Appendix

**Table 2-1** System of reverse transcription

|  |  |
| --- | --- |
| Ingredients | Dosage |
| RNA Template | 7μL |
| dNTP Mix | 4μL |
| Primer Mix | 2μL |
| 5×RT Buffer | 4μL |
| DTT | 2μL |
| HiFiScript | 1μL |

**Table 2-2** Reaction condition for cDNA synthesis

|  |  |  |
| --- | --- | --- |
| Steps | Temperature /℃ | Time /min |
| 1 | 42 | 20 |
| 2 | 85 | 5 |

**Table 2-3** The system of RT-qPCR reaction

|  |  |
| --- | --- |
| Component of reaction | Dosage |
| cDNA模板 | 1μL |
| Forward primer（10μM） | 0.5μL |
| Reverse primer（10μM） | 0.5μL |
| SYBR® Premix Ex TaqTM Ⅱ | 10μL |
| ROX Reference Dye II | 0.4 μL |
| ddH2O | 7.6μL |
| Total | 20 μL |

**Table 2-4** The program of RT-qPCR

|  |  |  |  |
| --- | --- | --- | --- |
| Steps | Temperature /℃ | Time /s | cycle |
| 1 | 95 | 30 |  |
| 2 | 95 | 5 |  |
| 3 | 60 | 30 | ×40 |
| 4 | 72 | 30 |  |
| 5 | 95 | 15 |  |
| 6 | 60 | 30 |  |
| 7 | 95 | 15 |  |

**Table2-5** Conditions for determination of pigment components by HPLC

|  |  |
| --- | --- |
| Detector | VWD（紫外） |
| Chromatographic column | C18 (VP-ODS, 150 L×4.6 μm) |

**Table 2-5** is continued

|  |  |
| --- | --- |
| Column temperature | 25℃ |
| Sample injection volume | 20 μL |
| Velocity of flow | 1mL/min |
| Wavelength of detection | 410nm |

**Table2-6** Determination of *Monascus* pigments

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| time | A% | B% | The flow rate (mL/min) | Maximum pressure (bar) |
| 0 | 40 | 60 | 1 | 400 |
| 12 | 40 | 60 | 1 | 400 |
| 25 | 10 | 90 | 1 | 400 |
| 27 | 10 | 90 | 1 | 400 |
| 30 | 40 | 60 | 1 | 400 |

**Table2-7** Primers for RT-qPCR of 8 related genes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Primer names | Sequence (5’-3’) | Function of genes | Tm/℃ | Length（bp） |
| *MpPKS5* | TTCTCCTCGCGGACAACACC | NR-PKS | 64 | 148 |
| ACTCGTTGCACCCATTCAGG | 62 |
| *mppG* | GCACTGTCGATGAACTGAGC | 氧化还原酶 | 62 | 129 |
| CGGCAACCATACTCCGACTC | 64 |
| *mppD* | CGCCACAACATCCTTCGTC | 丝氨酸水解酶 | 60 | 141 |
| GTGCATGTGCATGGGATGC | 60 |
| *mppE* | CTCGTTCAAGTTCAAGGGCC | 还原酶 | 62 | 182 |
| GGATGCTCTCCAATCCCTTG | 62 |
| *VeA* | CATGATGCCATCCCTTCCGC | 性发育激活剂 | 60 | 146 |
| ACAAGACCGACTCCGGATCG | 61 |
| *VosA* | ATGCCCGGGTCCGTATTTGG | 20 OG-Fe（Ⅱ）加氧酶超级家族蛋白 | 62 | 178 |
| TTGGATACAAGGCCGTCGCC | 60 |
| *LaeA* | CTTAATGCGATCCACCGGCC | 甲基转移酶 | 60 | 184 |
| GCAGGAGGCTGGATTTACAG | 57 |
| *GprD* | CTGGGACTCGCATCAGCATC | G蛋白偶联受体 | 59 | 181 |
| GCCAACTCAGTGTCGCGTAT | 59 |

**Table 3-1** Proportion of pigment peak area of three strains without additional nitrogen source and Gln

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | C100 | | Δ*MAreA* | | OE-*MAreA* | |
| no additional nitrogen source | Gln | no additional nitrogen source | Gln | no additional nitrogen source | Gln |

**Table 3-1** is continued

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| R1 | 3.538% | 7.325% | 2.205% | 8.776% | 4.269% | 7.462% |
| R2 | 5.386% | 7.166% | 2.253% | 8.222% | 4.107% | 7.209% |
| O1 | 19.063% | 14.956% | 20.58% | 15.616% | 18.457% | 15.909% |
| O2 | 26.38% | 22.621% | 31.292% | 20.898% | 28.783% | 21.662% |
| Y1 | 13.91% | 12.484% | 13.189% | 12.605% | 13.415% | 13.134% |
| Y2 | 3.953% | 4.035% | 4.069% | 3.746% | 4.073% | 3.918% |

**Table 3-2** Proportion of pigment peak area of three strains without additional nitrogen source and (NH4)2SO4

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | C100 | | Δ*MAreA* | | OE-*MAreA* | |
| no additional nitrogen source | (NH4)2SO4 | no additional nitrogen source | (NH4)2SO4 | no additional nitrogen source | (NH4)2SO4 |
| R1 | 3.538% | 11.729% | 2.205% | 12.217% | 4.269% | 16.91% |
| R2 | 5.386% | 14.102% | 2.253% | 13.688% | 4.107% | 21.544% |
| O1 | 19.063% | 12.485% | 20.58% | 14.229% | 18.457% | 6.296% |
| O2 | 26.38% | 19.056% | 31.292% | 20.366% | 28.783% | 11.063% |
| Y1 | 13.91% | 15.193% | 13.189% | 13.676% | 13.415% | 15.93% |
| Y2 | 3.953% | 5.463% | 4.069% | 4.554% | 4.073% | 6.22% |

**Table 3-3** Proportion of pigment peak area of three strains without additional nitrogen source and NaNO3

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | C100 | | Δ*MAreA* | | OE-*MAreA* | |
| no additional nitrogen source | NaNO3 | no additional nitrogen source | NaNO3 | no additional nitrogen source | NaNO3 |
| R1 | 3.538% | 2.353% | 2.205% | 3.408% | 4.269% | 3.067% |
| R2 | 5.386% | 2.758% | 2.253% | 3.423% | 4.107% | 3.567% |
| O1 | 19.063% | 19.769% | 20.58% | 21.431% | 18.457% | 20.023% |
| O2 | 26.38% | 28.965% | 31.292% | 28.87% | 28.783% | 28.592% |
| Y1 | 13.91% | 14.579% | 13.189% | 11.303% | 13.415% | 12.524% |
| Y2 | 3.953% | 4.552% | 4.069% | 3.828% | 4.073% | 4.083% |

**Table 3-4** Proportion of pigment peak area of three strains without additional nitrogen source and Urea

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | C100 | | Δ*MAreA* | | OE-*MAreA* | |
|  | no additional nitrogen source | Urea | no additional nitrogen source | Urea | no additional nitrogen source | Urea |
| R1 | 3.538% | 28.924% | 2.205% | 26.379% | 4.269% | 27.882% |
| R2 | 5.386% | 29.508% | 2.253% | 27.362% | 4.107% | 28.643% |
| O1 | 19.063% | - | 20.58% | - | 18.457% | - |
| O2 | 26.38% | - | 31.292% | - | 28.783% | - |
| Y1 | 13.91% | 10.863% | 13.189% | 13.035% | 13.415% | 12.077% |
| Y2 | 3.953% | 3.528% | 4.069% | 4.472% | 4.073% | 4.117% |