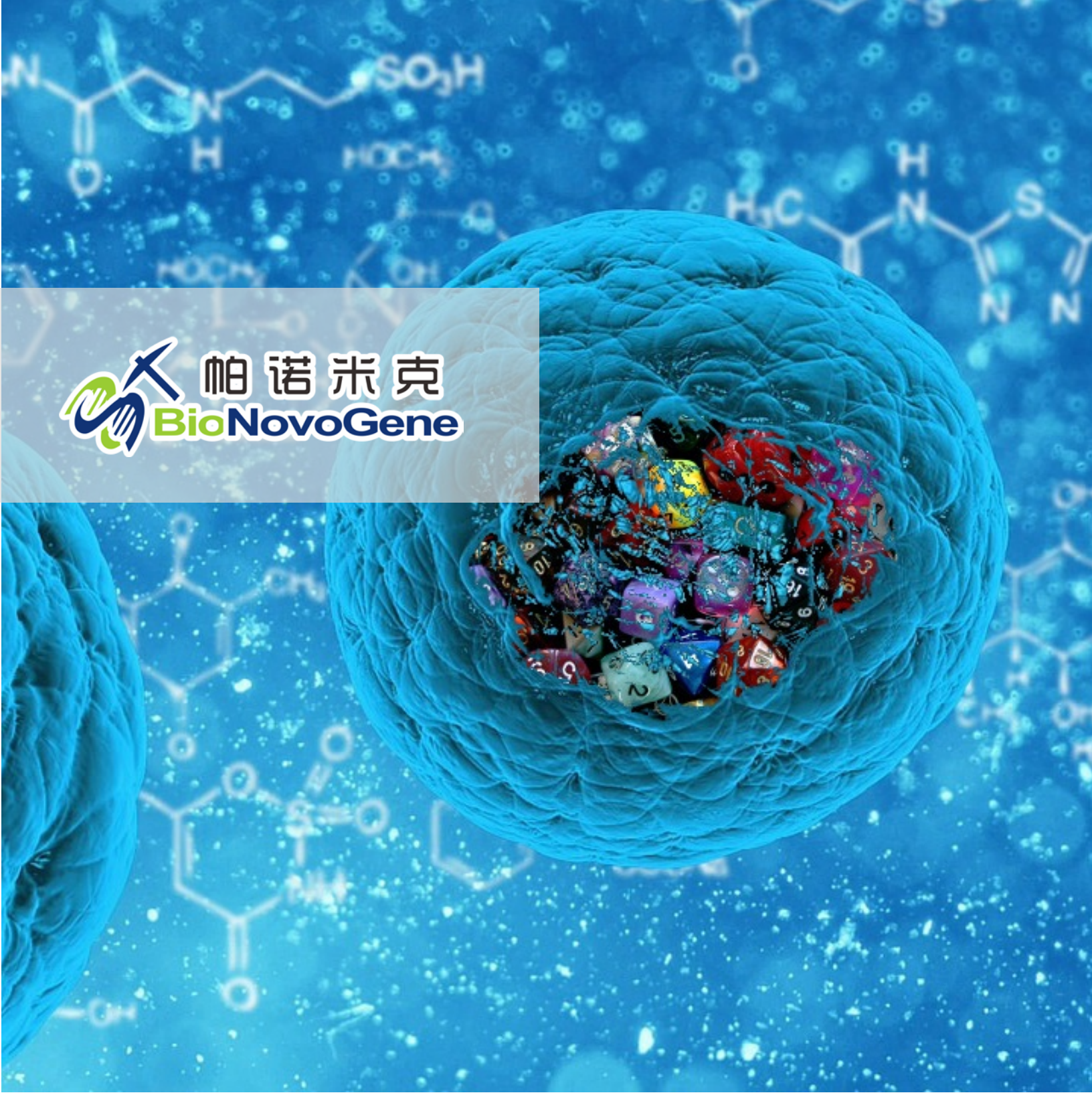


帕诺米克
BioNovoGene

代谢组学检测报告

第一部分 实验报告

项目单位：

联系人：

项目编号：

报告日期：

目　录

实验流程

帕诺米克自客户处收到样本以后，首先对样本进行质检；经确认符合送样要求后，随机抽取单个样本进行预实验，生成预实验报告；根据预实验结果调整实验方法，进行正式实验和生物信息学分析，最后生成最终的正式实验分析报告。

具体检测步骤如下所示：

1. 收到样本
↓
2. 样本质检
↓
3. 预实验
↓
4. 正式检测实验
↓
5. 实验报告
↓
6. 生物信息学分析
↓
7. 数据分析报告

实验报告负责人

代谢物提取：　【刘莹璐】
LC-MS 检测：　【谢丽丽】
报告撰写：　【谢丽丽】
报告质检：　【王艳丽】

请将与本报告相关的问题或建议通过电子邮件的方式发送至：project@bionovogene.com
或直接致电：　(0512) 6295 9105

试剂与仪器

代谢组学检测过程中所用试剂与仪器如以下表 1、表 2、表 3 所示。

为确保检测过程的可靠性与数据准确性，帕诺米克在进行代谢组学检测时所采用的试剂与仪器均为市场上性能优秀的产品。

表 1. 代谢组学检测主要试剂

名称	CAS	纯度	品牌
甲醇	67-56-1	≥99.0%	Thermo
乙腈	75-05-8	≥99.9%	Thermo
甲酸	64-18-6	LC-MS grade	TCI
甲酸铵	540-69-2	≥99.9%	Sigma
ddH2O			Millipore
L-Valine (1-13C)	81201-85-6	≥98%	CIL
L-Phenylalanine (1-13C)	81201-86-7	≥98%	CIL
Uracil (2-13C)	35803-45-3	≥98%	CIL
D-Fructose (1-13C)	108311-21-3	≥98%	CIL
Vitamin B3 (nicotinic acid, d4)	66148-15-0	≥98%	CIL
Choline chloride (trimethyl-d9)	61037-86-3	≥98%	CIL
N-(Carboxymethyl)-N,N,N-trimethyl-d9-ammonium Chloride	285979-85-3	99 atom % D	CDN
L-carnitine-d3 HCl (N-methyl-d3)	350818-62-1	99 atom % D	CDN

表 2. 代谢物提取仪器及设备

名称	品牌	型号
冷冻离心机	Eppendorf	H1650-W
混匀仪	Vortex Mixer	QL-866
真空浓缩仪	eppendorf	5305
滤膜	Jin Teng	0.22μm PTFE

表 3. 代谢组学检测仪器 LC-MS

名称	品牌	型号
液相色谱仪	Thermo	Vanquish
质谱仪	Thermo	Q Exactive

样本信息

样本采用全程冷链运输方式由客户处运送至我方实验室。

我公司共收到来自客户的【52】例血清样本，经核实确认，均符合送样要求，送样要求详情请参见《样品签收报告》。

实验方法

NIST 血清标准曲线校正溶液 [1-2]

1. 精确移取 NIST 血清 5，10，50，100，200，300 μL 一系列浓度梯度，按照表 4 方法加入补充液、内标和蛋白沉淀试剂；

2. 上述 6 个 NIST 样本充分混匀，12000 rpm 4 ℃离心 10 min，取全部上清浓缩干燥后，残渣中加入 150 μL 80%甲醇溶液（-20 ℃）复溶 NIST 样品，充分混匀后 12000 rpm 4 ℃再次离心 10 min，取上清液作为 NIST 标准曲线校正溶液。

表 4 NIST 血清标准曲线校正溶液的加样方法

编号	取样量(μL)	补充液 ddH2O(μL)	内标混标 (μL)	甲醇 (μL)（-20℃）	上清液 (μL)
NIST-1	5	95	100	400	500
NIST-2	10	90	100	400	500
NIST-3	50	50	100	400	500
NIST-4	100	0	100	400	500
NIST-5	200	0	100	800	1000
NIST-6	300	0	100	1200	1500

1. Transfer 5, 10, 50, 100, 200, 300 μL of NIST serum into centrifuge tubes. Add ddH2O, mixed internal standard solution and methanol according to Table. 4;

2. Vortex the six NIST serum, respectively. Centrifuge at 4 ℃ for 10 min at 12 000 rpm, and then transfer all the supernatant from each sample into another 2 mL centrifuge tube. Samples were concentrated to dry in vacuum. Dissolve samples with 150 μL of 80% methanol solution (-20 ℃), and centrifuge at 4 ℃ for 10 min at 12 000 rpm again to obtain the supernatant for LC-MS.

代谢物提取 [3-5]

1. 将所有样本在 4 ℃下融化【样品量不足按照等比例缩减】；
2. 精确移取血清 100 μL 于 2 mL 离心管中；
3. 加入 100 μL 内标混标和 400 μL 甲醇（-20℃），涡旋振荡 1 min；
4. 12000 rpm 4 ℃离心 10 min，取上清液 500 μL 至 2 mL 离心管中真空浓缩干燥；
5. 150 μL 80%甲醇溶液复溶，充分混匀后 12000 rpm 4 ℃再次离心 10 min，取上清液作为待测样本；
6. 自每个待测样本各取 20 μL 混合成 QC 样本，见图 1；（QC：quality control，用来校正混合样品分析结果的偏差以及由于分析仪器自身原因所造成的失误）
7. 用剩余待测样本进行 LC-MS 检测。

1. Thaw all samples at 4 ℃【The insufficient samples are reduced to an equal scale】;
2. Transfer 100 μL of each sample into 2 mL centrifuge tubes;
3. Add 100 μL of mixed internal standard solution and 400 μL of methanol (pre-cooled at -20 ℃), then vortex for 60 s;
4. Centrifuge at 4 ℃ for 10 min at 12000 rpm, and then transfer 500 μL of the supernatant from each sample into another 2 mL centrifuge tube. Samples were concentrated to dry in vacuum;
5. Dissolve samples with 150 μL of 80% methanol solution, and centrifuge at 4 ℃ for 10 min at 12000 rpm again to obtain the supernatant for LC-MS;
6. Take 20 μL from each sample to the quality control (QC) samples (Fig. 1)*;
(These QC samples were used to monitor deviations of the analytical results from these pool mixtures and compare them to the errors caused by the analytical instrument itself)
7. Use the rest of the samples for LC-MS detection.

上机检测

色谱条件

仪器采用 Thermo Vanquish，使用 ACQUITY UPLC® HSS T3 1.8 μm（2.1×150 mm）色谱柱，自动进样器温度设为 8 ℃，以 0.25 mL/min 的流速，40 ℃的柱温，进样 2 μL 进行梯度洗脱，流动相为正离子 0.1%甲酸水（A1）- 0.1%甲酸乙腈（B1）；负离子 5 mM 甲酸铵水（A3）-乙腈（B3）。梯度洗脱程序为 0~1 min，2% B1/B3；1~9 min，2%~50% B1/B3；9~12 min，50%~98% B1/B3；12~13.5 min，98% B1/B3；13.5~14 min，98%~2% B1/B3；14~20 min，2% B1 -正模式（14~17 min，2% B3 -负模式）[6]。

Chromatographic separation was accomplished in an Thermo Vanquish system equipped with an ACQUITY UPLC® HSS T3 (150 ×2.1 mm, 1.8 μm, Waters) column maintained at 40 ℃. The temperature of the autosampler was 8 ℃. Gradient elution of analytes was carried out with 0.1% formic acid in water (A1) and 0.1% formic acid in acetonitrile (B1) or 5 mM ammonium formate in water (A3) and acetonitrile (B3) at a flow rate of 0.25 mL/min. Injection of 2 μL of each sample was done after equilibration. An increasing linear gradient of solvent A (v/v) was used as follows: 0~1 min, 2% B1/B3; 1~9 min, 2%~50% B1/B3; 9~12 min, 50%~98% B1/B3; 12~13.5 min, 98% B1/B3; 13.5~14 min, 98%~2% B1/B3; 14~20 min, 2% B1-positive model (14~17 min, 2% B3-negative model).

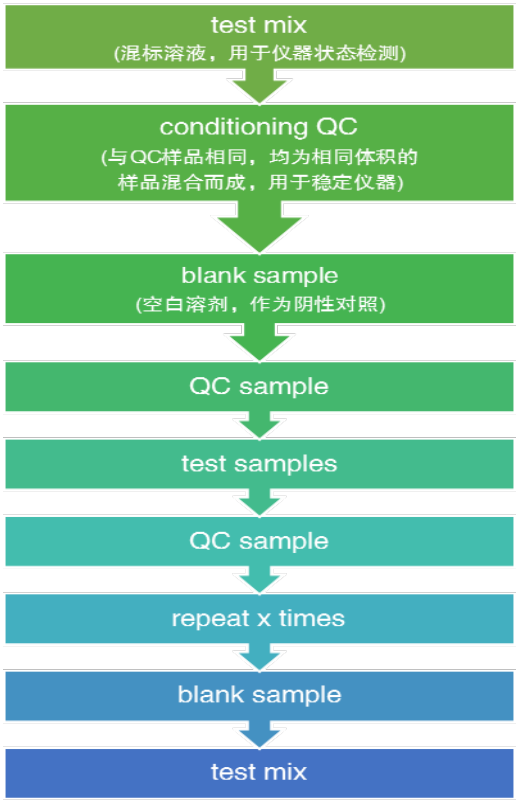
质谱条件

仪器使用 Thermo Q Exactive，电喷雾离子源（ESI），正负离子电离模式，正离子喷雾电压为 3.50 kV，负离子喷雾电压为 2.50 kV，鞘气 30 arb，辅助气 10 arb。毛细管温度 325 ℃，以分辨率 70 000 进行全扫描，扫描范围 81~1 000，并采用 HCD 进行二级裂解，碰撞电压为 30 eV，同时采用动态排除去除无必要的 MS/MS 信息[6]。

The ESI-MSn experiments were executed on the Thermo Q Exactive mass spectrometer with the spray voltage of 3.8 kV and -2.5 kV in positive and negative modes, respectively. Sheath gas and auxiliary gas were set at 30 and 10 arbitrary units, respectively. The capillary temperature was 325 ℃. The analyzer scanned over a mass range of m/z 81-1 000 for full scan at a mass resolution of 70 000. Data dependent acquisition (DDA) MS/MS experiments were performed with HCD scan. The normalized collision energy was 30 eV. Dynamic exclusion was implemented to remove some unnecessary information in MS/MS spectra.

LC-MS 检测进样顺序 [7]

检测分析结果请见《数据分析报告》



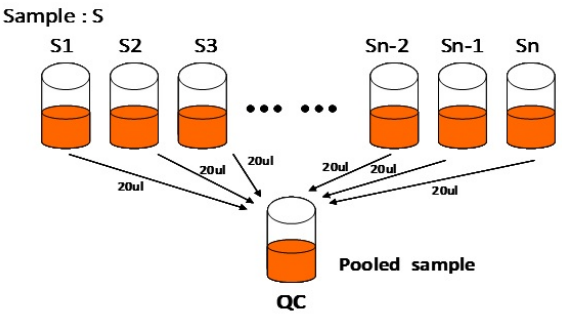


图 1. QC 样本由每个待测样本中各取 20 μL 混合得到，用于校正混合样品分析结果的偏差以及由于分析仪器自身原因所造成的失误

参考文献

1. Chen Y, Zhou Z, Yang W, et al. Development of a data-independent targeted metabolomics method for relative quantification using liquid chromatography coupled with tandem mass spectrometry[J]. Anal Chem, 2017, 89(13): 6954-6962.
2. Dunn W B, Broadhurst D, Begley P, et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry[J]. Nature Protocols, 2011, 6(7):1060-83.
3. Dunn, Warwick B. et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nature Protocols. 6, 1060-1083 (2011).
4. Zelena, Eva. et al. Development of a Robust and Repeatable UPLC-MS Method for the Long-Term Metabolomic Study of Human Serum. Analytical Chemistry. 81, 1357-64 (2009).
5. Sangster, T., Major, H., Plumb, R., Wilson, A. J., & Wilson, I. D. A pragmatic and readily implemented quality control strategy for HPLC-MS and GC-MS-based metabonomic analysis. Analyst. 131, 1075-1078 (2006).
6. The HUSERMET Project. Analysis of Serum Samples by UPLC-MS. 2008.
7. Want, E. J. et al. Global metabolic profiling of animal and human tissues via UPLC-MS. Nature Protocols. 8, 17-32 (2013).

苏州帕诺米克生物医药科技有限公司

地址：苏州市工业园区新平街 388 号 22 幢 2 层

网站：www.bionovogene.com

电话：(0512) 6295 9105