGGCTATTCAGTTTATGTTAGTTTTGGTCCACCTTCAAAAAAATTACGTGATCCTTTTGAAGAACACGAAGATTAAACAAGTTAAAAAGTACTATTTTTACAAGTGACTTCGGTGCCTCTGAGAACCCTAGTTATAGTGATATAAAATAACTAGCTAACTACTTTATATTTTTATGAAAGTCATTTTGTCGAGCATATAAACAAAAACAAAATTGCTATACTAGGCAGTCACAGTGCAACTGTCTCCGTCTCCTTAACCGAGAAAGGGTAAACGTCTTCGGTAAAGTAACAAACTTTAGTTATGTTAACTGCTTGCGAGTTAACCATTTTTTTTCCTCCGAAGGACAACAGTTGGCAGTTGCCAAACTTTAGTGGTCTAATATTTATATTAGGCAGTTGGCAGGCAACTGCACTGACGTCCCGAAGGGGAAGGGGTTTACTTACCTCCTAACGGAGTATATAAATAGAATAAAATTTATTTCCTGCGCTAGCAGATTTACATACTAGGATTTTAATACTCCGAAGGAGGCAGTGGCGGTACCACTGCCACTGGCGTCCTCCTTCCCCTTCGGGCAAATGCATTTTAGTGCCACTTAAGTTTACTTGCCTAGGCAGTTGGCAGGACGTCAGTGGCAGTGGTACCGCGACTGCCTATATTTATATACTCCTAAGTTTACTTGCCTAGGCAGTTGGCAGGCAACTGCCACTGACGTCCTTCCCCTTCCCCTTCGGGACGTCCCCTTACGGGAATATAAATATTAGTGGATATTTATATACTGCGATGTTTACATACTCCGAAGGAGGAGAGCTAGCAGTTGCCTGCCAACTGCCTAATATAAATATTGGGCAAGTAAACTTAGAATGTTTACATACTCCGAAGGAGGACGTCCCTTACGGGAATATAAATATTAGTGGCAGTGGTACCGCCACTGCCTCCTTCGGAGTATTAAAATCCTAGTATATAATATACCGTAAGGGACGTCCTCCGACGGTGGCAGTGGCGGTACCACTGCCACCGGCGTCCTAATATACATATTGAAGTATTTAAACCTGTTAGCGCACGCTCTAACGAGTCAGTAAACTTCCCTTTTGGGGCTTCTAGGCAGCGCATAAATTTTCTAGGACGAACGTCCACTGGCGTCTCGTAAGGAGCAGTGACAGGCCACTAATGTCCCCTTAATGGGTAAATAAATGGCTATCGTCTATCCATGAAGAGACCATATATTCCAGTAGCACCGTTATGATCCTCAAAGGGTAACACCATTTGTATAGTATTATGGTGAAATGCATCCCTTTCAGGGTAGATTTATATCTTACAG

**Plasmid pRY134aΔdxs**

This plasmid is identical to pRY134a except for the partial deletion of *dxs* gene. The deleted 141 bp from the *dxs* gene is underlined. Key: NcoI restriction site (highlighted in yellow), HA tag (highlighted in red) and two stop codons (highlighted in turquoise).

ATGCACATCAGCGAACTGACCCACCCCAATGAGTTAAAGGGTTTATCTATCCGTGAGCTGGAGGAAGTTTCCCGGCAAATCCGAGAAAAACATCTCCAAACTGTGGCCACCAGCGGCGGTCATCTGGGGCCTGGGTTGGGAGTGGTGGAACTGACGGTGGCCCTATATTCCACTCTTGATTTAGACAAAGACCGGGTGATTTGGGATGTGGGACACCAAGCCTATCCCCACAAAATGTTGACGGGACGTTACCATGATTTCCATACCCTGCGGCAAAAAGATGGGGTGGCTGGTTACCTCAAACGCTCTGAAAGTCGTTTTGACCATTTTGGTGCTGGGCACGCTTCCACAAGTATTTCCGCTGGTTTGGGCATGGCTTTAGCTCGAGATGCCAAGGGGGAAGATTTTAAAGTAGTTTCCATCATTGGCGATGGTGCTTTAACCGGGGGCATGGCCTTGGAGGCAATCAACCATGCCGGGCATTTGCCCCATACCAGACTAATGGTGATTCTCAATGACAATGAGATGTCCATTTCCCCCAACGTGGGAGCCATTTCCCGTTATTTAAATAAAGTCCGCCTCAGCAGTCCGATGCAGTTTTTGACGGACAATCTGGAGGAACAGATCAAACACTTGCCCTTTGTGGGGGATTCCCTCACCCCGGAGATGGAAAGGGTCAAGGAGGGAATGAAACGTCTTGTTGTCCCCAAAGTGGGAGCGGTGATTGAAGAACTAGGATTTAAATATTTTGGTCCCATTGACGGTCATAGTTTACAGGAATTAATCGATACCTTTAAACAAGCGGAAAAAGTGCCTGGTCCGGTGTTTGTCCATGTTTCCACCACCAAGGGCAAGGGTTATGACTTAGCCGAAAAGGATCAAGTAGGCTACCACGCCCAAAGTCCTTTCAACCTTTCCACCGGTAAGGCCTATCCTTCTAGTAAGCCGAAACCCCCCAGCTATTCCAAGGTTTTTGCCCATACGTTGACTACCCTGGCCAAGGAAAATCCCAACATTGTCGGTATCACGGCTGCTATGGCGACGGGGACAGGGCTAGATAAACTCCAGGCCAAGCTACCCAAGCAGTATGTGGACGTGGGCATTGCGGAACAACACGCAGTTACTTTGGCGGCGGGCATGGCCTGCGAGGGCATTCGTCCAGTGGTGGCCATCTATTCCACCTTTTTGCAACGGGGTTACGACCAGATTATCCATGACGTTTGTATTCAAAAATTACCCGTATTTTTCTGCCTAGACCGGGCGGGCATTGTGGGGGCGGATGGGCCCACCCACCAAGGGATGTATGACATTGCCTATCTCCGTTGCATTCCCAATCTGGTGCTGATGGCTCCCAAGGATGAAGCAGAGTTACAACAAATGCTGGTTACTGGGGTTAACTACACCGGTGGGGCGATCGCCATGCGTTATCCCCGGGGTAATGGCATCGGGGTGCCCCTGATGGAGGAAGGTTGGGAACCGCTGGAAATTGGTAAAGCTGAAATTCTCCGGTCGGGGGATGATGTGCTTCTGTTGGGCTACGGCTCCATGGTCTATCCCGCTCTGCAAACAGCGGAATTACTCCACGAACACGGCATCGAAGCAACGGTGGTTAATGCCCGCTTTGTTAAACCACTAGATACGGAATTAATTTTGCCCTTGGCCGAGCGCATCGGCAAAGTGGTGACCATGGAAGAAGGTTGCCTCATGGGGGGCTTTGGCTCAGCAGTGGCGGAGGCCCTGATGGATAACAACGTGTTGGTGCCCTTGAAACGTTTAGGGGTGCCCGACATACTTGTGGACCATGCCACTCCGGAACAATCCACCGTTGACCTTGGTTTAACCCCAGCCCAGATGGCGCAGAATATTATGGCTTCACTGTTCAAAACTGAAACTGAATCAGTTGTCGCTCCTGGAGTTAGTTACCCATACGATGTTCCAGATTACGCTTAATAA