

## Supporting Information

### Engineering *Saccharomyces cerevisiae* for geranylgeraniol overproduction by combinatorial design

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### Supplementary Table S1

Integration sites analyzed in 10 strains from 4 combinations with distinct GGOH production.

| Strain | GGOH (mg/L) | Integration site |
|--------|-------------|------------------|
| 03-1   | 70.78       | XII-2            |
| 03-7   | 124.53      | XVI-2            |
| 03-8   | 161.82      | XVI-1            |
| 04-1   | 41.22       | X-1              |
| 04-7   | 116.26      | XVI-2            |
| 04-8   | 145.19      | XV-1             |
| 09-1   | 36.32       | XII-2            |
| 09-8   | 127.59      | X-1              |
| 10-1   | 48.90       | X-1              |
| 10-8   | 133.39      | XVI-1            |

### Supplementary Table S2

2-level fractional factorial design for determining the notable factors in fermentation medium.

| Run | Glucose (g/L) | Yeast extract (g/L) | Peptone (g/L) | MgSO <sub>4</sub> (g/L) | KH <sub>2</sub> PO <sub>4</sub> (g/L) | GGOH/ (mg/L) |
|-----|---------------|---------------------|---------------|-------------------------|---------------------------------------|--------------|
| 1   | 50            | 1                   | 30            | 0                       | 10                                    | 298.96       |
| 2   | 10            | 1                   | 1             | 10                      | 0                                     | 15.02        |
| 3   | 50            | 1                   | 1             | 0                       | 0                                     | 28.25        |
| 4   | 50            | 15                  | 1             | 10                      | 0                                     | 372.85       |
| 5   | 10            | 15                  | 30            | 10                      | 0                                     | 92.01        |
| 6   | 50            | 15                  | 1             | 0                       | 10                                    | 180.01       |
| 7   | 50            | 1                   | 30            | 10                      | 0                                     | 243.28       |
| 8   | 10            | 1                   | 1             | 0                       | 10                                    | 18.26        |
| 9   | 50            | 15                  | 30            | 0                       | 0                                     | 360.65       |
| 10  | 10            | 1                   | 30            | 0                       | 0                                     | 48.94        |
| 11  | 10            | 1                   | 30            | 10                      | 10                                    | 64.81        |
| 12  | 50            | 15                  | 30            | 10                      | 10                                    | 418.28       |
| 13  | 10            | 15                  | 1             | 10                      | 10                                    | 102.87       |
| 14  | 50            | 1                   | 1             | 10                      | 10                                    | 20.43        |
| 15  | 10            | 15                  | 30            | 0                       | 10                                    | 62.39        |
| 16  | 10            | 15                  | 1             | 0                       | 0                                     | 86.17        |

### Supplementary Table S3

RSM with Box-Behnken design for medium optimization.

| Run | Glucose (g/L) | Yeast extract(g/L) | Peptone(g/L) | GGOH(mg/L) |
|-----|---------------|--------------------|--------------|------------|
| 1   | 50.0          | 15.5               | 30.5         | 424.09     |
| 2   | 90.0          | 30.0               | 30.5         | 147.12     |
| 3   | 50.0          | 1.0                | 1.0          | 20.43      |
| 4   | 10.0          | 15.5               | 60.0         | 105.59     |
| 5   | 50.0          | 30.0               | 1.0          | 293.56     |
| 6   | 90.0          | 1.0                | 30.5         | 139.63     |
| 7   | 50.0          | 15.5               | 30.5         | 426.28     |
| 8   | 90.0          | 15.5               | 1.0          | 105.65     |
| 9   | 50.0          | 1.0                | 60.0         | 269.93     |
| 10  | 10.0          | 1.0                | 30.5         | 64.81      |
| 11  | 50.0          | 30.0               | 60.0         | 169.06     |
| 12  | 50.0          | 15.5               | 30.5         | 437.29     |
| 13  | 90.0          | 15.5               | 60.0         | 152.92     |
| 14  | 10.0          | 15.5               | 1.0          | 102.87     |
| 15  | 10.0          | 30.0               | 30.5         | 59.12      |
| 16  | 50.0          | 15.5               | 30.5         | 402.16     |
| 17  | 50.0          | 15.5               | 30.5         | 397.82     |

### Supplementary Table S4

cassettes built in this study.

| Gene Name         | Cassette Description                                                                                                                                                                                                             |
|-------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>ura3</i>       | YPRC15up- <i>ura3</i> -CYC1t<br>delta1- <i>ura3</i> -CYC1t                                                                                                                                                                       |
| <i>ggppssa</i>    | CYC1t-TPI1p- <i>ggppssa</i> -TEF1t<br>CYC1t- HXT7p- <i>ggppssa</i> -TEF1t<br>CYC1t-TDH1p - <i>ggppssa</i> -TEF1t<br>CYC1t-TDH3p- <i>ggppssa</i> - TEF1t<br>TEF1t-GPM1p- <i>bts1-erg20</i> -TPI1t                                 |
| <i>bts1-erg20</i> | TEF1t- TDH2p - <i>bts1-erg20</i> -TPI1t<br>TEF1t- HXK2p - <i>bts1-erg20</i> -TPI1t<br>TEF1t- TEF1p - <i>bts1-erg20</i> -TPI1t                                                                                                    |
| <i>thmgr</i>      | TPI1t-PDC1p- <i>thmgr</i> -FBA1t- YPRC15dn<br>TPI1t-PDC1p- <i>thmgr</i> -FBA1t- delta2<br>TPI1t- PGK1p - <i>thmgr</i> -FBA1t- delta2<br>TPI1t- PGI1p - <i>thmgr</i> -FBA1t- delta2<br>TPI1t- TEF1p - <i>thmgr</i> -FBA1t- delta2 |

## Supplementary Table S5

Primers used in this study.

| Name                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Sequence (5'-3')                                                  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|
| <b>Primers for gene amplification in the gene cassettes construction step. The primers pair “gene name-promoter name-F” and “gene name-terminator name-R” were used for the Golden Gate restriction-ligation method, and the enzyme sites were marked with underline. And the primers pair “gene name-promoter name-F -Y” and “gene name-terminator name-R-Y” were used for yeast homologous recombination method.</b>                                                                                                                                          |                                                                   |
| GGPPSsa-TPI1p-F/<br>GGPPSsa-HXT7p-F                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | <u>GGTCTC</u> CAAAAATGTCTTACTTCGACAACACTACTTTAACG                 |
| GGPPSsa-TDH1p-F/<br>GGPPSsa-TDH3p-F                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | <u>GGTCTC</u> CCAAAATGTCTTACTTCGACAACACTACTTTAACG                 |
| GGPPSsa-TEF1t-R                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | <u>GGTCTC</u> CATTTTTATTTCCTTCTTCTAATGGTAAACTCTG                  |
| BE-GPM1p-F                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <u>GGTCTC</u> CAATAATGGAGGCCAAGATAGATGAGC                         |
| BE-TDH2p-F                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <u>GGTCTC</u> CCAAAATGGAGGCCAAGATAGATGAGC                         |
| BE-HXK2p-F                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <u>GGTCTC</u> CTAAAATGGAGGCCAAGATAGATGAGC                         |
| BE-TPI1t-R                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | <u>GGTCTC</u> CAATCCTATTTGCTTCTCTTGTAACCTTTGTTC                   |
| BE-TEF1p-F-Y                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | GAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGGAGG<br>CCAAGATAGATGAGC   |
| BE-TPI1t-R-Y                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | GAAGATAATATTTTTATATAATTATATTAATCCTATTTGCTTCTCTT<br>GTAAACTTTGTTC  |
| tHMGR-PDC1p-F                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <u>GGTCTC</u> CCAAAATGGACCAATTGGTGAAAACCTG                        |
| tHMGR-PGK1p-F                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <u>GGTCTC</u> CAACAATGGACCAATTGGTGAAAACCTG                        |
| tHMGR-PGI1p-F                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <u>GGTCTC</u> CAAAAATGGACCAATTGGTGAAAACCTG                        |
| tHMGR-FBA1t-R                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <u>GGTCTC</u> CTAACTTAGGATTTAATGCAGGTGACGG                        |
| tHMGR-TEF1p-F-Y                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | GAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAAATGGACC<br>AATTGGTGAAAACCTG  |
| tHMGR-FBA1t-R-Y                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | CTCATTAATAAAACTATATCAATTAATTTGAATTAACCTTAGGATTTA<br>ATGCAGGTGACGG |
| <b>Primers for amplification of the promoters / terminators and OE-PCR of the related fragments. A pair of primers “terminator-promoter-F” and “promoter-terminator-R” were used for amplification of the promoter fragment while the primers pair “promoter-terminator-F” and “terminator-promoter-R” were for amplification of the terminator fragment. For example, the primer pair “GPDt-PDC1p-F” and “PDC1p-FBA1t-R” were for amplification of the promoter PDC1p, which was subsequently to construct the empty cassette GPDt-PDC1p- FBA1t by OE-PCR.</b> |                                                                   |
| <b>CYC1t- (TPI1p/ HXT7p/ TDH1p/ TDH3p)- TEF1t</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                   |
| CYC1t-F                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | AAAACCTGCAGGCGGCGCCGCGCATGTAATTAGTTATGTCACGCTTAC                  |
| CYC1t-TPI1p-R                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | CAACCTGATGGGTTTCCTAGATATAAAAGCCTTCGAGCGTCCC                       |

|                                                  |                                                                                                                                                                       |
|--------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CYC1t-HXT7p-R                                    | CGAAATTGTTCTACGAGAAGTAAAGCCTTCGAGCGTCCC                                                                                                                               |
| CYC1t-TDH1p-R                                    | GTGATATAGAGTGTAATGAGCATATACAAAAGCCTTCGAGCGTCCC                                                                                                                        |
| CYC1t-TDH3p-R                                    | AAATGGCAGTATTGATAATGATAAACTAAAGCCTTCGAGCGTCCC                                                                                                                         |
| CYC1t-TPI1p-F                                    | GGGACGCTCGAAGGCTTTTATATCTAGGAACCCATCAGGTTG                                                                                                                            |
| TPI1p-TEF1t-R                                    | GAAAAGTCTTATCAATCTCCTTATTTGGAGACCGGTCTCCTTTTATGTTATGTATGTGTTTTTTGTAGT                                                                                                 |
| CYC1t-HXT7p-F                                    | GGGACGCTCGAAGGCTTTACTTCTCGTAGGAACAATTTTCG                                                                                                                             |
| HXT7p-TEF1t-R                                    | GAAAAGTCTTATCAATCTCCTTATTTGGAGACCGGTCTCCTTTTTGATTAATAAATAAAAAAACTTTTTG                                                                                                |
| CYC1t-TDH1p-F                                    | GGGACGCTCGAAGGCTTTTGTATATGCTCATTACACTCTATATCAC                                                                                                                        |
| TDH1p-TEF1t-R                                    | GAAAAGTCTTATCAATCTCCTTATTTGGAGACCGGTCTCCTTTGTTTTGTGTGTAAATTTAGTGAAG                                                                                                   |
| CYC1t-TDH3p-F                                    | GGGACGCTCGAAGGCTTTAGTTTATCATTATCAATACTGCCATTT                                                                                                                         |
| TDH3p-TEF1t-R                                    | GAAAAGTCTTATCAATCTCCTTATTTGGAGACCGGTCTCCTTTGTTGTTTATGTGTGTTTATTCG                                                                                                     |
| TPI1p-TEF1t-F                                    | ACTACAAAAACACATACATAAACTAAAAGGAGACCGGTCTCCAATAAAGGAGATTGATAAGACTTTTC                                                                                                  |
| HXT7p-TEF1t-F                                    | CAAAAAGTTTTTTTAATTTTAATCAAAAAGGAGACCGGTCTCCAATAAAGGAGATTGATAAGACTTTTC                                                                                                 |
| TDH1p-TEF1t-F                                    | CTTCACTAAATTTACACACAAAACAAAGGAGACCGGTCTCCTCAATAAAGGAGATTGATAAGACTTTTC                                                                                                 |
| TDH3p-TEF1t-F                                    | CGAATAAACACACATAAAACAAACAAAGGAGACCGGTCTCCTCAATAAGGAGATTGATAAGACTTTTC                                                                                                  |
| TEF1t-R                                          | CGCGGATCCGCGGCGCGGATAGCGCCGATCAAAGTA                                                                                                                                  |
| <b>TEF1t-(GPM1p/ TDH2p/ HXK2p/ TEF1p) -TPI1t</b> |                                                                                                                                                                       |
| TEF1t-F                                          | AAAACCTGCAGGCGCGGCGCGCAAATAAGGAGATTGATAAGACTTTTC                                                                                                                      |
| TEF1t-GPM1p-R                                    | CTTAAAGTCATACATTGCACGACTAGATAGCGCCGATCAAAGTATCATTCACTGGAAAAACCAATGATAGCGCCGATCAAAGTATGCTCTTCTATGGCGTTCAGATAGCGCCGATCAAAGTAGAAGAGTAAAAAAGGAGTAGAAACATTGATAGCGCCGATCAAA |
| TEF1t-TDH2p-R                                    | GTATGTAATATGTGTGTTTGTGTTG                                                                                                                                             |
| TEF1t-HXK2p-R                                    | TACTTTGATCGGCGCTATCTAGTCGTGCAATGTATGACTTTAAG                                                                                                                          |
| TEF1t-TEF1p-R                                    | GAAGATAATATTTTTATATAATTATATTAATCTGAGACCGGTCTCCTATTGTAATATGTGTGTTTGTGTTG                                                                                               |
| TEF1t-GPM1p-F                                    | TACTTTGATCGGCGCTATCATTGGTTTTTCCAGTGAATGA                                                                                                                              |
| GPM1p-TPI1t-R                                    | GAAGATAATATTTTTATATAATTATATTAATCGGAGACCGGTCTCATTTGTTTTGTTTGTGTTGTTGTGTGATG                                                                                            |
| TEF1t-TDH2p-F                                    | TACTTTGATCGGCGCTATCTGAACGCCATAGAAGAGCA                                                                                                                                |
| TDH2p-TPI1t-R                                    |                                                                                                                                                                       |
| TEF1t-HXK2p-F                                    |                                                                                                                                                                       |

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|                                                          |                                                                                        |
|----------------------------------------------------------|----------------------------------------------------------------------------------------|
| HXK2p-TPI1t-R                                            | GAAGATAATATTTTTATATAATTATATTAATCTGAGACCGGTCTCC<br>TTTATTTAATTAGCGTACTTATTATGTG         |
| TEF1t-TEF1p-F                                            | TACTTTGATCGGCGCTATCAATGTTTCTACTCCTTTTTTACTCTTC                                         |
| TEF1p-TPI1t-R                                            | TATAATTATATTAATCGTTTAAACTTTGTAATTAAAACTTAGATTA<br>GATTGCTATG                           |
| GPM1p-TPI1t-F                                            | CCAAACAAACACACATATTACAATAGGAGACCGGTCTCAGATTA<br>ATATAATTATATAAAAAATATTATCTTC           |
| TDH2p-TPI1t-F                                            | CATCACACAAACAAACAAACAAATGAGACCGGTCTCCGATTA<br>ATATAATTATATAAAAAATATTATCTTC             |
| HXK2p-TPI1t-F                                            | CACATAATAAGTACGCTAATTAAATAAAGGAGACCGGTCTCAGA<br>TTAATATAATTATATAAAAAATATTATCTTC        |
| TEF1p-TPI1t-F                                            | TAAGTTTTAATTACAAAGTTTAAACGATTAATATAATTATATAAAA<br>ATATTATCTTC                          |
| TPI1t-R                                                  | CGCGGATCCGCGGCGCGCTATATAACAGTTGAAATTTGGATAAGA<br>AC                                    |
| <b>TPI1t-(PDC1p/ PGK1p/ PGI1p/ TEF1p) -FBA1t- delta2</b> |                                                                                        |
| TPI1t-F                                                  | AAAACCTGCAGGCGGCGCGGATTAATATAATTATATAAAAAATATTAT<br>C                                  |
| TPI1t-PDC1p-R                                            | ATGCTCACCCAGTCGCATGTATATAACAGTTGAAATTTGGATAAG<br>AAC                                   |
| TPI1t-PGK1p-R                                            | TTGAAGTCAGGAATCTAAAATATATATAACAGTTGAAATTTGGAT<br>AAGAAC                                |
| TPI1t-PGI1p-R                                            | CAATACACCCACACCCACCTATATAACAGTTGAAATTTGGATAAG<br>AAC                                   |
| TPI1t-TEF1p-R                                            | GAAGAGTAAAAAAGGAGTAGAAACATTTATATAACAGTTGAAAT<br>TTGGATAAGAAC                           |
| TPI1t-PDC1p-F                                            | GTTCTTATCCAAATTTCAACTGTTATATACATGCGACTGGGTGAG<br>CAT                                   |
| PDC1p-FBA1t-R                                            | CATTAAAAAACTATATCAATTAATTTGAATTAACGGAGACCGGTC<br>TCCTTTGATTGATTTGACTGTGTTATTTTG        |
| TPI1t-PGK1p-F                                            | GTTCTTATCCAAATTTCAACTGTTATATATATTTTAGATTCCTGACT<br>TCAA                                |
| PGK1p-FBA1t-R                                            | CATTAAAAAACTATATCAATTAATTTGAATTAACGGAGACCGGTC<br>TCCTGTTTTATATTTGTTGTAAAAAGTAGATAATTAC |
| TPI1t-PGI1p-F                                            | GTTCTTATCCAAATTTCAACTGTTATATAGGTGGGTGTGGGTGTA<br>TTG                                   |
| PGI1p-FBA1t-R                                            | CATTAAAAAACTATATCAATTAATTTGAATTAACGGAGACCGGTC<br>TCCTTTTAGGCTGGTATCTTGATTCT            |
| TPI1t-TEF1p-F                                            | GTTCTTATCCAAATTTCAACTGTTATATAAATGTTTCTACTCCTTT<br>TTACTCTTC                            |
| TEF1p-FBA1t-R                                            | ATTAATTTGAATTAACGTTTAAACTTTGTAATTAAAACTTAGATTA<br>GATTGCTATG                           |

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|                 |                                                                                        |
|-----------------|----------------------------------------------------------------------------------------|
| PDC1p-FBA1t-F   | CAAAATAACACAGTCAAATCAATCAAAGGAGACCGGTCTCCGTT<br>AATTCAAATTAATTGATATAGTTTTTTAATG        |
| PGK1p-FBA1t-F   | GTAATTATCTACTTTTTACAACAAATATAAAACAGGAGACCGGTC<br>TCCGTAAATTCAAATTAATTGATATAGTTTTTTAATG |
| PGI1p-FBA1t-F   | AGAATCAAGATACCAGCCTAAAAGGAGACCGGTCTCCGTAAATT<br>CAAATTAATTGATATAGTTTTTTAATG            |
| TEF1p-FBA1t-F   | TAAGTTTTAATTACAAAGTTTAAACGTTAATTCAAATTAATTGATA<br>TAGTTTTTTAATG                        |
| FBA1t- Delta2-R | CACCTGCTTCATCAGCTGTAAAGATGAGCTAGGCTTTTGTA<br>AATATC                                    |
| FBA1t- Delta2-F | GATATTTTTACAAAAGCCTAGCTCATCTTTAACAGCTGATGAAGC<br>AGGTG                                 |
| Delta2-R        | CGCGGATCCGCGGCCGCGAGAACTTCTAGTATATTCTGTATACCT<br>AATAT                                 |

**Primers for verification of the gene insert and the geranylgeraniol pathway integration.**

|             |                             |
|-------------|-----------------------------|
| TPI1p-seq-F | GCAGCATAATTTAGGAGTTTAGTG    |
| HXT7p-seq-F | TATGCCAATACTTCACAATGTTTCG   |
| TDH1p-seq-F | TGGTGATAATGACCAAAC          |
| TDH3p-seq-F | AGTTCCCTGAAATTATTCCCCTAC    |
| TEF1p-seq-F | CTCTTTCGATGACCTCCCATTG      |
| GPM1p-seq-F | CTGTTGCCAGGGAGGGTG          |
| TDH2p-seq-F | TAGTACCCAGTGATCGCAGAC       |
| HXK2p-seq-F | GCACCTTCGCCACTGTC           |
| PDC1p-seq-F | CTCTTTCACTCTCCTTGCAATCAGAT  |
| PGK1p-seq-F | GCATAAATTGGTCAATGC          |
| PGI1p-seq-F | TACTTGCAATTCAGTGAATTTTAATAC |
| TEF1t-seq-R | CATTTGGACTGTCGCCTGTT        |
| FBA1t-seq-R | CTTCAGAAGAAAAGAGCCGACC      |
| PGK1t-seq-R | AGCGTAAAGGATGGGGAAAG        |
| CYC1t-seq-R | TCCTTCCTTTTCGGTTAGAGC       |

**Primers for verification of the integration site on the yeast chromosome. The format of the reverse primer names were “chromosome- $\delta$  site number-length of amplicon-R”. The numbers in the parentheses represented the percentage of similarity of the corresponding chromosome  $\delta$  site sequence with the sequence we used for integration.**

|                   |                                    |
|-------------------|------------------------------------|
| thmgr-Delta-F     | GGTCCGTCACCTGCATTAAATCC            |
| I-1-1590-R (98%)  | CAACTACTTAGTCGTGATACAACAGTATGGATAG |
| IV-1-1382-R (95%) | CAATCGTCCTCTCTTGGCATAGTAGACACAG    |
| IV-2-1650-R (94%) | TATTGACTGAACGAATGCTGAGTAGGCGAGG    |
| IV-3-1488-R (92%) | TACTGTGATTTTGAATACTGGAATAGGGG      |
| IV-4-1527-R (92%) | GTTTGAAAGGTCTTTGGCACAGAACTTCG      |

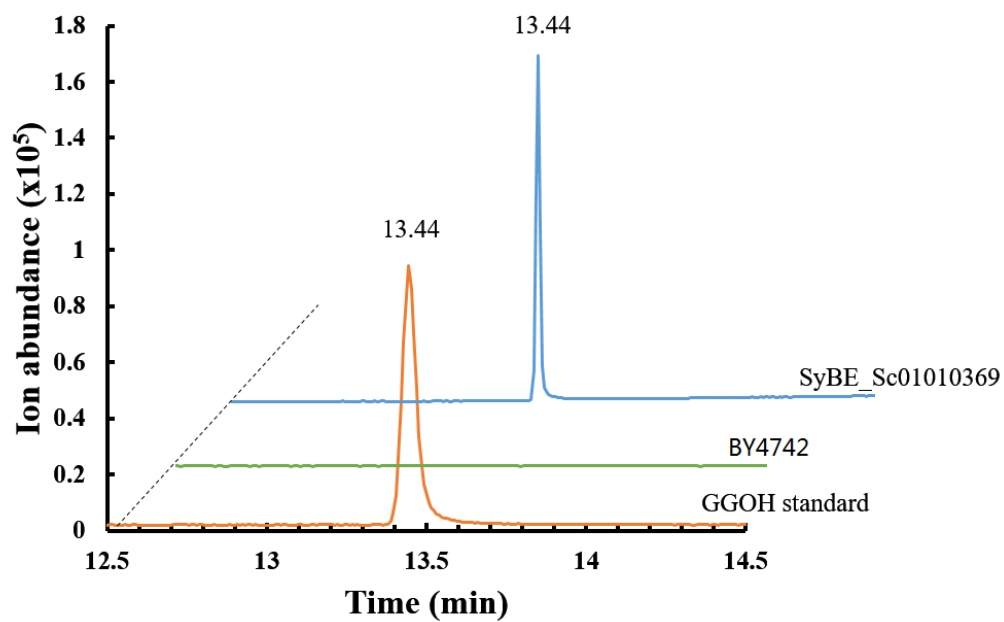
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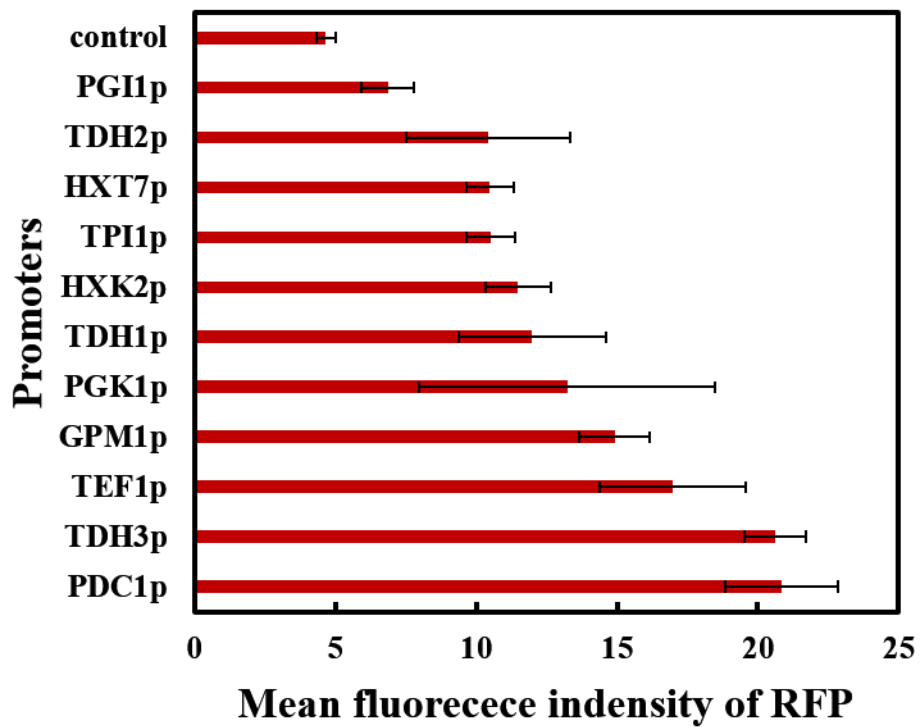
|                     |                                        |
|---------------------|----------------------------------------|
| IV-5-1432-R (91%)   | GATAGGTAACCTCTTGTGAATAAGTGTTGGG        |
| V-1-1713-R (98%)    | CTGTTAGTCCATCAATATACGCCATCTTTT         |
| V-2-1590-R (91%)    | TCCCGAGAAATACTACAACGACAGTGATAC         |
| VII-1-1396-R (93%)  | GCTAAATAATCTAAAGGGAAACAGCCCCAAAAC      |
| VII-2-1545-R (93%)  | TCTCAACATTCCCCCATTTTTCAAAGGTAATT       |
| X-1-1397-R (94%)    | TGCTGATGTGATGACAAAACCTCTTCCGAT         |
| X-2-1532-R (93%)    | CGAGTAAATACGCCTCCTTTTTATCTGTGAATGAC    |
| XII-1-1543-R (98%)  | CACCCACTACACTACTCGGTCAGGCTCTTAC        |
| XII-2-1570-R (94%)  | CATTATCTTATTACATTATCAATCCTTCCATTTCAGC  |
| XII-3-1473-R (94%)  | GTATAAACCTGACTTAGAAGACTTTTTTGATGC      |
| XII-4-1592-R (91%)  | CGTATTAATTGTTGGGATTTTCGTTGTTGATATTAGGT |
| XIII-1-1447-R (98%) | CTTAGCCACTAAGGAATCACCGAATAGTAAAAG      |
| XIII-2-1457-R (91%) | GCGAACAATAGAATCCCAACGGTTATCAAAT        |
| XIV-1-1510-R (94%)  | CAACTCGTGCTCTGCTGGCTATAAAAGGGGT        |
| XIV-2-1539-R (91%)  | CGATTAACAGTCGCACGCCTTAACCAACTT         |
| XV-1-1481-R (92%)   | TGTCAAAATAGCCGCCCAACTTCTACGCCC         |
| XVI-1-1785-R (98%)  | TGGACAGTTGTTACAGTTGCCCGTGAAGGT         |
| XVI-2-1398-R (95%)  | AACCGCTTCCACATCTGAATCATATCTCCG         |
| XVI-3-1805-R (93%)  | GAAAATCACTAAGCCACAAAAACAATAAAG         |
| XVI-4-1653-R (91%)  | GTCACCCTGTAAGGCTTTCTACTCTATCAC         |

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**Supplementary Fig. S1.** GC-MS results for GGOH standard, host cell BY4742, and SYBE\_Sc01010369.

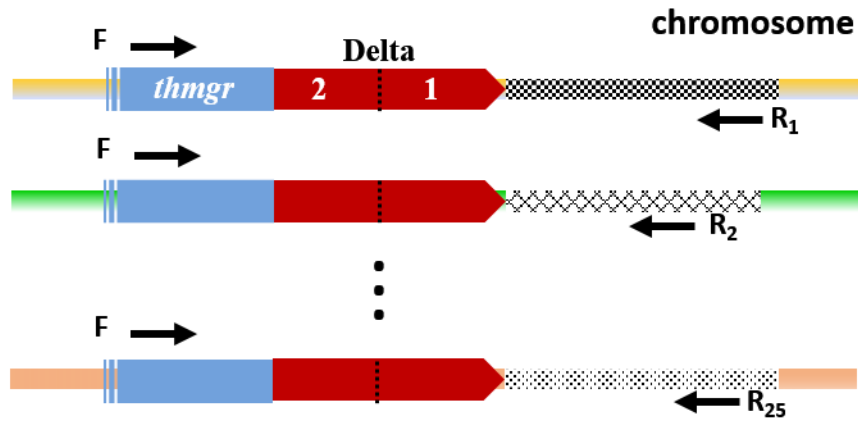


**Supplementary Fig. S2.** Promoters strength characterization in our study. X-axis showed the mean fluorescence intensity of RFP while Y-axis listed the names of the promoters. The experiments were carried out in 96 well plate. Error bars represented the standard deviations from three independent experiments. The control was wild strain without RFP expression.

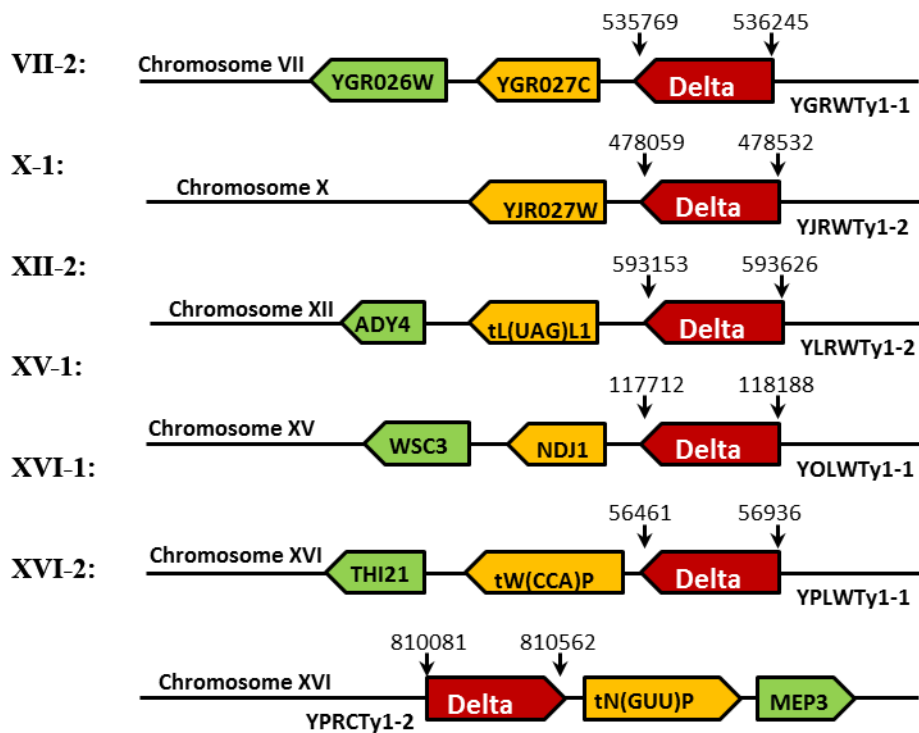


**Supplementary Fig. S3.** Integration site detection. (a) Schematic diagrams of the integration sites detection. The genomic DNA of the chassis BY4742 was used as the negative control. (b) 6 verified integration sites by PCR.

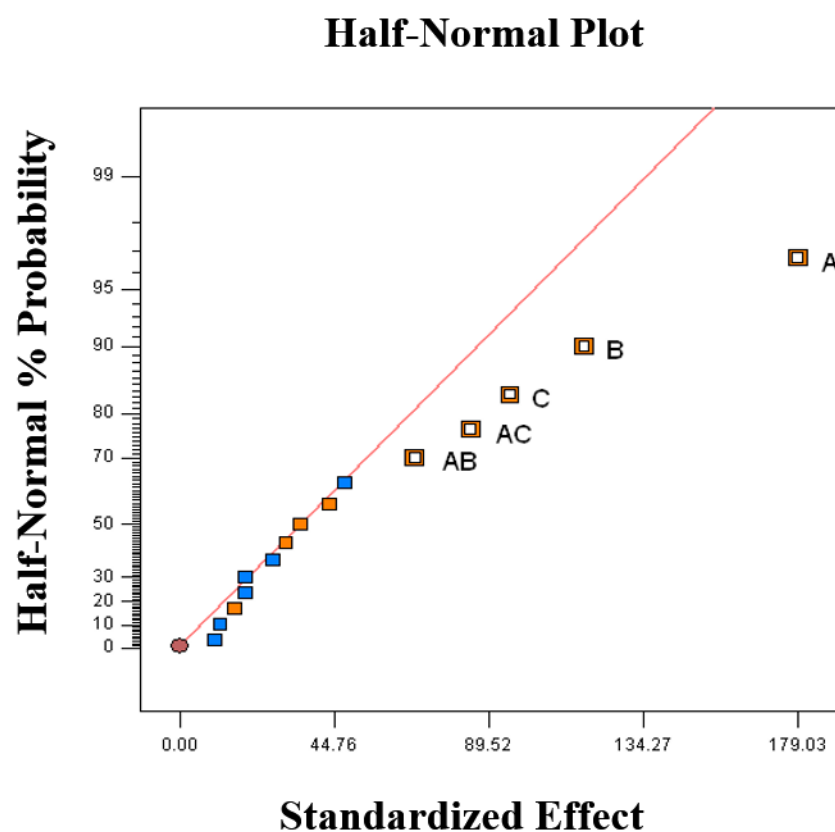
(a)



(b)



**Supplementary Fig. S4.** Half-normal probability plot of the results of the 2-level fractional factorial design. The factors glucose (A), yeast extract (B) and peptone (C) displayed far off the near zero line (red line) on the plot were considered to be the notable ones, while  $\text{MgSO}_4$  and  $\text{KH}_2\text{PO}_4$  which were not marked were displayed on or close to the near zero line and were defined as unimportant factors.



**Supplementary Equation S1.** The fitted equation (in terms of coded values) for the prediction of geranylgeraniol production (mg/L). A, B and C stand for glucose, yeast extract and peptone, respectively.

$$\text{Geranylgeraniol} = 417.53 + 26.62 * A + 21.76 * B + 21.87 * C + 3.29 * A * B + 11.14 * A * C - 93.5 * B * C - 193.17 * A^2 - 121.69 * B^2 - 107.60 * C^2$$