

	plant NGM 2	plant NGM 2 Pro	plant NGM 3 Pro
platform	OE 120, Thermo	OE 120, Thermo	Orbitrap Astral, Thermo
column	C18	C18+Amide	C18+Amide
internal standard	6	6	6
database (inhouse)	20,000+	20,000+	20,000+
database (Public library)	20,000+	20,000+	20,000+
database (MetDNA3 theoretical DB)	47,000+	76,000+	76,000+
annotation algorithms	(MetDNA 3)	(MetDNA 3)	(MetDNA 3)
discovery of unknown metabolites	NO	YES	YES
targeted custom compounds*	NO	YES	YES
identification confidence level	MSI, level 1-3.1	MSI, level 1-3.2	MSI, level 1-3.2
quality control (QC)	1. The peak height difference of internal standard is within 30%. 2. No obvious internal standard detected in blank samples. 3. RSD of internal standard response in QC samples < 10%. 4. QC samples cluster in two-dimensional PCA plot. 5. The distribution of QC samples is within 2-fold STD for PCA1. 6. The distribution of QC samples is within 2-fold STD for PCA2. 7. Intra-batch QC correlation > 0.85.	1. The peak height difference of internal standard is within 30%. 2. No obvious internal standard detected in blank samples. 3. RSD of internal standard response in QC samples < 5%. 4. QC samples cluster in two-dimensional PCA plot. 5. The distribution of QC samples is within 2-fold STD for PCA1. 6. The distribution of QC samples is within 2-fold STD for PCA2. 7. Intra-batch QC correlation > 0.95. 8.Retention time error of internal standard for RTQC within 30 s. 9.R ² > 0.9 after RTQC correction.	1. The peak height difference of internal standard is within 30%. 2. No obvious internal standard detected in blank samples. 3. RSD of internal standard response in QC samples < 5%. 4. QC samples cluster in two-dimensional PCA plot. 5. The distribution of QC samples is within 2-fold STD for PCA1. 6. The distribution of QC samples is within 2-fold STD for PCA2. 7. Intra-batch QC correlation > 0.95. 8.Retention time error of internal standard for RTQC within 30 s. 9.R ² > 0.9 after RTQC correction.
number of detected metabolites (wheat leaves)	#level 1+2+3 intotal 2300+	#level 1+2+3 intotal 5600+	#level 1+2+3 intotal 9900+
	#level 1>1200	#level 1>2200	#level 1>2900

Level 1: Metabolites in samples match authentic standards in MS1, MS2 and retention time (RT).

Level 2: Metabolites in samples match public databases with respect to MS1 and MS2.

Level 3: Metabolites in samples match theoretical databases for MS1, MS2 and predicted retention time (RT).

°C

Level 3.1: Predicted known metabolites Level

3.2: Predicted novel metabolites Level

4.1: Metabolites identified solely by MS1 Level

4.2: Unknown compounds

* When clients can provide Biotree with a list of metabolites of interest, then Biotree scientists configure the instrumental method during testing to specifically monitor the peaks of these compounds.