**Supplementary Table 1** Mean Ct values of 7 candidate reference genes in different samples of *Cannabis*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gene | Different organsof wild *Cannabis*Mean Ct±SD | Different organsof cultivated *Cannabis*Mean Ct±SD | Leaves(of fivedevelopment stages) of wild *Cannabis*Mean Ct±SD | Leaves(of fivedevelopment stages)of cultivated *Cannabis*Mean Ct±SD | All samplesMean Ct±SD |
| *ACT2* | 17.96±0.90 | 18.17±0.82 | 18.24±0.62 | 18.11±0.32 | 18.12±0.69 |
| *18S rRNA* | 21.24±0.92 | 21.33±0.35 | 20.83±0.50 | 21.11±0.51 | 21.13±0.62 |
| *GAPDH* | 17.34±5.32 | 17.85±5.52 | 13.76±0.83 | 13.91±0.73 | 15.68±4.16 |
| *UBQ* | 17.86±0.87 | 17.66±0.51 | 18.99±0.32 | 18.27±0.30 | 18.19±0.74 |
| *TUB* | 17.39±2.23 | 17.68±1.49 | 19.90±0.67 | 20.05±0.58 | 18.75±1.85 |
| *PP2A* | 19.02±0.58 | 19.28±0.56 | 20.22±0.50 | 20.05±0.31 | 19.64±0.70 |
| *EF1α* | 19.77±0.47 | 19.74±0.41 | 20.11±0.46 | 19.96±0.33 | 19.89±0.44 |

SD: standard deviation.



**Figure S1** Exon-intron organization of *ACT2* in the study. The black boxes, blue and green lines in the gene structure diagram represent exons, introns and UTR, respectively. The arrows indicate the position of forward and reverse primers, and follow their number on behalf of the gene location of primer. The numbers below the gene structure refer to the size of the genomic PCR products.



**Figure S2** Amplification results of candidate genes with cDNA and genomic DNA of Cannabis, respectively. (A) Agarose electrophoresis analysis of the PCR products of seven reference genes and two target genes in *Cannabis*. M: Trans DNA Marker; Lane :1 *EF1α*, 2 *UBQ*,3 *PP2A*, 4 *ACT2*, 5 *18S rRNA*,6 *TUB*,7 *GAPDH*,8 *OLS*,9 *MDS*; (B) Amplification results of *ACT2* with 6 samples to assay RNA extraction quality. M: Trans DNA Marker; 1-3: genomic DNA as templates; 4-6: cDNA added 10ng genomic DNA as templates.

A:





 



B:

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Gene | *ACT2* | *18S rRNA* | *GAPDH* | *UBQ* | *TUB* | *PP2A* | *EF1α* |
| Dissociation temperature | 76.9 | 78.4 | 76.6 | 82.0 | 80.2 | 79.3 | 81.4 |

**Figure S3** Dissociation curve of seven candidate reference genes(A) and temperature (B) of candidate reference genes.