# Supplemental Material

## A toolkit for rapid CRISPR-*Sp*Cas9 assisted construction of hexose-transport-deficient *Saccharomyces cerevisiae* strains

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# Supplemental Material 1

## List of primers used in this study

| Primer nr | Plasmid | Sequence 5'--> 3' | Purpose |
|---|---|---|---|
| **Plasmid construction** | | | |
| **6005** | pROS10/pROS14/pROS16 | GATCATTTATCTTTCACTGCGGAGAAG | Amplification of pROS backbone |
| | | | |
| **9572** | sgRNA 7 (target *HXT9,11, 12*) | TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATAAATGATCGGGGCAATCAACTTTTACTAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG | sgRNA |
| **9573** | sgRNA 5 (target *HXT13,15.16*) | TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATAAATGATCTGGTCATATTTGCAAGTATCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG | sgRNA |
| **9574** | sgRNA 6 (target *HXT2)* | TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATAAATGATCTTTTCTTCTTCGCTATTAGTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG | sgRNA |
| **9575** | sgRNA 3 (target *HXT8*) | TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATAAATGATCGAAATAATCACATAACATACGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG | sgRNA |
| **9576** | sgRNA 8 (target *HXT10*) | TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATAAATGATCTTACTATCAACAATAACTAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG | sgRNA |
| **9577** | sgRNA 4 (target *HXT14*) | TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATAAATGATCAACACATTTGAATTTATTATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG | sgRNA |
| **9579** | sgRNA 9 (target *MPH2,3*) | TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATAAATGATCAAAGTACAGTATAAAAGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG | sgRNA |
| **9585** | sgRNA 10 (target MAL11) | TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATAAATGATCTGTAATATCTTAATGCTAAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG | sgRNA |
| **10513** | sgRNA 2 (target *GAL2*) | TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATAAATGATCTTATTTATGTGAGGTGAATAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG | sgRNA |
| **11754** | sgRNA 1 (target HXT1,3-7) | TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATAAATGATCGATAACTATTTCTTCTACTAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG | sgRNA |
| **13616** | sgRNA 11 (target STL1) | TGCGCATGTTTCGGCGTTCGAAACTTCTCCGCAGTGAAAGATAAATGATCATGTTCACCCCAATATTTATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG | sgRNA |
| **Plasmid confirmation** | | | |
| **4034** | For B intergenic | CACCTTTCGAGAGGACGATG | Primer A |
| **5941** | LP crRNA rv | GCTGGCCTTTTGCTCACATG | Primer B |
| **11662** | sgRNA 7check | TAGTAAAAGTTGATTGCCCC | |
| **11663** | sgRNA 5 check | GATACTTGCAAATATGACCA | |
| **11664** | sgRNA 6 check | CACTAATAGCGAAGAAGAAAAG | |
| **11665** | sgRNA 3 check | CGTATGTTATGTGATTATTTCG | |
| **11666** | sgRNA 8 check | CTTAGTTATTGTTGATAGTAAG | |
| **11667** | sgRNA 4 check | CATAATAAATTCAAATGTGTTG | |
| **11668** | sgRNA 2 check | TCAACAAACGTTGAAATACT | |
| **11669** | sgRNA 9 check | CTCACTTTTATACTGTACTTTG | |
| **11670** | sgRNA 10 check | CTTTAGCATTAAGATATTACAG | |
| **11756** | sgRNA 1 check | CTAGTAGAAGAAATAGTTATCG | |

| | | | |
|---|---|---|---|
| **Sanger Sequencing primers** | | | |
| **2758** | | GTTCACGTCGCACCTATATCTG | Confirmation of CRISPR plasmid |
| **6097** | | GGCTATACCTATCCGTCTACG | Confirmation of CRISPR plasmid |
| **8556** | | ATCAGATCCACTAGTGGC | Confirmation of CRISPR plasmid |
| **2918** | | ATGAAGCACAGATTCTTCGTTG | Confirmation of CRISPR plasmid |
| **Repair oligonucleotide** | | | |
| **9522** | HXT1,4,5 FW | CCTACTTTTTTCCGAACATCTTCTTGTAAAATGGTTGATCATCATGCATTAGATCATCAGTCTTTCTTTACCTCTAATATATTCTTTTTTCTAGAAAAATAATATTTTTTGCTGGTTTTA | |
| **9523** | HXT1,4,5 RV | TAAAACCAGCAAAAAATATTATTTTTCTAGAAAAAAGAATATATTAGAGGTAAAGAAAGACTGATGATCTAATGCATGATGATCAACCATTTTACAAGAAGATGTTCGGAAAAAAGTAGG | |
| **9525** | HXT3,6,7 FW | AGCGGGATACAGAAAAAGAAGATATTTCCCATTTCAAAAAGGCTCTACTATATCCTTACCAAGACTCTAGGGGGATCGCC | Amplification of *ARS4* from pRS416 |
| **9526** | HXT3,6,7 RV | GGTCATTATACTGACCGCCATTATAATGACTGTACAACGACCTTCTGGAGAAAGAAACAAGCGCCTCGTATCTTTTAATG | Amplification of *ARS4* from pRS416 |
| **9528** | HXT2 FW | TTACTATCAAGATACCGTAGAAAAGAAAAAGAACCGGGGATGAATAATAACAAAACGGGCGAGATTATACTTAAACTAGCACTGATTTTTTTAAGGCTAATGGCTACTAATACTTTAATA | |
| **9529** | HXT2 RV | TATTAAAGTATTAGTAGCCATTAGCCTTAAAAAAATCAGTGCTAGTTTAAGTATAATCTCGCCCGTTTTGTTATTATTCATCCCCGGTTCTTTTTCTTTTCTACGGTATCTTGATAGTAA | |
| **9532** | HXT8 FW | TATTTTAGATTGCATTTTTTATAAGTCACTTTTAGTTAGCTCAGAACGCCAGCAAAAACCCCCGTCAGGACTACCAAAATATAACAAAGTATTGATTATCGTCAACGCTTTATAGATCAC | |
| **9533** | HXT8 RV | GTGATCTATAAAGCGTTGACGATAATCAATACTTTGTTATATTTTGGTAGTCCTGACGGGGGTTTTTGCTGGCGTTCTGAGCTAACTAAAAGTGACTTATAAAAAATGCAATCTAAAATA | |
| **9535** | HXT9 FW | TTGGAAAAAATAATCATTGCACAATTGAGTACTAAAAGCTTTCGTATCTTACCCAATATCTCTTCCGGTTTTTAGTAACTGGAAAAAAATACATGAACTTAATTATTTATTATATACAAT | |
| **9536** | HXT9 RV | ATTGTATATAATAAATAATTAAGTTCATGTATTTTTTTCCAGTTACTAAAAACCGGAAGAGATATTGGGTAAGATACGAAAGCTTTTAGTACTCAATTGTGCAATGATTATTTTTTCCAA | |
| **9538** | HXT10 FW | TCATCAATTTCGGCTGTCTGGATGGCGACAACGAGAGGAAGCTTGCTTGCCATGATAGGAATCAGGCAATGTAAGGTCAAACTTGTACCATCGACATATATAATGCTTTGAGATATAAGT | |
| **9539** | HXT10 RV | ACTTATATCTCAAAGCATTATATATGTCGATGGTACAAGTTTGACCTTACATTGCCTGATTCCTATCATGGCAAGCAAGCTTCCTCTCGTTGTCGCCATCCAGACAGCCGAAATTGATGA | |
| **9542** | HXT11 FW | TTACATAATCATTTCTCCGTGCGAGTTATAAGTGCTTTTCTCGTGCATCCCTTGGCACTTTCTTCTAGTTTTCGGTAAATTGGTAAAAAAGCAAAAAAAAAAAATTGACGAAACTAATTCT | Also used to repair *HXT12* |
| **9543** | HXT11 RV | AGAATTAGTTTCGTCAATTTTTTTTTTTTGCTTTTTTACCAATTTACCGAAAACTAGAAGAAAGTGCCAAGGGATGCACGAGAAAAGCACTTATAACTCGCACGGAGAAATGATTATGTAA | Also used to repair *HXT12* |
| **9547** | HXT13 FW | CCTTTTACCAATTTAATAATGTCTAGGATTTATCGCTGTACTGCCAAATGCTTCACAACGGAGTGCATATGTTTGTCTTGATAGGCAACATTGTATATTATAGTTTACATAATAATGTGT | |
| **9548** | HXT13 RV | ACACATTATTATGTAAACTATAATATACAATGTTGCCTATCAAGACAAACATATGCACTCCGTTGTGAAGCATTTGGCAGTACAGCGATAAATCCTAGACATTATTAAATTGGTAAAAGG | |
| **9551** | HXT14 FW | ACTAACAAAGAATTTGTTAACGTATTCTTAGGAAGTAAAGTACTTCAATTATTGCTAGAACAGTAACTAAGTGGCCGTGAGGACTGACCACATTTTCTTTACATACAGTAAAGTAAATAA | |
| **9552** | HXT14 RV | TTATTTACTTTACTGTATGTAAAGAAAATGTGGTCAGTCCTCACGGCCACTTAGTTACTGTTCTAGCAATAATTGAAGTACTTTACTTCCTAAGAATACGTTAACAAATTCTTTGTTAGT | |
| **9555** | HXT15,16 FW | GATTCCACCTTAAAAGACGACAATAGTAACTTTGTCCTTGATCTGGGTTACTAAATCAGCACATAGGTCCACTTGAGTGCTTATGGAAGATCCTTAAAAATGCATTTCCAGGAACGTAAC | |

|  |  |  |  |
|---|---|---|---|
| **9556** | HXT15,16 RV | GTTACGTTCCTGGAAATGCATTTTTAAGGATCTTCCATAAGCACTCAAGTGGACCTATGTGCTGATTTAGTAACCCAGATCAAGGACAAAGTTACTATTGTCGTCTTTTAAGGTGGAATC |  |
| **9563** | GAL2 FW | GGATTGAAAATTTGGTGTTGTGAATTGCTCTTCATTATGCACCTTATTCAATTATCATCAGATAACATGCTCTGCCATCCTTTGTTCACCGAGCAAAATTAAAAACGCAAAATGAATTGT |  |
| **9564** | GAL2 RV | ACAATTCATTTTGCGTTTTTAATTTTGCTCGGTGAACAAAGGATGGCAGAGCATGTTATCTGATGATAATTGAATAAGGTGCATAATGAAGAGCAATTCACAACACCAAATTTTCAATCC |  |
| **9565** | MPH2,3 FW | CATCATATGTTGAAAAATCAAGCTGGAATTTATTGGTGGATCTTTCATCTCCATTTATACTCATAGTAACAGTCTGGGCACTCACTTCGTACATCCCTTATACATTTAAGTGCAATTTTA |  |
| **9566** | MPH2,3 RV | TAAAATTGCACTTAAATGTATAAGGGATGTACGAAGTGAGTGCCCAGACTGTTACTATGAGTATAAATGGAGATGAAAGATCCACCAATAAATTCCAGCTTGATTTTTCAACATATGATG |  |
| **9569** | MAL11 (AGT1) FW | AGTAATCAAGAGTGTACCCAACTTTAACGAACTCAGTAAAAAATAAGGAATGTCGACATCTGCCAGGTCAACCGTATTCTCAATTAACTGTTACTTTAAATGTCCATACACTTAATAAAA |  |
| **9570** | MAL11 (AGT1) RV | TTTTATTAAGTGTATGGACATTTAAAGTAACAGTTAATTGAGAATACGGTTGACCTGGCAGATGTCGACATTCCTTATTTTTTACTGAGTTCGTTAAAGTTGGGTACACTCTTGATTAC |  |
| **13617** | STL1 FW | CTAGCATACAAGTTAGAATAAATAAAAAATAGAAAAATAGAACATAGAAAGTTTTAGACCTTCATACACAACTCACAAATCATATTTTGTAATTTACTTAAGAAAGCATTTTGCGGGGTT |  |
| **13618** | STL1 RV | AACCCCGCAAAATGCTTTCTTAAGTAAATTACAAAATATGATTTGTGAGTTGTGTATGAAGGTCTAAAACTTTCTATGTTCTATTTTTCTATTTTTTATTTATTCTAACTTGTATGCTAG |  |
| **Diagnostic primers** |  |  |  |
| **5198** | E-FW | TGTCGAACGAGGGCATGAAG | *HXT1,4,5* outside coding region FW |
| **9524** | Hxt1,4,5_dg rv | ATGCTAGTCGAACGGTTCTC | *HXT1,4,5* outside coding region RV |
| **9586** | gRNA_Hxt1,3-7_ check fw | TTTAACATTGGTTGTGCCAT | *HXT1,4,5* inside coding region RV |
| **9527** | Hxt3,6,7_dg fw | CCTTTGGATTGGCACCTTTG | *HXT3,6,7* outside coding region FW |
| **9221** | HXT3_rv_3 | GACTGTACAACGACCTTCTG | *HXT3,6,7* outside coding region RV |
| **9231** | HXT7_rv_1 | CCCACCATCTTTCGAGATCC | *HXT3,6,7* inside coding region RV |
| **9530** | Hxt2_dg fw | ACAGGTCAGTTAAGGCACAG | *HXT2* outside coding region FW |
| **9531** | Hxt2_dg rv | ACGCCATTGATGCTTGAAGG | *HXT2* outside coding region RV |
| **8887** | fw_GAL2_5 | GTCTTCATGGGCTGTTTG | *HXT2* inside coding region FW |
| **9072** | SeqR_ima5_block5 | CTCCGTCTAGGCATTTACGCTTTC | *HXT8* outside coding region FW |
| **9534** | Hxt8_dg rv | GGGAAATTGCCAAGGGAATC | *HXT8* outside coding region RV |
| **9063** | SeqF_ima5_block1 | CAGCCAATGGCGCTTCCCAC | *HXT8* inside coding region RV |
| **9062** | SeqR_ima3_block5 | GAGCGTAAACAACCCTCGATCC | *HXT9* and *HXT12* outside coding region FW |
| **9537** | dg rv | TCTGTTCACCCTGTCAACTC | *HXT9* outside coding region RV |
| **9589** | gRNA_Hxt9,11,12_ check fw | GGGGCAATCAACTTTTACTA | *HXT9* inside coding region RV |
| **9540** | Hxt10_dg fw | CCCATACGCAAATAAACAGT | *HXT10* outside coding region FW |
| **9541** | Hxt10_dg rv | ACTGCGCCTCTCAATCTATG | *HXT10* outside coding region RV |
| **11661** | HXT1,3-7 check RV | TTTGGCCATAATCTAGTGAC | *HXT10* inside coding region RV |
| **9061** | SeqR_ima2_block5 | GTAGTCCCACGACAAAGTACG | *HXT11* outside coding region FW |
| **9546** | Hxt12_dg rv | ATTCCGTGGATCGCCATGTG | *HXT11* and *HXT12* outside coding region RV |
| **9047** | SeqF_ima2_block1 | GCGGATGTATTATTAACACCTGAC | *HXT11* inside coding region RV |
| **9049** | SeqF_ima3_block1 | ACCGGACATGATATTGGGTAAG | *HXT12* inside coding region RV |
| **9549** | Hxt13_dg fw | TGGTACGAGCTCTGGTTGTC | *HXT13* outside coding region FW |
| **9550** | Hxt13_dg rv | GTTAAGGGCGGTAAAGATCC | *HXT13* outside coding region RV |
| **9591** | gRNA_Hxt13,15-17_ check fw | TGGTCATATTTGCAAGTATC | *HXT13* inside coding region FW |

|  |  |  |  |
|---|---|---|---|
| **9553** | Hxt14_dg fw | TCGGTAATCCATGTCGTCTG | *HXT14* outside coding region FW |
| **9554** | HXT15_dg rv | ACCCTTCCACATGAGGAAACTG | *HXT14* outside coding region RV |
| **9557** | Hxt15,16_dg fw | TTGTGGAGTATTGCCAGAGG | *HXT15* and *HXT16* outside coding region FW |
| **10315** | Hxt15,16 del check RV | CGGTTACTACCACAGTTAGG | *HXT15* and *HXT16* outside coding region RV |
| **10937** | pTDH3_HXT15_inside_fw | ACGGAGTGGTGATGAAGATG | *HXT15* and *HXT16* inside coding region FW |
| **9567** | Mph2,3_dg fw | TCCAGAGGTTATTGTGGGAG | *MPH2* and *MPH3* outside coding region FW |
| **9568** | Mph2,3_dg rv | GAGCAACTCTCCCGTATATG | *MPH2* and *MPH3* outside coding region RV |
| **1243** | MPH2/MPH3 Probe RV | GTCTTTCCGGCAGTTTCTGGTAGG | *MPH2* and *MPH3* inside coding region RV |
| **1470** | AGT1-KO upstream | GCGAGTTGCAAGAATCTCTACG | *MAL11* outside coding region FW |
| **1471** | AGT1-KO downstream | GATGACGACCACATGGGTTTG | *MAL11* outside coding region RV |
| **4848** | Mal11 D123N rv | GTAGTAGACACTAATATGGACCACAGG | *MAL11* inside coding region RV |
| **13619** | STL1 dg fw | TGGCTGCTTCGCGATAGTAG | *STL1* outside coding region FW |
| **13620** | STL1 dg rv | GGTTTTTGCGGTGTCCCATG | *STL1* outside coding region RV |

## Supplemental material 2

### PCR confirmation of the deletion of the hexose transporter genes

| Gene(s) | Forward primer | Reverse primer | Size gene out[1] (bp) | Size gene in[2] (bp) |
|---|---|---|---|---|
| ***MPH2, MPH3*** | 9567 | 9568 | 565 | 3407 |
| ***MAL11*** | 1472 | 1471 | 1115 | 3327 |
| ***GAL2*** | 8883 | 8892 | 281 | 2292 |
| ***HXT1, HXT4, HXT5*** | 5198 | 9524 | 661 | 9914 |
| ***HXT3, HXT6, HXT7*** | 9527 | 9221 | 926 | 11546 |
| ***HXT2*** | 9530 | 9531 | 720 | 3086 |
| ***HXT8*** | 9072 | 9534 | 902 | 2903 |
| ***HXT9*** | 9062 | 9537 | 742 | 2446 |
| ***HXT10*** | 9540 | 9541 | 499 | 3189 |
| ***HXT11*** | 9061 | 9546 | 917 | 3061 |
| ***HXT12*** | 9062 | 9546 | 872 | 3037 |
| ***HXT13*** | 9549 | 9550 | 680 | 3192 |
| ***HXT14*** | 9553 | 9554 | 681 | 2369 |
| ***HXT15, HXT16*** | 9557 | 10315 | 604 | 3377 |
| ***STL1*** | 13619 | 13920 | 768 | 2478 |

**Table S2.1** – Primer pairs used for diagnostic PCR and expected size of the amplicons if the targeted genes are deleted or left intact. Primers bind outside of the deleted region.

[1] Expected size of the amplicon if the gene is correctly deleted

[2] Expected size of the amplicon in the control strain, without deletion

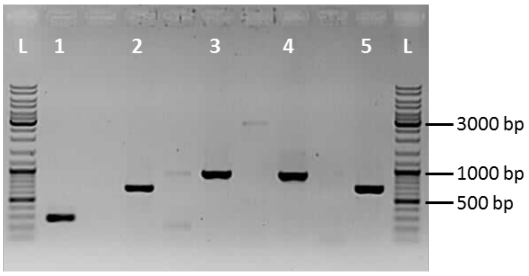
### IMX1521

**Table S2.2**

| Lane on gel | Gene(s) |
|---|---|
| **1** | *GAL2* |
| **2** | *HXT1,4,5* |
| **3** | *HXT3,6,7* |
| **4** | *HXT8* |
| **5** | *HXT14* |

**Figure S2.1**

Confirmation of hexose transporter genes deletions by PCR in strain IMX1521. The lanes marked with L contain GeneRuler DNA Ladder Mix. The content of each lane is described in Table S2.2. The primers used for the PCR and expected band sizes are listed in Table S2.1. All bands displayed the expected size.



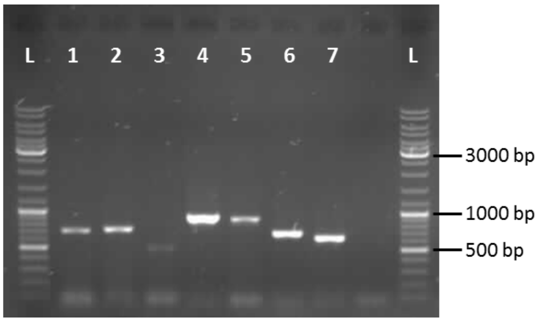
## IMX1541

### Table S2.3

| Lane on gel | Gene(s) |
|---|---|
| **1** | *HXT2* |
| **2** | *HXT9* |
| **3** | *HXT10* |
| **4** | *HXT11* |
| **5** | *HXT12* |
| **6** | *HXT13* |
| **7** | *HXT15,16* |

**Figure S2.2**

Confirmation of hexose transporter genes deletions by PCR in strain IMX1541. The lanes marked with L contain GeneRuler DNA Ladder Mix. The content of each lane is described in Table S2.3. The primers used for the PCR and expected band sizes are listed in Table S2.1. All bands displayed the expected size.



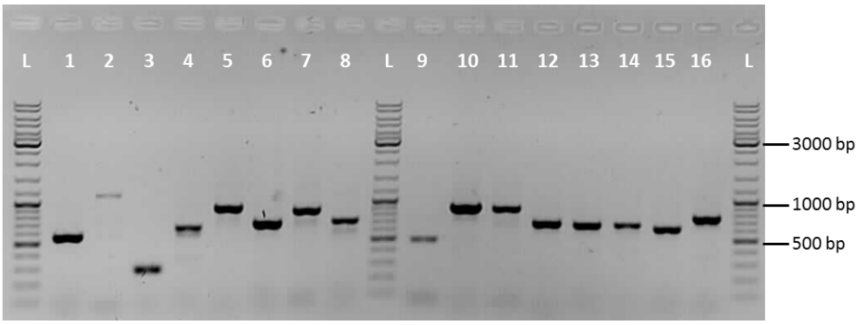
## IMX1812

### Table S2.4

| Lane on gel | Gene(s) |
|---|---|
| **1** | *MPH2,3* |
| **2** | *MAL11* |
| **3** | *GAL2* |
| **4** | *HXT1,4,5* |
| **5** | *HXT3,6,7* |
| **6** | *HXT2* |
| **7** | *HXT8* |
| **8** | *HXT9* |
| **9** | *HXT10* |
| **10** | *HXT11* |
| **11** | *HXT12* |
| **12** | *HXT13* |
| **13, 14** | *HXT14* |
| **15** | *HXT15,16* |
| **16** | *STL1* |

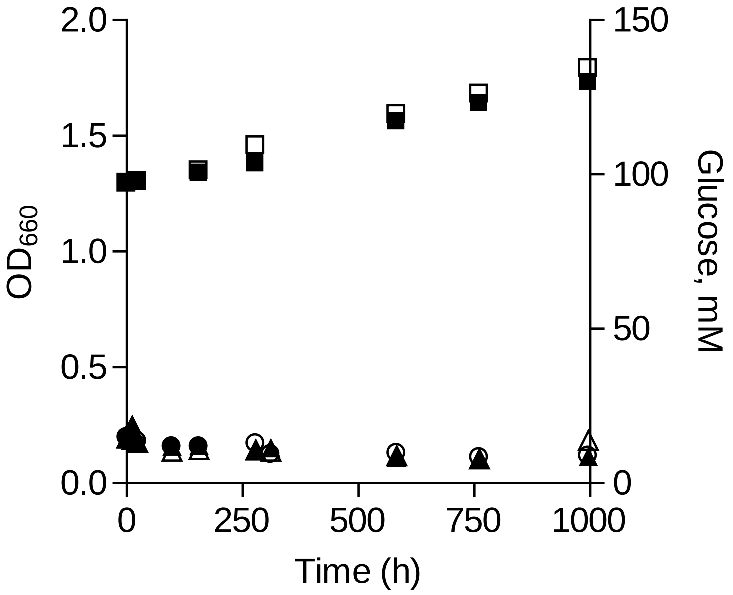
**Figure S2.3**

Confirmation of hexose transporter genes deletions by PCR in strain IMX1812. The lanes marked with L contain GeneRuler DNA Ladder Mix. The content of each lane is described in Table S2.4. The primers used for the PCR and expected band sizes are listed in Table S2.1. All bands displayed the expected size.



## Supplemental material 3

**Phenotypic characterization of IMX1812 and EBY.VW4000 during growth with glucose as sole carbon source.**



Optical density was monitored as well as glucose and ethanol concentration during aerobic growth in shake flasks with chemically defined medium supplemented with 2% glucose. Filled and empty symbols represent IMX1812 and EBY.VW4000 respectively. Glucose is represented by square symboles, while rectangles and circles represent OD. Two independent culture replicates are shown for each strain for OD measurements, a single culture is shown for glucose concentration. Increase in glucose concentration resulted from water evaporation over time. Ethanol was not detected throughout the cultivation.

## Supplemental material 4

### Expected impact of hexose transporter genes deletion on chromosome size

| Chr. | Deleted genes | Expected chromosome size (bp) | | | | | Expected size difference between IMX672 and IMX1812 |
|---|---|---|---|---|---|---|---|
| | | CEN.PK113-7D | IMX672 | IMX1521 | IMX1541 | IMX1812 | |
| **IV** | *HXT3-6-7*<br>*HXT15*<br>*STL1, MPH2* | 1504372 | 1504372 | **1493752** | **1490979** | **1486427** | -17945 |
| **VII** | *MAL11* | 1123019 | 1123019 | 1123019 | 1123019 | **1120807** | -2212 |
| **XV** | *HXT11* | 1085503 | **1085316** | 1085316 | **1083172** | 1083172 | -2144 |
| **XII** | *GAL2* | 1032974 | 1032974 | **1030963** | | | -2011 |
| **XVI** | | 950369 | 950369 | 950369 | | | |
| **XIII** | *HXT2* | 912802 | 912802 | 912802 | **910436** | 910436 | -2366 |
| **II** | | 806478 | 806478 | 806478 | | | 0 |
| **XIV** | *HXT14* | 765137 | 765137 | **763449** | | | -1688 |
| **X** | *HXT8*<br>*HXT16, HXT9*<br>*MPH3* | 727362 | 727362 | **725362** | **720885** | **718043** | -9319 |
| **XI** | | 678882 | 678882 | 678882 | | | 0 |
| **V** | *HXT13* | 577587 | **582226** | 582226 | **579726** | 579726 | -2500 |
| **VIII** | *HXT1-4-5* | 550966 | 550966 | **541713** | | | -9253 |
| **IX** | *HXT12* | 440269 | 440269 | 440269 | **438125** | 438126 | -2144 |
| **III** | | 348163 | 348163 | 348163 | | | 0 |
| **VI** | *HXT10* | 272224 | 272224 | 272224 | **269534** | 269534 | -2690 |
| **I** | | 208852 | 208852 | 208852 | | | 0 |

Chromosome sizes of CEN.PK113-7D are derived from Salazar *et al.* (Salazar *et al. FEMS Yeast Res* 2017;**17**:10.1093/femsyr/fox074). In IMX672, the slight reduction in chromosome XV as compared to CEN.PK113-7D results from deletion of *HIS3*. Additionally, integration of *Sp*Cas9 plus Clonat in the *CAN1* locus explains the increase in chromosome V size in IMX672 as compared to CEN.PK113-7D.
The colour code indicates in which round of transformation the deletion is made, and therefore the chromosome size reduced.