# The screening and identification of DNA barcode sequences for *Rehmannia*

**Hongying Duan, Wanshen Wang, Yunpeng Zeng, Mengmeng Guo, Yanqing Zhou***

## Supplementary information

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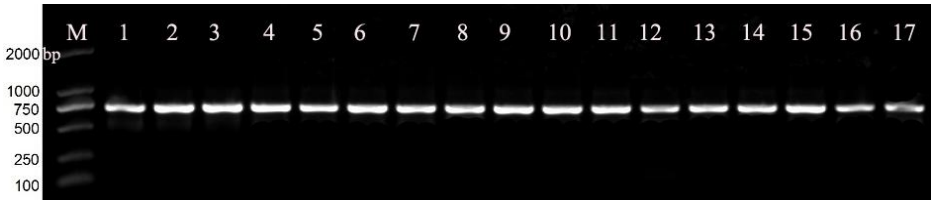


Fig.S1 PCR amplification results of ITS in *Rehmannia*

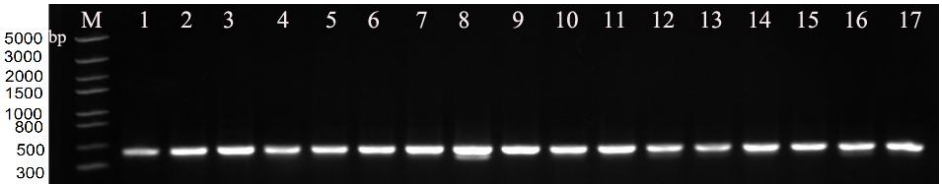


Fig.S2 PCR amplification results of ITS2 in *Rehmannia*

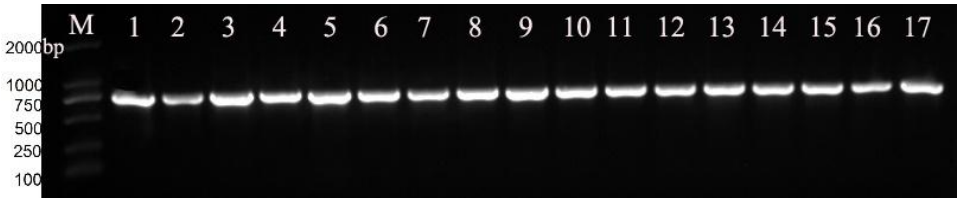


Fig.S3 PCR amplification results of *rbcL* in *Rehmannia*

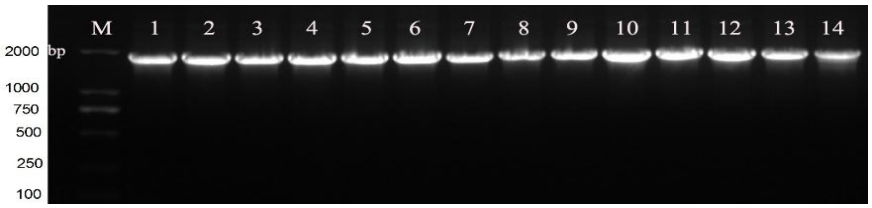


Fig.S4 PCR amplification results of *matK* in *Rehmannia*

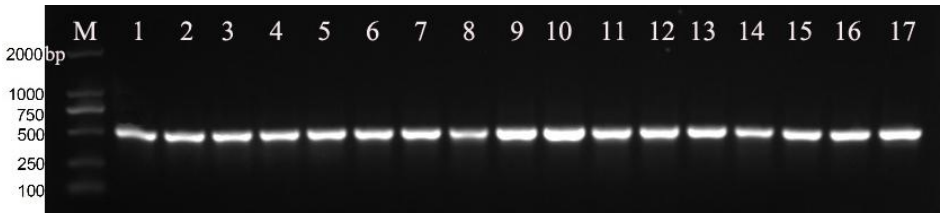


Fig.S5 PCR amplification results of *psbA-trnH* in *Rehmannia*

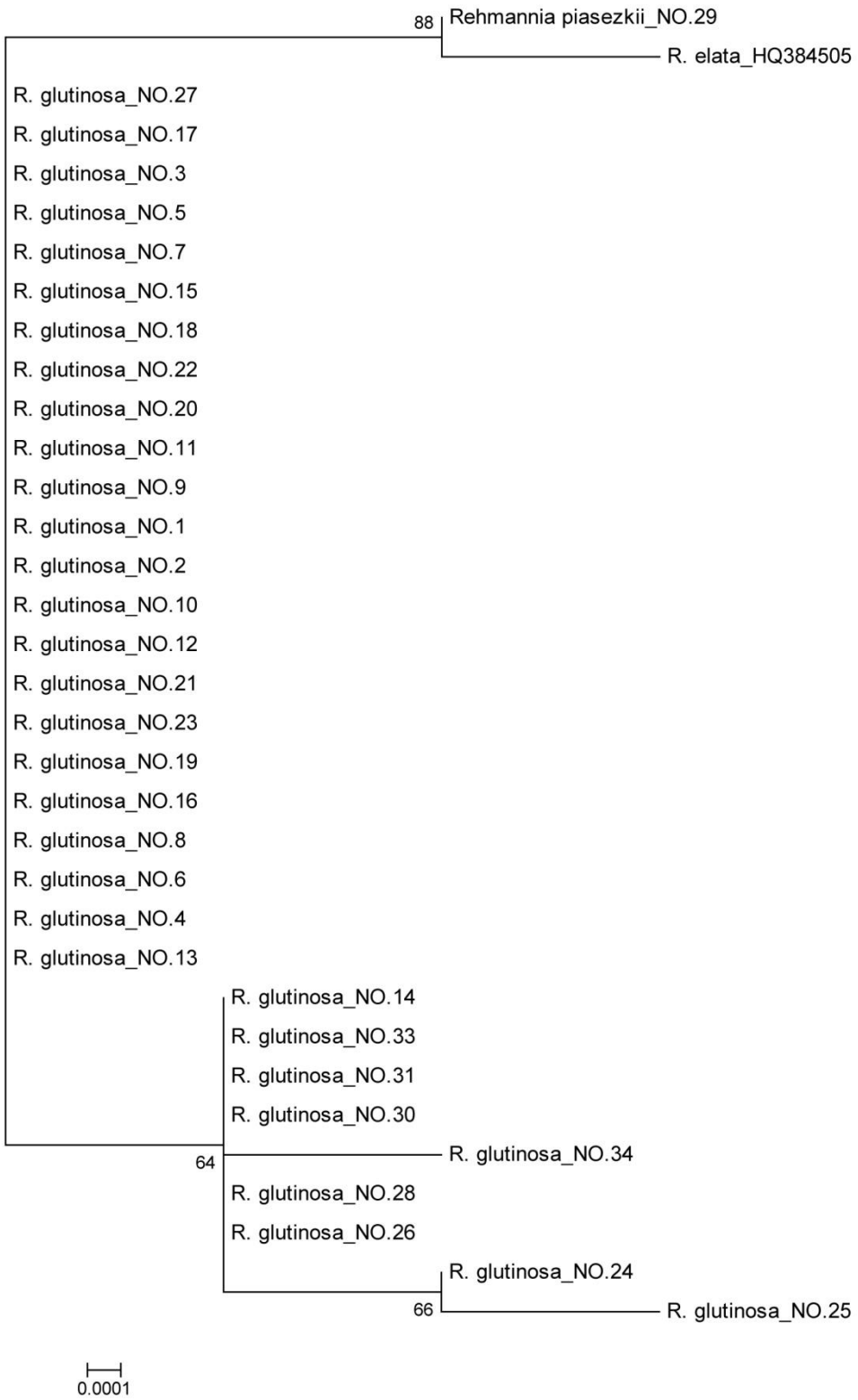


Fig.S6 Phylogenetic tree of *Rehmannia* based on *matK*

The bootstrap scores (1000 replicates) were shown (≥50%) for each branch.

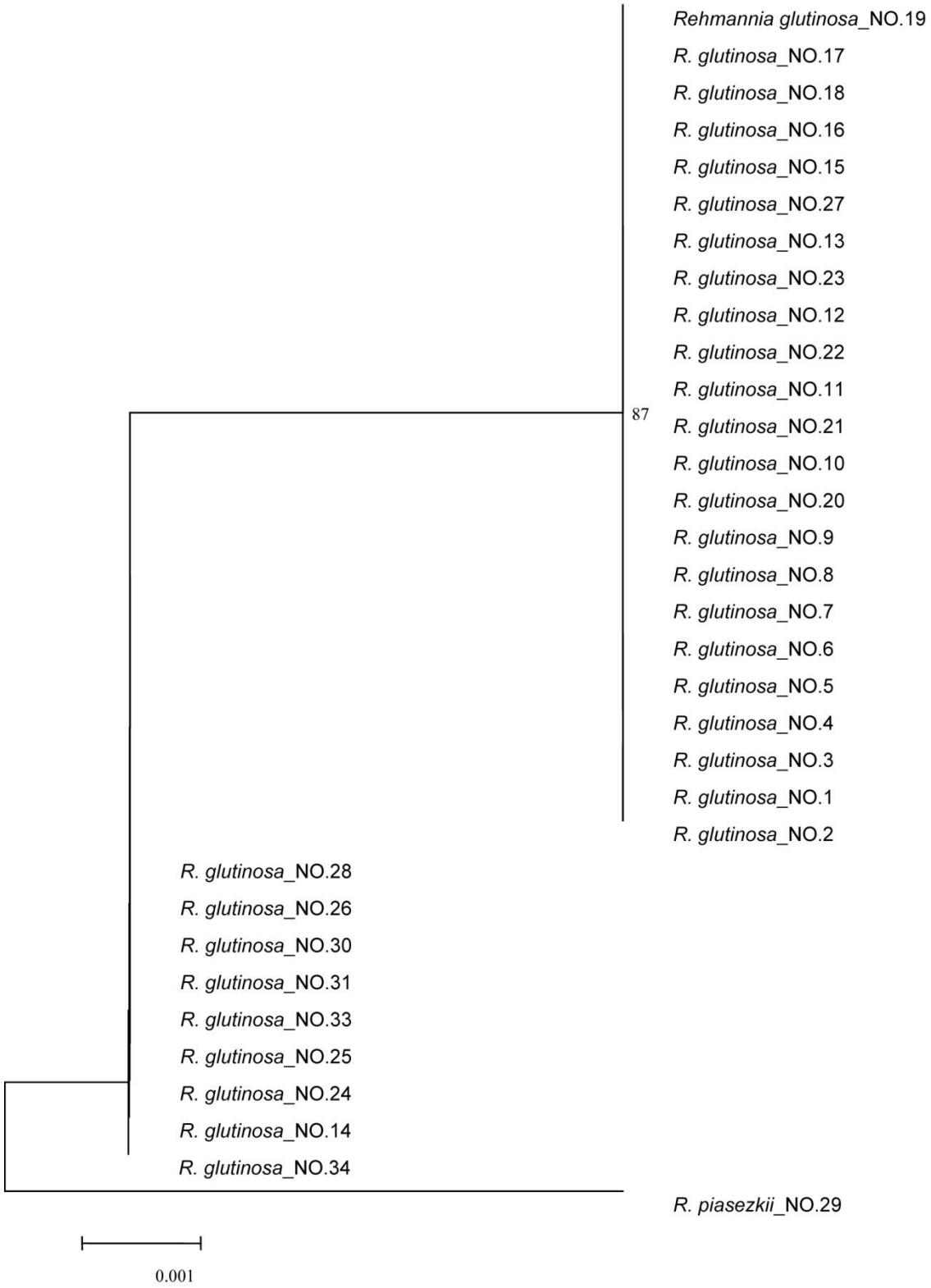


Fig.S7 Phylogenetic tree of *Rehmannia* based on *psbA-trnH*

The bootstrap scores (1000 replicates) were shown (≥50%) for each branch.

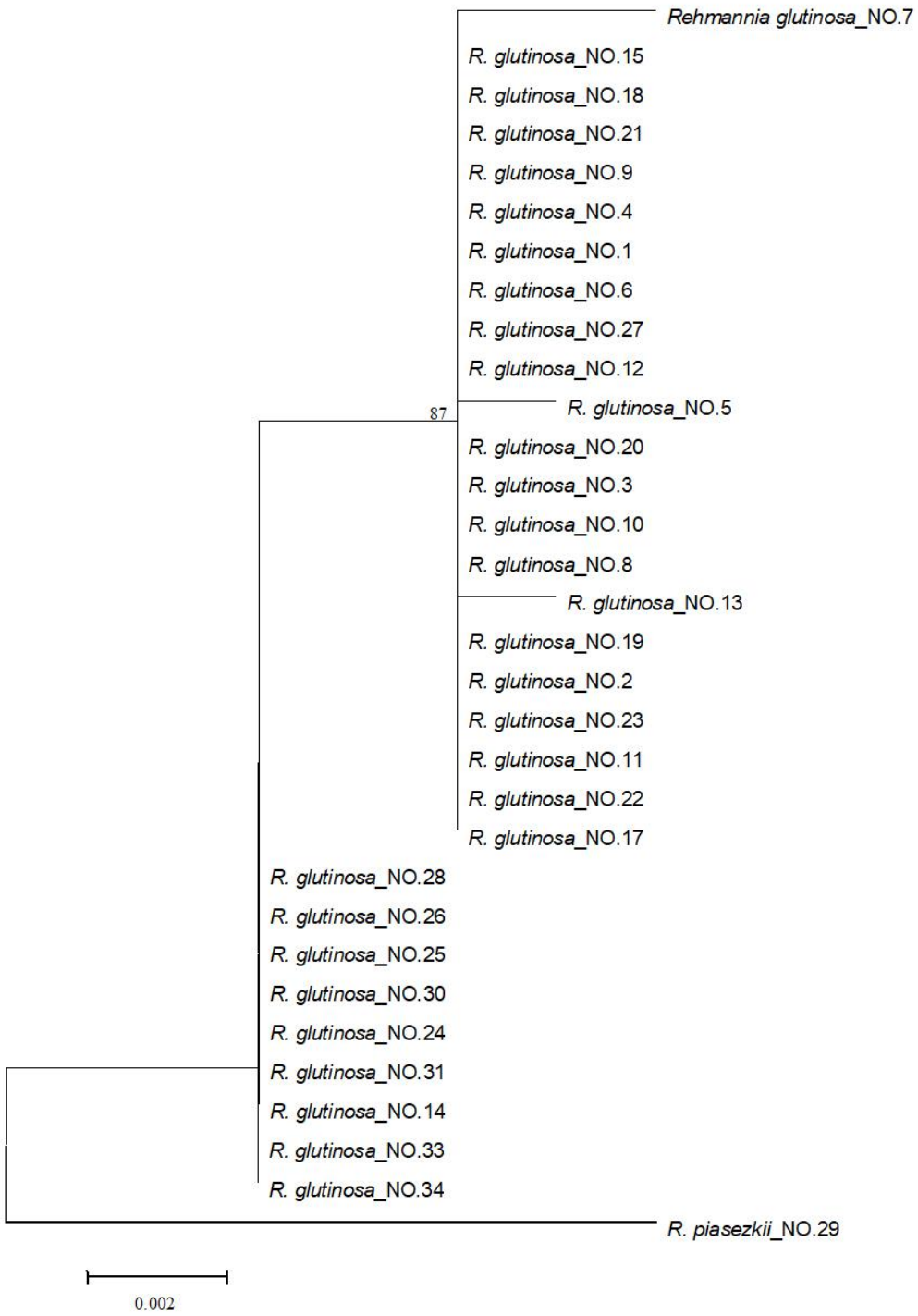


Fig.S8 Phylogenetic tree of *Rehmannia* based on ITS2+*psbA-trnH*

The bootstrap scores (1000 replicates) were shown (≥50%) for each branch.

Table S1 The efficiency of PCR amplification for candidate barcodes

| Marker | Amplification rate | Sequencing rate | Sequences obtained rate |
|---|---|---|---|
| ITS | 100 | 100 | 100 |
| ITS2 | 100 | 100 | 100 |
| *rbcL* | 100 | 100 | 100 |
| *matK* | 100 | 100 | 100 |
| *psbA-trnH* | 100 | 100 | 100 |

Table S2 The similarity comparison of candidate barcodes in *Rehmannia* by BLAST

| Marker | NO. | Species | ID | Identity% |
|---|---|---|---|---|
| ITS | 1 | *R. glutinosa* cultivar Dihuang 85-5 | EU787017 | 100 |
| | | *R. glutinosa* voucher XZ-2004-04-005 | EF363674 | 100 |
| | | *R. glutinosa* voucher HAAS-DH85-5 | EU810384 | 100 |
| | 14/28 | *R. glutinosa* voucher PS1518MT01 | FJ980430 | 100 |
| | | *R. glutinosa* cultivar No.1 Shennongshan | FJ770237 | 100 |
| | | *R. glutinosa* voucher XZ-2004-04-005 | EF363674 | 99 |
| | 29 | *R. piasezkii* voucher XZ-2004-04-001 | EF363670 | 99 |
| | | *R. piasezkii* | DQ069316 | 99 |
| *matK* | | *R. elata* | HQ384505 | 98 |
| *rbcL* | | *R. chingii* | EF544598 | 99 |
| | | *R. glutinosa* | AJ247615 | 99 |
| | | *R. elata* | HQ384874 | 100 |
| | | *R. henryi* | FJ172722 | 100 |
| | | *R. piasezkii* | FJ172721 | 99 |
| | | *R. glutinosa* | FJ172725 | 100 |

Table S3 Wilcoxon signed rank test for interspecific variations between different sequences

| $W^+$ | $W^-$ | Inter relative ranks，n，*P* value | Result |
|---|---|---|---|
| ITS2 | ITS | $W^+$=231533，$W^-$=11720，n=699，*P*=0.000 | ITS2>ITS |
| ITS | *psbA-trnH* | $W^+$=496，$W^-$=0，n=31，*P*=0.000 | ITS>*psbA-trnH* |
| *psbA-trnH* | *matK* | $W^+$=528，$W^-$=0，n=32，*P*=0.000 | *psbA-trnH*>*matK* |
| *rbcL* | *matK* | $W^+$=0，$W^-$=2145，n=65，*P*=0.000 | *rbcL*<*matK* |

Table S4 Wilcoxon signed rank test for intraspecific variations between different sequences

| $W^+$ | $W^-$ | Inter relative ranks，n，*P* value | Result |
|---|---|---|---|
| ITS2 | ITS | $W^+$=638743，$W^-$=807107，n=2151，*P*=0.000 | ITS2<ITS |
| ITS | *psbA-trnH* | $W^+$=25273.5，$W^-$=27052.5，n=465，*P*=0.593 | ITS=*psbA-trnH* |
| *matK* | *psbA-trnH* | $W^+$=242.5，$W^-$=25863.5，n=496，*P*=0.000 | *matK*<*psbA-trnH* |
| *rbcL* | *matK* | $W^+$=460，$W^-$=21068，n=496，*P*=0.000 | *rbcL*< *matK* |

Table S5 Primers used in this study

| Marker | Primer | Direction | Sequence 5’→3’ |
|---|---|---|---|
| ITS | T5 | F | GGAAGTAAAAGTCGTAACAAGG |
| | T4 | R | TCCTCCGCTTATTGATATGC |
| ITS2 | T3 | F | GCATCGATGAAGAACGCAGC |
| | T4 | R | TCCTCCGCTTATTGATATGC |
| *rbcL* | 1F | F | ATGTCACCACAAACAGAAAC |
| | 724R | R | TCGCATGTACCTGCAGTAGC |
| | 636F | F | GCGTTGGAGAGATCGTTTCT |
| | 1368R | R | CTTTCCAAATTTCACAAGCAGCA |
| *rbcL* | 5’F | F | ATGTCACCACAAACAGAAACTAAAGC |
| | z895R | R | ACCATGATTCTTCTGCCTATCAATAACTGC |
| | z674F | F | TTTATAAATCACAAGCCGAAACTGGTGAAATC |
| | 3’R | R | CTTTTAGTAAAAGATTGGGCCGAG |
| *matK* | 1F | F | ACTGTATCGCACTATGTATCA |
| | trnk2R | R | AACTAGTCGGATGGAGTAG |
| | trnk3914F | F | GGGGTTGCTAACTCAACGG |
| *psbA-trnH* | trnHf_05 | F | CGCGCATGGTGGATTCACAATCC |
| | psbA 3’f | R | GTTATGCATGAACGTAATGCTC |

Table S6 PCR reaction conditions used in this study

| Marker | Primer | Condition |
|---|---|---|
| ITS | T5 | 94℃ 3min，30cycles（94℃ 30s，46℃ 30s，72℃ 60s），72℃ |
| | T4 | 10min |
| ITS2 | T3 | 94℃ 3min，30cycles（94℃ 30s，48℃ 30s，72℃ 60s），72℃ |
| | T4 | 10min |
| *rbcL* | 5’F | 94℃ 4min，30cycles（94℃ 30s，60℃ 30s，72℃ 60s），72℃ |
| | z895R | 10min |
| | z674F | 94℃ 4min，30cycles（94℃ 30s，52℃ 30s，72℃ 60s），72℃ |
| | 3’R | 10min |
| *rbcL* | 1F | 94℃ 3min，30cycles（94℃ 30s，50℃ 30s，72℃ 1min），72℃ 10min |
| | 724R | |
| | 636f | 94℃ 3min，30cycles（94℃ 30s，50℃ 30s，72℃ 1min），72℃ 10min |
| | 1368r | |
| *matK* | 1F | 94℃ 5min，30cycles（94℃ 90s，48.6℃ 2min，72℃ 1min），72℃ 15min |
| | trnk 2R | |
| | trnk 3914F | 94℃ 4min，35cycles（94℃ 30s，48℃ 1min，72℃ 2min），72℃ 10min |
| | trnk 2R | |
| *psbA-trnH* | psbA 3’f | 94℃ 4min，35cycles（94℃ 30s，55℃ 30s，72℃ 1min），72℃ 10min |
| | trnHf_05 | |