

Table S1. Primers used for the amplification of the *N.benthamiana* xylosyltransferases XT1 (Niben101Scf04205Ctg025) and XT2 (Niben101Scf04551Ctg021) regions.

| | |
|------------|---|
| XT1_F | 5'-AACCACTTTTCCTCGTCGGAAA-3' |
| XT1_R | 5'-TAACTATTCAACTAAAGCTCAAACAG-3' |
| XT2_F | 5'-AACCACTTTTCCTTGTCGGAAA-3' |
| XT2_R | 5'-GGAATGAAATTAACCACTTCAGG-3' |
| XT12BsaI_F | 5'-GCGGGTCTCAGGAGCCCTAATGTTGCTTGGAGATC-3' |
| XT12BsaI_R | 5'-GCGGGTCTCAAGCGCCGTCTAAGGTTCAATTTGAGTAGC-3' |

Table S2. List of forward and reverse primers used to construct the targets.

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|--------------------|---------------------------------|
| gRNA XT1_F | 5'-ATTGAAAACACCGTCTTCGGAGA-3' |
| gRNA XT1_R | 5'-AAACTCTCCGAAGACGGTGTTTT-3' |
| gRNA XT2_F | 5'-ATTGAAAATTGGGAAAAAACTAG-3' |
| gRNA XT2_R | 5'-AAACCTAGTTTTTTCCCAATTTT-3' |
| gRNA1 pNOS_F | 5'-ATTGAGACTCTAATTGGATACCG-3' |
| gRNA1 pNOS_R | 5'-AAACCGGTATCCAATTAGAGTCT-3' |
| gRNA2 pNOS_F | 5'-ATTGACGTTCCATAAATCCCCT-3' |
| gRNA2 pNOS_R | 5'-AAACAGGGGAATTTATGGAACGT-3' |
| gRNA3 pNOS_F | 5'-ATTGACTTTTGAACGCGCAATAA-3' |
| gRNA3 pNOS_R | 5'-AAACTTATTGCGCGTTCAAAAGT-3' |
| gRNA4 pNOS_F | 5'-ATTGCCACTGAGCCGCGGTTTC-3' |
| gRNA4 pNOS_R | 5'-AAACGAAACCCGCGGCTCAGTGG-3' |
| gRNA5 pNOS_F | 5'-ATTGGGACAAGCCGTTTTACGTT-3' |
| gRNA5 pNOS_R | 5'-AAACAACGTAAAACGGCTTGTC-3' |
| gRNA FT5447tRNA_F* | 5'-GTGCACCCAAAAGAAATGGTCCAAT-3' |
| gRNA FT5447tRNA_R* | 5'-AAACATTGGACCATTTCTTTTGGGT-3' |
| gRNA FT1272tRNA_F* | 5'-GTGCACCAATAAGCAATGGCGCAAT-3' |
| gRNA FT1272tRNA_R* | 5'-AAACATTGCGCCATTGCTTATTGGT-3' |
| gRNA FT2631tRNA_F* | 5'-GTGCACCGATAAACAATGGCGCAAT-3' |
| gRNA FT2631tRNA_R* | 5'-AAACATTGCGCCATTGTTTATCGGT-3' |
| gRNA XT4205tRNA_F* | 5'-GTGCAGAAAACACCGTCTTCGGAGA-3' |
| gRNA XT4205tRNA_R* | 5'-AAACTCTCCGAAGACGGTGTTTTCT-3' |
| gRNA XT4551tRNA_F* | 5'-GTGCAGAAAATTGGGAAAAAACTAG-3' |
| gRNA XT4551tRNA_R* | 5'-AAACCTAGTTTTTTCCCAATTTTCT-3' |

*Primers used on the assembly of GBoligomers on level 0.

Table S3. List of GBelements generated in this work.

| Level -1 GBparts | | |
|-------------------------|--|--------------------|
| GBdatabase ID | Name | |
| GB1205 | tRNA-gRNA position [D1_2] | |
| GB1206 | tRNA-gRNA position [2_n-1] | |
| GB1207 | tRNA-gRNA position [n] | |
| GB1208 | tRNA-gRNA position [D1_n-1] | |
| GB1209 | tRNA-gRNA position [M1_2] | |
| GB1210 | tRNA-gRNA position [M1_n-1] | |
| Level 0 GBparts | | |
| GBdatabase ID | Name | Category |
| GB0273 | ppcoCas9 | B3-B4-B5 |
| GB0575 | phCas9 | B3-B4-B5 |
| GB0645 | psgRNA | B6b-C1 |
| GB1001 | pAtU6-26 | A1-A2-A3-B1-B2c |
| GB1041 | pdCas9 | B3-B4-B5 |
| GB1079 | pdCas9 | B3-B4 |
| GB1175 | pNLS-BRD | B5 |
| GB1184 | pOsU3 | A1-A2-A3-B1-B2d |
| GB1185 | pNLS-SRDX | B5 |
| GB1186 | p3xNLS-VP64 | B5 |
| GB1187 | p3xNLS-EDLL | B5 |
| GB1204 | pAtU6-1 | A1-A2-A3-B1-B2c |
| GB1211 | ptRNA-target5447_5494_17626FT-gRNA[D1_2] | Other |
| GB1212 | ptRNA-target1272FT-gRNA[2_n-1] | Other |
| GB1213 | ptRNA-target2631FT-gRNA[n] | Other |
| GB1214 | ptRNA-target4205XT-gRNA[D1_n-1] | Other |
| GB1215 | ptRNA-target4551XT-gRNA[n] | Other |
| Level 1 GB TUs | | |
| GBdatabase ID | Name | % accuracy* |
| GB0639 | pEGB2 α 2 35s:hCas9:tNOS | 100% |
| GB0576 | pEGB3 α 2 35s:pcoCas9:tNOS | 50% |
| GB1104 | pEGB3 α 1 U626:gRNAXT4551:sgRNA | 100% |
| GB1105 | pEGB3 α 2 U626:gRNAXT4205:sgRNA | 100% |
| GB1172 | pEGB3 α 2 35s:hCas9:BRD:tNOS | 100% |
| GB1177 | pEGB3 α 1 U626:gRNA1pNOS:sgRNA | 100% |
| GB1178 | pEGB3 α 2 U626:gRNA1pNOS:sgRNA | 100% |
| GB1179 | pEGB3 α 1 U626:gRNA2pNOS:sgRNA | 100% |
| GB1180 | pEGB3 α 1 U626:gRNA4pNOS:sgRNA | 100% |
| GB1188 | pEGB3 α 2 35s:hCas9:SRDX:tNOS | 100% |
| GB1189 | pEGB3 α 2 35s:hCas9:VP64:tNOS | 100% |
| GB1190 | pEGB3 α 2 35s:hCas9:EDLL:tNOS | 100% |
| GB1191 | pEGB3 α 2 35s:dCas9:tNOS | 100% |
| GB1192 | pEGB3 α 1 U626:gRNA3pNOS:sgRNA | 100% |
| GB1193 | pEGB3 α 1 U626:gRNA5pNOS:sgRNA | 100% |
| GB1194 | pEGB3 α 2 U626:gRNA3pNOS:sgRNA | 100% |
| GB1195 | pEGB3 α 2 U626:gRNA5pNOS:sgRNA | 100% |
| GB1196 | pEGB3 α 2 U626:gRNA2pNOS:sgRNA | 75% |
| GB1197 | pEGB3 α 2 U626:gRNA4pNOS:sgRNA | 100% |
| GB1221 | pEGB3 α 1 U626:gRNA1pDFR:sgRNA | 100% |

| Level >1 GB Modules | | |
|---------------------|---|------|
| GBdatabase ID | Name | |
| GB1064 | pEGB3Ω2 U6-26:target4551XT:sgRNA-35s:pcoCas9:tNOS | 75% |
| GB1106 | pEGB3Ω1 tNOS:nptII:pNOS-U626:target4205XT:sgRNA | 100% |
| GB1107 | pEGB3Ω2 U6-26:target4551XT:sgRNA-35s:hCas9:tNOS | 100% |
| GB1108 | pEGB 3α1 tNOS:nptII:pNOS-U626:target4205XT:sgRNA-U626:target4551XT:sgRNA-35s:hCas9:tNOS | 100% |
| GB1116 | pEGB3α1 pNOS:Luciferase:tNOS-SF-35S:Renilla:tNOS-35S:P19:tNOS-SF | 100% |
| GB1198 | pEGB3Ω1 U626:gRNA1pNOS:sgRNA-U626:gRNA2pNOS:sgRNA | 100% |
| GB1199 | pEGB3Ω1 U626:gRNA1pNOS:sgRNA-U626:gRNA4pNOS:sgRNA | 100% |
| GB1200 | pEGB3Ω1 U626:gRNA2pNOS:sgRNA-U626:gRNA4pNOS:sgRNA | 100% |
| GB1201 | pEGB3Ω2 U626:gRNA4pNOS:sgRNA-SF | 100% |
| GB1202 | pEGB3α1 U626:gRNA1pNOS:sgRNA-U626:gRNA2pNOS:sgRNA-U626:gRNA4pNOS:sgRNA | 100% |
| GB1216 | pEGB3α1 U626:tRNA-target1FT-gRNA:tRNA-target2FT:gRNA:tRNA-target3FT-gRNA | 100% |
| GB1217 | pEGB3α2 U626:tRNA-target1XT-gRNA:tRNA-target2XT:gRNA | 100% |
| GB1218 | pEGB3Ω1 U626:tRNA-gRNA(x3withFTtargets)-U626:tRNA-gRNA(x2withXTtargets) | 100% |
| GB1219 | pEGB3Ω1 U626:gRNA3pNOS:sgRNA-U626:gRNA5pNOS:sgRNA | 100% |
| GB1220 | pEGB3α1 U626:gRNA3pNOS:sgRNA-U626:gRNA5pNOS:sgRNA-U626:gRNA4pNOS:sgRNA | 100% |
| GB1222 | pEGB3α1 U626:tRNA-gRNA(x3withFTtargets)-U626:tRNA-gRNA(x2withXTtargets)-35s:hCas9:tNOS | 100% |

* Cloning accuracy expressed as the % of colonies showing the correct restriction pattern (number of colonies assayed 2-4).