*In silico* design of *Saccharomyces cerevisiae* strains for improved production of chondroitin

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**Table S1.** Primers used in this study.

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| **Purpose** | **Name** | **Template** | **Sequence (5΄ → 3΄)** | **Restriction enzyme** |
| Cloning of *Giuae* in pSP-GM1 (PGK1) | Gi\_uae\_Fw1 | pUC57\_Giuae | AAAGGATCCAAAAAAATGCATCACCATCACCATCACCAGAATCATTCCTTCTTGG | *Bam*HI |
| Gi\_uae\_Rv1 | AAAACTCGAGCTATAATCCAATACCTTTTACATC | *Xho*I |
| Cloning of *Btchsy* in pSP-GM1 (PGK1) | Bt\_chsy1\_Fw | pUC57\_Btchsy1 | AAAGGATCCAAAAAAATGCATCACCATCACCATCACGCTGTCCGAGGTCGAAGAGC | *Bam*HI |
| Bt\_chsy1\_Rv | AAAACTCGAGTTAAGCCGTGCGGACTGAGC | *Xho*I |
| Cloning of *Giuae* in pSP-GM1\_(PTEF) | Giuae\_tefp\_Fw | pSP-GM1\_Giuae | ATTTGCGGCCGCAAAACAATGCATCACCATCACCAT | *Not*I |
| Giuae\_tefp\_Rv | AACGAGCTCCTATAATCCAATACCTTTTACATC | *Sac*I |
| *Zmugd* cloning in pSP-GM1\_tefp\_*Giuae* | Zm\_psp\_Fw | pSP-GM1\_Zmugd | AAAGGATCCAAAAAAATGCATCACCATCACCATCACCGCATTACCATGATCGGTTCC | *Bam*HI |
| Zm\_psp\_Rv | AAAGCTAGCTTACCCTGCATTGGGTCGAC | *Nhe*I |
| *Btchsy* cloning in pBEVY | Btchsy\_pBEVY\_Fw | pSP-GM1\_Btchsy | AACGCGGATCCAAAACAATGCATCACCATCAC | *Bam*HI |
| Btchsy\_pBEVY\_Rv | AACGCCGTCGACTTAAGCCGTGCGGACTGAGC | *Sal*I |
| *kfoA* cloning in pSP-GM1\_Zmugd | kfoA\_psp\_Fw | pETM6\_kfoCA | ATTTGCGGCCGCAAAACAATGAATATATTAGTTACAGGTG | *Not*I |
| kfoA\_psp\_Rv | AACGAGCTCTTAAATATAACCATTTGGGTTT | *Sac*I |
| *kfoC* cloning in pBEVY-L | kfoC\_pBEVY\_Fw | pETM6\_kfoCA | AACGCGGATCCAAAACAATGAGTATTCTTAATCAAGC | *Bam*HI |
| kfoC\_pBEVY\_Rv | AACGCCGTCGACTTATAAATCATTCTCTATTTTTTCCC | *Sal*I |
| *Colony PCR and sequencing* | | | | |
|  | tADH1\_Fw | pSP-GM1\_tefp\_Giuae / pSP-GM1\_Zmugd\_kfoA | CCTAAGAGTCACTTTAAAATTTGT |  |
|  | TEF1p\_Rv | CGTACCACTTCAAAACACC |  |
|  | PGK1p\_FW | pSP-GM1\_Zmugd / pSP-GM1\_Giuae\_Zmugd | GTGATCTCCAGAGCAAAGT |  |
|  | CYCt\_REV | CTTCGAGCGTCCCAAAAC |  |
|  | tADH1\_Fw | pBEVY\_Btchsy / pBEVY\_kfoC | CCTAAGAGTCACTTTAAAATTTGT |  |
|  | pGAL\_middle\_Rv | ATTATTCCCCTACTTGACTAATAAG |  |
|  | Bt\_chsy1\_middle | pBEVY\_Btchsy | CGGTAAATGTTTAAGAGAGATG |  |
|  | kfoC\_pBEVY\_Rv | pBEVY\_kfoC | AACGCCGTCGACTTATAAATCATTCTCTATTTTTTCCC |  |

**Table S2.** Codon-optimized gene sequences (5΄ → 3΄) for *Saccharomyces cerevisiae.*

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| >Chondroitin synthase (chsy1) (2412 bp GC%: 41,54; CAI: 0,74) |
| ATGGCTGTCCGAGGTCGAAGAGCATGGTTAAGCGTTTTGCTGGGTTTAGTTCTTGGTTTTGTCTTAGCCTCTAGACTCGTTTTGCCGAGGGCTTCTGAGTTAAAAAGAGCAGGCCCACGGAGAAGAGCTAGTCCAGAGGCTTGTAGGCCGGGTCAGGCAGCAGCCGTACCATTAGCTGGAGGTGCTAGAGGAGATGCGAGAGGCCAAAGATTATGGCCCCACGGGGAAGCTCAAGACGGTGTTCCCCGCGACCGCAATTTCTTATTCGTAGGAGTCATGACCGCTCAAAAGTATCTACAAACGAGAGCTGTGGCTGCTTTCAGAACTTGGTCAAAAACTATCCCAGGCAAGGTCGAATTTTTCTCATCTGAAGGTTCTGATACCAGTATTAGTATTCCTGTGGTCCCATTGCGAGGTGTTGATGATTCATATCCGCCTCAGAAAAAATCATTCATGATGTTAAAATATATGCATGATCATTACTTGGATAAGTACGAATGGTTCATGAGAGCTGACGATGATGTTTATATTAAAGGTGACAGATTGGAATCATTCTTAAGATCGCTCAACAGCAGCGAGCCTTTGTTCTTGGGACAGACCGGCTTAGGAACCACTGAAGAAATGGGAAAACTTGCTCTAGAACCCGGTGAAAACTTTTGCATGGGAGGCCCCGGCGTTATTATGAGCAGAGAAGTATTGAGAAGAATGGTACCTCACATCGGTAAATGTTTAAGAGAGATGTACACTACTCACGAGGATGTAGAGGTGGGTCGCTGCGTTAGAAGATTTGCTGGTGTACAATGCGTCTGGTCGTACGAAATGCAACAACTCTTTTATGAAAATTATGAACAAAATAAAAAAGGTTACATACGTGATTTACACAATTCCAAGATACATAGAGCCATCACCTTACATCCTAACAAAAATCCTCCATATCAATATCGTTTGCACAGCTATATGCTTTCTAGAAAAATCGCTGAACTGAGACATCGTACTATCCAACTGCATCGCGAGATTGTCCTTATGTCCAAGTACAGTAATACGGAAGTTCATAAAGAGGATTTGCAATTAGGTATTCCACCGTCTTTCATGAGATTTCAACCAAGACAAAGAGAAGAAATTTTGGAATGGGAATTTCTAACCGGTAAGTATTTATATAGTGCAGCCGATAGCCAGCCCCCACGTAGAGGTATGGACAGCGCCCAAAGAGAAGCATTGGATGATATTGTTATGCAAGTTATGGAAATGATAAATGCCAATGCCAAAACTAGAGGGAGAATTATAGATTTCAAAGAAATTCAATACGGTTATCGCCGTGTTAATCCGATGTACGGGGCTGAATATATACTGGATTTGTTGTTGCTTTACAAGAAACATAAAGGTAAGAAAATGACAGTTCCAGTTAGAAGACATGCCTATTTGCAACAAACTTTTTCAAAAATTCAGTTTGTTGAACATGAAGAACTTGATGCAAAGGAATTGGCGAATAAAATTAATCAAGAATCCGGCTCCCTGTCTTTCCTGTCCAATTCATTGAAAAAGTTGGTGCCATTTCAACTTCCCGGGAGTAAAAACGAACACAAAGAACCTAAGGAGAAAAAGATTAATATTTTAATCCCCCTGAGCGGAAGATTTGACATGTTTGTCCGTTTCATGGGAAACTTTGAAAAGACATGTTTAATTCCGAATCAAAACGTGAAACTGGTTGTCTTATTGTTTAATTCTGATTCGAACCCTGACAAAGCCAAGCAAGTGGAACTTATGCGTGATTACAGAATTAAATATCCCAAGGCCGATATGCAAATTTTGCCGGTGTCAGGTGAGTTTTCCAGAGCCTTGGCATTGGAGGTGGGCAGTTCTCAATTTAACAATGAGAGTCTTTTGTTTTTCTGTGATGTTGACTTAGTCTTCACGGCCGAGTTTTTGCAACGTTGTAGAGCTAACACTGTCTTAGGCCAACAAATTTACTTCCCTATTATCTTCTCACAATATGATCCTAAAATTGTCTACTCCGGCAAAGTTCCAAGTGATAATCACTTTGCTTTTACTCAGAAAACTGGTTTTTGGAGGAATTATGGATTTGGAATTACGTGTATATACAAAGGGGATTTAGTGAGGGTGGGTGGCTTTGATGTGTCCATCCAGGGTTGGGGTCTGGAAGATGTCGATTTATTCAACAAAGTCGTTCAAGCAGGGCTTAAAACTTTCAGGAGCCAGGAAGTTGGCGTGGTCCACGTACACCATCCTGTTTTTTGTGATCCGAACTTAGATCCTAAGCAATACAAAATGTGTTTGGGCAGTAAAGCCTCCACTTATGGTAGCACTCATCAATTGGCAGAAATGTGGTTAGAAAAAAACGATCCCAATTACTCTAAGTCGAGTAACAACAACGGCTCAGTCCGCACGGCTTAA |
| >UDP-N-acetylglucosamine 4-epimerase (UAE) (1158 bp GC%: 42,31; CAI: 0,77) |
| ATGCAGAATCATTCCTTCTTGGGTAAAACGGTCTTGATCACAGGCGGTTGTGGTTTCATTGGATCTCATTTTGTAGAGGCTTGTCATGTTCTCGGTATGACCGTCTATGTTTTAGATAACCTCAGTTCTGGTAAGAATGTATTTAAGACAACATCTGATTGTTCTTCATCATTGGTTTACACTATCGGTGACATTAGAGACAAGGCTATCTTCTCTAGACTTCCGCAGAAAATTGACTTCGTCATTCATTTGGCCGCAGCTGTTTCTGTTGCCGAATCAGTAACAAATCCGCAGAAATATATGTTAACCAATGTTGAGGGTAGTAGAAACGTTTTCCAGTATGCTGTTGACGCAAAGGCTTCAGCCGTCTTAAGTGCTTCTACTGCTGCATATTACGGCGATTGTGGTAAGTCAGCTATTACCGAGGCTTTCCCCTATGGTGGCATTTCTCCATACGCTGAAAGTAAGATGGAGATGGAGAGATTAGGAGCCGAATTTCAAAAGACATCTCGTTGCAGATTCATTTTCTGCAGATTTTTCAATGTCTACGGCCCTAGACAGGACCCCTCCTCGCCCTACACTGGAGTTATGAGTATTTTTATGGACAGGTGCGCCGCTAGAAAACCGATTACAATCTTTGGTACCGGCGAGCAAACAAGAGATTTTGTTTTCATCAAGGACTTAATTGTCGCTGCCATTAACTTATTAGGTCAATTGGATAAGTTTCCTATTGGAGCAGATGCCGTGCAACAGAACGATCCAGAAGAAGTCCAAAGGTCCGCATATACCGGTGAAGGTGTGTACCCAACTGTTTTCAACATCGGATCGGGAATTTCGATCAGTGTCAATGAGCTTGCTGAATTAGCCAAAATTGTTTCTGGTAGACATGAAGTGGAAATTGTTCATGGAGAACCACGTAGTGGAGACATATTGCATTCATTATCCGATTGCACTAGGATTAGAAACGCTACTGGATGGAGCGCATCTACTACTCTTAGAGTTGGTATGTCTGAAACTTGGGGATGGGCTGCTGGCGAAATTAGTTACTTGTCTGGTGACTTGGTAAGAGTGTTGGAAAACGAATTAAAGATTGATGGGGTTTCAGTAGCAAAATCTCTATGTGGTAAAGATGCAGATGTAAAAGGTATTGGATTATAG |