

Enhancement of Astaxanthin Biosynthesis in Oleaginous Yeast *Yarrowia lipolytica* via Microalgal Pathway

Larissa Ribeiro Ramos Tramontin ¹, Kanchana Rueksomtawin Kildegaard ¹, Suresh Sudarsan ¹ and Irina Borodina *

¹ The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kemitorvet 220, 2800 Kgs. Lyngby, Denmark; lartra@biosustain.dtu.dk (L.R.R.T.); kanchana@biophero.com (K.R.K.); sursud@biosustain.dtu.dk (S.S.)

* Correspondence: irbo@biosustain.dtu.dk

Supplementary figures

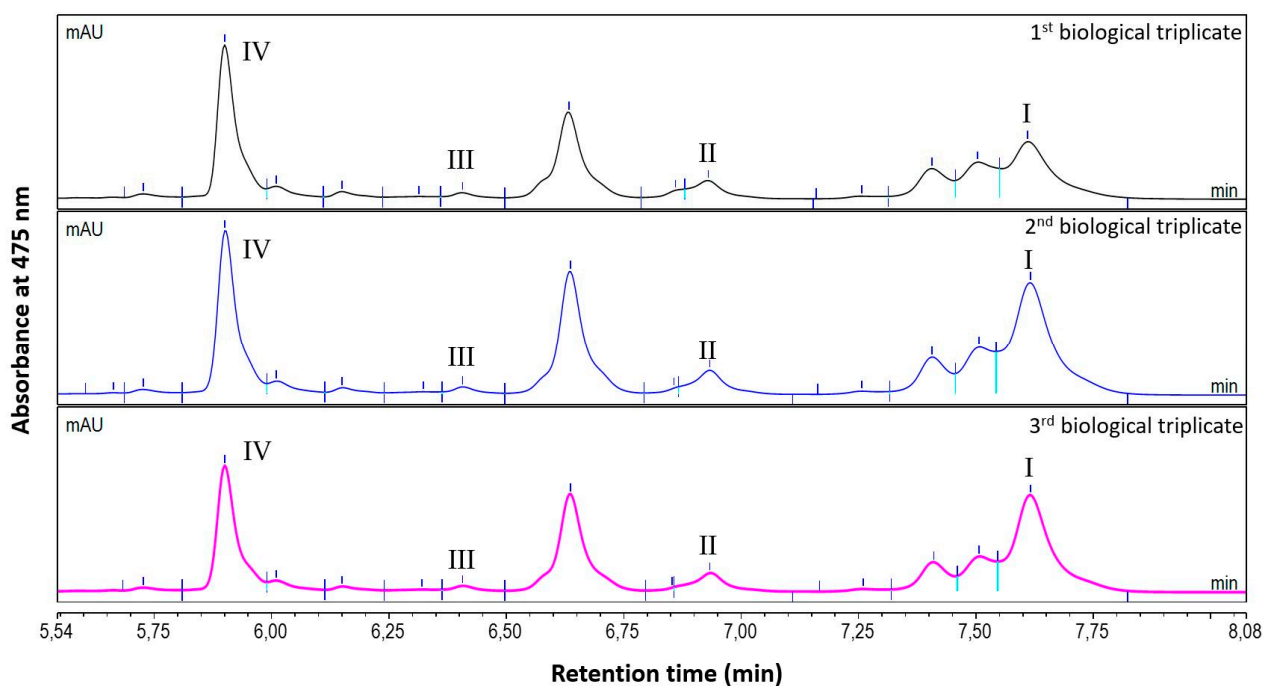


Figure S1. HPLC profile of carotenoids produced after 168h of fed-batch fermentation of ST7976 (iso 3). I: β -carotene; II: echinenone; III: canthaxanthin; IV: astaxanthin.

Supplementary tables

Table S1. Production of carotenoids by the strains generated in this study. Titers were quantified by HPLC analysis. Values are the average of the triplicates. The molar ratio (β -ketolase: β -hydroxylase) is indicated between brackets. The isolate number is indicated after the abbreviation 'iso'.

Genotype	Strain ID	β -carotene (mg/L)	\pm	Echinenone (mg/L)	\pm	Cantaxanthin (mg/L)	\pm	Astaxanthin (mg/L)	\pm	Dry cell (mg)	\pm
Wild type	ST3683	0	0	0	0	0	0	0	0	13.93	1.38
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i>	ST6899	88.85	11	0	0	0	0	0	0	21.00	1.35
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> <i>XdCrtE</i> \uparrow	ST7433	132.02	11.28	0	0	0	0	0	0	18.13	1.16
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow	ST7434	330.73	66.48	0	0	0	0	0	0	14.67	2.69
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow - <i>CrtW</i> \uparrow \uparrow \uparrow	ST7906	30.47	1.48	109.37	4.97	13.83	0.86	0	0	18.37	0.260
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow - <i>HpBKT</i> \uparrow \uparrow \uparrow	ST7972	82.73	14.15	55.87	3.53	30.85	2.65	0	0	18.47	0.291
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow - <i>CrtW</i> \uparrow \uparrow \uparrow - <i>PaCrtZ</i> \uparrow \uparrow \uparrow	ST7927 (1:3) iso 6	174.22	7.50	13.02	0.21	4.67	0.12	17.56	0.19	16.20	0.058
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow - <i>PSQS1_50bp:PsCrtW</i> \uparrow - <i>PaCrtZ</i> \uparrow <i>PrTefintron-</i> <i>PaCrtZ</i> \uparrow \uparrow \uparrow	ST7400	45.08	2.31	27.58	3.11	2.21	0.29	15.80	0.83	13.83	1.82
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PSQS1_50bp:SQS1</i> <i>PsCrtW</i> \uparrow - <i>PaCrtZ</i> \uparrow	ST7403	22.66	2.80	22.17	5.20	1.70	0.41	18.10	0.64	13.87	0.28
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow - <i>HpBKT</i> \uparrow \uparrow \uparrow - <i>PaCrtZ</i> \uparrow	ST7973 iso 4	25.22	1.41	42.79	1.92	9.28	1.01	10.24	0.10	16.37	0.29
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow - <i>HpBKT</i> \uparrow \uparrow \uparrow - <i>PaCrtZ</i> \uparrow	ST7973 iso 5	51.96	11.72	38.91	3.80	13.60	3.11	9.38	0.62	16.70	0.15
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow - <i>HpBKT</i> \uparrow \uparrow \uparrow - <i>PaCrtZ</i> \uparrow	ST7973 iso 6	67.87	7.29	29.66	3.07	15.95	1.47	9.80	0.53	16.10	0.23
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow - <i>HpBKT</i> \uparrow \uparrow \uparrow - <i>PaCrtZ</i> \uparrow	ST7973 iso 12	75.01	11.55	35.05	1.47	15.61	2.11	10.77	0.20	16.17	0.12
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow - <i>HpBKT</i> \uparrow \uparrow \uparrow - <i>PaCrtZ</i> \uparrow	ST7973 iso 16	46.69	3.28	71.56	3.97	18.70	1.37	8.37	0.31	16.07	0.27
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow - <i>HpBKT</i> \uparrow \uparrow \uparrow - <i>PaCrtZ</i> \uparrow	ST7973 iso 28	24.58	4.37	51.15	0.89	15.67	0.40	5.23	0.42	15.90	0.45
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow - <i>HpBKT</i> \uparrow \uparrow \uparrow - <i>PaCrtZ</i> \uparrow	ST7973 iso 29	17.22	2.02	62.45	5.71	18.55	2.70	4.75	0.21	16.97	0.93
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow - <i>HpBKT</i> \uparrow \uparrow \uparrow - <i>HpCrtZ</i> \uparrow	ST7974 iso 1	44.28	2.24	53.94	2.43	6.48	0.57	16.23	0.32	20.97	3.18
<i>XdCrtI</i> \uparrow - <i>XdCrtYB</i> \uparrow <i>XdCrtE</i> \uparrow - HMG1 \uparrow <i>PrSQS_50bp:SQS1</i> GGPPS7 \uparrow - <i>HpBKT</i> \uparrow \uparrow \uparrow - <i>HpCrtZ</i> \uparrow	ST7974 iso 7	91.92	2.77	49.49	1.02	9.12	0.49	10.85	0.08	17.03	0.03

<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑	ST7974 iso 11	39.99	2.3 1	47.58	3.5 4	3.43	0.5 3	20.41	0. 83	17.67	0.1 9
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑	ST7974 iso 14	30.77	1.9 8	41.28	2.0 7	2.19	0.3 2	19.62	0. 27	17.67	0.2 7
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑	ST7974 iso 16	31.88	1.2 6	39.50	1.7 3	1.81	0.3 2	18.50	0. 40	17.53	0.6 2
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑	ST7974 iso 20	50.22	3.6 1	44.12	2.3 6	8.11	0.7 2	16.29	0. 24	17.77	0.4 7
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:1) iso 3	28.63	1.7 2	48.50	2.5 0	20.94	0.8 8	3.77	0. 12	16.97	0.5 3
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:1) iso 4	39.72	7.8 0	48.09	2.0 7	14.91	1.1 3	9.51	0. 09	16.83	0.2 3
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:1) iso 5	16.47	1.1 9	51.58	2.8 7	25.21	1.2 1	1.05	0. 01	17.57	1.2 5
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:1) iso 6	14.77	1.1 6	54.91	1.8 7	33.50	1.1 3	0.00	0. 00	18.07	2.0 2
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:1) iso 7	27.79	2.7 3	50.21	1.3 3	23.81	1.1 5	2.09	0. 18	16.17	0.3 2
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:1) iso 8	32.13	1.6 0	56.51	3.3 0	20.00	1.5 6	4.56	0. 14	15.73	0.4 4
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:1) iso 9	10.55	0.2 4	63.34	1.5 6	33.34	1.0 5	1.21	0. 12	16.50	0.6 0
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:2) iso 1	177.09	15. 37	135.54	8.3 6	88.62	3.3 9	0.00	0. 00	16.70	0.6 0
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:2) iso 3	19.88	2.0 1	55.16	2.4 1	32.01	2.1 9	0.00	0. 00	15.67	0.0 7
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:2) iso 6	32.39	4.1 6	48.00	3.1 2	11.98	1.5 6	11.02	0. 11	15.70	0.3 1
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:2) iso 7	17.84	1.5 3	63.11	3.2 3	20.53	1.0 5	0.00	0. 00	15.93	0.1 5
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:2) iso 11	231.41	25. 97	43.72	3.2 7	18.90	1.3 9	0.00	0. 00	14.70	0.1 7
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:2) iso 12	141.87	73. 55	32.19	3.5 7	15.89	1.3 3	12.86	0. 31	15.90	0.4 9
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:2) iso 14	18.52	3.6 5	64.70	3.6 5	28.81	1.4 8	0.00	0. 00	15.93	0.3 2
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:3) iso 1	33.27	1.5 5	57.80	4.8 3	29.79	2.4 3	8.97	0. 64	17.23	0.0 3
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:3) iso 2	201.69	54. 85	55.25	3.1 6	25.83	1.3 3	1.88	0. 24	17.10	0.6 5
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:3) iso 3	12.70	2.1 2	81.20	10. 36	45.24	6.7 2	0.38	0. 38	17.20	0.2 5
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:3) iso 5	14.75	1.0 8	105.79	8.4 8	52.00	4.1 8	2.12	0. 07	17.07	0.2 2
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:3) iso 6	23.31	2.5 6	113.38	10. 32	35.18	1.9 3	0.33	0. 33	15.07	0.2 2
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1</i> <i>GGPPS7</i> ↑ - <i>HpBKT</i> ↑↑↑ - <i>PaCrtZ</i> ↑↑↑	ST7975 (1:3) iso 11	4.62	0.6 8	135.25	7.1 4	39.31	1.7 2	1.65	0. 19	15.73	0.8 8

<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>PaCrIZ</i> ↑ ↑ ↑	ST7975 (1:3) iso 15	29.30	0.8 2	35.81	12. 80	22.44	1.3 6	13.48	0. 39	16.37	0.2 9
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:1) iso 3	163.32	12. 75	32.32	5.7 3	0.89	0.5 1	44.34	1. 40	14.80	0.4 6
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:1) iso 9	221.91	25. 67	28.62	4.9 6	7.14	1.4 6	29.63	1. 40	16.07	0.2 0
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:1) iso 11	186.55	7.7 9	26.65	4.4 7	7.30	1.4 1	26.04	1. 64	14.90	0.3 1
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:1) iso 12	135.50	26. 42	28.42	5.7 6	7.40	1.8 1	27.86	1. 34	15.97	0.6 1
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:1) iso 13	146.95	29. 73	29.80	2.1 3	7.10	1.2 2	26.94	0. 25	15.83	0.1 8
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:1) iso 14	23.69	1.8 8	79.04	11. 96	35.10	0.8 7	12.28	0. 93	16.13	0.5 8
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:1) iso 20	65.13	2.5 0	19.89	1.1 2	3.27	1.6 8	27.60	2. 83	18.03	0.5 4
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:2) iso 3	188.16	28. 54	94.39	9.7 7	48.11	1.7 9	0.00	0. 00	15.03	1.0 1
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:2) iso 11	225.80	33. 68	95.30	1.7 9	42.12	1.8 8	0.43	0. 43	17.23	0.6 2
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:2) iso 12	96.81	23. 34	50.48	8.6 2	4.20	0.9 1	41.37	1. 35	17.00	0.2 0
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:2) iso 13	24.87	1.7 9	65.38	2.8 9	37.65	0.8 0	0.00	0. 00	16.60	0.3 1
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:2) iso 14	117.23	35. 00	51.42	3.4 6	16.31	1.1 2	23.45	1. 53	16.50	0.7 6
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:2) iso 15	137.55	19. 51	72.54	10. 99	49.55	2.4 2	0.00	0. 00	17.20	0.3 6
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:2) iso 16	238.94	7.6 5	47.46	2.9 4	2.61	0.3 2	42.92	0. 25	17.43	0.3 0
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:3) iso 3	269.62	45. 75	96.81	13. 00	51.04	4.4 2	4.61	1. 99	17.33	0.9 2
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:3) iso 10	358.35	25. 18	61.70	22. 75	14.93	1.2 2	25.28	3. 07	15.77	0.2 2
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:3) iso 11	206.45	46. 96	147.01	14. 31	35.63	1.1 7	13.00	1. 00	15.67	0.3 8
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:3) iso 12	225.81	17. 94	60.42	0.6 4	60.17	2.7 8	3.21	0. 27	14.80	0.1 5
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:3) iso 15	253.72	5.1 0	19.00	2.8 5	27.91	0.7 5	18.01	0. 78	19.00	0.4 0
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:3) iso 17	400.36	15. 44	96.09	32. 36	35.65	0.6 7	12.84	1. 17	16.10	0.4 9
<i>XdCrI</i> ↑ - <i>XdCrYB</i> ↑ <i>XdCrE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS</i> 50bp: <i>SQSI</i> GGPPS7 ↑ - <i>HpBKT</i> ↑ ↑ ↑ - <i>HpCrIZ</i> ↑ ↑ ↑	ST7976 (1:3) iso 18	165.23	23. 04	11.86	7.9 3	15.36	10. 32	10.27	0. 91	19.77	0.1 9

<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑	ST7926 iso 4	38.53	3.1 3	175.83	15. 85	10.88	3.2 0	7.56	1. 49	15.43	0.8 3
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑	ST7926 iso 5	47.81	3.8 6	192.87	2.9 0	7.76	0.5 1	12.71	0. 16	19.70	0.2 5
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑	ST7926 iso 8	204.15	62. 72	123.55	2.9 1	11.42	0.3 6	1.95	0. 10	17.97	0.1 5
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑	ST7926 iso 9	1038.46	47. 05	0.14	0.0 1	0.00	0.0 0	0.00	0. 00	21.23	0.0 3
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑	ST7926 iso 10	31.35	2.3 5	199.24	7.5 6	14.73	1.7 7	10.40	1. 69	15.73	0.2 7
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑	ST7926 iso 12	146.57	5.4 9	78.15	13. 97	11.80	0.3 3	3.03	0. 19	17.00	0.2 3
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑	ST7925 iso 1	3.07	0.0 9	55.36	0.3 0	15.61	0.2 9	0.00	0. 00	14.57	0.1 7
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑	ST7925 iso 2	3.66	0.5 8	39.99	6.6 6	0.61	0.6 1	10.84	1. 50	13.40	0.5 3
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7627 (1:1) iso 1	4.50	1.5 5	156.90	23. 50	12.25	3.3 1	11.90	0. 66	17.07	0.2 9
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7627 (1:1) iso 2	5.60	1.0 3	99.09	2.2 0	4.74	2.3 7	16.74	0. 33	16.63	0.2 2
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7627 (1:1) iso 4	8.47	3.3 5	147.93	4.9 4	21.53	1.2 9	7.46	1. 20	13.87	0.2 2
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7627 (1:1) iso 7	53.77	11. 38	64.60	3.2 7	4.91	0.4 7	12.49	0. 50	16.87	0.3 2
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7627 (1:2) iso 2	2.78	0.9 1	143.08	16. 37	8.50	1.8 4	12.93	0. 56	15.77	0.8 4
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7627 (1:2) iso 3	3.81	0.3 7	14.65	1.6 4	0.56	0.3 0	4.69	0. 78	15.97	1.4 7
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7627 (1:2) iso 4	11.91	1.5 4	72.51	12. 17	5.76	2.1 0	17.28	0. 92	14.43	0.9 5
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7627 (1:2) iso 5	25.46	1.4 9	64.55	4.9 5	3.19	0.4 5	18.81	1. 30	15.33	0.7 8
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7627 (1:3) iso 1	30.71	6.0 4	98.48	18. 05	8.82	2.6 3	15.23	0. 97	16.03	0.9 3
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7627 (1:3) iso 2	146.12	20. 67	0.00	0.0 0	0.47	0.2 3	32.86	1. 20	15.13	0.9 5
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7627 (1:3) iso 5	303.73	15. 75	10.43	0.7 3	0.77	0.0 7	8.13	0. 30	14.43	0.8 4
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7627 (1:3) iso 6	136.57	37. 55	0.00	0.0 0	0.48	0.2 4	44.98	4. 72	14.40	1.0 5
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7928 (1:1) iso 6	13.27	1.6 2	134.15	16. 49	2.69	0.6 0	18.90	2. 52	16.13	0.8 1
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>PaCrtZ</i> ↑ ↑ ↑	ST7928 (1:1) iso 10	7.14	1.6 2	107.96	28. 06	3.37	0.9 9	13.55	4. 78	14.80	1.9 3

<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑ ↑ ↑	ST7928 (1:1) iso 11	17.97	0.3 9	63.90	10. 95	0.20	0.1 0	19.90	4. 11	15.50	0.9 6
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑ ↑ ↑	ST7928 (1:1) iso 15	18.63	0.6 2	50.15	7.4 2	1.19	0.1 8	17.91	3. 44	14.13	0.6 9
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑ ↑ ↑	ST7928 (1:2) iso 1	19.74	1.9 2	136.16	4.0 4	7.48	1.4 8	17.39	0. 37	14.10	0.6 7
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑ ↑ ↑	ST7928 (1:2) iso 4	4.39	0.1 3	62.06	14. 82	1.34	0.3 0	18.05	4. 21	15.20	1.0 6
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑ ↑ ↑	ST7928 (1:2) iso 5	51.55	13. 13	53.42	11. 68	1.59	0.3 3	16.60	3. 31	14.00	1.7 6
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑ ↑ ↑	ST7928 (1:2) iso 8	26.94	8.3 5	60.49	18. 83	0.17	0.1 0	18.77	4. 61	15.67	1.2 0
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑ ↑ ↑	ST7928 (1:3) iso 2	14.57	2.6 9	26.06	5.0 5	0.07	0.0 7	15.90	3. 03	14.57	0.5 2
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑ ↑ ↑	ST7928 (1:3) iso 9	30.24	2.6 1	22.17	1.6 4	0.00	0.0 0	14.36	0. 79	14.20	0.2 3
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑ ↑ ↑	ST7928 (1:3) iso 10	10.60	1.9 6	61.02	10. 06	0.00	0.0 0	19.74	1. 74	15.73	0.5 2
<i>XdCrtI</i> ↑ - <i>XdCrtYB</i> ↑ <i>XdCrtE</i> ↑ - <i>HMG1</i> ↑ - <i>PrSQS_50bp:SQS1 GGPPS7</i> ↑ - <i>PsCrtW</i> ↑ ↑ ↑ - <i>HpCrtZ</i> ↑ ↑ ↑	ST7928 (1:3) iso 12	11.83	3.6 2	75.97	20. 06	0.00	0.0 0	23.48	4. 47	17.30	1.4 0

Table S2. List of *Yarrowia lipolytica* engineered strains. Single black arrow (↑) represents a single integration event; triple black arrow (↑ ↑ ↑) represents multiple integration events.

Strain ID	Genotype	Parent strain	Integrated Plasmid	Reference
ST6899	ST6899 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 loop out HphMX)			[23]
ST7400	XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ - PrSQS1_50bp:PsCrtW ↑ -PaCrtZ ↑ - PrTefintron-PaCrtZ ↑ ↑ ↑			[23]
ST7403	XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ - PrSQS1_50bp:SQS1 PsCrtW ↑ -PaCrtZ ↑			[23]
ST7433	ST7433 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 XdCrtE ↑)	ST6899	pCfB7646	This study
ST7434	ST7434 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 GGPPS7 ↑)	ST6899	pCfB7645	This study
ST7906	ST7906 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 GGPPS7 ↑ PsCrtW ↑ ↑ ↑)	ST7434	pCfB7063	This study
ST7925	ST7925 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 GGPPS7 ↑ PsCrtW ↑ ↑ ↑ PaCrtZ ↑)	ST7906	pCfB7815	This study
ST7926	ST7926 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 GGPPS7 ↑ PsCrtW ↑ ↑ ↑ HpCrtZ ↑)	ST7906	pCfB7883	This study
ST7927	ST7927 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 GGPPS7 ↑ PsCrtW ↑ ↑ ↑ PaCrtZ ↑ ↑ ↑)	ST7434	pCfB7063 and pCfB7064	This study
ST7928	ST7928 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 GGPPS7 ↑ PsCrtW ↑ ↑ ↑ HpCrtZ ↑ ↑ ↑)	ST7434	pCfB7063 and pCfB7884	This study
ST7972	ST7972 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 GGPPS7 ↑ HpBKT ↑ ↑ ↑)	ST7434	pCfB8027	This study
ST7973	ST7973 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 GGPPS7 ↑ HpBKT ↑ ↑ ↑ PaCrtZ ↑)	ST7972	pCfB7815	This study
ST7974	ST7974 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 GGPPS7 ↑ HpBKT ↑ ↑ ↑ HpCrtZ ↑)	ST7972	pCfB7883	This study
ST7975	ST7975 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 GGPPS7 ↑ HpBKT ↑ ↑ ↑ PaCrtZ ↑ ↑ ↑)	ST7434	pCfB8027 and pCfB7064	This study
ST7976	ST7976 (XdCrtI ↑ - XdCrtYB ↑ XdCrtE ↑ - HMG1 ↑ PrSQS_50bp:SQS1 GGPPS7 ↑ HpBKT ↑ ↑ ↑ HpCrtZ ↑ ↑ ↑)	ST7434	pCfB8027 and pCfB7884	This study

Table S3. List of plasmids used in this study. The plasmids were constructed by USER cloning, inserting BioBricks into parental plasmid.

ID	Description	Parental plasmid	Inserted BioBricks	Reference
pCfB4784	pIntE-2-NatMX-TPex20-TLip2			[39]
pCfB5219	pIntE-4-HphMX-TPex20-TLip2			[39]
pCfB5313	prDNA-Ura3d1-TPex20-TLip2			[23]
pCfB7063	prDNA-Ura3d1-PrTefintron->PsCrtW-TLip2	pCfB5313	BB02093, BB02220	[23]
pCfB7064	prDNA-Ura3d1-PrTefintron-PaCrtZ-TLip2	pCfB5313	BB02093, BB02221	[23]
pCfB7645	pIntF-2-NatMX-PrFBA1->SsGGPPS7-TLip2	pCfB4784	BB01559, BB02142	This study
pCfB7646	pIntF-2-NatMX-PrFBA1->XdCrtE-TLip2	pCfB4784	BB01559, BB01569	This study
pCfB7815	pIntE-4-HphMX-TPex20-PrTefintron->PaCrtZ-TLip2	pCfB5219	BB02093, BB02221	This study
pCfB7883	pIntE-4-HphMX-TPex20-PrTefintron->HpCrtZ-TLip2	pCfB5219	BB02564, BB02563	This study
pCfB7884	prDNA-Ura3d1-TPex20-HpCrtZ-<PrTefintron-TLip2	pCfB5313	BB02564, BB02563	This study
pCfB8027	prDNA-Ura3d1-TPex20-HpBKT-<PrTefintron-TLip2	pCfB5313	BB02209, BB02566	This study

Table S4. List of primers used in this study. Underlined sequences represent overhangs used for USER cloning.

Primer ID	Description	Sequence, 3' → 5'
PR-15523	PrYIFBA1_fw (PR-15523)	<u>CGTGC GAU</u> AACAGTGTACGCAGTACTATAGAGG
PR-15524	PrYIFBA1_rev (PR-15524)	<u>ATGACAGA</u> UTGTGTGATGTGTAGTTTAGATTTTCG
PR-18214	PrTefintron_USER_rv	<u>AGTACTGCAAAA</u> AGUGCTG
PR-21242	SsGGPPs7_fw	<u>ATCTGTCAU</u> GCCACA ATGGTCGCACAAACTTTC
PR-21243	SsGGPPs7_rv	<u>CACGCGAU</u> TTAATGCTGACGACGTGTG
PR-18928	PrTefintron_USER <-_U1_fw	<u>CACGCGAU</u> AGAGACCGGGTTGG
PR-20673	PsCrtWGene2_Fw	<u>ACTTTTTGCAGTACUA</u> ACCGCAGTCTGCCACGCTCTGCCAAG
PR-20675	PaCrtZGene2_Fw	<u>ACTTTTTGCAGTACUA</u> ACCGCAGCTGTGGATCTGGAACGCCCTGATCG
PR-20676	PaCrtZGene2_Rv	<u>CACGCGAU</u> T TACTTGCCAGAGGCGGGCTCGT
PR-20677	PsCrtWGene2_Rv	<u>CACGCGAU</u> T TAGGCGGTGTGCGCCCTTAGTTTCGAG
PR-21079	HpCrtZ_Ylop_forfusionPrTefintron_U2_rv	<u>CACGCGAU</u> TTATCGCTTAGACCAGTCC
PR-21419	HpBKT_forfusionPrTefintron_USER_E coRI U1 fw	<u>ACTTTTTGCAGTACUA</u> ACCGCAGCACGTGGCCTCTGCTCTG

PR-21421	HpCrtZ_forfusionPrTefintron_USER_U 2 fw	<u>ACTTTTTGCAGTACUAACCGCAG</u> CTGTCTAAGCTGCAGTCT
PR-21422	PrTefintron_USER_SfaAI_BamHI- > U2 fw	<u>CGTGCGAU</u> GCGATCGC CCGG GGATCC AGAGACCGGGTTGGCGGC
PR-21423	HpBKT_forfusionPrTefintron_USER_S faAI U1 rv	<u>CGTGCGAU</u> GCGATCGC TTAGGCCAGAGCGGGGAC
PR-7043	TJOS-25F (PR-7043)	<u>ATCTGTCAU</u> ATGGATTACGCGAACATCCTC
PR-7044	TJOS-25R (PR-7044)	<u>CACGCGAU</u> TCACAGAGGGATATCGGCTAG

Table S5. List of BioBricks used in this study. BioBricks were obtained by PCR, using the template and primers as indicated in the table.

Biobrick ID	Description	Template	Forward primer	Reverse primer	Reference
BB01559	PrY1FBAI->	gDNA <i>Y. lipolytica</i>	PR-15523	PR-15524	[23]
BB01569	XdcrtE->	p1763 (YB1/E)	PR-7043	PR-7044	[23]
BB02093	PrTefintron_USER	gDNA <i>Y. lipolytica</i>	PR-10595	PR-18214	[23]
BB02142	GGPPs7	GeneString PL-6778 (SsGGPS7)	PR-21242	PR-21243	This study
BB02209	PrTefintron_USER <-	gDNA <i>Y. lipolytica</i>	PR-18928	PR-18214	[23]
BB02220	PscrtW	Synthetic gene string from GeneArt	PR-20673	PR-20677	[23]
BB02221	PacrtZ	Synthetic gene string from GeneArt	PR-20675	PR-20676	[23]
BB02563	HpcrtZ	Synthetic gene string from GeneArt p7642 (HpCrtZ ylop)	PR-21421	PR-21079	This study
BB02564	Promoter for HpcrtZ	gDNA <i>Y. lipolytica</i>	PR-21422	PR-18214	This study
BB02566	HpBKT_forfusionPrTefintr on USER SfaAI<-	GeneString p7641 (HpBKTm ylop)	PR-21419	PR-21423	This study

Supplementary sequences

Sequences of synthetic genes used in the study.

>GGPPs7 (geranylgeranyl pyrophosphate synthase from *Synechococcus* sp. codon-optimized for *Y. lipolytica*).

ATGGTCGCACAACTTTCAACCTGGATACCTACTTATCCCAAAGACAACAACAAGTTGAAGAGGCC
CTAAGTGCTGCTCTTGTGCCAGCTTATCCTGAGAGAATATACGAAGCTATGAGATACTCCCTCCTGG
CAGGTGGCAAAGATTAAGACCTATCTTATGTTTAGCTGCTTGCGAATTGGCAGGTGGTTCTGTTGA
ACAAGCCATGCCAACTGCGTGTGCACTTGAAATGATCCATACAATGCTACTAATTCATGATGACCTG
CCAGCCATGGATAACGATGATTTTCAGAAGAGGAAAGCCAACTAATCACAAAGGTGTTTCGGGGAAGA
TATAGCCATCTTAGCGGGTGATGCGCTTTTAGCTTACGCTTTTGAACATATTGCTTCTCAAACAAGAG
GAGTACCACCTCAATTGGTGCTACAAGTTATTGCTAGAATCGGACACGCCGTTGCTGCAACAGGCCT
CGTTGGAGGCCAAGTCGTAGACCTTGAATCTGAAGGTAAAGCTATTTCCCTTAGAAACATTGGAGTAT
ATCACTCACATAAGACTGGAGCCTTGCTGGAAGCATCAGTTGTCTCAGGCGGTATTCTCGCAGGGG
CAGATGAAGAGCTTTTGGCCAGATTGTCTCATTACGCTAGAGATATAGGCTTGGCTTTTCAAATCGTC
GATGATATCCTGGATGTTACTGCTACATCTGAACAGTTGGGGAAAACCGCTGGTAAAGACCAGGCA

GCCGCAAAGGCAACTTATCCAAGTCTATTGGGTTTAGAAGCCTCTAGACAGAAAGCGGAAGAGTTG
ATTCAATCTGCTAAGGAAGCCTTAAGACCTTACGGTTCACAAGCAGAGCCACTCCTAGCGCTGGCA
GACTTCATCACACGTCGTCAGCATTA

>**crtW** (β -ketolase from *Paracoccus* sp. codon-optimized for *Y. lipolytica*)

ATGTCTGCCCACGCTCTGCCCAAGGCCGACCTGACCGCCACCTCTCTGATCGTGTCTGGCGGCATCA
TTGCCGCCTGGCTGGCCCTGCATGTGCACGCCCTGTGGTTTCTGGACGCCGCTGCTACCCCATCCTG
GCTATCGCCAACTTCTGGCCTGACCTGGCTGTCTGTGGGCCTGTTTATTATCGCCCACGACGCCAT
GCACGGCTCTGTGGTGCCCGGACGACCCCGAGCCAACGCCGCCATGGGCCAGCTGGTGCTGTGGCT
GTACGCCGGCTTCTCTTGGCGAAAGATGATCGTGAAGCACATGGCCCACCACCGACACGCCGGCAC
CGACGACGACCCTGACTTCGACCACGGCGGACCCGTCGGATGGTACGCCCGATTTCATCGGCACCTA
CTTCGGCTGGCGAGAGGGCCTGCTGCTGCCCGTGATCGTGACCGTGTACGCCCTGATCCTGGGCGAC
CGATGGATGTACGTGGTGTCTGGCCCCTGCCTTCTATCCTGGCCTCTATCCAGCTGTTTCGTGTTCCGC
ACCTGGCTGCCCCACCGACCTGGCCACGACGCTTTCGCCGACCGACACAACGCCCGATCTTCTCGAA
TCTCTGACCCCGTGTCTCTGCTGACCTGCTTCCACTTCGGCGGCTACCACCACGAGCACCATCTGCAC
CCCACCGTGCCTTGGTGGCGACTGCCCTTACTCGAACTAAGGGCGACACCCGCTAA

>**PactZ** (β -hydroxylase from *Pantoea ananatis* codon-optimized for *Y. lipolytica*)

ATGCTGTGGATCTGGAACGCCCTGATCGTGTTCGTGACCGTGATCGGCATGGAAGTGATCGCCGCTC
TGGCCCACAAGTACATCATGCACGGCTGGGGCTGGGGATGGCACCTGTCTCACCACGAGCCTCGAA
AGGGCGCCTTCGAGGTGAACGACCTGTACGCCGTGGTGTTCGCTGCCCTGTCTATCCTGCTGATCTA
CCTGGGCTCTACCGGCATGTGGCCCCTGCAGTGGATCGGAGCCGGCATGACCGCCTACGGCCTGCTG
TACTTCATGGTGCACGACGGCCTGGTCCACCAGAGATGGCCCTTCCGGTACATTCCCCGAAAGGGCT
ACCTGAAGCGACTGTACATGGCCCACCGAATGCACCACGCCGTGCGAGGCAAGGAAGGCTGCGTTT
CTTTCGGCTTCTGTACGCACCTCCTCTGTCTAAGCTGCAGGCTACCCTGCGAGAGCGACACGGCGC
TCGAGCCGGCGCTGCCAGAGATGCCCAAGGCGGCGAGGACGAGCCCGCCTCTGGCAAGTAA

>**HpctrZ** (β -hydroxylase from *H. pluvialis* codon-optimized for *Y. lipolytica*)

ATGCTGTCTAAGCTGCAGTCTATCTCTGTGAAGGCCCGACGAGTCGAGCTGGCCCCGAGACATCACC
GACCTAAGGTGTGCCTGCACGCCAGAGATGTTCTCTGGTGCGACTGCGAGTGGCTGCTCCCCAGAC
CGAGGAAGCCGTGGGCACCCAGCAGGCCGCTGGCGCTGGCGACGAGCACTCTGCCGACGTGGCCC
TGCAGCAGCTGGACCGAGCCATTGCCGAGCGACGAGCCCGACGAAAGCGAGAGCAGCTGTCTTAC
CAGGCTGCCGCTATCGCCGCCTCTATCGGCGTGTCTGGAATCGCCATCTTCGCCACCTACCTGCGATT

CGCCATGCACATGACCGTCGGCGGAGCTGTGCCCTGGGGCGAAGTGGCTGGCACCCCTGCTGCTGGT
GGTCGGAGGGCGCCCTCGGCATGGAAATGTACGCCCATAACGCCACAAGGCCATCTGGCACGAGTC
GCCCCTCGGCTGGCTGCTGCACAAGTCTCATCACACCCCTCGAACCGGACCTTTGAGGCCAACGAC
CTGTTCCGCATCATCAACGGACTGCCCGCCATGCTGCTGTGTACCTTCGGCTTCTGGCTGCCAACGT
GCTGGGCACCGCCTGCTTCGGAGCCGGCCTGGGCATCACCCCTGTACGGCATGGCCTACATGTTTCGT
CACGACGGCCTGGTGCACCGACGATTCCCCACCGGACCTATCGCTGGACTGCCCTACATGAAGCGA
CTGACCGTGGCTCACCAGCTGCACCACTCTGGCAAGTACGGCGGAGCCCCTTGGGGCATGTTCTGG
GACCTCAAGAGCTGCAGCACATCCCCGGTGCCGCCGAAGAGGTCGAGCGACTGGTGCTCGAGCTGG
ACTGGTCTAAGCGATAA

HpBKT (β -ketolase from *H. pluvialis* codon-optimized for *Y. lipolytica*)

ATGCACGTGGCCTCTGCTCTGATGGTCGAGCAGAAGGGCTCTGAGGCCGCTGCCTCTTCTCCCGACG
TGCTGCGAGCCTGGGCTACCCAGTACCACATGCCTTCCGAGTCCTCTGACGCCGCTCGACCCGCTCT
GAAGCACGCCTACAAGCCTCCAGCCTCCGACGCCAAGGGCATCACCATGGCTCTGACCATCATCGG
AACCTGGACCGCCGTGTTCTGACGCCATCTTCCAGATTGACTGCCACCTCTATGGACCAGCTG
CACTGGCTGCCCGTGTCTGAGGCCACCGCTCAGCTGCTCGGCGGATCTTCTTCTCTGCTGCACATTGC
CGCCGTCTTTATCGTGCTCGAGTTCCTGTACACCGGCCTGTTTCATCACCACTCACGACGCCATGCACG
GCACCATTGCTCTGCGACACCGACAGCTGAACGACCTGCTGGGCAACATCTGCATCTCCCTGTACGC
CTGGTTCGACTACTCTATGCTGCGACGAAAGCACTGGGAGCACCACAACCACACCGGCGAGGTCGG
CAAGGACCCCGACTTCCACAAGGGCAACCCCGGACTGGTGCCCTGGTTCGCCTCTTTCATGTCTCT
TACATGTCTCTGTGGCAGTTCGCCCAGTGGCCTGGTGGGCCGTCGTCATGCAGATGCTGGGCGCTC
CCATGGCCAACCTGCTGGTGTTCATGGCCGCTGCTCCCATCCTGTCCGCCTTCCGACTGTTCTACTTC
GGCACCTACCTGCCTCACAAGCCCGAGCCTGGACCTGCCGCCGATCTCAGGACATGGCCTGGTTC
CGAGCCAAGACCTCTGAGGCTTCTGACGTGATGTCTTTCCTGACCTGCTACCACTTCGACCTGCATTG
GGAGCATCATCGATGGCCCTACGCTCCCTGGTGGCAGCTCCCTCACTGCCGACGACTGTCTGGCCGA
GGACTGGTCCCCGCTCTGGCCTAA