MALBAC® 单细胞全基因组扩增试剂盒

【产品名称】

通用名：MALBAC® 单细胞全基因组扩增试剂盒

英文名：MALBAC® Single Cell WGA Kit

【规格及货号】

规格	货号
10 测试/盒	KT110700110
50 测试/盒	KT110700150

【预期用途】

本产品可用于单细胞或其他微量样本中 DNA 提取和全基因组扩增，扩增产物可用于实时定量 PCR、高通量测序等技术平台，也可用于多种下游实验如：基因点突变分析检测，单核苷酸多态性（SNP）基因分型，基因拷贝数（CNV）分析，微阵列比较基因组杂交（Array CGH），SNP 芯片技术。

【实验原理】

本产品可实现高度均一的全基因组扩增，其核心技术为多次退火环状循环扩增（MALBAC）技术，该技术利用独特的具有链置换活性的 DNA 聚合酶进行准线性的全基因组预扩增，再通过 PCR 技术进行指数式扩增，为下游分析提供充足的实验材料。

【主要组成成分】

组分	标签名	管盖颜色	规格	
			10 测试	50 测试
细胞裂解液	Cell Lysis Buffer	蓝色	50 μL×1 管	250 μL×1 管
细胞裂解酶	Cell Lysis Enzyme	蓝色	5 μL×1 管	25 μL×1 管
预扩增缓冲液	Pre-Amp Buffer	绿色	300 μL×1 管	750 μL×2 管
预扩增酶	Pre-Amp Enzyme	绿色	10 μL×1 管	50 μL×1 管
扩增缓冲液	Amplification Buffer	红色	300 μL×1 管	750 μL×2 管
扩增酶	Amp Enzyme	红色	8 μL×1 管	40 μL×1 管

注：同一组分不同批号不能混用。

【自备物品】

1. 试剂：无核酸酶水、1×PBS 缓冲液。
2. 仪器：PCR 仪、微量分光光度计、混匀仪、微型离心机。

【储存条件及有效期】

试剂盒于-25～-15℃保存；可以按需求分装保存，避免反复冻融。

有效期为 24 个月。生产日期和失效日期见外包装盒。

【产品特点】

1. 单细胞经扩增后可获得 2～5 μg 的 DNA 产物。
2. 单管操作、3 步完成、仅需 4 小时、中间产物无需纯化。
3. 可从流式分选出的细胞或 0.5 pg 级 DNA 中扩增出全基因组 DNA，且成功率达 95%以上。
4. 可在 AT-GC 富集区得到准确、高重复度的连续扩增结果。
5. 全基因组覆盖度高，仅存在<10%的等位基因丢失。

【适用领域】

MALBAC 单细胞全基因组扩增试剂盒的适用范围十分广泛，多种原始材料和微量样本均可通过该试剂盒进行全基因组扩增，包括基因组 DNA、新鲜或干燥的血液、新鲜或冷冻的组织、司法痕量物证等材料。本产品适用的领域有：

1. 人和动物生物学研究：生物标志物研究（CNVs、SNVs）；体外受精胚胎植入前基因筛查和诊断（PGS/PGD）；转基因动物基因分型；胚胎和干细胞单精子、卵细胞研究，神经元细胞等研究。
2. 肿瘤研究：体细胞遗传变异分析；肿瘤进化和发展；肿瘤干细胞；循环肿瘤细胞(CTCs)。

上海亿康医学检验所有限公司
电话：400-921-3918
官网：www.yikongenomics.com
地址：上海市奉贤区望园路1698弄聚惠领域26号

3. 微生物研究：宏基因组学研究；微生物基因分型。

【样本要求】

1. 样本起始量：本试剂盒是针对单细胞的全基因组扩增设计的，同时适用于起始模板量为单染色体或范围在 0.5 pg～1 ng 级的微量基因组 DNA。
2. 样本收集方式：通过流式细胞仪分选，缓冲液稀释，显微操作和激光显微切割等方式获得的单细胞均可利用本试剂盒进行全基因组扩增。此外，本试剂盒也适用于经过多聚甲醛固定或激光显微切割的微量的组织细胞样本。
3. 样本清洗：为避免细胞准备过程中外源 DNA 的污染，建议在实验前对细胞进行清洗，清洗液为不含 Mg^{2+} 和 Ca^{2+}的 1×PBS 溶液，需注意，确保后续实验中的 PBS 溶液的体积不超过 2 μL。

【实验准备】

1. 预扩增样本准备区的隔离

- 为避免样本受外界因素干扰或 DNA 扩增产物的污染，在扩增前，预扩增样本的准备过程需在单独隔离的实验室或专门工作区完成，并预备专用的实验材料和仪器，例如，移液器、移液管、PCR 管、1.5 mL 的离心管、离心管架、离心机、涡旋混匀仪和实验服等。
- 请将 DNA 扩增产物与预扩增试剂分开储存；所有的下游分析处理，如 DNA 纯化、测序前的准备工作，请在另一实验室中进行。

2. 对照组 DNA 样本(5 μL) 配制

- 阳性对照配制：用无核酸酶水将 DNA 存储液稀释为 30 pg/μL 的 DNA，取 30 pg/μL DNA 溶液 1 μL，加入含 4 μL Cell Lysis Buffer 的 PCR 管中，作为阳性对照。
- 阴性对照配制：取 1 μL 无核酸酶水，加入到含 4 μL Cell Lysis Buffer 的 PCR 管中，作为阴性对照。

【使用方法】

使用前注意：

1. 使用前请将细胞裂解酶、预扩增酶和扩增酶置于冰浴中，其他组分请在用前置于冰上解冻。
2. 由于单细胞全基因组扩增实验全程在同一 PCR 管中进行且反应体积较小，因此加液操作时，移液器吸头不要接触管中液体，避免将单细胞或 DNA 裹挟出反应体系。
3. 移液时，请沿管壁小心添加，勿吹打 PCR 管中液体。
4. 反应前，请进行短暂离心（微型离心机，2000 转，3-4 秒），确保反应体系中的液体混和均匀。

步骤一：细胞裂解

1. 根据反应的数量 N，按照下表制备细胞裂解混合液：

试剂名称	体积
Cell Lysis Buffer	5 μL×（N+1）
Cell Lysis Enzyme	0.5 μL×（N+1）
总体积	5.5 μL×（N+1）

2. 将单细胞收集在含有 5 μL 细胞裂解混合液的 PCR 管中（含有单细胞样本的 PBS 溶液体积不得超过 2 μL）。
3. 在预热的 PCR 仪中孵育样本，按照下表设置反应参数：

循环	温度	时间
1	50℃	50 min
	80℃	10 min
	4℃	Forever

步骤二：MALBAC 预扩增

4. 根据反应的数量 N，按照下表制备预扩增混合液。

试剂名称	体积
Pre-Amp Buffer	30 μL×（N+1）
Pre-Amp Enzyme	1 μL×（N+1）
总体积	31 μL×（N+1）

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5. 在 5 μL 的细胞裂解产物或对照组 DNA 样本中加入 30 μL 预扩增混合液（此时反应总体积为 35 μL）。
6. 在 PCR 仪中进行扩增，反应条件如下：

循环	温度	时间
1	94℃	3 min
8	20℃	40 sec
	30℃	40 sec
	40℃	30 sec
	50℃	30 sec
	60℃	30 sec
	70℃	4 min
	95℃	20 sec
	58℃	10 sec
1	4℃	Forever

步骤三：指数式扩增

7. 根据反应的数量 N，按照下表制备扩增混合液。

试剂名称	体积
Amplification Buffer	30 μL×（N+1）
Amp Enzyme	0.8 μL×（N+1）
总体积	30.8 μL×（N+1）

8. 在 35 μL 的预扩增产物中加入 30 μL 扩增混合液（此时溶液总体积为 65 μL）。
9. 在 PCR 仪中进行扩增，反应条件如下：

循环	温度	时间
1	94℃	30sec
17*	94℃	20sec
	58℃	30sec
	72℃	3 min
1	4℃	Forever

*说明：对于 100 pg 的 gDNA，我们建议设定 14 个循环；对于流式分选等方式获得的单细胞，设定 17 个循环；对于单染色体，设定 19-21 个循环；对于其他种类的细胞或其他方式获得的样本，建议在 PCR 前，对循环次数进行优化。

10. 扩增产物保存于-20℃冰箱，留待后续实验使用。

【产品性能指标】

1. 琼脂糖凝胶电泳检测片段长度：取 5 μL 扩增产物进行琼脂糖凝胶电泳（1%琼脂糖凝胶，110V 恒压，25～35 min），扩增产物大小为 300～2000bp。
2. 纯化产物定量：对扩增产物进行纯化，纯化产物总量为 2～5 μg。

【参考文献】

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【问题解决方案】

问题	原因	措施
无扩增产物	样本在收集过程中丢失	重新收集样本，确保操作准确
	起始样本中含有聚合酶酶抑制剂	建议实验前应使用不含 Ca^{2+}、Mg^{2+}、Mo^{2+}或肝素等成分的 1× PBS 缓冲液对细胞进行清洗，确保样本中不含有聚合酶抑制剂
	酶失活	试剂盒中所有的组份都应该在-25～-15℃的条件下保存；试剂复融过程需在冰上进行，避免反复冻融
扩增产物含量低	样本中含有聚合酶抑制剂	建议实验前应使用不含 Ca^{2+}、Mg^{2+}、Mo^{2+}或肝素等成分的 1× PBS 缓冲液对细胞进行清洗，确保样本中不含有聚合酶抑制剂
	DNA 降解	避免细胞因储存方法或准备方法不当造成 DNA 的降解，影响扩增效率
阴性对照产生了与单细胞全基因组扩增相似的产物	试剂被外源性 DNA 污染	避免试剂盒的组份与扩增产物接触，并使用无核酸酶和无核酸污染的实验耗材进行单细胞全基因组扩增，建议实验前应使用不含 Ca^{2+}、Mg^{2+}、Mo^{2+}或肝素等成分的 1×PBS 缓冲液对细胞进行清洗，确保不含有外源 DNA 污染。
	操作台被外源性 DNA 污染	使用防核酸污染试剂彻底清理操作台
	阴性对照溶液被外源性 DNA 污染	使用新阴性对照

【注意事项】

1. 操作前必须仔细检查所有样品，应排除污染的样品。
2. 产品直接接触皮肤或眼睛可能造成轻微伤害，操作注意安全防护，穿戴防护衣物、一次性手套、口罩；所有直接接触过样本的物品应进行消毒后丢弃或再次使用，同时防止操作者脱落细胞的污染。
3. 使用前，试剂应混合均匀，尽量避免反复冻融。
4. 所使用的接触试剂的材料均要求干燥、洁净，以防止污染。操作过程中涉及的耗材为一次性使用，不得重复使用。
5. 实验人员必须进行专业培训，严格按照说明书操作。
6. 样本应视为存在潜在的生物危害，实验结束后作为潜在传染源处理。
7. 减少准备过程中 DNA 的污染，聚合酶抑制剂的影响。
8. 避免样本因储存方法或准备方法不当造成的 DNA 降解。
9. 请严格按照试剂盒储存条件存放试剂盒各个组分，防止由于储存不当导致的试剂失效或其他不良结果。
10. 本试剂盒仅供科研使用，不用于临床诊断和其他用途。
11. 本试剂盒必须严格按照说明书进行操作，由于不按说明书操作引起的损失和伤害（相关法律特别要求的除外），本公司不承担由此引发的任何责任。

【基本信息】

生产企业：亿康基因科技有限公司
地址：上海：上海市徐汇区田林路 888 号科技绿洲一期 1 楼 102 室，200233
北京：北京市海淀区永丰产业基地永澄北路 2 号院 1 号楼绿海大厦 C 座 302 室，100094
江苏：江苏省泰州市药城大道一号 TQB 大楼 5 楼，225300
苏州：江苏省苏州市工业园区星湖街 218 号生物纳米园 B7 楼 201 室，215000
邮箱：technical@yikongenomics.com（技术支持）
info@yikongenomics.com（咨询）
order@yikongenomics.com（销售）
电话：+86-10-5091-7399（国际及中国港澳台），400-688-9230（中国大陆境内）
传真：+86-10-5091-7374
网址：www.yikongenomics.com

【版本号】190118.1
【识别码】PME051

MALBAC® Single Cell WGA Kit Manual

PRODUCT NAME

Universal Name: MALBAC® Single Cell WGA Kit

SIZE / CAT. NO.

Size	Cat. No.
10 rxns	KT110700110
50 rxns	KT110700150

INTENDED USES

The amplified products by the MALBAC Single Cell WGA Kit can be used on various analytical platforms such as real-time PCR (qPCR) and next-generation sequencing. It is also applicable to other downstream experiments and analyses: point mutation detection by Sanger sequencing; SNP genotyping ; CNV profiling and aneuploidy detection; Array CGH; SNP array **.**

BASIC PRINCIPLE

MALBAC Single Cell WGA Kit generates highly uniform amplification across the entire genome. The method is based on the patented technology – MALBAC (Multiple Annealing and Looping Based Amplification Cycles). MALBAC utilizes a mixture of highly processive DNA polymerases with strong strand displacement activity to carry out quasilinear pre-amplification cycles of the genome, followed by exponential amplification (regular PCR) to yield sufficient amount of genomic DNA for various downstream analyses.

COMPONENTS

Component	Cap Color	Voume & Quantity	
		10 rxns	50 rxns
Cell Lysis Buffer	Blue	50 μL * 1	250 μL * 1
Cell Lysis Enzyme	Blue	5 μL * 1	25 μL * 1
Pre-Amp Buffer	Green	300 μL * 1	750 μL * 2
Pre-Amp Enzyme	Green	10 μL * 1	50 μL * 1
Amplification Buffer	Red	300 μL * 1	750 μL * 2
Amp Enzyme	Red	8 μL * 1	40 μL * 1

EQUIPMENT & CONSUMABLES SUPPLIED BY USERS

1. Consumables: Nuclease-free water, 1×PBS Buffer.
2. Equipment: Thermal cycler, Micro spectrophotometer, Vortexer, Microcentrifuge.

STORAGE & PERIOD OF VALIDITY

Store Condition: -25～-15℃; avoid repeated freeze-thaw cycles.
Period of Validity: 24 months; MFD / EXP: On the package.

PRODUCT FEATURES

1. 2 to 5 μg DNA generated from single cell whole genome amplification.
2. tube, 3-step, 4-hr process. No purification required for intermediate products.
3. 95% amplification success rate for single cells sorted by flow cytometry or starting genomic DNA of > 0.5pg.
4. Reproducible locus representation and consistent amplification efficiency in both AT- and GC-rich regions.
5. High coverage with the locus dropout rate less than 10%.

RESEARCH AREAS

MALBAC Single Cell WGA Kit is an innovative tool for whole genome amplification from various starting materials including genomic DNA, cultured cells, sperm, oocyte, semen stains, fresh or dried blood, fresh or frozen tissue and other trace forensic evidence. Applicable research fields include:

1. **Human and applied animal biology:** Biomarker study (CNVs, SNVs); pre-implantation genetic screening and diagnostic (PGS/PGD); genotyping of transgenic animals; embryo, single sperm genotyping, stem cell and neuron research.
2. **Cancer research:** Somatic mutation analysis; tumor heterogeneity, development/evolution; cancer stem cells; circulating tumor cells (CTCs).
3. **Microbiology:** Metagenomics; microbial genotyping.

SAMPLE SPECIFICATION

上海亿康医学检验所有限公司
电话：400-921-3918
官网：www.yikongenomics.com
地址：上海市奉贤区望园路1698弄聚惠领域26号

1. **Sample Amount:** MALBAC Single Cell WGA Kit is specifically designed for single-cell whole genome amplification. The kit is also suitable for samples that is a single chromosome or 0.5 pg to 1 ng of genomic DNA.
2. **Collection Method:** MALBAC Single Cell WGA Kit is compatible with the following collection methods: flow cytometer sorting, dilution by buffer, micromanipulation, laser capture microdissection, buccal cells, biopsy samples. The kit is also suitable for the tissue samples obtained by paraformaldehyde fixation and laser capture microdissection.
3. **Pre-treatment of Samples:** Cell washing is strongly recommended prior to the experiment to avoid the contamination of exogenous DNA during cell preparation. Use Ca^{2+} and Mg^{2+} free 1xPBS solution for washing. Wash buffers containing Mg^{2+}, Ca^{2+} must be avoided. Note that the volume of PBS solution carried over with the cell sample into the amplification should not exceed 2 µL.

PREPARATION BEFORE EXPERIMENT

1. Isolation of Pre-Amplification Sample Preparation Working Area

- Prior to amplification, sample preparation should be performed in a designated laboratory or a working station with lab materials and equipment solely for pre-amplification such as pipettes, pipette tips, PCR tubes, 1.5 mL microcentrifuge tubes, tube racks, PPE, etc.
- The amplified DNA products should be stored separately from the pre-amplification reagents to avoid cross contamination. Other downstream analysis or treatment, such as DNA purification, preparation before sequencing, should be processed in another laboratory.

2. Preparation of Controls

- Positive Control: Dilute the concentration of the genomic DNA to 30 pg/µL with nuclease-free water. Add 1 µL of the 30 pg/µL genomic DNA solution to 4 µL of Cell Lysis Buffer in a PCR tube or well.
- Negative Control: Add 1 µL nuclease-free water to 4 µL of Cell Lysis Buffer in a PCR tube or well.

PROTOCOL

Note:

1. All reactions described in the procedures of MALBAC Single Cell WGA Kit take place in the same tube where the single cell has been isolated and lysed.
2. To avoid accidental removal of the cell (or parts of the cellular genome), carefully add an appropriate amount of reaction mix onto the inner wall of the tube, without disturbing the liquid.
3. Briefly centrifuge the mixture afterwards (2000rpm, 3 to 4 seconds). Transfer Cell Lysis Enzyme, Pre-Amp Enzyme and Amp Enzyme tubes to ice just before use, and other components can be thawed on ice before use.

Step 1: Cell Lysis

1. Prepare the Cell Lysis Reaction Mix according to the following table and thoroughly mix.

Component	Volume
Cell Lysis Buffer	5 µL*（N+1）
Cell Lysis Enzyme	0.5 µL*（N+1）
Total Volume	5.5 µL*（N+1）

Note: N denotes the number of reaction.

2. Transfer samples into 5 µL of the prepared Cell Lysis Reaction Mix in a PCR tube or well (The volume of PBS solution carried over with the sample should not exceed 2 µL). Centrifuge briefly. Do not vortex.
3. Incubate the sample(s) in a thermal cycler with heated lid using the following parameters tabulated below.

Cycle	Temperature	Time
1	50℃	50 min
	80℃	10 min
	4℃	Forever

Step 2: MALBAC Pre-Amplification

4. Prepare the MALBAC Pre-Amp Reaction Mix according to the following table and thoroughly mix.

Component	Volume
Pre-Amp Buffer	30 µL*（N+1）
Pre-Amp Enzyme	1 µL*（N+1）
Total Volume	31 µL*（N+1）

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5. Add 30 μL of freshly-prepared MALBAC Pre-Amp Reaction Mix to each 5 μL of completed lysed samples and control groups(total volume should be 35 μL). Mix by vortexing and centrifuge briefly.
6. Incubate the sample(s) in a thermal cycler with heated lid using the following parameters tabulated below.

Cycle	Temperature	Time
1	94℃	3 min
8	20℃	40 sec
	30℃	40 sec
	40℃	30 sec
	50℃	30 sec
	60℃	30 sec
	70℃	4 min
	95℃	20 sec
	58℃	10 sec
1	4℃	Forever

Step 3: Exponential Amplification

7. Prepare the Amp Reaction Mix according to the following table and thoroughly mix.

Component	Volume
Amplification Buffer	30 μL*（N+1）
Amp Enzyme	0.8 μL*（N+1）
Total Volume	30.8 μL*（N+1）

8. Add 30 μL of freshly-prepared Amp Reaction Mix to 35 μL of the Pre-Amp products(total volume should be 65 μL). Mix by vortexing and centrifuge briefly.
9. Incubate the sample(s) in a thermal cycler with heated lid using the following parameters tabulated below.

Cycle	Temperature	Time
1	94℃	30sec
17*	94℃	20sec
	58℃	30sec
	72℃	3 min
1	4℃	Forever

* Cycle number can be optimized according to sample types. 14 cycles is recommended for 100 pg of gDNA or equivalent amount . 17 cycles is recommended for a single cell sorted by flow cytometry. 19 to 21 cycles is recommended for single chromosome. For other types of samples, optimization of cycle number is suggested.

10. When the thermal cycling program is done, the amplified product can be stored at -20℃ for later use.

PRODUCT TECHNICAL SPECIFICATION

1. **Agarose Gel Electrophoresis :** Run 5 μL of the amplification product on a 1% agarose gel (110V, 25-35 min). A DNA smear ranging from 300 to 2000 bp will be observed.
2. **Quantification :** Purify the amplified DNA product and quantify it. The final yield weighs 2～5 μg.

LITERATURE REFERENCES

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上海亿康医学检验所有限公司
电话：400-921-3918
官网：www.yikongenomics.com
地址：上海市奉贤区望园路1698弄聚惠领域26号

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TROUBLESHOOTING GUIDE

Issue	Potential Cause	Solution
No amplified product	Sample loss during embryo biopsy	Redo the embryo biopsy and make sure the biopsy samples are transferred completely to the cell lysis buffer. Avoid accidental removal of the genetic materials.
	Sample containing polymerase inhibitors	Polymerase inhibitors carried over with the starting materials can sometimes cause low amplification efficiency. Cell washing is strongly recommended to minimized no-cellular contamination. 1X PBS solution that contains no Ca^{2+}, Mg^{2+}, Mo^{2+} or heparin can be used for washing.
	Inactive enzymes	All components should be stored at -25～-15℃. All enzymes and buffers should be freshly prepared and thoroughly mixed before use. Avoid repeated freeze/thaw cycles for all enzymes and buffers.
Low amplified product	Sample containing polymerase inhibitors	Polymerase inhibitors carried over with the starting materials can sometimes cause low amplification efficiency. Cell washing is strongly recommended to minimized no-cellular contamination. 1X PBS solution that contains no Ca^{2+}, Mg^{2+}, Mo^{2+} or heparin can be used for washing. The volume of the washing buffer carried over to the cell lysis step should not exceed 1 μL in order to obtain optimal amplification efficiency.
	Degradation of genomic DNA	Avoid DNA degradation due to the improper cell storage or template preparation.
Amplified products in negative (no-template) control	Reagents contaminated by exogenous DNA	Keep kit reagents and the amplified DNA at designated storage space. Aliquot the reagents after the first use. Use sterile and nuclease-free tubes and filter tips to set up the reactions.
	Work area contaminated by external DNA	Clean the work space thoroughly by DNA and RNA decontamination reagents.
	Negative control groups contaminated by external DNA	Use new negative control groups.

NOTES

1. Check all the samples carefully. Exclude the contaminated sample(s).
2. Direct contact with skin or eyes may cause minor injury. Wear personal protection equipment including lab coat, disposable gloves and face mask when handling the sample(s) and the reagent(s). Properly dispose of all the goods which have contact with the sample after sterilization. Avoid the contamination of cells shed from the operator.
3. Buffer should be mixed thoroughly before use and repeated freeze/thaw should be avoided.
4. All the materials used must be dried and clean for contamination control. The consumables used during the experiment are for one-time use and cannot be reused.
5. The operator should be given professional training and perform the experiment according to the manual strictly.
6. The sample(s) should be treated as biohazard and be disposed of as a source of infection after experiment.
7. Minimize the influence of DNA contamination and enzyme inhibitors during sample preparation.
8. Avoid DNA degradation caused by improper storage or preparation.
9. Please store the components of the kit strictly as the instruction of the manual to avoid loss of effect or other adverse consequence caused by incorrect storage.
10. The kit is only for research use, not for diagnosis and other purposes.
11. The kit should be used strictly as the content of the manual. Yikon Genomics Co., Ltd. will take no responsibility caused by improper or incorrect uses unless otherwise specified by relative regulations and

上海亿康医学检验所有限公司
电话：400-921-3918
官网：www.yikongenomics.com
地址：上海市奉贤区望园路1698弄聚惠领域26号

laws.

MANUFACTURE BASIC INFORMATION

Registration Unit/Manufacture: Yikon Genomics Co., Ltd
Address:
Shanghai Office: Room 102, No.1, Lane 888, Tianlin Road, Hi-Tech Building, Shanghai Business Park 1, Caohejing Development Zone, Shanghai, 200233, China
Beijing Office: C bl℃k 3 layer 302, Yongfeng industrial base, Cheng Wing Road, No. 2 Building, No. 1 hospital green building, Haidian District, Beijing, 100094, China
Jiangsu Office: 5th Floor, Building TQB, No.1 China Medical City Avenue, Taizhou, Jiangsu, 225300, China
Suzhou Office: Room 201, No. B7, SIP BioBay, 218 Xinghu Road, Suzhou Industrial Park, Suzhou, Jiangsu, 215000, China
E-mail: technical@yikongenomics.com (technical support)
info@yikongenomics.com (consultation)
order@yikongenomics.com (order)
Tel: +86-10-5091-7399 (International, HongKong, Macau and Taiwan), 400-688-9230 (Mainland China)
Fax: +86-10-5091-7374
Web: www.yikongenomics.com

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