博淼生物
BIOMIAO BIOLOGICAL
-SINCE2009-
Your own Laboratory
您的专属实验室

Massarray技术在基因检测领域的应用

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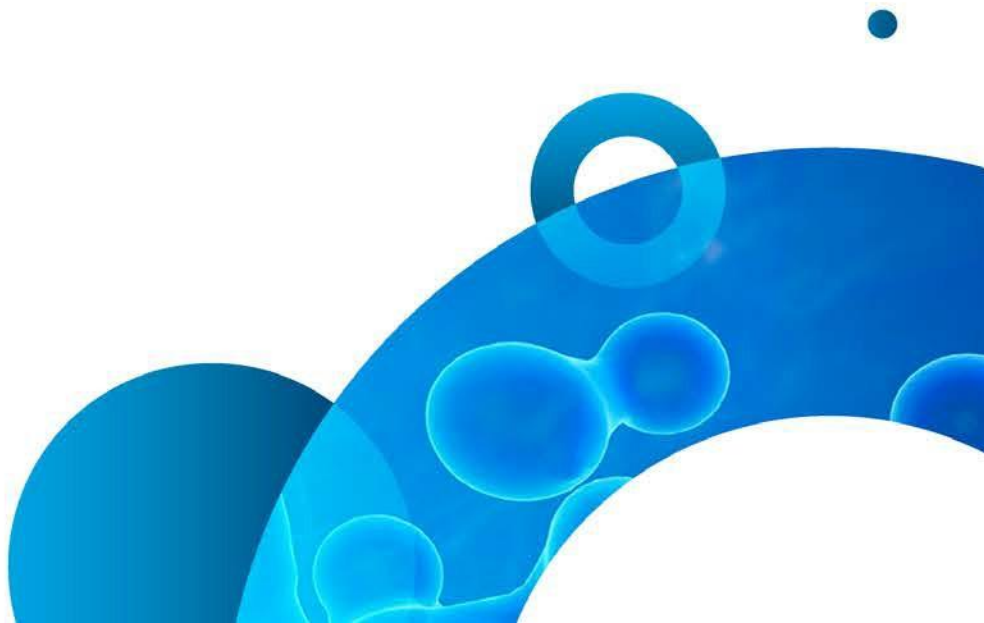

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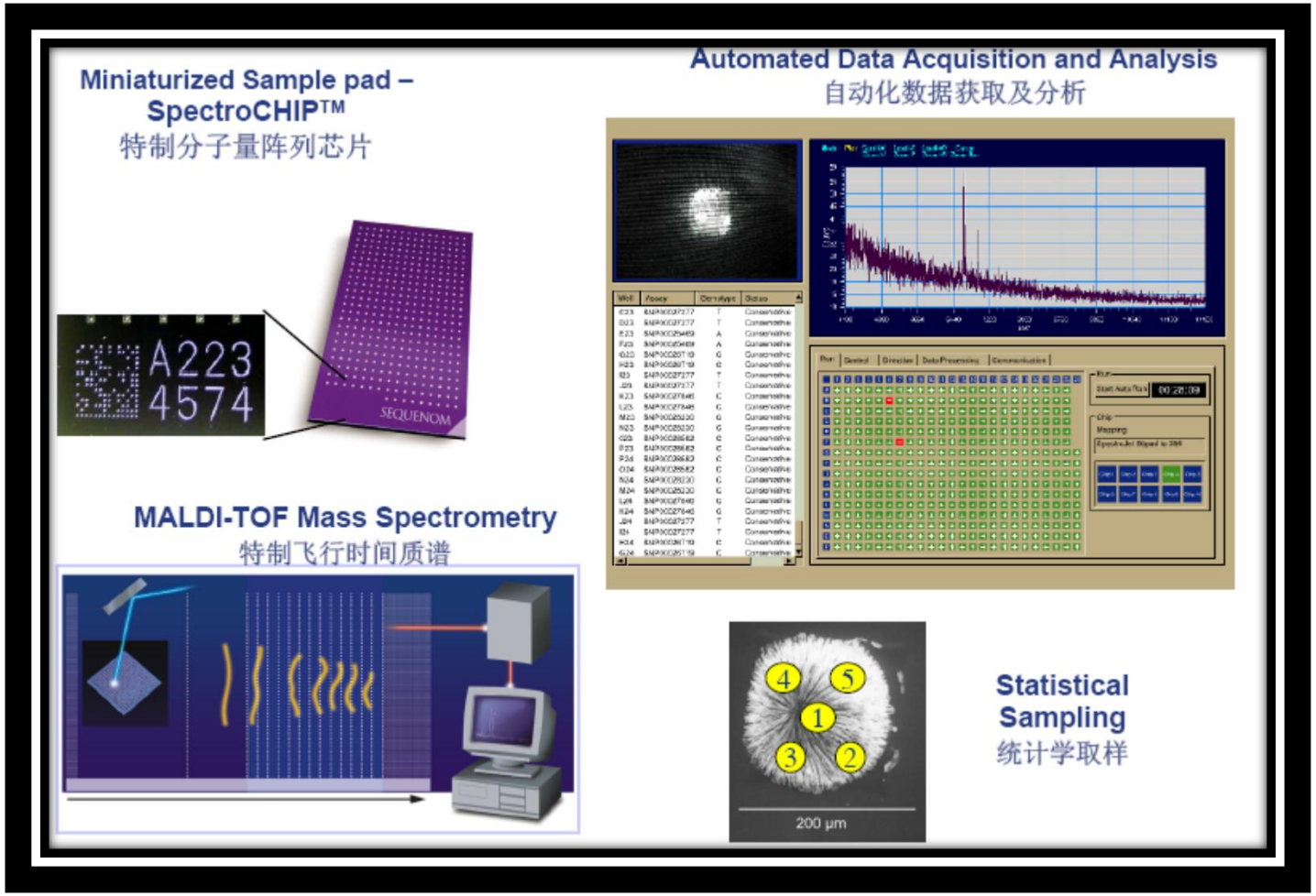
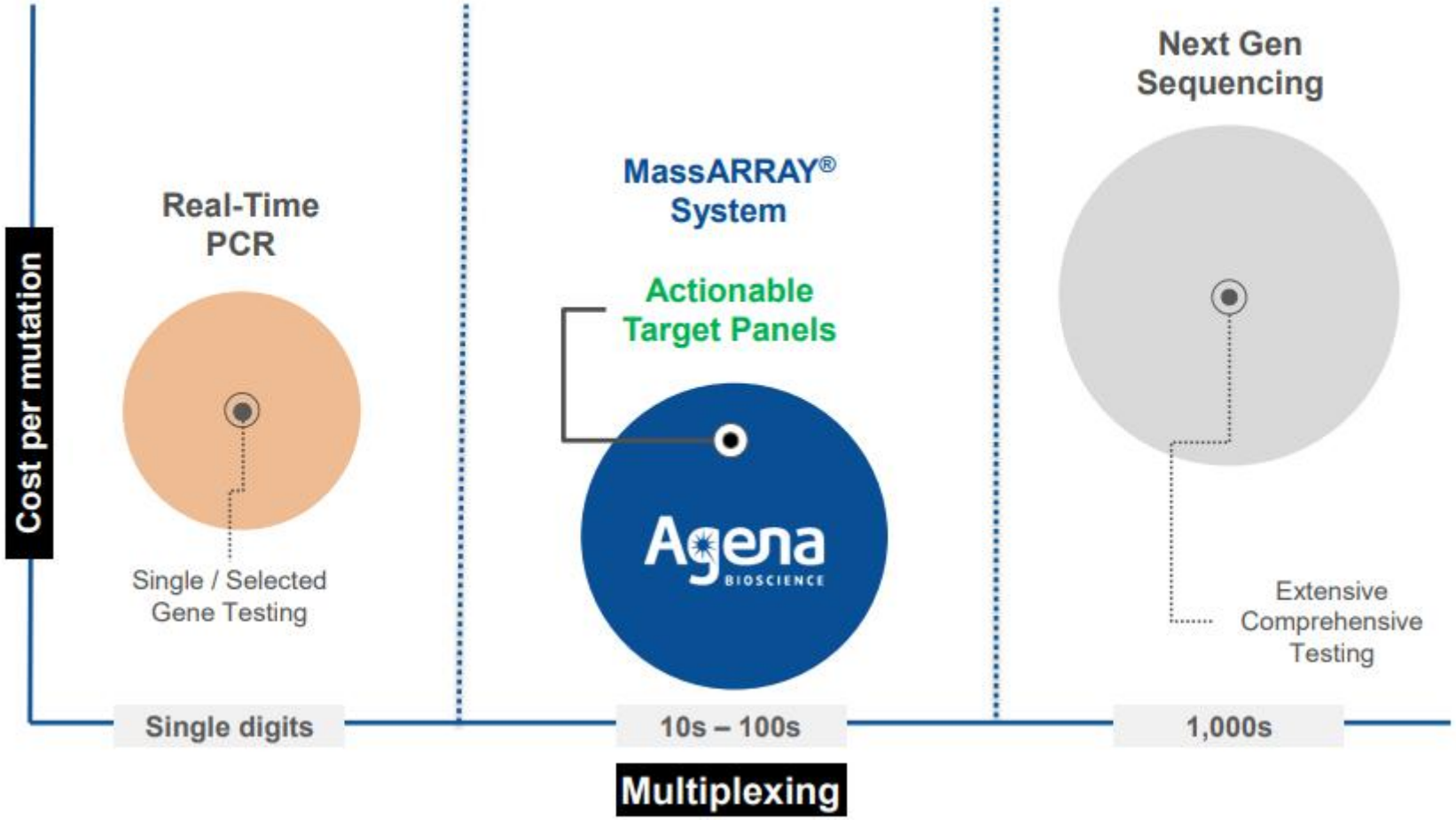


中通量SNP及DNA定量检测金标准技术

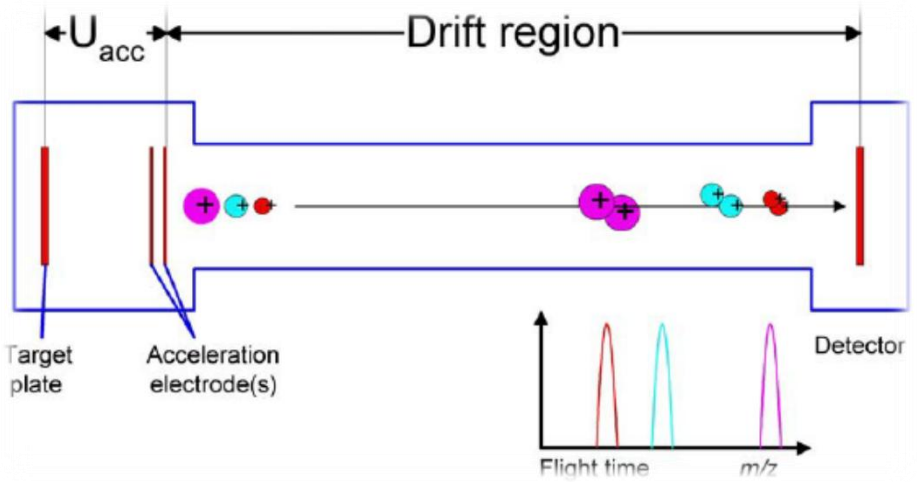


01

Massarray技术SNP分型技术原理

技术原理

- 质谱仪通过对物质分子量（质荷比）进行检测，达到区分、鉴别物质的目的
- SNP位点两个等位基因之间存在分子量差异，通过实验将差异放大，然后以质谱仪进行检测即可



$$E = \frac{1}{2}mv^2$$

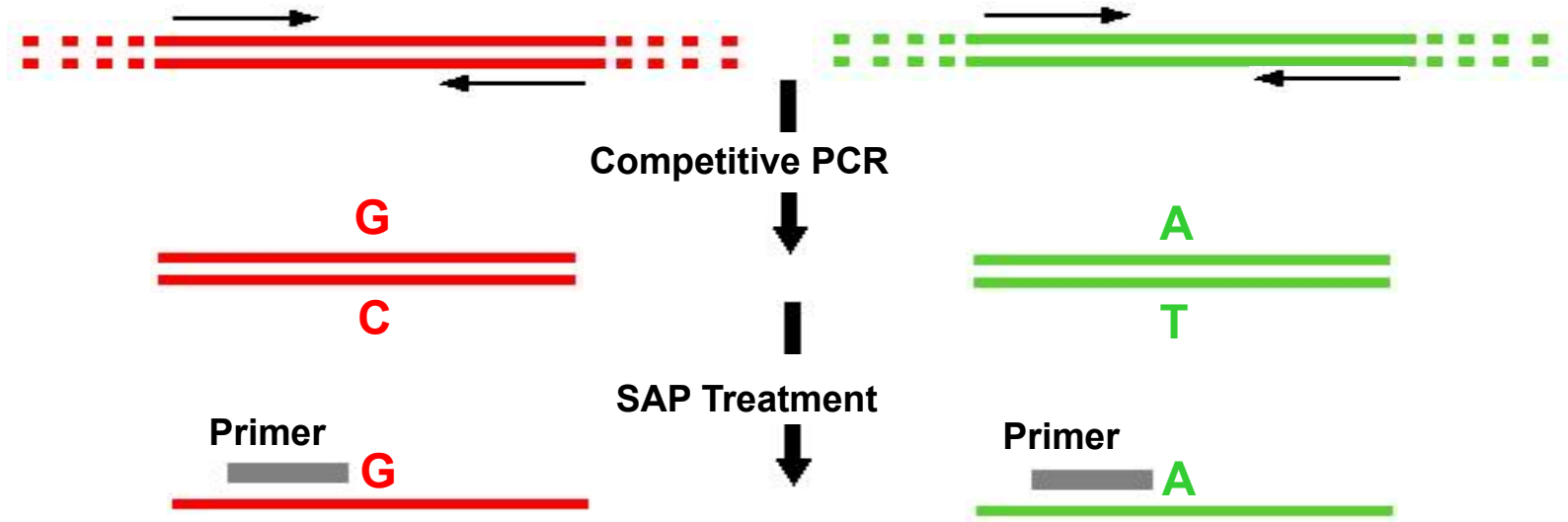
dAMP = 313.2Da
dCMP = 289.2Da
dGMP = 329.2Da
dTMP = 304.2Da

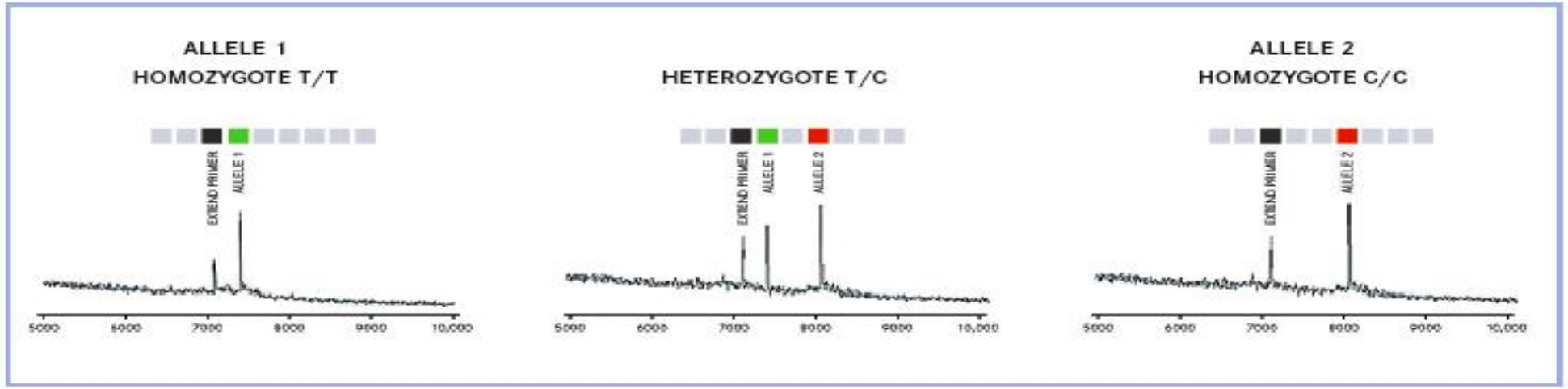
技术原理

Genomic DNA

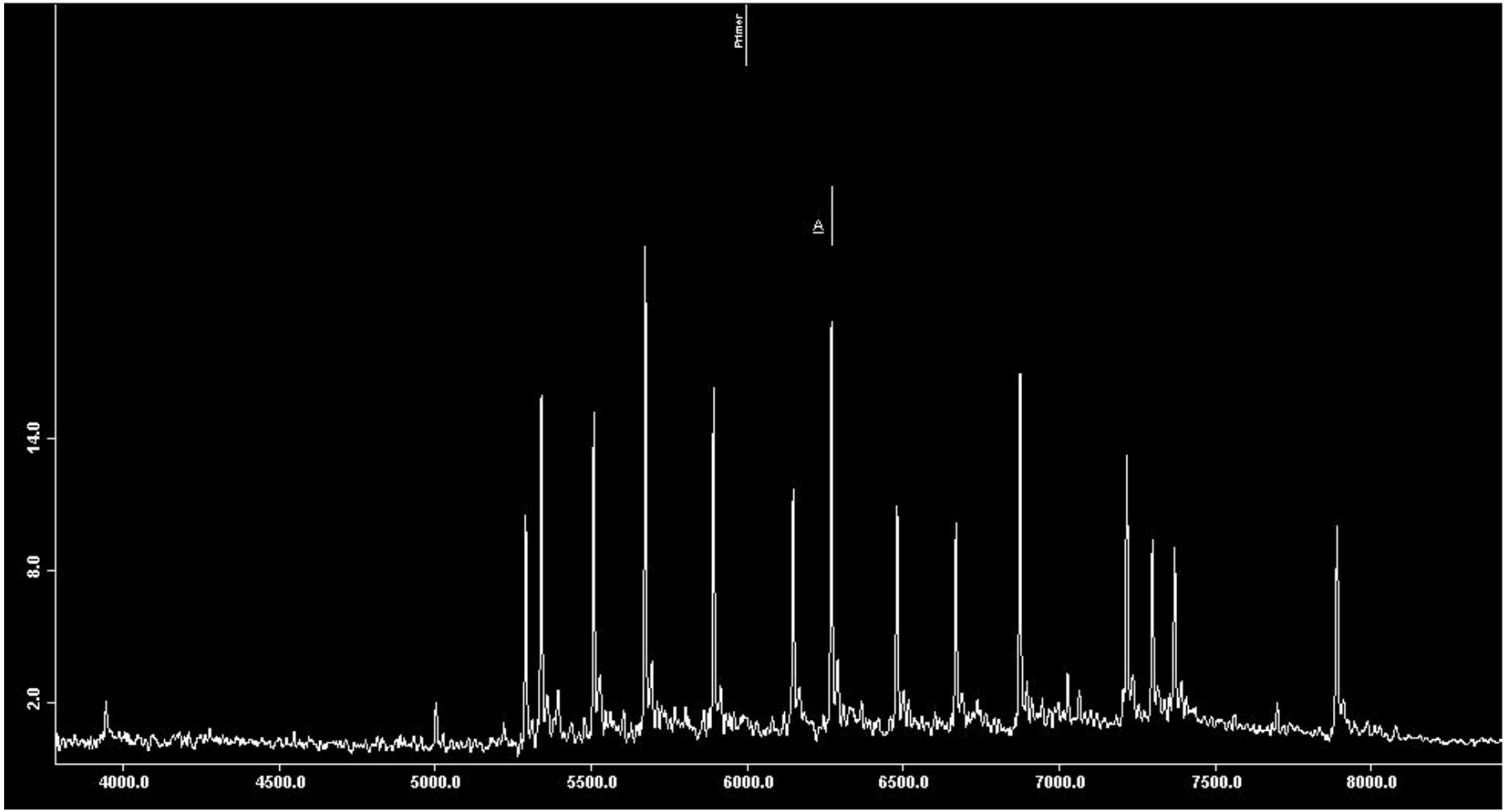
PCR Amplification

Primer Extension

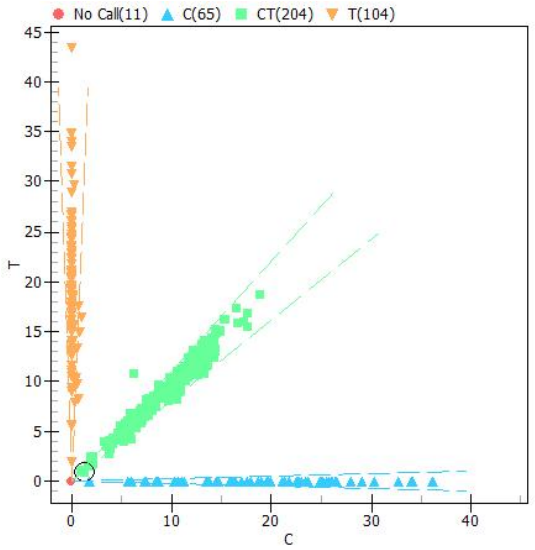




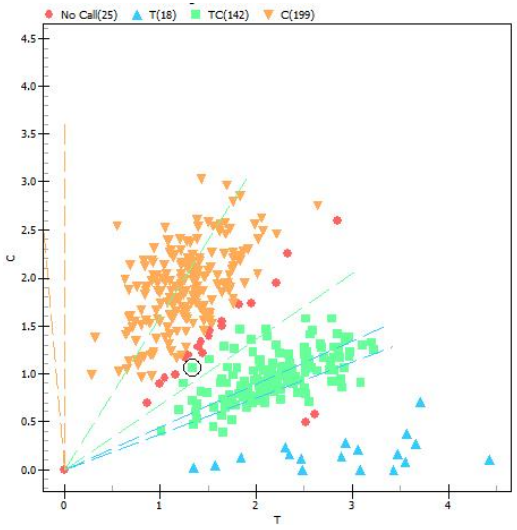
技术原理

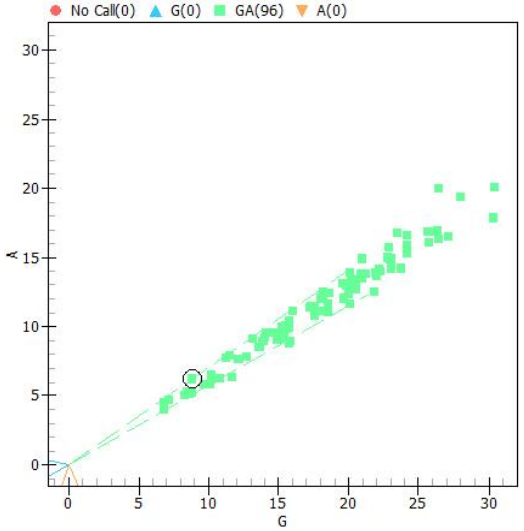

Primer
A
14.0
8.0
2.0
4000.0
4500.0
5000.0
5500.0
6000.0
6500.0
7000.0
7500.0
8000.0

技术原理



聚类质控良好，数据准确





聚类质控不佳，数据不准确，与同源序列干扰、非特异性扩增等因素有关

技术原理

提供序列标准格式

rs1：

CTTCAACTCCTGGGCTCAGGCTCAAGTGATCCTCCGACCTCGGCCTCCTGAAGTGCTGGGATTACAGGCATGAGCCACTGTGTCTGG
CCACAATACACAC**[T/G]**TGACTGTCATTTATAAACTCAAATGACTCAGCTATCAAGATGCCAAATTGGATTTTAGAGAGCCTCCTCCTA
GGCACCTGATACTTTCATGCTTGGCTTA

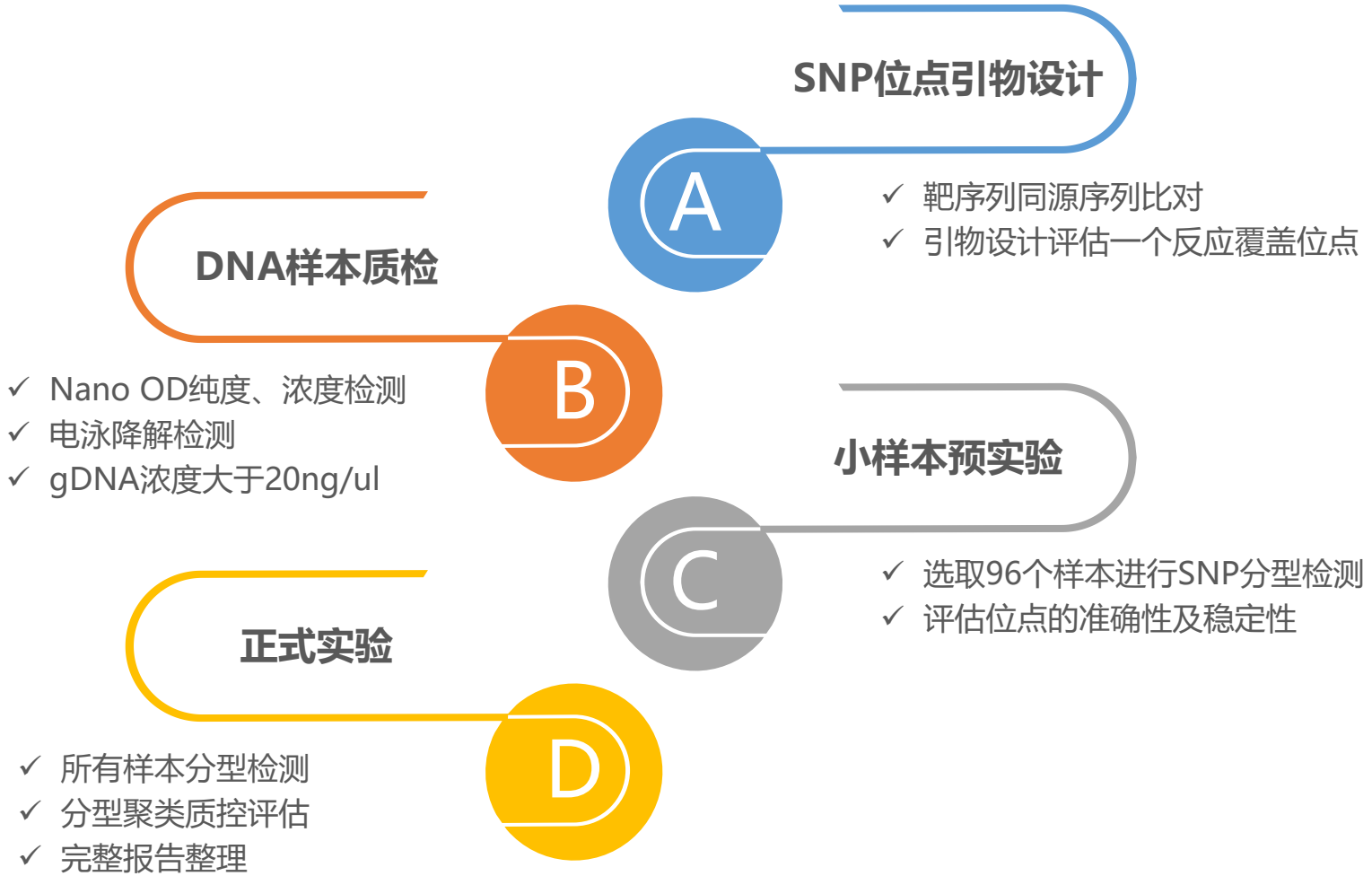
SNP位点Massarray引物序列

SNP_ID	2nd-PCRP	1st-PCRP	UEP_SEQ
rs1	ACGTTGGATGTTGTGTGCTCACACATCTGC	ACGTTGGATGGCAGTCTCTACCTTCCAAAG	TGCCCTCTCTTGCCC
rs2	ACGTTGGATGTCCTTCCCTCTGACACATAG	ACGTTGGATGTAGACATGAGGGCTGCAAGG	ATGTCCAGTGCCCTG
rs3	ACGTTGGATGAAAGGTTCTTGCAGACGTCC	ACGTTGGATGTTACTGAAGGGTCCTGAGTG	GACGTCCTCCAAGACA
rs4	ACGTTGGATGTTTGGAGTCTGAGGGATCTG	ACGTTGGATGAACAGGACAAGCAATCCACC	AGGGATCTGGGTTCTG
rs5	ACGTTGGATGTGGAGAAATGGCAGATGTGG	ACGTTGGATGCAGTCACATCTTAAAGGCCC	GCAGATGTGGTGGAAA
rs6	ACGTTGGATGAGTGGCTCACACCTGCAATC	ACGTTGGATGAGGCTGGTCTCAAAATCCTG	aACCTGCAATCCCAACAC
rs7	ACGTTGGATGCCTGCACTCCATCCTGAGTA	ACGTTGGATGCAGGAAATTCTGGTGGTTTT	CCATCCTGAGTAACAGAG
rs8	ACGTTGGATGCCTGAGATGCTAAGAGATCC	ACGTTGGATGCATGTGGGAACCACATTCTG	aTCCTGTGATGGGAAAAG
rs9	ACGTTGGATGCCTTGCTTTTCCAACTTGGG	ACGTTGGATGCACCTTGATTGCAGAACAGC	gATAGCTCCCACAAAGAAG
rs10	ACGTTGGATGTGAGTGTGAAGGGCAGAAAG	ACGTTGGATGTGCTTCTCATCTCTCAAGGC	gaagGGCTTCAAGGAGGAG
rs11	ACGTTGGATGTTAAATTTCTGAAGCCCAGG	ACGTTGGATGCAGACTGTATTTTCCTCATGC	ccacGCCCAGGTATTCTGAT

技术优势

- ✓ 基于分子量差异，而非荧光信号进行检测，准确性更高；
- ✓ 检测通量高，一个反应可以覆盖近30个SNP位点，适用于同一个样本300个位点以内的样本检测，成为GWAS stageII大样本分型最经典的技术工具；
- ✓ 成本低廉，适用于大样本群体的SNP分型；
- ✓ 检测周期短，整个流程8个小时；
- ✓ 属于SNP分型技术的金标准之一。

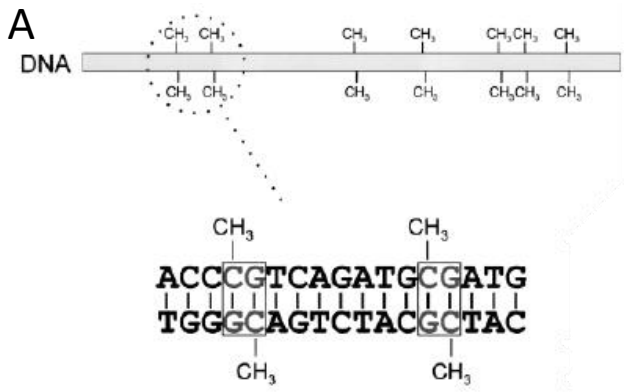
技术服务流程

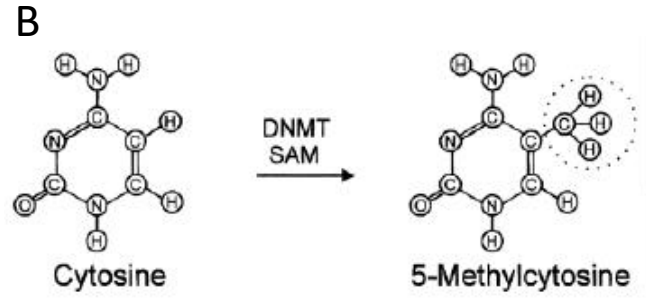

SNP位点引物设计
A
✓ 靶序列同源序列比对
✓ 引物设计评估一个反应覆盖位点
DNA样本质检
B
✓ Nano OD纯度、浓度检测
✓ 电泳降解检测
✓ gDNA浓度大于20ng/ul
小样本预实验
C
✓ 选取96个样本进行SNP分型检测
✓ 评估位点的准确性及稳定性
正式实验
D
✓ 所有样本分型检测
✓ 分型聚类质控评估
✓ 完整报告整理

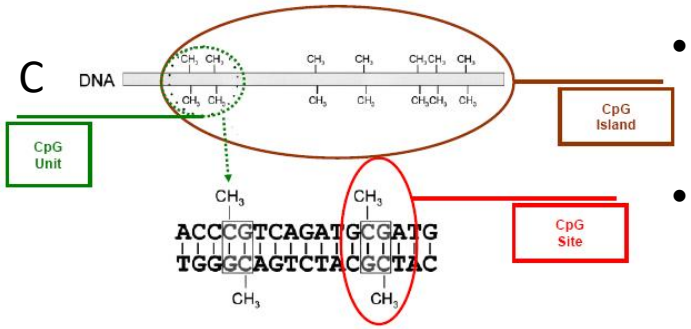
02

Massarray技术DNA甲基化定量技术原理

技术原理

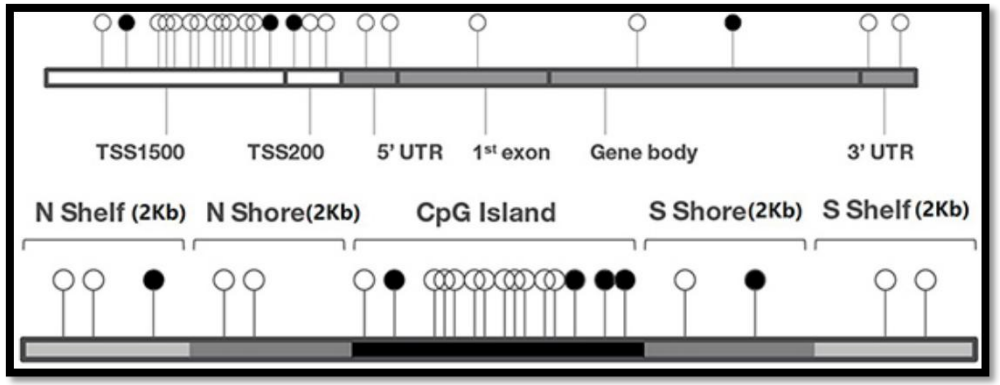




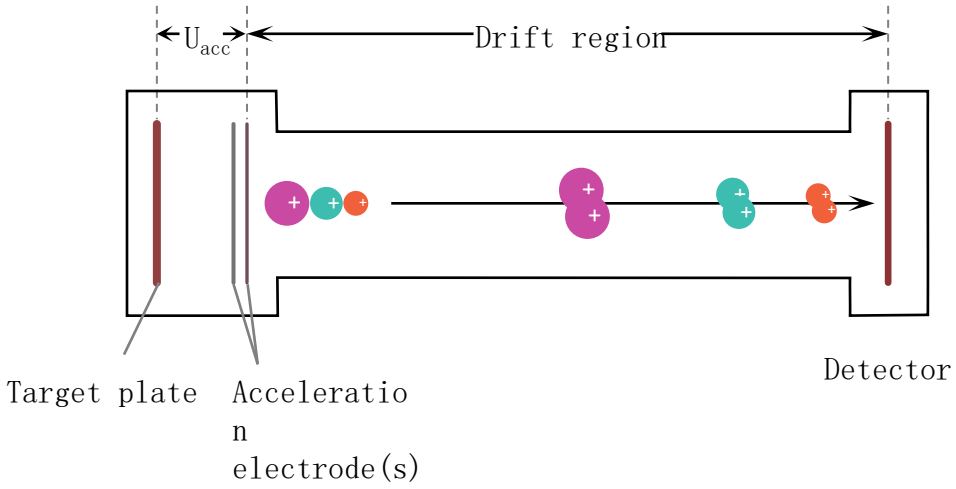


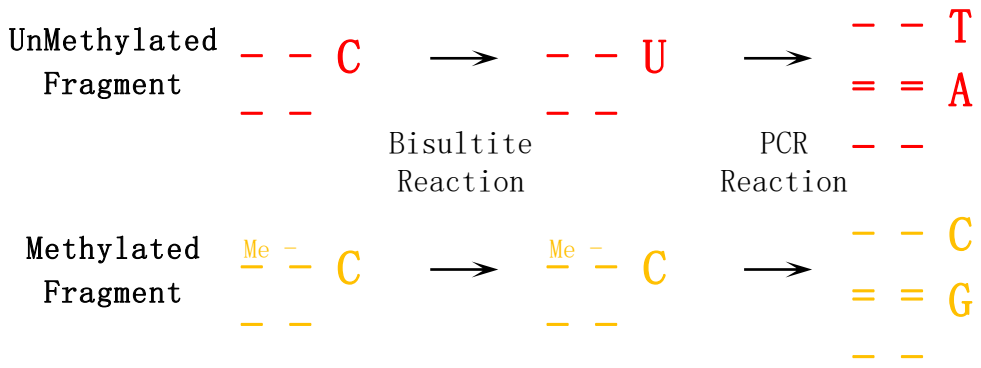
- 甲基化通常发生于功能相关的二核苷酸CpG
- 通常集群分布在所谓的CpG岛内
- CpG岛通常位于启动子区域，具有调控染色质结构及基因表达的功能

区域划分：CpG island、island shores、island shelves、Opensea



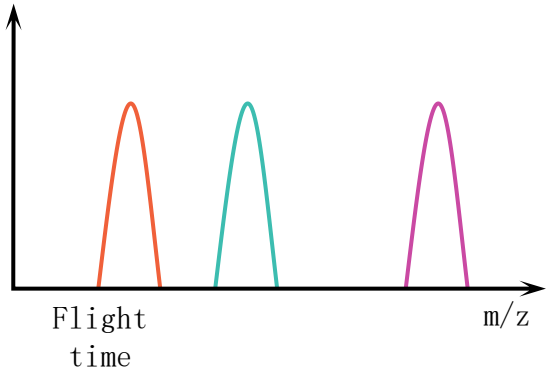
技术原理

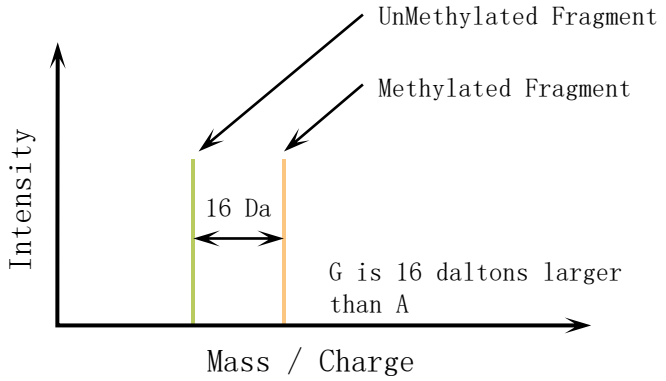




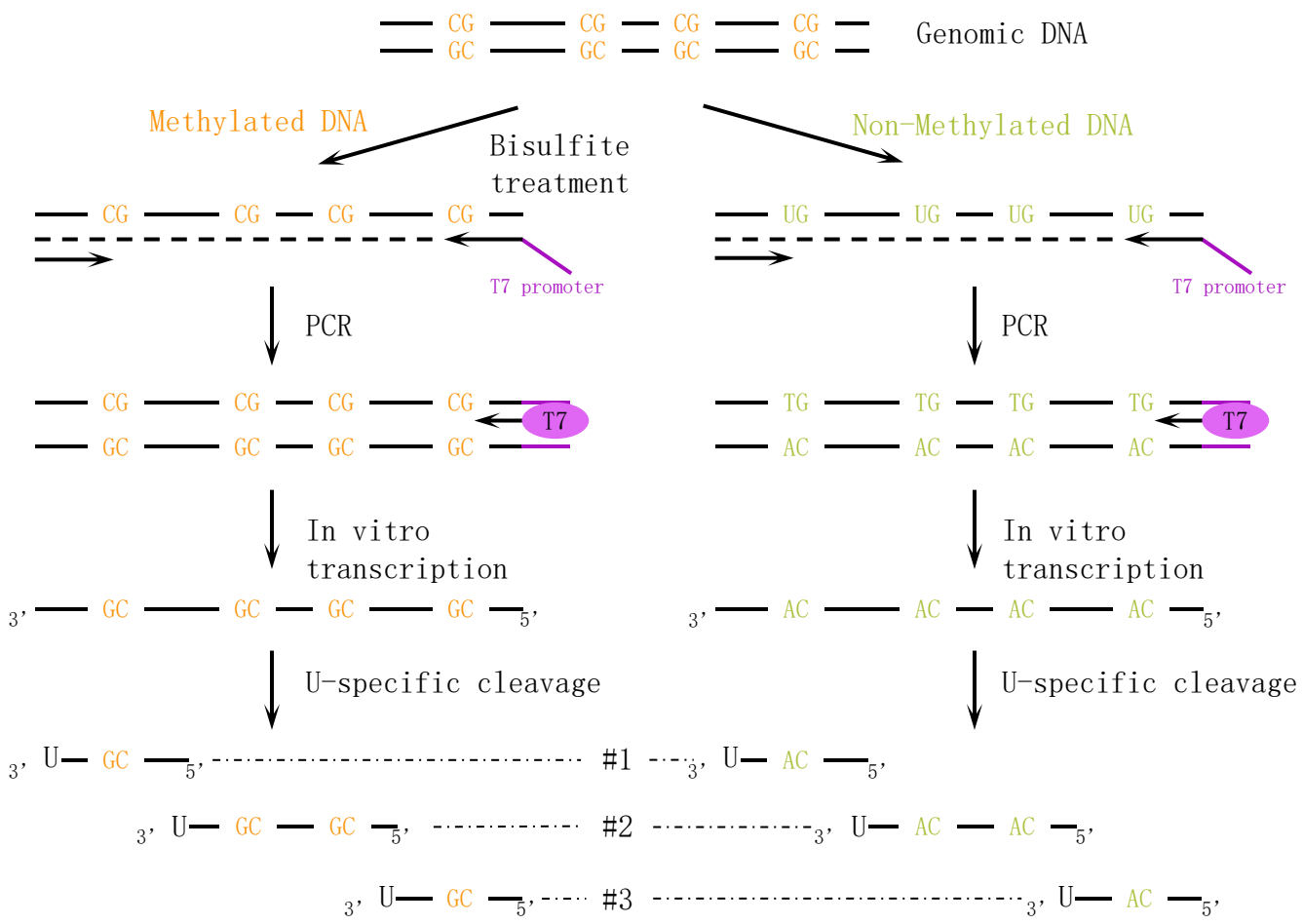
$$E = \frac{1}{2}mv^2$$

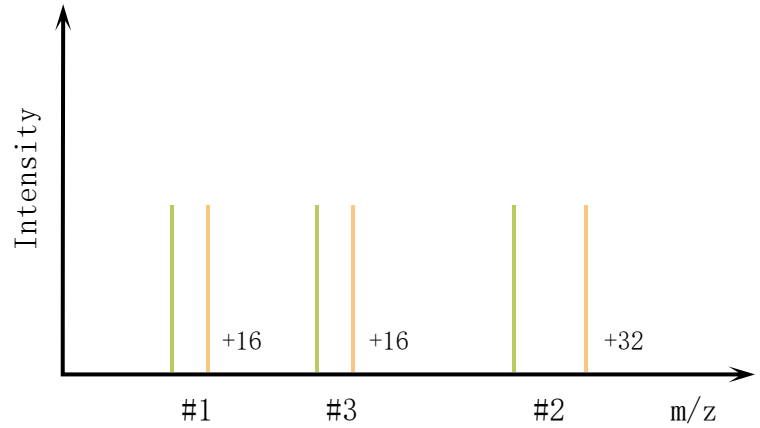
dAMP = 313.2Da
dGMP = 329.2Da
dCMP = 289.2Da
dTMP = 304.2Da



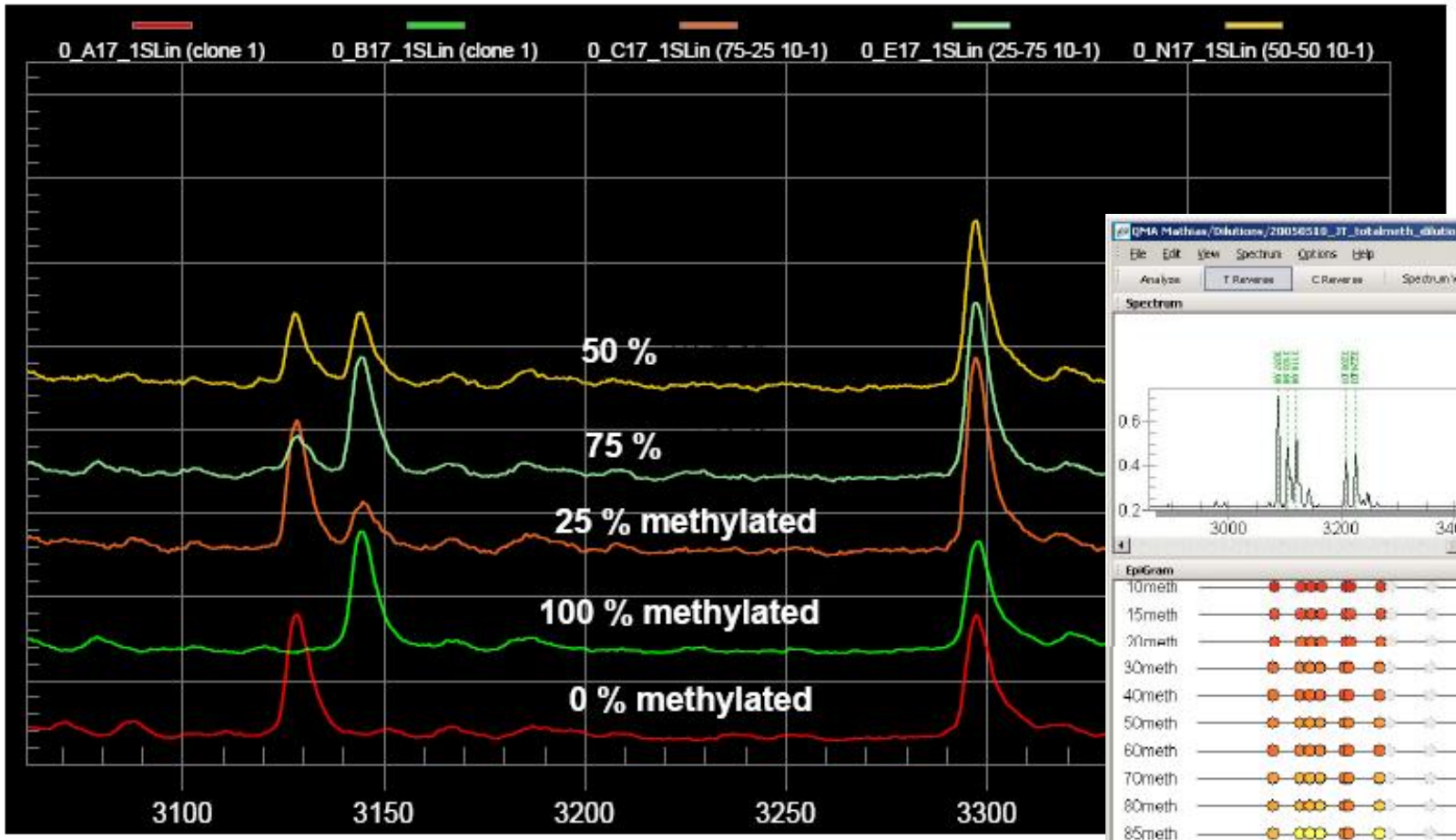


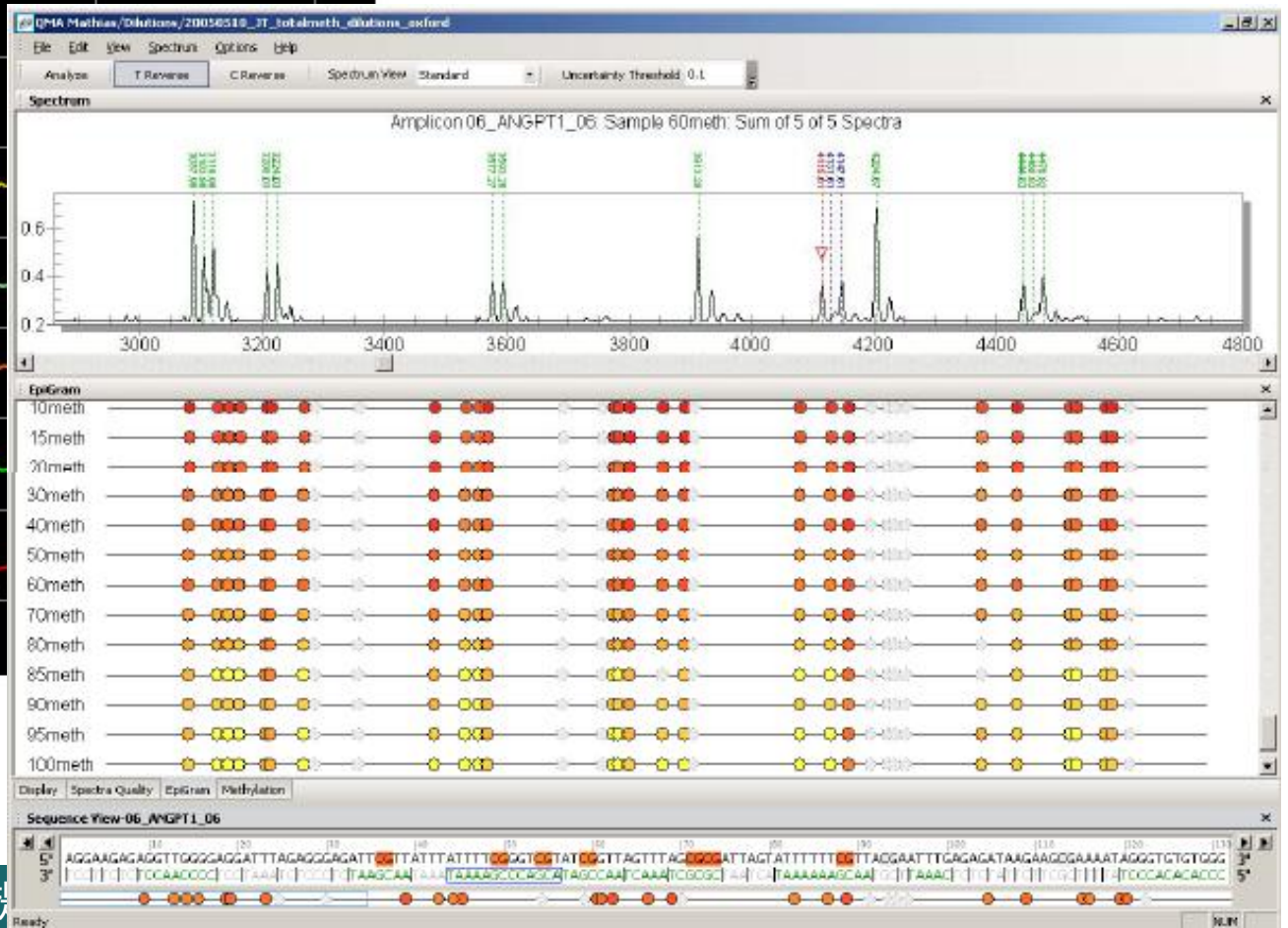
技术原理


Genomic DNA
Methylated DNA
Non-Methylated DNA
Bisulfite treatment
T7 promoter
T7 promoter
PCR
PCR
T7
T7
In vitro transcription
In vitro transcription
U-specific cleavage
U-specific cleavage
#1
#2
#3

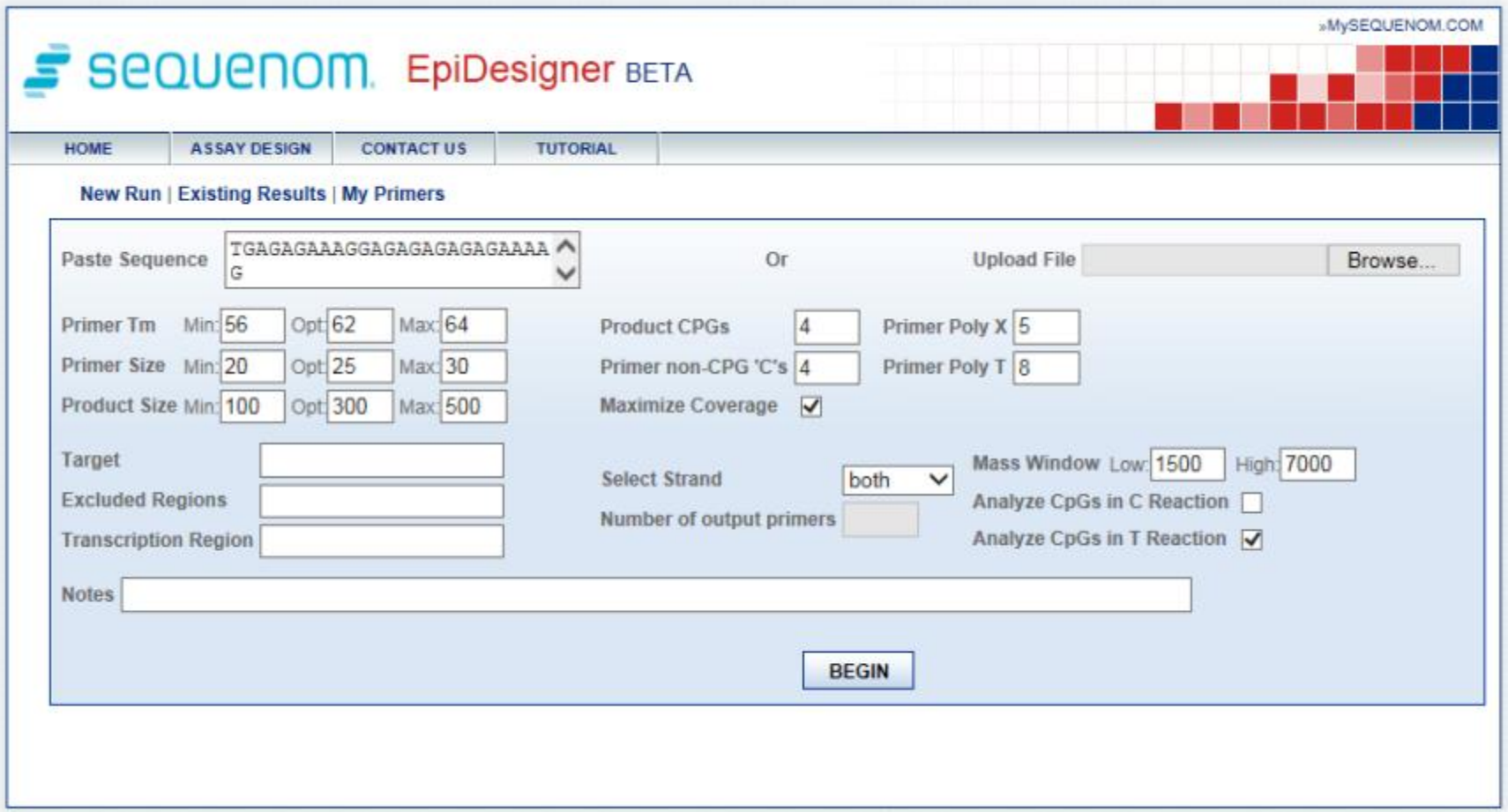

Intensity
+16
+16
+32
#1
#3
#2
m/z

技术原理

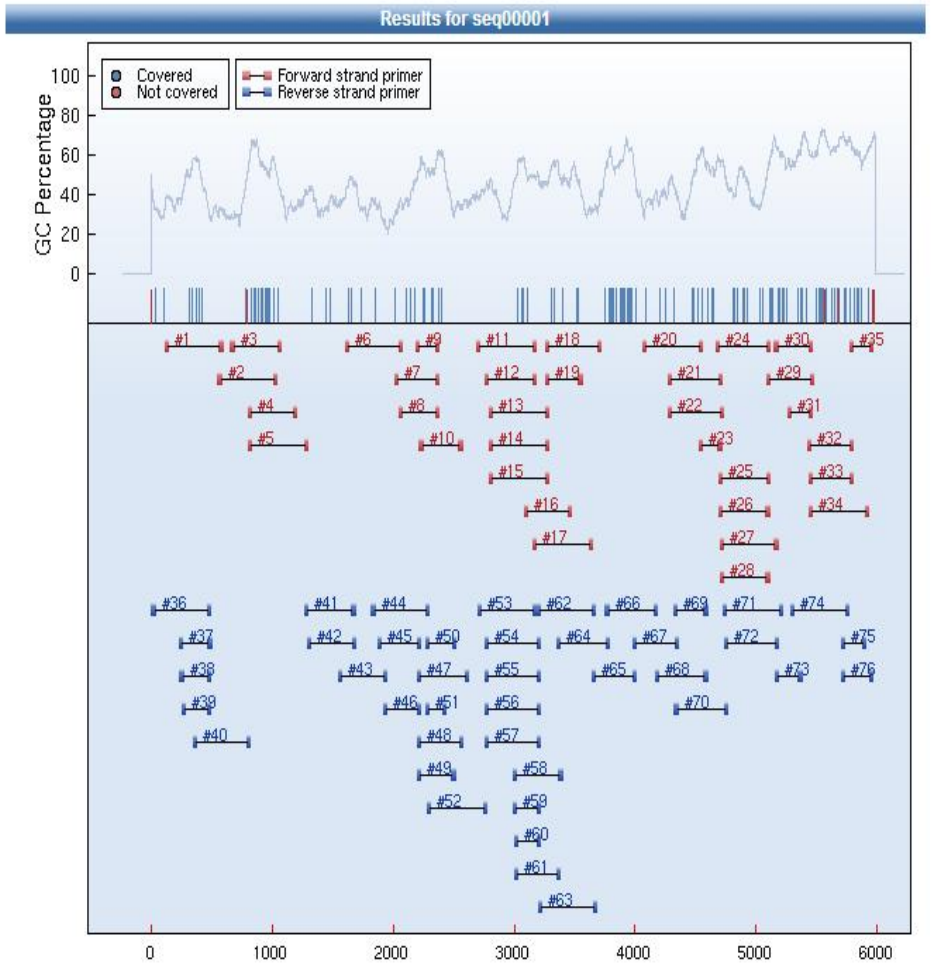

0_A17_1SLin (clone 1)
0_B17_1SLin (clone 1)
0_C17_1SLin (75-25 10-1)
0_E17_1SLin (25-75 10-1)
0_N17_1SLin (50-50 10-1)
50 %
75 %
25 % methylated
100 % methylated
0 % methylated
3100
3150
3200
3250
3300


Spectrum
Amplicon 06_ANGPT1_06: Sample 60meth: Sum of 5 of 5 Spectra
EpiGram
10meth
15meth
30meth
40meth
50meth
60meth
70meth
80meth
85meth
90meth
95meth
100meth
Sequence View-06_ANGPT1_06

技术原理


»MySEQUENOM.COM
sequenom EpiDesigner BETA
HOME
ASSAY DESIGN
CONTACT US
TUTORIAL
New Run | Existing Results | My Primers
Paste Sequence
TGAGAGAAAGGAGAGAGAGAGAAAA
G
Or
Upload File
Browse...
Primer Tm Min: 56 Opt: 62 Max: 64
Primer Size Min: 20 Opt: 25 Max: 30
Product Size Min: 100 Opt: 300 Max: 500
Product CPGs 4
Primer non-CPG 'C's 4
Maximize Coverage
Primer Poly X 5
Primer Poly T 8
Target
Excluded Regions
Transcription Region
Select Strand both
Number of output primers
Mass Window Low: 1500 High: 7000
Analyze CpGs in C Reaction
Analyze CpGs in T Reaction
Notes
BEGIN

技术原理



（CG为连续检测位点，灰色标记的 CpG 无法检测）：

tcccccaggctggagtgcagtggtgCGatctCGgctcactgcaagctcCGcctccCGggttcaCGCGattctc
ctgcctcagcctccCGagtagctgggactacaggtgccCGccaccaCGcccagctaatttttttgtatttttagtag
agaCGgggtttcacCGtgttaccCGggatggtctCGatctcctgacctcatgatccaccttcctCGgcctcccaa
agtgctgggattataggcatgagccacCGtgcctggtgaaatggacaaattcttagcaaaataaagcagactaa
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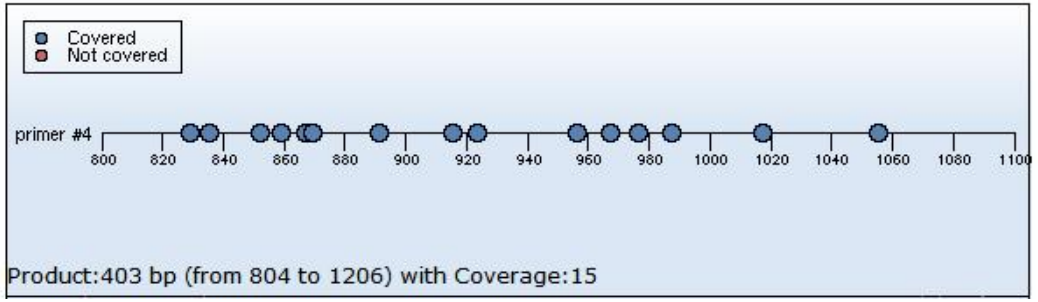


引物信息

Primer	Start	Size	Tm	GC%	C's
5'端引物	804	25	60.66	40	7
3'端引物	1206	25	59.91	32	5
PRODUCT Size: 403, No of CpG's : 15, Coverage : 15					

5'端引物序列：aggaagagag TTTTTTAGGTTGGAGTGTAGTGGTG

3'端引物序列 ：cagtaatacgactcactatagggagaaggct CCAATAAAACCACCTAAATTTTCCT

- 注释：3'端引物加有 T7 启动子标签和 8 个碱基的插入序列；5'引物添加了 10 碱基的标签，平衡正反向引物的 Tm 值。

技术优势

中通量：适用1-20个片段的中通量验证，样本数量不受限

长片段：覆盖约400-600bp长度的区域位点，属于最长的locus-specific assays

高准确：扩增子内单个CpG sites进行准确定量

数据简便：可视化数据产出

技术服务流程

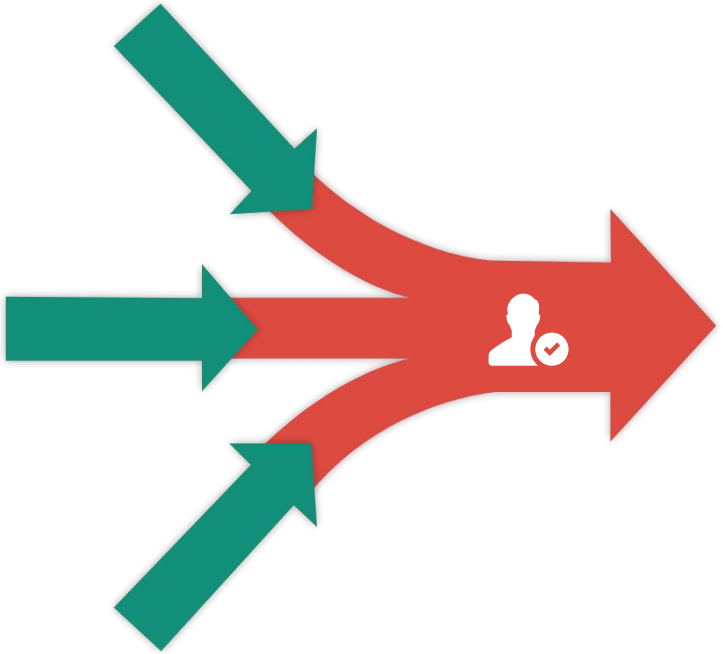

DNA甲基化位点区域引物设计
A
✓ 候选区域CpG island评估
✓ Epityper引物设计评估
DNA样本质检
B
✓ Nano OD纯度、浓度检测
✓ 电泳降解检测
✓ gDNA总量>500ng，浓度>50ng/ul
引物PCR预实验
C
✓ 采用标准品进行PCR扩增
✓ 确认最佳的退火温度等条件
正式实验
D
✓ 所有样本DNA甲基化定量检测
✓ 完整报告整理

不同检测技术对比

Quantitative comparison of DNA methylation assays for biomarker development and clinical applications JULY 2016 ***Nature biotechnology***

思路设计

- ✓ 选取6种global assays技术&21种locus-specific assays
- ✓ 32个样本，来自7个不同城市的18个实验室独立检测
- ✓ 数据灵敏度、重复性、准确性、通量参数对比



三个性能最佳的检测技术：

- ✓ Among the absolute DNA methylation assays, AmpliconBS and Pyroseq showed the best all-round performance, closely followed by EpiTyper
- ✓ Global arrsays present lower accuracy

最佳技术对比

- ✓ Pyroseq can work well even on minute amounts of highly fragmented DNA
- ✓ EpiTyper provides the highest sample throughput
- ✓ AmpliconBS is the best choice for assaying dozens of genomics regions in parallel

03

SNP与DNA甲基化联合研究策略概述

Omics roles

博淼生物 BIO MIAO BIOLOGICAL SINCE 2009

基因组学（Genomics）
—What is possible

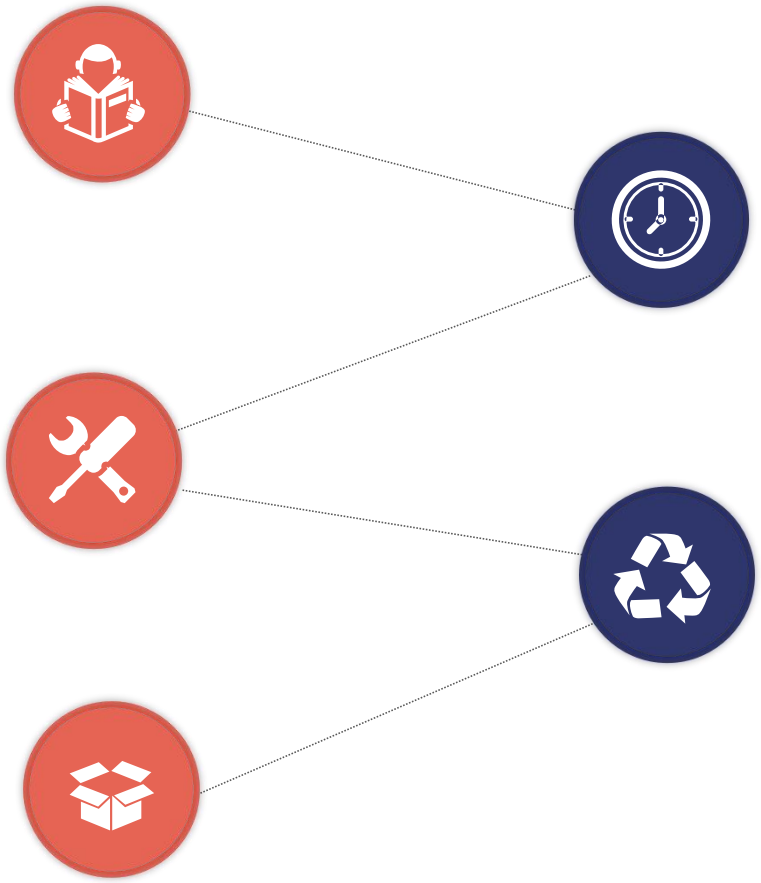
- ✓ 胚系细胞SNP、CNV、InDel等遗传变异
- ✓ 体细胞mutation、InDel等环境突变变异

转录组学（Transcriptomics）
—What appears to be happening

- ✓ 转录本类型：mRNA、miRNA、lncRNA、cirRNA、piRNA等
- ✓ 转录定位：表达、融合、剪切、ceRNA等

代谢组学（Metabolomics）
—What has happened

- ✓ 脂质组、氨基酸类、胆汁酸类、神经递质类等等
- ✓ 宿主代谢物、微生物代谢物



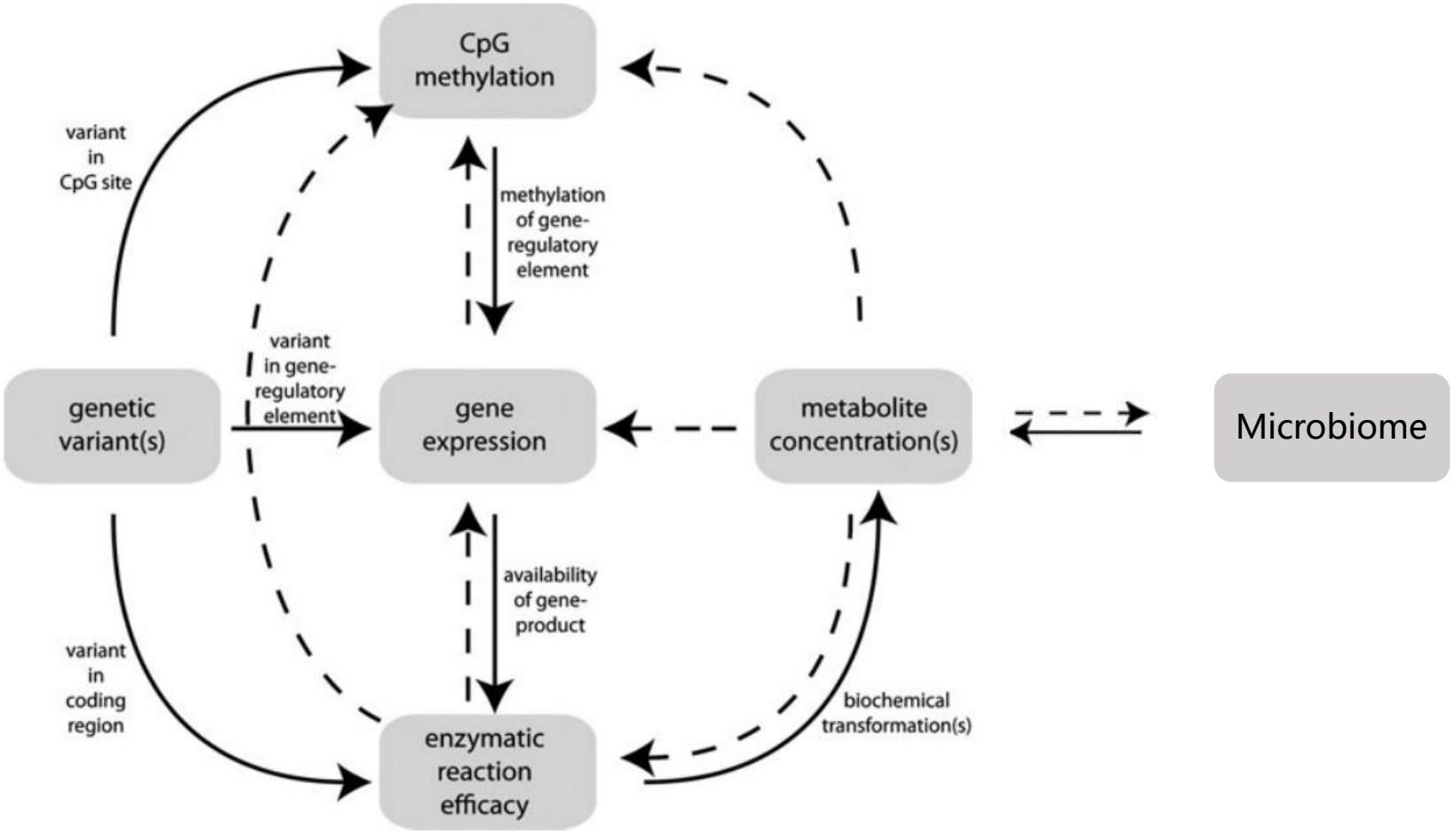
表观基因组学（Epigenomics）
—What appears to be happening

- ✓ 基因组：ATAC、HiC
- ✓ DNA甲基化：5mC、5hmC、6mA等
- ✓ RNA甲基化：m6A等

蛋白质组学（Proteinomics）
—What is happening

- ✓ 功能蛋白质：酶、转录因子等
- ✓ 修饰蛋变质：糖基化、乙酰化等

Omics network



meQTL的科研意义

87% of cis-eQTL SNPs, 82% of cis-pQTL SNPs, and 59% of GWAS Catalog SNPs are also cis-meQTL variants

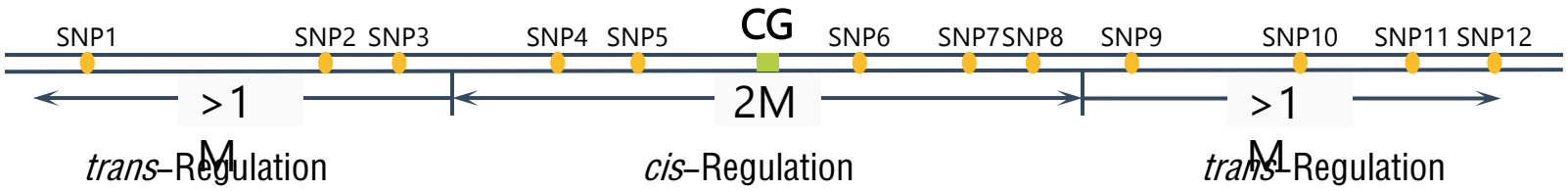
		cis-meQTL			*trans*-meQTL		
	Total SNPs	N of the overlap	Fold Change	P-value	N of the overlap	Fold Change	P-value
cis-eSNPs FHS (1e-7)	460,536	399,670	1.66	0	84,591	2.33	0
cis-eSNPs Eur-Meta	398,524	356,500	1.71	0	70,810	2.25	0
cis-pQTLs FHS-1000g	12,401	10,176	1.57	0	2,045	2.09	0
pQTLs FHS-1000g	19,942	16,660	1.6	0	4,428	2.81	0
cis-pQTLs NC2016	376	318	1.62	0	53	1.78	1.63E-05
pQTLs NC2016	456	385	1.61	0	68	1.89	1.79E-07
metabolism QTLs	135	111	1.57	8.38E-14	26	2.44	6.52E-06
GWAS Catalog	32,260	19,000	1.13	0	3078	1.21	0

meQTL的基础理论

SNP-meth遗传调控

Allele–specific methylation (ASM) { Associated with methylation of a nearby CpG site
SNP itself destroyed a CpG site by changing the C or G

Haplotype–specific methylation (HSM) { Associated with methylation of a nearby CpG site

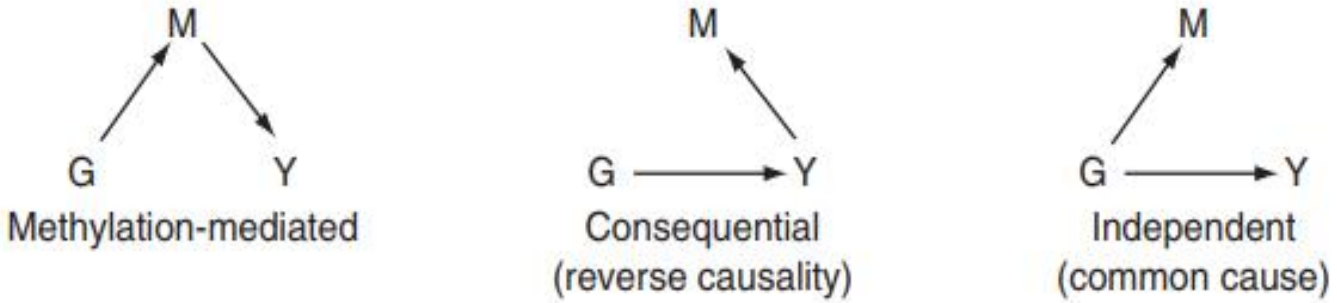


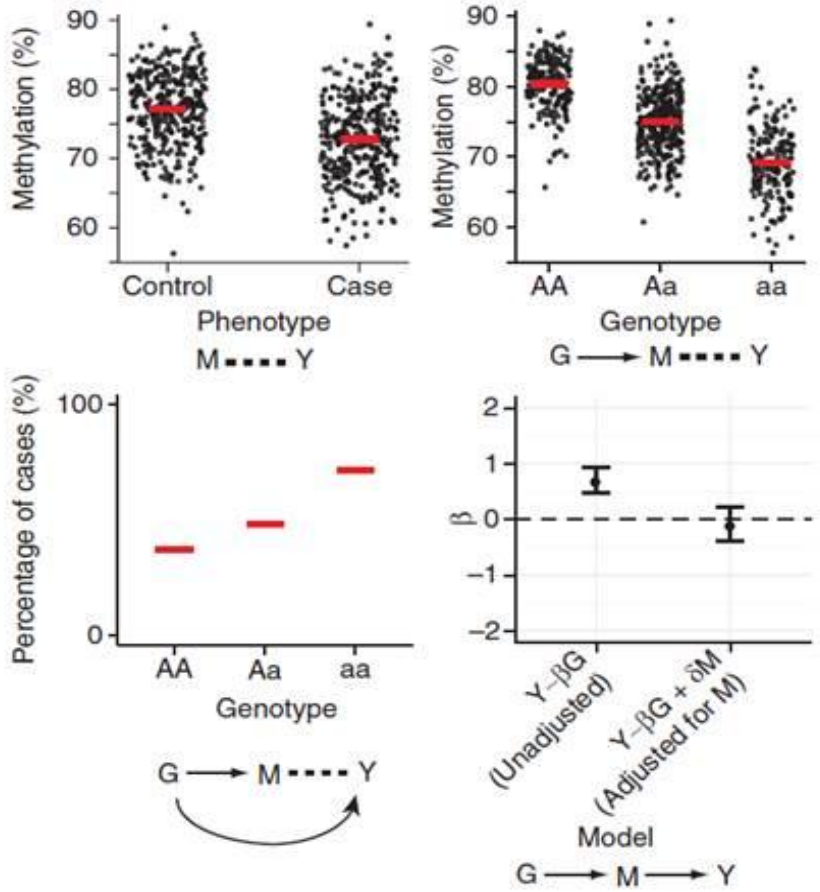
cis–Regulation: cisacting regulation by DNA elements in or adjacent to each CpG site

trans–Regulation: trans–acting regulation by factors from the genomic regions distant from the CpG sites, including from different chromosomes.

SNP-Meth-Phenotype CIT因果效应分析

SNP-meth-Phenotype因果效应





Two-step epigenetic mendelian randomization: A strategy for establishing the causal role of epigenetic processes in pathways to disease

SNP联合DNA甲基化研究路径

SNP分型检测

- ✓ 全基因组SNP分型检测
- ✓ 靶向SNP位点分型检测

科研设置

- ✓ 科研目标：表观遗传分子调控机制研究
- ✓ 样本类型：外周血、组织等

生物信息分析

- ✓ 关联分析mQTL（cis-/trans-）及遗传调控网络network构建
- ✓ SNP-Meth-phenotype CIT中介效应分析
- ✓ Meth-phenotype的MR随机化因果推断分析

DNA甲基化检测

- ✓ 全DNA甲基化组检测
- ✓ 靶向DNA甲基化位点检测

博淼技术服务项目一览图

博淼生物
BIO MIAO BIOLOGICAL
SINCE 2009

基因组学服务

- GWAS芯片/WES/Target NGS
- Massarray /Multi-PCR NGS/Taqman /KASP SNP分型
- 微生物基因组

表观基因组学服务

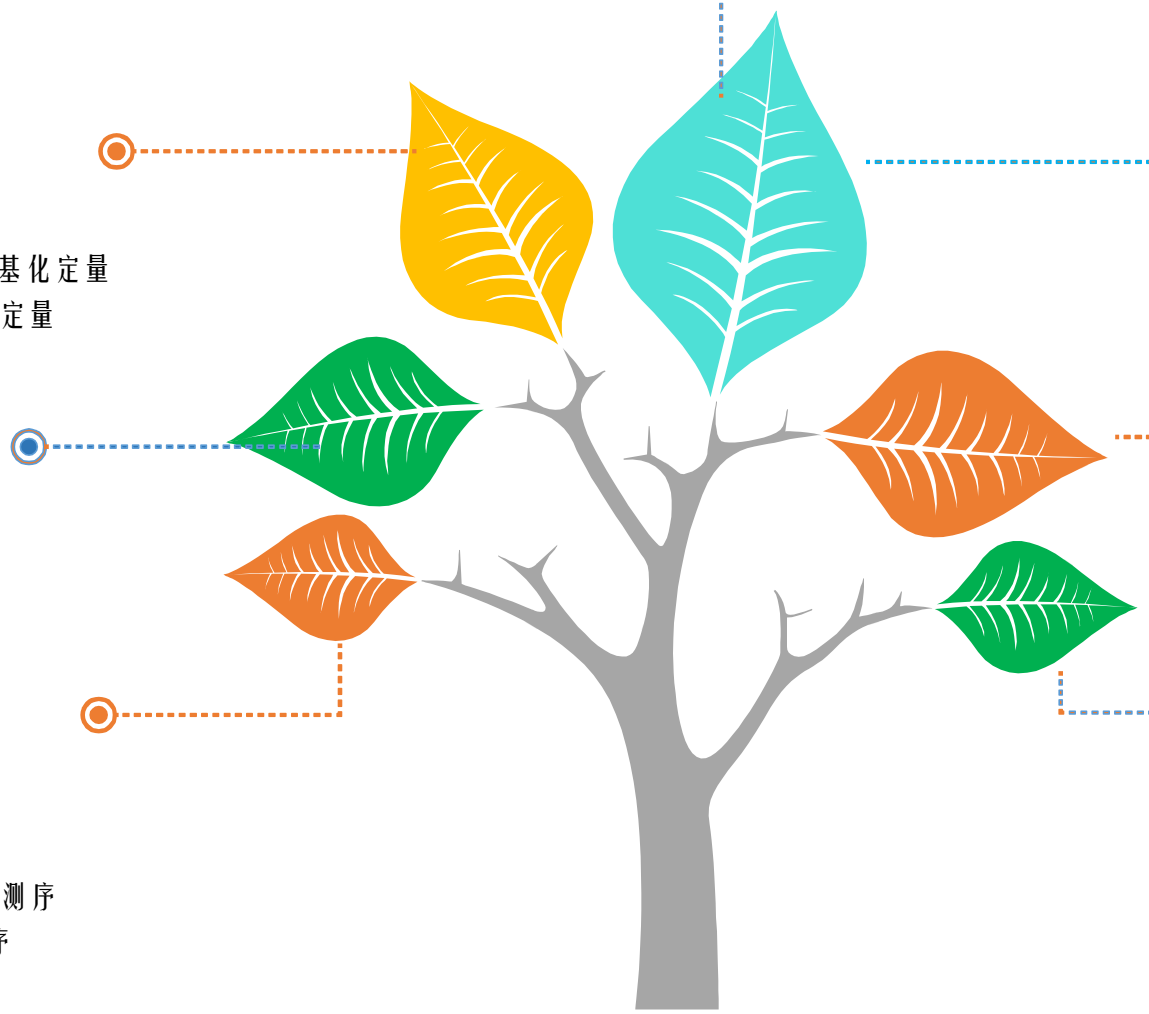
- EWAS芯片
- Multi-PCR NGS靶向DNA甲基化定量
- Massarray 靶向DNA甲基化定量

转录组学服务

- 转录组NGS/表达谱芯片
- RT-qPCR靶向转录本定量

单细胞组学服务

- 单细胞转录组测序
- 单细胞免疫组库测序
- 单细胞ATAC测序&转录组测序
- 单细胞表面蛋白/抗原测序
- 空间转录组测序



代谢组学服务

- 非靶向代谢组
- 非靶向脂质组
- 靶向代谢组

蛋白质组学服务

- DIA定量蛋白质组
- TMT标记定量/Label free非标定量蛋白质组
- 修饰蛋白质组

多组学联合研究服务

- GWAS&多组学技术服务
- EWAS&多组学技术服务
- 微生物基因组&代谢组技术服务
- 蛋白质组&代谢组技术服务

感谢各位的聆听

Your own Laboratory

——您的专属实验室