

Supplementary materials for

Engineering *Y. lipolytica* as a chassis for *de novo* synthesis of five aromatic-derived natural products and chemicals

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Supplementary Table

Table 1. Strains and plasmids used in this study

Names	Characteristics	Reference
Strains		
po1g	Wild-type strain W29 (ATCC20460) derivate; W29 $\Delta matA \Delta xpr2-332 \Delta axp-2 \Delta leu2-270$ pBR platform	1
po1f	po1g derivate; Further deletion of gene <i>ura3</i> ; po1g $\Delta ura3$	1
po1fk	po1f derivate; Further deletion of gene <i>ku70</i> ; po1f $\Delta ku70::loxP$	2
YL0	po1fk pYLP'	This work
YL1	po1fk pYLP'-yIPAR4	This work
YL2	po1fk pYLP'-yIARO10	This work
YL3	po1fk pYLP'-yIPHA2	This work
YL4	po1fk pYLP'-yIARO7	This work
YL5	po1fk pYLP'-yIPAR4-yIARO10-yIPHA2-yIARO7	This work
YL6	po1fk pYLP'-yIPAR4-yIARO10-yIPHA2-yIARO7-ScARO7 ^{G141S}	This work
YL7	po1fk derivate; Further integration of genes <i>yIPAR4</i> , <i>yIARO10</i> , <i>yIPHA2</i> , <i>yIARO7</i> , and <i>ScARO7^{G141S}</i> at <i>YALI0E30965g</i> site; po1fk <i>yIPAR4 yIARO10 yIPHA2 yIARO7 ScARO7^{G141S}::loxP</i>	This work
YL8	YL7 pYLP'-yIARO1	This work
YL9	YL7 pYLP'-yIARO2	This work
YL10	YL7 pYLP'-yIARO3	This work
YL11	YL7 pYLP'-yIARO4	This work
YL12	YL7 pYLP'-yIARO5	This work
YL13	YL7 pYLP'-yIARO1-yIARO2	This work
YL14	YL7 pYLP'-yIARO1-yIARO2-yIARO3-yIARO4-yIARO5	This work
YL15	YL7 pYLP'-yIARO1-yIARO2-scARO4 ^{K229L}	This work
YL16	YL7 pYLP'-yIARO1-yIARO2-ecaroG ^{L175D}	This work
YL17	YL7 pYLP'-yIARO1-yIARO2-ecaroG ^{S180F}	This work
YL18	po1fk derivate; Further deletion of genes <i>yITYR1</i> ; po1fk $\Delta yITYR1::loxP$	This work
YL19	YL18 derivate; Further Deletion of genes <i>yITRP2</i> , and <i>yITRP3</i> ; po1fk $\Delta yITYR1 \Delta yITRP2 \Delta yITRP3::loxP$	This work
YL20	YL19 derivate; Further Deletion of genes <i>yIARO8</i> , and <i>yIARO9</i> ; po1fk $\Delta yITYR1 \Delta yITRP2 \Delta yITRP3 \Delta yIARO8 \Delta yIARO9::loxP$	This work
YL21	YL18 pYLP'-yIPAR4-yIARO10-yIPHA2-yIARO7-ScARO7 ^{G141S}	This work
YL22	YL19 pYLP'-yIPAR4-yIARO10-yIPHA2-yIARO7-ScARO7 ^{G141S}	This work
YL23	YL20 pYLP'-yIPAR4-yIARO10-yIPHA2-yIARO7-ScARO7 ^{G141S}	This work
YL24	YL20 derivate; Further integration of genes <i>yIARO1</i> , <i>yIARO2</i> , <i>yIARO3</i> , <i>yIARO4</i> , <i>yIARO5</i> , <i>scARO4^{K229L}</i> , <i>aroG^{S180F}</i> at <i>YALI0E30965g</i> and <i>ku70</i> sites; po1fk $\Delta yITYR1 \Delta yITRP2 \Delta yITRP3 \Delta yIARO8 \Delta yIARO9 yIARO1 yIARO2 yIARO3 yIARO4 yIARO5 scARO4K229L aroGS180F::loxP$	This work
YL25	YL24 pYLP'-yIPAR4-yIARO10-yIPHA2-yIARO7-ScARO7 ^{G141S}	This work
YL26	YL24 pYLP'-yITKT-yIPAR4-yIARO10-yIPHA2-yIARO7-ScARO7 ^{G141S}	This work
YL27	YL24 pYLP'-bbxfpK-yIPAR4-yIARO10-yIPHA2-yIARO7-ScARO7 ^{G141S}	This work

YL28	YL24 pYLP'- <i>acxpkA</i> - <i>yIPAR4</i> - <i>yIARO10</i> - <i>yIPHA2</i> - <i>yIARO7</i> - <i>ScARO7</i> ^{G141S}	This work
YL29	YL24 derivative; Further integration of genes <i>bbxpfK</i> , <i>yIPAR4</i> , <i>yIARO10</i> , <i>yIPHA2</i> , <i>yIARO7</i> , <i>ScARO7</i> ^{G141S} at pBR platform; po1fk $\Delta yITR1$ $\Delta yITR2$ $\Delta yITR3$ $\Delta yIARO8$ $\Delta yIARO9$ <i>yIARO1</i> <i>yIARO2</i> <i>yIARO3</i> <i>yIARO4</i> <i>yIARO5</i> <i>scARO4</i> ^{K229L} <i>aroG</i> ^{S180F} <i>bbxpfK</i> <i>yIPAR4</i> <i>yIARO10</i> <i>yIPHA2</i> <i>yIARO7</i> <i>ScARO7</i> ^{G141S} :: <i>Leu</i>	This work
YL30	YL24 derivative; Further integration of genes <i>acxpkA</i> , <i>yIPAR4</i> , <i>yIARO10</i> , <i>yIPHA2</i> , <i>yIARO7</i> , <i>ScARO7</i> ^{G141S} at pBR platform; po1fk $\Delta yITR1$ $\Delta yITR2$ $\Delta yITR3$ $\Delta yIARO8$ $\Delta yIARO9$ <i>yIARO1</i> <i>yIARO2</i> <i>yIARO3</i> <i>yIARO4</i> <i>yIARO5</i> <i>scARO4</i> ^{K229L} <i>aroG</i> ^{S180F} <i>acxpkA</i> <i>yIPAR4</i> <i>yIARO10</i> <i>yIPHA2</i> <i>yIARO7</i> <i>ScARO7</i> ^{G141S} :: <i>Leu</i>	This work
YL31	YL24 derivative; Further integration of genes <i>yITKT</i> , <i>BbxpfK</i> , and <i>Acxpk</i> at 26s rDNA site; po1fk $\Delta yITR1$ $\Delta yITR2$ $\Delta yITR3$ $\Delta yIARO8$ $\Delta yIARO9$ <i>yIARO1</i> <i>yIARO2</i> <i>yIARO3</i> <i>yIARO4</i> <i>yIARO5</i> <i>scARO4</i> ^{K229L} <i>aroG</i> ^{S180F} <i>yITKT</i> <i>bbxpfK</i> <i>acxpk</i> :: <i>loxP</i>	This work
YL32	YL31 pYLP'- <i>yIPAR4</i> - <i>yIARO10</i> - <i>yIPHA2</i> - <i>yIARO7</i> - <i>ScARO7</i> ^{G141S}	This work
YL33	YL31 derivative; Further deletion of gene <i>yIPYK</i> ; po1fk $\Delta yITR1$ $\Delta yITR2$ $\Delta yITR3$ $\Delta yIARO8$ $\Delta yIARO9$ $\Delta yIPYK$ <i>yIARO1</i> <i>yIARO2</i> <i>yIARO3</i> <i>yIARO4</i> <i>yIARO5</i> <i>scARO4</i> ^{K229L} <i>aroG</i> ^{S180F} <i>yITKT</i> <i>bbxpfK</i> <i>acxpk</i> :: <i>loxP</i>	This work
YL34	YL33 pYLP'- <i>yIPAR4</i> - <i>yIARO10</i> - <i>yIPHA2</i> - <i>yIARO7</i> - <i>ScARO7</i> ^{G141S}	This work
YL35	YL33 derivative; Further integration of genes <i>yIPAR4</i> , <i>yIARO10</i> , <i>yIPHA2</i> , <i>yIARO7</i> , <i>ScARO7</i> ^{G141S} at pBR platform; po1fk $\Delta yITR1$ $\Delta yITR2$ $\Delta yITR3$ $\Delta yIARO8$ $\Delta yIARO9$ $\Delta yIPYK$ <i>yIARO1</i> <i>yIARO2</i> <i>yIARO3</i> <i>yIARO4</i> <i>yIARO5</i> <i>scARO4</i> ^{K229L} <i>aroG</i> ^{S180F} <i>yITKT</i> <i>bbxpfK</i> <i>acxpk</i> <i>yIPAR4</i> <i>yIARO10</i> <i>yIPHA2</i> <i>yIARO7</i> <i>ScARO7</i> ^{G141S} :: <i>Leu</i>	This work
YL36	YL31 derivative; Further integration of genes <i>yIPAR4</i> , <i>yIARO10</i> , <i>yIPHA2</i> , <i>yIARO7</i> , <i>ScARO7</i> ^{G141S} at pBR platform; po1fk $\Delta yITR1$ $\Delta yITR2$ $\Delta yITR3$ $\Delta yIARO8$ $\Delta yIARO9$ <i>yIARO1</i> <i>yIARO2</i> <i>yIARO3</i> <i>yIARO4</i> <i>yIARO5</i> <i>scARO4</i> ^{K229L} <i>aroG</i> ^{S180F} <i>yITKT</i> <i>bbxpfK</i> <i>acxpk</i> <i>yIPAR4</i> <i>yIARO10</i> <i>yIPHA2</i> <i>yIARO7</i> <i>ScARO7</i> ^{G141S} :: <i>Leu</i>	This work
YL37	YL35 derivative; Further deletion of genes <i>yIALD2</i> and <i>yIALD3</i> ; po1fk $\Delta yITR1$ $\Delta yITR2$ $\Delta yITR3$ $\Delta yIARO8$ $\Delta yIARO9$ $\Delta yIPYK$ $\Delta yIALD2$ $\Delta yIALD3$ <i>yIARO1</i> <i>yIARO2</i> <i>yIARO3</i> <i>yIARO4</i> <i>yIARO5</i> <i>scARO4</i> ^{K229L} <i>aroG</i> ^{S180F} <i>yITKT</i> <i>bbxpfK</i> <i>acxpk</i> <i>yIPAR4</i> <i>yIARO10</i> <i>yIPHA2</i> <i>yIARO7</i> <i>ScARO7</i> ^{G141S} :: <i>Leu</i>	This work
YL38	YL37 derivative; Further deletion of genes <i>yIHPD</i> ; po1fk $\Delta yITR1$ $\Delta yITR2$ $\Delta yITR3$ $\Delta yIARO8$ $\Delta yIARO9$ $\Delta yIPYK$ $\Delta yIALD2$ $\Delta yIALD3$ $\Delta yIHPD$ <i>yIARO1</i> <i>yIARO2</i> <i>yIARO3</i> <i>yIARO4</i> <i>yIARO5</i> <i>scARO4</i> ^{K229L} <i>aroG</i> ^{S180F} <i>yITKT</i> <i>bbxpfK</i> <i>acxpk</i> <i>yIPAR4</i> <i>yIARO10</i> <i>yIPHA2</i> <i>yIARO7</i> <i>ScARO7</i> ^{G141S} :: <i>Leu</i>	This work
YL39	YL33 derivative; Further integration of genes <i>rgTAL</i> and <i>yITYR1</i> at pBR platform; po1fk $\Delta yITR1$ $\Delta yITR2$ $\Delta yITR3$ $\Delta yIARO8$ $\Delta yIARO9$ $\Delta yIPYK$ <i>yIARO1</i> <i>yIARO2</i> <i>yIARO3</i> <i>yIARO4</i> <i>yIARO5</i> <i>scARO4</i> ^{K229L} <i>aroG</i> ^{S180F} <i>yITKT</i> <i>bbxpfK</i> <i>acxpk</i> <i>rgTAL</i> <i>yITYR1</i> :: <i>Leu</i>	This work
YL40	YL39 derivative; Further deletion of genes <i>yIPHA2</i> ; po1fk $\Delta yITR1$ $\Delta yITR2$ $\Delta yITR3$ $\Delta yIARO8$ $\Delta yIARO9$ $\Delta yIPYK$ $\Delta yIPHA2$ <i>yIARO1</i> <i>yIARO2</i> <i>yIARO3</i> <i>yIARO4</i> <i>yIARO5</i> <i>scARO4</i> ^{K229L} <i>aroG</i> ^{S180F} <i>yITKT</i> <i>bbxpfK</i> <i>acxpk</i> <i>rgTAL</i> <i>yITYR1</i> :: <i>Leu</i>	This work
YL41	YL33 derivative; Further integration of genes <i>VioA</i> , <i>VioB</i> , <i>VioC</i> , <i>VioD</i> , and <i>VioE</i> at pBR platform; po1fk $\Delta yITR1$ $\Delta yITR2$ $\Delta yITR3$ $\Delta yIARO8$ $\Delta yIARO9$ $\Delta yIPYK$ <i>yIARO1</i> <i>yIARO2</i> <i>yIARO3</i> <i>yIARO4</i> <i>yIARO5</i> <i>scARO4</i> ^{K229L} <i>aroG</i> ^{S180F} <i>yITKT</i> <i>bbxpfK</i> <i>yITR2</i> <i>yITR3</i> <i>acxpk</i> <i>VioA</i> <i>VioB</i> <i>VioC</i> <i>VioD</i> <i>VioE</i> :: <i>Leu</i>	This work
YL42	YL41 derivative; Further integration of genes <i>yITR5</i> , <i>yITR4</i> , <i>yITR3</i> , <i>yITR2</i> , and	This work

yITRP1 at *YALIOE03212g* site; *po1fk ΔyITYR1 ΔyITRP2 ΔyITRP3 ΔyIARO8 ΔyIARO9 ΔyIPYK yIARO1 yIARO2 yIARO3 yIARO4 yIARO5 scARO4^{K229L} aroG^{S180F} yITKT bbx_{fpk} acxpk yITRP5 yITRP4 yITRP3 yITRP2 yITRP1 yITRP2 yITRP3 VioA VioB VioC VioD VioE::Leu*

pof1kV *po1fk* derivate; Further integration of genes *VioA*, *VioB*, *VioC*, *VioD*, and *VioE* at *pBR* This work

Plasmids

<i>pYLXP'</i>	YaliBrick plasmid	3
<i>pYLXP'-loxP-ura</i>	<i>pYLXP'</i> containing the <i>loxP-URA-loxP</i> cassette	4
<i>pYLXP'-loxP-hygr</i>	<i>pYLXP'</i> containing the <i>loxP-hygr-loxP</i> cassette	4
<i>pYLXP'-Cre</i>	<i>pYLXP'</i> containing gene <i>Cre</i>	4
<i>pYLXPs'</i>	<i>pYLXP'</i> derivate; <i>NotI</i> site was mutated to <i>SnaBI</i> site	This work
<i>pURLA</i>	<i>Ku70</i> site integration plasmid	This work
<i>pURLK</i>	<i>YALIOE30965g</i> site integration plasmid	This work
<i>pURLD</i>	<i>YALIOE03212g</i> site integration plasmid	This work
<i>prDNAloxP</i>	26s rDNA site integration plasmid	4
<i>pYLXP'-yIPAR4</i>	<i>pYLXP'</i> containing gene <i>yIPAR4</i>	
<i>pYLXP'-yIARO10</i>	<i>pYLXP'</i> containing gene <i>yIARO10</i>	
<i>pYLXP'-yIPHA2</i>	<i>pYLXP'</i> containing gene <i>yIPHA2</i>	This work
<i>pYLXP'-yIARO7</i>	<i>pYLXP'</i> containing gene <i>yIARO7</i>	This work
<i>pYLXP'-yIPAR4-yIARO10-yIPHA2-yIARO7</i>	<i>pYLXP'</i> containing gene <i>yIPAR4</i> , <i>yIARO10</i> , <i>yIPHA2</i> , and <i>yIARO7</i>	This work
<i>pYLXP'-yIPAR4-yIARO10-yIPHA2-yIARO7-ScARO7^{G141S}</i>	<i>pYLXP'</i> containing gene <i>yIPAR4</i> , <i>yIARO10</i> , <i>yIPHA2</i> , <i>yIARO7</i> , and <i>ScARO7^{G141S}</i>	This work
<i>pURLA-yIPAR4-yIARO10-yIARO7-yIPHA2-scARO7^{G141S}</i>	<i>pURLA</i> containing gene <i>yIPAR4</i> , <i>yIARO10</i> , <i>yIPHA2</i> , <i>yIARO7</i> , and <i>ScARO7^{G141S}</i>	This work
<i>pYLXP'-yIARO1</i>	<i>pYLXP'</i> containing gene <i>yIARO1</i>	5
<i>pYLXP'-yIARO2</i>	<i>pYLXP'</i> containing gene <i>yIARO2</i>	This work
<i>pYLXP'-yIARO3</i>	<i>pYLXP'</i> containing gene <i>yIARO3</i>	This work
<i>pYLXP'-yIARO4</i>	<i>pYLXP'</i> containing gene <i>yIARO4</i>	This work
<i>pYLXP'-yIARO5</i>	<i>pYLXP'</i> containing gene <i>yIARO5</i>	This work
<i>pYLXP'-yIARO1-yIARO2</i>	<i>pYLXP'</i> containing gene <i>yIARO1</i> and <i>yIARO2</i>	This work
<i>pYLXP'-yIARO1-yIARO2-yIARO3-yIARO4-yIARO5</i>	<i>pYLXP'</i> containing gene <i>yIARO1</i> , <i>yIARO2</i> , <i>yIARO3</i> , <i>yIARO4</i> , and <i>yIARO5</i>	This work
<i>pYLXP'-yIARO1-yIARO2-scARO4^{K229L}</i>	<i>pYLXP'</i> containing gene <i>yIARO1</i> , <i>yIARO2</i> , <i>scARO4^{K229L}</i>	This work
<i>pYLXP'-yIARO1-yIARO2-ecaroG^{L175D}</i>	<i>pYLXP'</i> containing gene <i>yIARO1</i> , <i>yIARO2</i> , <i>ecaroG^{L175D}</i>	This work
<i>pYLXP'-yIARO1-yIARO2-ecaroG^{S180F}</i>	<i>pYLXP'</i> containing gene <i>yIARO1</i> , <i>yIARO2</i> , <i>ecaroG^{S180F}</i>	This work
<i>pYLXP'-loxP-hygr-ΔyITYR1</i>	<i>pYLXP'-loxP-hygr</i> containing gene <i>yITYR1</i> deletion cassette	This work
<i>pYLXP'-loxP-hygr-ΔyITRP2</i>	<i>pYLXP'-loxP-hygr</i> containing gene <i>yITRP2</i> deletion cassette	This work
<i>pYLXP'-loxP-hygr-ΔyITRP3</i>	<i>pYLXP'-loxP-hygr</i> containing gene <i>yITRP3</i> deletion cassette	This work
<i>pYLXP'-loxP-hygr-ΔyIARO8</i>	<i>pYLXP'-loxP-hygr</i> containing gene <i>yIARO8</i> deletion cassette	This work
<i>pYLXP'-loxP-hygr-ΔyIARO9</i>	<i>pYLXP'-loxP-hygr</i> containing gene <i>yIARO9</i> deletion	This work

	<i>cassette</i>	
pURLA-y/ARO2-y/ARO3-y/ARO4-y/ARO5-scARO4 ^{K229L} -aroG ^{S180F}	pURLA containing gene y/ARO2, y/ARO3, y/ARO4, y/ARO5, scARO4 ^{K229L} , and aroG ^{S180F}	This work
pURLK-y/ARO1	pURLK containing gene y/ARO1	This work
pYLXP'-y/TKT-y/PAR4-y/ARO10-y/PHA2-y/ARO7-ScARO7 ^{G141S}	pYLXP' containing gene y/TKT, y/PAR4, y/ARO10, y/PHA2, and y/ARO7	This work
pYLXP'-bbxfpK-y/PAR4-y/ARO10-y/PHA2-y/ARO7-ScARO7 ^{G141S}	pYLXP' containing gene bbxfpK, y/PAR4, y/ARO10, y/PHA2, and y/ARO7	This work
pYLXP'-acxpkA-y/PAR4-y/ARO10-y/PHA2-y/ARO7-ScARO7 ^{G141S}	pYLXP' containing gene acxpkA, y/PAR4, y/ARO10, y/PHA2, and y/ARO7	This work
prDNAloxP-y/TKT-bbxfpK-acxpk	prDNAloxP containing gene y/TKT, bbxfpK, and acxpk	This work
pYLXP'-loxP-ura-y/IPYK	pYLXP'-loxP-ura containing gene y/IPYK deletion cassette	This work
pYLXP'-loxP-hygr-y/ALD2	pYLXP'-loxP-hygr containing gene y/ALD2 deletion cassette	This work
pYLXP'-loxP-hygr-y/ALD3	pYLXP'-loxP-hygr containing gene y/ALD3 deletion cassette	This work
pYLXP'-loxP-hygr-y/HPD	pYLXP'-loxP-ura containing gene y/HPD deletion cassette	This work
pYLXP'-rgTAL-y/TYR1	pYLXP' containing gene rgTAL and y/TYR1	This work
pYLXP'-rgTAL-y/TYR1-VvSTS1-Pc4CL2	pYLXP' containing gene rgTAL, y/TYR1, VvSTS1, and Pc4CL2	This work
pYLXP'-loxP-hygr-y/PHA2	pYLXP'-loxP-hygr containing gene y/PHA2 deletion cassette	³
pYLXP'-VioDCBAE	pYLXP' containing gene VioA, VioB, VioC, VioD, and VioE	This work
pYLXP'-y/TRP2-y/TRP3-VioDCBAE	pYLXP' containing gene y/TRP2, y/TRP3, VioA, VioB, VioC, VioD, and VioE	This work
pYLXP'-loxP-hygr-y/ARO7	pYLXP'-loxP-hygr containing gene y/ARO7 deletion cassette	This work

Table 2. Primers used in this study

Primers	Sequence
TRP2_Dw-F	tagcgagacaataacggaggaTTGGAGAGTGTGAGCTCTCGTTC
TRP2_Dw-R	gttacatccttttatcagacataGAGTAGGAATGCTTCCGATGTACGC
TRP2_Up-F	ggcatccctaaatttgatgaaagATCCCATTGTTGGTTGATGCCC
TRP2_Up-R	taatgtatgctatacgaagttatGGTGGTGTAGTTCGGGGGTG
TRP3_Dw-F	gctagcgagacaataacggaggaAGGCGATGAAGATGCACTTCAT
TRP3_Dw-R	ttacatccttttatcagacataAATTAACAGGGTCACACGAGCTCT
TRP3_Up-F	gcacccctaaatttgatgaaagGCTGCCAGAGTGCAATTTCTCG
TRP3_Up-R	taatgtatgctatacgaagttatTGTGGAGTAAGTGAAGCCGTTGAG
TYP1_Dw-F	gctagcgagacaataacggaggaGACACACTTGCAGGTCTAAAAGTTCC
TYP1_Dw-R	ttacatccttttatcagacataCCTCCGAAGAGGCTCTCAAAATGA
TYP1_Up-F	ggcatccctaaatttgatgaaagGGACAGAGTGTCCAACAAGCCAAT
TYP1_Up-R	aatgtatgctatacgaagttatcGTTGTAGAGCGTGGCGAAAAGT
ALD3_Dw-F	gctagcgagacaataacggaggaAGGCCGTCCACATTAACCTGG
ALD3_Dw-R	gttacatccttttatcagacataCTGCTGCAACCAGCCCTACAAA
ALD3_Up-F	gcacccctaaatttgatgaaagCAAGAAGGGATAAAAATGGAAACTCGGTCT
ALD3_Up-R	taatgtatgctatacgaagttatACTTGCACTAGGTTAGCAGCGAC
ALD2_Dw-F	gctagcgagacaataacggaggaACGATGAGCGAACGAATCGTCT
ALD2_Dw-R	gttacatccttttatcagacataTCTGTTGGATTCTAGGGAAGTGTTCCTG
ALD2_Up-F	ggcatccctaaatttgatgaaagCCGCTCTCAAGTGTCTGAAAGTTGAAT
ALD2_Up-R	taatgtatgctatacgaagttatATATTTAGAGTTCGGGATAAAGTTCAATGT
ALD2_Cas-F	CCGCTCTCAAGTGTCTGAAAGTTGAAT
ALD2_Cas-R	TCTGTTGGATTCTAGGGAAGTGTTCCTG
TYP1_Cas-F	GGACAGAGTGTCCAACAAGCCAAT
TYP1_Cas-R	CTCCGAAGAGGCTCTCAAAATGA
TRP3_Cas-F	GCTGCCAGAGTGCAATTTCTCG
TRp3_Cas-R	AATTAACAGGGTCACACGAGCTCT
TRP2_Cas-F	ATCCCATTGTTGGTTGATGCCC
TRP2_Cas_R	GAGTAGGAATGCTTCCGATGTACGC
ALD3_Cas-F	CAAGAAGGGATAAAAATGGAAACTCGGTCT
ALD3_Cas-R	CTGCTGCAACCAGCCCTACAAA
TYP1_DwChk-R	AGGATAAGAAGGCCAAGGCTTCT
TYP1_UpChk-F	ATGGATCTAAACGCTGGCGTCT
TRP2_DwChk-R	CATTGATGCACGCATCATTCCC
TRP2_UpChk-F	AAGTAGTAGGACAAGGGGTTGGC
ALD2_DwChk-R	ACTCCTCTCTAGACTCCTCCTGTTCT
ALD2_UpChk-F	GTGTCTCCATCACATGACCACAATC
TRP3_DwChk-R	ACGGTAAACCTCACCTGATCCG
TRP3_UpChk-F	CTCTTCGACTGTTGGCTCTGTCTC
ALD3_DwChk-R	TAGCCTCGTTAATGCACCGAGT
ALD3_UpChk-F	GGGAATGCTCCATTGAGATGATGGA
ARO1_Ing_R(Ku70)	CCTAGtcctcggtattgtctcgggacacgggcatctcacttgc

ARO1_Int_F(ku70)	tagcatatacattatacgaagttattgaaagcctaggagcagacagagac
ku70-Dw-R(NotI)	acatccttttatcagacataggcggccgcAGTGAACGACCAAGACTAAAGGGTG
ku70_Up-F(NotI)	ggcatccctaaatttgatgaaaggcggccgcTGTACCATTTCTACCCGGGGTCTG
PHA2_DwChkR	AGAATACCTTGATTCTGGCCACCG
PHA2_UpChkF	TCTGGCCGAGTTCAAGCTCCA
PHA2_CasF	TACCATCACCGACCCAGAGACCA
PHA2_CasR	TGCCGCATGCCATTGAGCTA
PHA2_DwF	gctagcagacaataacggaggaCTTAGACGGTTCAGCGTTTCTGT
PHA2_DwR	tacatccttttatcagacataTGCCGCATGCCATTGAGCTA
PHA2_UpF	atccctaaatttgatgaaagTACCATCACCGACCCAGAGACCA
PHA2_UpR	atgtatgctatacgaagttatGATGTGTAATGTGTGTGATCAAGTGTGC
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ORI1001_R	tggatctaaggttcgtactcaacactcac
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HPD_CheckR	TATTCGCACGACACTGACATTTAAGGC
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HPD_CasR	CTTCCCCTCCTGCTCACCCC
HPD_Dw_F	cgagacaataacggaggaTCATCTCTAGAGACGAGGCGTGC
HPD_Dw_R	agcttgccctatgttacatccttttatcagacataCTTCCCCTCCTGCTCACCCC
HPD_Up_F	ccctaaatttgatgaaagCCGTACGTAAGAGACTGCCATAAGT
HPD_Up_R	atgtatatacgaagttatGTTGTTGGTGGTTATTTGTTGTGTGTCA
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EcAroG(L175D)_2R	ggacaggccatggaactagtcggtaccttaccgcgacgcgcttttac
EcAroG(L175G)_1R	ctgatgcatcttcgcggtgc
EcAroG(L175G)_2F	gcaccgcgaagatgcatcag
SaARO7(G141S)_2R	aggccatggaactagtcggtaccttactcttccaaccttcttagcaagtattccac
ScARO7(G141S)_1F	gcactttttgcagtactaaccgcaggatttcacaaaaccagaaactgttttaaatctac
ScARO7(G141S)_1R	aacagaagagaagttattcttatcatcaccatctc
ScARO7(G141S)_2F	agagatggtgatgataagaataacttcttctgtt
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EcAroG(S180F)_2F	tggcatcagggttttctgtccg
EcAroG(S180F)_2R	gggacaggccatggaactagtcggtaccttaccgcgacgcgcttttac
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ScARO4(K229L)_1R	accatgcaaagtaacacccatgaaat
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ScARO4(K229L)_2R	ggccatggaactagtcggtaccctatttcttgtaacttcttctttgtctgacagc
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ARO7_UpChkF	CTTCTGGTTTACTCCGATACGGGGA
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ARO7_CasR	TCTACCGAGGCATCATGCCAC
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ARO7_UpF	ctaaatttgatgaaagcgccgcCACACGCTTGATATATATTTATCAAGTTTTTTC
ARO7_UpR	aatgtatgctatacgaagtattTCCGGCGAATTTGGGCAGA
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Ace_UpF	gcataccctaaatttgatgaaagcctaggGCTATTCTTACGGTGTACAGTTACGAGCA
Ace_UpR	gtatgctatacgaagtattTGTGTGAGATGGGGTAGTACGGAA
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Lac_UpF	atccctaaatttgatgaaagcctaggCTTACGACGAGCACGCTTCTGAC
Lac_UpR	tatgctatacgaagtattGGTCGTAGTGGTTTGTGGAGGT
Ace_DwChkR	CTCCTTTGTTGGCGAAGATTCCG
Ace_UpChkF	GTTGTTTGACGGCGTTTGACAAG

yI TRP1_F	accagcacttttgcagtactaaccgcaggactttctctactcttcgacatgtctacat
yI TRP1_R	cgtggggacaggccatggaactagtcggtaccttaccctggcgttttgacaaac
NotI/SnaBI_R	cagatccactattggcctattacgtaggatctgctgcggtaaagctc
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Ace_UpChkF	GTTGTTTGACGGCGTTTGACAAG
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aroG_ChkF	tcaaaaatggcaccgcggtacga
scARO7_ChkR	ctcctactcaagctttgcaaacattctat
yI ARO7_ChkF	GATGTCATCAACAACCTGGCTCTTGATACTAA
RgTAL_ChkF	gtgtcgctgatcgatcaacacttc
VvSTS1_ChkR	ggaacttctgcagtaataatctcttgacgaatg
VioC_ChkR	cctggatcagcaccgacttgc
VioD_ChkF	ttcatgaccctgagccacgacc
yI TRP3_ChkF	tcggctacaacggagaggcc
yI TRP5_ChkR	gaggcttgccctccagtgtactttc

Table 3. Screening of high-performance strains

Strains	Clone numbers					
	1	2	3	4	5	6
2PE (mg/L)						
YL0	3.12	1.25	3.52	4.31	1.28	2.42
YL1	6.01	4.91	4.69	4.21	4.77	6.07
YL2	16.84	15.01	7.35	7.10	9.64	7.02
YL3	9.42	6.59	4.95	9.83	7.05	10.15
YL4	4.95	2.80	1.96	7.84	8.61	4.95
YL5	19.61	13.44	5.10	13.52	5.98	52.78
YL6	45.91	52.52	8.09	7.27	5.55	3.57
YL7	104.73	81.06	79.70	32.71	71.87	31.50
YL8	27.91	17.36	33.02	31.25	36.47	36.82
YL9	18.11	16.79	30.81	17.79	21.56	21.48
YL10	77.92	65.63	28.93	56.42	32.85	62.82
YL11	73.92	29.68	69.43	42.72	19.54	43.14
YL12	19.72	35.18	18.27	47.19	22.55	33.85
YL13	51.58	81.08	38.18	49.33	68.51	89.08
YL14	50.46	70.93	51.87	44.12	14.01	17.48
YL15	191.54	259.54	151.93	171.49	317.40	310.66
YL16	17.21	15.55	31.19	57.69	19.64	15.98
YL17	155.96	124.23	161.78	168.41	181.59	171.66
YL21	80.20	44.95	25.87	46.62	49.24	55.69
YL22	299.98	328.41	317.85	310.65	280.67	323.90
YL23	19.00	400.26	340.45	411.12	417.22	360.08
YL25	290.53	470.77	540.94	283.13	297.29	366.72
YL26	436.87	336.87	334.81	302.29	389.76	377.62
YL27	100.08	70.98	154.52	128.54	63.19	175.35
YL28	90.79	18.46	47.54	116.80	66.47	166.55
YL29	368.29	61.30	52.03	125.03	33.88	57.65
YL30	402.60	282.74	278.75	609.87	353.52	311.73
YL32	371.70	11.87	205.22	282.22	298.26	140.18
YL34	36.23	64.83	9.48	37.47	16.25	11.91
YL35	452.16	796.15	620.45	771.47	313.86	605.25
YL36	163.99	492.29	454.35	201.74	380.01	609.48
YL37	667.31	1156.35	853.28	418.57	247.00	768.40
YL38	741.23	102.09	939.73	1156.93	577.68	499.90
p-Coumaric acid (mg/L)						
YL39	61.23	121.14	153.39	218.98	81.33	214.99
YL40	21.27	6.28	4.93	189.55	236.40	323.41
Resveratrol (mg/L)						
YLRes	8.39	3.54	2.36	7.31	5.93	2.54
Violacein (mg/L)						

YL41	69.18	59.85	169.91	15.48	53.98	202.04
YL42	121.93	61.80	56.75	157.30	54.61	291.17
po1fkV	89.87	65.14	71	62	77.22	95.11

Data in red color indicates the highest production titer screened from the pooled yeast colonies.

Table 4 The comparison of 2PE and other aromatic production by *de novo* pathway in microorganisms

Strains	Substrate	Titer	Cultivation	Reference
2-PE production by <i>de novo</i> pathway in microorganisms				
<i>S. cerevisiae</i> BY4741	glucose	0.10 g/L	Shake flask	6
<i>S. cerevisiae</i> BY4741	glucose	0.41 g/L	Shake flask	7
<i>S. cerevisiae</i>	glucose	1.59 g/L	2-L Bioreactor	8
<i>E. coli</i> DG02	glucose	1.02 g/L	Shake flask	9
<i>E. coli</i> NST74	glucose	1.94 g/L	Shake flask	10
<i>E. coli</i> MG1655	glucose	0.18 g/L	Shake flask	11
<i>Enterobacter sp.</i> CGMCC 508	glucose	0.34 g/L	Shake flask	12
<i>E. coli</i> BP-42	glucose	0.29 g/L	Shake flask	13
<i>E. coli</i> BW25113(DE3)	glucose	0.94 g/L	Shake flask	14
YL25	glucose	2.43 g/L	Shake flask	This work
<i>p</i>-coumaric acid production by <i>de novo</i> pathway in microorganisms				
<i>S. cerevisiae</i>	glucose	12.5 g/L	1-L Bioreactor	15
<i>E. coli</i>	glucose	0.97 g/L	Shake flask	16
<i>S. cerevisiae</i> W303-1A	glucose	0.01 g/L	Shake flask	17
<i>S. cerevisiae</i>	glucose	1.93 g/L	96-deep well plate	18
YL40	glucose	0.59 g/L	Shake flask	This work
Violacein production by <i>de novo</i> pathway in microorganisms				
<i>Janthinobacterium lividum</i>	glycerol	1.83 g/L	2-L Bioreactor	19
<i>Chromobacterium violaceum</i>	-	0.15 g/L	Shake flask	20
<i>Y. lipolytica</i>	glucose	0.07 g/L	Shake flask	21
YL42	glucose	0.37 g/L	Shake flask	This work

Supplementary Methods

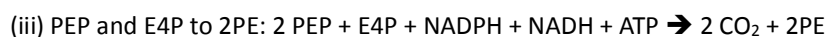
Integrative plasmid constructed in this work

Compared to *S. cerevisiae*, the genetic toolbox in *Y. lipolytica* is still less developed, due to the unclear genetic backgrounds and complexity of the non-homologous end joining mechanism⁵. To overcome these limitations, we constructed three genomic integration plasmids, namely pUrLA, pUrLK, and pHyLD, to assemble and deposit very long gene fragments to the chromosome. Plasmid maps and sequence for pUrLA, pUrLK, and pHyLD have been appended in this supplementary file.

Supplementary Notes

Note1. Deduction of the stoichiometry of 2PE biosynthesis and the theoretical yield calculation

For deducing the stoichiometry of 2PE biosynthesis by *de novo* pathway from glucose, we divided the 2PE synthesis pathway to three parts, including (i) glucose to PEP; (ii) glucose to E4P; and (iii) PEP and E4P to 2PE. According to the reactions annotated by KEGG (<https://www.kegg.jp/>), we got three stoichiometry equations:



Thus, the stoichiometry of 2PE biosynthesis by De Novo pathway from glucose is $(3.5 \text{ Glc} + 3 \text{ ATP} \rightarrow 5 \text{ CO}_2 + 2 \text{ 2PE})$.

Subsequently, we established a global stoichiometric model of 2PE biosynthesis and assumed that production of 1 mol of 2PE needs x mol of glucose. Thus, the overall stoichiometrics will be:



Thus, the yield of 2-PE (g/g_{glucose}) is

$$Y_{2-PE} = \frac{122}{180x} \quad (1)$$

Furthermore, to assess the carbon conversion efficiency, we introduced the respiratory quotient (RQ):

$$\text{RQ} = \frac{6x-8}{6x-10} \quad (2)$$

Thus, the yield of 2-PE could be solved as

$$Y_{2-PE} = \frac{122}{180x} = \frac{122}{180} \cdot \frac{3n-3}{5n-4} \quad (3)$$

As Shown in the metabolic model (SFigure1), the stoichiometrics (Eqn. 3) suggest that the theoretically maximum Y_{2-PE} is 0.4436 g/g_{glucose}.

Supplementary Figures

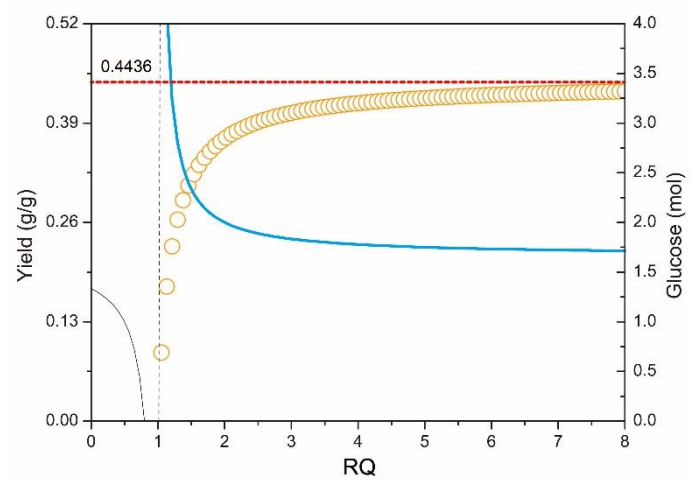


Figure S1. Mathematical models of 2-PE yield

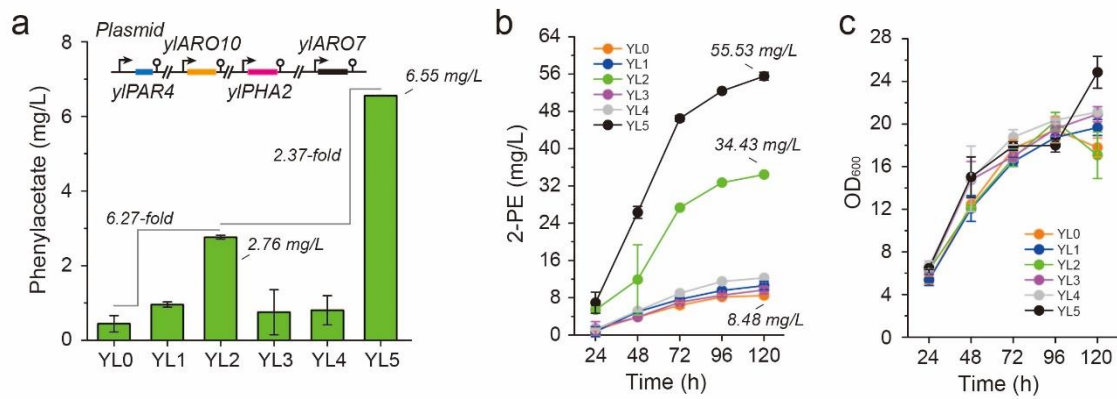


Figure S2. (a-c) Phenylacetate titer, time profiles of 2-PE titer and cell growth of strains carrying the 2-PE pathway, including genes *yIPAR4* (*YALI0D07062g*, encoding phenylacetaldehyde reductase), *yARO10* (*YALI0D06930g*, encoding phenylpyruvate decarboxylase), *yIPHA2* (*YALI0B17336g*, encoding prephenate dehydratase) and *yARO7* (*YALI0E17479g*, encoding chorismate mutase). All experiments were performed in triplicate and error bars represent standard deviations (SD).

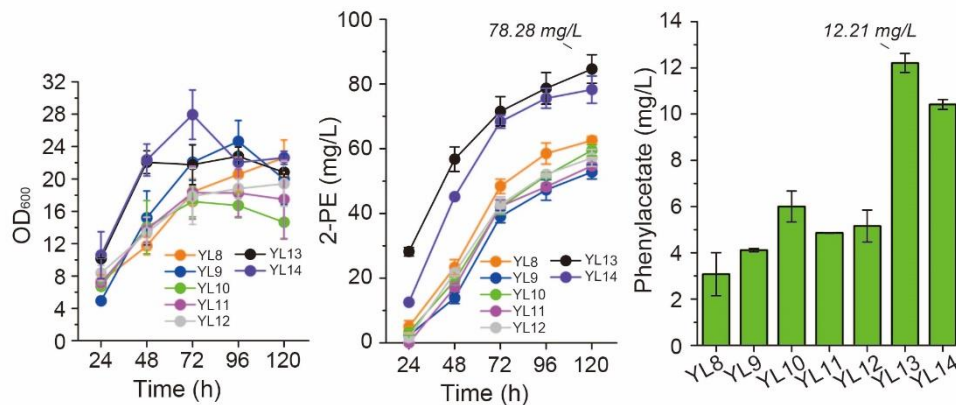


Figure S3. Phenylacetate, 2-PE and cell growth profile of strains overexpressing genes *yARO1* (*YALI0F12639g*, encoding pentafunctional protein), *yARO2* (*YALI0D17930g*, encoding bifunctional chorismate synthase), *yARO3* (*YALI0B20020g*, encoding DAHP synthase), *yARO4* (*YALI0B22440g*, encoding DAHP synthase), and *yARO5* (*YALI0C06952g*, encoding DAHP synthase); All experiments were performed in triplicate and error bars represent standard deviations (SD).

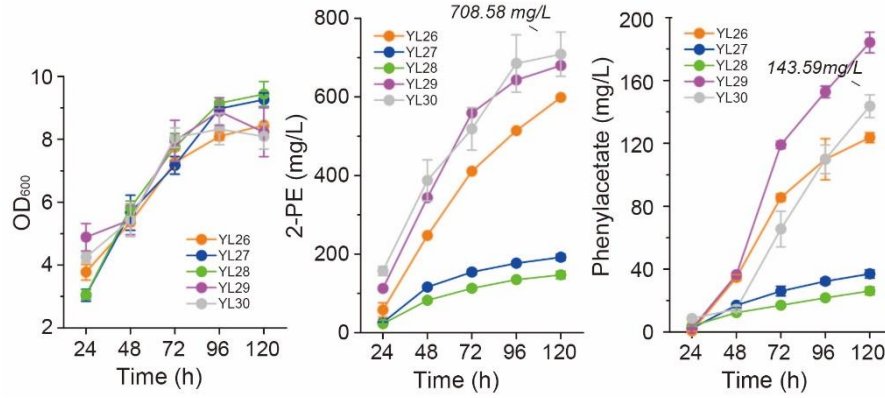


Figure S4. Time profiles of 2-PE, cell growth, and phenylacetate of strains overexpressing transketolase *yITKT*, phosphoketolases *BbxfpK* and *AcxpkA*; All experiments were performed in triplicate and error bars represent standard deviations (SD).

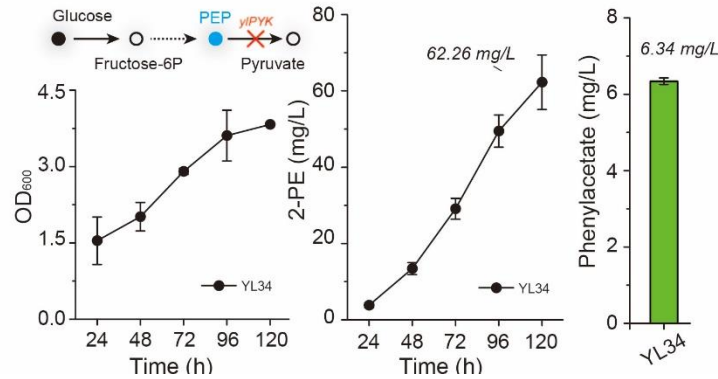


Figure S5. Time profile of 2-PE, cell growth, and phenylacetate of strains with pyruvate kinase *yIPYK* deletion in CSM medium with feeding of acetate. All experiments were performed in triplicate and error bars represent standard deviations (SD).

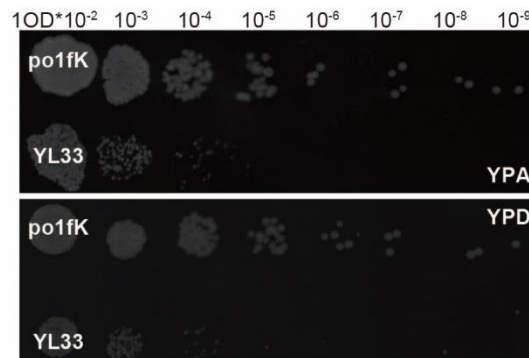


Figure S6. Cell growth on plate (24h) of *Y. lipolytica* with gene *yIPYK* deletion. *po1fK*, *po1fK Δku70::loxP*; *YL33*, *po1fK ΔyITYR1 ΔyITRP2 ΔyITRP3 ΔyIARO8 ΔyIARO9 ΔyIPYK yIARO1 yIARO2 yIARO3 yIARO4 yIARO5 scARO4K229L aroGS180F*

y/TKT bbxfpK acxpk::loxP; YPD medium, containing glucose 40.0 g/L, yeast extract 10.0 g/L, peptone 20.0 g/L; YPA medium, YPD with sodium acetate 5.0 g/L.

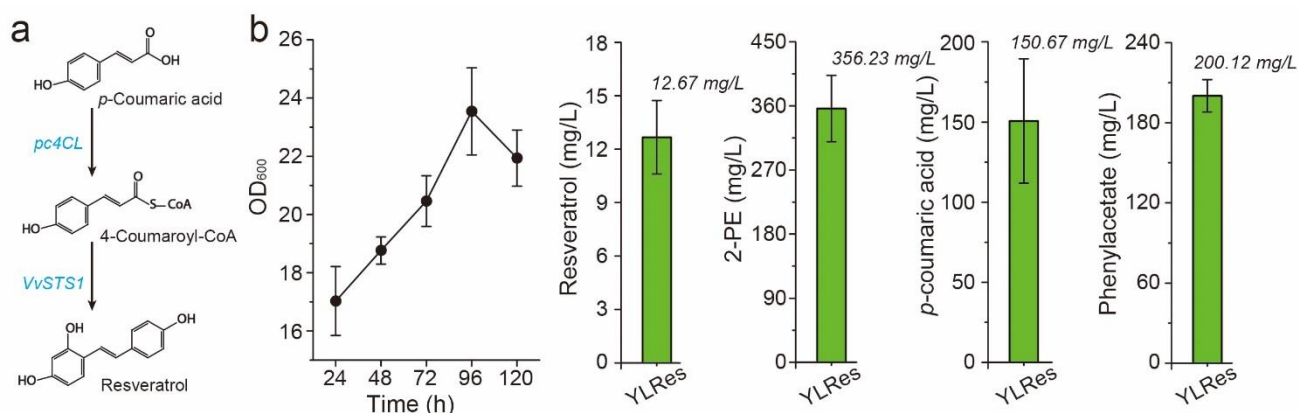


Figure S7. Resveratrol production by strain YLRes. (a) The catalytic steps form *p*-coumaric acid to resveratrol; *pc4CL*, 4-coumarate-CoA ligase from *Petroselinum crispum*; *VvSTS1*, resveratrol synthase from *Vitis vinifera*; (b) Cell growth, resveratrol titer, 2-PE titer, *p*-coumaric acid titer and phenylacetate titer of strain YLRes. All experiments were performed in triplicate and error bars show standard deviation (SD). All experiments were performed in triplicate and error bars represent standard deviations (SD).

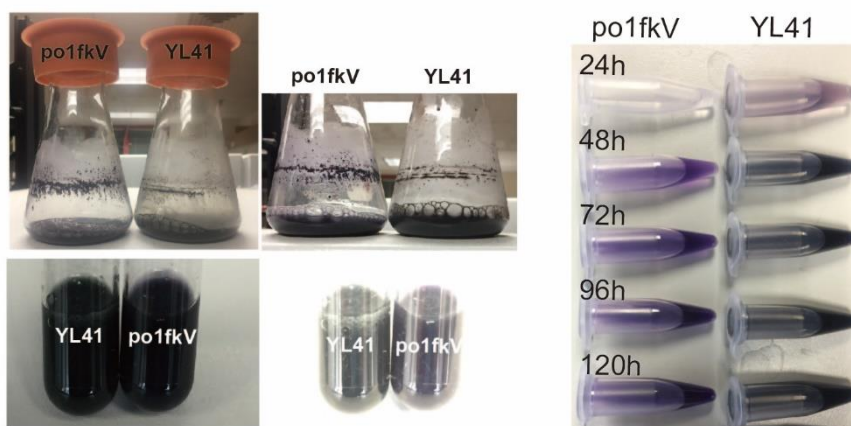


Figure S8. Shaking flask cultivation and extraction of violacein produced by strains YL41 and po1fkV. (a) Cell cultural of shaking flask cultivation. The extraction process is that 0.20 mL of fermentation culture was mixed with 5-fold volume of ethyl acetate and appropriate glass beads, vortexed at 30 °C for 24 h.

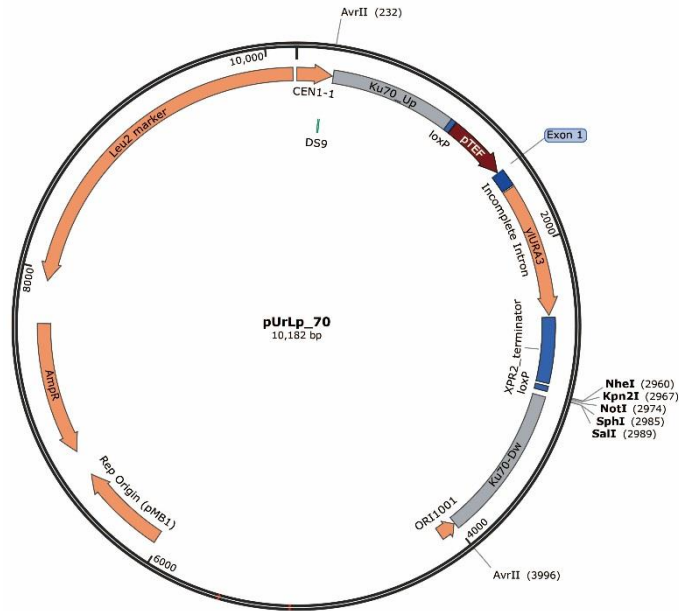


Figure S9. Plasmid pUrLp70 map. The multiple clone sites include *NheI*, *Kpn2I*, *NotI*, *SphI*, and *SallI*. After assembling the desired genes, the integration cassettes could be obtained by digested plasmid with enzyme *AvrII*.

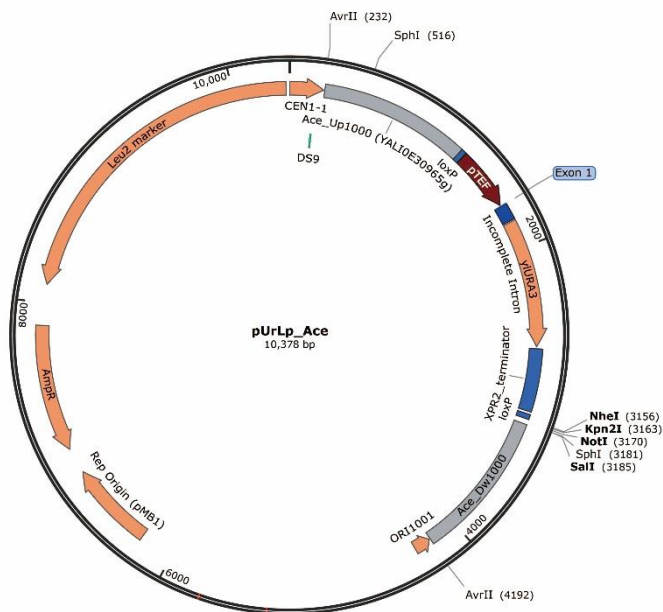


Figure S10. Plasmid pUrLpAce map. The multiple clone sites include *NheI*, *Kpn2I*, *NotI*, and *SallI*. After assembling the desired genes, the integration cassettes could be obtained by digested plasmid with enzyme *AvrII*.

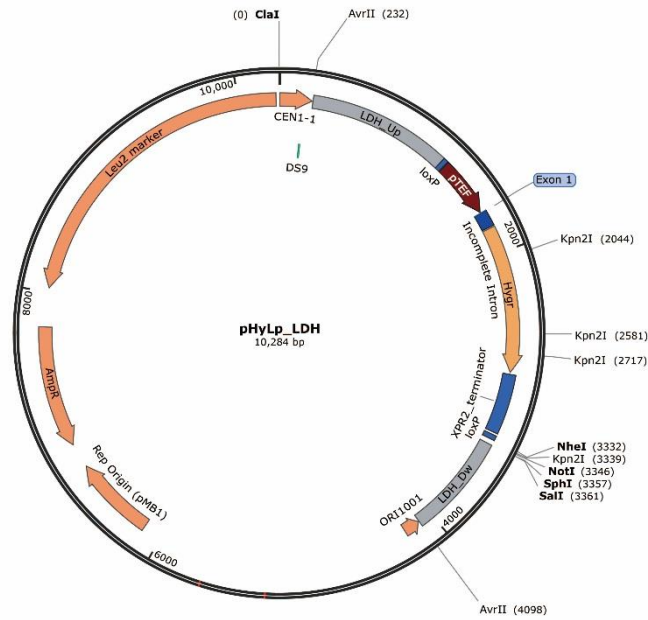


Figure S11. Plasmid pUrLDH map. The multiple clone sites include *NheI*, *NotI*, *SphI*, and *SalI*. After assembling the desired genes, the integration cassettes could be obtained by digested plasmid with enzyme *AvrII*.

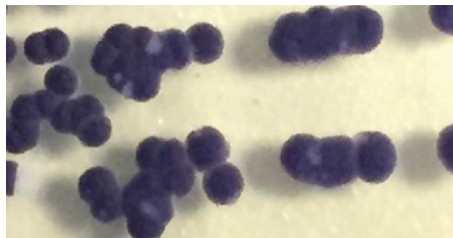


Figure S12. Yeast colonies (poOfkV) with integrating violacein pathway in genome

DNA Sequences

Plasmid pUrLA sequences:

```
1      CGATGCTTTT CGTAGATAAT GGAATACAAA TGGATATCCA GAGTATACAC ATGGATAGTA
61     TACACTGACA CGACAATTCT GTATCTCTTT ATGTAACTA CTGTGAGGCG TTAAATAGAG
121    CTTGATATAT AAAATGTTAC ATTTACACAGT CTGAACTTTT GCAGATTACC TAATTTGGTA
181    AGATATTAAT TATGAACTGA AAGTTGATGG CATCCCTAAA TTTGATGAAA GCCTAGGGAG
241    AACTGCTCCT GTGAATCTCT TAACGAACAC AGTCGCTCAA CCAATGTTGG TGATGAAGAT
301    GTCAAAAACA ACGCGGAGCC TACCCCGGAA GTGGGGGGTC AAACCTGAGA CAGATCGCGT
361    ACCGCTGCA TACCTGCAAT GCAACCATCA TGCTACTTGT AGTGTTGCAG GGCCCGATGT
421    TCCACCGAAG CATTTTCATTG GTGGATCACC CACTAGTTTG AACTGGTATG ATCTCTGTTT
481    TTGTTTTGTC AATTGCATAA CCATGTTGGA GGGCCATGTT TATTTACCCC CCACGCCCTT
541    GCTCTCACAG CTATTTTTC GCCCGTGTCT CACACCGTCG GGGTGGTTTT AGTTTGTGAT
601    TCAATTACTG CTGACCGGCG TGTTCTGCCC CACTCGCTAC TCAACACACC CACCGCCACT
661    GCTACACTGC GCCACTAGTC TGACGGATTT GCTTGCAATC CTCCAATATG TAGCTTACAA
721    CACGTTTTTA AGCGCCGTCA CATATAATTA ATTTAATCTG CATTTTTCTA TCTCTCGCTT
781    GCCCGTAGTA TTACGTGAAT CAACTAGAAC ATATGGGGAG CTTCTGTTGC TGTTTCTCCA
841    ACTGCAATTA TGTCTACTAC AAGTAGTATA TATTTGACCA ACCGGTTATT GAACCCTACT
901    GGCTGTATTC TGGGGGAATA CATTGCATAG ATATGCACGT AAGTGGGGGT GTCTTTCTCG
961    CGCCATTGTA CTAAGAATGA CCCCCACGTC TCATTCTGGG GCAACCAGAG TCACAGCGAA
1021   GGGATATATA GCTCAAGCTA GTCTTTAATA CACTTCCTCT TTTCTGACAT TTGATCTTTC
1081   ACAACCGTCC TCGCTGAGCG CTTTGTACTT ACTGTAAGGG ACTCCTCTCT GTGCAACTCT
1141   ATTACTCACT CCGCAAGCCA CGAATCTACA ACTACCGATA CTCTGAATTG GCATAGGGTC
1201   TCTTTCCATT CTTGATACAT TCACACATCC AGTTTCGCCA TAACTTCGTA TAGCATACAT
1261   TATACGAAGT TATGACGACA GAGACCGGGT TGGCGGCGCA TTTGTGTCCC AAAAAACAGC
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1861   GTTCTGATC GAAAAGTTCG ACAGCGTCTC CGACCTGATG CAGCTCTCGG AGGGCGAAGA
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2401   CCTCGTGCAC GCGGATTTTC GCTCCAACAA TGTCTTGACG GACAATGGCC GCATAACAGC
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10081 TAAACCTCCG AAGATTGTGA CTCAGGTAGT GCGGTATCGG CTAGGGACCC AAACCTTGTC
10141 GATGCCGATA GCGCTATCGA ACGTACCCAG CCGGCCGGGA GTATGTCGGA GGGGACATAC
10201 GAGATCGTCA AGGGTTTGTG GCCAACTGGT AAATAAATGA TGAATCAGGC GACGACGGAA
10261 TTCTCATGTT TGACAGCTTA TCAT

Plasmid pUrLK sequences:

1 CGATGCTTTT CGTAGATAAT GGAATACAAA TGGATATCCA GAGTATACAC ATGGATAGTA
61 TACACTGACA CGACAATTCT GTATCTCTTT ATGTAACTA CTGTGAGGCG TTAAATAGAG
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Plasmid pUrLA sequences:

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