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Your own Laboratory
您的专属实验室

遨游基因组学研究

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基因检测技术概述

芯片技术

02

- ✓ GWAS芯片
- ✓ EWAS芯片
- ✓ mRNA&LncRNA&miRNA&cirRNA表达谱芯片
- ✓ CNV芯片

02 04

新一代测序技术

01

- ✓ 全转录组测序
- ✓ 全外显子组测序
- ✓ 全基因组甲基化测序
- ✓ 全RNA甲基化测序
- ✓ 全基因组重测序
- ✓ 微生物基因组&转录组测序
- ✓ 单细胞测序

验证性技术

03

- ✓ Massarray技术—SNP分型和DNA甲基化定量
- ✓ Rt-qPCR技术——Taqman和SYBGreen基因转录本定量
- ✓ Sanger测序技术
- ✓ Kasp技术——SNP分型
- ✓ 焦磷酸测序技术
- ✓ 数字PCR技术

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博淼技术服务项目

表观组学服务

- EWAS芯片
- 全转录组m6A测序
- Massarray DNA甲基化位点定量

转录组学服务

- 全基因组表达谱芯片-mRNA+miRNA+LncRNA+cirRNA
- RT-qPCR靶向转录本定量

单细胞组学服务

- 单细胞表达谱组测序
- 单细胞免疫组库测序

基因组学服务

- GWAS芯片
- CNV芯片
- Massarray Taqman KASP SNP分型
- MLPA CNV检测

微生物基因组学服务

- 16S/18S扩增子测序
- 宏基因组测序

代谢组学服务

- 非靶向代谢组
- 非靶向脂质组
- 代谢流
- 定制代谢组

基因分型经典与创新科研路线

- GWAS经典方案设计

- GWAS与NGS技术联合方案设计

- GWAS多组学整合方案设计

- 候选基因SNP方案设计

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基因分型经典与创新科研路线——SNP分型检测技术概述

GWAS芯片

- ✓ 中华芯片：约90万个位点，覆盖中国人群SNP密度最高的芯片
- ✓ ASA芯片：约75万位点，以东亚人群数据为依据，价格低廉

Masarray SNP分型

适用于位点数量10-300个SNP分型检测

全外显子组/靶基因捕获NGS测序

更多适用于大家系样本，筛选功能性稀有/罕见突变

Taqman、KASP SNP分型

适用于位点数量1-10个SNP分型检测。

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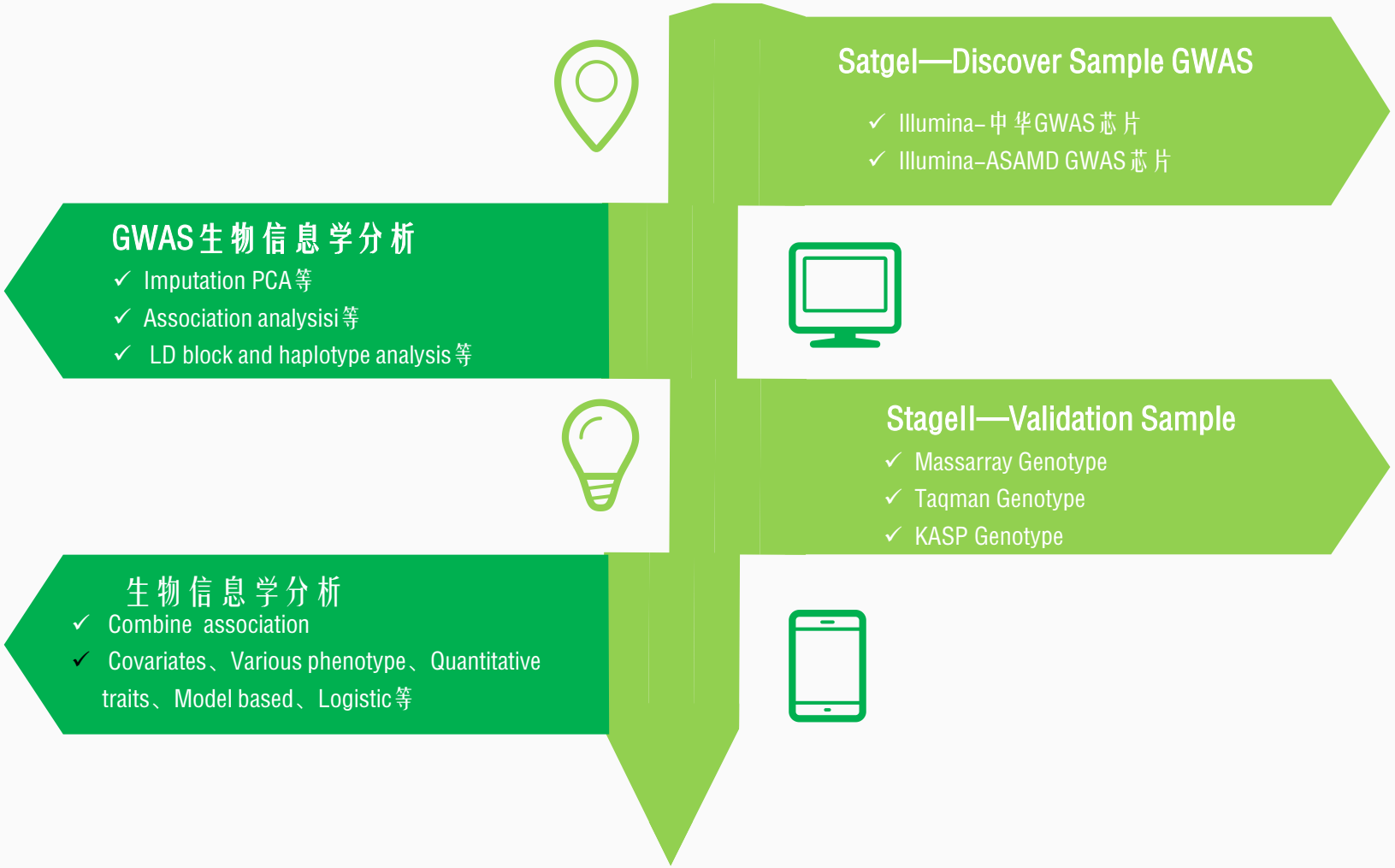
GWAS经典方案设计



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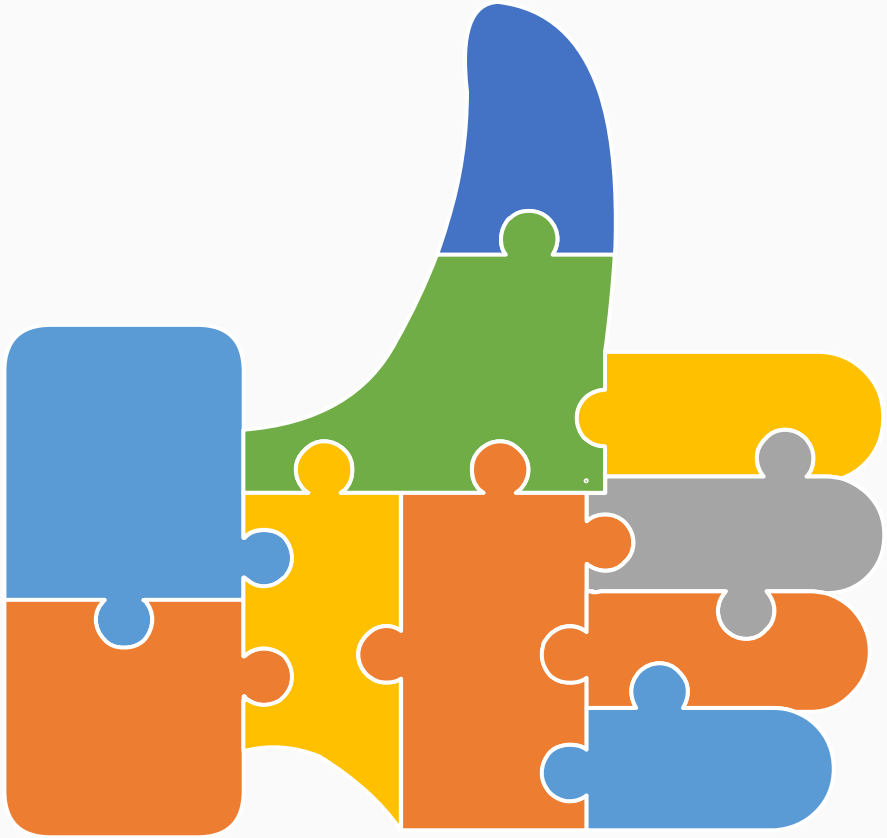
适合中国人群GWAS芯片介绍



- **中华芯片**

✓ 位点数量：90万个
✓ 中国人群，MAF>1%，覆盖率最高
✓ Imputation位点数量>1000万个

- **ASAMD芯片**

✓ 位点数量：74万个
✓ 东亚人群，MAF>1%
✓ Imputation位点数量>650万个
✓ 价格低廉
✓ 兼具科研及转化应用双重价值

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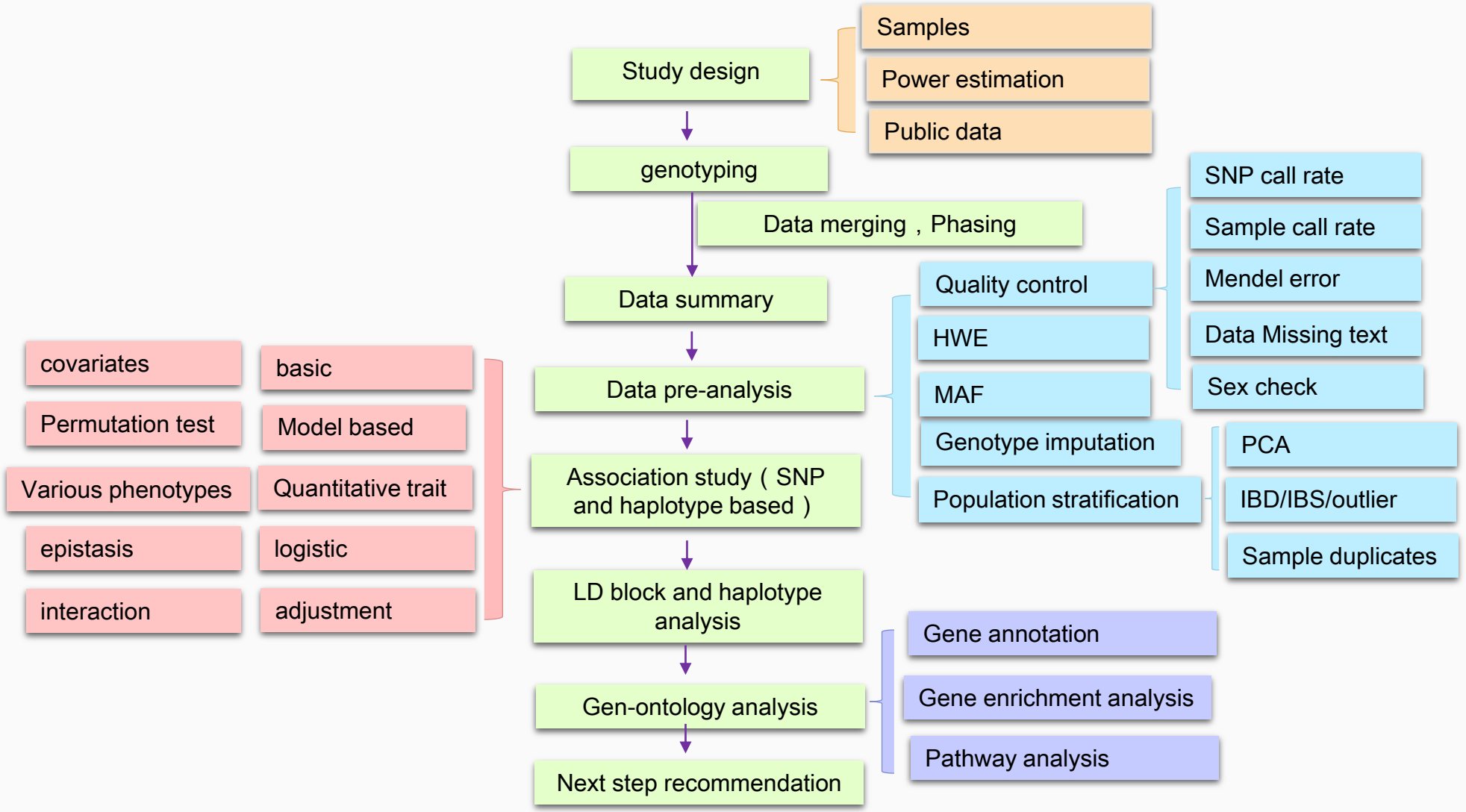
GWAS生物信息学分析流程



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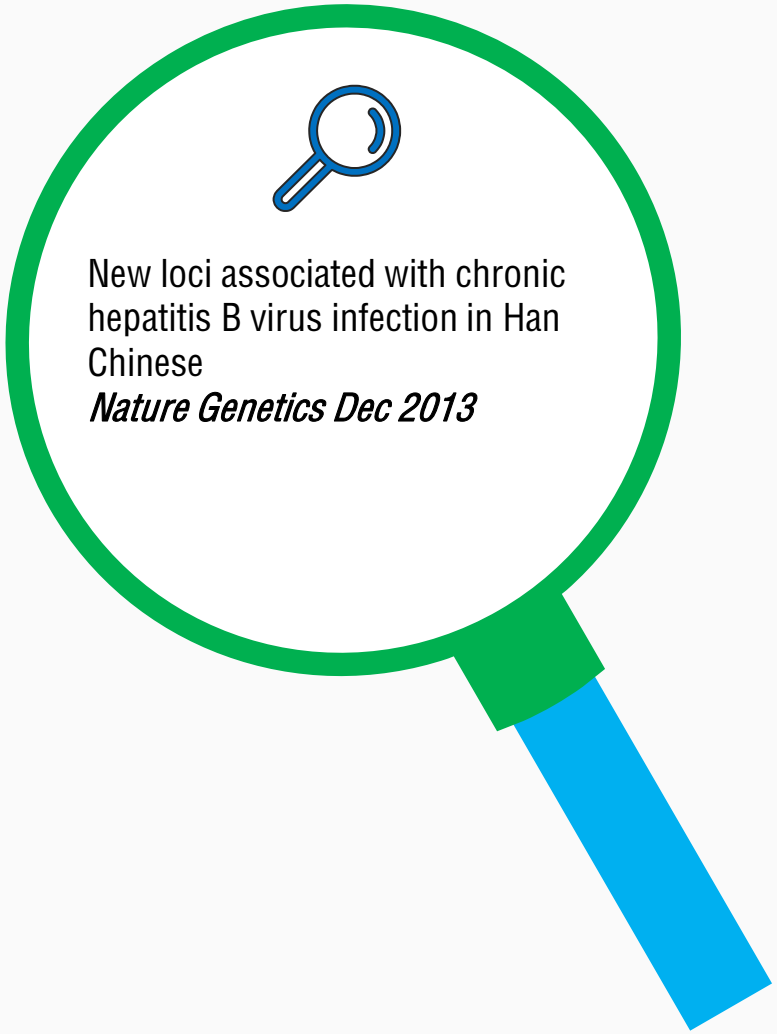
GWAS经典文章路线分享



Discover–GWAS检测

- ✓ 中华GWAS芯片
- ✓ 951 HBV carriers and 937 individuals who had naturally cleared HBV infection

Replication 检测

- ✓ Massarray SNP Genotype
- ✓ 2248 cases and 3051 controls and additional replications with 1982 HBV carriers and 2622 controls from the general population

生物信息学分析

- ✓ In the combined analysis, all three loci reached genome–wide significance for association with chronic HBV infection

变异位点分子功能学预测讨论

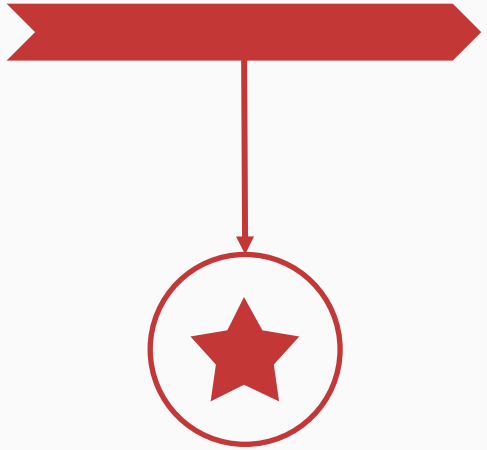
- ✓ rs7453920 ——intron,A transcriptome study showed that the A allele of rs7453920 was associated with higher HLA–DQ mRNA levels in circulating monocytes, which are critical for mounting immune responses。Additionally, the A allele in HLA–DQB2 is consistent with a PPARγ–binding site, but the G allele is not
- ✓ rs3130542 —— near HLA–C， The A allele of rs3130542 was associated with lower expression of HLA–C36
- ✓ Rs4821116——intron, associated with higher expression of UBE2L3

博淼GWAS代表文章

A functional copy–number variation in MAPKAPK2 predicts risk and prognosis of lung cancer *AJHG 2012*

TaqMan real-time quantitative PCR (qPCR) method according to the protocol of Applied BioSystems (catalog no. Hs01173160, Applied BioSystems, Foster City, CA, USA)[11] and validated the results with the AccuCopy assay (a multiple competitive real-time PCR) by Genesky Bio-Tech (Shanghai, China) and Affymetrix Genome-Wide Human SNP Array 6.0 by Bio Miao Biological Technology (Beijing) in 200 randomly selected samples (Figures S1 and S2);[12] the results were 97.5% or 98.0% concordant.

We detected three kinds of the g.CNV-30450 (i.e., two, three, and four copies) in blood samples, and found a significant difference in the distributions of the g.CNV-30450 genotypes between individuals with lung cancer and unaffected controls ($p = 2.26 \times 10^{-6}$) (Table 1). After



Discovery of susceptibility loci associated with tuberculosis in Han Chinese *Hum Mol Genet. 2017*

We collected blood samples from the TB and control groups. Samples were stored at −80 °C. Genomic DNA was extracted from 400 μl EDTA anti-coagulated whole blood samples using QIAamp DNA Blood Midi Kits (Qiagen, Duesseldorf, Germany). The DNA sample concentration was 50 ng/μlin the discovery stage and 10 – 30 ng/μl in the replication stage. DNA samples were quantified using a NanoDrop ND-1000 spectrophotometer (Thermo, MA, USA) and agarose gel electrophoresis. In the discovery stage, 1008 cases and 1538 control samples were genotyped by Bio Miao Biological Technology (Beijing, China) using the HumanOmniZhongHua-8 v1.1 BeadChip (Illumina, CA, USA) in accordance with the manufacturer's specifications. The investigators were blinded to the group allocation of chips during the genotyping. Genotypes of 900, 015 SNPs were analysed using the genotyping module of GenomeStudio v3.0 (Illumina). In total, 2526 DNA samples were successfully genotyped at a call rate >99.8%; the genotype call threshold (boundary for calling a genotype relative to its associated cluster) was 0.15. The genotype reoccurrence rate for 12 duplicated individuals was 99.99% on average.

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GWAS与NGS技术联合方案设计

GWAS研究——家系样本

✓ 家系中各代的case样本

外显子组测序

✓ 至少两代直系遗传的case样本
✓ 家系中的control样本/1000Genomes及ESP数据作为对照

生物信息学分析

✓ Linkage analysis
✓ Consegregation analysis
✓ Mutation prediction analysis

细胞/动物模型分子功能验证

筛选功能意义的突变进行invitro及invivo分子功能机制研究

GWAS研究——散发样本

✓ 大样本GWAS检测，基于Common SNP发现易感区域及基因
✓ Meta analysis易感区域及基因

外显子组测序/靶基因捕获测序

✓ 全外显子组测序，与上述GWAS易感基因进行稀有/罕见致病突变挖掘
✓ 根据上述GWAS易感基因通过基因捕获进行NGS更深度测序，挖掘稀有/罕见致病突变

Massarray/Taqman/KASP突变验证

针对上述发现的高频、低频、罕见突变进行大人群、多中心验证

细胞/动物模型分子功能验证

筛选功能意义的突变进行invitro及invivo分子功能机制研究

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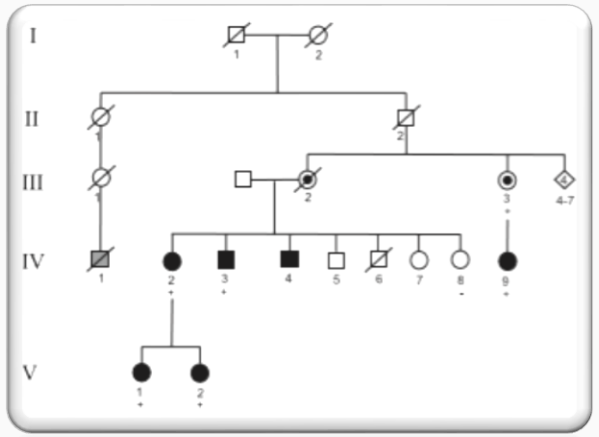
GWAS与NGS经典文章路线分享

Exome Sequencing Identifies a Novel Variant in ACTC1 Associated With Familial Atrial Septal Defect
Canadian Journal of Cardiology 2014

GWAS检测

- ✓ IV–2 V–1 V–2 IV–9 III–3五个case样本
- ✓ Illumina Omni 2.5M array



Exome sequence检测

- ✓ IV–2 V–1两个case样本
- ✓ Agilent SureSelect Human All Exon 50Mb

Validation 检测

- ✓ 84个散发case样本
- ✓ sanger测序技术验证上述筛选的潜在致病突变

结论

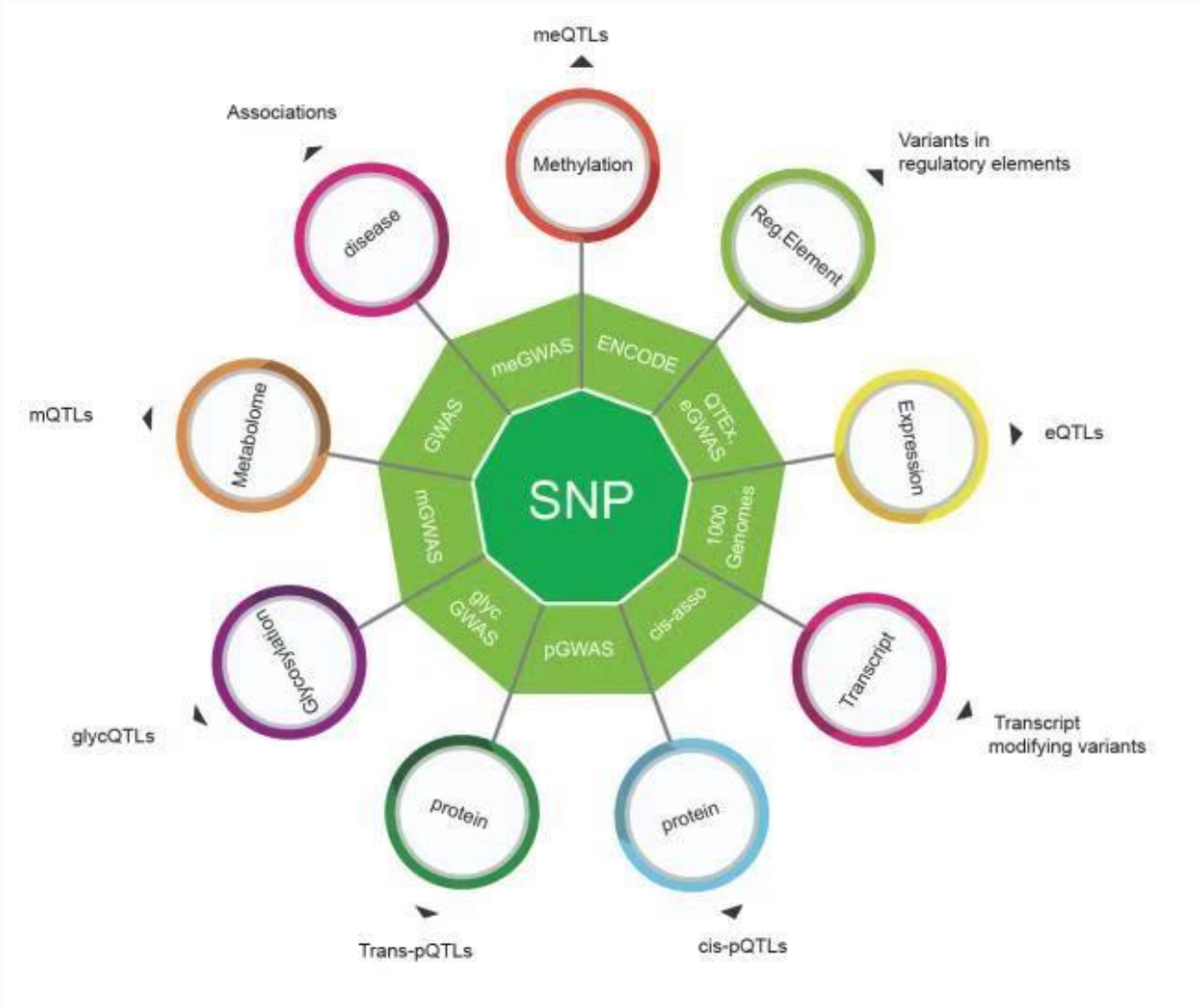
- ✓ p.M178L mutation was identified as benign by half of the mutation prediction algorithms tested. PolyPhen–2, MutationAssessor, and PROVEAN predicted that the p.M178L substitution will be benign or neutral in its effect on protein function

GWAS与NGS经典文章路线分享——重要结论展示

Algorithm	p.M178L Score	p.M178L Prediction	p.M123V Score	p.M123V Prediction	p.E101K Score	p.E101K Prediction
PolyPhen-2	0.035	Benign	0.011	Benign	0.788	Possibly damaging
PROVEAN	0.306	Neutral	−2.55	Deleterious	−2.919	Deleterious
MutationAssessor	−0.82	Neutral functional impact	3.785	High functional impact	2.84	Medium functional impact
SNPs&GO	7	Disease	9	Disease	9	Disease
Align-GVGD	14.3	Less likely to interfere with function	20.52	Less likely to interfere with function	56.87	More likely to interfere with function
MutationTaster	15	Disease-causing	21	Disease-causing	56	Disease-causing

Results of mutation prediction algorithms for 3 mutations in ACTC1

GWAS多组学整合方案设计



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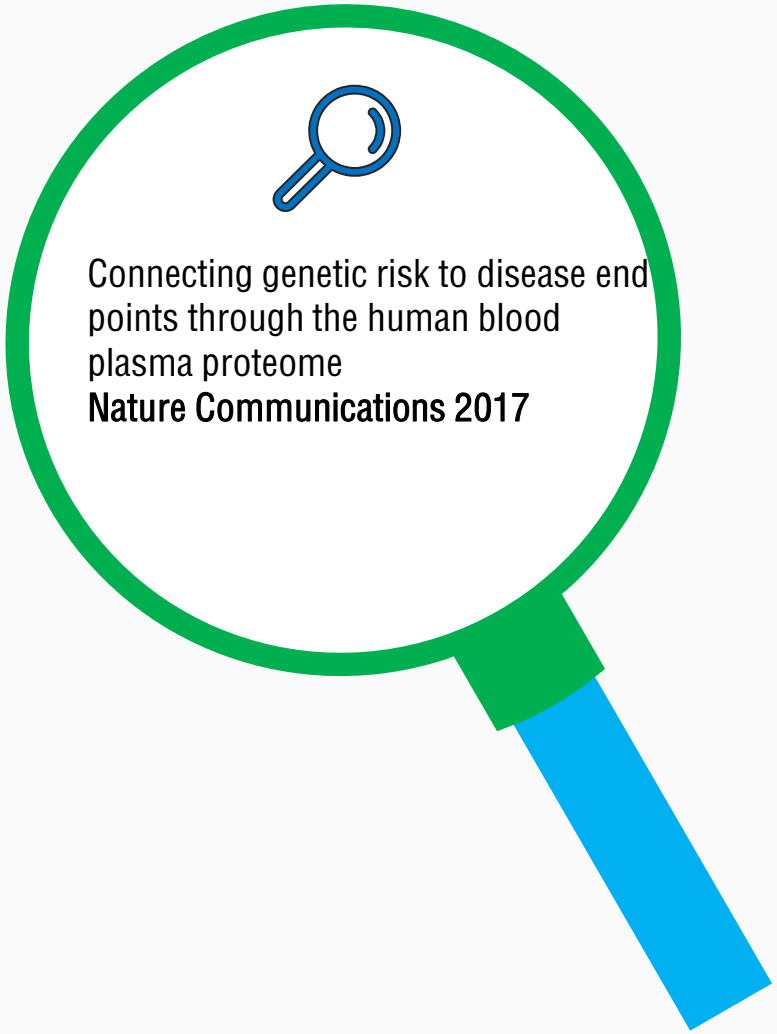
GWAS多组学经典文章路线分享



GWAS检测

- ✓ Illumina Omni 2.5+Affy GWAS
- ✓ 3788 samples+359 samples

Proteomics measurements

- ✓ the SOMAscan platform was used to quantify protein levels
- ✓ 1000 Samples

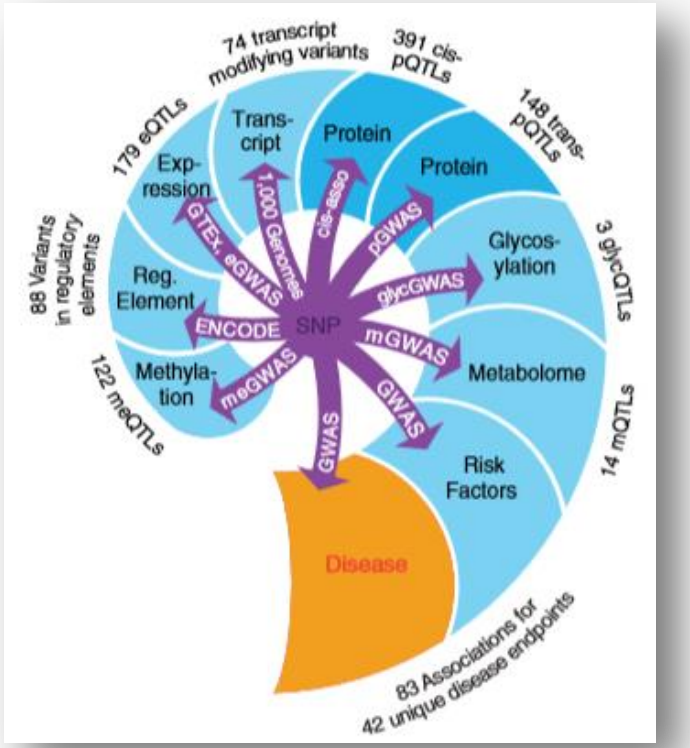
Methylation+Metabolomics+mRNA sequencing

- ✓ Data was downloaded from the BIOS QTL browser
- ✓ GWAS associations with mQTLs were obtained using the GWAS–server
- ✓ RNA sequencing data was downloaded from EBI

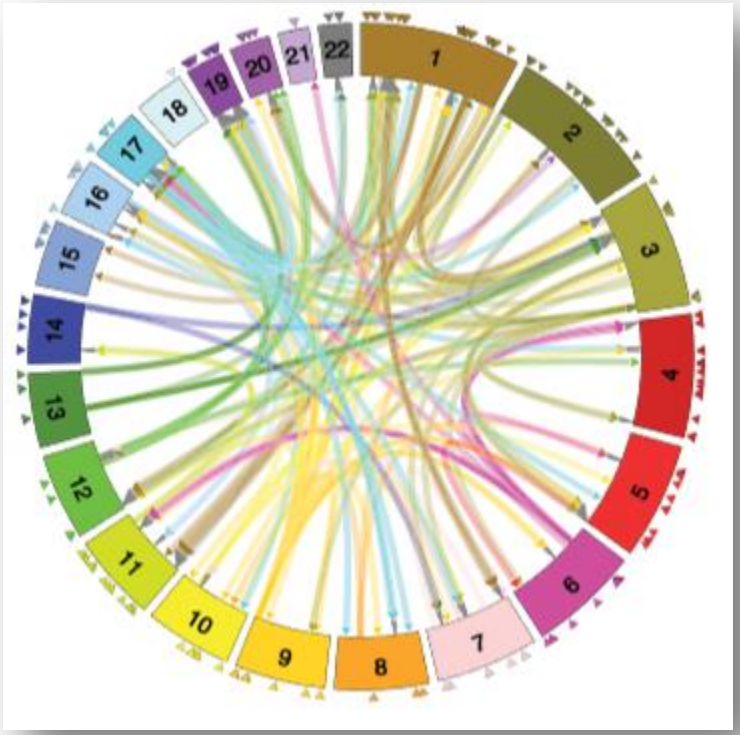
结论

- ✓ Fifty–five of the replicated pQTLs are located in trans
- ✓ overlap with 57 genetic risk loci for 42 unique disease end points
- ✓ a genome–proteome network and provide an interactive web–tool for interrogations.

GWAS多组学经典文章路线分享——重要结论展示

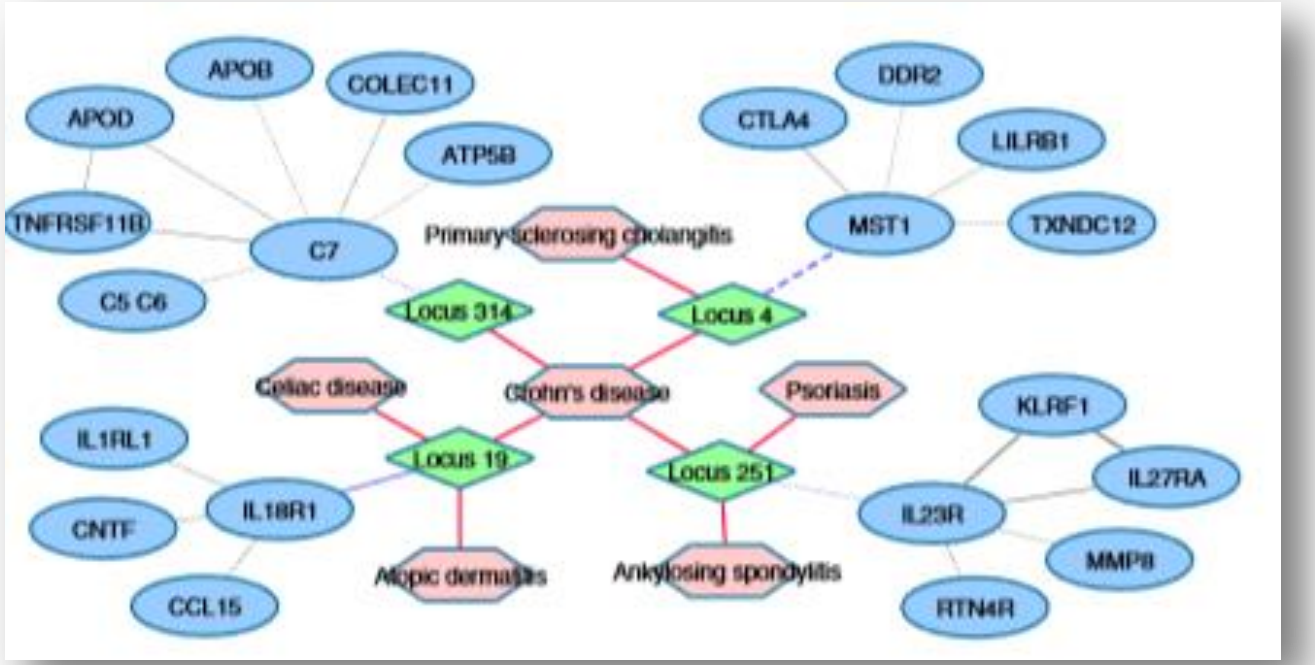


Data sources integrated into the network



Circular plot of all cis– and trans–associations

GWAS多组学经典文章路线分享——重要结论展示



Example of a genome–proteome–disease sub–network， This example revealed four risk loci that associated with plasma levels of C7, MST, IL23R and IL18R, respectively. These four proteins all have a major role in auto–immune disorders

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候选基因SNP方案设计

01 确定候选基因

- ✓ 全基因组芯片及NGS筛选的差异基因
- ✓ 表型机制相关的功能基因
- ✓ GEO/TCGA分析的调控基因
- ✓ 文献报道的基因多态性易感基因

02 候选SNP位点筛选

- ✓ 1000Genomes/Hapmap数据库
- ✓ Functional Region SNP/tagSNP筛选
- ✓ Validated/Hot SNP筛选
- ✓ Function prediction analysis

03 样本分型检测

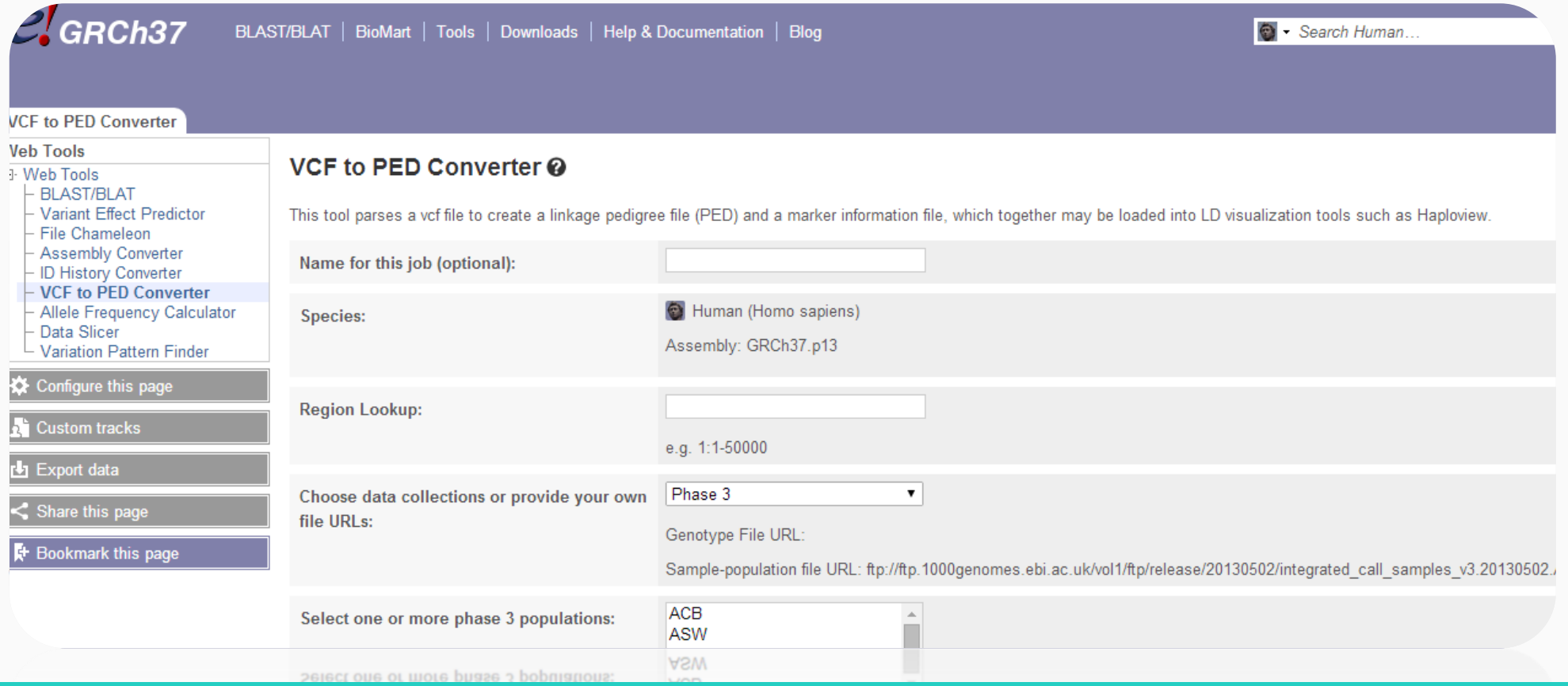
- ✓ Massarray Genotype
- ✓ Taqman Genotype
- ✓ KASP Genotype

04 生物信息学分析

Covariates 、Various phenotype、Quantitative traits、Model based、Logistic等

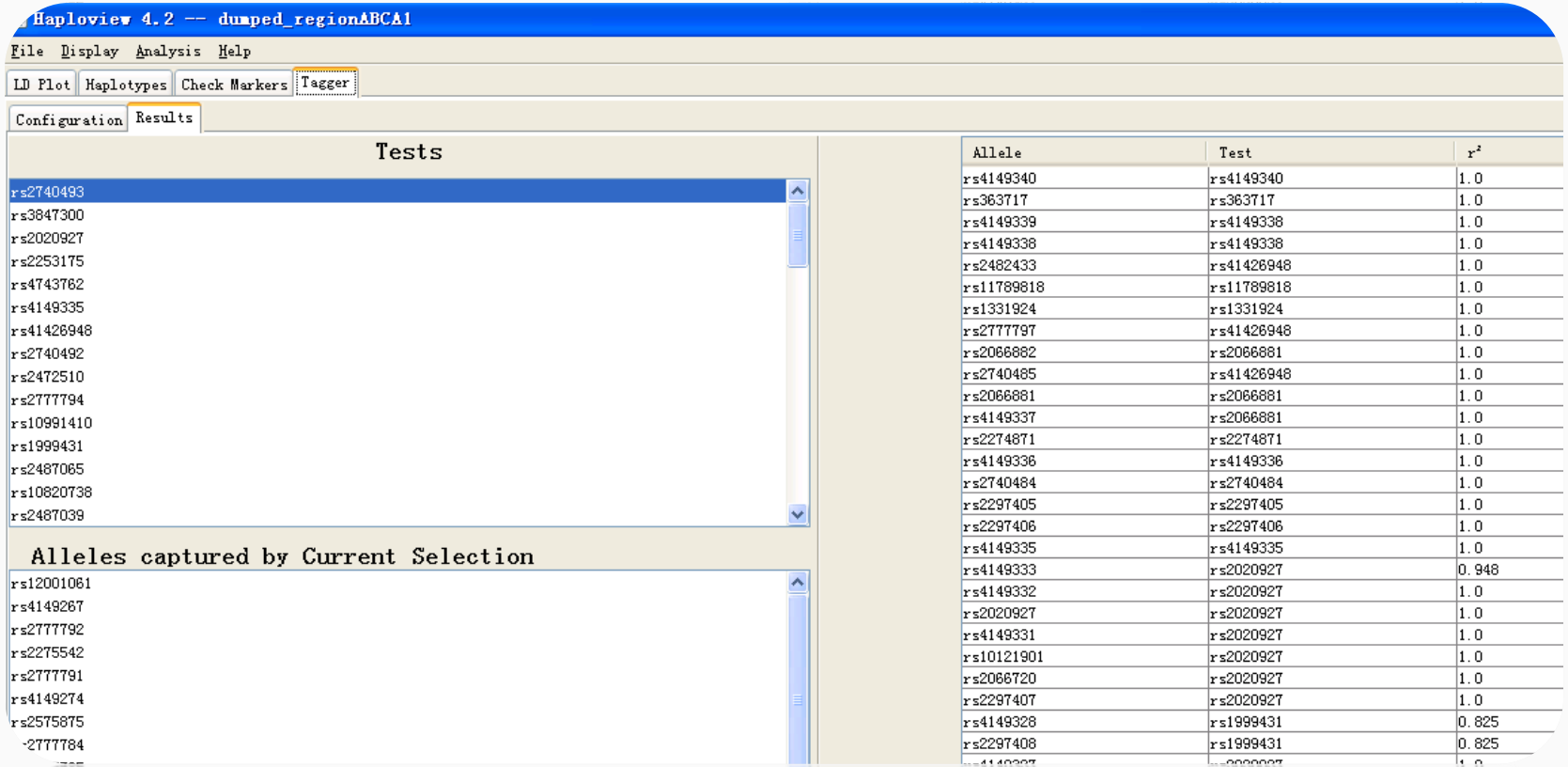
候选基因SNP方案设计路线展示

基于1000Genomes CHB/CHS数据的tagSNP筛选



候选基因SNP方案设计路线展示

通过Haploview进行tagSNP分析



候选基因SNP方案设计路线展示

基于Hapmap CHB数据的tagSNP筛选

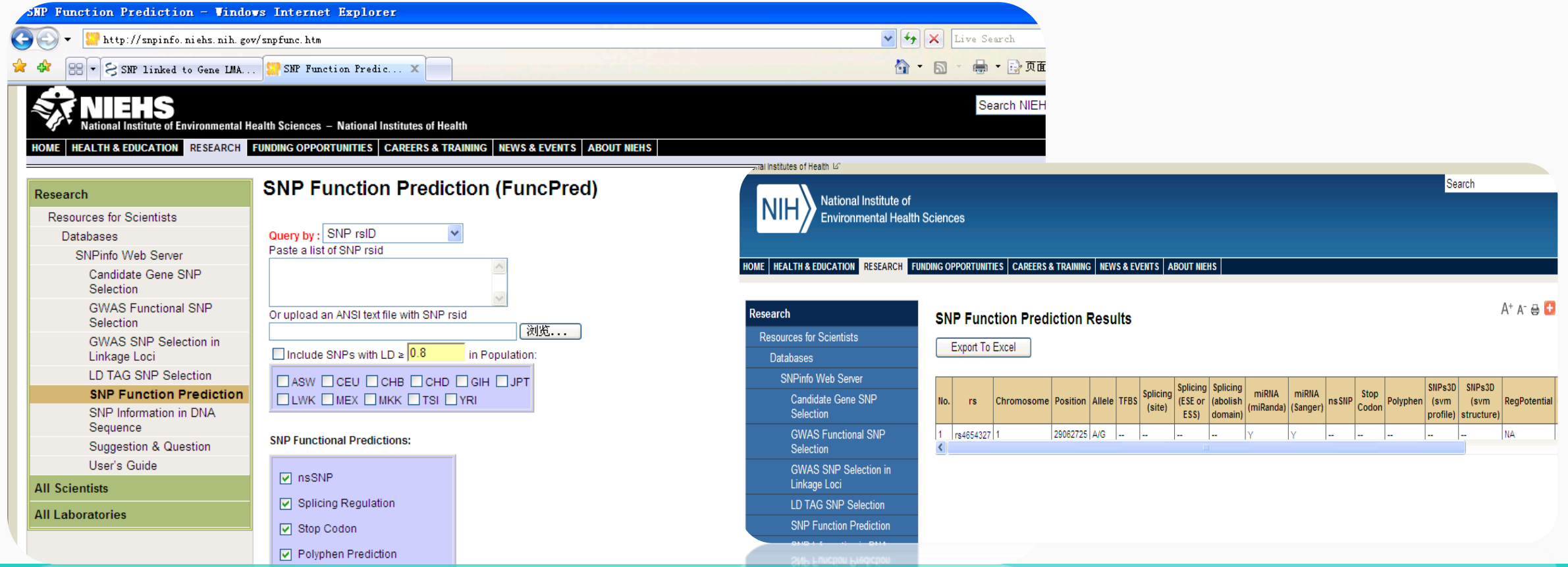
候选基因SNP方案设计路线展示

基于dbSNP数据库进行functional region SNP筛选

Number of variant consequences		Type	Description
0	-	Transcript ablation	A feature ablation whereby the deleted region includes a transcript feature (SO:0001893)
4	Show	Splice donor variant	A splice variant that changes the 2 base region at the 5' end of an intron (SO:0001575)
1	Show	Splice acceptor variant	A splice variant that changes the 2 base region at the 3' end of an intron (SO:0001574)
0	-	Stop gained	A sequence variant whereby at least one base of a codon is changed, resulting in a premature stop codon, leading to a shortened transcript (SO:0001587)
0	-	Frameshift variant	A sequence variant which causes a disruption of the translational reading frame, because the number of nucleotides inserted or deleted is not a multiple of three (SO:0001589)
0	-	Stop lost	A sequence variant where at least one base of the terminator codon (stop) is changed, resulting in an elongated transcript (SO:0001578)
0	-	Initiator codon variant	A codon variant that changes at least one base of the first codon of a transcript (SO:0001582)
0	-	Transcript amplification	A feature amplification of a region containing a transcript (SO:0001889)
0	-	Inframe insertion	An inframe non synonymous variant that inserts bases into in the coding sequence (SO:0001821)
0	-	Inframe deletion	An inframe non synonymous variant that deletes bases from the coding sequence (SO:0001822)
0	-	Missense variant	A sequence variant, that changes one or more bases, resulting in a different amino acid sequence but where the length is preserved (SO:0001583)
7	Show	Splice region variant	A sequence variant in which a change has occurred within the region of the splice site, either within 1-3 bases of the exon or 3-8 bases of the intron (SO:0001630)
0	-	Incomplete terminal codon variant	A sequence variant where at least one base of the final codon of an incompletely annotated transcript is changed
0	-	Synonymous variant	A sequence variant w
0	-	Stop retained variant	A sequence variant w remains (SO:0001567
0	-	Coding sequence variant	A sequence variant that changes the coding sequence (SO:0001580)
0	-	Mature miRNA variant	A transcript variant located with the sequence of the mature miRNA (SO:0001620)
0	-	5 prime UTR variant	A UTR variant of the 5' UTR (SO:0001623)
0	-	3 prime UTR variant	A UTR variant of the 3' UTR (SO:0001624)
142	Show	Non coding transcript exon variant	A sequence variant that changes non-coding exon sequence in a non-coding transcript (SO:0001792)
1228	Show	Intron variant	A transcript variant occurring within an intron (SO:0001627)
0	-	NMD transcript variant	A variant in a transcript that is the target of NMD (SO:0001621)
1375	Show	Non coding transcript variant	A transcript variant of a non coding RNA gene (SO:0001619)
451	Show	Upstream gene variant	A sequence variant located 5' of a gene (SO:0001631)
488	Show	Downstream gene variant	A sequence variant located 3' of a gene (SO:0001632)
2314	Show	ALL	All variations

候选基因SNP方案设计路线展示

进行Function prediction analysis筛选

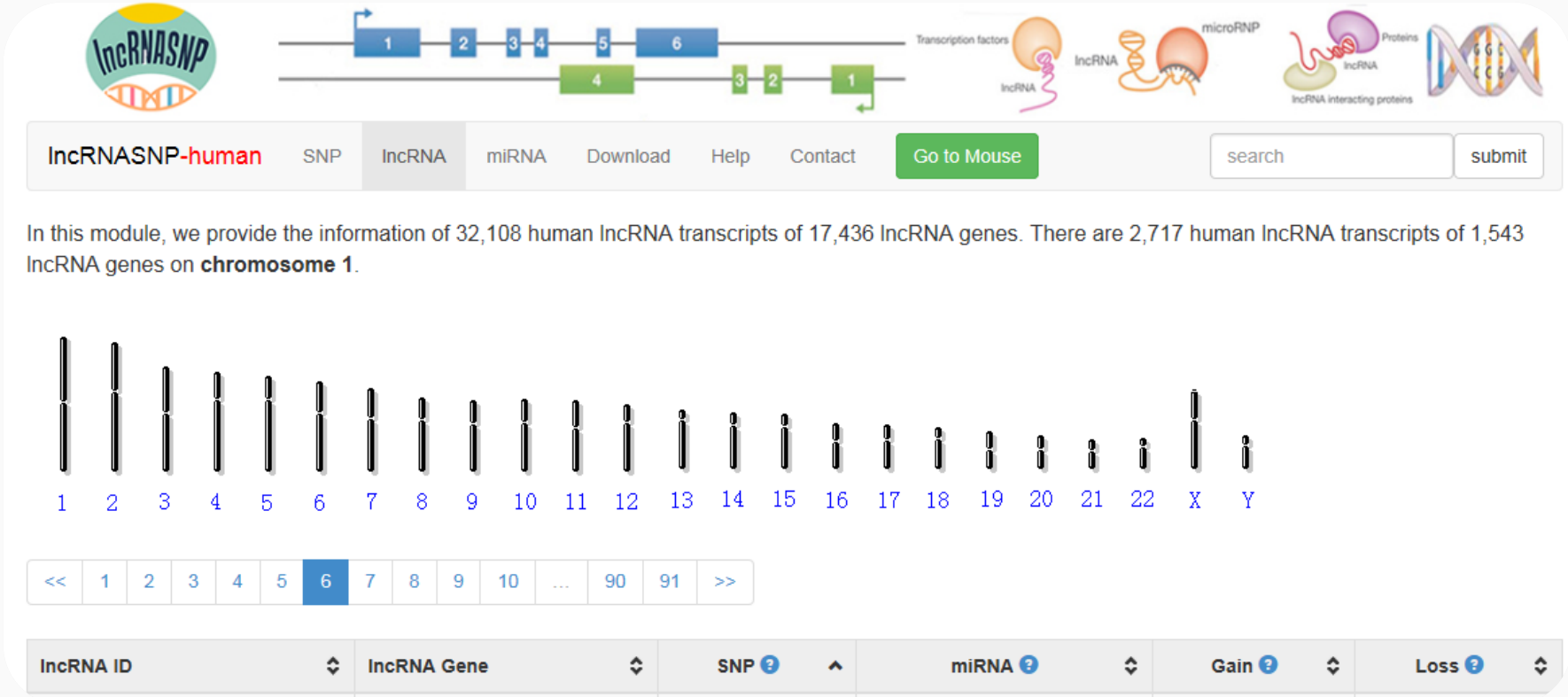


候选基因SNP方案设计路线展示

通过google/pubmed进行validated/Hot SNP筛选

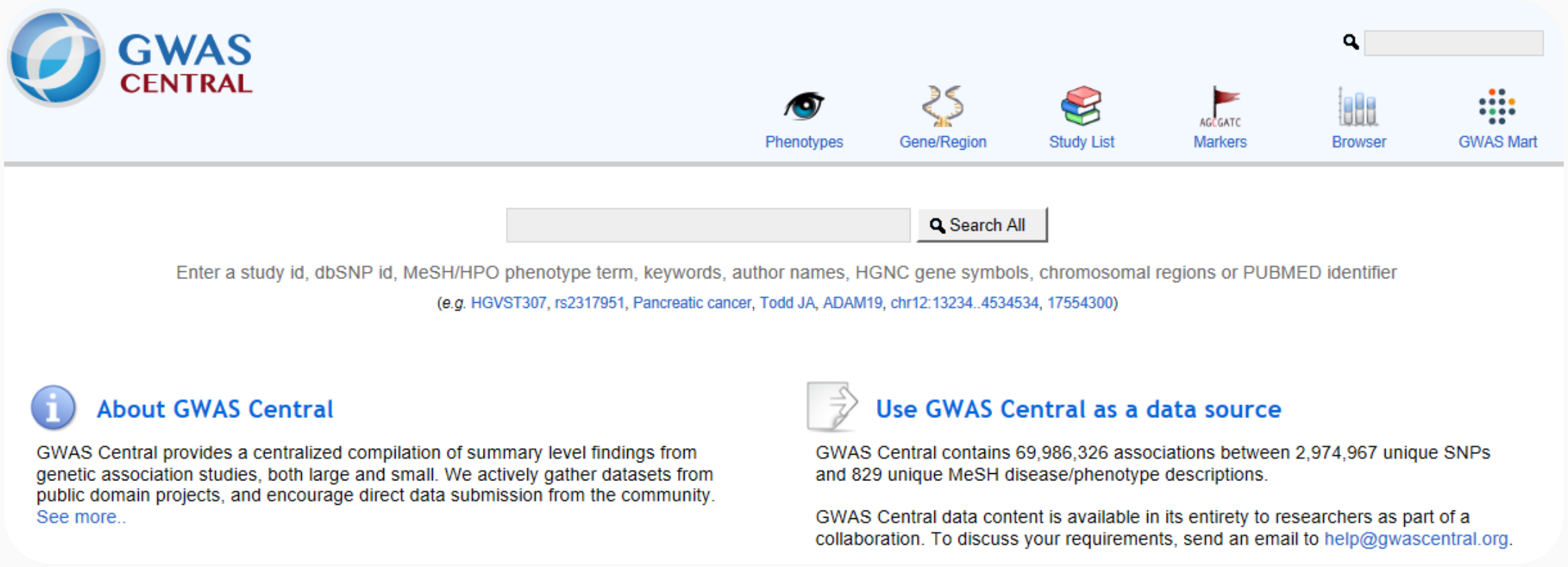
候选基因SNP方案设计路线展示

基于ceRNA机制进行功能lncRNA-miRNA相关SNP筛选



候选基因SNP方案设计路线展示

基于GWAS数据库筛选



候选基因SNP方案设计路线展示

针对药物基因组SNP研究领域的数据库——CPIC

Genes-Drugs

CPIC assigns CPIC levels to genes/drugs with (1) PharmGKB Clinical Annotation Levels of Evidence of 1A, 1B, 2A and 2B, or (2) a PharmGKB PGx level for FDA-approved drug labels of "actionable pgx", "genetic testing recommended", or "genetic testing required", or (3) based on nomination to CPIC for consideration.

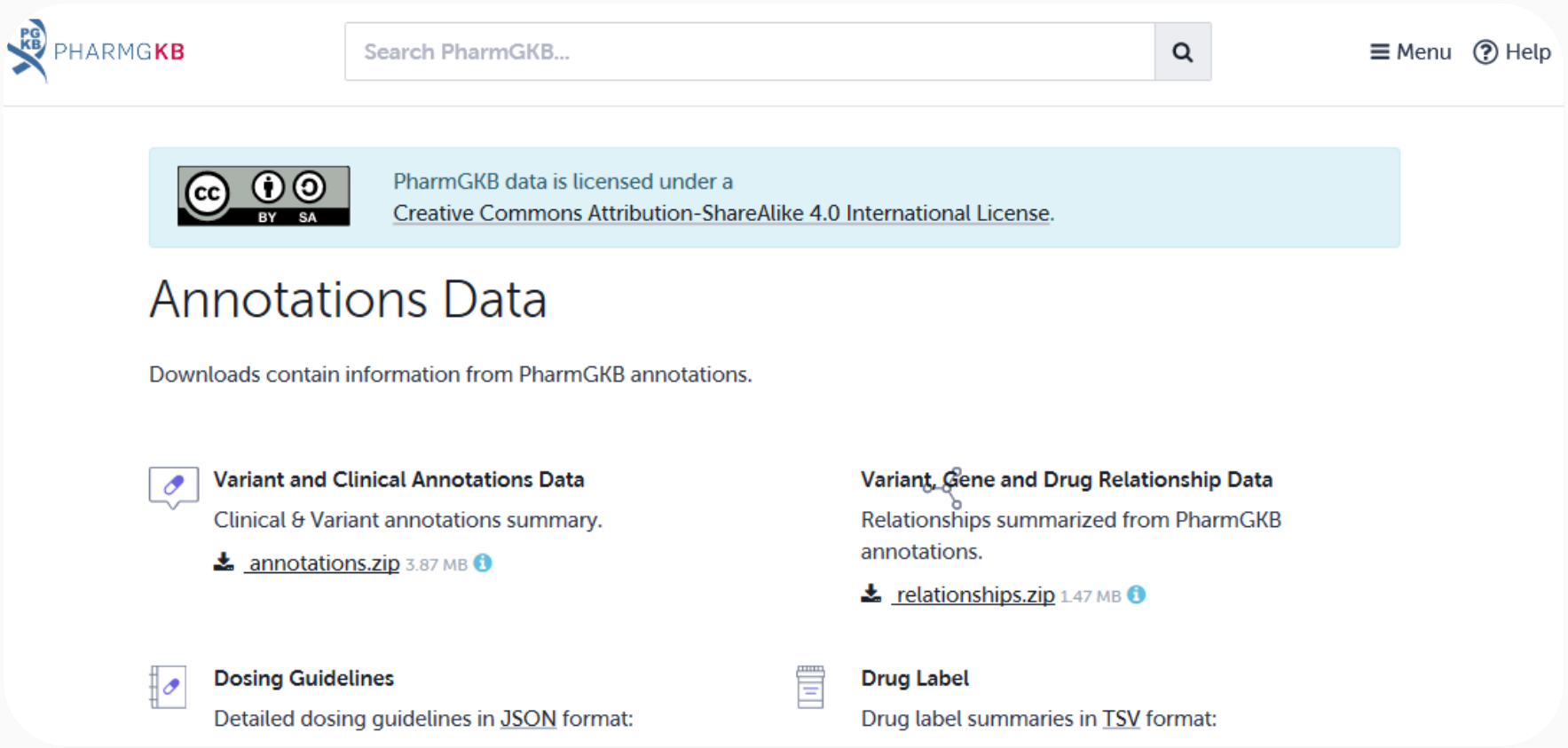
- View CPIC's process for assigning CPIC levels
- View CPIC's levels for genes/drugs
- View CPIC's process for prioritizing CPIC guidelines

CPIC invites feedback on existing and planned gene/drug guidelines.

Download Table (CSV)

候选基因SNP方案设计路线展示

针对药物基因组SNP研究领域的数据库——PharmGKB



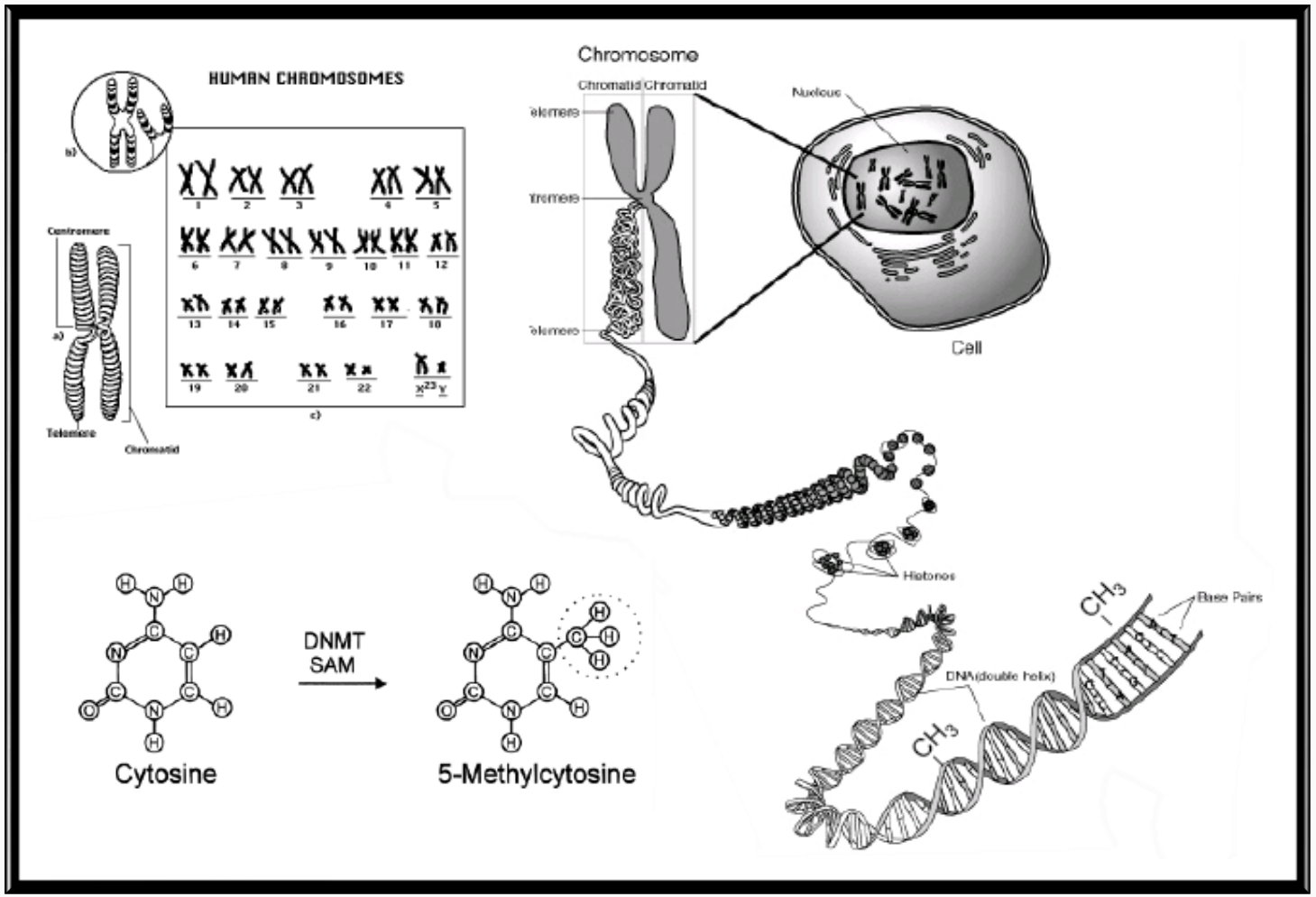
表观遗传学经典与创新科研路线

- EWAS经典方案设计

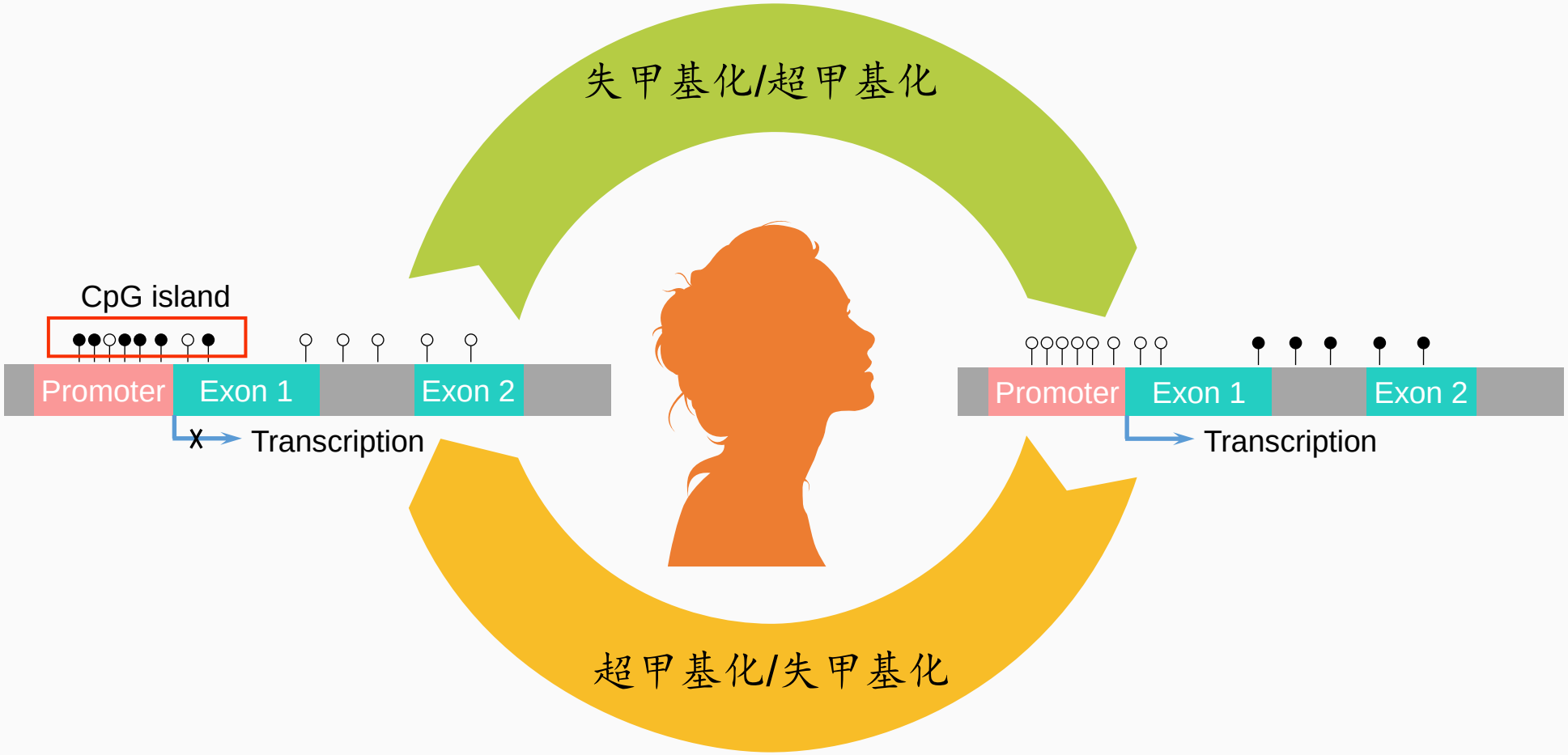
- EWAS与GWAS、代谢组学整合方案设计

- 候选基因DNA甲基化方案设计

表观遗传学经典与创新科研路线——基础理论



表观遗传学经典与创新科研路线——基础理论



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表观遗传学经典与创新科研路线——DNA甲基化检测技术概述

全基因组DNA甲基化芯片检测

- ✓ 采用Illumina 850K芯片
- ✓ 约85万个CpG sites
- ✓ 价格低廉、准确性高

全基因组DNA甲基化NGS检测

- ✓ 约550万个CpG sites
- ✓ 价格昂贵、数据准确性不足

Massarray靶向位点甲基化检测

- ✓ 一次性覆盖400-600bp区域
- ✓ 芯片/NGS二期关联位点验证金标准技术
- ✓ 价格低廉、准确性高

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EWAS经典方案设计

全基因组甲基化检测

✓ 850K全基因甲基化芯片

生物信息学分析

✓ 数据预处理
✓ 差异CpG site分析、聚类分析等
✓ 显著基因分析等

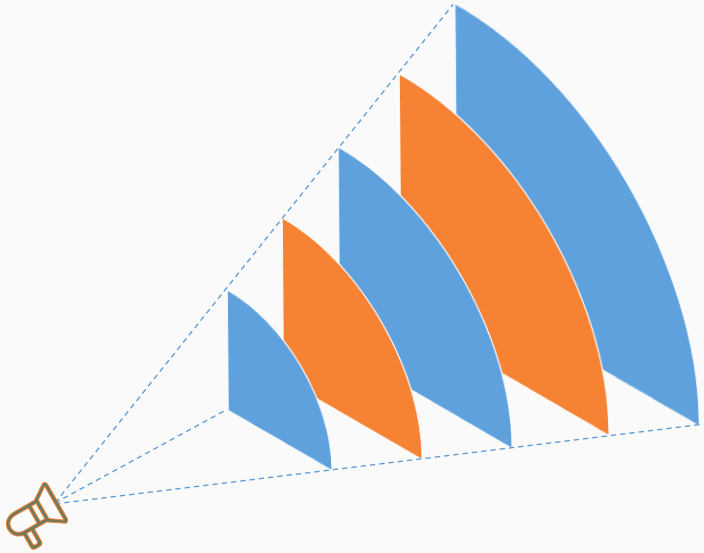
二期样本replication验证

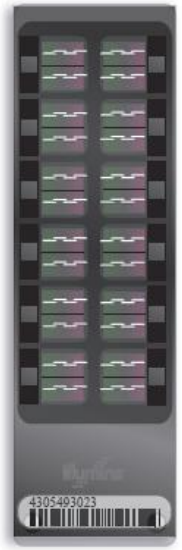
✓ Massarray技术

功能预测及验证

✓ 转录因子预测
✓ 与基因转录表达、剪切的功能机制研究

EWAS芯片介绍





- **Infinium MethylationEPIC BeadChip**

✓ Over 850,000 methylation sites

✓ Coverage gene promoter、5UTR、first exon、gene body、3UTR

✓ miRNA promoter regions

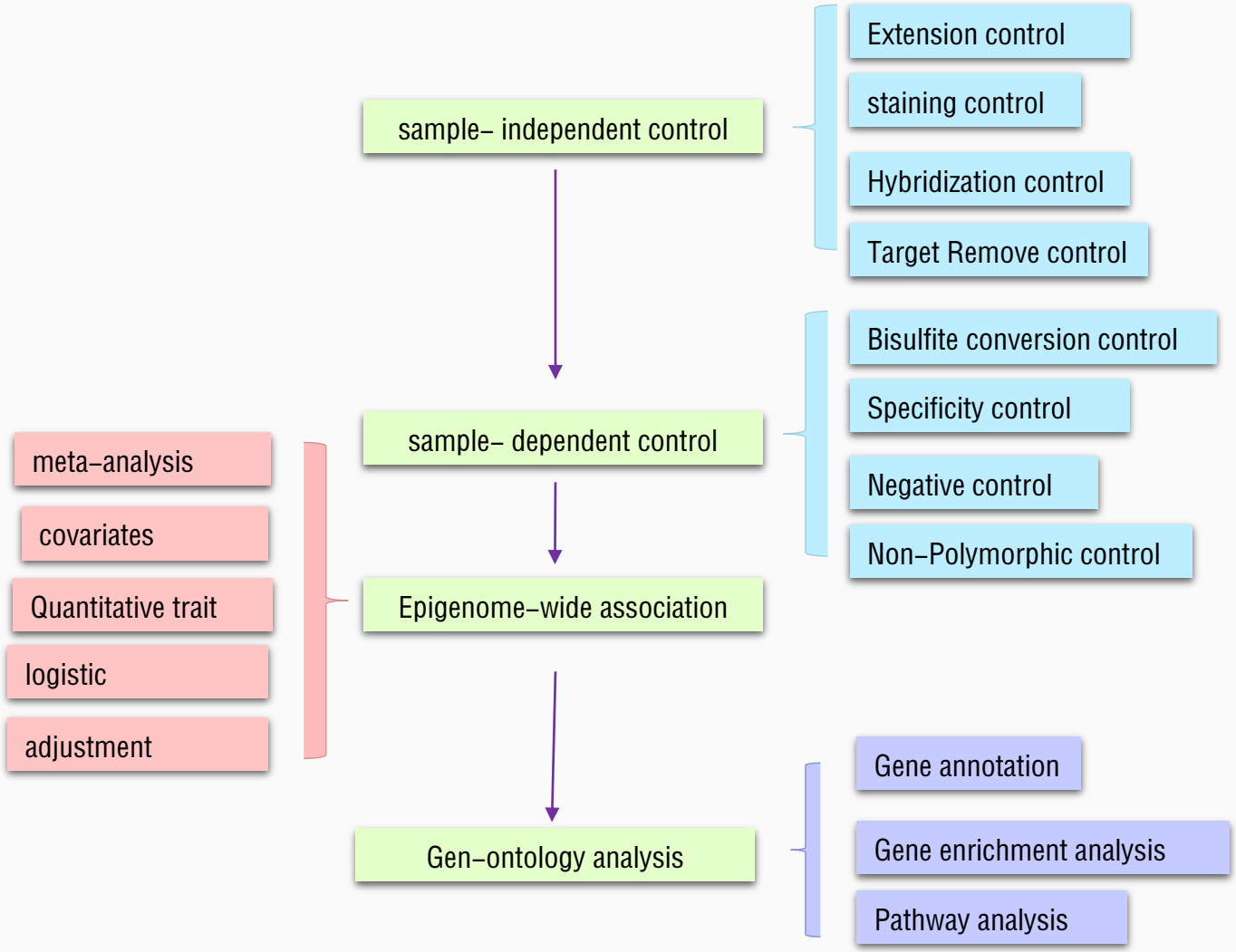
✓ DNase hypersensitivity sites

✓ CpG island

✓ Compatible with FFPE Sample

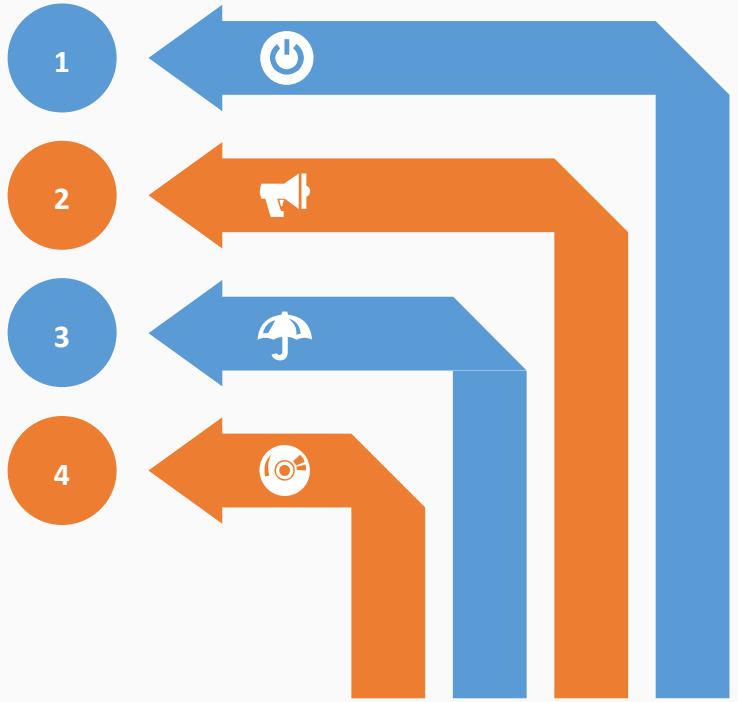
……

EWAS生物信息学分析流程



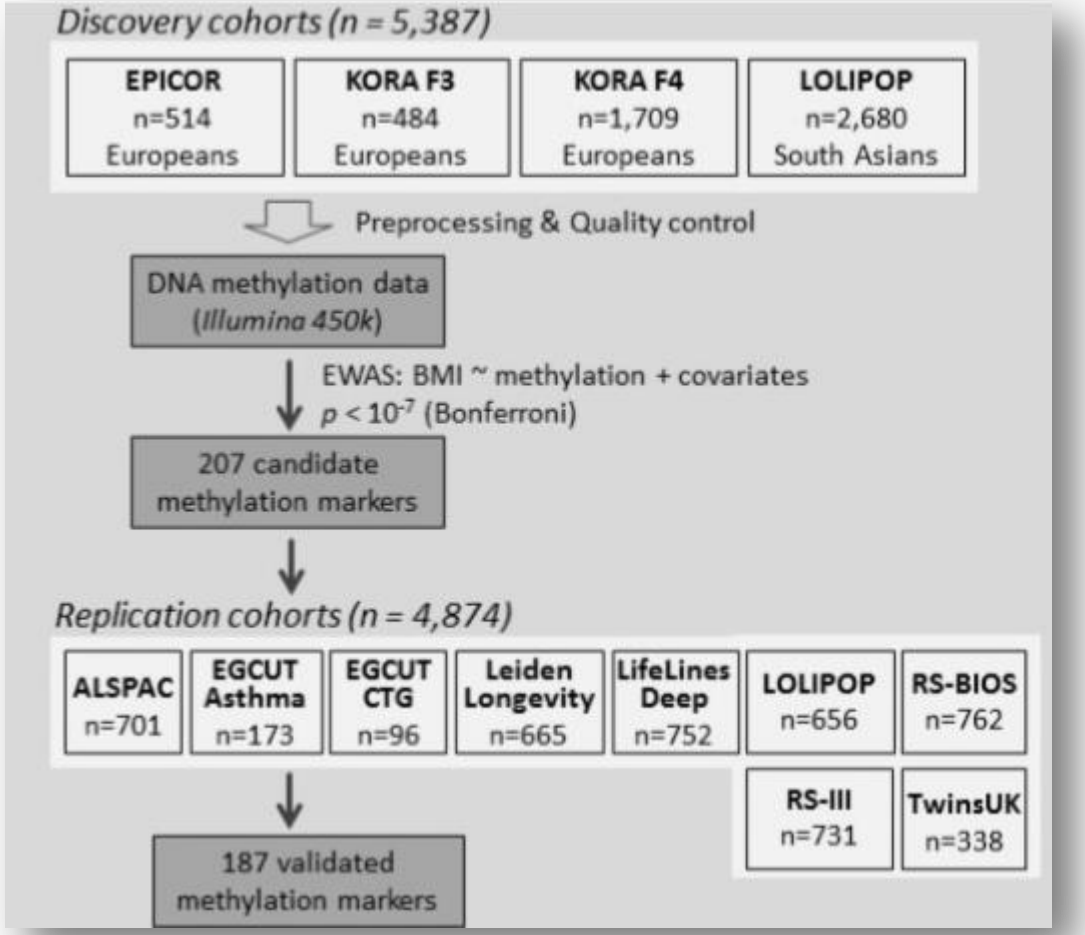
EWAS replication位点筛选策略

- ✓ 结合Delta β和p-values分析数据筛选差异显著的CG位点
- ✓ 分析差异显著及数量集中分布的基因功能，以与研究表型功能机制相关为优先
- ✓ 基因位置选择，优先选择CpG位点位于Promoter区（包括TSS1500、TSS200），以及5’UTR区的芯片位点，且以其中CpGisland为最优先
- ✓ 对芯片高密度覆盖区域数据进行DMR（Differentially Methylated Regions）分析，选择差异显著的DMR，并进行相关基因的功能分析



EWAS经典文章路线分享

Epigenome–wide association study of body mass index, and the adverse outcomes of adiposity
Nature2017



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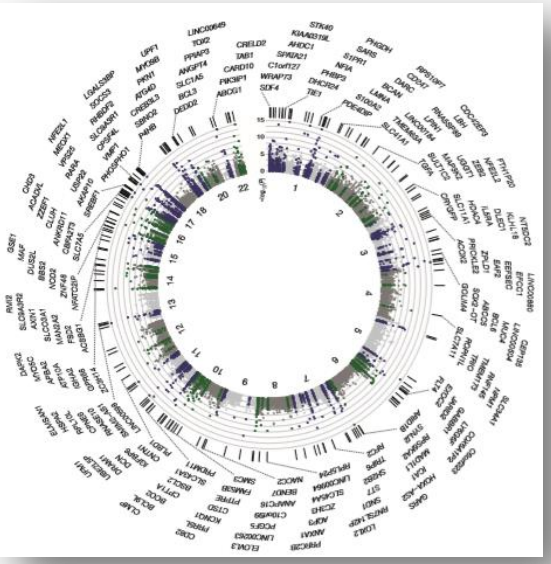
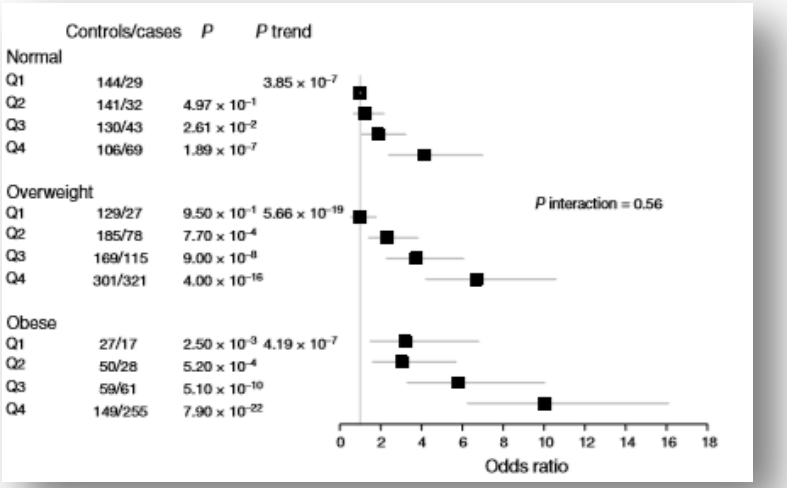
EWAS经典文章路线分享

Genetic association and causality (blood)	CpG → SNP ---> BMI GRS ---> CpG	KORA F3/F4 n=2,193	LOLIPOP n=1,841	
Longitudinal analyses (blood)	Baseline Meth → Follow-up Δ BMI Δ Meth	KORA S4/F4 n=1,435	LOLIPOP n=1,513	
Cross-tissue methylation		Adipose n=542	Isolated adipocyte n=48	Other n=6
Functional genomics and gene expression		Blood n=1,785	Adipose n=499	Liver n=70
Gene set enrichment analysis				
Metabolic traits and type 2 diabetes	Baseline Meth → Follow-up Incident T2D	LOLIPOP n=2,680	KORA n=1,697/400	



Circos plot of the epigenome–wide association of DNA methylation in blood with BMI



Relative risk of incident type 2 diabetes by quartile of methylation risk score amongst Indian Asians

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EWAS与GWAS联合方案设计

全基因组甲基化EWAS检测

- ✓ 850K全基因甲基化芯片

全基因组SNP GWAS检测

- ✓ 中华芯片
- ✓ ASAMD芯片

全转录组检测

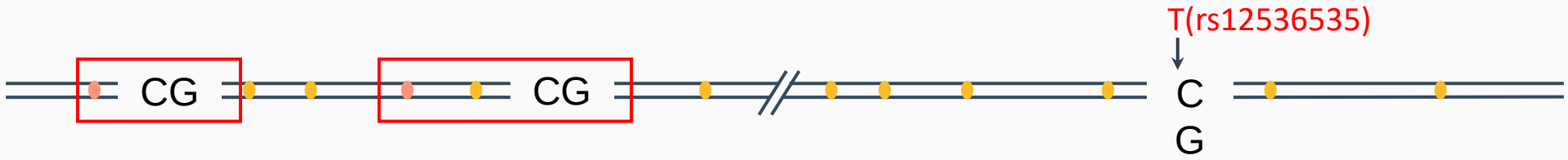
- ✓ 转录组NGS测序
- ✓ 全基因组表达谱新品

生物信息学分析

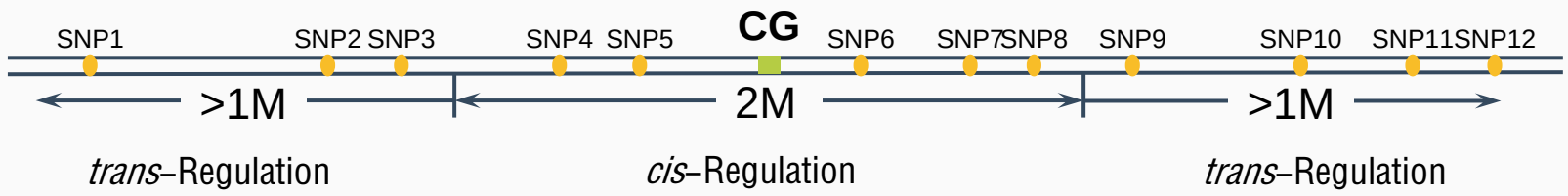
- ✓ SNP与甲基化遗传调控分析
- ✓ SNP与转录组（expression\splice）遗传调控分析
- ✓ 甲基化与转录组（expression\splice）遗传调控分析

EWAS与GWAS联合方案设计

SNP与甲基化关系



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



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.

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EWAS与GWAS联合方案经典文章路线分享

GWAS检测

- ✓ 99,994 individuals of East Asian (n = 31,516), European (n = 35,352) and South Asian (n = 33,126) ancestry
- ✓ identified 19 previously unreported loci

SNP Replication 检测

- ✓ 133,052 individuals (48,268 East Asian, 68,456 European and 16,328 South Asian)
- ✓ Twelve of the 19 SNPs reached both P < 0.05 in replication testing and P < 1 × 10–9 in the combined analysis of data from across all stages

EWAS检测+CpG site Replication 检测

- ✓ 1,904 South Asians
- ✓ All 28 leading CpG sites showed replication in further testing among 4,780 Euro and South Asian

GWAS–EWAS生物信息学分析

- ✓ Twenty–eight of the 35 sentinel blood pressure SNPs were associated with one or more methylatio markers at P < 3.8 × 10–6
- ✓ three–way relationships between the sentinel SNPs, their leading CpG sites and blood pressure among the 6,757 Europeans and South Asians

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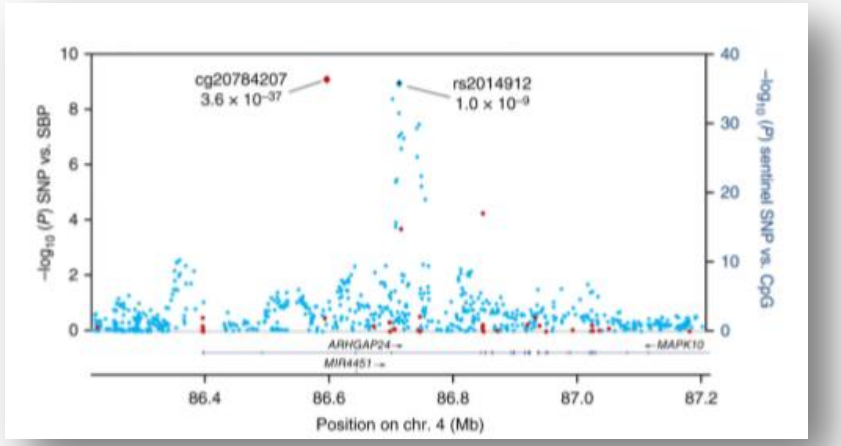
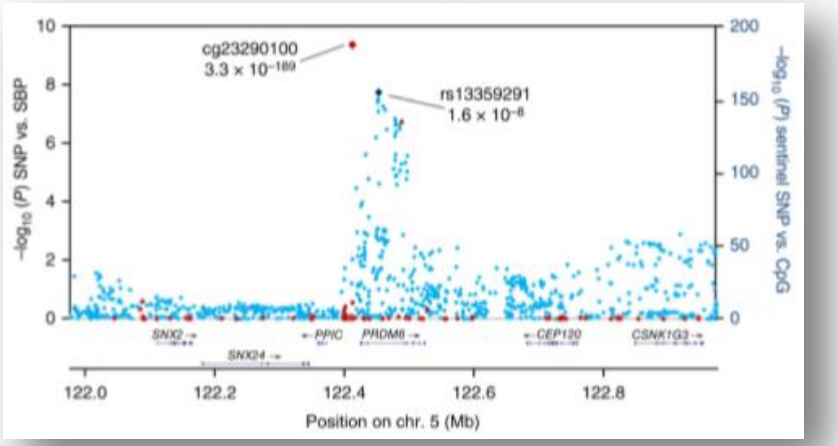
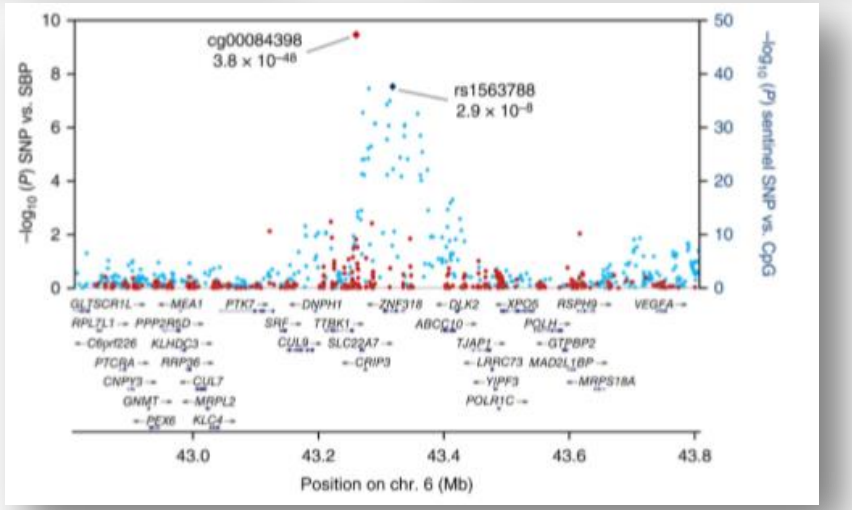
EWAS与GWAS联合方案经典文章路线分享

- ✓ next–generation sequencing to fine map DNA methylation at all CpG sites within 1 kb on either side of the leading 450K CpG site in 168 samples
- ✓ The sentinel blood pressure SNP at the AMH locus (rs740406) had a directionally consistent effect on methylation at 29 of the 34 CpG sites assayed
- ✓ Of the 34 CpG sites assayed, we found that 28 had a positive relationship with blood pressure ($P = 2 \times 10–4$, sign test), and 10 were associated with blood pressure at $P < 0.05$
- ✓ methylation levels in blood provide a surrogate for patterns of methylation in other tissues
- ✓ weighted genetic risk scores comprising known and new variants predicted increased left ventricular mass by electrocardiographic criteria, circulating levels of NT–proBNP (a marker of heart function), clinical coronary heart disease, and cardiovascular and all–cause mortality ($P = 0.04$ to $8.6 \times 10–6$)

EWAS与GWAS联合方案经典文章路线分享——重要结论展示







Associations of SNPs with SBP in the trans–ancestry GWAS (blue markers; n = 99,994) and of sentinel SNP with methylation at nearby CpG sites (red markers; n = 2,664) are shown. The identities of the sentinel SNP and most closely associated CpG site are provided

EWAS与GWAS联合方案经典文章路线分享——重要结论展示

Sentinel SNP	Chr.	EA	Lead CpG	CpG position (bp)	SNP-CpG distance (bp)	SNP–CpG[a]		Nearest gene to CpG	Relation to gene (CpG)	CpG–eQTL[b]	
						Effect	*P*			Effect	*P*
rs880315	1	T	cg02903756	10,750,680	46,186	−0.17	7.0×10^{-24}	*CASZ1*	Body	0.09	2.5×10^{-2}
rs12567136	1	T	cg05228408	11,865,352	18,379	0.6	2.8×10^{-248}	*MTHFR*	5′ UTR	2.34	6.5×10^{-4}
rs1344653	2	A	cg13996430	19,741,587	−10,742	−0.12	7.0×10^{-14}	*OSR1*	Intergenic	0.20	2.4×10^{-1}
rs1275988	2	T	cg19115882	26,919,145	−4,781	−0.3	1.8×10^{-74}	*KCNK3*	Body	0.25	1.5×10^{-4}
rs7629767	3	T	cg02108620	42,002,230	41,279	0.57	2.1×10^{-741}	*ULK4*	5′ UTR	−0.1	4.4×10^{-1}
rs13149993	4	A	cg05452645	81,117,647	40,898	−0.26	3.7×10^{-47}	*PRDM8*	5′ UTR	0.03	5.8×10^{-1}
rs2014912	4	T	cg20784207	86,597,598	118,072	−0.27	9.7×10^{-51}	*ARHGAP24*	Body	−0.51	2.4×10^{-1}
rs7733331	5	T	cg24363955	32,788,467	40,379	−0.22	1.6×10^{-41}	*NPR3*	Upstream	0.09	5.9×10^{-1}
rs13359291	5	A	cg23290100	122,435,626	40,831	−0.88	6.8×10^{-372}	*PRDM6*	Body	−0.05	4.4×10^{-1}
rs9687065	5	A	cg18129178	148,520,854	−129,714	−0.45	2.0×10^{-138}	*ABLIM3*	TSS	−0.07	3.5×10^{-1}
rs11960210	5	T	cg22790839	157,883,933	−66,299	−0.28	3.1×10^{-65}	*EBF1*	Intergenic	−0.11	1.7×10^{-1}
rs1563788	6	T	cg00084398	43,249,983	58,380	−0.42	5.0×10^{-139}	*TTBK1*	Body	0.06	5.3×10^{-1}
rs17080102	6	C	cg02784464	151,121,916	−117,146	0.27	7.2×10^{-29}	*PLEKHG1*	Body	0	3.0×10^{-2}
rs10260816	7	C	cg12244052	45,961,469	48,631	−0.08	4.6×10^{-6}	*IGFBP3*	Upstream	0.59	7.6×10^{-15}
rs731141	10	A	cg10751070	96,143,568	−244,887	0.14	8.3×10^{-16}	*TBC1D12*	Intergenic	0.1	5.2×10^{-2}

CpG sites associated in cis with the sentinel blood pressure SNPs

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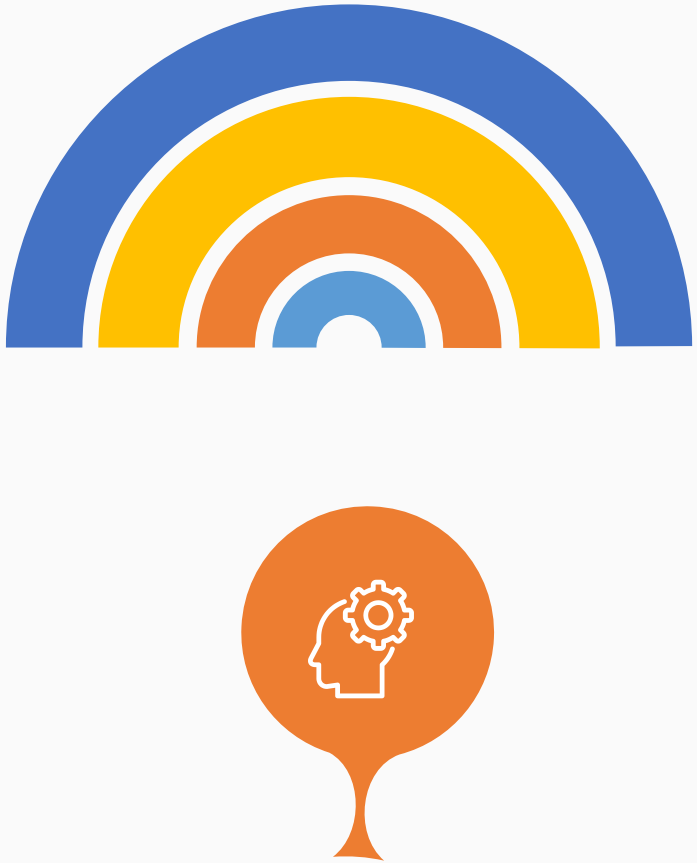
EWAS与代谢组联合方案设计



全基因组甲基化EWAS检测

- ✓ 850K全基因甲基化芯片
- ✓ Ilumina Infinium人甲基化450 BeadChip

代谢组检测

- ✓ LC-MS非靶向代谢组
- ✓ LC-MS靶向代谢组
- ✓ NMR脂蛋白检测
- ✓ Biocrates 试剂盒

数据处理

- ✓ 甲基化差异位点和差异代谢物分析
- ✓ 相关性分析
- ✓ 线性模型

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EWAS与代谢组联合方案经典文章路线分享

Epigenetics meets metabolomics: an epigenome–wide association study with blood serum metabolic traits
Human molecular genetics 2014

EWAS检测

- ✓ Ilumina Infinium Human Methylation 450 BeadChip
- ✓ 1805 participants

Metabolomics 检测

- ✓ Biocrates kit, LC–MS/MS, GC–MS, NMR
- ✓ 1700 subjects

数据处理

- ✓ A linear model with covariates age, gender, BMI and white WBC for association between DNA methylation and log–transformed metabolite concentrations

遗传和非遗传因素甲基化-代谢型相关

- ✓ Eight loci (ACADS, PYROXD2, NAT8, ACADM, OPLAH, FADS1, UGT1A and SULT2A1) were found harboring previously reported SNP–metabotype associations.
- ✓ Seven distinct loci (UGT2B15, TXNIP, DHCR24, MYO5C, ABCG1, SLC25A22 and CPT1A) and two loci groups (STEROIDS, VINYLPHENO) were driven unidentified external factor.

EWAS与代谢组联合方案经典文章路线分享——重要结论展示

Table 1. CpG – metabotype associations limited to loci that also show a strong association with a genetic variant

Locus name	CpG	Chr	Pos	Metabolic trait	Beta	r^2	P-value	N	r^2 (N_{Epi})	Fragment	# Samples with SNP
ACADS	cg24768164	12	121 163 261	Butyrylcarnitine	2 0.998	0.221	2.0×10^{-108}	1744	0.907 (35)	CpG_9	0
PYROXD2	cg26690318	10	100 167 465	X-12092	2.171	0.138	2.2×10^{-60}	1725	0.904 (31)	CpG_14	0
NAT8	cg13584399	2	73 907 327	N-acetylornithine	2 0.950	0.120	8.9×10^{-52}	1731	Not analyzed		—
ACADM	cg10523679	1	76 189 770	Hexanoylcarnitine	2 0.456	0.065	1.8×10^{-30}	1749	0.954 (31)	CpG_4	2
OPLAH	cg06239191	8	145 163 136	5-oxoproline	0.813	0.056	8.0×10^{-25}	1737	0.872 (32)	CpG_1	0
FADS1	cg11250194	11	61 601 937	PC aa C38:4	11.41	0.054	1.0×10^{-24}	1781	0.653 (35)	CpG_5	0
UGT1A	cg26799339	2	234 664 336	bilirubin (Z,Z)	2 0.973	0.054	2.9×10^{-24}	1706	Not analyzed		—
SULT2A1	cg00365481	19	48 362 237	X-11440	1.358	0.0363	3.9×10^{-20}	1742	Not analyzed		—

Table 2. CpG – metabotype associations after correction for genetic effects and exclusion of inflated loci

Locus name	CpG	Chr	Pos	Metabolic trait	Beta'	r^2	P-value	N	r^2 (N_{Epi})	Fragment	# samples with SNP
UGT2B15	cg09189601	4	69 514 031	X-11491	2 0.865	0.087	2.69×10^{-27}	1283	Not analyzed		–
TXNIP	cg19693031	1	145 441 552	Chylo-A	2 0.996	0.038	1.11×10^{-21}	1771	0.842 (41)	CpG_5	0
DHCR24	cg17901584	1	55 353 706	PC ae C36:5	4.001	0.036	3.65×10^{-18}	1780	0.744 (41)	CpG_5	0
MYO5C	cg06192883	15	52 554 171	Glycine	2 0.659	0.030	1.61×10^{-15}	1744	0.257 (41, n.s.)	CpG_4	31
ABCG1	cg06500161	21	43 656 587	SM C16:0	2 0.817	0.008	1.04×10^{-14}	1781	0.507 (33)	CpG_2.3	21
SLC25A22	cg09441501	11	798 350	Arg	2 1.000	0.035	1.66×10^{-14}	1780	Not analyzed		–
CPT1A	cg00574958	11	68 607 622	VLDL-A	2 1.000	0.025	9.23×10^{-14}	1773	0.332 (41)	CpG_5	1
SLC7A11 (STEROIDS)	cg06690548	4	139 162 808	A-diol	2 0.980	0.071	6.83×10^{-39}	1746	0.123 (40, n.s.)	CpG_2	0
PHGDH (STEROIDS)	cg14476101	1	120 255 992	A-diol	2 0.929	0.035	6.50×10^{-21}	1742	0.205 (41, n.s.)	CpG_2	4
LOC100132354 (STEROIDS)	cg18120259	6	43 894 639	A-diol	2 0.932	0.023	1.10×10^{-14}	1747	0.667 (41)	CpG_3	0
SLC1A5 (STEROIDS)	cg22304262	19	47 287 778	A-diol	2 0.954	0.022	6.49×10^{-14}	1744	0.608 (41)	CpG_11	13
cg13526915 (STEROIDS)	cg13526915	14	24 164 078	A-diol	2 0.924	0.020	3.15×10^{-13}	1746	0.181 (31, n.s.)	CpG_3	15
AHRR (VINYLPHENOL)	cg05575921	5	373 378	4-vs	2 0.953	0.107	3.52×10^{-49}	1709	0.977 (41)	CpG_3	0
ALPPL2 (VINYLPHENOL)	cg21566642	2	233 284 661	4-vs	2 0.945	0.079	7.03×10^{-37}	1706	Not analyzed		–
F2RL3 (VINYLPHENOL)	cg03636183	19	17 000 585	4-vs	2 0.977	0.063	5.63×10^{-30}	1708	Not analyzed		–
cg06126421 (VINYLPHENOL)	cg06126421	6	30 720 080	4-vs	2 0.952	0.048	4.12×10^{-25}	1709	0.951 (41)	CpG_4	0
RARA (VINYLPHENOL)	cg19572487	17	38 476 024	4-vs	2 0.887	0.034	6.12×10^{-16}	1707	0.822 (40)	CpG_2	0
GFI1 (VINYLPHENOL)	cg09935388	1	92 947 588	4-vs	2 0.899	0.030	3.01×10^{-15}	1709	0.816 (42)	CpG_4.5.6	0
TPM1 (VINYLPHENOL)	cg10403394	15	63 349 192	4-vs	6.198	0.013	4.63×10^{-13}	1709	0.934 (41)	CpG_5.6	0
cg23079012 (VINYLPHENOL)	cg23079012	2	8 343 710	4-vs	2 0.996	0.026	9.07×10^{-13}	1709	0.870 (35)	CpG_5.6	14

Legend as in Table 1; 4-androsten-3beta, 17beta-diol disulfate (A-diol); 4-vinylphenol sulfate (4-vs); cases where no statistically significant correlation between methylation readouts from the Infinium HumanMethylation450 BeadChip and the EpiTYPER system was observed are marked as 'n.s.'. Loci in the VINYLPHENOL group that are marked as reported by Zeilinger (10) in association with smoking.

博淼生物
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候选基因DNA甲基化方案设计

候选基因筛选

- ✓ mRNA/miRNA/lncRNA/cirRNA

筛选重要甲基化区域

- ✓ 启动子及1st-Exon区域分析
- ✓ 分析CpG island/shore/shelf区域
- ✓ 转录因子、SNP分析

CpG site定量检测

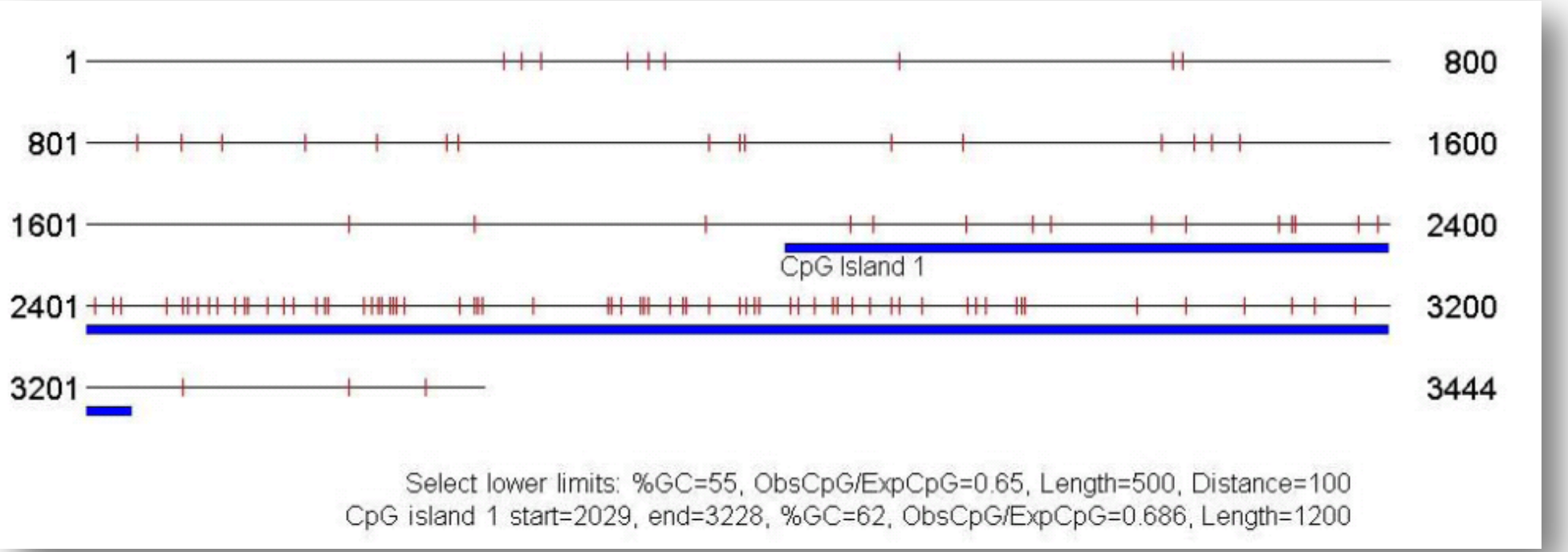
- ✓ Massarray技术

生物信息学分析

- ✓ 关联分析、聚类分析
- ✓ 甲基化与转录组（expression\splice）功能调控分析

候选基因甲基化方案设计路线展示

通过http://cpgislands.usc.edu/，寻找符合特定参数要求的CpG island基因序列区域

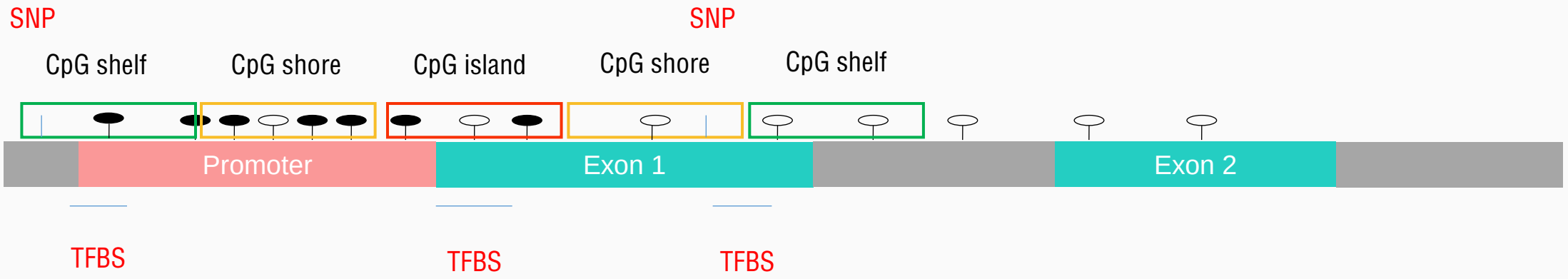


候选基因甲基化方案设计路线展示

通过jaspar.genereg.net/search针对候选区域进行转录因子预测

```
1 TTTGCCAACG TTAAGGACGC GCAGCTGTGA CACAGCCCCA GGAAGTCCAG entry          score
                                <-----          M00048 ADR1     95.4
                     ---------->                M00175 AP-4     93.8
                     <----------                M00176 AP-4     91.9
                     <----------                M00175 AP-4     91.7
                                ---------->     M00032 c-Ets-   90.2
                     ---------->                M00176 AP-4     89.2
               <----------                      M00263 StuAp    87.7
                          ------->              M00253 cap      86.7
```

候选基因甲基化方案设计路线展示



CpG shore：CpG island UP/Down 2KB

CpG shelf： CpG shore UP/Down 2KB

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候选基因甲基化与SNP联合方案经典文章路线分享

A genetic association study of DNA methylation levels in the DRD4 gene region finds associations with nearby SNPs

Behavioral and Brain Functions

样本选择

- ✓来自30个家系的89个个体相关淋巴母细胞系
- ✓18个脑组织

候选基因选择

- ✓ DRD4基因
- ✓精神疾病易感基因

甲基化检测区域及SNP筛选

- ✓ +/−10kb of the promoter region内的5个SNP位点
- ✓该区域中的甲基化位点

实验结论

- ✓有4个SNP位点与该区域甲基化水平关联
- ✓有2个位点在脑组织中也存在与该区域甲基化水平关联
- ✓DRD4目标区域甲基化在淋巴母细胞系与脑组织中相似
- ✓SNP通过调控甲基化来发挥其功能机制

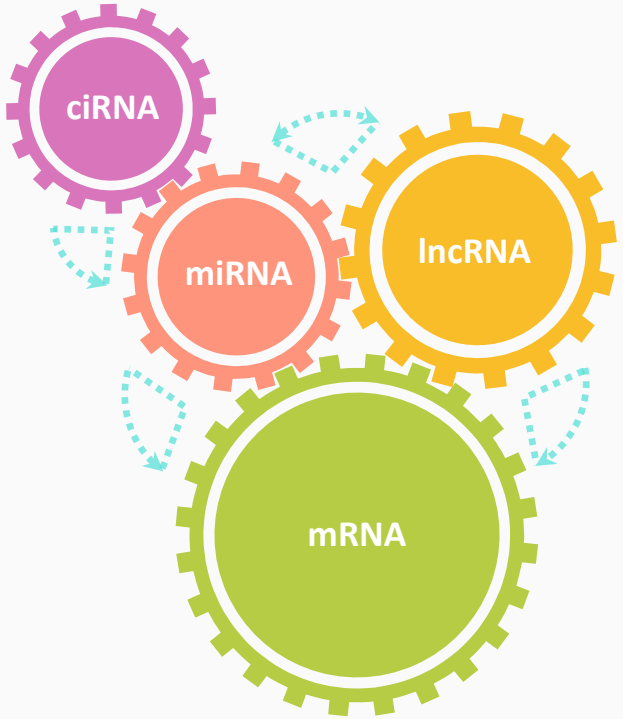
转录组研究——ceRNA功能调控机制

circular RNA

招募U1 snRNP促进基因转录起始
吸附miRNA，调控mRNA

long non-coding RNA

参与X染色体的异染色质化
吸附miRNA，调控mRNA
细胞核亚结构的结构骨架
作为染色质重塑的调控因子



micro RNA

不完全互补——阻遏调节基因翻译
完全互补——靶向切割mRNA

message RNA

转录并翻译成蛋白质，行使其相应功能

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ceRNA功能调控机制方案设计

样本类型选择

- ✓ 癌 vs 癌旁
- ✓ 用药前后
- ✓ 不同发育阶段

……

全转录组检测

- ✓ LncRNA芯片+miRNA芯片
- ✓ totalRNA NGS+miRNA NGS

ceRNA调控网络预测

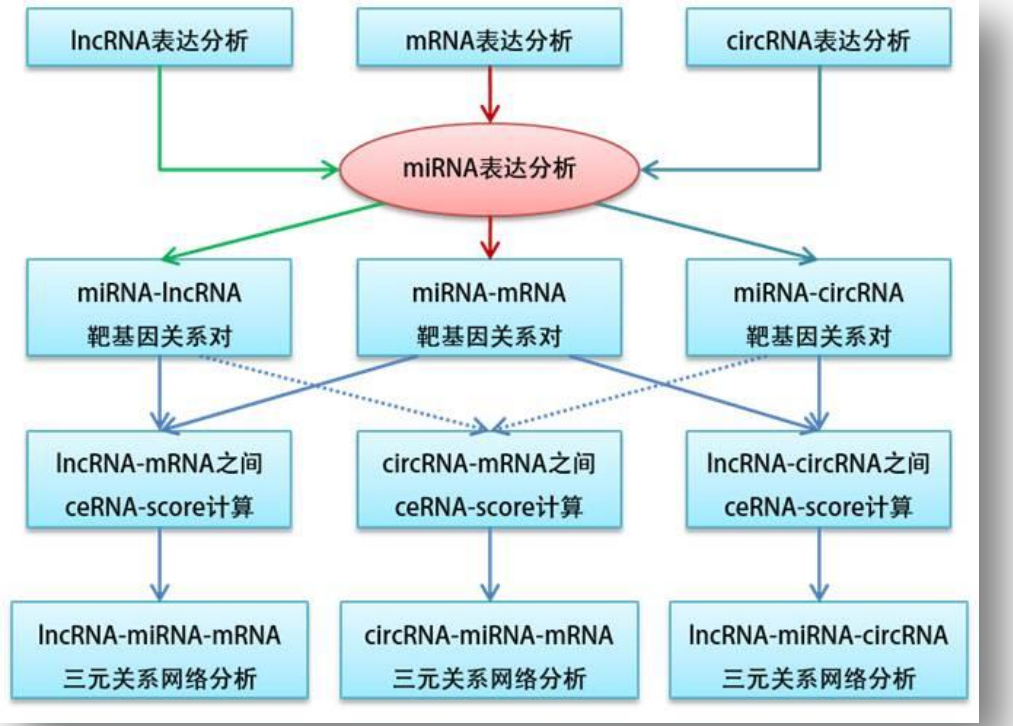
- ✓ lncRNA–miRNA–mRNA调控分析
- ✓ cirRNA–miRNA–mRNA调控分析
- ✓ LncRNA–miRNA–cirRNA调控分析

ceRNA调控关系与功能验证

- ✓ ceRNA对目的基因的影响
（RNA及蛋白水平、竞争结合区域验证）
- ✓ ceRNA对miRNA依赖性
（miRNA结合位点、与miRNA的调控关系）
- ✓ 功能验证（细胞水平、动物模型、临床水平）

ceRNA功能调控机制方案——生物信息分析流程

- ✓ 利用碱基序列预测microRNA–target关系对
- ✓ 利用表达值预测microRNA–target关系对
- ✓ 综合碱基序列和表达值预测结果
- ✓ ceRNA_score原理预测ceRNA
- ✓ 利用表达值预测ceRNA
- ✓ 综合ceRNA_score和表达值预测结果
- ✓ lncRNA功能注释（KEGG、GO）
- ✓ 绘制网络图



ceRNA生物信息分析流程——miRanda软件预测MRE

miRanda软件用来预测基因组碱基序列中的microRNA识别位点

名称	意义
miRNA_name	microRNA名字
Target_name	microRNA作用目标序列的名字
Score	得分
Energy	相互作用能量值
miR_Start.miR_end	MRE在miRNA上的起始结束位置
mRNA_start.mRNA_end	MRE在mRNA上的起始结束位置
MRE_length	MRE长度

ceRNA生物信息分析流程——miRNA-target共表达

根据miRNA和target（lncRNA或mRNA）的表达值，计算出miRNA-target关系对的皮尔森相关系数。从中筛选出表达上具有负相关的miRNA-target调控关系。表达值相关系数的P值不高于0.05，且皮尔森相关系数绝对值不低于0.7，则认为具有相关性

名称	意义
pValue	target与miRNAs表达值相关系数的P值。
correlation	target与miRNAs表达值的相关系数。
miRNA_name	miRNA名字
...	样本名
FoldChange	miRNA的差异表达情况，差异倍数
p-value	miRNA的差异表达情况，P值
Target_name	调控目标名字（lncRNA或mRNA）
...	样本名
FoldChange	target在本次实验中的差异表达情况
p-value	target在本次实验中的差异表达情况，P值

ceRNA生物信息分析流程——ceRNA_score原理预测ceRNA

如果lncRNA对miRNA有调控关系，而该miRNA对mRNA也有调控关系，那么该lncRAN就有可能作为该mRNA的ceRNA产生调控作用。lncRNA作为预测的ceRNA，我们会从两个方面进行约束。lncRNA-mRNA必须有共同的miRNA，且相对高的MRE密度

名称	意义
mRNA	mRNA信息
lncRNA	lncRNA信息
ceRNA_score	预测的ceRNA关系得分
#MRE_for_share_miRNA	lncRNA和共同miRNA的MRE数量
#MRE_for_lncRNA_miRNA	lncRNA所有的MRE数量
pvalue	ceRNA预测的P值
#shared_miRNA	共同miRNA的数量
miRNAs	共同miRNA名字

ceRNA生物信息分析流程——利用表达值预测ceRNA

根据mRNA和LncRNA的表达值，计算出mRNA-LncRNA关系对的皮尔森相关系数。从中筛选出表达上具有负相关的mRNA-LncRNA调控关系。表达值相关系数的P值不高于0.05，且皮尔森相关系数绝对值不低于0.7，则认为具有相关性

名称	意义
mRNA_name	mcroRNA名字
lncRNA_name	LncRNA名字
Score	得分
Energy	相互作用能量值

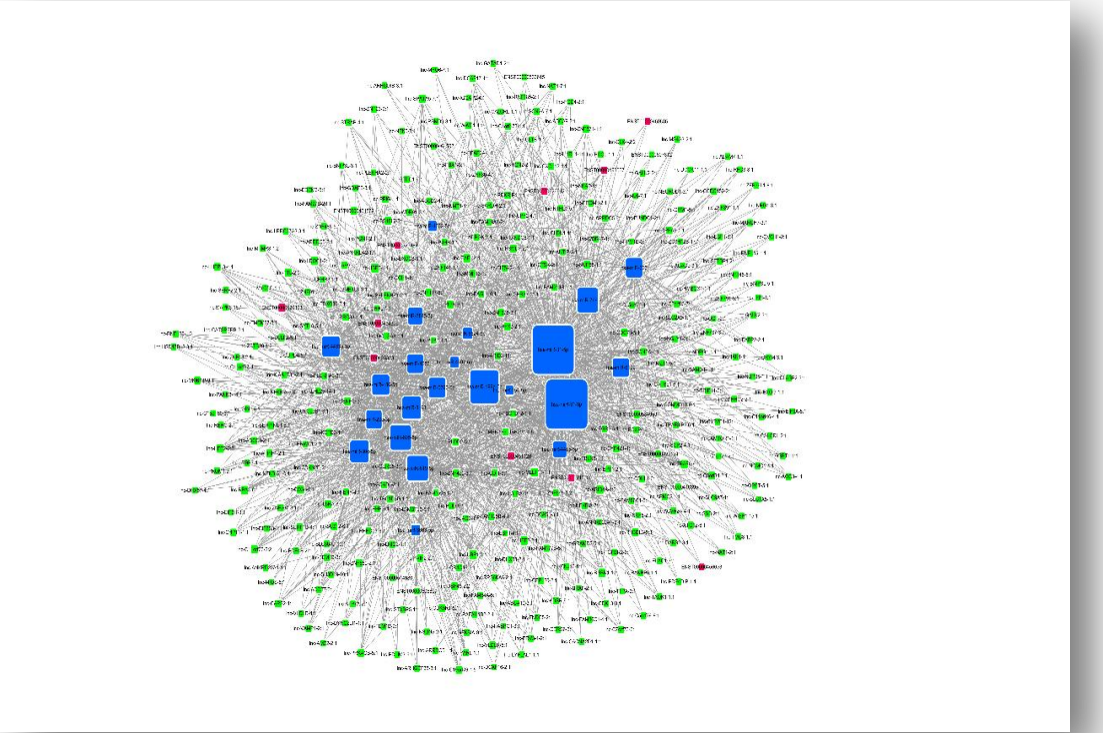
ceRNA生物信息分析流程——lncRNA功能注释

对于每一个差异表达的lncRNAs，计算得到有ceRNA关系的编码基因，用超几何分布检验方法计算每个GO或KEGG条目中差异基因富集的显著性，计算的结果会返回一个富集显著性的p值，小的p值表示差异gene在该GO或KEGG条目中出现了富集

名称	意义
enrichmentTerm	GO ID
ListHits	注释为某特定GO term的差异表达gene数目
ListTotal	所有gene中具有GO注释的数目
PopHits	差异表达gene中具有GO注释的gene数目
PopTotal	所有gene中注释为某特定GO条目的数目
p.value	P值
q.value	Q值（矫正后的p值）
enrichmentIDs	GeneSymbol
GO_ID	GO ID
Evidence	可信等级
Qualifier	Qualifier信息
GO_term	GO ID描述
PubMed	相关PubMed ID号
Category	GO数据库中类别

ceRNA生物信息分析流程——ceRNA调控网络图

将预测的ceRNA结果关系对，按照ceRNA_score和共享miRNA进行从大到小进行排序。过滤掉共享miRNA数量过少（小于7），p值大于0.05的记录。取最可靠的前20和50条分别进行网络图的绘制



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ceRNA调控机制经典文章路线分享

Non-coding RNAs participate in the regulatory network of CLDN4 via ceRNA mediated miRNA evasion
Nature communications 2017

样本选择

✓StageI—6对GC癌组织及癌旁组织

✓StageII—104对GC癌组织及癌旁组织

检测技术

✓ LncRNA+mRNA芯片、miRNA芯片+RNA-seq

重要结论

✓ 通过芯片数据分析，其中表达显著的CLDN4为肿瘤发展的关键基因，并通过软件预测及验证实验，miR-596 与 miR-3620-3p调控该基因的表达

✓ 通过体外细胞功能实验，证实CLDN4分子功能意义，并与两条miRNA的调控机制

✓ 通过104对样本的CLDN4 及两条miRNA的表达验证，均与上述数据一致

✓ 通过芯片数据的ceRNA调控分析，推断lncRNA-TUBB2A and lncRNA-KRTAP5-AS1与上述miRNA存在调控关系，由此针对两条miRNA、LncRNA过表达细胞及各自对应的对照细胞进行RNA-seq，验证两条LncRNA过表达均可增加CLDN4 的表达

✓ 通过携带两条LncRNA的荧光素酶报告基因的载体与miRNA进行共表达，验证对应的调控关系符合理论推测

✓通过RIP实验验证，lncRNA-KRTAP5-AS1可以结合miR-596 与 miR-3620-3p，而，lncRNA-TUBB2A可以结合miR-3620-3p

✓通过体外GC细胞功能实验，证实两条lncRNA的分子功能机制，及与对应的miRNA通过ceRNA调控机制对于GC细胞的影响

✓通过小鼠体内研究上述的ceRNA调控机制，证实CLD4可以小鼠体内肿瘤的生长和转移，由于ceRNA调控，miR-596 和miR-3620-3p可以抑制，而lncRNA-KRTAP5-AS1与lncRNA-TUBB2A可以促进。

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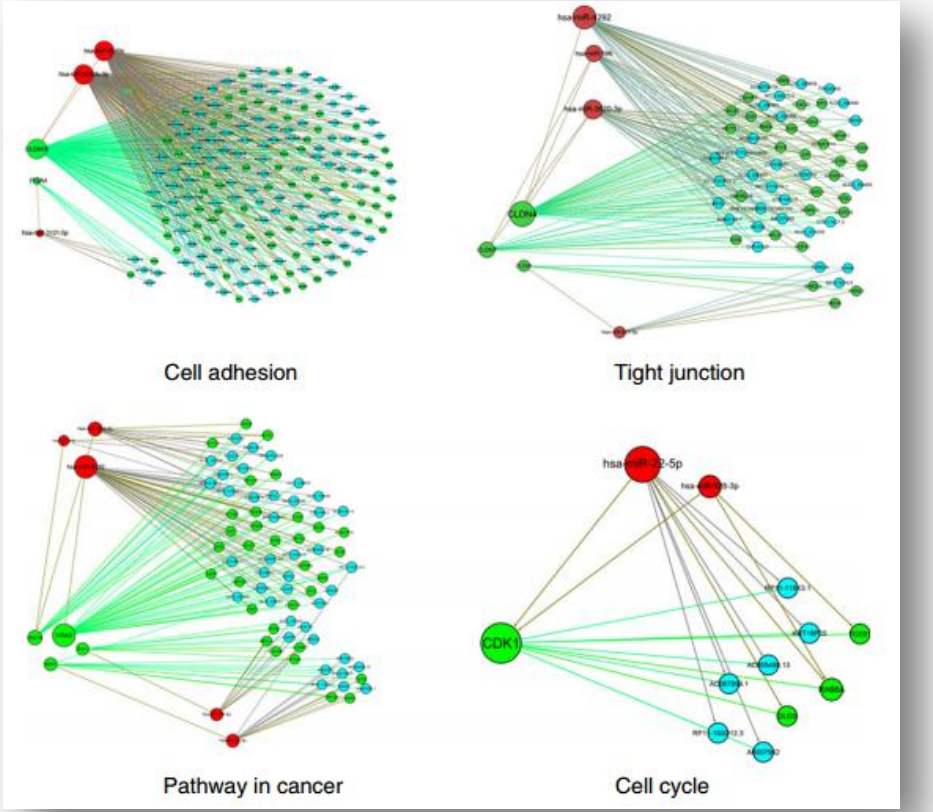
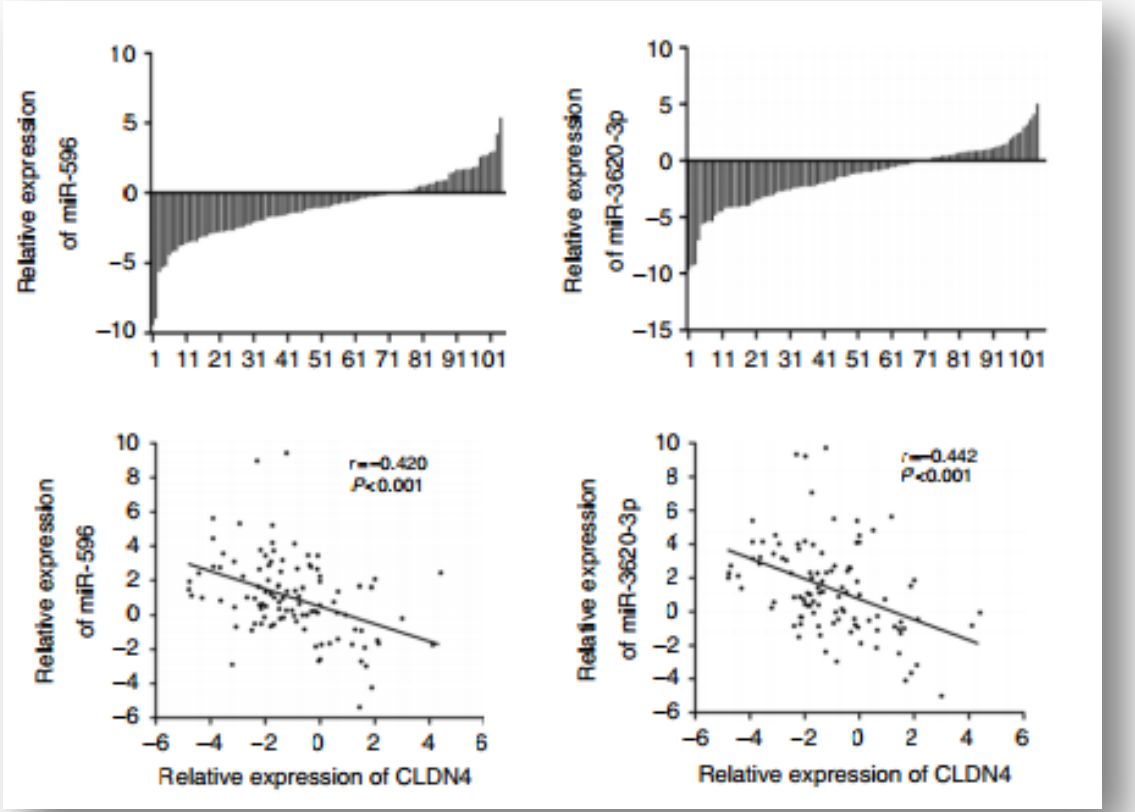
ceRNA调控机制经典文章路线分享——重要结论展示



The mRNA–lncRNA –miRNA networks in the GC



The relative expression levels of miR–596 and miR–3620–3p in human GC tissues compared with their matched non–tumorous adjacent tissues
The correlation between CLDN4 transcriptional levels and miR–596 or miR–3620–3p transcriptional levels were measured in the same set of patients by Spearman correlation analysis

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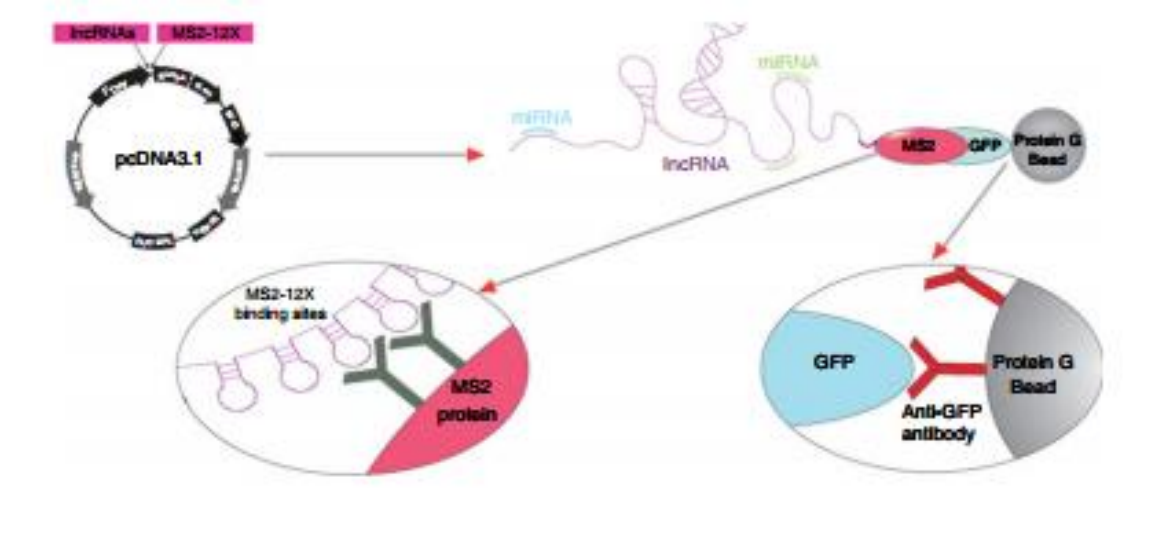
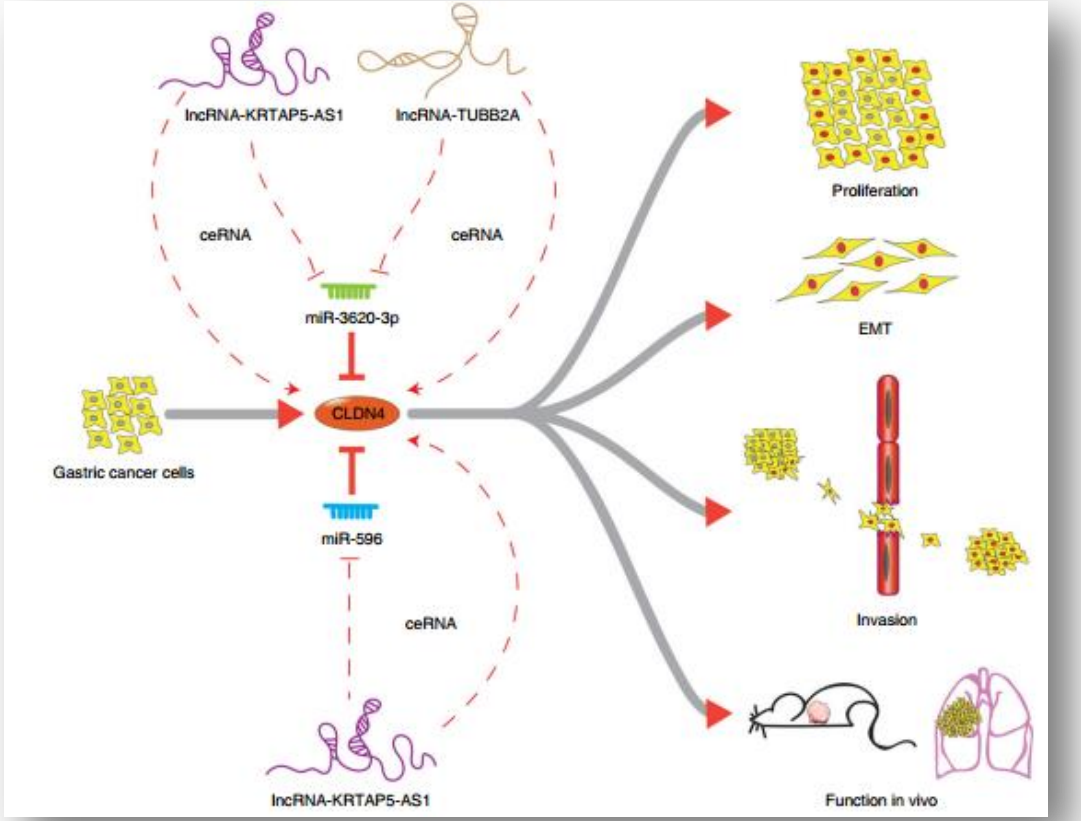
ceRNA调控机制经典文章路线分享——重要结论展示





The schematic diagram and real–time PCR results of the MS2–RIP method used to identify the binding between lncRNAs and miRNAs in both SGC–7901 and AGS cells

The mechanism graph of the regulatory network and function of CLDN4. CLDN4 could promote proliferation, metastasis or EMT processes of GC, which could be inhibited by miR–596, miR–3620–3p and enhanced by lncRNA–KRTAP5–AS1, lncRNA–TUBB2A as ceRNAs

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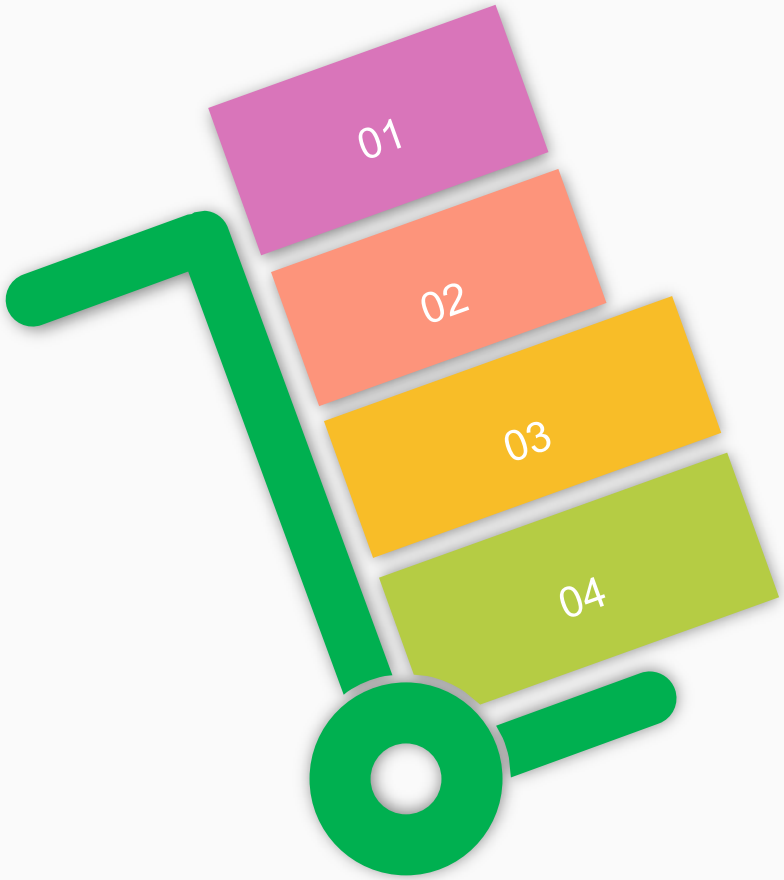
转录组经典研究路线——miRNA分子功能机制研究



全基因组miRNA检测

- ✓ miRNA表达谱芯片
- ✓ miRNA NGS测序

生物信息学分析

- ✓ 卡方差异分析
- ✓ 调控靶基因预测

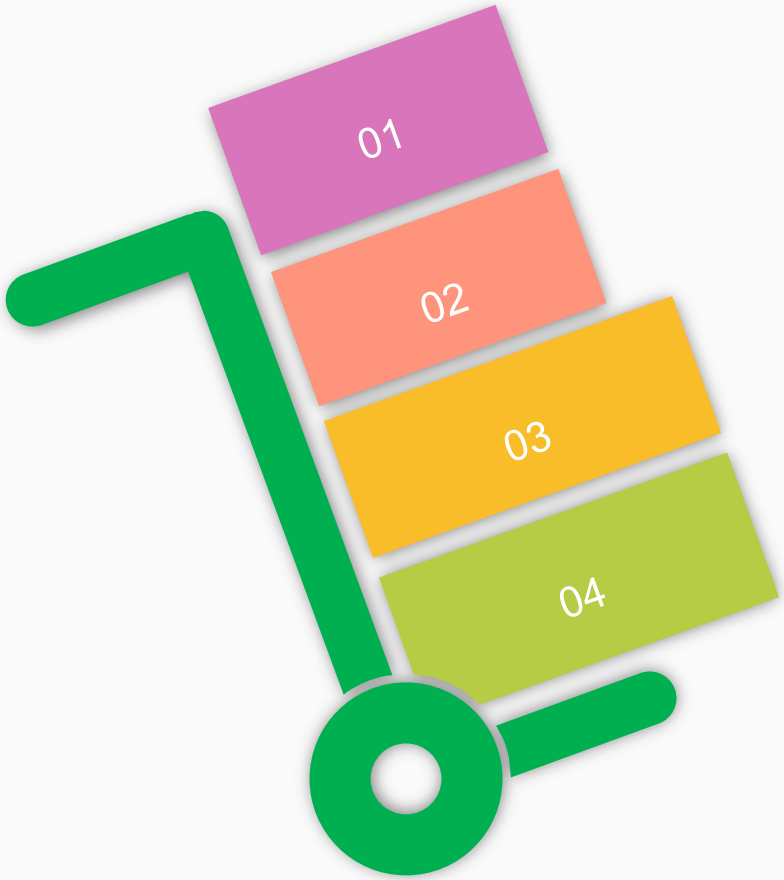
二期样本显著miRNA定量检测

- ✓ 大样本差异miRNA RT-qPCR

miRNA分子调控机制功能研究

- ✓ 细胞水平in vitro miRNA-mRNA靶基因功能验证
- ✓ 动物体内in vivio功能验证

转录组经典研究路线——miRNA遗传标记物研究



全基因组miRNA检测

- ✓ miRNA表达谱芯片
- ✓ miRNA NGS测序

生物信息学分析

- ✓ 卡方差异分析

Training and validation stage

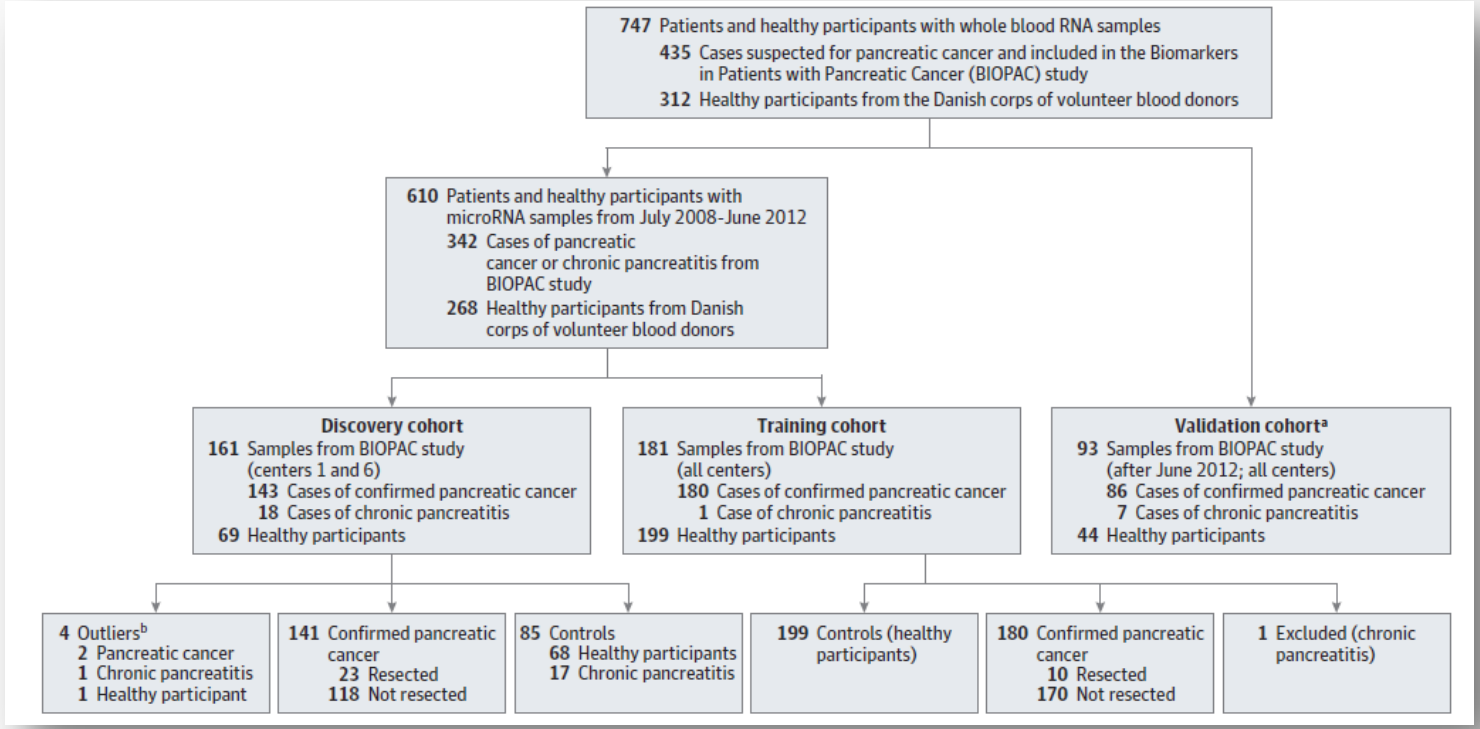
- ✓ 大样本、多中心RT-qPCR

Logistic regression model

- ✓ Bivariable and multivariable

miRNA遗传标记物研究经典文章路线分享

MicroRNA Biomarkers in Whole Blood for Detection of Pancreatic Cancer
JAMA 2014



Index I:

miR–150 + miR–636 – miR–145 – miR–223.

Index II:

6.9275 – (0.2134 × miR–122) – (0.3560 × miR–34a) – (0.8577 × miR–145) + (1.0043 × miR–636) – (0.6725 × miR–223) + (0.7018 × miR–26b) – (0.3233 × miR–885–5p) + (1.1304 × miR–150) – (0.2204 × miR–126*) – (0.1730 × miR–505)

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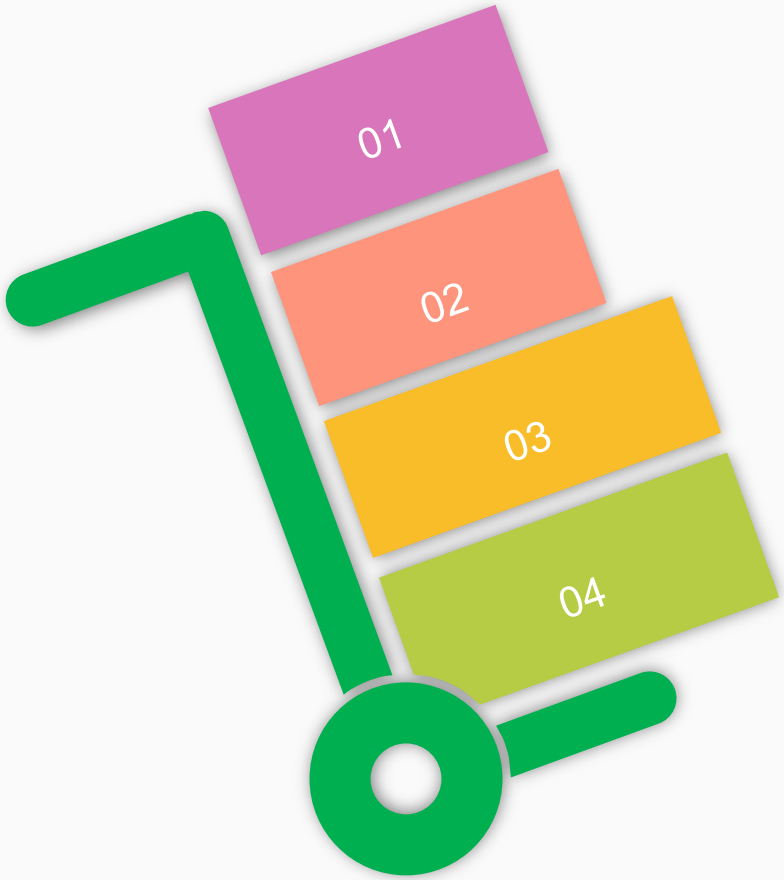
转录组经典研究路线——miRNA分子遗传调控机制研究



全基因组miRNA检测

- ✓ miRNA表达谱芯片
- ✓ miRNA NGS测序

生物信息学分析

- ✓ 表达差异分析、聚类分析
- ✓ 靶基因预测分析、GO/Pathway分析

miRNA CpG遗传调控机制

- ✓ miRNA调控区域甲基化检测

miRNA SNP遗传调控机制

- ✓ pre-miRNA/pri-miRNA/mature-miRNA SNP
- ✓ miRNA-mRNA/miRNA-LncRNA binding site

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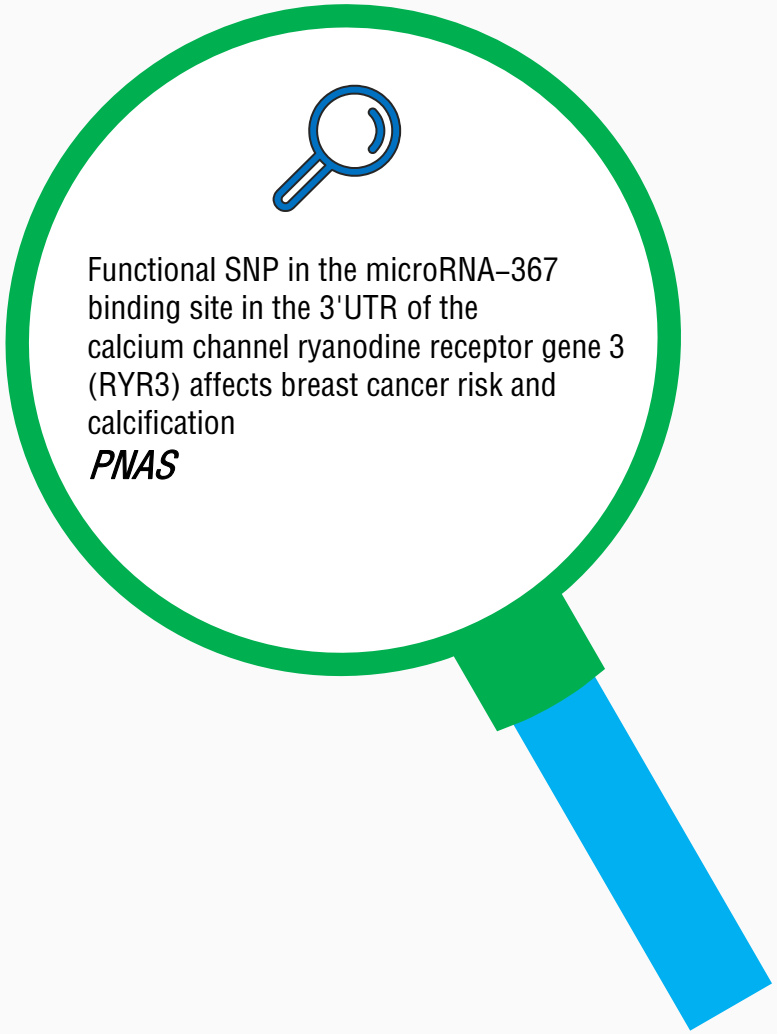
miRNA分子遗传调控机制研究经典文献——miRNA相关SNP研究



Functional SNP in the microRNA–367 binding site in the 3'UTR of the calcium channel ryanodine receptor gene 3 (RYR3) affects breast cancer risk and calcification

PNAS

样本选择

✓ 1,532 breast cancer cases 1,600 healthy Chinese women

位点选择

✓ miR–367 exists in the 3'UTR of RYR3 rs1044129 A→G, is present in this binding region

实验结论

✓ rs1044129 is a unique SNP that resides in a miRNA–gene regulatory loop that affects breast cancer risk, calcification, and survival.

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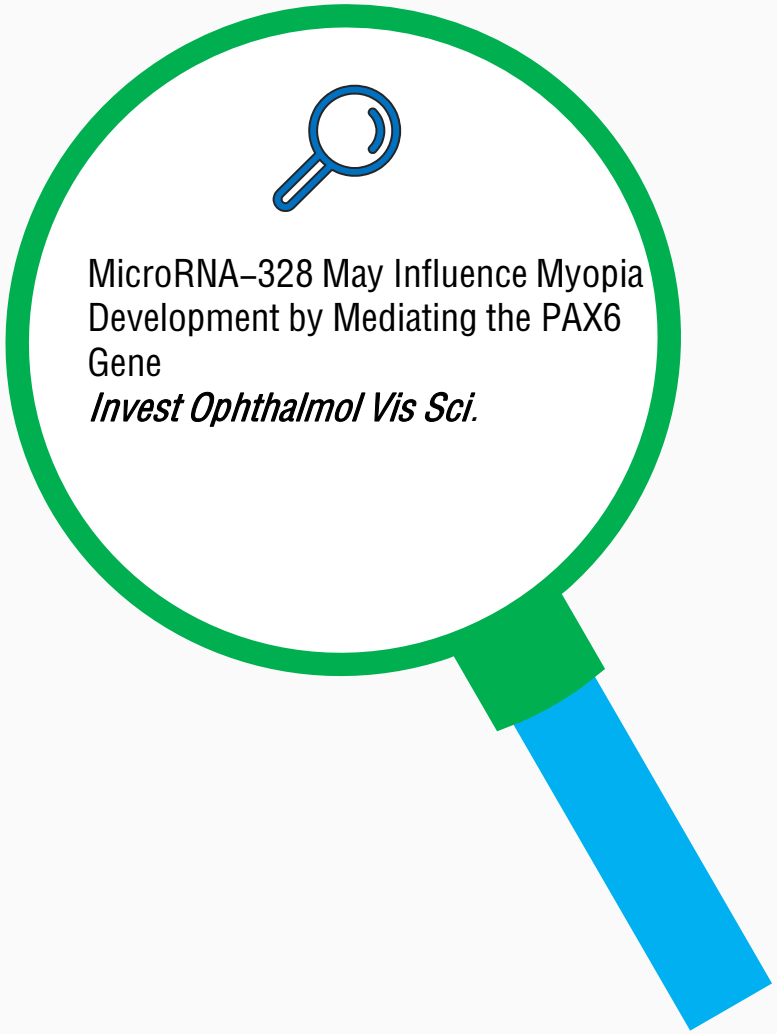
miRNA分子遗传调控机制研究经典文献——miRNA SNP分子功能机制研究



MicroRNA-328 May Influence Myopia Development by Mediating the PAX6 Gene

Invest Ophthalmol Vis Sci.

方案设计

- ✓ rs662702是否存在MicroRNA-328与PAX6基因结合的区域
- ✓ 验证rs662702、MicroRNA-328与视黄酸RA在近视中的作用

检测技术

- ✓ 用荧光素酶检测（luciferase assay）证实MicroRNA-328与PAX6基因相结合
- ✓ 克隆构建rs662702不同的突变体，分别验证与MicroRNA-328的结合效率
- ✓ 检测敲出PAX6基因是否对与视网膜色素细胞（RPE）和巩膜细胞产生影响，以及其它近视相关基因的表达
- ✓ 视黄酸（RA）对MicroRNA-328表达的影响

实验结论

- ✓ rs662702的野生型是MicroRNA-328与PAX6基因（3-UTR）的结合位
- ✓ PAX6基因的低表达促使RPE细胞的繁殖，降低巩膜细胞的繁殖。同时，RPE细胞中的TGF-Beta3基因与巩膜细胞中的MMP2基因表达升高，巩膜细胞中的collagen I and integrin β1 降低
- ✓ 视黄酸（RA）增加了MicroRNA-328的表达，从而降低了PAX6基因的表达

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miRNA分子遗传调控机制研究经典文献——miRNA相关甲基化研究

样本选择

✓ 50例NSCLC的肿瘤组织及癌旁组织，108例NSCLC的肿瘤组织及癌旁组织进行二期验证

方案设计

- ✓ MeDIP-chip进行全基因组甲基化分析，筛选差异甲基化区域
- ✓ 二期样本验证甲基化区域的差异性
- ✓ 差异化基因功能机制

实验结论

✓ 通过数据分析，发现有39个与肿瘤调控相关的miRNA基因存在显著甲基化水平差异

✓ 其中将六个miRNA基因，miR-10b、miR-1179、miR-137、miR-572、miR3150b和miR-129-2作为靶向区域，采用108例NSCLC的肿瘤组织及癌旁组织进行二期验证

✓ 通过miRNAWalk2.0进行miRNA调控靶基因预测，筛选与肿瘤发病有关的基因。经过来源于Cancer Genome Atlas数据库中超1000例NSCLC样本的RNA-seq分析，发现上述较多miRNA调控的靶基因在肿瘤组织中表达上调

✓ 基于上述数据，在体外进行了miRNA-1179与靶向基因CCNE1的调控机制研究，证明前者抑制后者的表达

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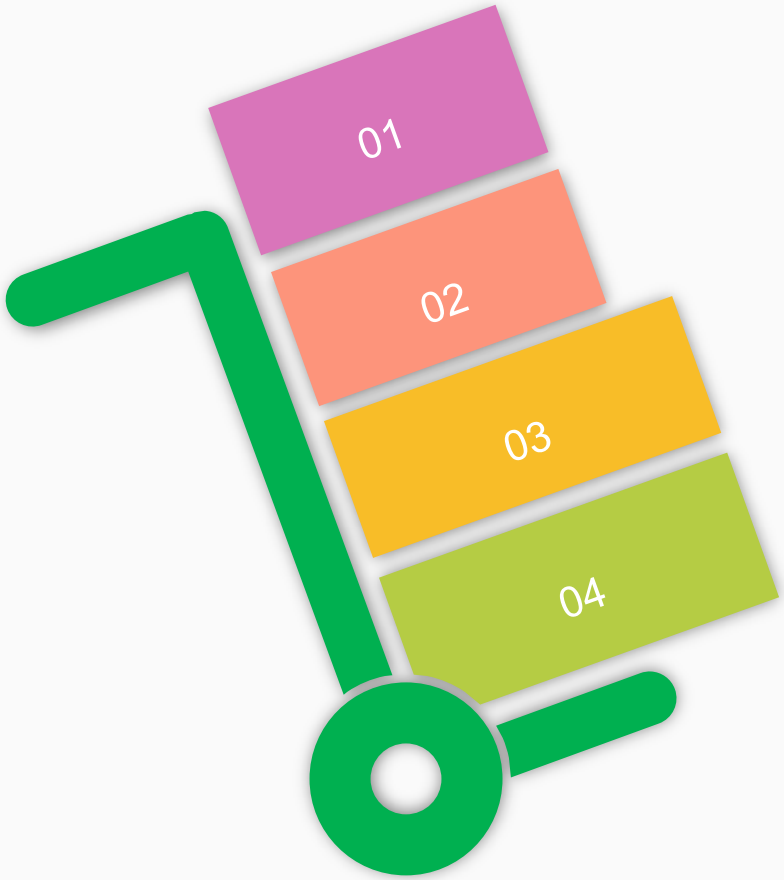
转录组经典研究路线——LncRNA分子遗传调控机制研究



全基因组LncRNA检测

- ✓ LncRNA表达谱芯片
- ✓ LncRNA NGS测序

生物信息学分析

- ✓ 表达差异分析、聚类分析
- ✓ 靶基因预测分析、GO/Pathway分析

miRNA CpG遗传调控机制

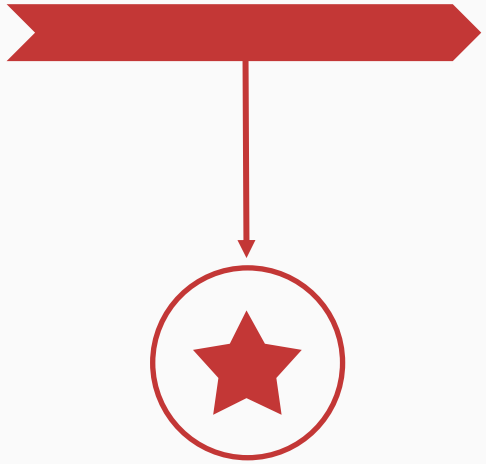
- ✓ miRNA调控区域甲基化检测

miRNA SNP遗传调控机制

- ✓ pre-miRNA/pri-miRNA/mature-miRNA SNP
- ✓ miRNA-mRNA/miRNA-LncRNA binding site

A genetic polymorphism in l*incRNA–uc003opf.1* is associated with susceptibility to esophageal squamous cell carcinoma in Chinese populations ***Carcinogenesis 2013***

between patients and controls in the genotype frequencies for the rs11752942A>G site in the *lincRNA-uc003opf.1* exon. Compared with the rs11752942AA genotype, AG and GG genotypes had a significantly reduced risk of ESCC (adjusted odds ratio = 0.73; 95% confidence interval = 0.63–0.84). Biochemical analysis demonstrated that, when compared with the A allele, the rs11752942G allele could markedly attenuate the level of *lincRNA-uc003opf.1* both *in vivo* and *in vitro* by binding micro-RNA-149*, thereby affecting cell proliferation and tumor growth. These findings indicated that functional polymorphism rs11752942A>G in *lincRNA-uc003opf.1* exon might be a genetic modifier for the development of ESCC.

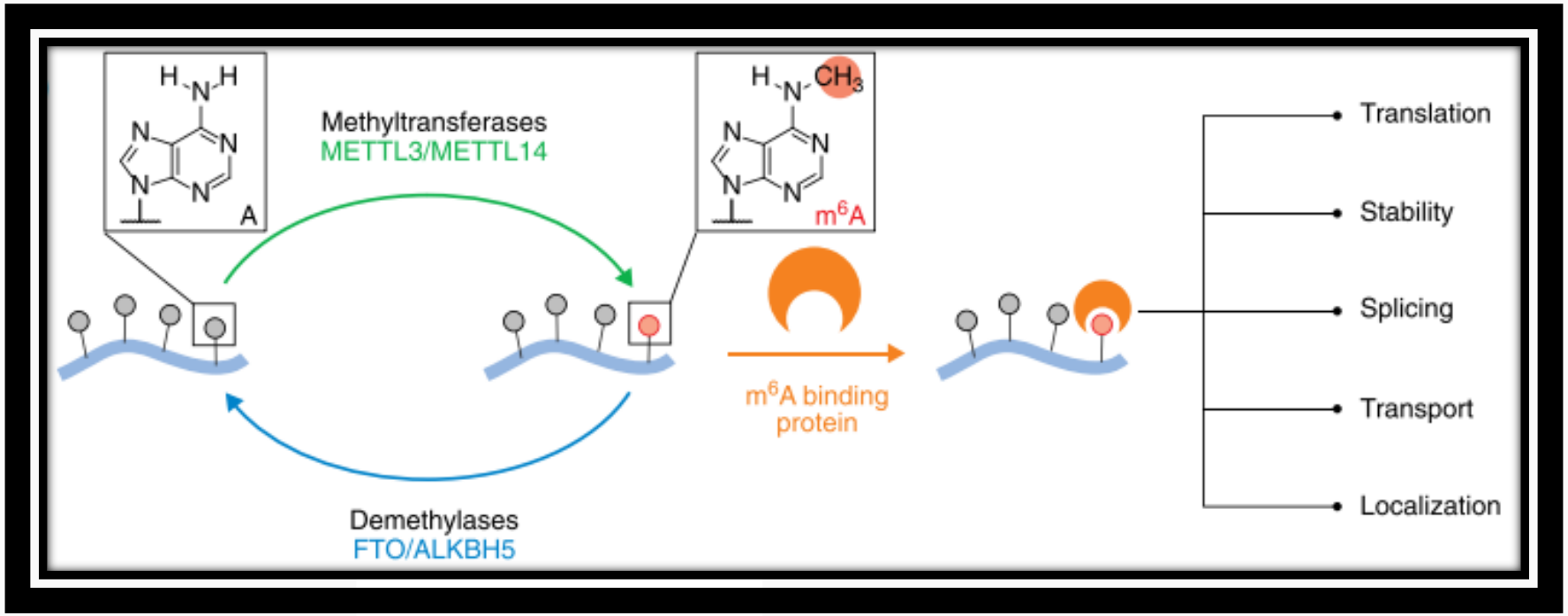


Increased Levels of the Long Intergenic Non–Protein Coding RNA POU3F3 Promote DNA Methylation in Esophageal Squamous Cell Carcinoma Cells ***Gastroenterology 2014***

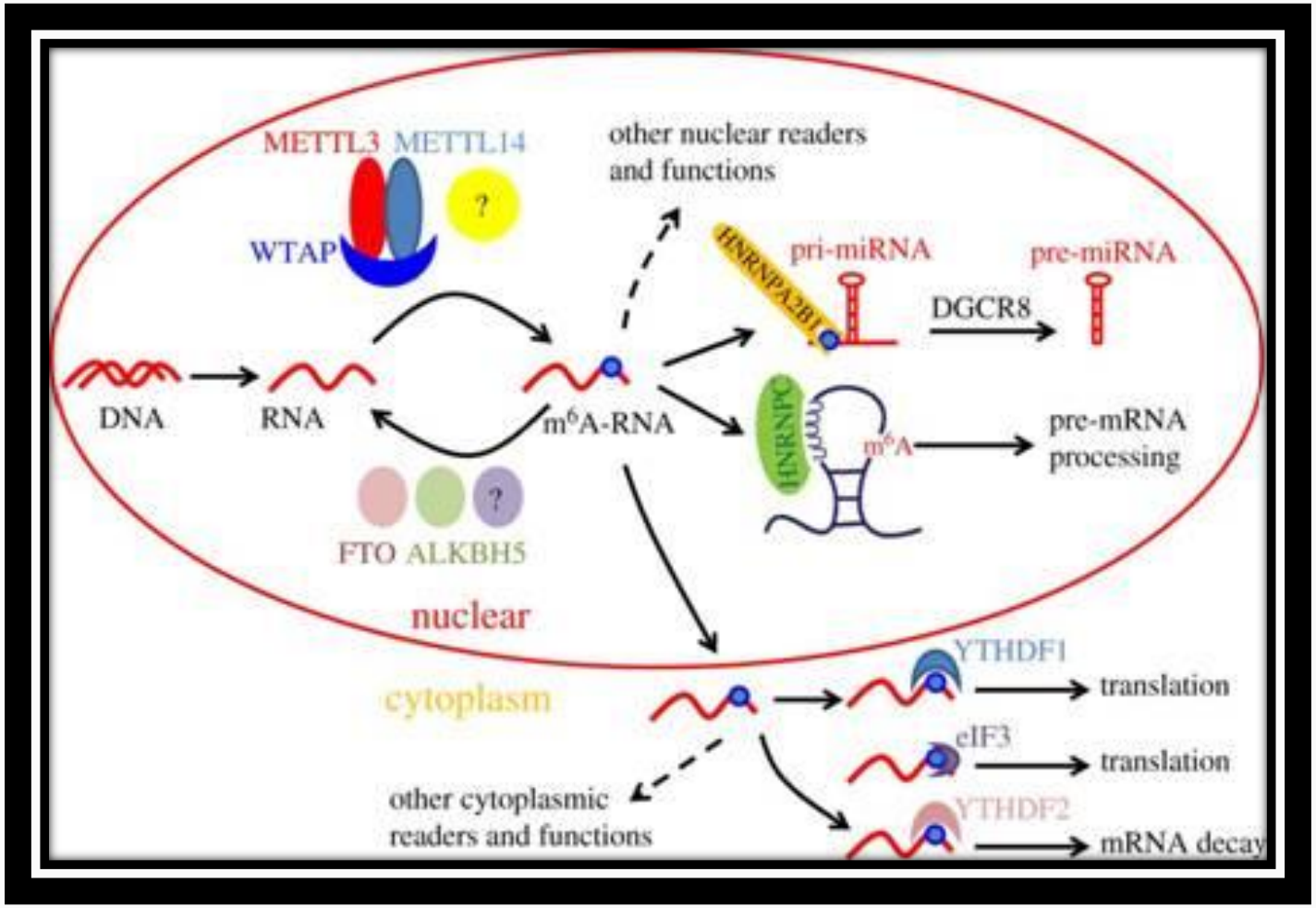
Quantitative Methylation Analysis

We designed primers for the *POU3F3* gene to cover the region with the most CpG sites. Our selected amplicon was located in the promoter region of the gene. The mass spectra were collected using a MassARRAY Compact MALDI-TOF (Sequenom; BioMiao Biological Technology, Beijing, China) and the spectra's methylation ratios were generated by the EpiTYPER software (Sequenom, San Diego, CA).

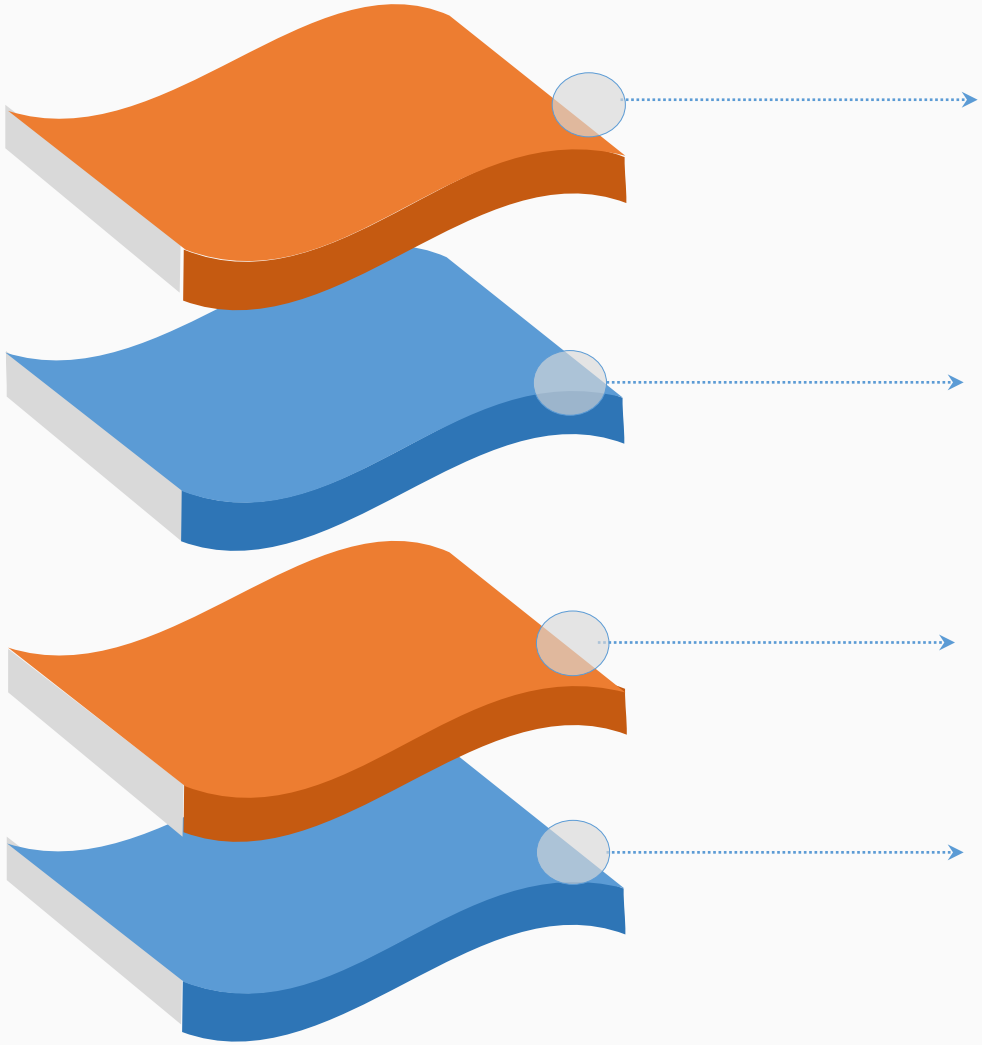
表观遗传学RNA甲基化“m6A”科研路线——基础理论



表观遗传学RNA甲基化“m6A”科研路线——基础理论



表观遗传学RNA甲基化“m6A”科研方案设计——修饰图谱



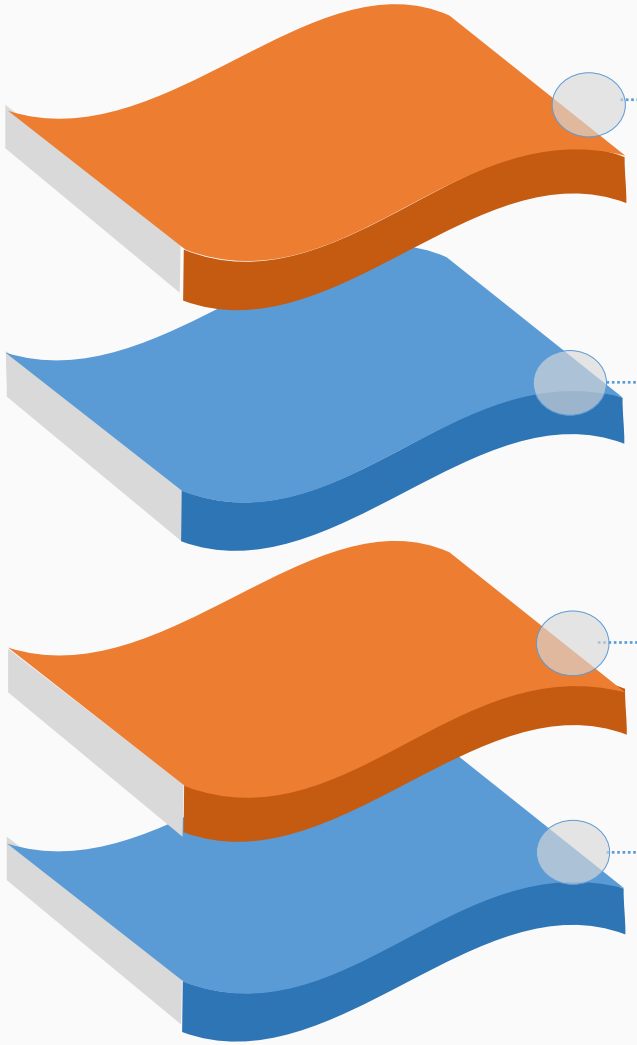
样本类型
- ✓ 细胞/组织/外周血

检测技术
- ✓ m6A-IP-seq
- ✓ RNA-seq

生物信息学分析
- ✓ m6A修饰谱分析
- ✓ m6A修饰差异基因分析
- ✓ meRIP-seq与RNA-seq关联分析（m6A与mRNA、miRNA、LncRNA表达关联分析； m6A与RNA可变剪切关联分析）
- ✓ MeRIP-seq与蛋白质组、代谢组联合分析

m6A位点验证
- ✓ m6A-RT-qPCR
- ✓ miCLIP-seq

表观遗传学RNA甲基化“m6A”科研方案设计——甲基化酶功能机制研究



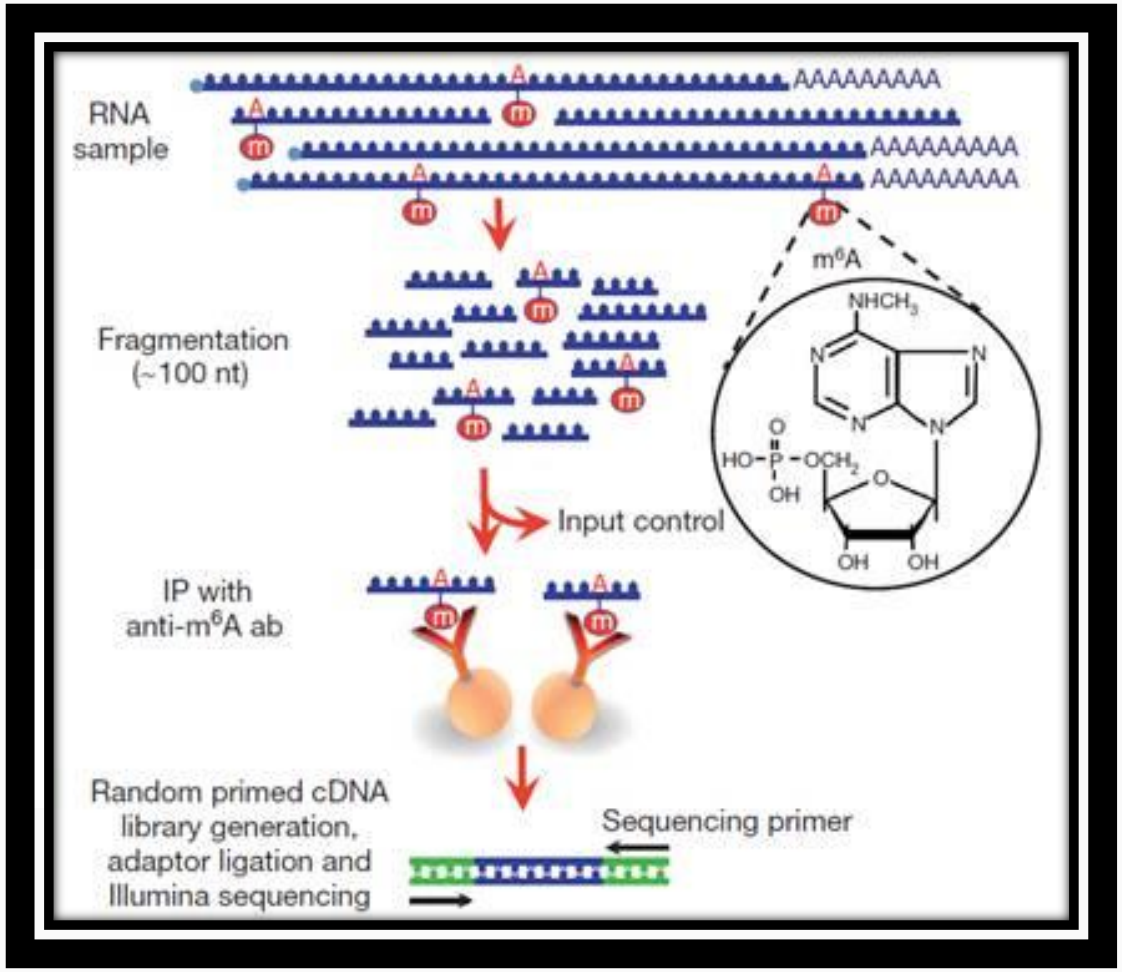
Step1
- ✓ m6A-seq+RNA-seq
- ✓ 差异m6A peak及差异基因筛选

Step2
- ✓ 干扰和过表达甲基化酶相关基因，检测整体RNA的m6A水平
- ✓ RNA-seq+miRNA-seq，分析上述对于mRNA或miRNA整体的影响，并着重研究第一步中筛选的m6A有差异的靶基因

Step3
- ✓ 对靶基因进行干扰或过表达，分析是否能够对甲基化酶异常表达后的表型进行恢复
- ✓ 对靶基因上的motif进行点突变后进一步确认直接受到甲基化酶调控

Step1
- ✓ 鉴定新型的甲基化酶

m6A-seq技术流程介绍



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BIOMIAO BIOLOGICAL
-SINCE2009-

m6A-seq样本准备要求

样本类型

- ✓ 细胞
- ✓ 新鲜组织
- ✓ 外周血
- ✓ RNA样品

样本量

- ✓ 细胞样本->10^7个细胞
- ✓ 组织样本>1g
- ✓ total RNA>500ug
- ✓ mRNA>30ug
- ✓ 外周血>1ml

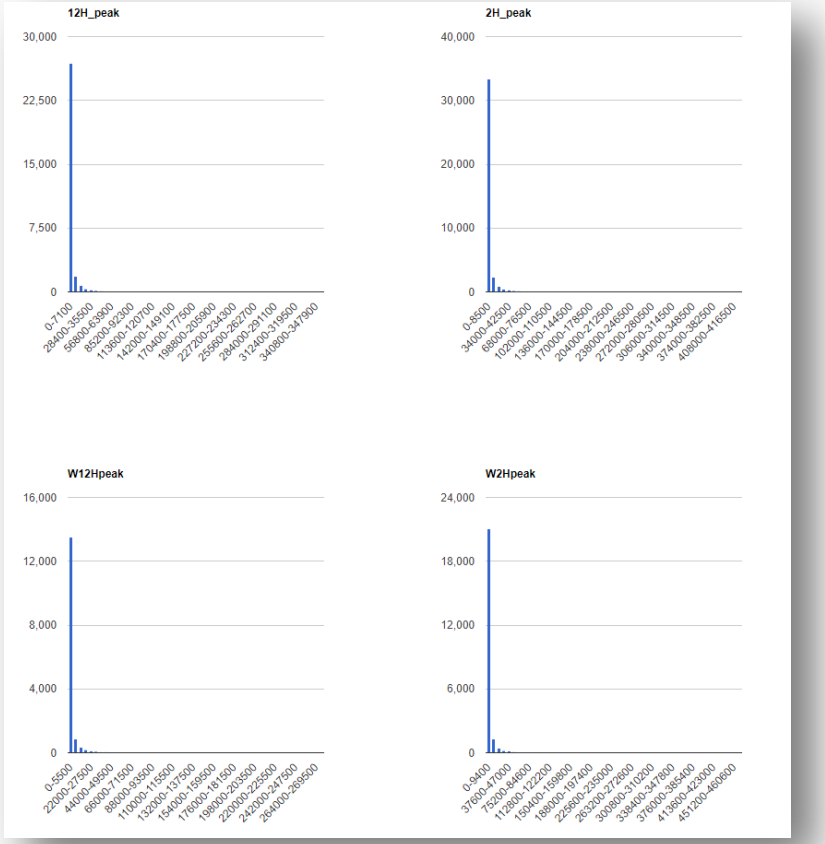
样品质量

- ✓ RNA无明显降解
- ✓ OD260/280为1.8-2.2
- ✓ 浓度>500ng/ul
- ✓ 28S:18S>1.5
- ✓ RIN>7

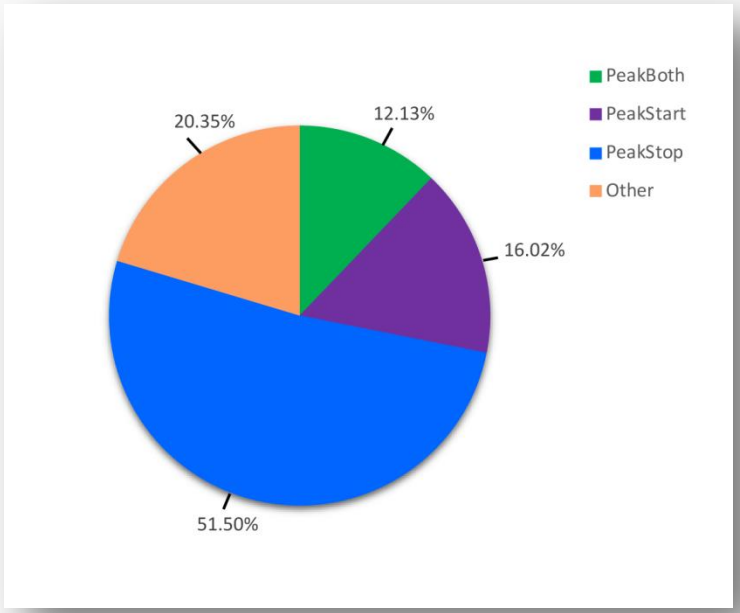
样本保存

- ✓ 细胞样本或新鲜组织块用TRIZOL或RNA保护剂处理，放置在-80度
- ✓ 全血样本需要分离白细胞，然后TRIZOL裂解并保存-80度
- ✓ RNA样本可溶于乙醇或RNA-free的超纯水中，并保存-80度。样本保存期间避免反复冻融

m6A-seq生物信息学数据分析内容展示



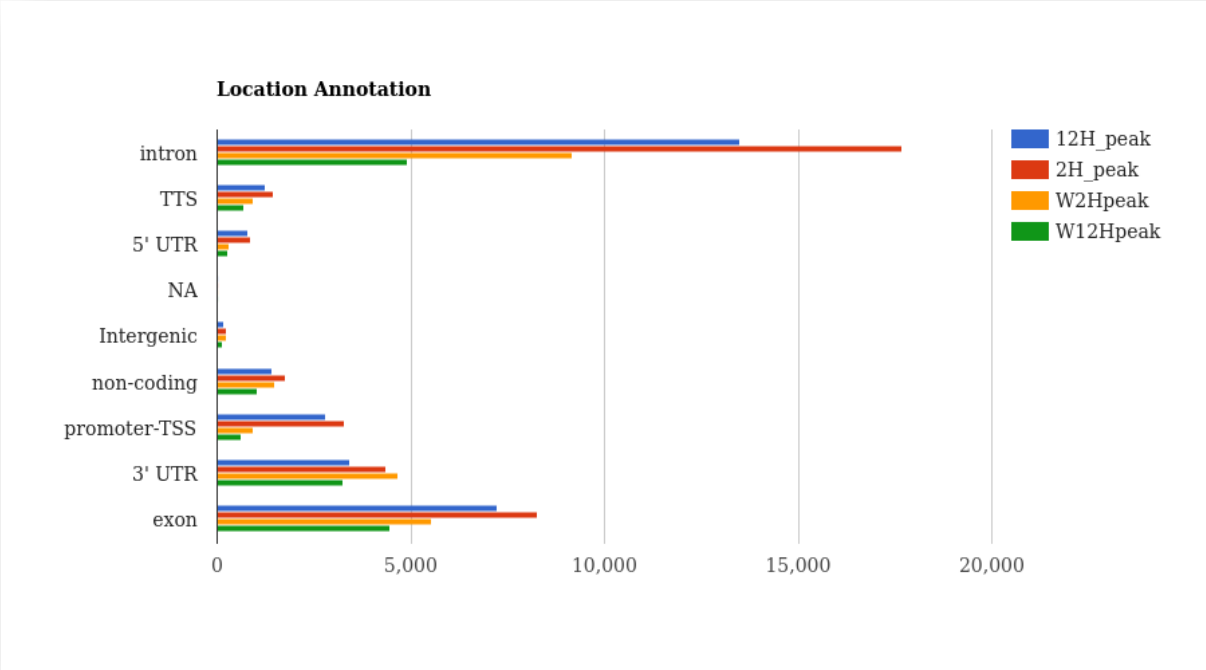
m6A Peak长度概述

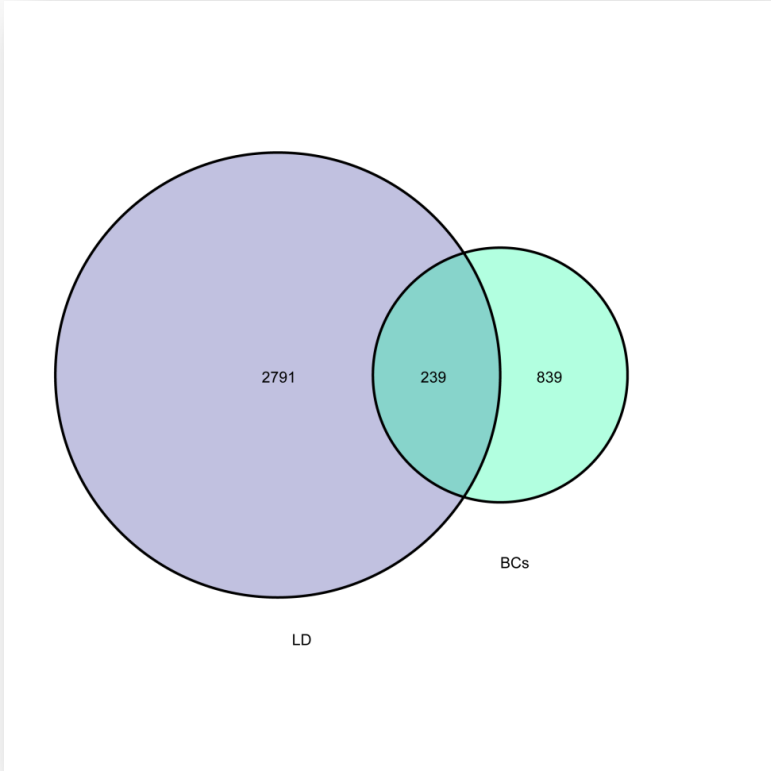


m6A Peak在基因元件中分布概述

peak关联基因特征pie图，针对Peak关联上的基因，看它的stop-coden、start-coden是否有m6A富集区域（peak），以此将gene分为4类：PeakStart (m6A peaks around start codon), PeakStop (m6A peaks around stop codon), PeakBoth (m6A peaks around both start and stop codons) and others

m6A-seq生物信息学数据分析内容展示





Peak 分布条形图，纵坐标表示各元件，横坐标表示落在基因元件中的peak数量

两样本peak韦恩图，两样品peak overlap 占50%以上则定义为common peak，其他的定义为special peak。

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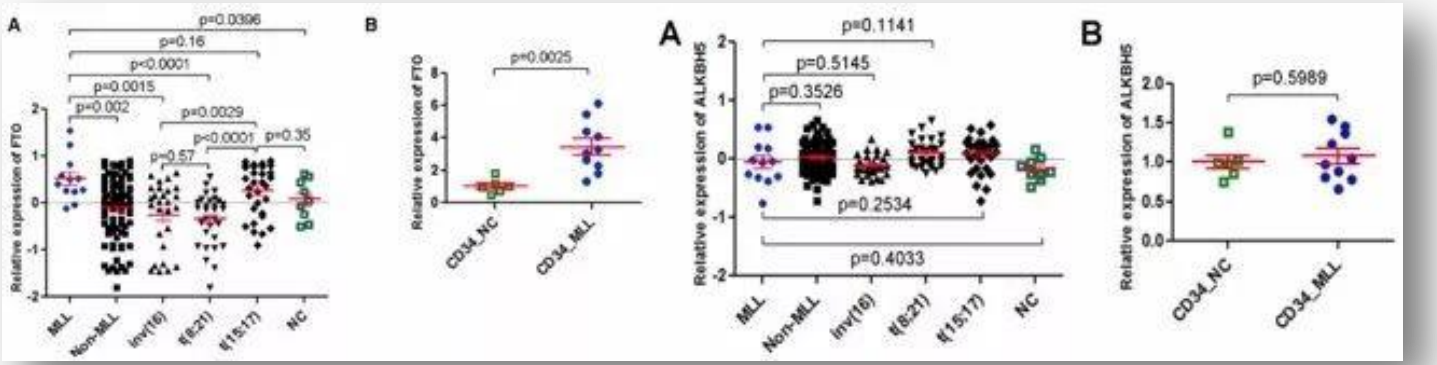
-SINCE2009-

m6A甲基化酶功能机制研究经典文献

FTO Plays an Oncogenic Role in Acute Myeloid Leukemia as a N6–Methyladenosine RNA Demethylase
CANCER CELL 2017

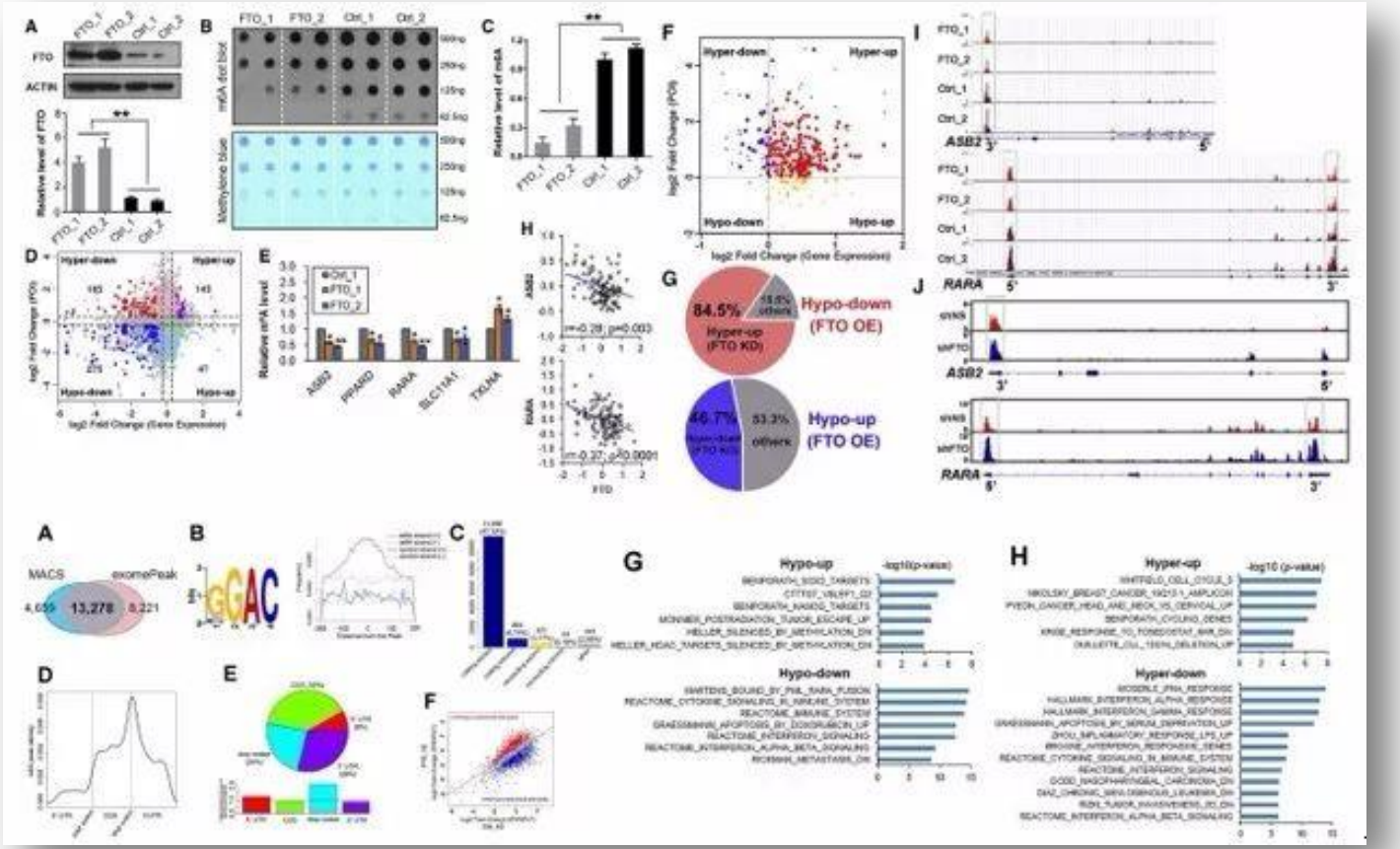
对MLL病人的CD34+骨髓细胞进行qPCR验证发现，FTO表达量显著高于普通CD34+细胞。作者还对另一种去甲基化酶ALKBH5进行了分析和qPCR验证，结果发现AML和对照没有差异



m6A甲基化酶功能机制研究经典文献

转录组测序和m6A-seq揭示FTO对靶基因存在负调控：

- ✓ 对FTO过表达的mono-mac-6（MLL-AF9AML）细胞系进行转录组测序和m6A-seq，并用空载体细胞作为对照。与对照的细胞系相比，FTO表达量提高了4~5倍，而m6A修饰水平显著降低；
- ✓ 鉴定出13278个m6A peak有交集，其中GGACmotif显著富集，大部分都位于外显子上，并且在3’端的终止密码子附近富集程度较高；
- ✓ 通过比较，发现有2785个m6A peak显著下降而3180个m6A peak显著上升，在2780个去甲基化转录本中，发现275个转录本（超过85%）显著下降而47个转录本显著上调。所以AML细胞系中，这些去甲基化转录本可能就是受到FTO负调控的靶基因。



m6A甲基化酶功能机制研究经典文献

使用m6A-seq对FTO敲低的MA9/FLT3-ITD白血病细胞进行测序：
FTO过表达的mono-mac-6细胞系中超过90%的去甲基化转录本在FTO敲低的MA9/FLT3-ITD白血病细胞系中m6A甲基化水平上升。

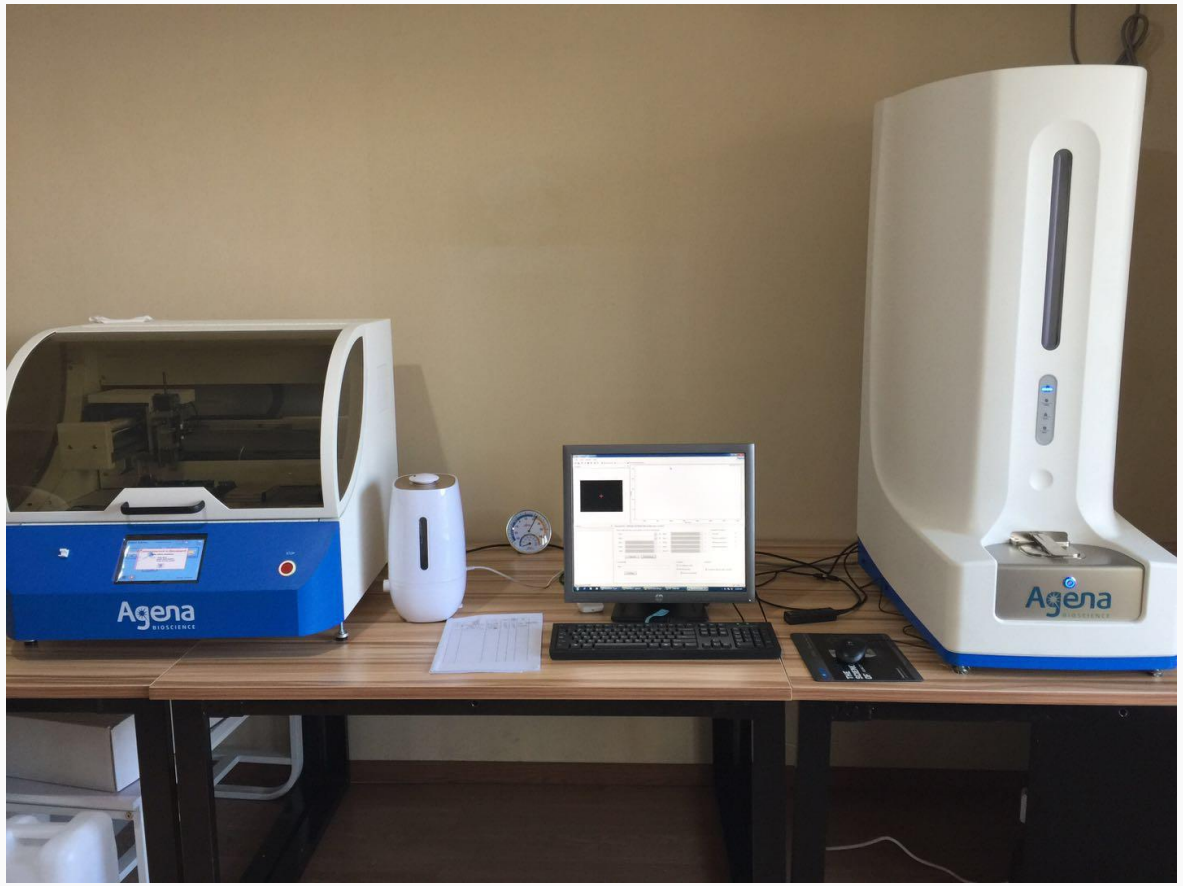
Table S3 (Related to Figure 5). The patterns of the m^{6}A level and RNA transcript abundance changes of the 11 m^{6}A-Hypo genes (shown in Table S2) in human AML cells with *FTO* overexpression or knockdown

Gene	In *FTO*-overexpressing MONOMAC-6 cells relative to the control MONOMAC-6 cells	In *FTO*-knockdown *MA9/FLT3*-ITD cells relative to the control *MA9/FLT3*-ITD cells (with expected patterns?)
ASB2	Hypo-down	Hyper-Up (Yes)
KCNG1	Hypo-down	Hyper-Up (Yes)
PPARD	Hypo-down	Hyper-Up (Yes)
RAB17	Hypo-down	Hyper-Up (Yes)
RARA	Hypo-down	Hyper-Up (Yes)
SLC11A1	Hypo-down	Hyper-down (No)
SLCO4A1	Hypo-down	Hyper-up (Yes)
TBC1D9	Hypo-down	ND[a] (-)
C21orf59	Hypo-up	Hyper-down (Yes)
MZF1	Hypo-up	Hyper-down (Yes)
TXLNA	Hypo-up	Hyper-up (No)

博淼品牌服务之——iScan技术

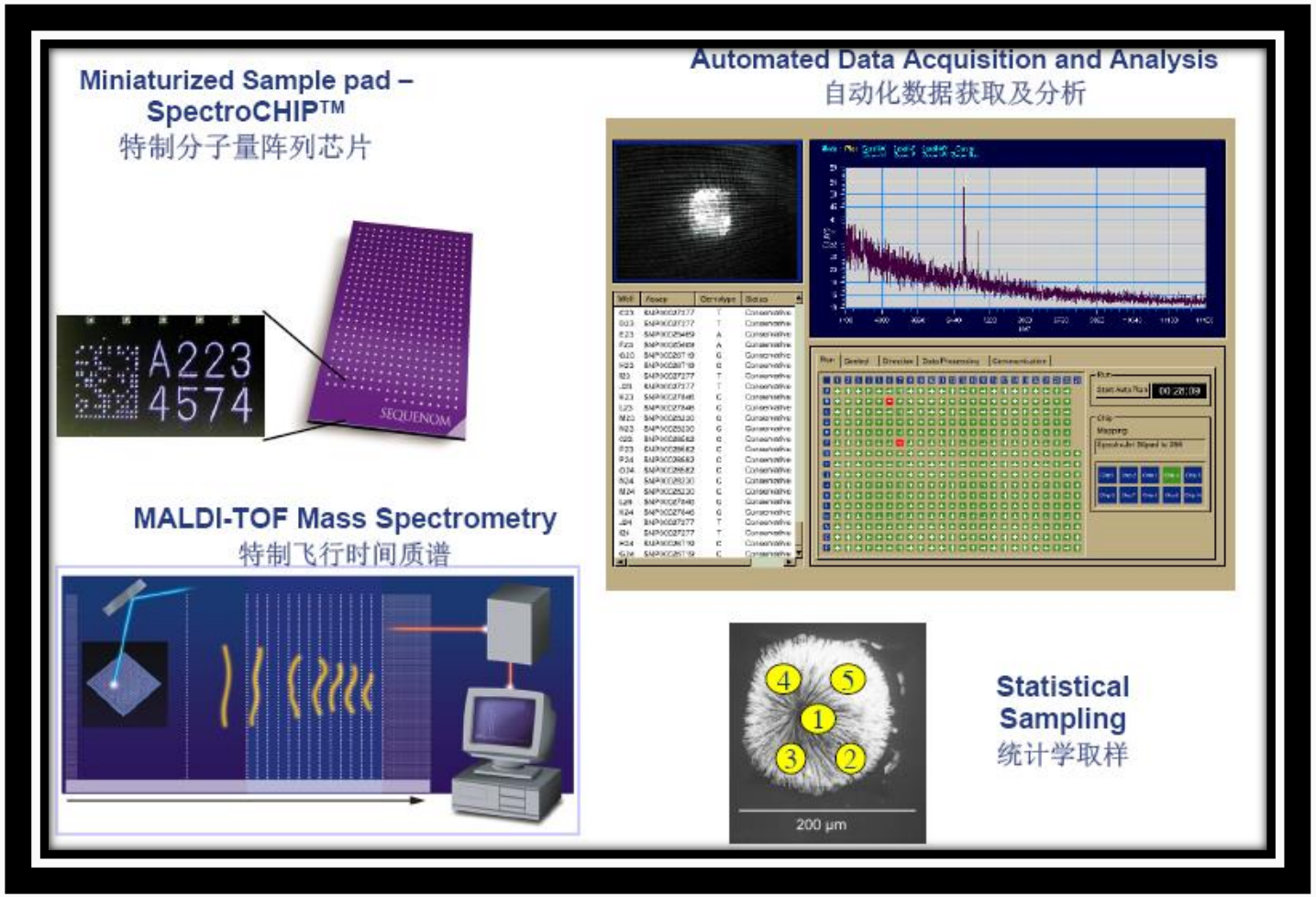
- GWAS芯片：中华芯片、ASA芯片
- 甲基化芯片：850K芯片

博淼品牌服务之——Massarray技术



- 候选SNP位点分型
- 候选甲基化位点定量检测

Massarray技术原理

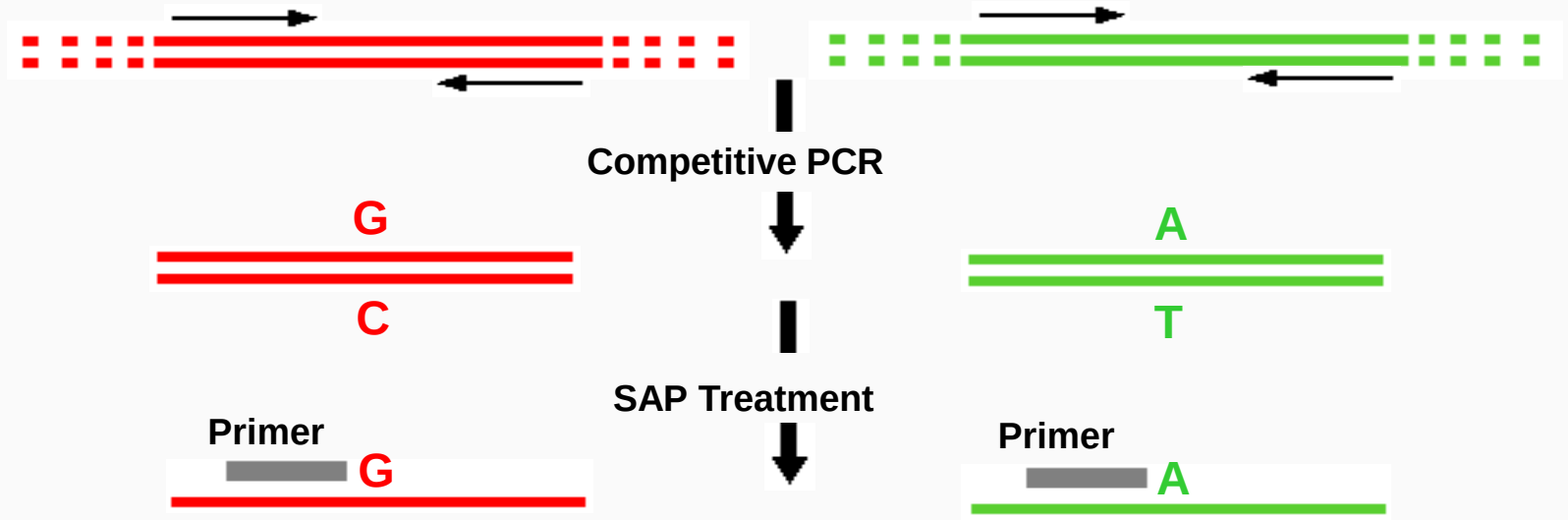


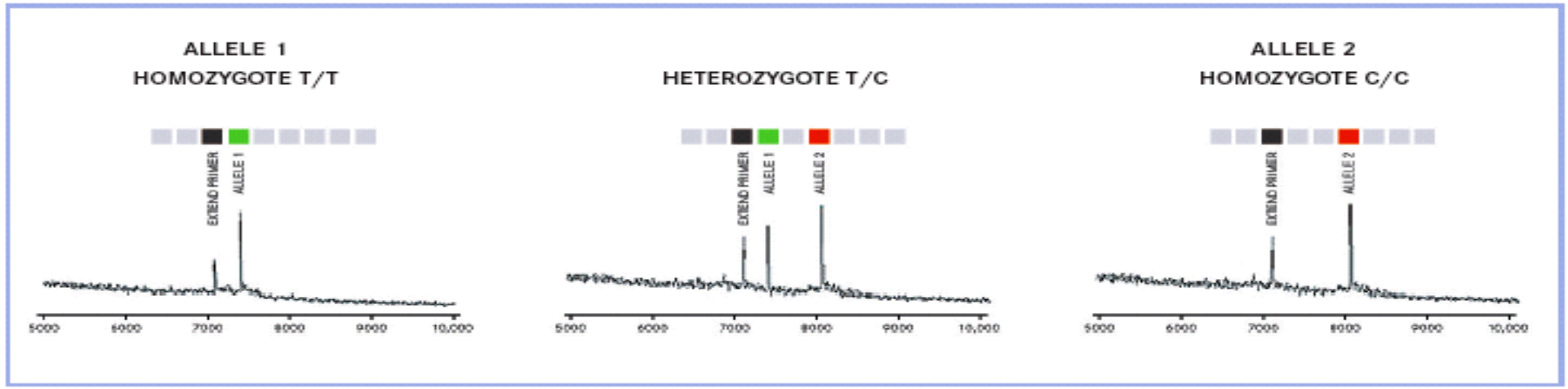
Massarray技术原理——SNP分型

Genomic DNA

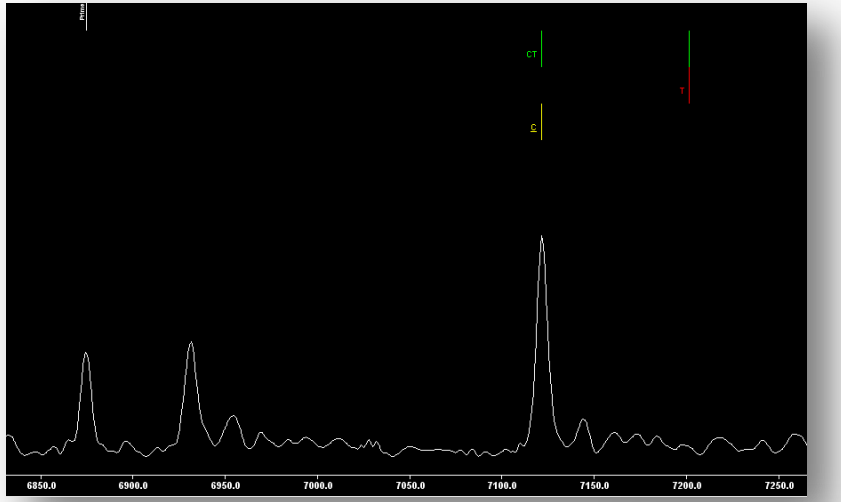
PCR Amplification

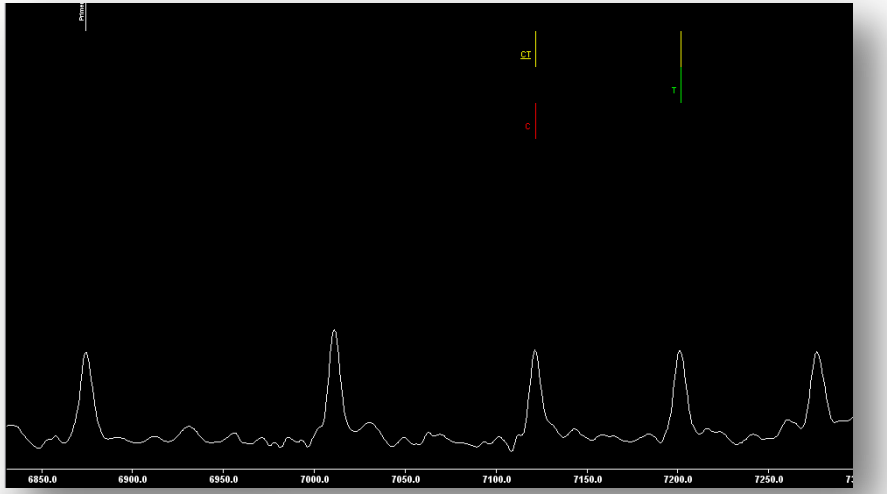
Primer Extension

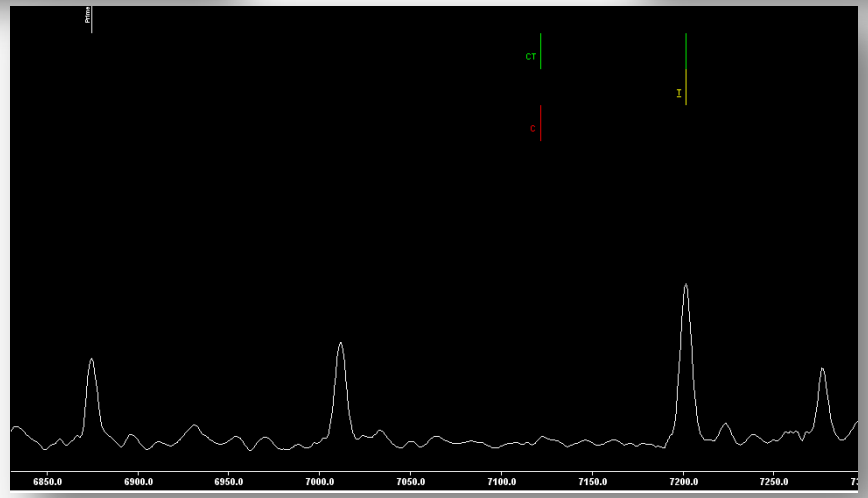




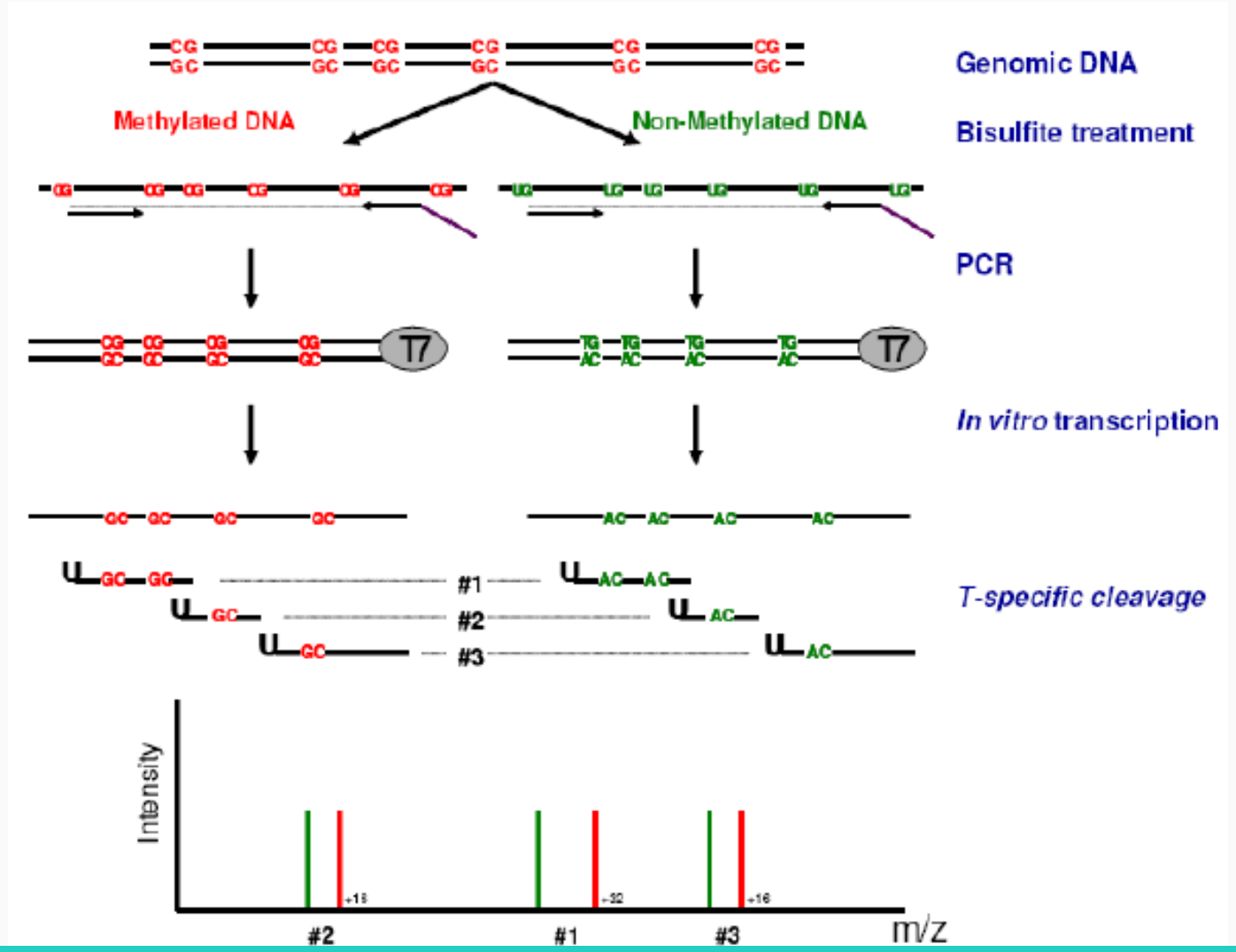
Massarray技术原理——SNP分型



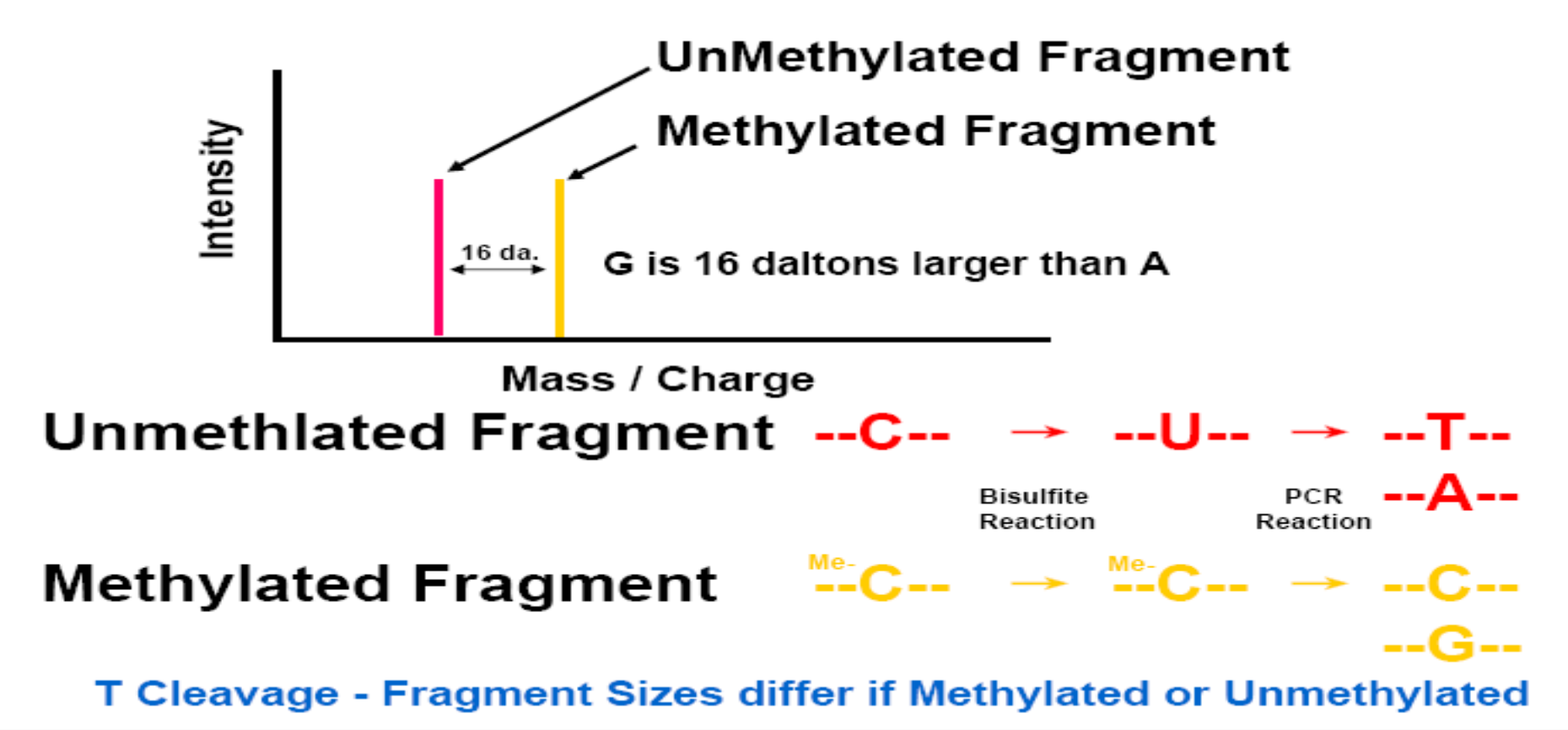




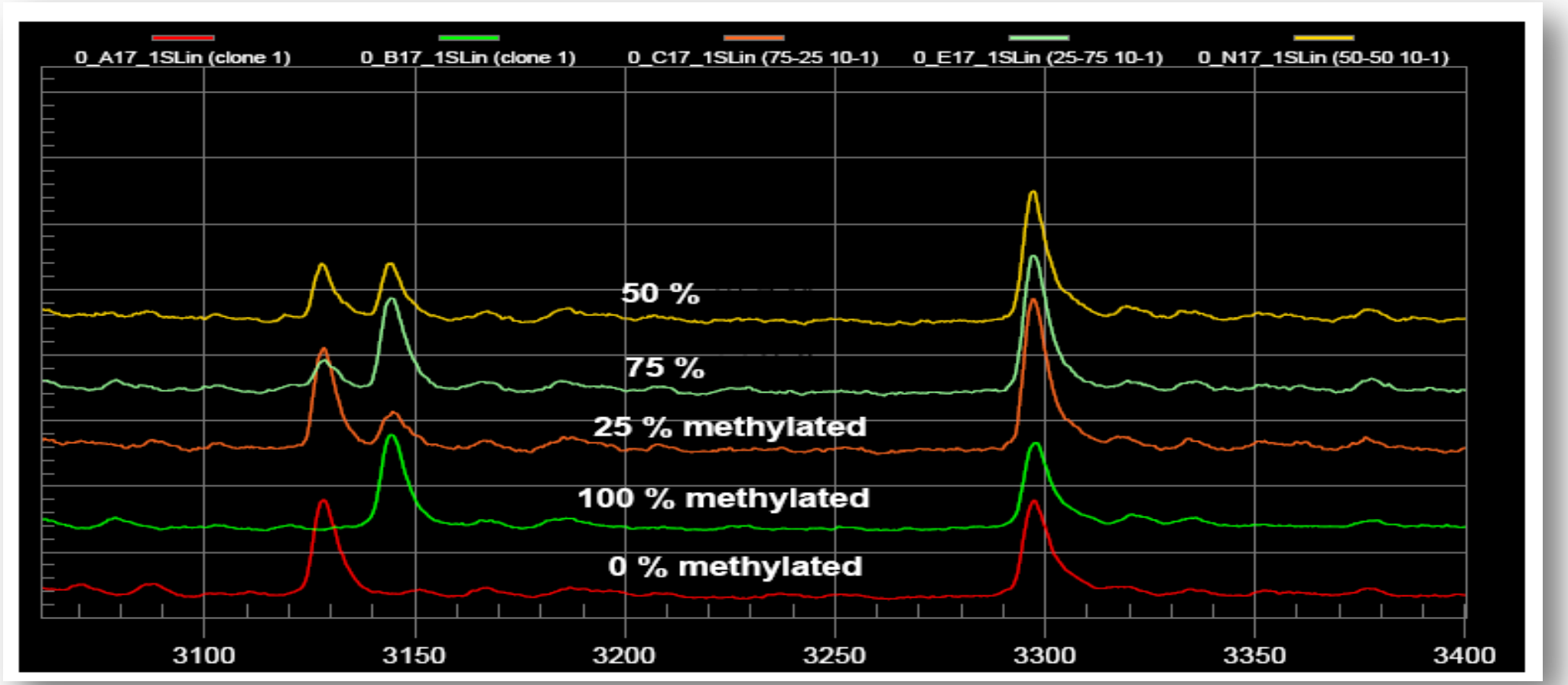
Massarray技术原理——DNA甲基化定量



Massarray技术原理——DNA甲基化定量



Massarray技术原理——DNA甲基化定量



博淼生物代表文章一览

•The relationship between the CYP2D6 polymorphisms and tamoxifen efficacy in adjuvant endocrine therapy of breast cancer patients in Chinese Han population **International journal of cancer** 2018

•A novel SNP in promoter region of RP11–3N2.1 is associated with reduced risk of colorectal cancer J*ournal of Human Genetics***volume 63**, pages47–54 (2018)

•Discovery of susceptibility loci associated with tuberculosis in Han Chinese **Human Molecular Genetics** 2017

•A genome–wide association study of cognitive function in Chinese adult twins **Biogerontolgy** 9 August 2017

•BRCA1 missense polymorphisms are associated with poor prognosis of pancreatic cancer patients in a Chinese population ***Oncotarget.*** 2017 May 30

• Effect of AMP–activated protein kinase subunit alpha 2 (*PRKAA2*) genetic polymorphisms on susceptibility to type 2 diabetes mellitus and diabetic nephropathy in a Chinese population **Journal of Diabetes** 13 July 2017

•Genome–wide association study identifies multiple susceptibility loci for craniofacial microsomia.***Nat Commun***. 2016 Feb

•Identification of Genetic and Environmental Factors Predicting Metabolically Healthy Obesity in Children: Data From the BCAMS Study ***J Clin Endocrinol Metab***. 2016 Apr **IF=6.02**

•The Mkk7 p.Glu116Lys Rare Variant Serves as a Predictor for Lung Cancer Risk and Prognosisi in Chinese ***PLOS Genetics***, 2016 March **IF=7.528**

• Sequence variation in Mature MircroRNA–499 Confers Unfavorable Prognosis of Lung Cancer Patients Treated with Platinum–Based Chemotherapy ***Clinical Cancer Research*** **IF=8.722**

•*STAT1* single nucleotide polymorphisms and susceptibility to immune thrombocytopenia **Autoimmunity** February 24, 2015

•Trans–ancestry genome–wide association study identifies 12 genetic loci influencing blood pressure and implicates a role for DNA methylation. **Nat Genet. 2015 Nov;47(11):1282–93 IF=29.352**

•Increased Levels of the Long Intervening ncRNA POU3F3 Promote DNA Methylation in Esophageal Squamous Cell Carcinoma ***Gastroenterology*** 2014 Mar **IF=12.88**

• Effects of a Functional Variant c. 353T> C in Snai1 on Risk of Two Contextual Diseases: COPD and Lung Cancer ***American journal of respiratory and critical care medicine*** 2014 January 15 **IF=11.080**

•RNA sequencing reveals the complex regulatory network in the maize kernel ***NATURE COMMUNICATIONS*** 17 Dec 2013 **IF=10.03**

• A sequence polymorphism in miRNA–608 predicts recurrence after radiotherapy of nasopharyngeal carcinoma ***Cancer Res***. 2013 Jun 24 **IF=7.856**

•PKC λ is critical in AMPA receptor phosphorylation and synaptic incorporation during LTP ***EMBO.J*** 2013 Mar 19 **IF=10.124**

•A genome wide association study of genetic loci that influence tumour biomarkers cancer antigen 19–9, carcinoembryonic antigen and α fetoprotein and their associations with cancer risk ***Gut.*** 2013 Jan 7. **IF=10.614**

•A novel SNP in promoter region of RP11–3N2.1 is associated with reduced risk of colorectal cancer ***Journal of Human Genetics2018***

•Genetics of Obesity Traits: A Bivariate Genome–Wide Association Analysis ***Front Genet.2018***

• Effect of *CYP2C19*, *UGT1A8*, and *UGT2B7* on valproic acid clearance in children with epilepsy: a population pharmacokinetic model **European Journal of Clinical Pharmacology2018**

•A variant in *KCNQ1* gene predicts metabolic syndrome among northern urban Han Chinese *women* ***BMC Medical Genetics2018***

10 YEARS

+3000 Res Pro

+200 Uni/Hos

+500 Papers

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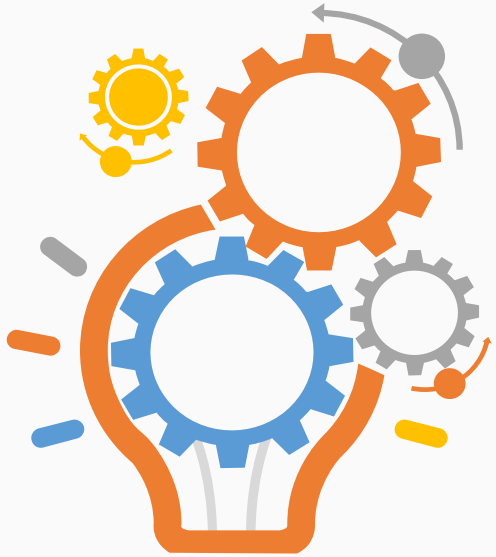

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