**Optimized sampling protocol for mass spectrometry-based Metabolomics in *Streptomyces***

List of supplementary Tables in supplementary file 1

**Table S1.** Details of quenching solutions and extraction procedures. Q1-Q5 denotes 5 quenching experiments and E1-E3 denotes 3 extraction procedures.

**Table S2**. The gradient and flow rate for the determination of nucleotides.

**Table S3.** The gradient and flow rate for the determination of coenzymes.

**Table S4.** Reproducibility is assessed by %RSD in different extraction and quenching conditions.

**Table S5.** Intracellular metabolites in *Streptomyces* ZYJ-6. Based on IsoaB and Meth60 as the quenching solution and thawing-freezing (TF) in liquid nitrogen and cryostat at -30℃ within 50%(v/v) methanol for three cycles as extraction solutions. IsoaB: isoamylol with base solution 5:1(v/v) at -30℃ and Meth60: 60% methanol at -30℃, respectively. Base solution was made up of acetone: ethanol= 1:1 (mol/mol). Multiples were calculated by IsoaB/Meth60.

**Table S1**

Details of quenching solutions and extraction procedures. Q1-Q5 denotes 5 quenching experiments and E1-E3 denotes 3 extraction procedures.

|  |  |  |
| --- | --- | --- |
| Numbers | Quenching solution A:B=5:1, v/v | Extraction |
|  | A | B | Abbreviation |  | Abbreviation |
| Q1 | Acetone | Acetone: ethanol=1:1, mol/mol | AceB | Thawing-Freezing | TF |
| Q2 | Isoamylol | Acetone: ethanol=1:1, mol/mol | IsoaB | Thawing-Freezing | TF |
| Q3 | Propanol | Acetone: ethanol=1:1, mol/mol | ProB | Thawing-Freezing | TF |
| Q4 | Methanol | Acetone: ethanol=1:1, mol/mol | MethB | Thawing-Freezing | TF |
| Q5 | Methanol 60%(v/v) | Methanol 60%(v/v) | Meth60 | Thawing-Freezing | TF |
| E1 | Isoamylol | Acetone: ethanol=1:1, mol/mol | IsoaB | Boiling ethanol | BE |
| E2 | Isoamylol | Acetone: ethanol=1:1, mol/mol | IsoaB | Thawing-Freezing | TF |
| E3 | Isoamylol | Acetone: ethanol=1:1, mol/mol | IsoaB | Grinding | G |

**Table S2**

The gradient and flow rate for the determination of nucleotides.

|  |  |  |  |
| --- | --- | --- | --- |
| Time  | A | B | Rate |
| [min] | [%] | [%] | [mL/min] |
| 0 | 90 | 10 | 0.1 |
| 5 | 77 | 23 | 0.1 |
| 8 | 77 | 23 | 0.1 |
| 8.1 | 90 | 10 | 0.2 |
| 19 | 90 | 10 | 0.2 |
| 19.1 | 90 | 10 | 0.1 |
| 20 | 90 | 10 | 0.1 |

**Table S3**

The gradient and flow rate for the determination of coenzymes.

|  |  |  |  |
| --- | --- | --- | --- |
| Time  | A | B | Rate |
| [min] | [%] | [%] | [mL/min] |
| 0 | 90 | 10 | 0.2 |
| 5 | 60 | 40 | 0.2 |
| 8 | 60 | 40 | 0.2 |
| 10 | 90 | 10 | 0.2 |
| 15 | 90 | 10 | 0.2 |

**Table S4**

Reproducibility is assessed by %RSD in different extraction and quenching conditions.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | IsoaB | ProB | MethB | AceB | Meth60 | BE | TF | G |
| AA | 2.17  | 2.57  | 4.80  | 7.97  | 3.28  | 1.32  | 1.64  | 1.74  |
| OA | 12.81  | 12.70  | 13.93  | 17.11  | 14.78  | 9.64  | 5.08  | 7.85  |
| PS | 15.49  | *16.75*  | 15.29  | 16.62  | 17.62  | 15.46  | 10.34  | 14.96  |
| SA | 6.93  | 7.58  | 12.15  | 8.52  | 8.06  | 6.30  | 5.84  | 6.02  |
| Average | 9.35  | 9.90  | 11.54  | 12.55  | 10.94  | 8.18  | 5.72  | 7.64  |

**Table S5**

Intracellular metabolites in *Streptomyces* ZYJ-6. Based on IsoaB and Meth60 as the quenching solution and thawing-freezing (TF) in liquid nitrogen and cryostat at -30℃ within 50%(v/v) methanol for three cycles as extraction solutions. IsoaB: isoamylol with base solution 5:1(v/v) at -30℃ and Meth60: 60% methanol at -30℃, respectively. Base solution was made up of acetone: ethanol= 1:1 (mol/mol). Multiples were calculated by IsoaB/Meth60.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Number | Metabolites | IsoaB | Meth60 | Multiples |
| [-] | [-] | [μmol gDCW-1] | [μmol gDCW-1] | [-] |
| 1 | 6PG | 6.73  | 1.58  | 4.26  |
| 2 | AceCoA | 0.44  | 0.60  | 0.73  |
| 3 | ADP | 0.89  | 0.65  | 1.36  |
| 4 | aKG | 57.24  | 4.96  | 11.54  |
| 5 | AMP | 3.80  | 5.27  | 0.72  |
| 6 | Asn | 15.29  | 3.71  | 4.12  |
| 7 | Asp | 5.05  | 2.49  | 2.03  |
| 8 | ATP | 4.94  | 0.13  | 38.40  |
| 9 | Cit | 2.84  | 0.88  | 3.23  |
| 10 | CoA | 21.60  | 12.67  | 1.70  |
| 11 | Ery | 7.25  | 0.62  | 11.67  |
| 12 | F6P | 3.12  | 1.72  | 1.82  |
| 13 | FBP | 1.65  | 0.70  | 2.35  |
| 14 | Fum | 6.51  | 1.18  | 5.51  |
| 15 | G3P | 5.63  | 2.76  | 2.04  |
| 16 | G6P | 6.95  | 3.92  | 1.77  |
| 17 | Gln | 170.85  | 38.22  | 4.47  |
| 18 | Glu | 211.34  | 114.51  | 1.85  |
| 19 | Gly | 17.75  | 8.93  | 1.99  |
| 20 | Ile | 50.28  | 7.25  | 6.94  |
| 21 | IMP | 2.54  | 1.82  | 1.39  |
| 22 | Leu | 33.49  | 4.89  | 6.86  |
| 23 | M6P | 9.96  | 6.06  | 1.64  |
| 24 | Mal | 4.84  | 0.07  | 72.05  |
| 25 | Manl | 94.25  | 89.74  | 1.05  |
| 26 | Met | 0.36  | 0.16  | 2.21  |
| 27 | Oaa | 26.05  | 3.24  | 8.04  |
| 28 | Orn | 21.17  | 4.37  | 4.85  |
| 29 | OX | 89.01  | 2.22  | 40.02  |
| 30 | PEP | 0.13  | 0.08  | 1.67  |
| 31 | Phe | 5.03  | 1.22  | 4.11  |
| 32 | Pro | 24.06  | 2.71  | 8.89  |
| 33 | Pyr | 4.60  | 1.05  | 4.38  |
| 34 | R5P | 22.61  | 1.50  | 15.04  |
| 35 | RL5P | 8.55  | 1.08  | 7.94  |
| 36 | S7P | 5.77  | 2.94  | 1.96  |
| 37 | SucCoA | 0.04  | 0.06  | 0.63  |
| 38 | Ser | 14.49  | 3.67  | 3.95  |
| 39 | Ska | 0.38  | 0.17  | 2.19  |
| 40 | Suc | 8.11  | 1.39  | 5.82  |
| 41 | T6P | 0.84  | 0.36  | 2.30  |
| 42 | Thr | 65.69  | 8.97  | 7.32  |
| 43 | Val | 96.67  | 11.34  | 8.53  |
| 44 | Xyl | 0.27  | 0.04  | 6.26  |