单细胞基因组学图谱绘制及分析技术

中国科学院北京基因组研究所
(国家生物信息中心)
蔡 军

2025年5月

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- **单细胞基因组变异图谱绘制及分析技术**

  - **体细胞基因组变异**

  - 体细胞基因组变异图谱绘制和分析技术

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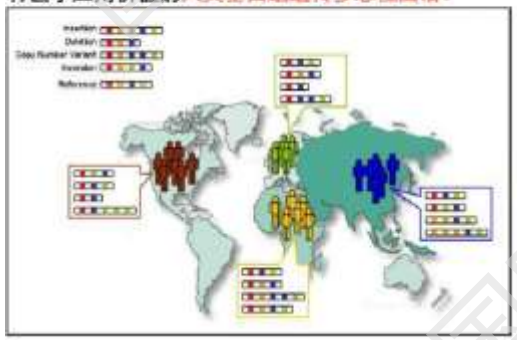
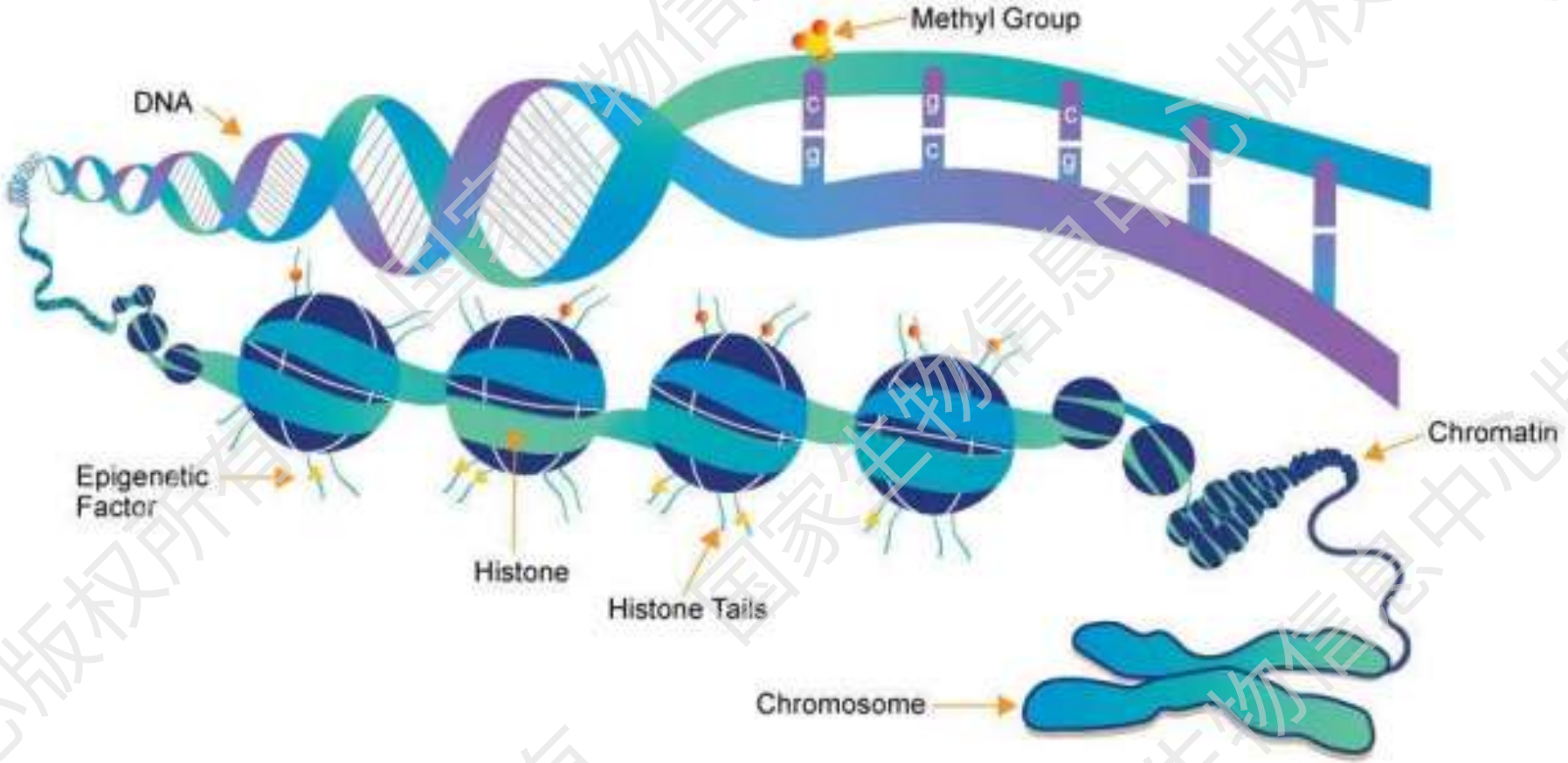
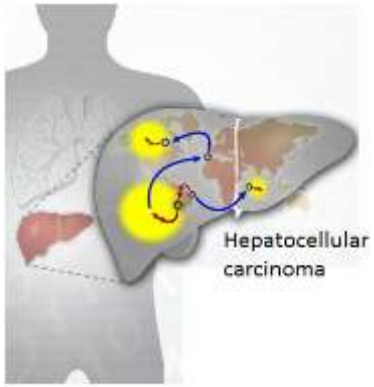
基因组学图谱概貌

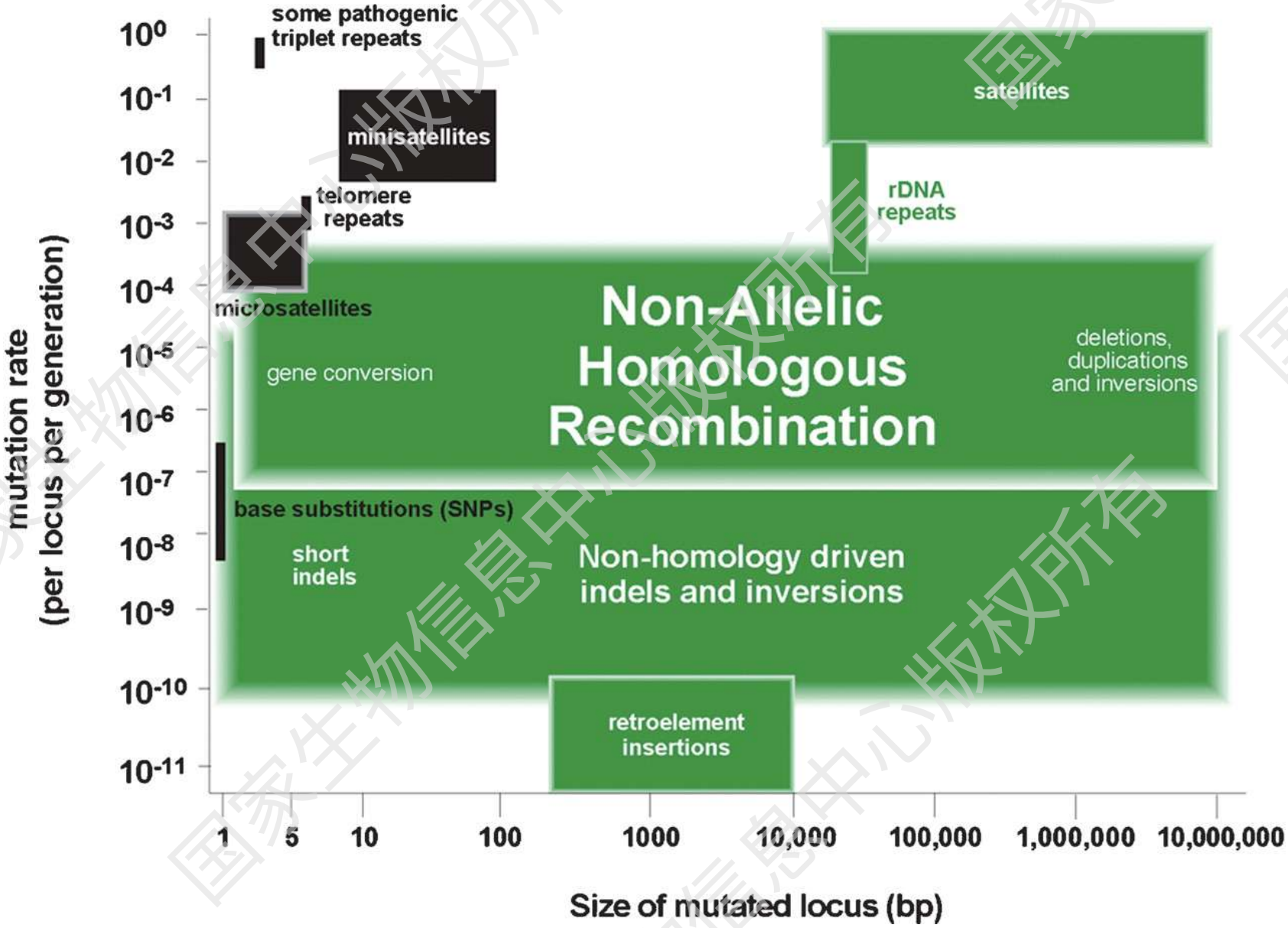
“国际千人基因组计划”自2008年1月22日启动，测序的总任务为1200个人（故称为千人基因组计划），旨在绘制迄今为止最详尽、最有医学应用价值的人类基因组遗传多态性图谱。







基因组遗传变异



Different classes of mutation operating in the human genome.
Freeman et al. Genome Res 2006

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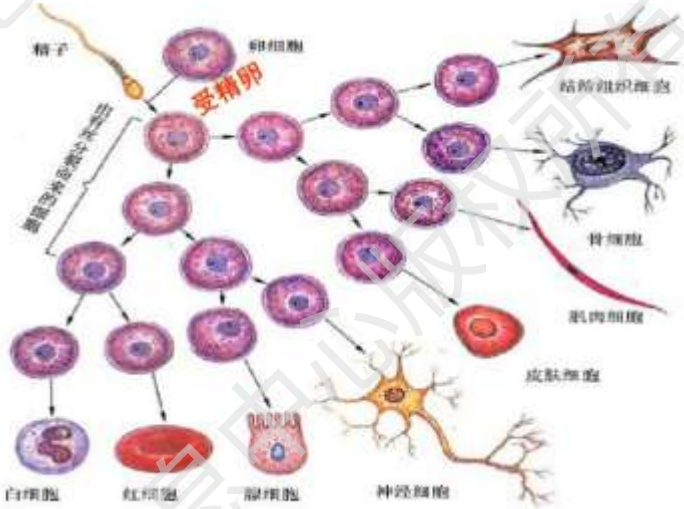
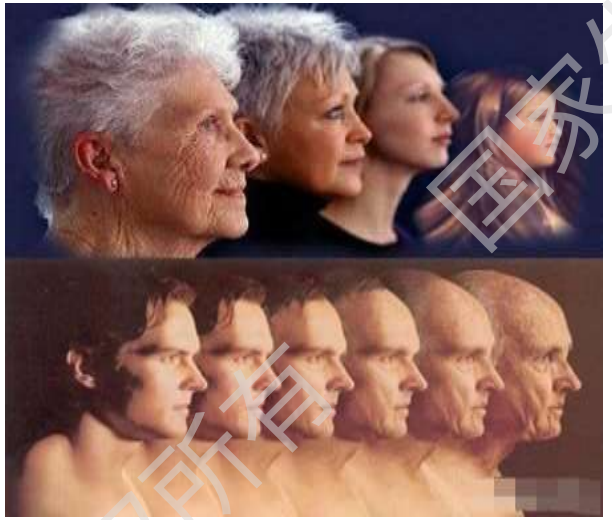
体细胞基因组遗传变异

- 细胞演化的观点：从演化的角度看待正常细胞的增殖和肿瘤的发生、发展
  - 细胞从哪里来到哪里去
  - 细胞群体的异质性
  - 细胞的克隆演化

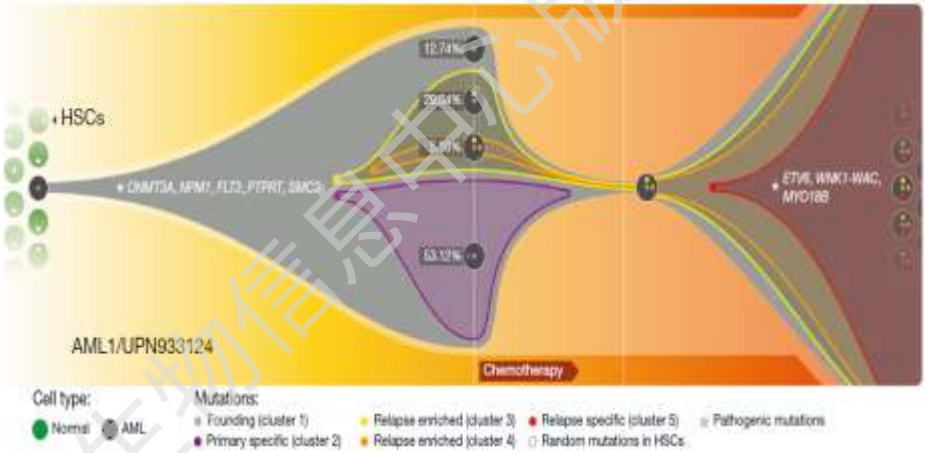




期望 -> 现实

正常细胞：
细胞群体高度异质
（没有细胞克隆）；

肿瘤细胞：
细胞群体同质化
（单一的细胞克隆）



Li Ding, Timothy J. Ley, et al. Nature. 2011

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体细胞基因组变异

- 来源和特征

与胚系突变的异同、突变频率

- 特定的检测技术

高异质性、细胞微起始量

- 用于识别细胞群体的异质性和细胞谱系关系

突变率->记录细胞从哪里来到哪里去

- 细胞类型特异性的表型和致病效应

体细胞突变eQTL

突变的累积与克隆膨胀：肿瘤组织中的体细胞突变可检测

需求：

解析单细胞基因组变异

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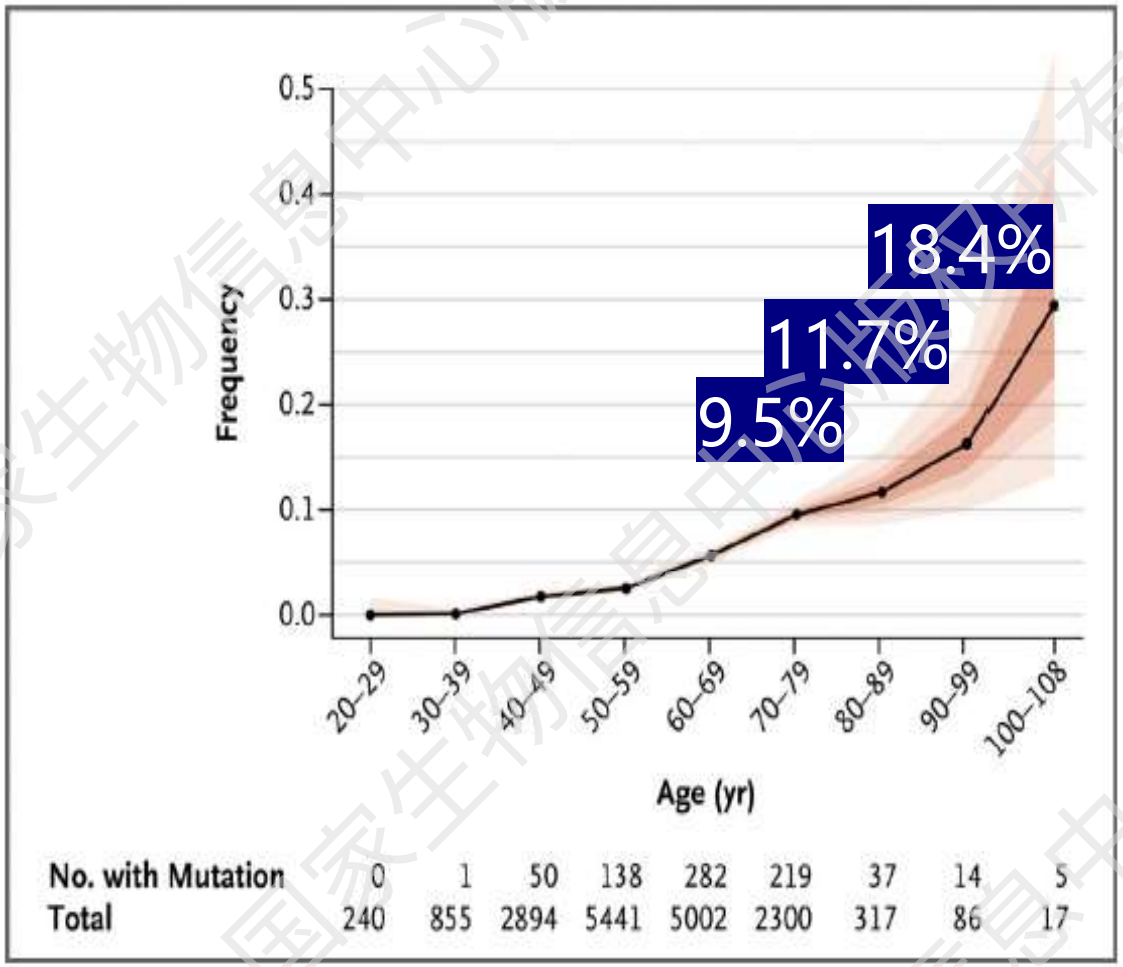
随着年龄的增长，克隆体细胞突变的频率增加



在40岁以下的人群中，可检测到的体细胞突变是罕见的，但频率随年龄的增加而明显增加

Figure 1. Prevalence of Somatic Mutations, According to Age

Colored bands, in increasingly lighter shades, represent the 50th, 75th, and 95th percentiles.

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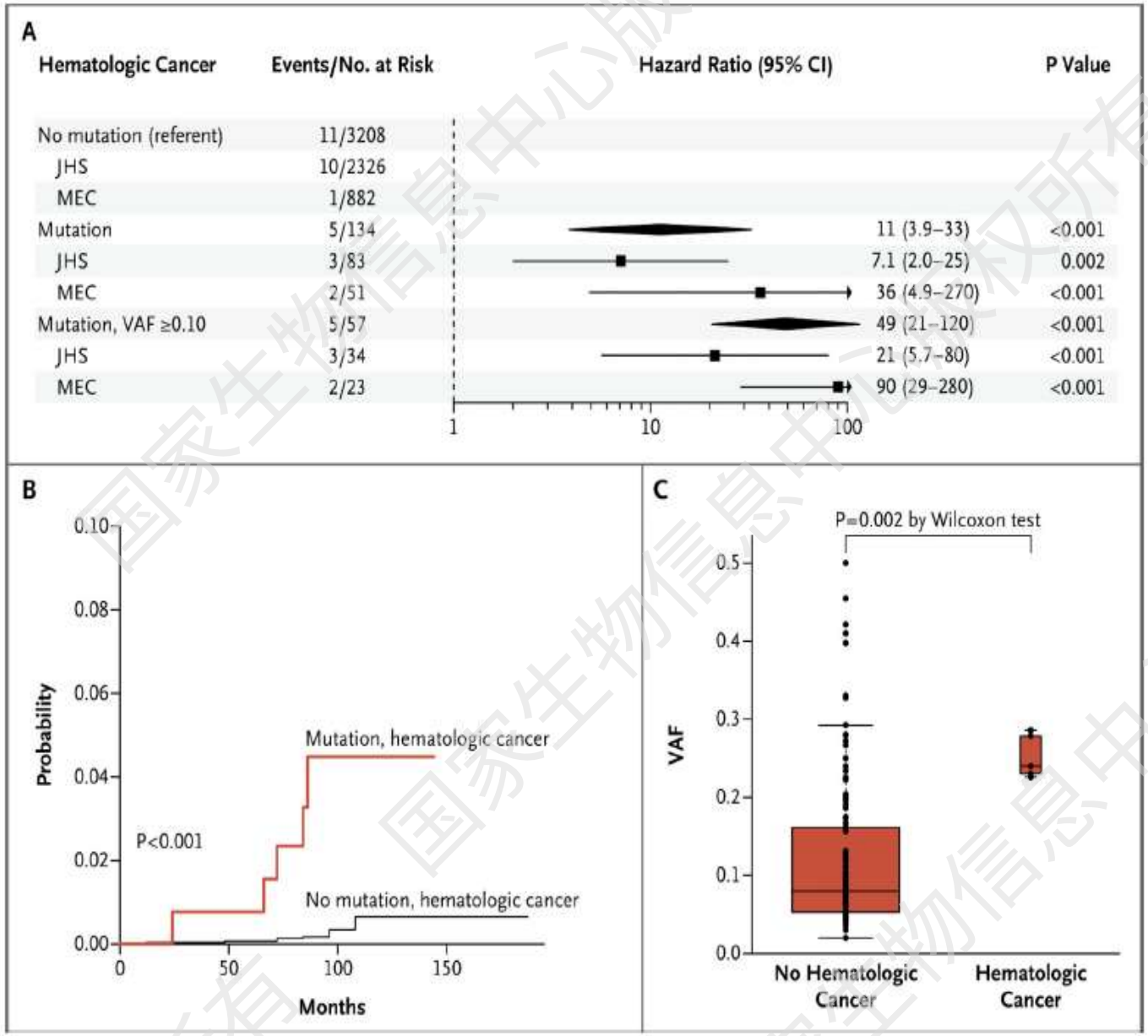
体细胞突变导致的血液癌症的总体风险



其他风险：

心血管死亡包括致命性中风(出血性或缺血性中风)和致命性心肌梗死

- **单细胞基因组变异图谱绘制及分析技术**

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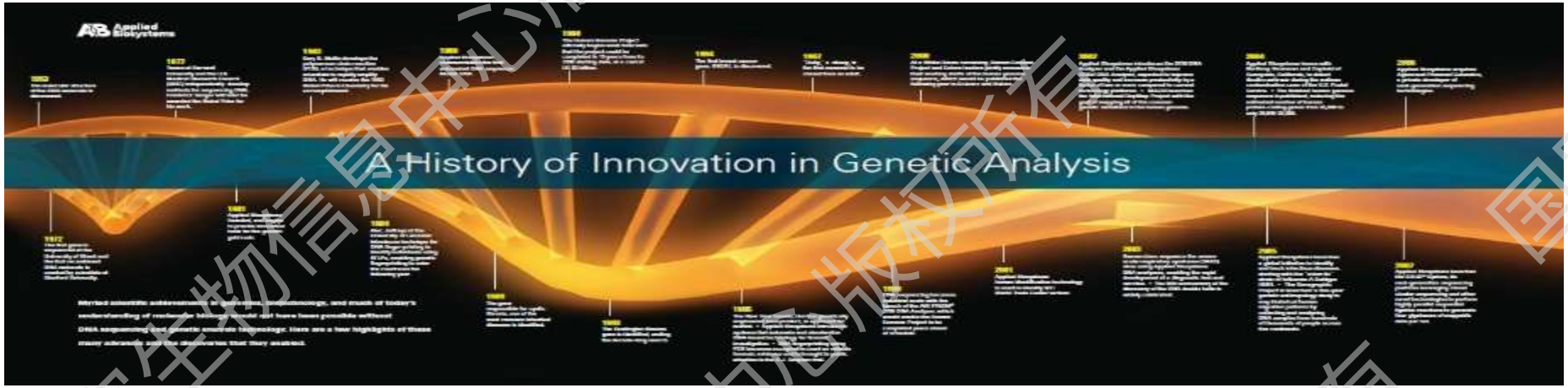
Next-generation sequencing technologies



第一代测序平台

第二代测序平台

第三代测序平台

AND MORE.....

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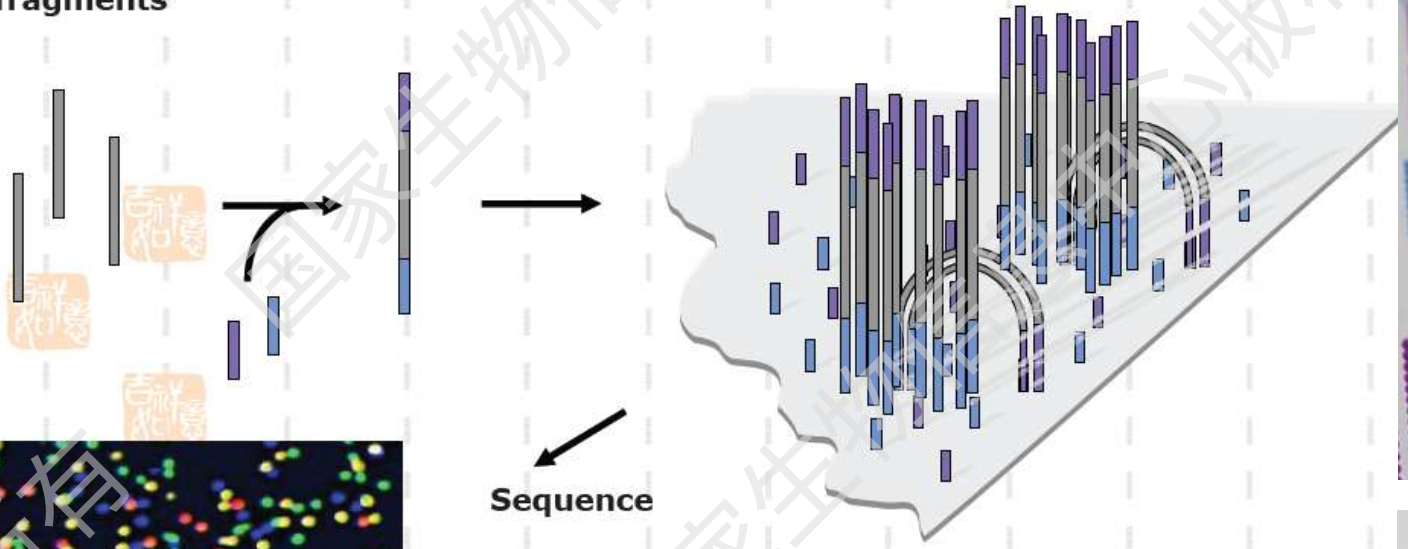
第二代测序平台测序原理

Clonal Single Molecule Arrays

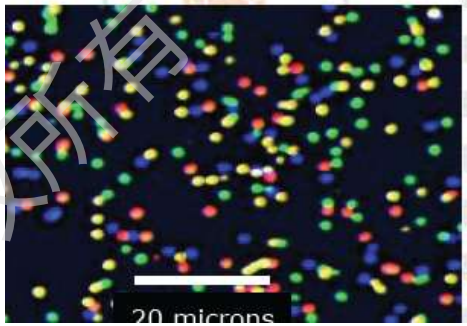
Attach single molecules to surface
Amplify to form clusters

Prepare DNA fragments

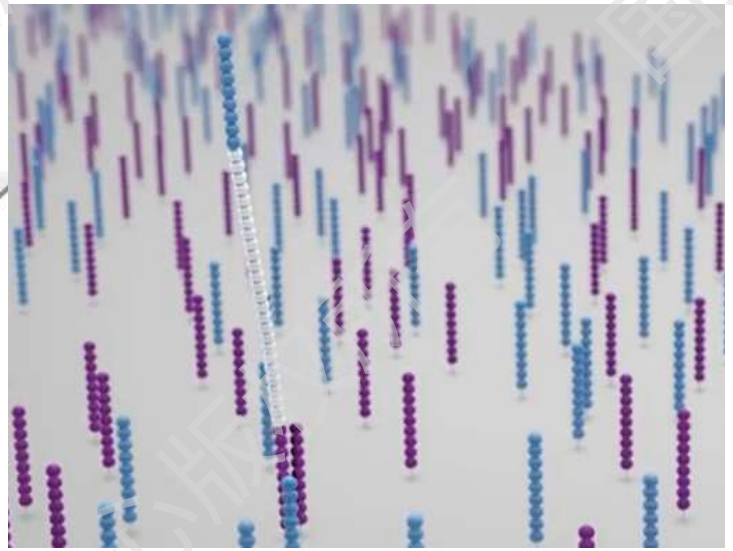
Ligate adapters

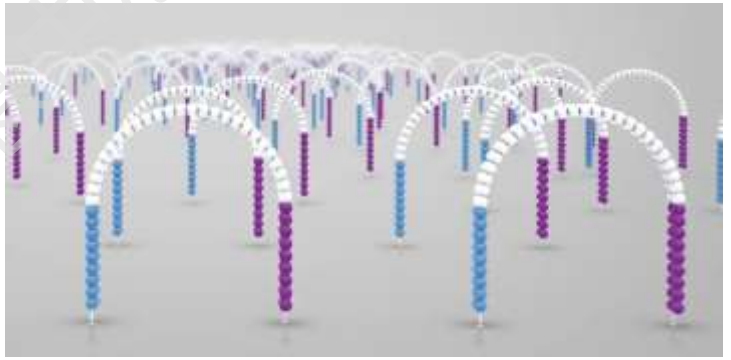


Sequence

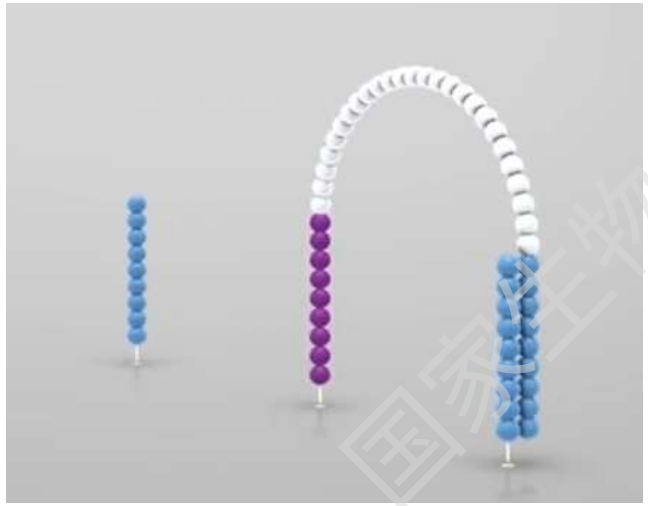


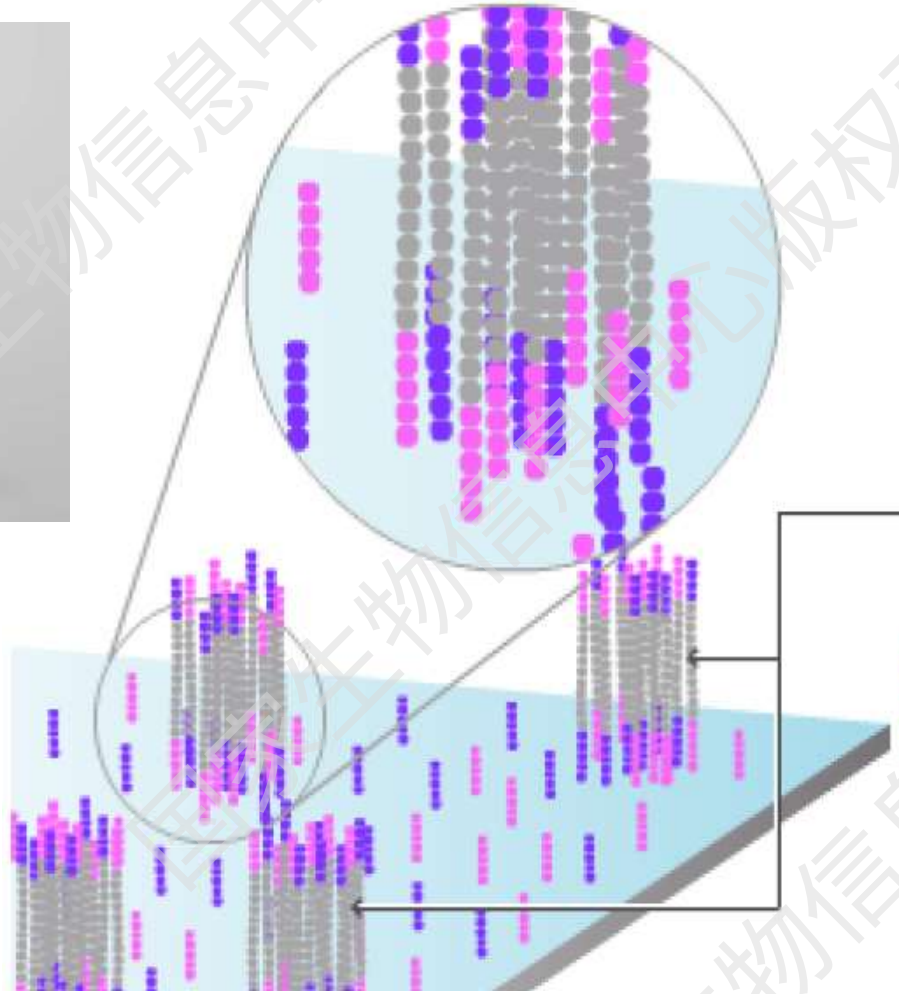
~1000 molecules per ~ 1 µm cluster
~1000 clusters per 100 µm square
~40 million clusters per experiment





(I) Amplification Clusters





One-molecule template forms one cluster

Only one set of primers are used (B- or R-primer)

Clusters

经过30轮扩增，每个单分子得到了1000倍扩增，成为单克隆“DNA簇群”

Completion of amplification

On completion, several million dense clusters of double stranded DNA are generated in each channel of the flow cell.

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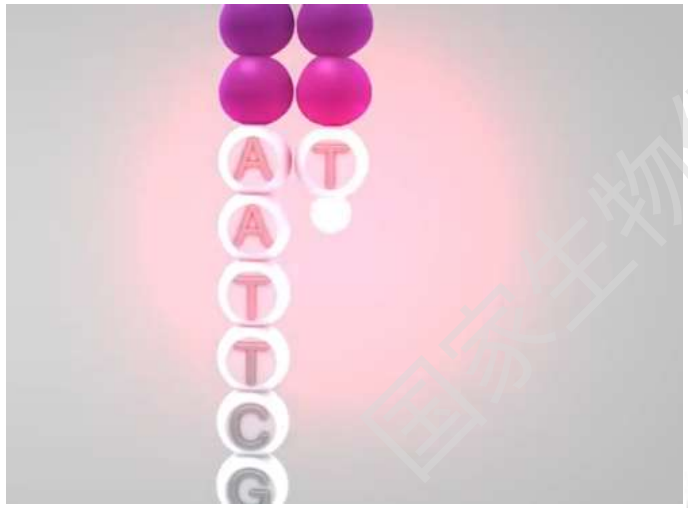
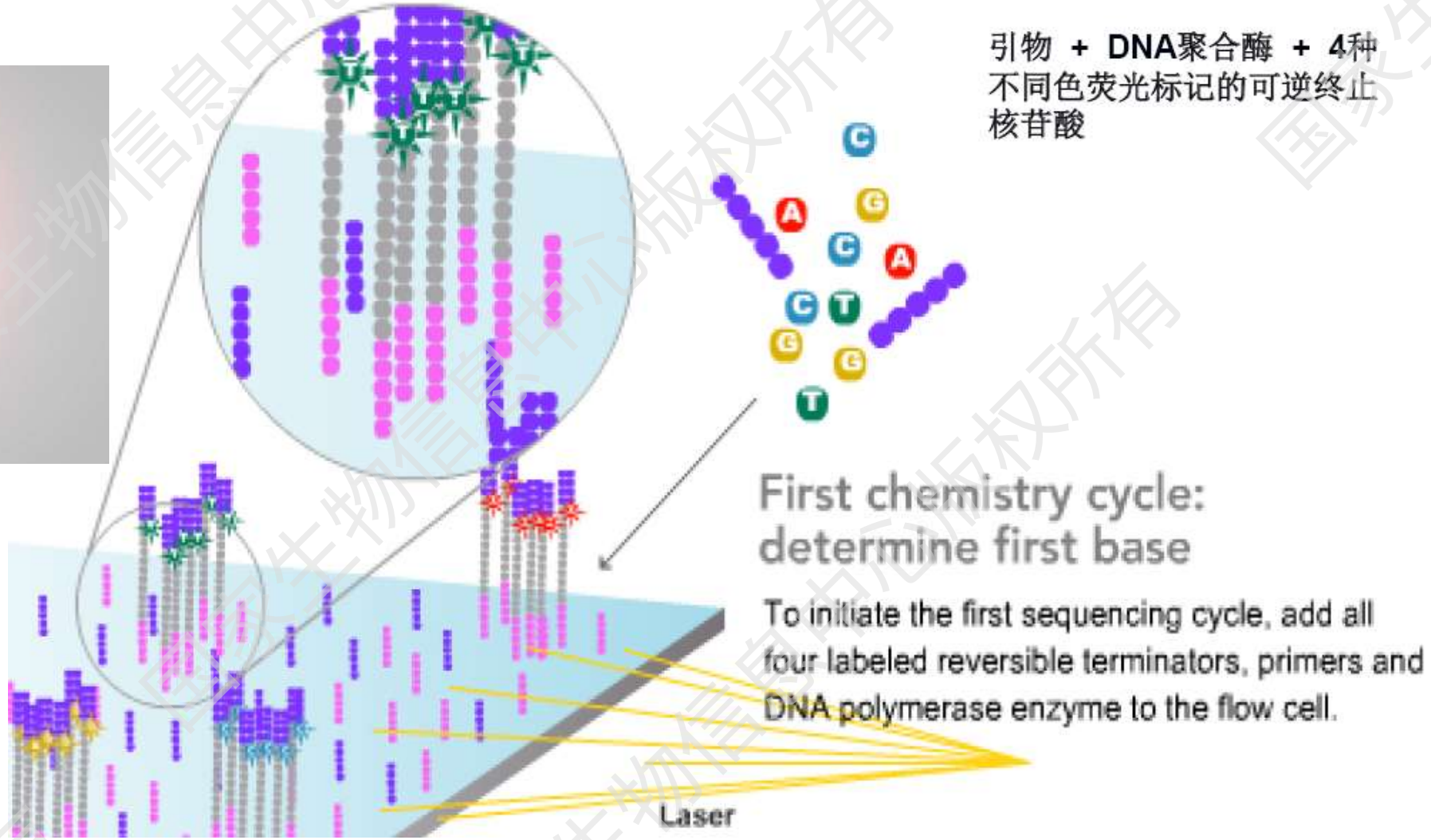
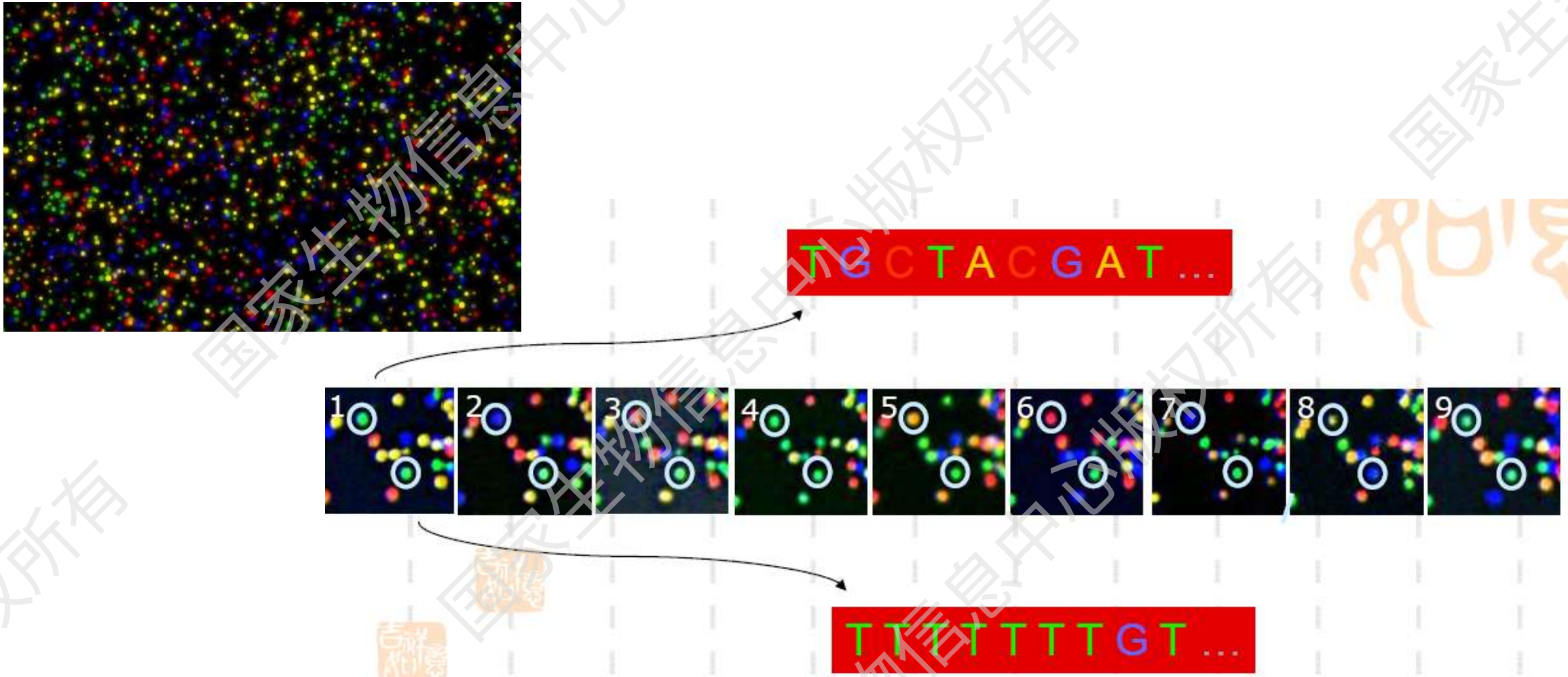
(II) Simultaneous Synthesis





(III) Imaging and Repeat Cycles



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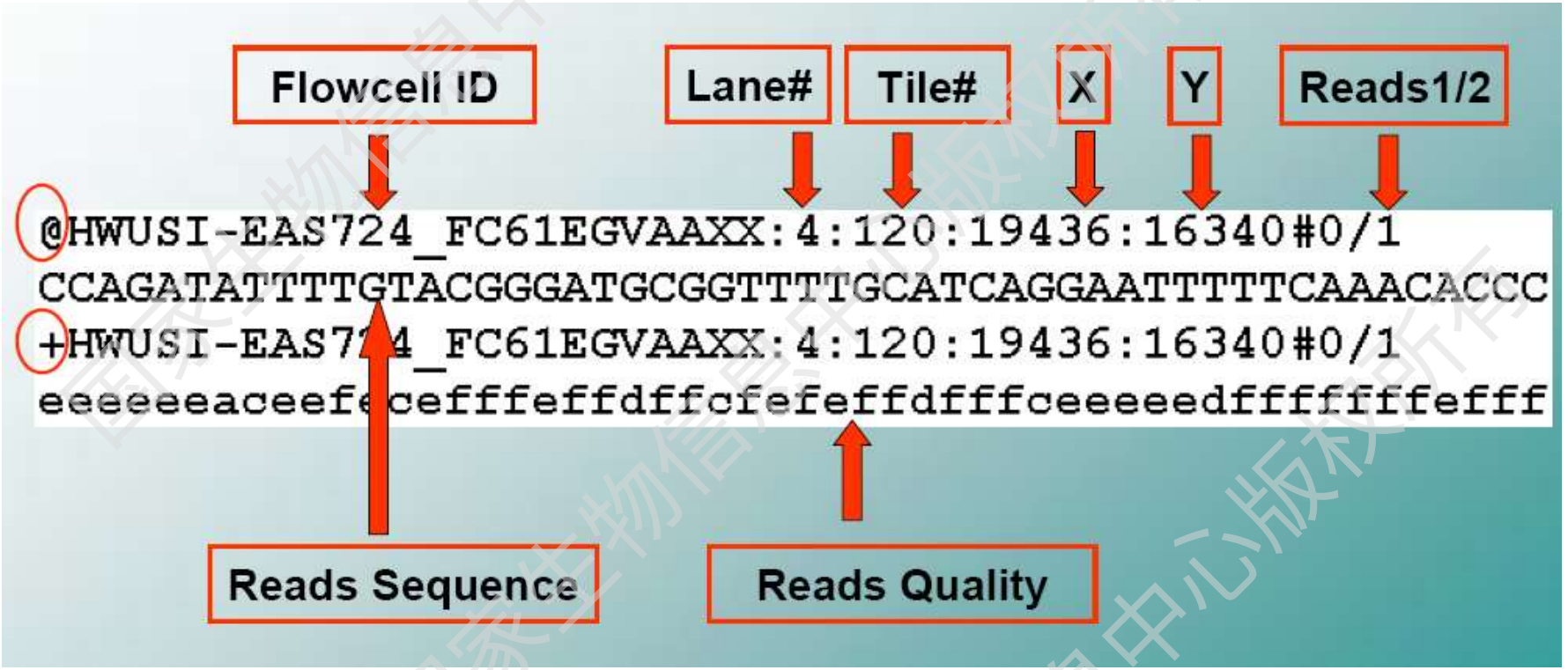
(IV) Raw data and fastq format

PF(pass filtering) :每个read的前25个碱基中，“质量不好的”碱基只允许有1个；如果超过1个，整条read即被抛弃。



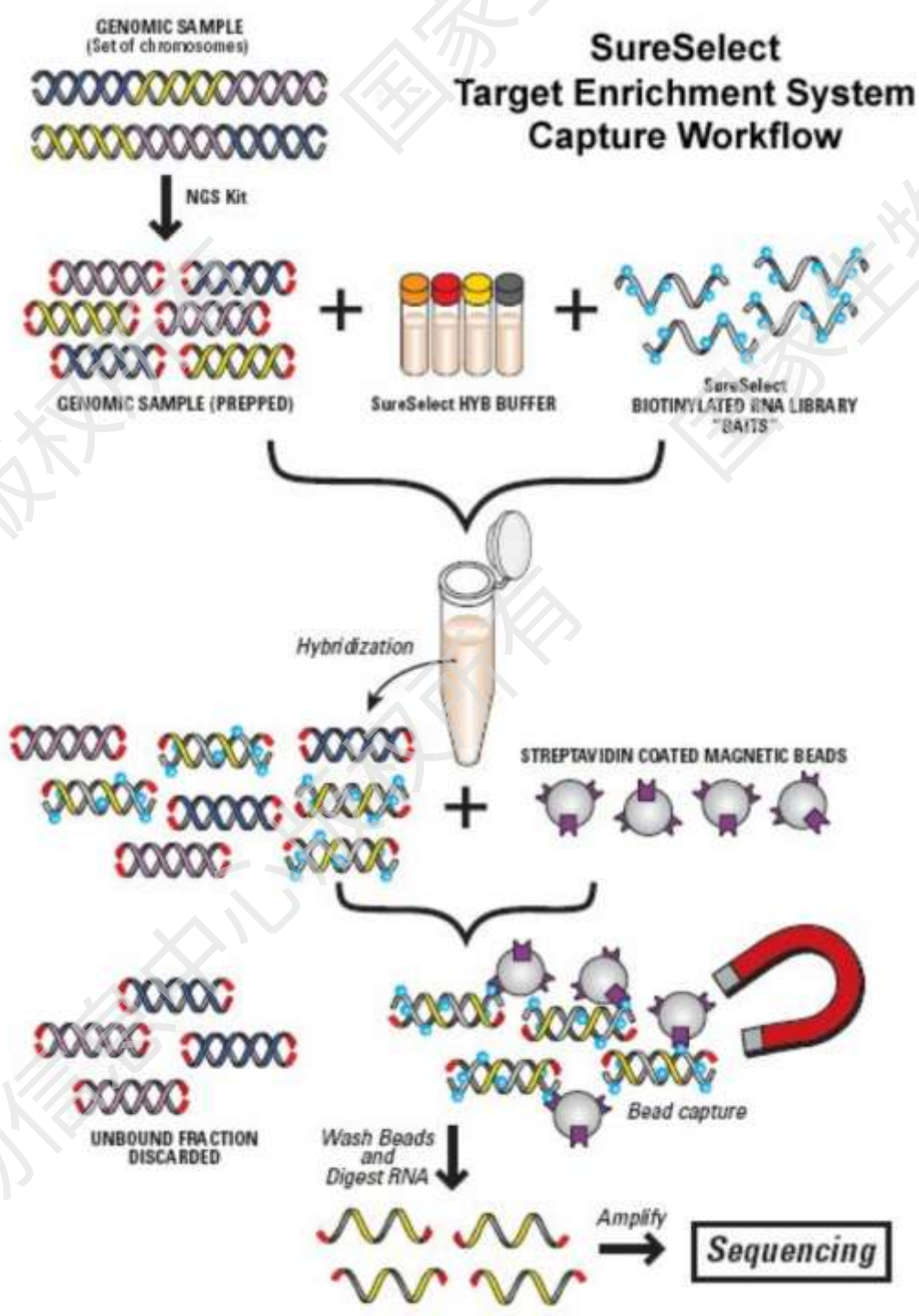
一个荧光信号被判读为某一个碱基，存在概率P发生错误。每一个碱基用QV值进行质量评估，定义为：**QV = -10 x lg P或-10 x lg P/(1-P)。**

如果碱基识别的准确率是99.9%，则错误率是0.1%，QV是30。Qv=ASII(char)-33。

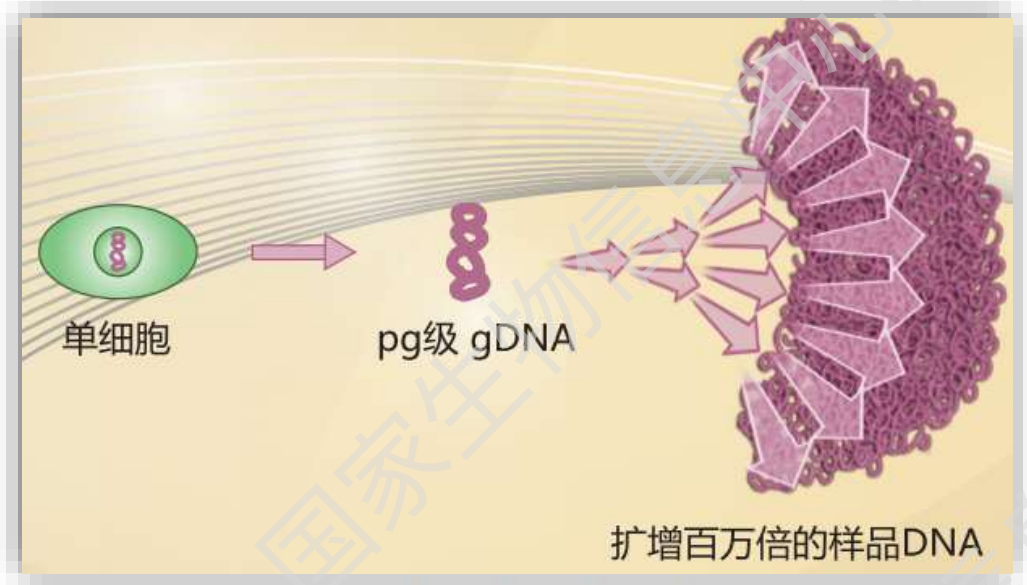
建库策略-> 不同的组学图谱

Sequencing or Re-sequencing:

- Whole genome data
- Probe capture测序
- RAD 酶切测序



Single cell technology



- 均一性　准确进行拷贝数筛查
- 覆盖度
- 脱扣率
- 扩增成功率

Table 1. single cell methods summary

method	example	advantage	disadvantage
Single Cell DNA Amplification	MDA MALBAC DR-Seq G&T-seq	direct; comprehensive solution	low coverage; high PCR error; validation limit
Single Cell Cell Culture	organoid technology	high coverage; low PCR error; validation	cell type limit

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MDA(multiple displacement amplifcation)

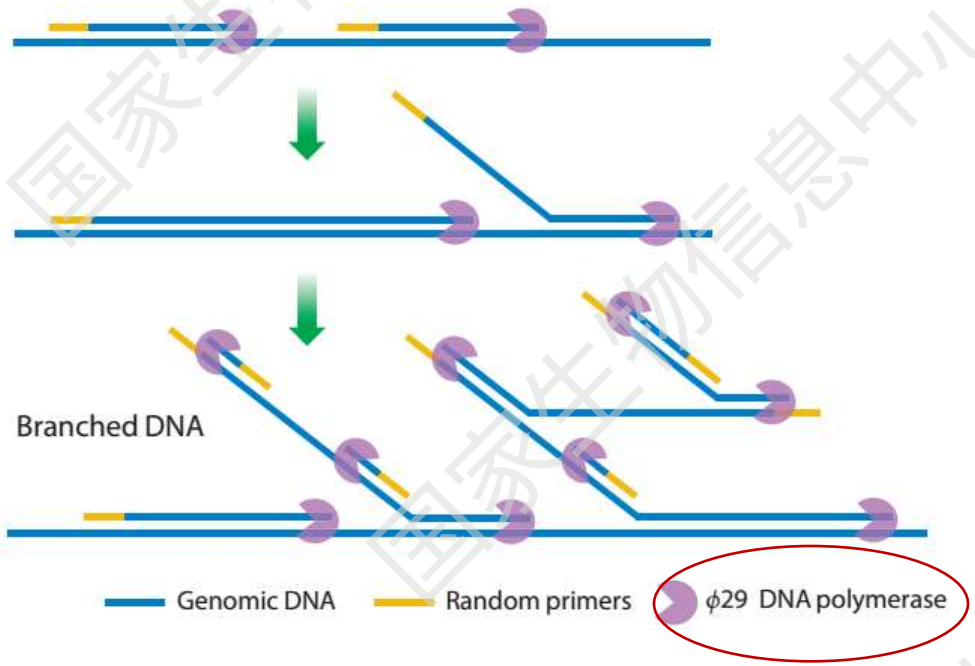
Comprehensive human genome amplification using multiple displacement amplification

2002 PNAS

Frank B. Dean*, Seiyu Hosono*, Linhua Fang, Xiaohong Wu, A. Fawad Faruqi, Patricia Bray-Ward, Zhenyu Sun, Qiuling Zong, Yuefen Du, Jing Du, Mark Driscoll, Wanmin Song, Stephen F. Kingsmore, Michael Egholm, and Roger S. Lasken†

多重置换扩增技术

恒温扩增



优点：极大改善了之前基于PCR的扩增偏好导致的基因组覆盖度低、不均一的情况。

缺点：基因组覆盖度还是比较低，存在一定的非特异性扩增问题。

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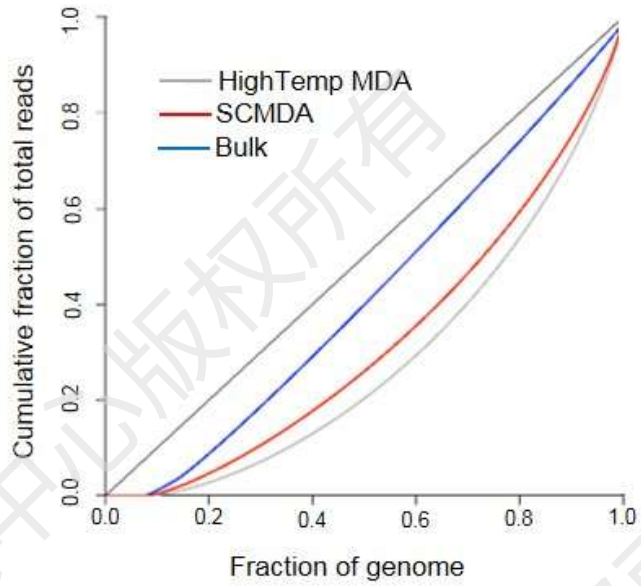
SCMDA (single-cell multiple displacement amplifcation)

2017 Nature Methods

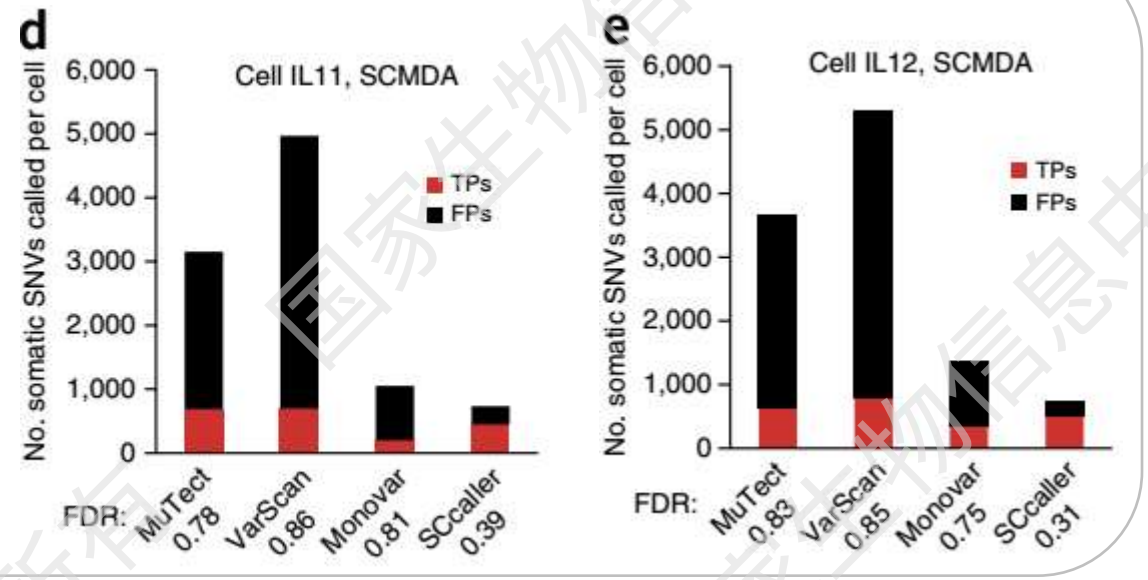
Accurate identification of single-nucleotide variants in whole-genome-amplified single cells

Xiao Dong[1,4], Lei Zhang[1,4], Brandon Milholland[1,4], Moonsook Lee[1], Alexander Y Maslov[1], Tao Wang[2] & Jan Vijg[1,3]



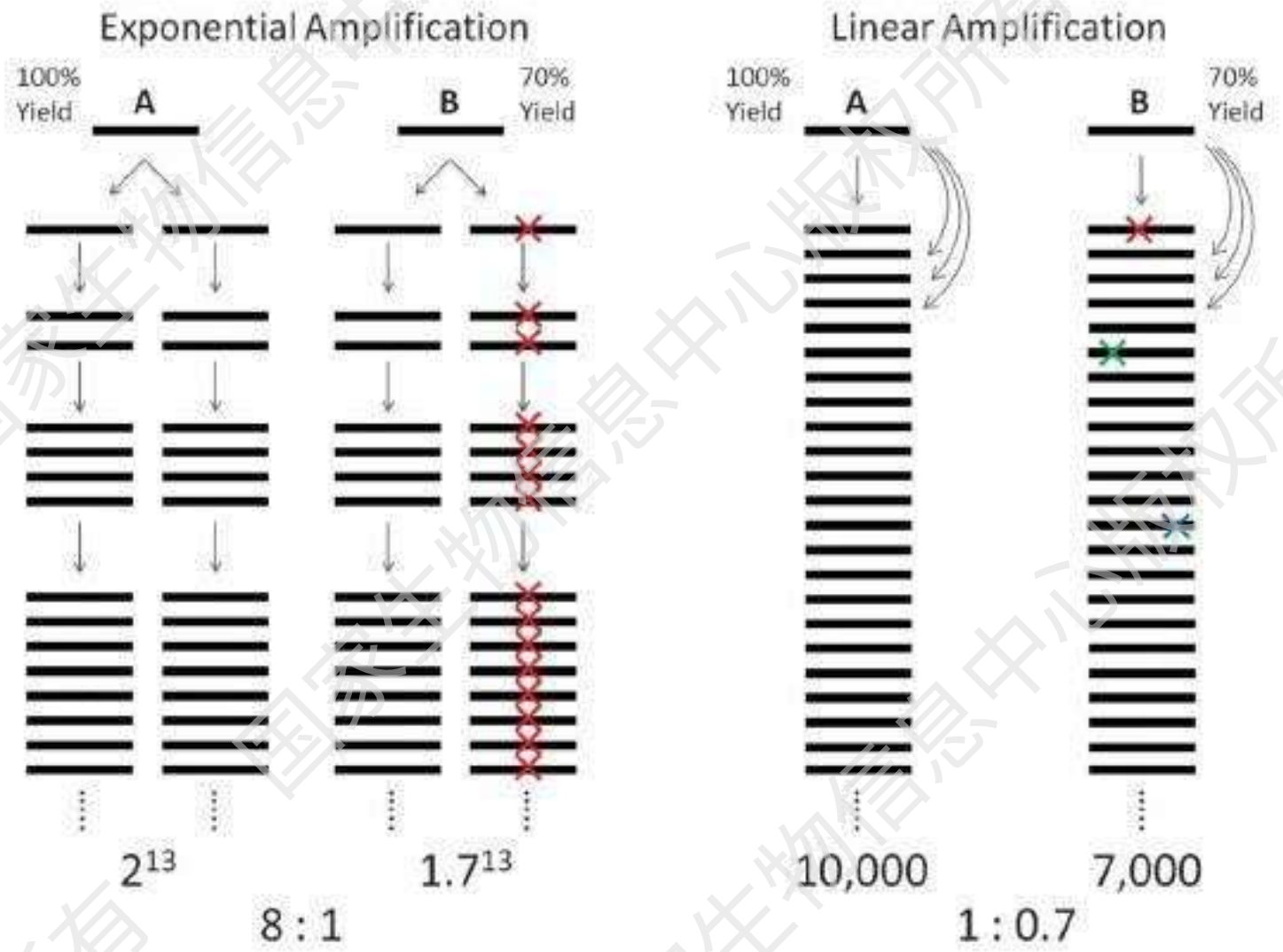
改良版的多重置换扩增技术
SNV calling方法

SCcaller



进一步提高了基因组覆盖度(depth大于等于5的reads数占85%)；显著降低了false positive rate。

指数扩增 VS. 线性扩增



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MALBAC(multiple annealing and looping-based amplification cycles)

Genome-Wide Detection of Single-Nucleotide and Copy-Number Variations of a Single Human Cell

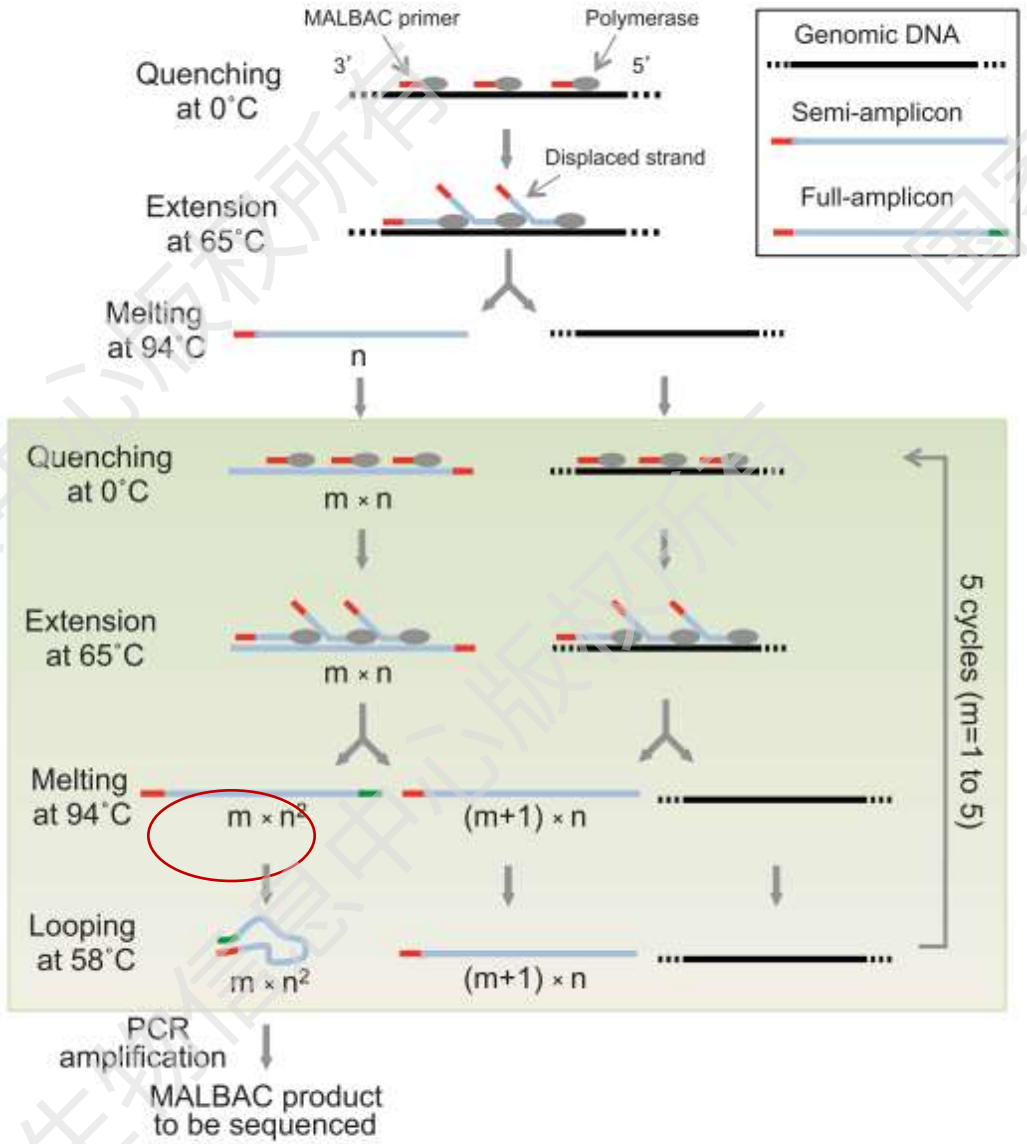
Chenghang Zong,[1]* Sijia Lu,[1]*† Alec R. Chapman,[1,2]* X. Sunney Xie[1]‡

2013 Science

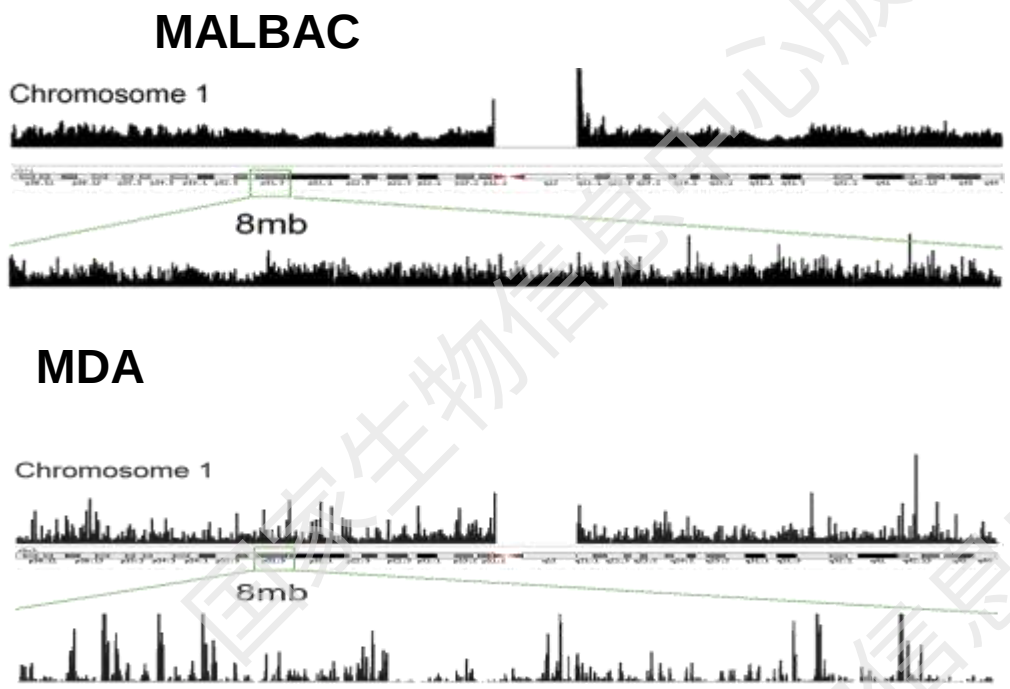
多次退火环状循环扩增技术

优点：进一步提高了基因组覆盖度(93%)，并减少了扩增错误。

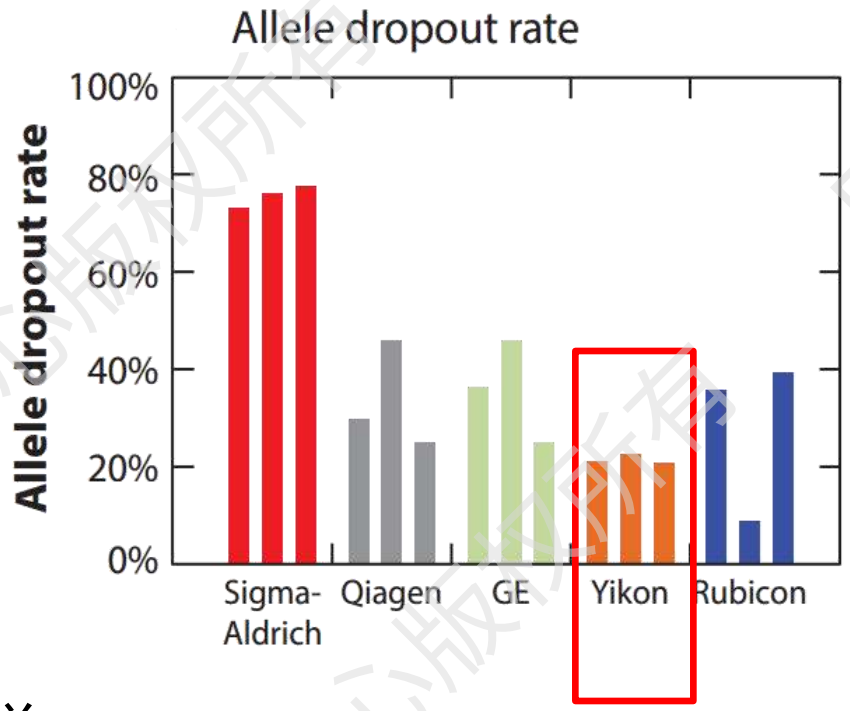
缺点：引入非特异性的引物，还是存在一定的非特异性扩增问题



扩增覆盖度及均一性比较



ADO比较



MALBAC:~ 93% 的基因组能够被扩增，扩增均一性好，可进行CNV分析

MDA: 50-70%的基因组能够被扩增,扩增均一性差，不适合进行高分辨率CNV分析

Lei Huang, et al. Annu Rev Genomics Hum Genet. 2015

Chenghang Zong, Science, 2012

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LIANTI (linear amplification via transposon insertion)

2017 Science

RESEARCH

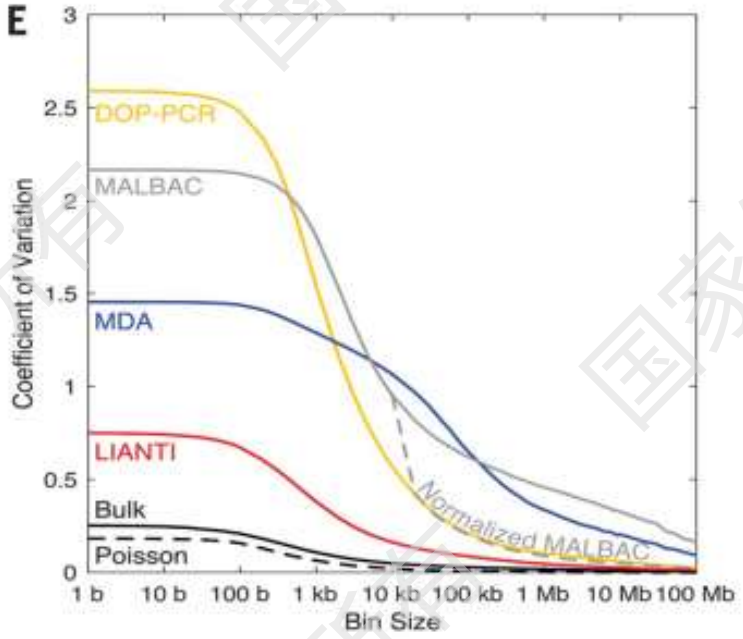
REPORT

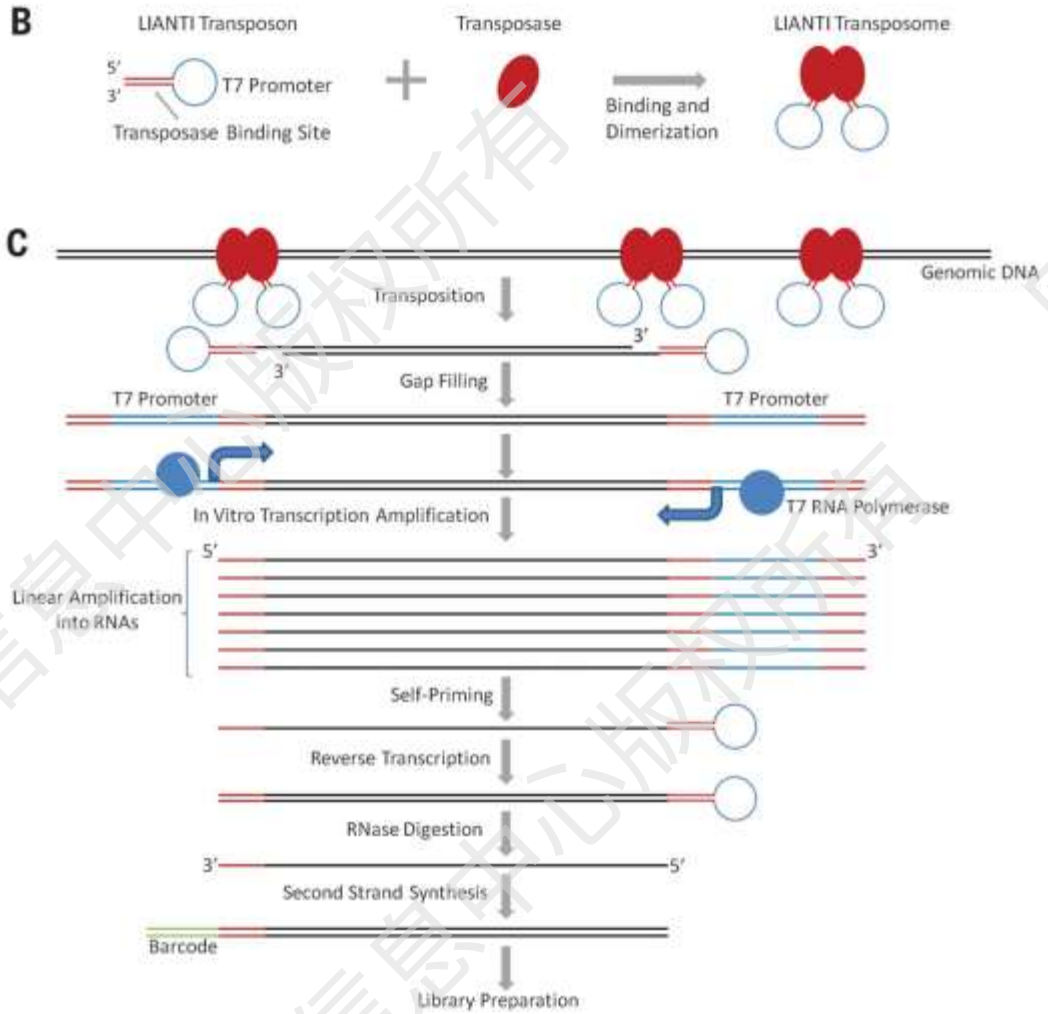
SINGLE-CELL GENOMICS

Single-cell whole-genome analyses by Linear Amplification via Transposon Insertion (LIANTI)

Chongyi Chen,[1*] Dong Xing,[1*] Longzhi Tan,[1*] Heng Li,[2*] Guangyu Zhou,[1] Lei Huang,[1,3,4] X. Sunney Xie[1,3,4†]

通过转座子插入实现线性扩增



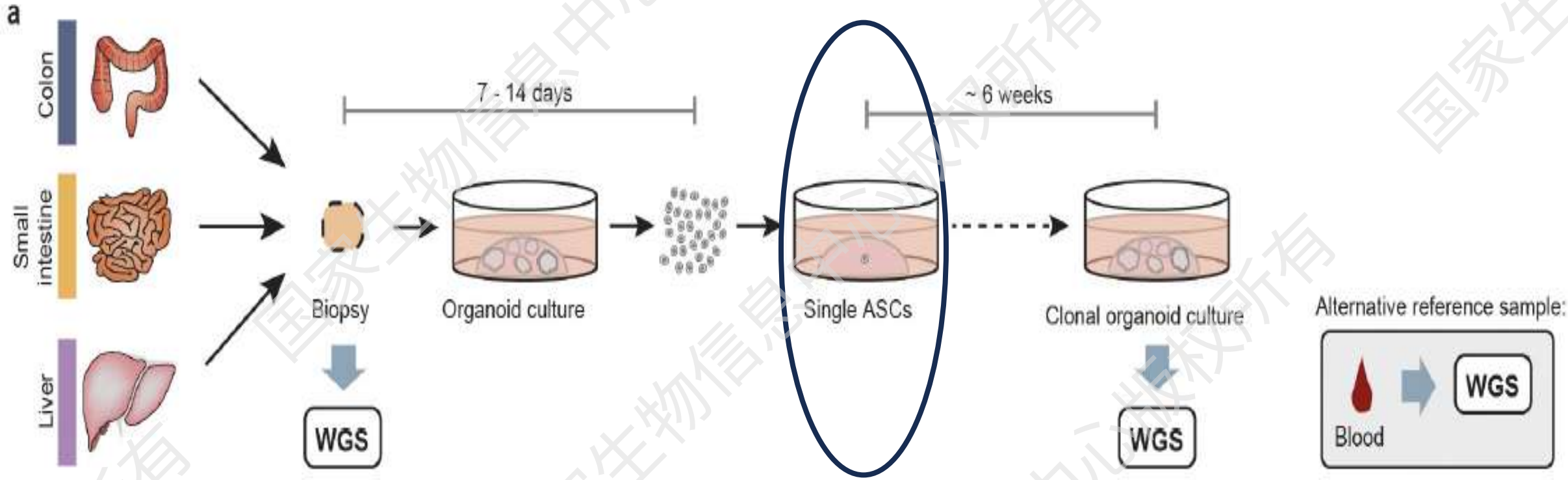


进一步提高了基因组覆盖度(97%)，提高了均一性。

LIANTI 文章中对目前几种single-cell DNA seq方法的比较

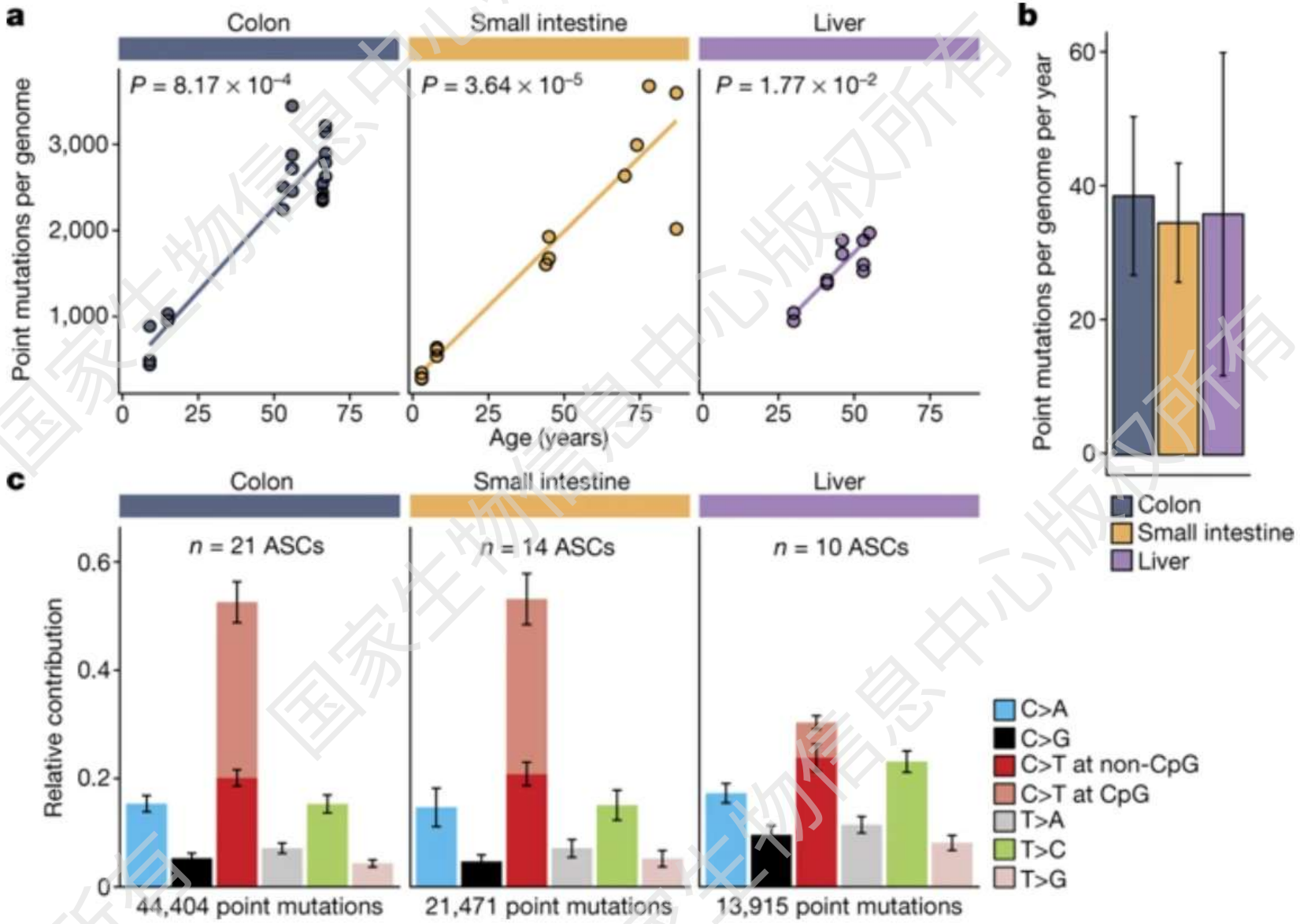
Method	Sample	Raw Data (Gb)	Coverage (%)	Allele Dropout Rate (ADO)	False Negative Rate (FNR)
Bulk	Bulk1*	119	*Bulk1 is the standard for comparison		
	Bulk2	138	99.9	0	0.003
LIANTI	BJ1	86	97.0	0.175	0.459
	BJ2	99	96.6	0.186	0.463
	BJ3	65	91.4	0.299	0.620
MDA (Qiagen)	Q1	82	88.1	0.329	0.609
	Q5	84	80.0	0.438	0.673
	Q9	90	91.8	0.249	0.540
MALBAC (Yikon)	YK1	91	72.0	0.477	0.708
	YK2	96	72.6	0.443	0.673
	YK5	96	73.4	0.436	0.659
DOP-PCR (Sigma)	S3	83	47.9	0.765	0.871
	S4	82	45.0	0.801	0.910
	S5	86	41.1	0.816	0.901
MDA (GE)	GE2	96	90.6	0.304	0.615
	GE4	129	92.0	0.281	0.588
	GE10	90	76.5	0.444	0.706
MALBAC-like (Rubicon)	R3	82	56.9	0.646	0.821
	R7	82	68.4	0.494	0.758
	R9	86	54.1	0.684	0.843

Organoid culture strategy and mutation load in stem cells

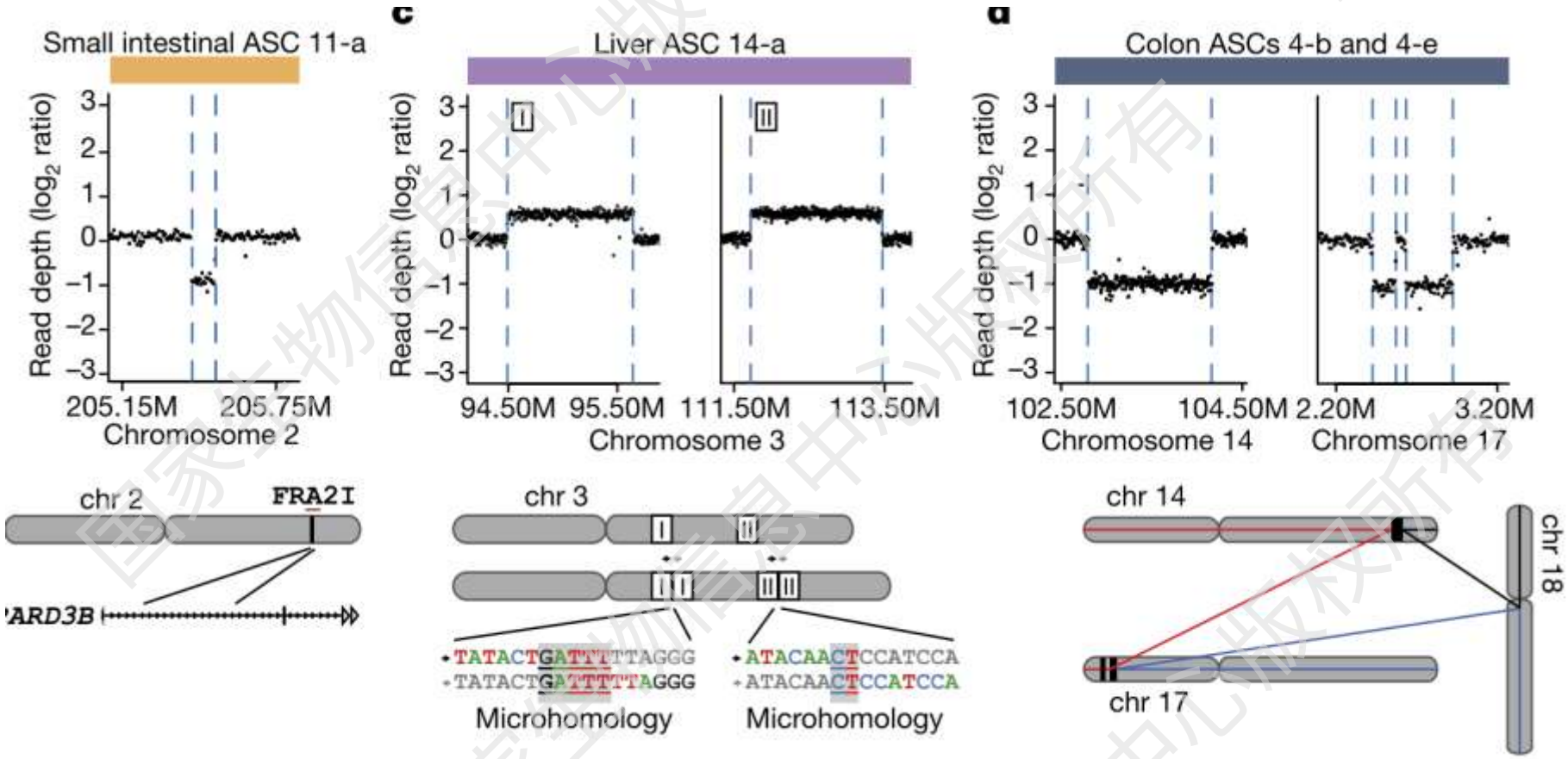


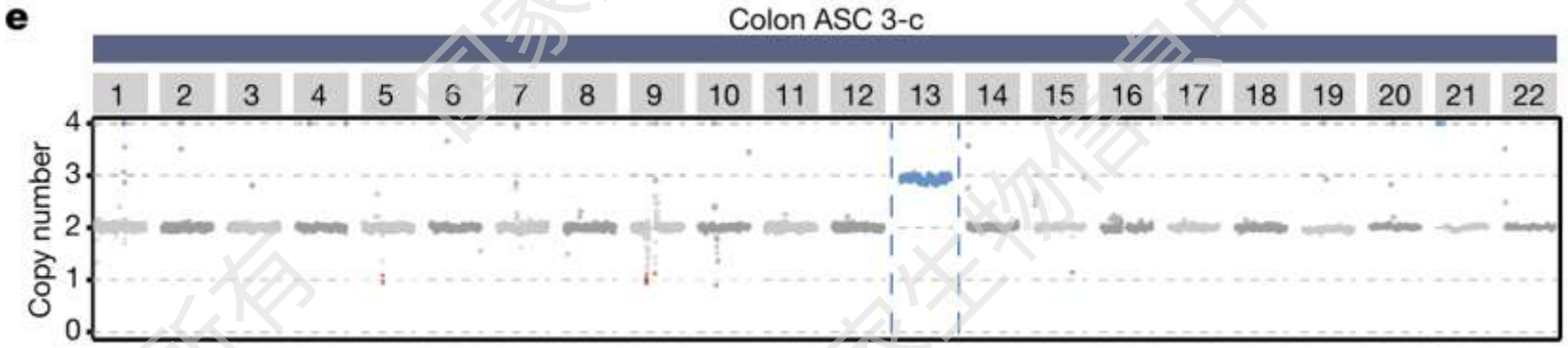
Boxtel,Nature,2016

Age-associated accumulation of somatic point mutations in human ASCs

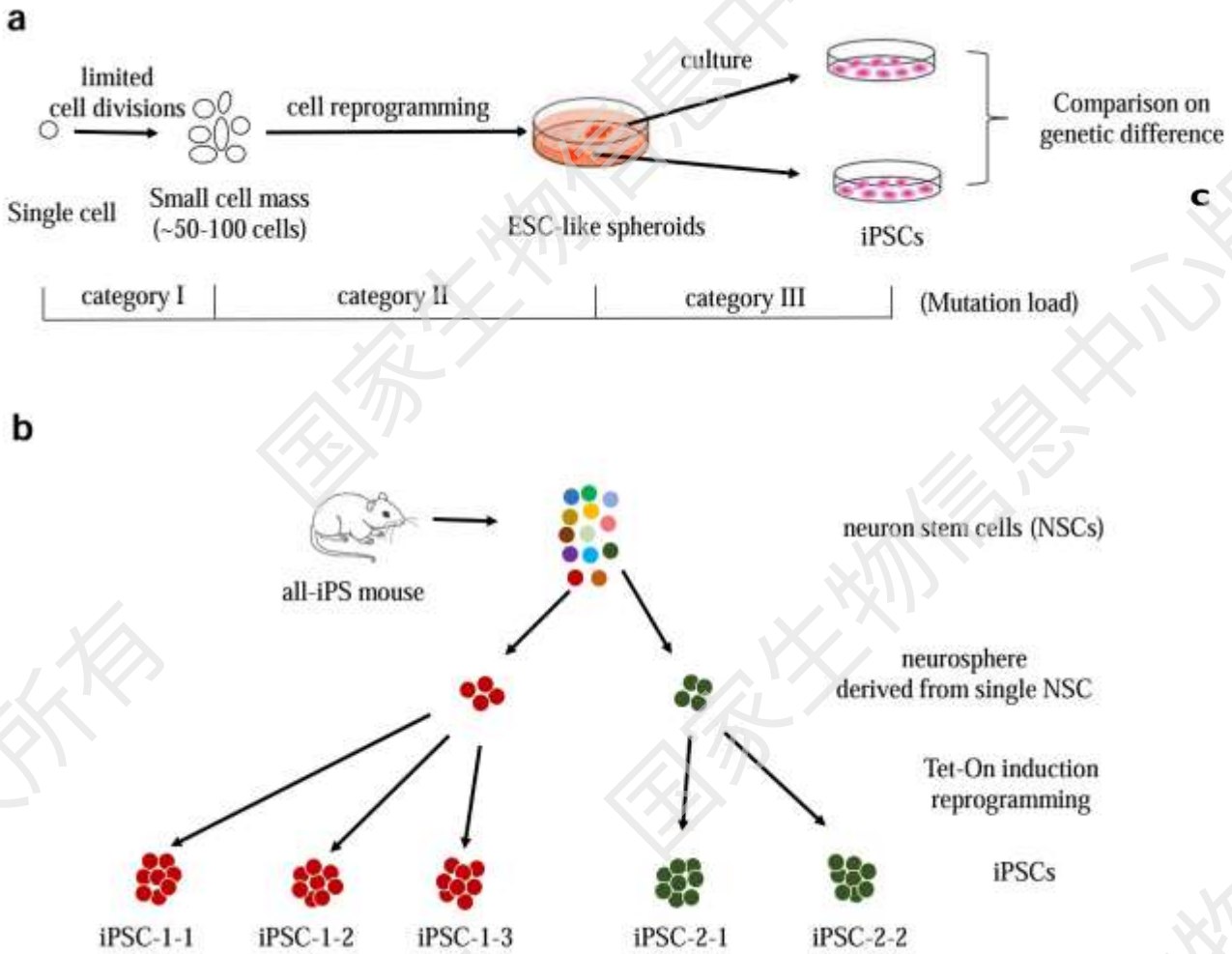


structural variation in normal ASCs



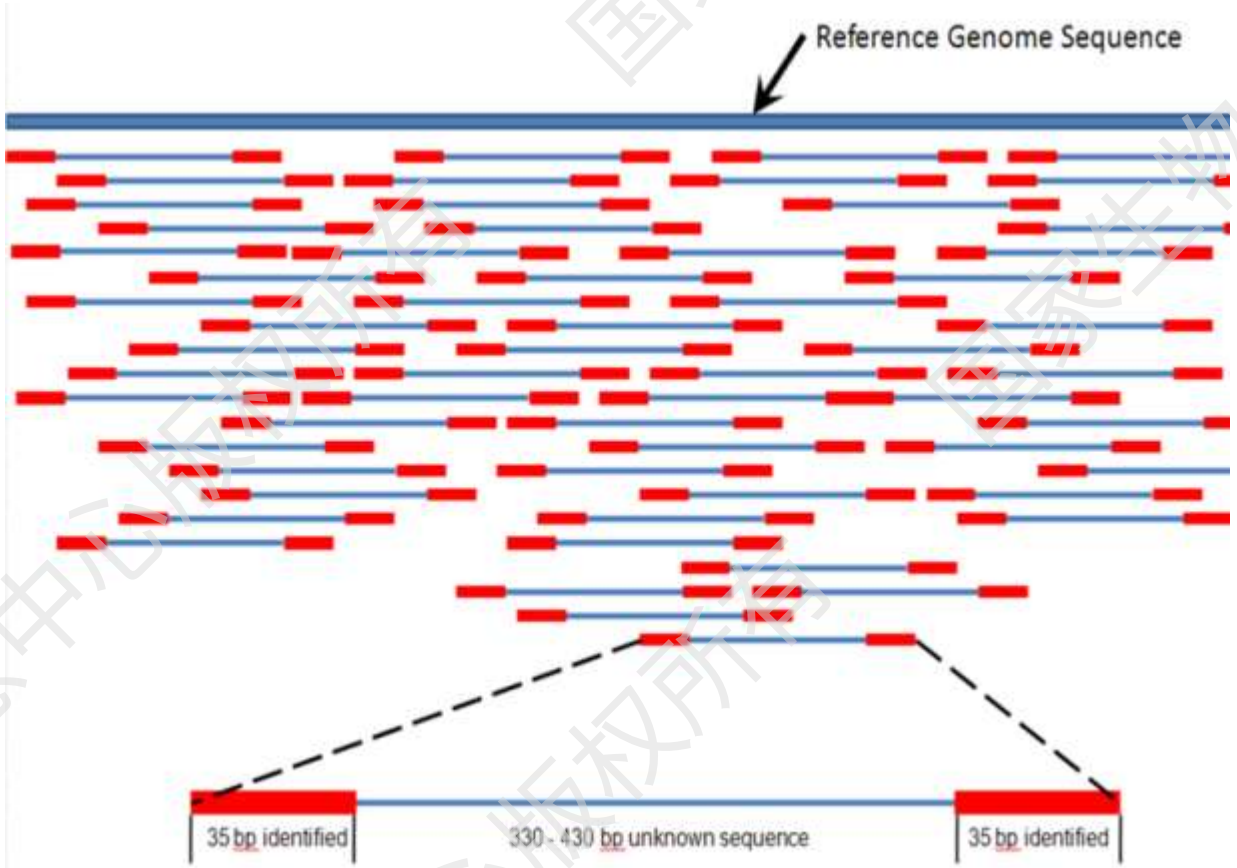


A new strategy for cultural cloning



•Re-sequencing or de novo sequencing

– Reads mapping （BWA, BOWTIE，SAMtools）
– Reads assembly

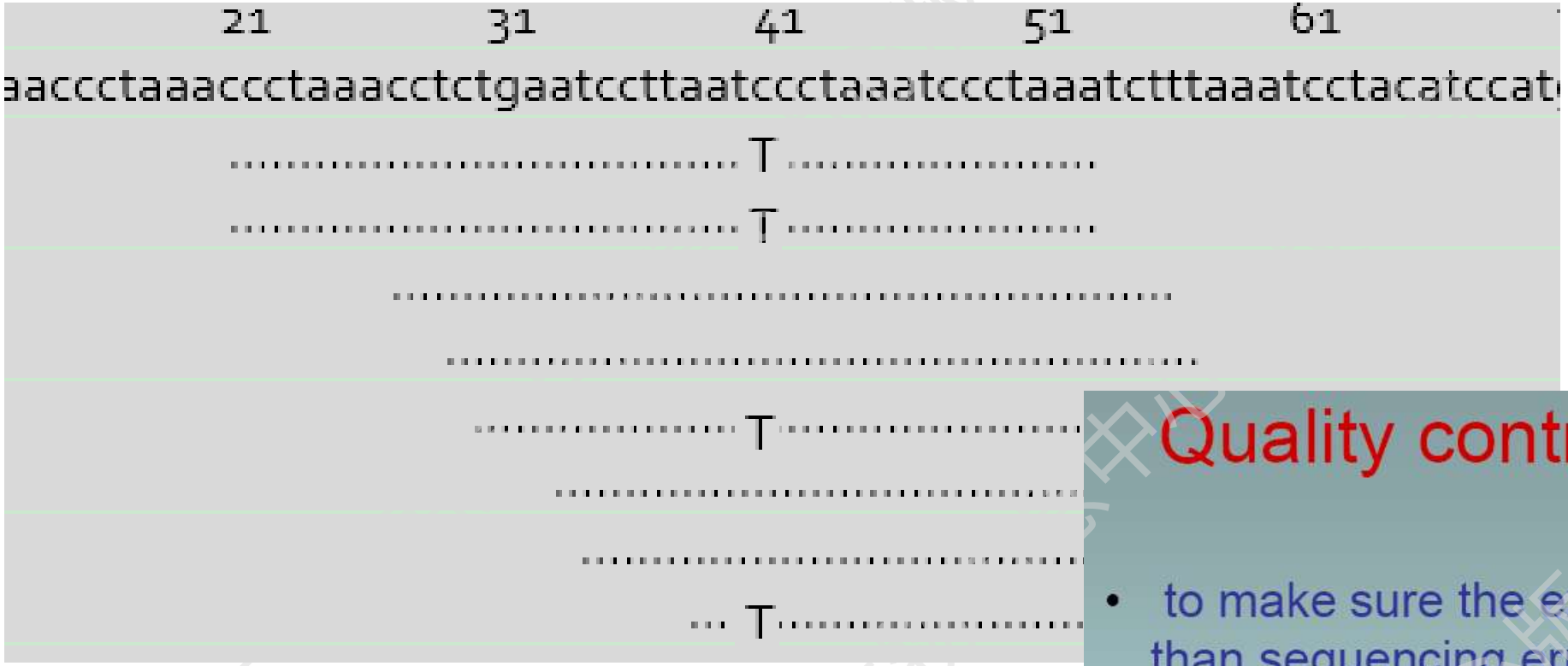


• Index reference sequences
bwa index-a is/bwtsw ref.fa
– is: <2Gb
– bwtsw:>2Gb

•Mapping
bwa aln ref.fa short_read.fq> aln_sa.sai

•Output alignments in the SAM format
bwa samse ref.fa aln_sa.sai short_read.fq> aln.sam
bwa sampe ref.fa aln_sa1.sai aln_sa2.sai read1.fq read2.fq > aln.sam

单核苷酸变异的基因组测序检测



单细胞基因组单核苷酸变异

大量假阳性 、假阴性

De novo or not

Quality control of sequence

- to make sure the existence of SNPs rather than sequencing error
- How?

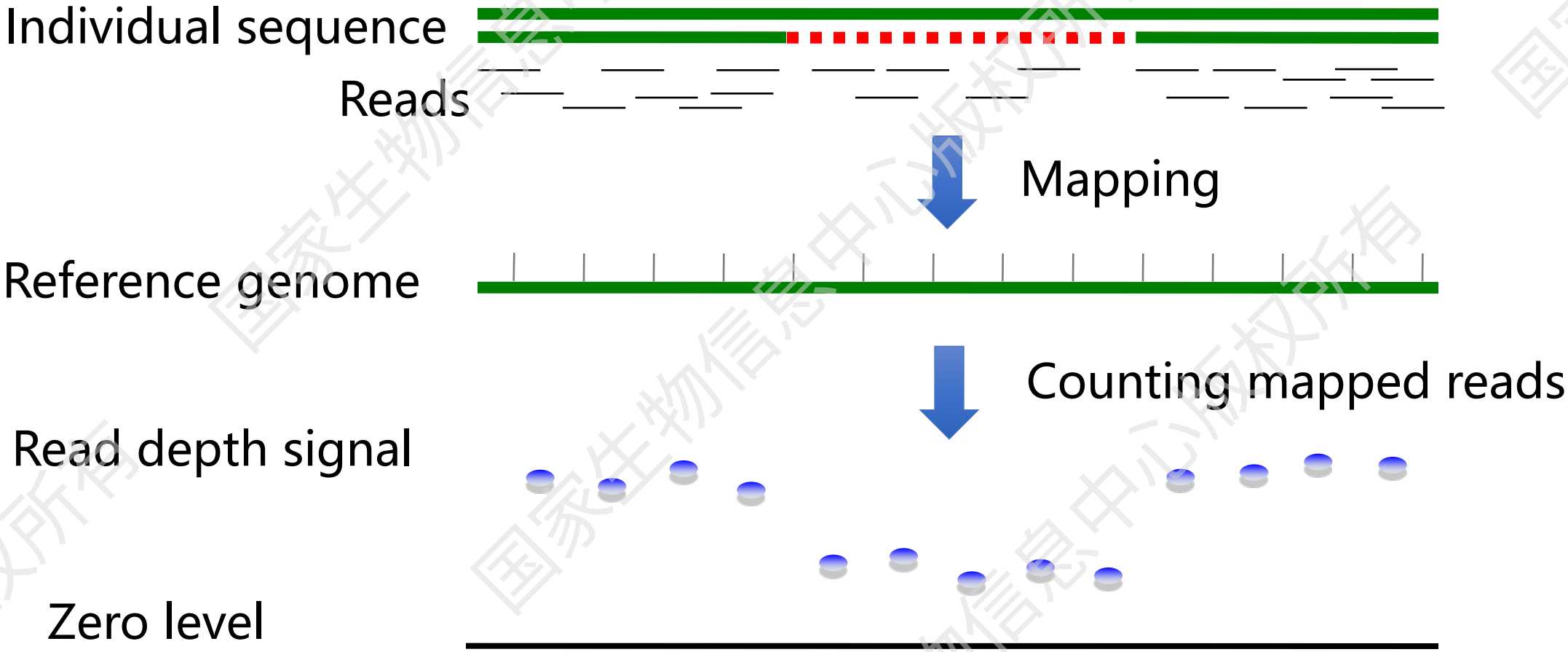
  the quality of SNP position

  the quality of neighboring bases

  the alignment condition of flanking sequence

  the number of SNPs in one read

拷贝数变异的基因组测序检测



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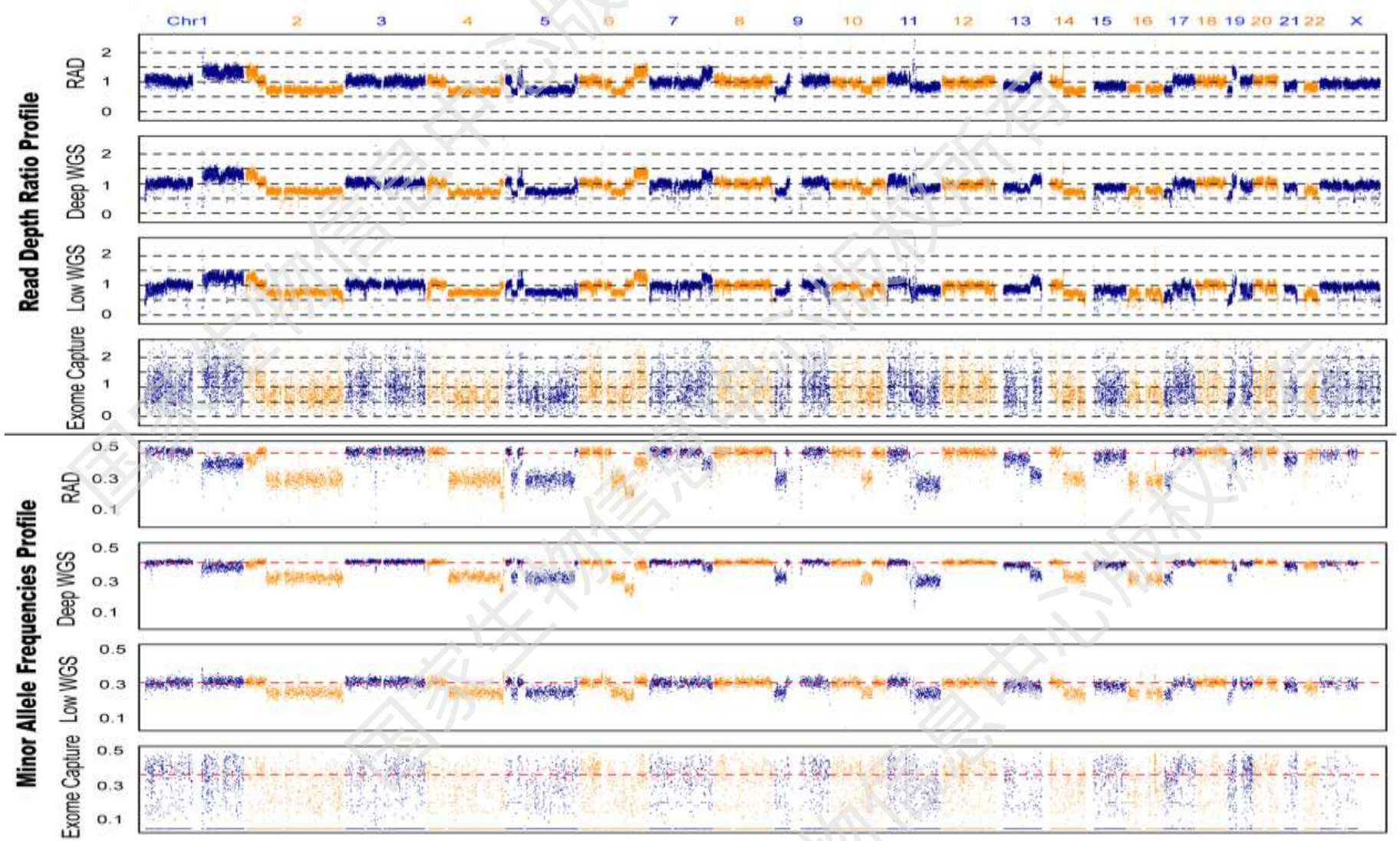
CNVs



Zheng, Bioinformatics,2013

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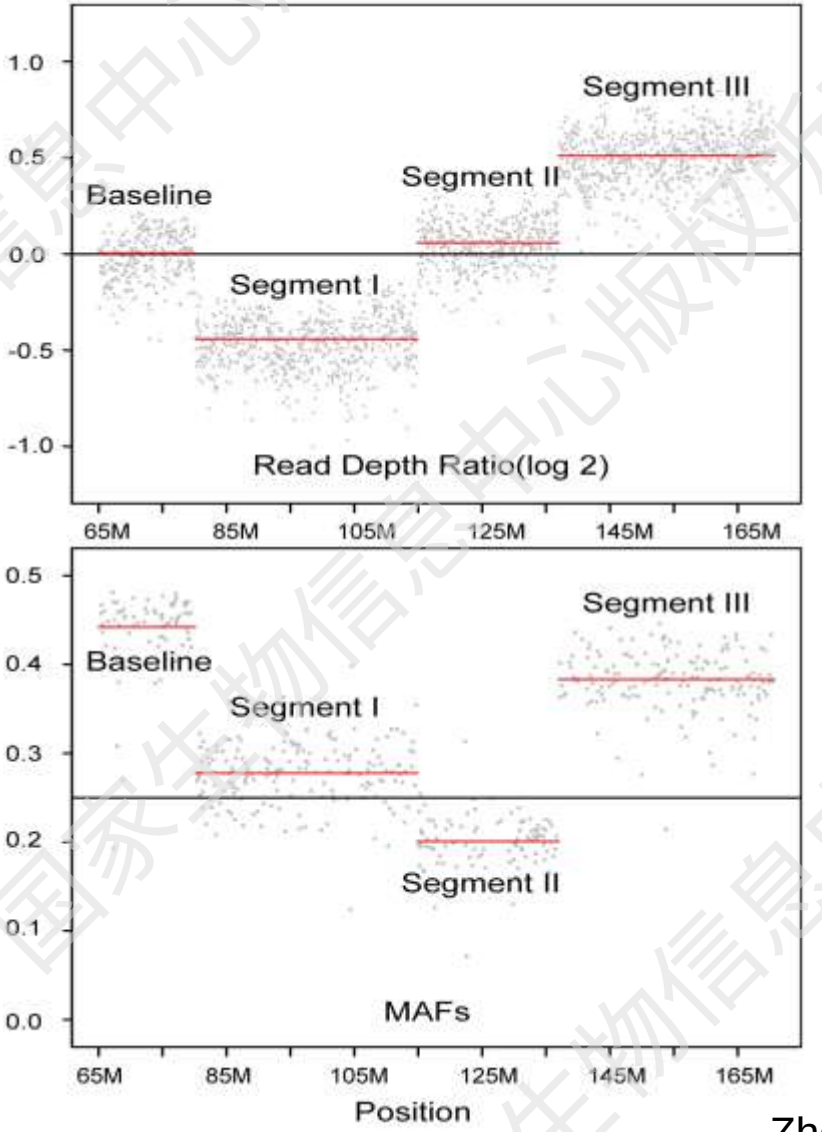
Inferring CNVs by combining read depth and allele frequency



Zheng, Bioinformatics,2013

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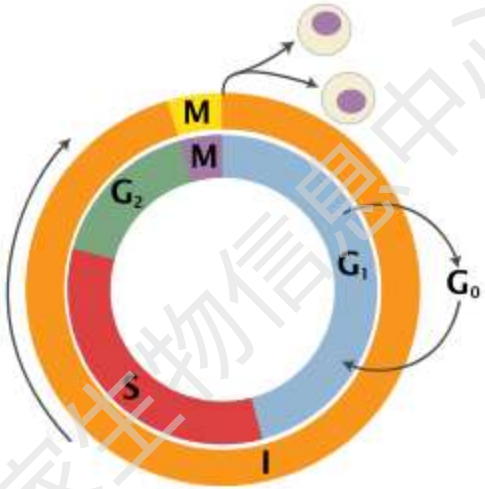
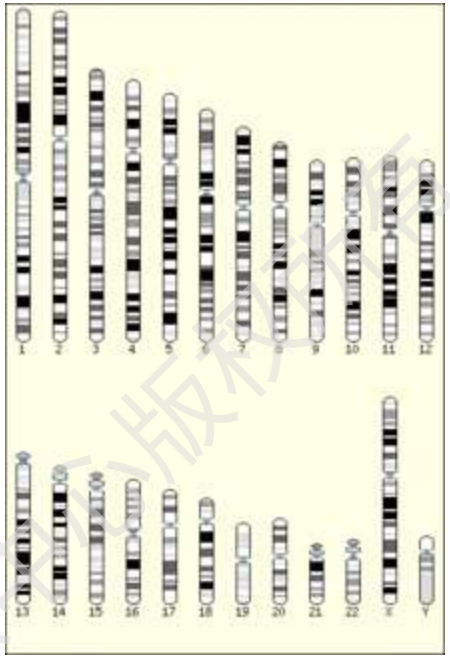
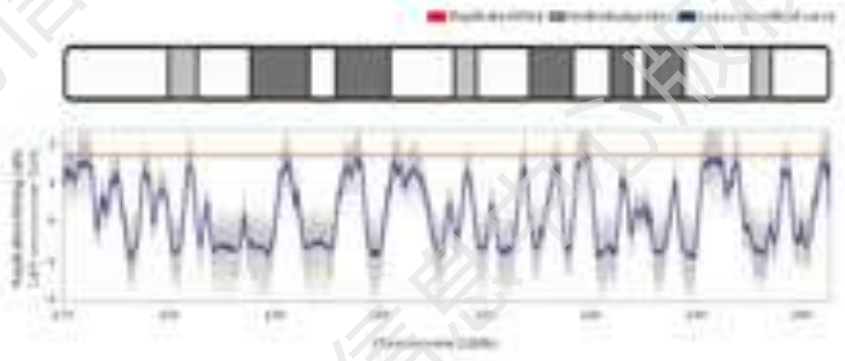
一种假阳性CNV的产生





The temporal order of replication of all the genomic segments, is called replication-timing.



From www.replicationdomain.org

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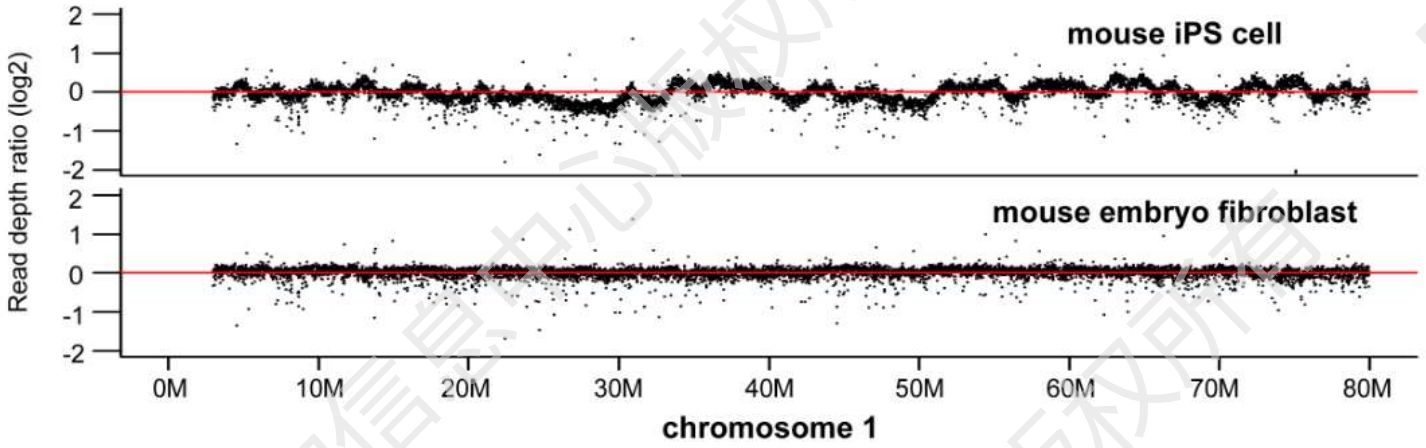
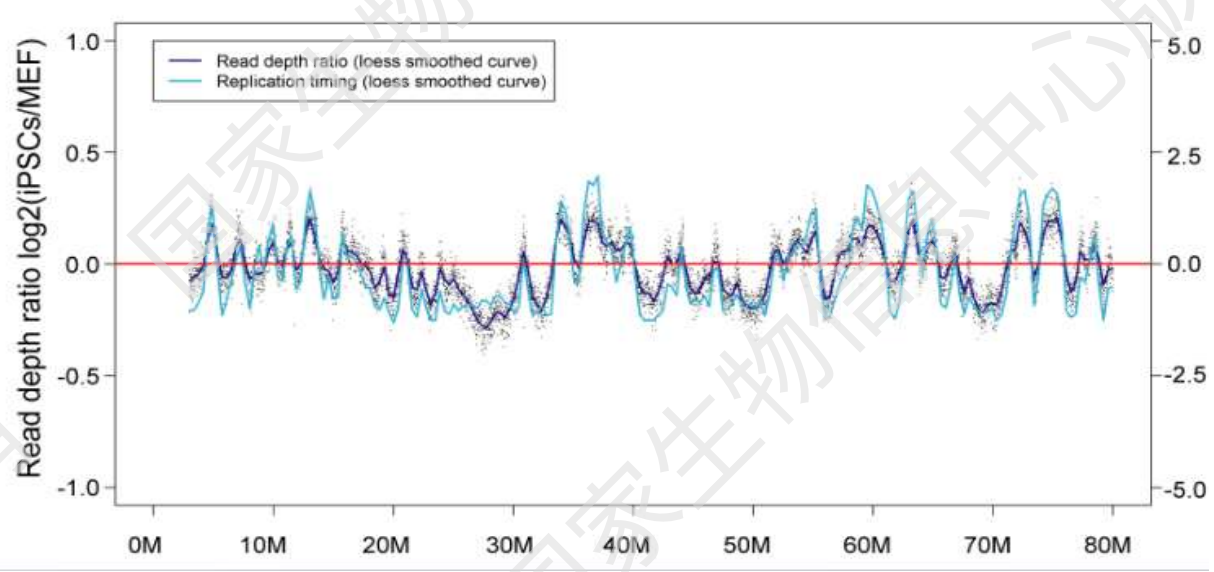
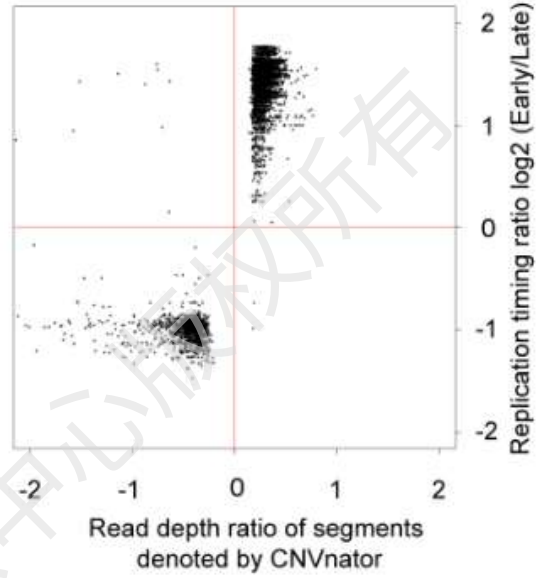




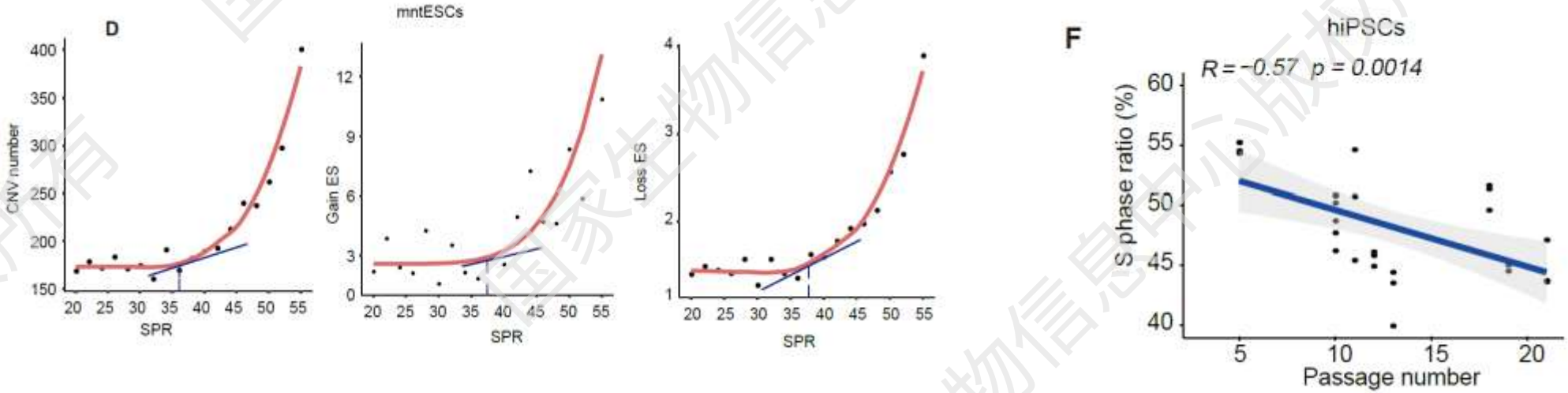


The read counts profile is highly consistent with the replication timing in mouse IPSCs.

Advanced Science,2025

Early S phase is extended, in particular mouse iPSCs.

Cell ID	Species	Cell Type	Percentage (%)				
			G0/G1 Phase	Early S Phase	Late S Phase	G2/M Phase	Total
APC	Mouse	Differentiated cell	46.20	5.86	9.06	35.50	96.62
MEF	Mouse	Differentiated cell	59.20	7.42	11.40	21.20	99.22
S-2-1	Mouse	iPS cell	15.10	39.10	35.40	8.27	97.87
S-2-14	Mouse	iPS cell	17.30	36.70	35.40	9.15	98.55
S-2-19	Mouse	iPS cell	18.40	38.30	33.20	8.97	98.87
6-2-8	Mouse	iPS cell	16.30	47.10	27.80	6.50	97.70
6-2-15	Mouse	iPS cell	17.60	46.60	31.00	3.01	98.21
6-1-16	Mouse	iPS cell	25.10	26.00	27.50	16.80	95.40
GM05659	Human	Differentiated cell	76.50	3.54	0.83	17.10	97.97
GM08399	Human	Differentiated cell	79.50	5.68	2.52	9.87	97.57
LT-4	Human	iPS cell	44.70	18.10	12.30	21.60	96.70
LT-16	Human	iPS cell	38.90	18.20	16.00	24.10	97.20



Extended S phase induced pseudo copy number variations.

Advanced Science,2025

- **单细胞表观基因组图谱绘制及分析技术**

  - **表观基因组**

  - **单细胞表观基因组图谱绘制和分析技术**

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AGE

OXIDATIVE STRESS

GENETIC POLYMORPHISM

NUTRITION

homocysteine/ SAH

DNA methylation

CpG islands

promoter

structural

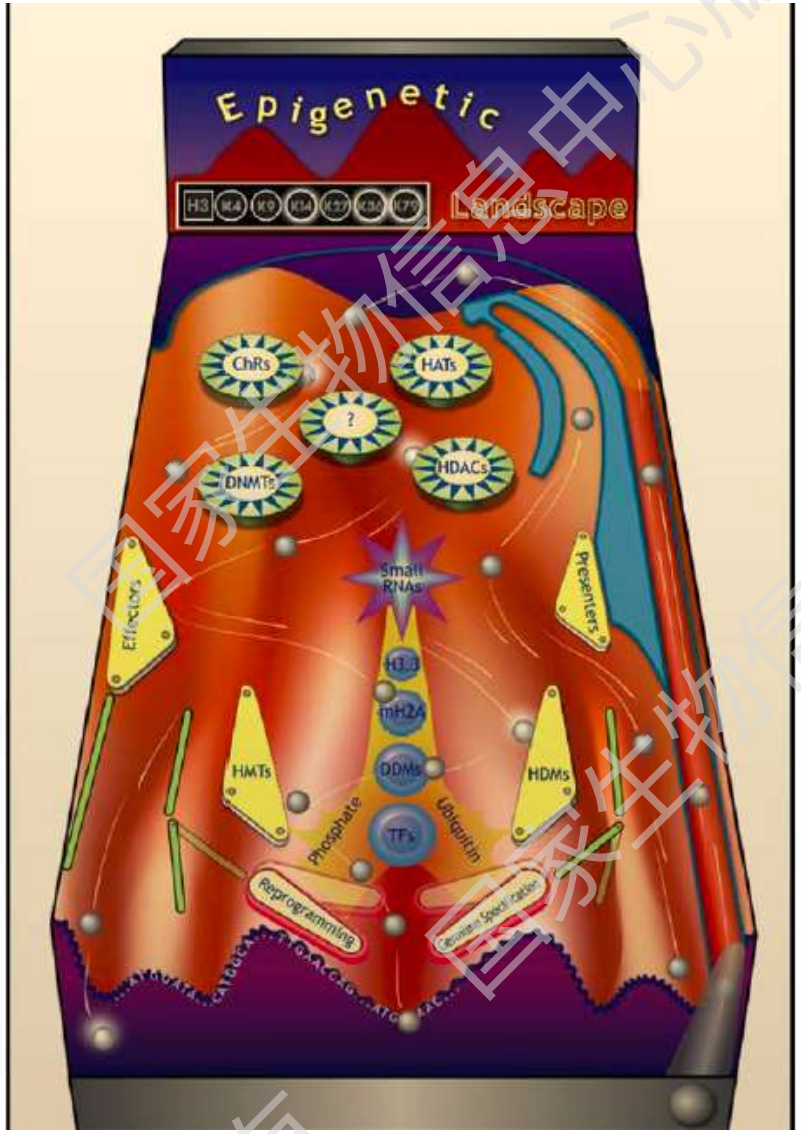
chromatin remodeling and decondensation

DNA damage

DNA stability

transcription

表观遗传学分子机器概览



Known factors that regulate epigenetic phenomena are shown directing the complex movement of pinballs (cells) across the elegant landscape proposed by Waddington. No specific order of molecular events is implied; as such a sequence remains unknown. Effector proteins recognize specific histone modifications, while presenters are proposed to impart substrate specificity for histone-modifying enzymes. The representative histone variants H3.3 and macroH2A are involved in transcriptional activation or repression. For simplicity, other proteins are not shown.

ChR, chromatin remodelers;
DNMTs, DNA methyltransferases;
DDMs, DNA demethylases;
HATs, histone acetyltransferases;
HDACs, histone deacetylases;
HMTs, histone methyltransferases;
HDMs, histone demethylases;
TFs, transcription factors.

Goldberg AD, Allis CD, Bernstein E. *Cell* 128, 635-8 (2007)

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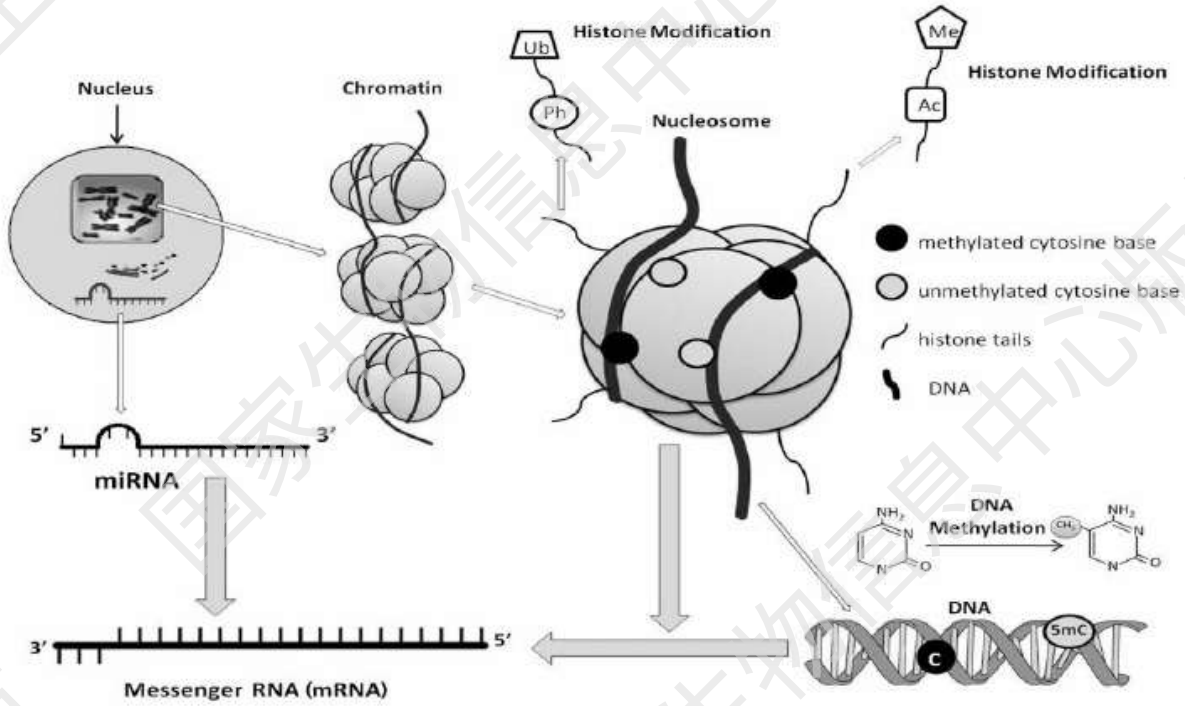
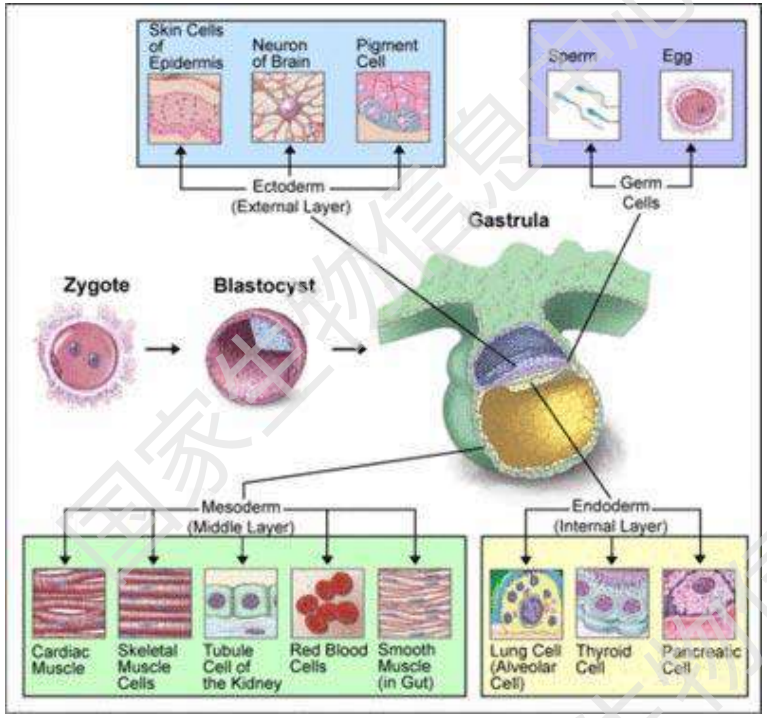
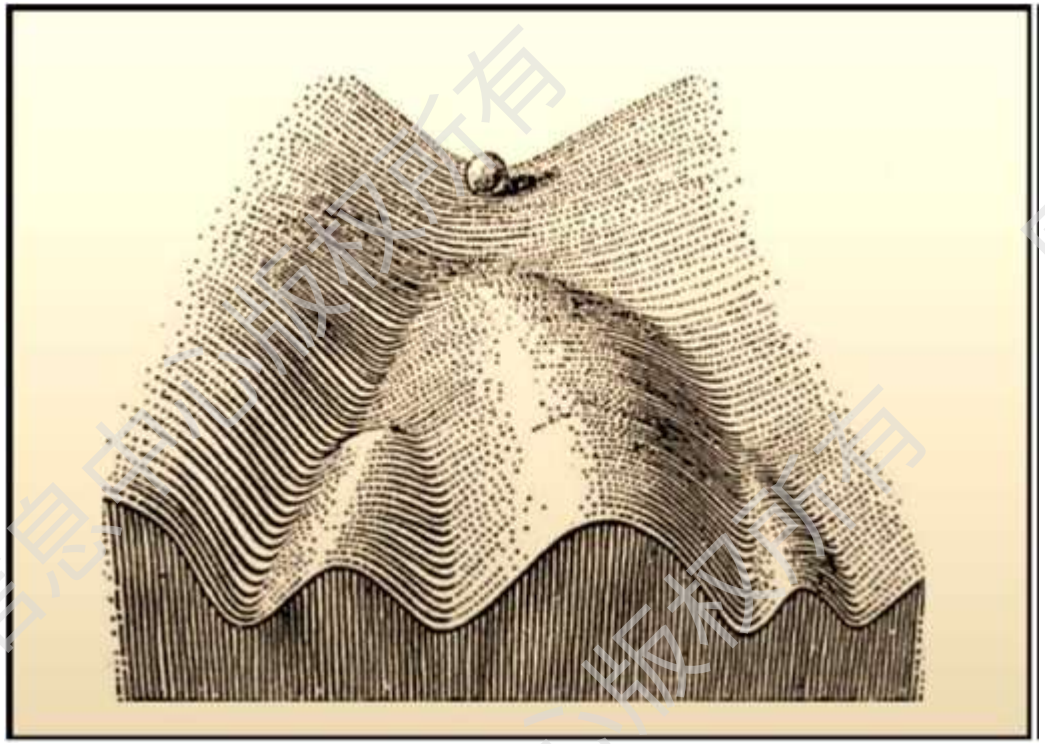
表观基因组

Epigenetics are inheritable and reversible chemical or structural changes to DNA or DNA-bound RNA and proteins that modulate gene expression without changing the DNA sequence.



细胞的发育、分化及其表观遗传学解释





In1957,Conrad Waddington proposed the concept of an epigenetic landscape to represent the process of cellular decision-making during development. At various points in this dynamic visual metaphor, the cell (represented by a ball) can take specific permitted trajectories, leading to different out comes or cell fates.

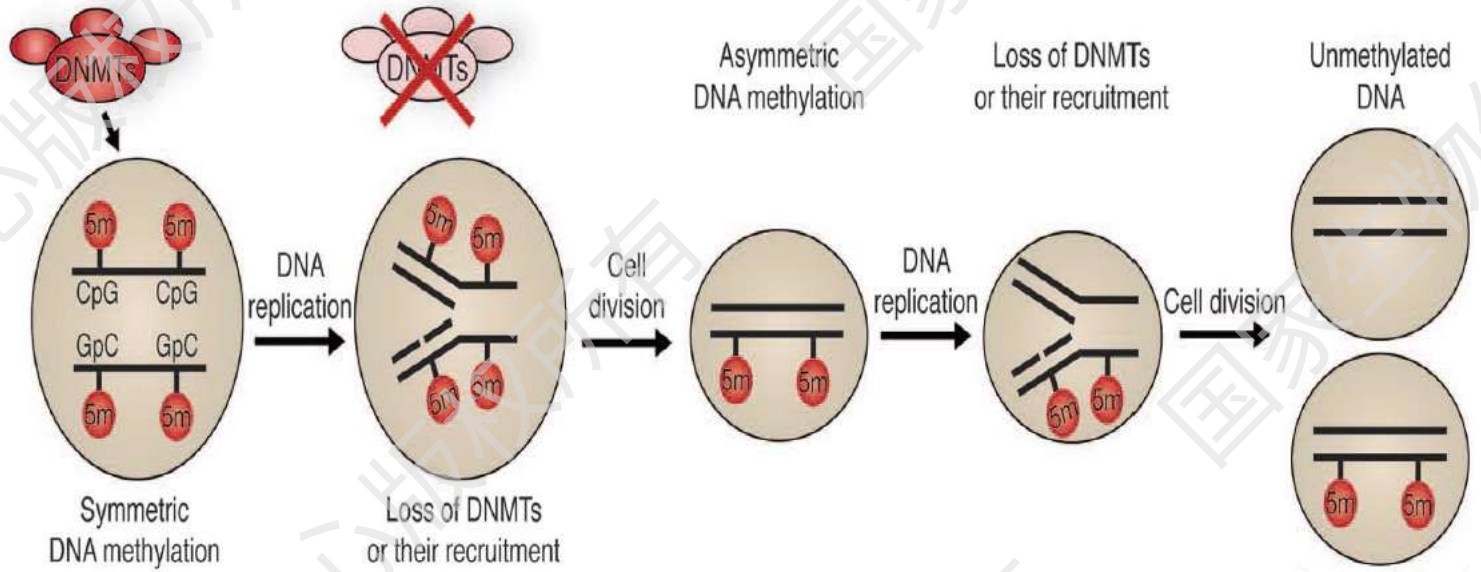
Goldberg AD, Allis CD, Bernstein E. *Cell* 128, 635-8 (2007)

DNA Methylation, DNA甲基化

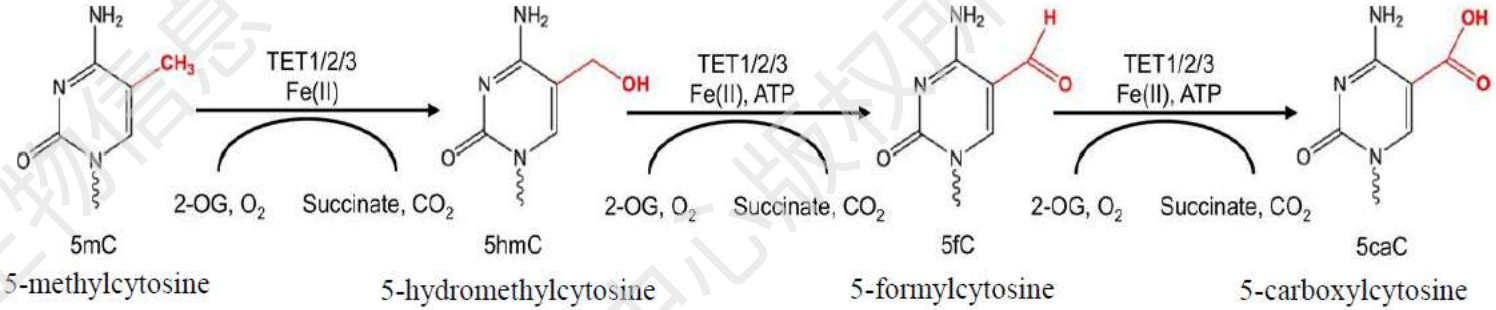
DNA甲基化是由DNA甲基转移酶(DNA Methyl-Transferase, DNMT)催化S-腺苷甲硫氨酸作为甲基供体，将胞嘧啶转变为5-甲基胞嘧啶(mC)的一种反应。

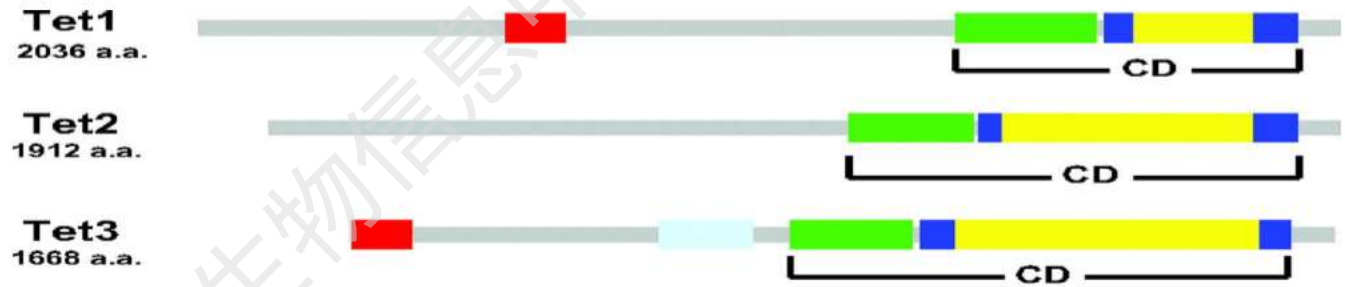
在哺乳动物中**mC**约占**C**总量的**2-7%**，一般说来，**DNA**的甲基化会抑制基因的表达。

肿瘤发生时，肿瘤细胞全基因组甲基化程度仅为正常细胞的**20-60%**，是重要的临床诊断指征。



Passive DNA demethylation





Active DNA demethylation (Tet)

- **单细胞表观基因组图谱绘制及分析技术**

  - 表观基因组

  - **单细胞表观基因组图谱绘制和分析技术**

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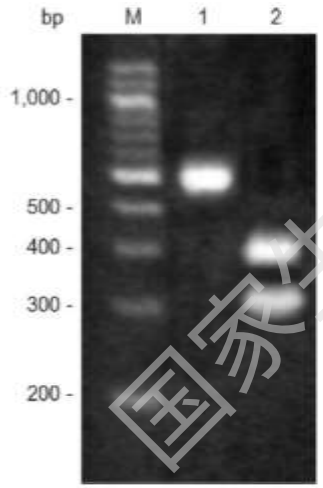
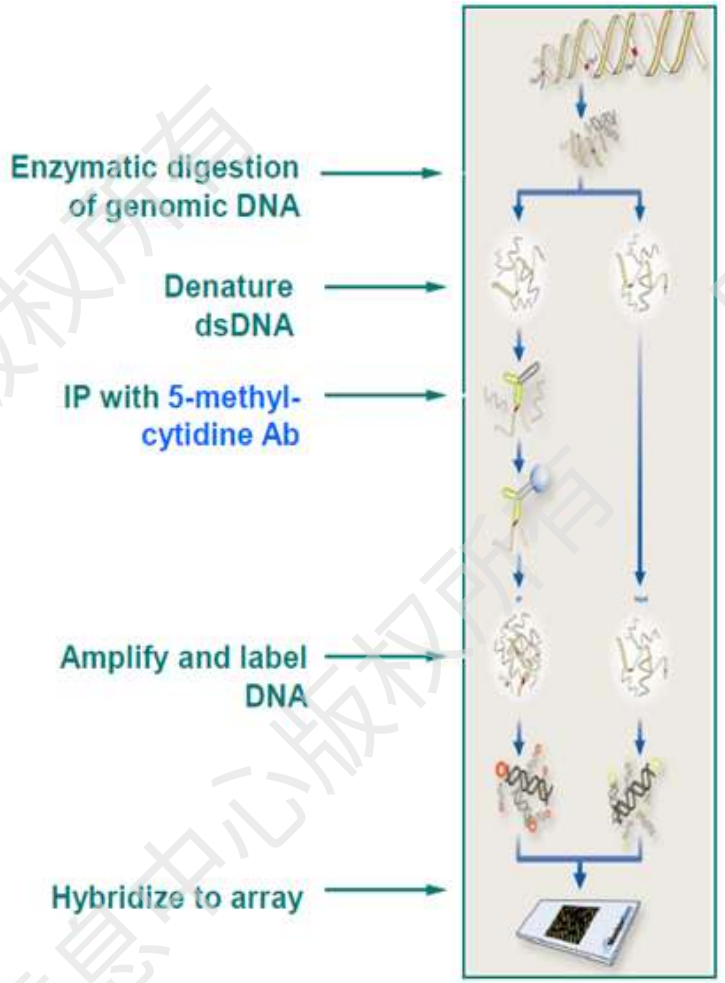
- Methylation-sensitive restriction enzyme digestion



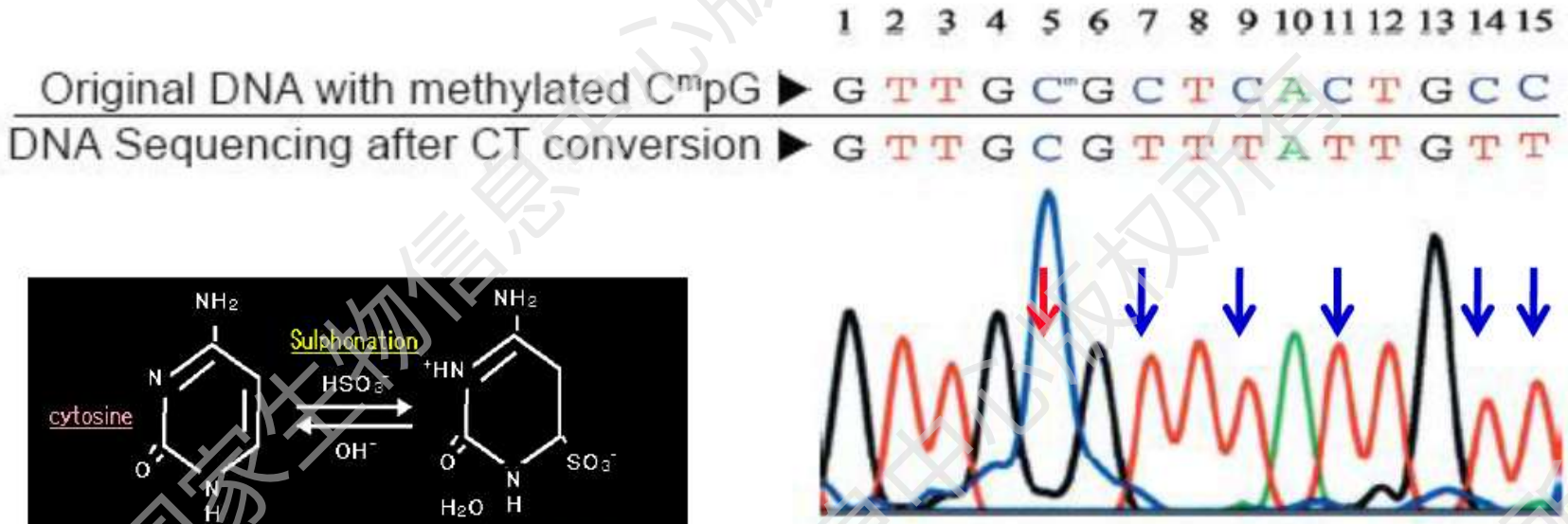
Methylated Sequence	Cleaved by	Not Cleaved by
m4CCGG	MspI (C/CGG)	HpaII (C/CGG)
Cm5CGG	MspI (C/CGG)	HpaII (C/CGG)
Cm4CGG	MspI (C/CGG)	HpaII (C/CGG)
CCm5CGGG	XmaI (C/CCGGG)	SmaI (CCC/GGG)
Gm6ATC	Sau3AI (/GATC)	MboI, NdeII (/GATC)
GATm5C	MboI, NdeII (/GATC)	Sau3AI (/GATC)
GATm4C	MboI (/GATC)	Sau3AI (/GATC)
GGTACm5C	KpnI (GGTAC/C)	Acc65I (G/GTACC)

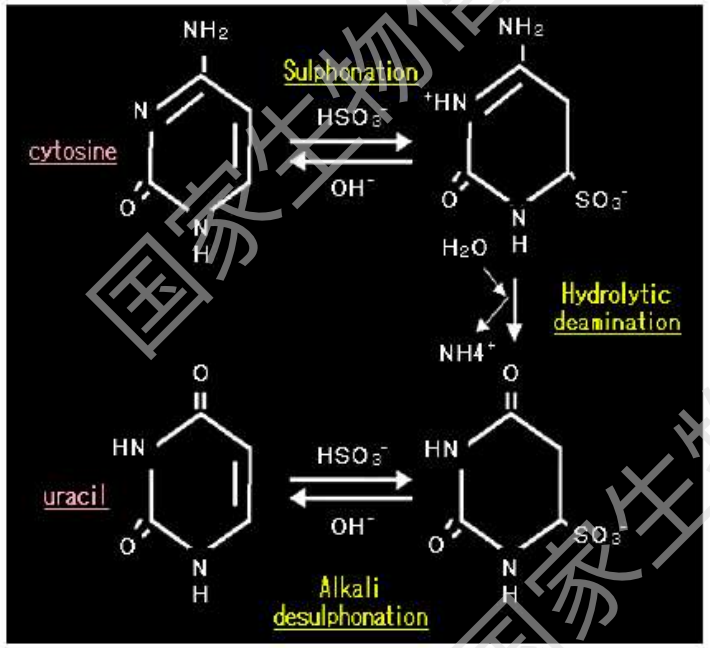
酶切DNA甲基化位点



抗体富集DNA甲基化位点

Bisulfite DNA甲基化测序





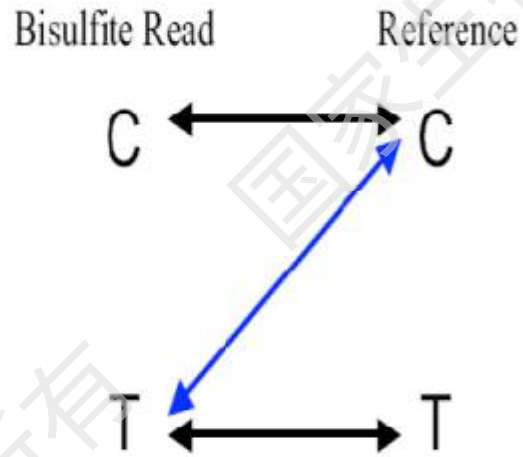
Sequence comparison
(after bisulfite treatment)

```
@HWUSI-EAS762_20100507:1:1:1156:11760#0/1
TATGATTTTTTAGGAAGGGTTGAAGTTGATTAGTTTTTTTAGAAAAATAAATAAAAATAAATAAATAAAAAAATATTTAA
+HWUSI-EAS762_20100507:1:1:1156:11760#0/1
bbbbbbbbbbbbbbbbbbbbbbbbbbbbbbb^bbbbbbaa[bbbba\aaaVbbaaaSaaZP`aZP``Z`]]]P[Ua`aa
```

BSMAP

http://code.google.com/p/bsmap/

- 原理



Reference

Watson >>CT**ACGGCT**GT>>

Crick >>AC**AGCCGT**AG>>

Possible Bisulfite Reads

From BSW >>**ACGGTT**>>

From BSWR >>**AACCGT**>>

From BSC >>**AGTCGT**>>

From BSCR >>**ACGACT**>>

Read from BSW >>**ACGGTT**>>

Match

Watson >>CT**ACGGCT**GT>>

Read from BSC >>**AGTCGT**>>

Match

Crick >>AC**AGCCGT**AG>>

Read from BSWR >>**AACCGT**>>

Reverse

>>**ACGGTT**>>

Match

Watson >>CT**ACGTCT**GT>>

Read from BSCR >>**ACGACT**>>

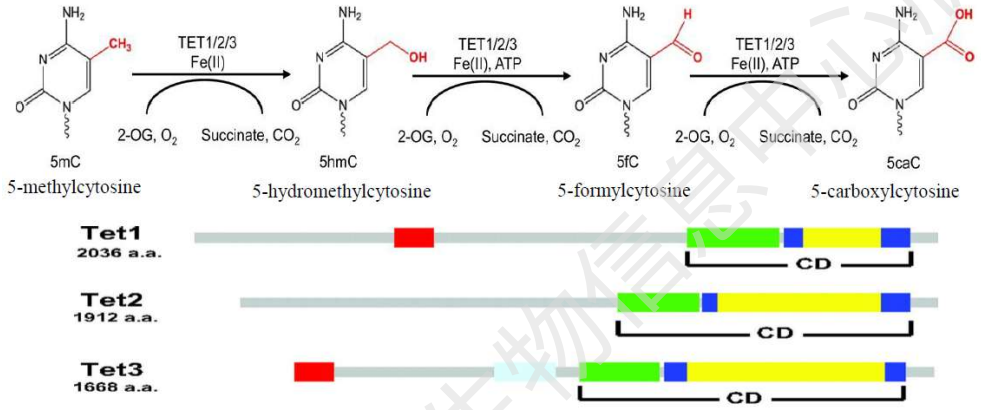
Reverse

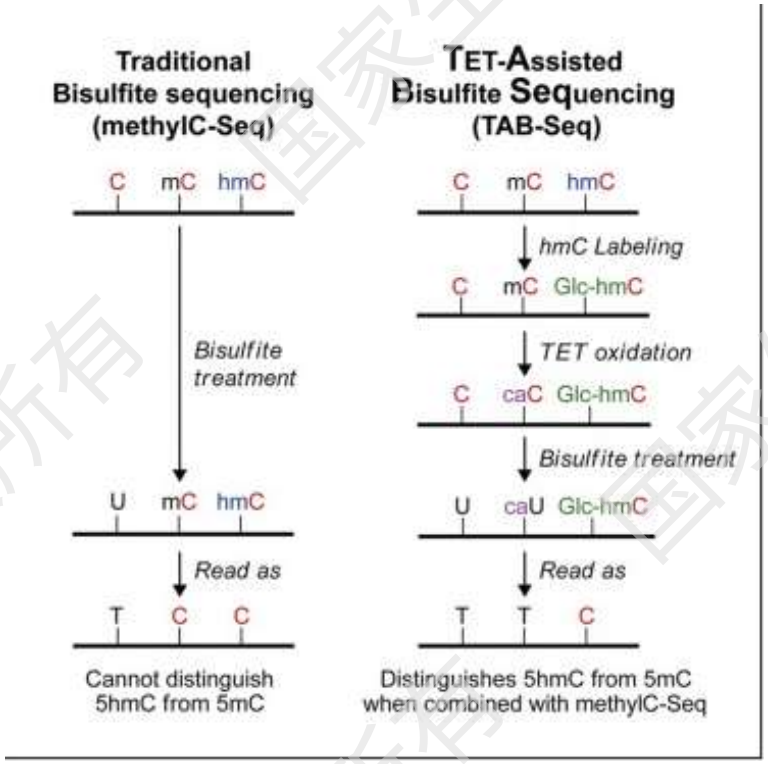
>>**AGTCGT**>>

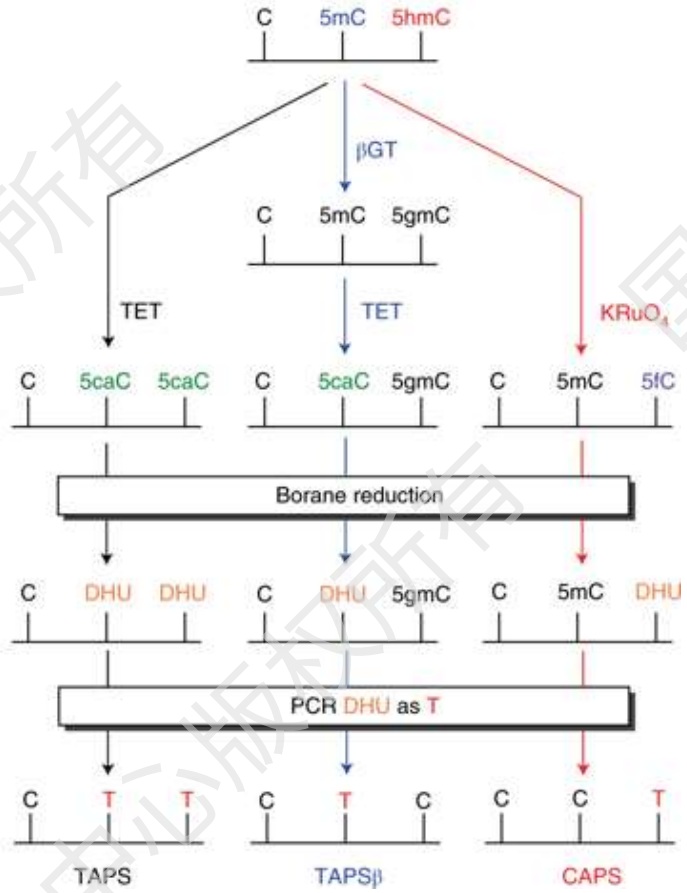
Match

Crick >>AC**AGCCGT**AG>>

Bisulfite-free direct detection of 5-methylcytosine and 5-hydroxymethylcytosine at base resolution



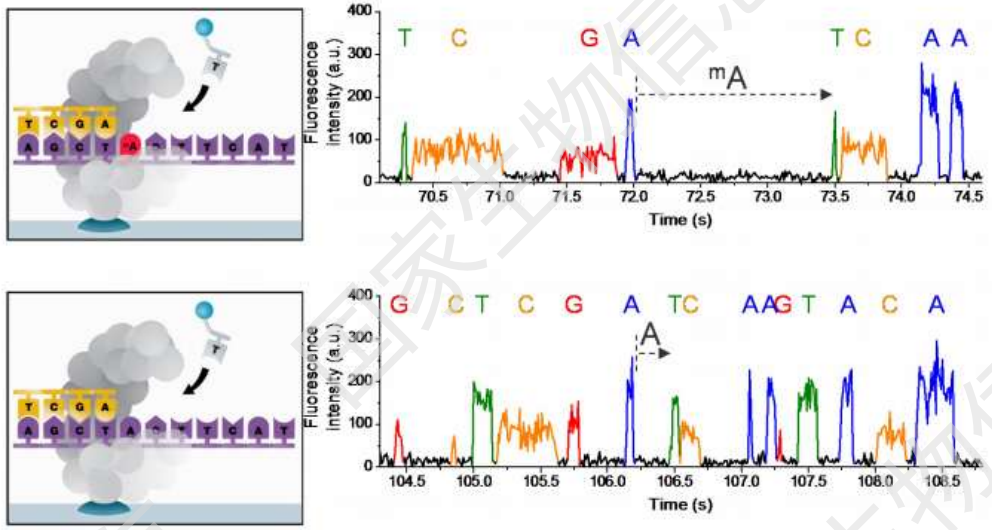




e

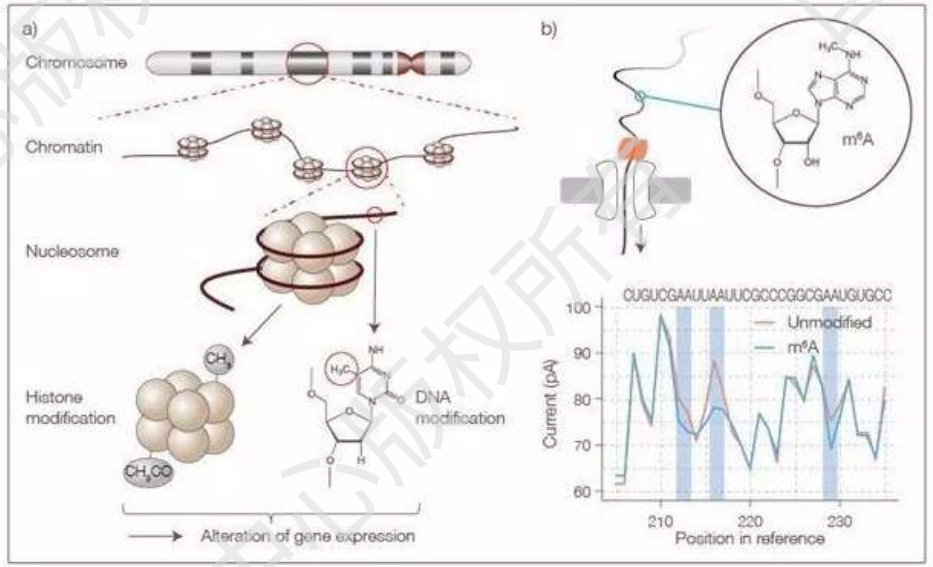
Base	BS	TAB-Seq	oxBS	TAPS	TAPSβ	CAPS
C	T	T	T	C	C	C
5mC	C	T	C	T	T	C
5hmC	C	C	T	T	C	T

第三代测序仪：*Base Modification Detection*



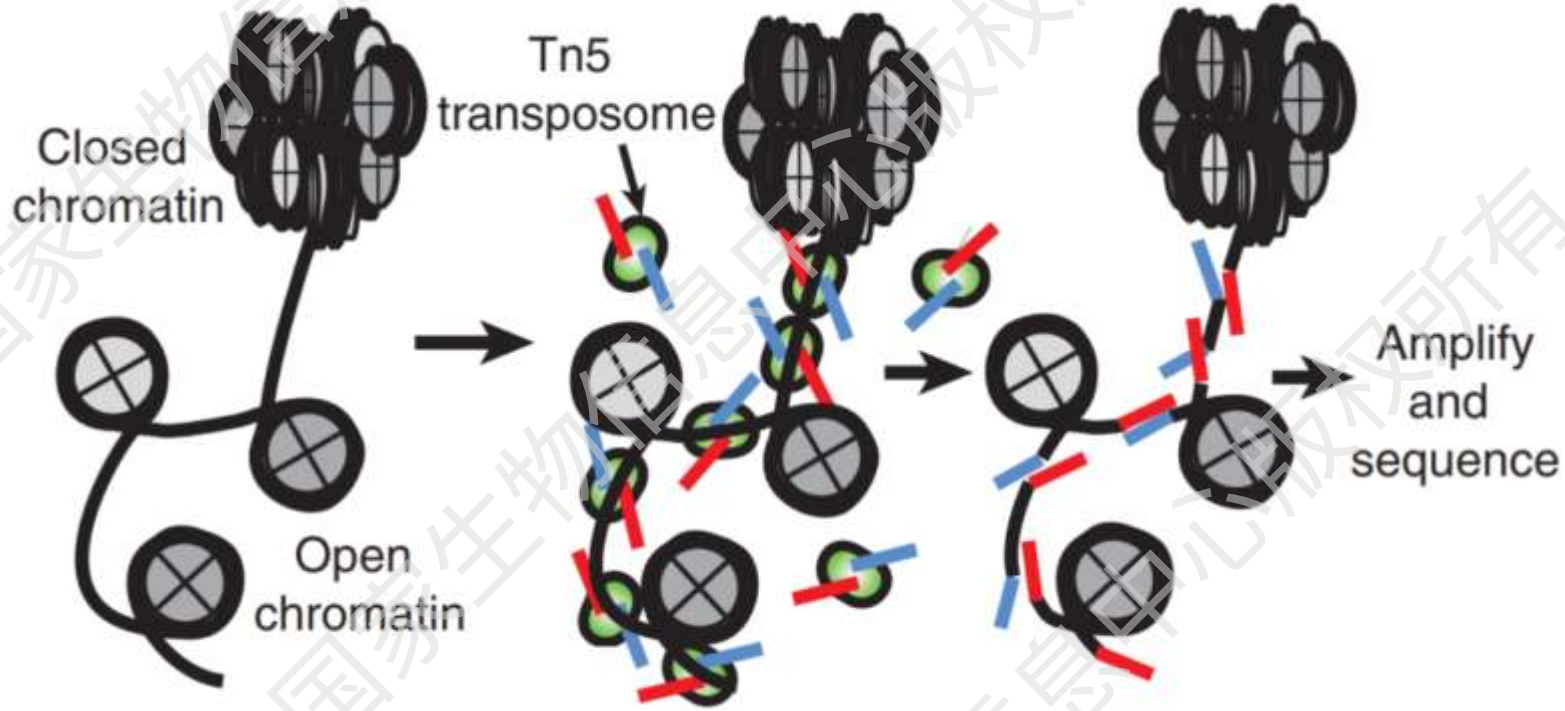
- Differentiation between modified and non-modified bases
  - Epigenetics, DNA damage, new, novel modifications
- Direct observation (*e.g.* no bisulfite)

修饰信息的保留



- 被修饰的碱基通过纳米孔时产生的电流变化会有一定差异，这种差异会体现在fast5文件中并被碱基识别软件识别出来

ATAC-seq （transposase-accessible chromatin using sequencing）建库：检测染色体开放区域



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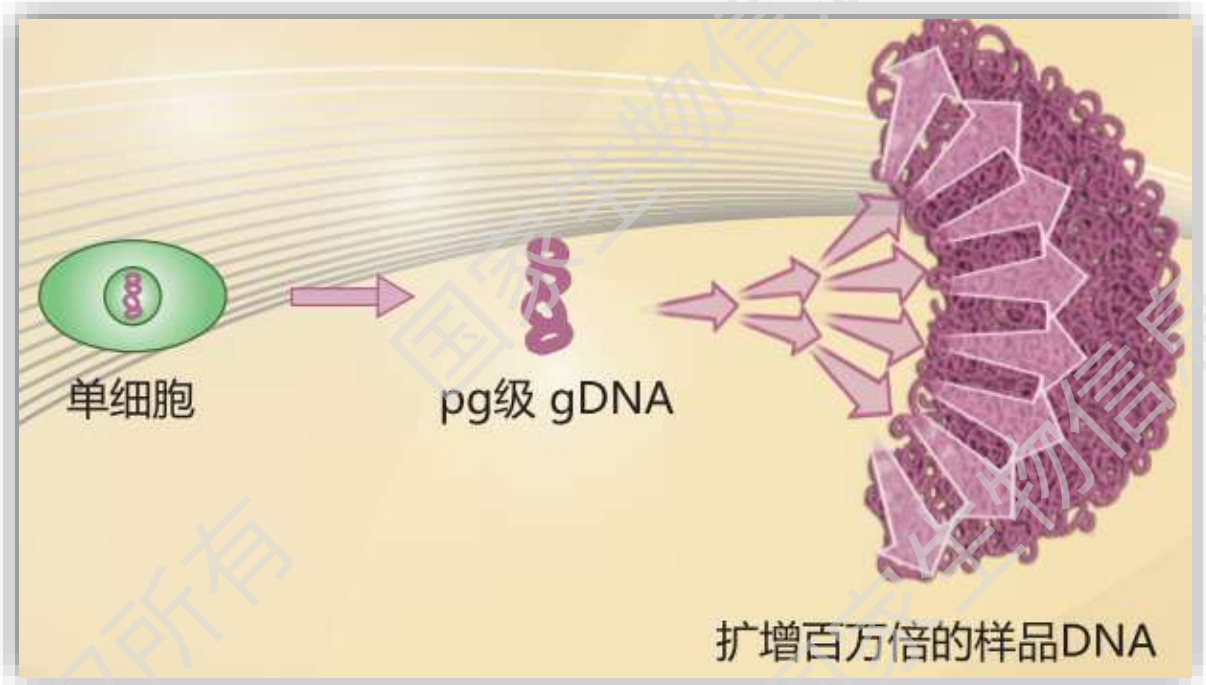
Bulk测序技术的单细胞化



单细胞表观基因组技术-单细胞处理

高通量单细胞表观基因组技术-barcode

高效加接头- Tn5

One tube操作（减少DNA损失）

三代长读长测序加持

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- **单细胞基因组图谱的应用**

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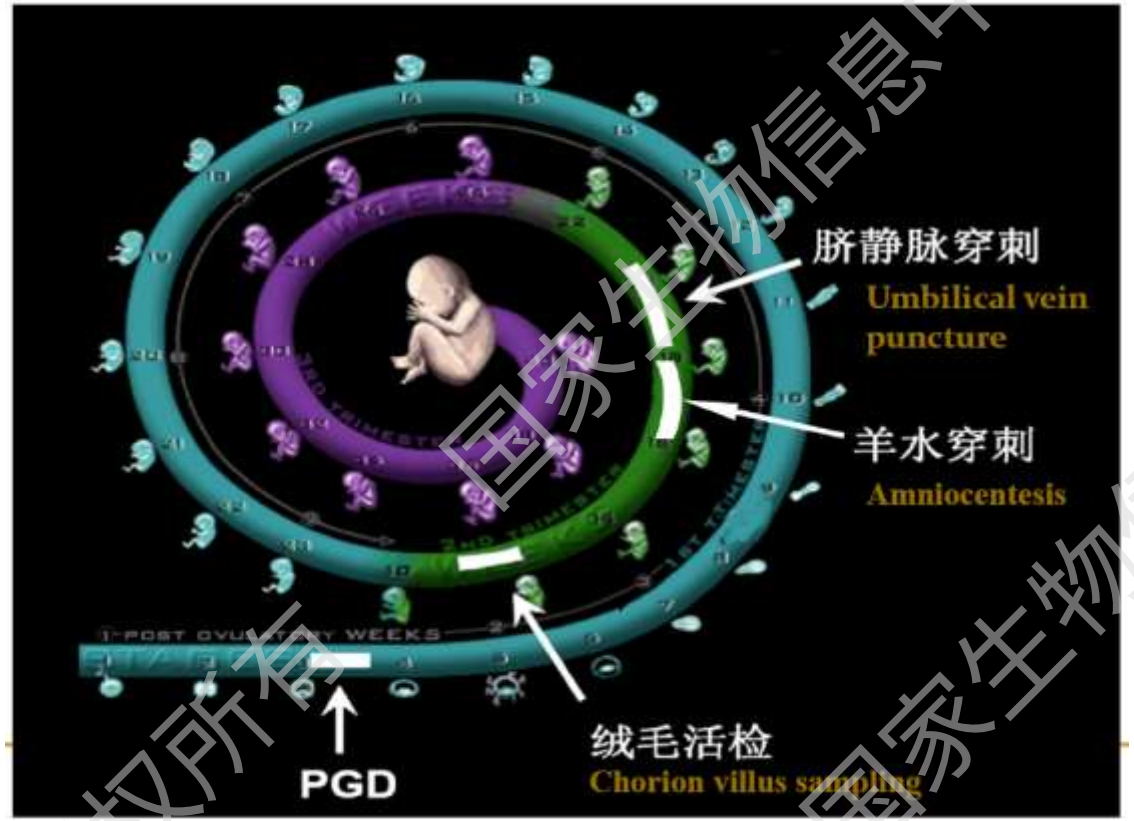
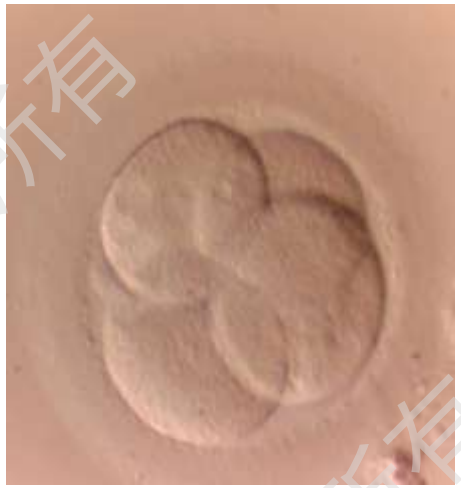
人类生殖过程的遗传学检测阶段



胚胎培养和胚胎发育

Day 1 stage



Day 2 stage

Day 3 stage

Day 4 stage

Day 5 stage – 囊胚期

取样时间点

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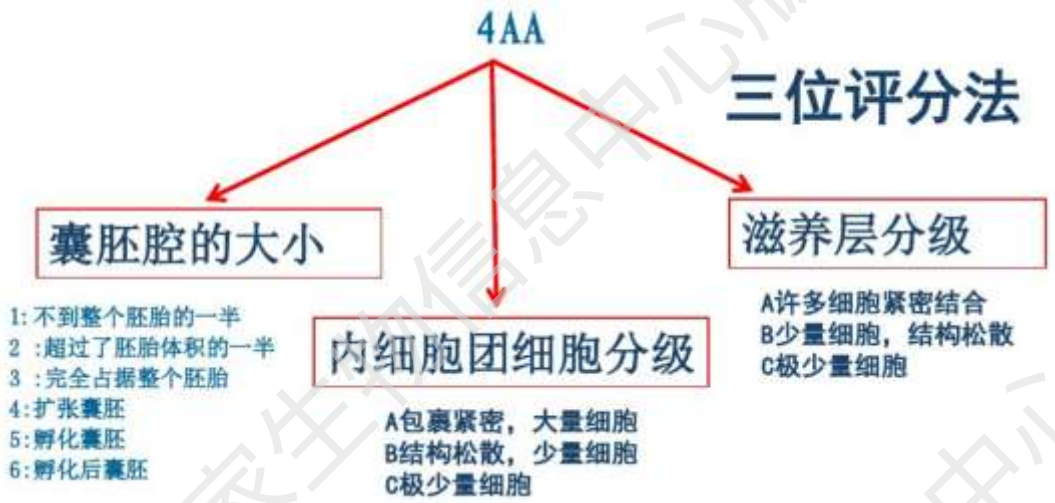
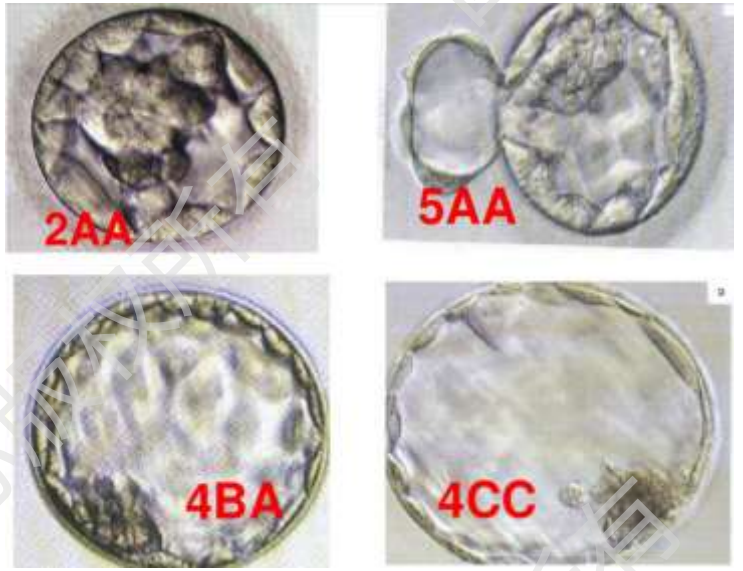
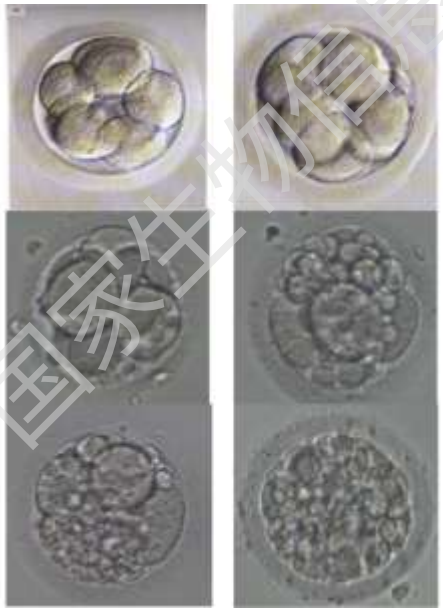
胚胎评估





- 胚胎评价标准
  - 细胞数
  - 碎片：无碎片
    - 碎片<10%
    - 碎片10－30%
    - 碎片30－50%
    - 碎片>50%
  - 卵裂球均匀程度



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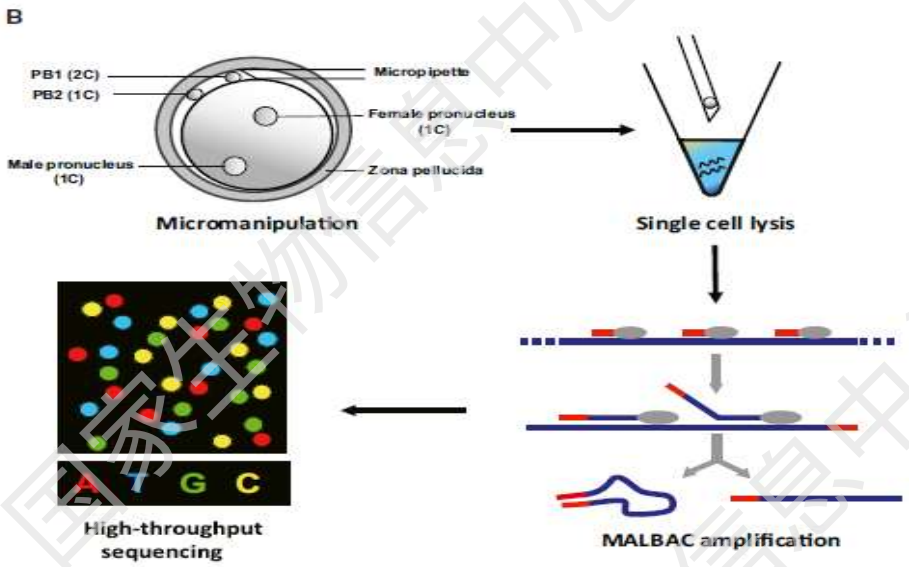
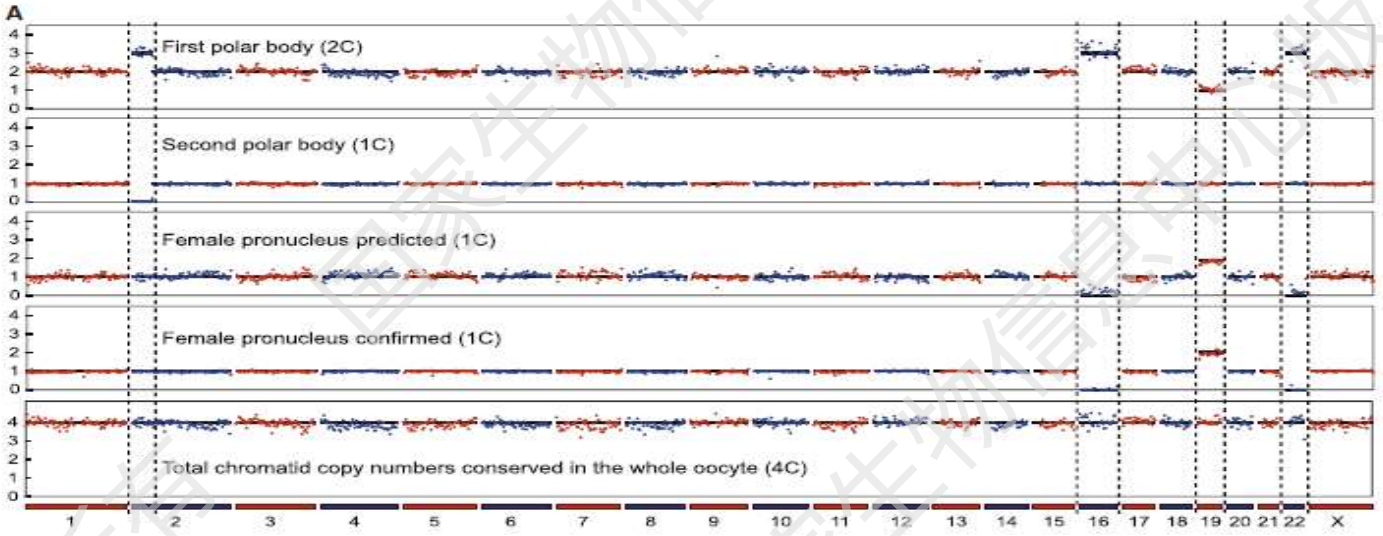
卵细胞染色体整倍性检测

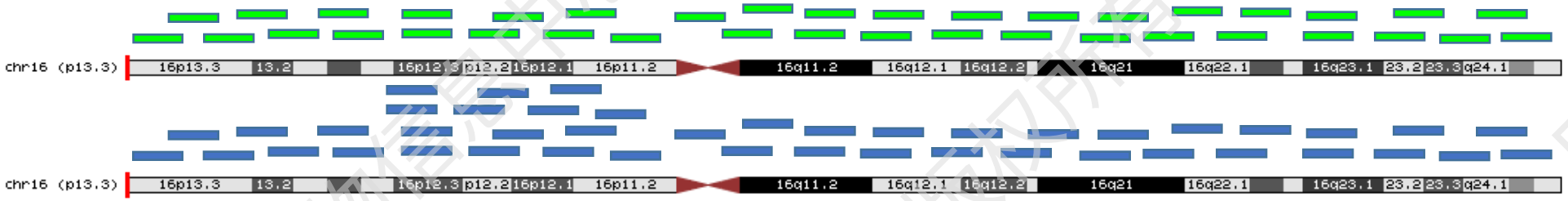
检测第一极体和第二极体染色体，从而推测卵细胞染色体整倍性





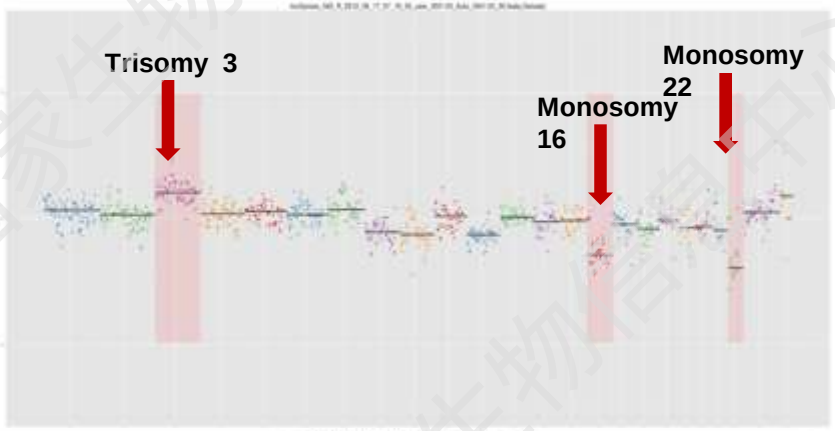
遗传学检测—原理

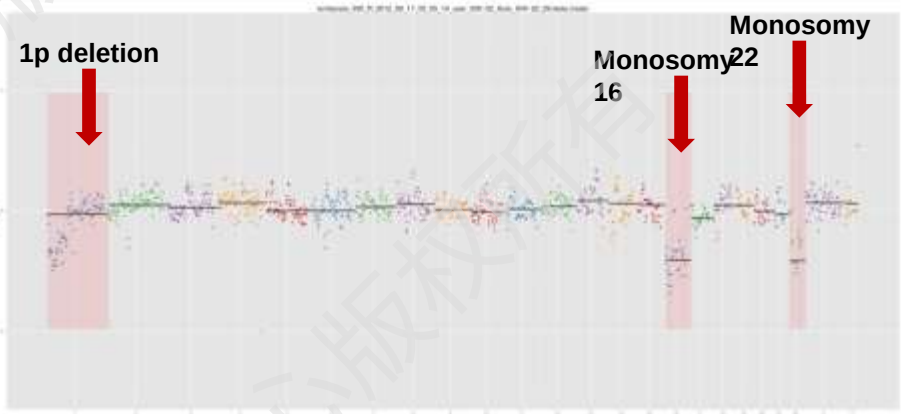
- 正常和染色体重复的测序数据对比

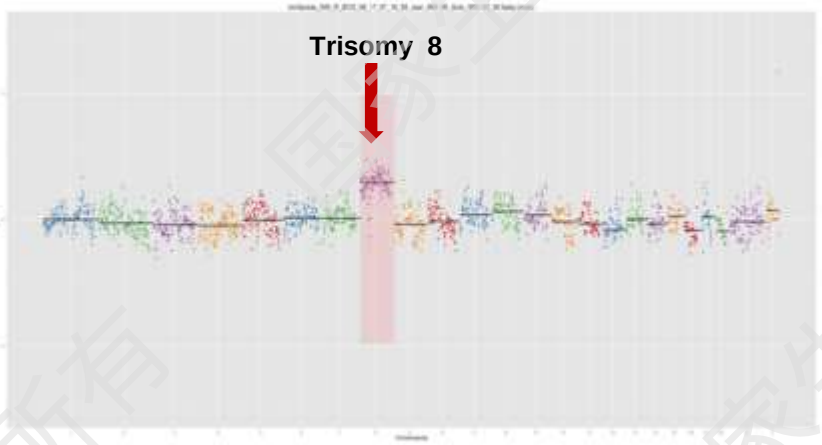


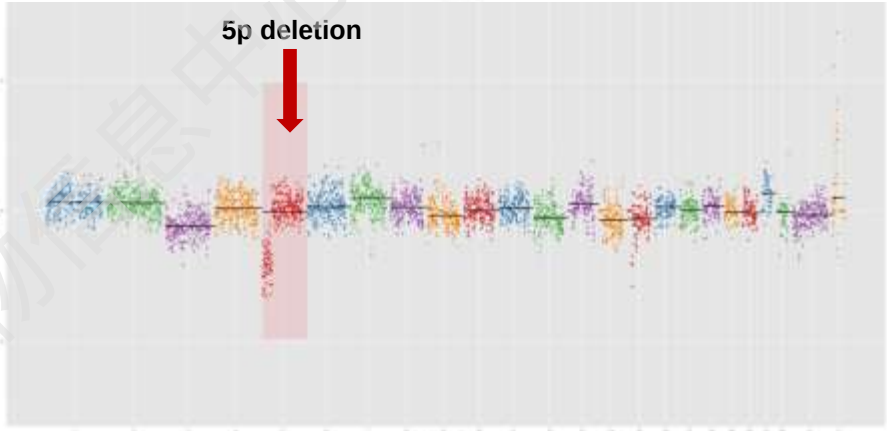
低覆盖度基因组测序

–拷贝数分析



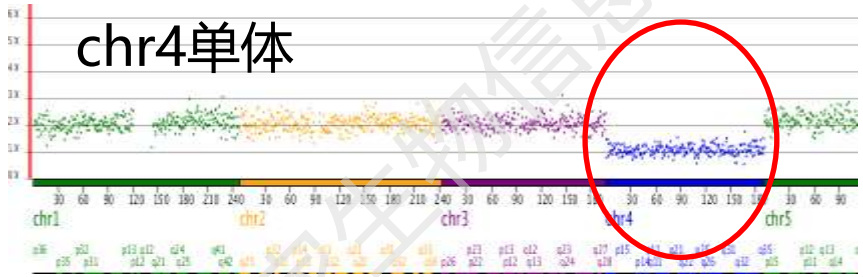


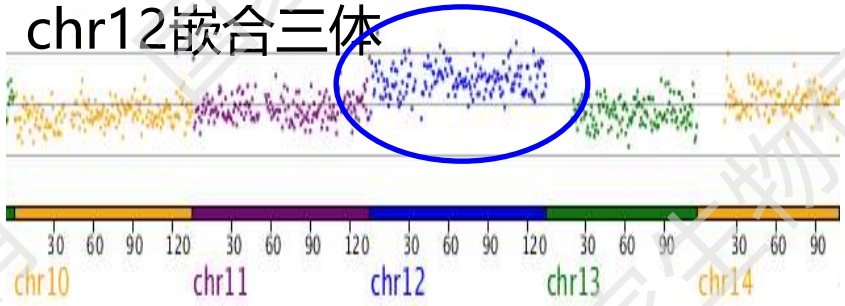




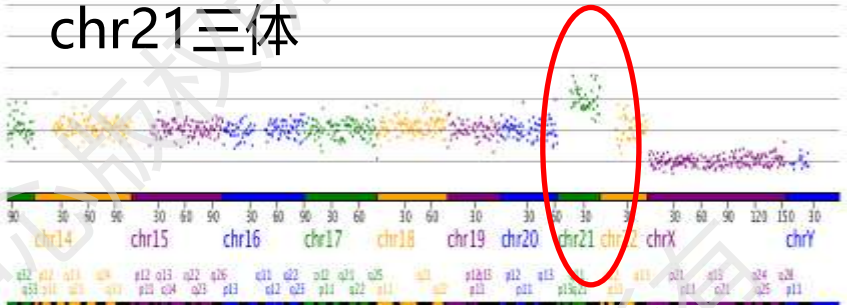
胚胎嵌合分析　原理+示例

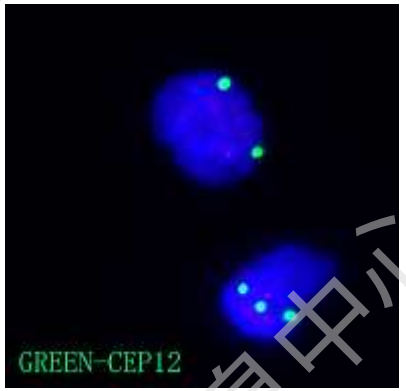
- 嵌合胚胎含有不同核型的细胞系（两种及两种以上）；
- 不同核型的细胞测序得到的序列数目不同；





测序结果指示：chr12数据整体上移，但并未达到三体标准阈值，推测囊胚存在嵌合性的chr12三体细胞。





该囊胚解冻后进行FISH验证，发现21%细胞为chr12三体，其余细胞为正常二体。

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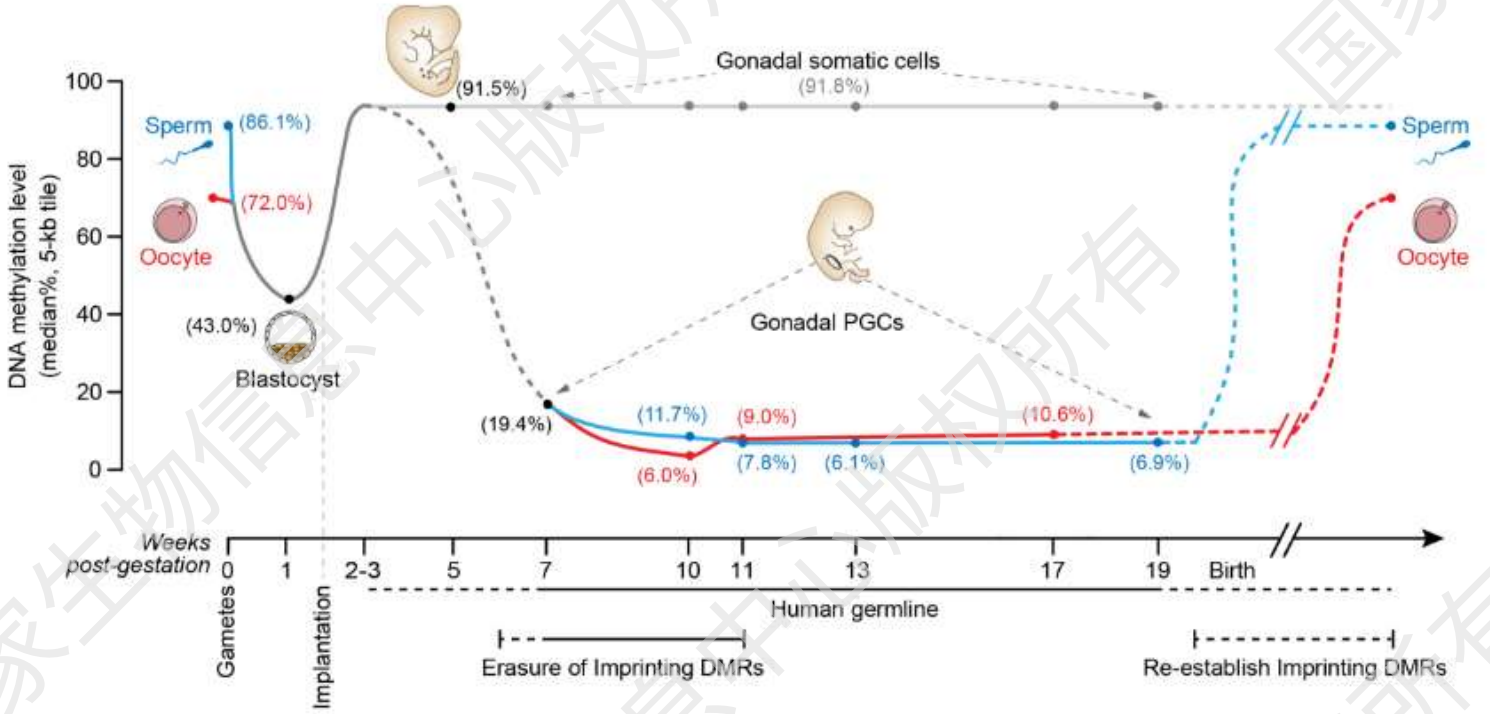
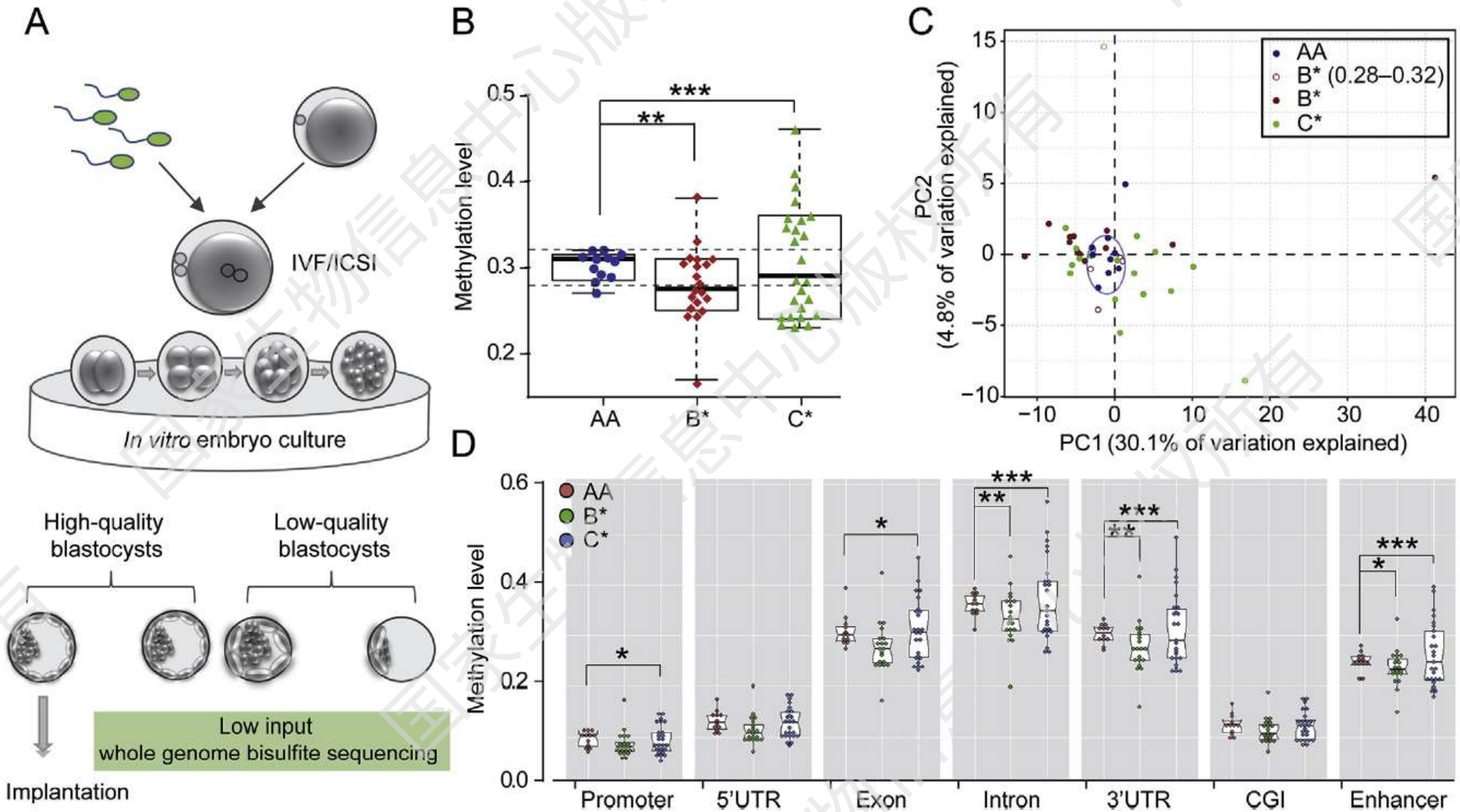
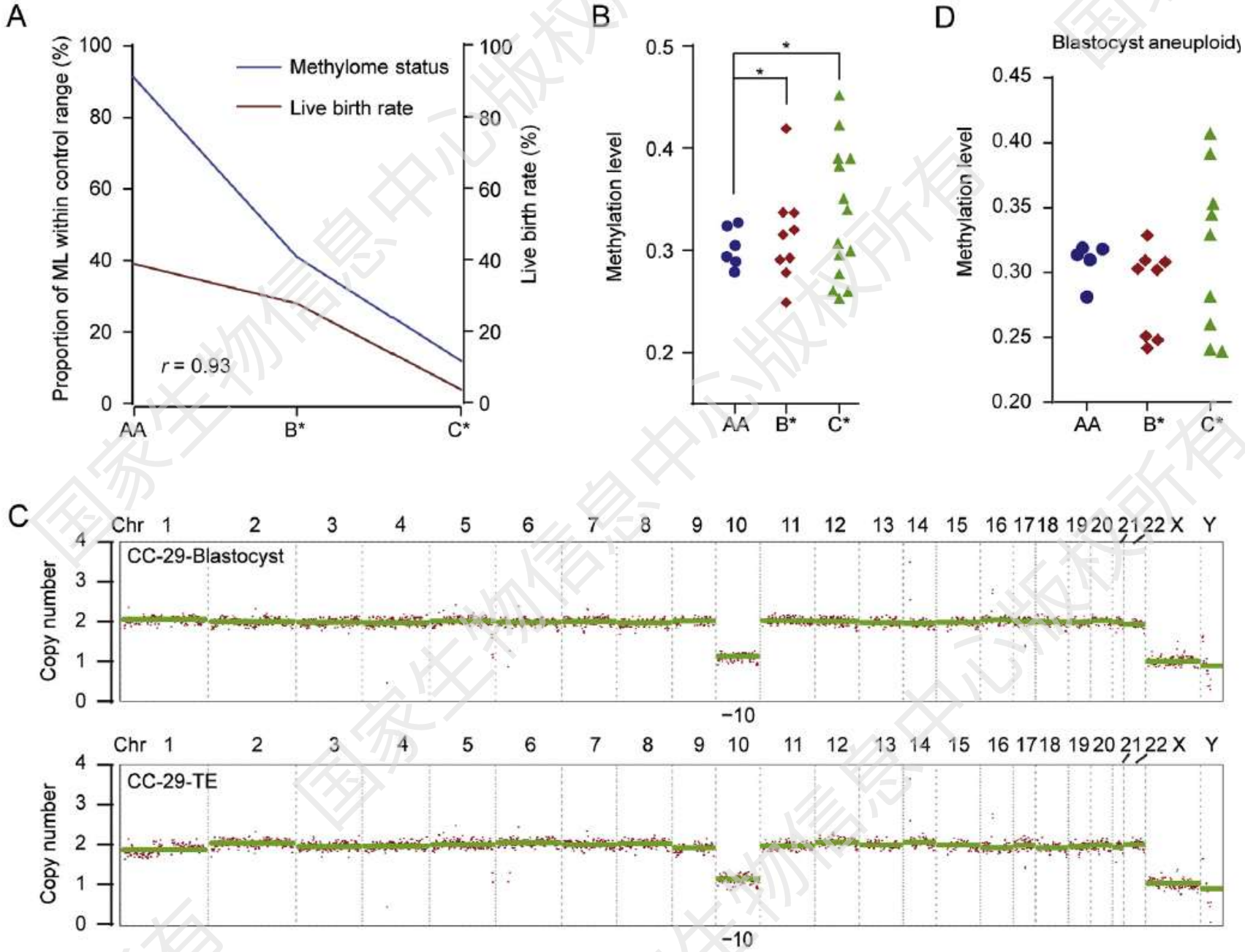


人类生殖细胞（精子、卵母细胞及原始生殖细胞）、植入前胚胎以及着床后胚胎体细胞的DNA甲基化水平示意图

人类植入前胚胎的DNA甲基化特征：精卵结合后，父母的表观遗传记忆信息（基因组DNA甲基化）被大规模的擦除，而印记基因的甲基化得以精确维持、重复序列元件上也保留了大量DNA甲基化，从而传递给子代；胚胎着床后，基因组DNA重新甲基化。

PGC产生于胚胎发育的早期，是发育为成熟的精子和卵母细胞的前体细胞。人类原始生殖细胞在发育过程中会经历大规模的DNA甲基化擦除，在胚胎发育到第10-11周时，DNA甲基化水平降至最低点，仅有7%左右保留下来。是人类所有已知类型的体内正常细胞中DNA甲基化程度最低的细胞类型。


A
IVF/ICSI
In vitro embryo culture
High-quality blastocysts
Low-quality blastocysts
Low input whole genome bisulfite sequencing
Implantation
B
Methylation level
0.5
0.4
0.3
0.2
***
**
AA
B*
C*
C
PC2 (4.8% of variation explained)
PC1 (30.1% of variation explained)
AA
B* (0.28–0.32)
B*
C*
D
Methylation level
AA
B*
C*
Promoter
5'UTR
Exon
Intron
3'UTR
CGI
Enhancer


A
Proportion of ML within control range (%)
Live birth rate (%)
Methylome status
Live birth rate
r = 0.93
AA
B*
C*
B
Methylation level
*
*
AA
B*
C*
D
Blastocyst aneuploidy
Methylation level
AA
B*
C*
C
Chr 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
CC-29-Blastocyst
Copy number
−10
CC-29-TE
Copy number
−10

Haplotype Assembly and Inference

来自父方的染色体: ATAGCCTATTTCCAGGAGTCGAAGAC

来自母方的染色体: ATAGCGTATTTCCAGGAGTCGTAGAC

单体型 1→ C C A

单体型 2→ G C T

基因型→ {C,G} {C,C} {A,T}

- **单体型组装(Haplotype Assembly)**
  - 从较小的SNP片断来组装单体型
  - 优点：比较精确
  - 缺点：技术实现较难、费用昂贵、速度慢
- **单体型推断(Haplotype Inference)**
  - 从群体的基因型来推断单体型
  - 优点：技术实现容易、基因型容易获取
  - 缺点：不精确

非家系群体/家系(merlin)

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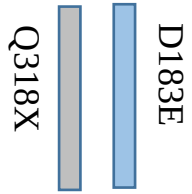
辅助生殖单基因病技术原理-单体型 （拒绝ADO ）

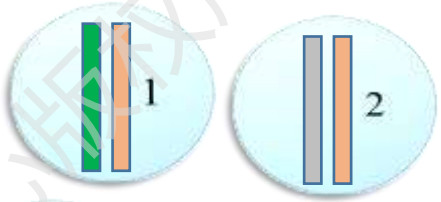
单体型是位于一条染色体特定区域的一组相互关联，
并倾向于以整体遗传给后代的单核苷酸多态的组合。

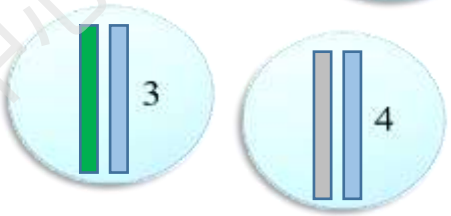
Mother
9437

Father
9438



Daughter
9436





Genotype

Father		Mother		Child	
C	T	C	T	C	C
A	G	A	A	A	G

Haplotype

Father		Mother		Child	
C	T	C	T	C	C
G	A	A	A	G	A

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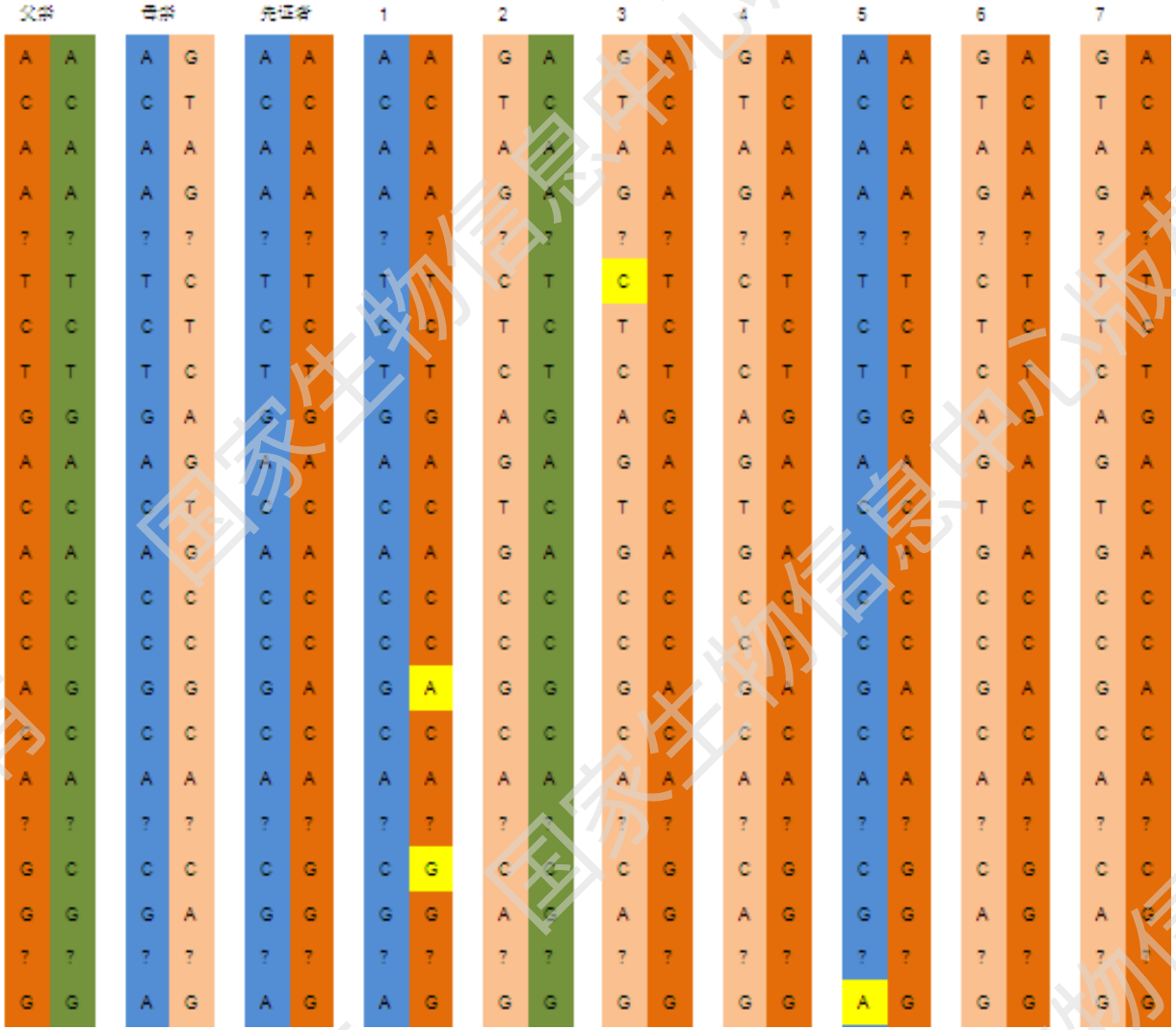
PGD单基因病　α-地贫　缺失型

α-地贫检测结果

病型	缺失型单基因病
疾病	α-地贫
父亲	正常
母亲	SEA +3.7 Del
胚胎1	3.7 Del
胚胎2	SEA Del
胚胎3	SEA Del
胚胎4	3.7 Del
胚胎5	SEA Del
胚胎6	3.7 Del
胚胎7	3.7 Del

		父亲		母亲		胚胎1		胚胎2		胚胎3		胚胎4		胚胎5		胚胎6		胚胎7	
		F1	F2	M1	M2	F1	M1	F1	M2	F1	M2	F2	M1	F1	M2	F2	M1	F2	M1
chr16	189971	C	C	T	C	C	T	C	C	C	C	T	T	C	C	C	C	C	C
chr16	207731	C	G	C	C	C	C	C	C	C	C	G	C	C	C	G	C	G	C
chr16	212649	C	T	C	C	C	C	C	C	C	C	T	C	C	C	T	C	T	C
chr16	218735	G	A	A	A	G	A	G	G	G	G	A	A	G	G	A	A	A	A
chr16	221057	C	T	T	T	C	T	C	C	C	C	T	T	C	C	T	T	T	T
chr16	221126	T	C	C	C	T	C	T	T	T	T	C	C	T	T	C	C	C	C
chr16	223735	G	G			G	G	G	G	G	G	G	G	G	G	G	G	G	G
chr16	224619	C	T			C	C	C	C	C	C	T	T	C	C	T	T	T	T
chr16	232773	T	G	T	T	T	T					T	T	G	T				
chr16	232787	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
chr16	234632	C	G	G	G	C	G	C	C	C	C	G	G	C	C	G	G	G	G
chr16	235579	T	C	C	C	T	C	T	T	T	T	C	C	T	T	C	C	C	C
chr16	240280	C	A	A	A	C	A	C	C	C	C	A	A	C	C	A	A	A	A
chr16	241210	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
chr16	244785	A	G	G	G	A	G	A	A			G	G	A	A	G	G	G	G
chr16	272349	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
chr16	280860	[C,T]	[T,C]	[T,C]	[C,T]	[C,T]	[T,C]	C	C	C	C	T	T	C	C	T	T	T	T
chr16	304514	T	C	C	C	T	C	C	C	T	C	C	C	T	T	C	C	C	C
chr16	336660	A	A	G	A	G	G	A	A	A	A	A	G	A	A	G	G	A	G
chr16	404750	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
chr16	571904	C	C	C	T	C	C	T	T	C	T	C	C	C	T	C	C	C	C
chr16	899974	G	A	G	G	G	G	A	A	G	G	A	G					A	G
chr16	1114458	A	A	A	G	A	A	A	A	A	A	A	G			G	G	A	G

PGD　HLA配型



HLA配型　5号胚胎与先证者单体型完全一致

HLA配型检测结果

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单基因病辅助生殖遗传学检测-MARSALA

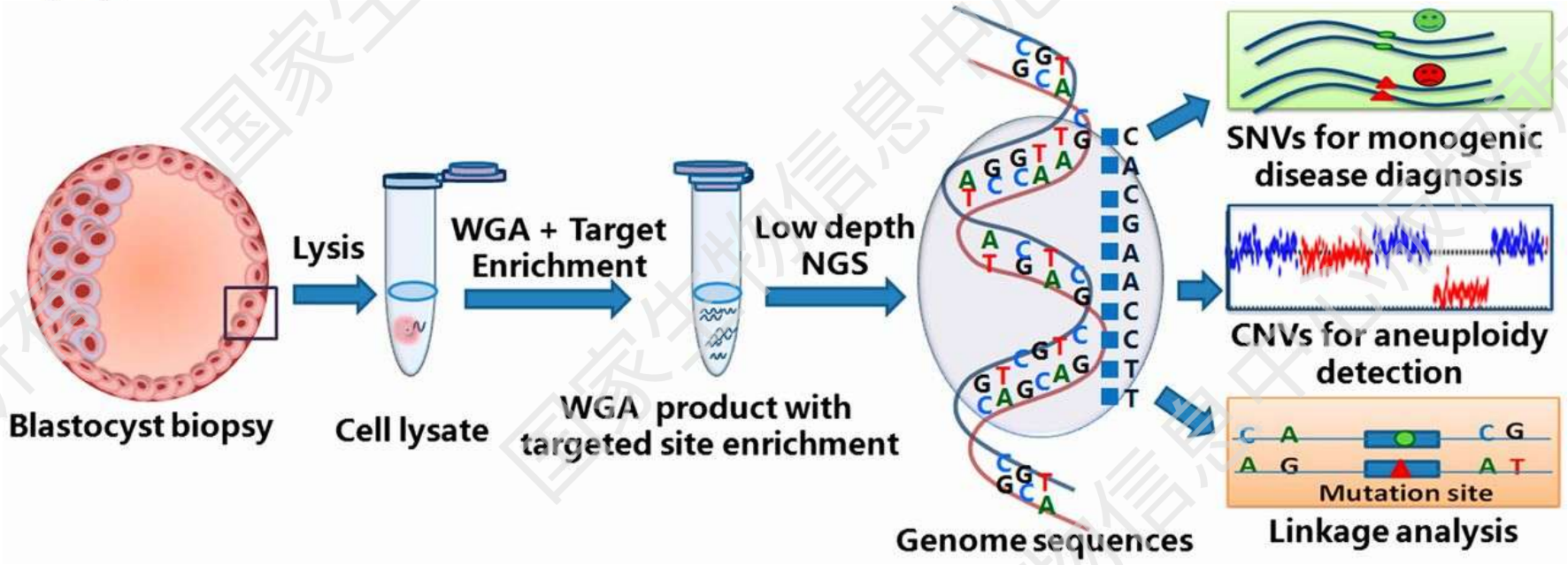
Live births after simultaneous avoidance of monogenic diseases and chromosome abnormality by next-generation sequencing with linkage analyses

Liying Yan, Lei Huang, Liya Xu, Jin Huang, Fei Ma, Xiaohui Zhu, Yaqiong Tang, Mingshan Liu, Ying Lian, Ping Liu, Rong Li, Sijia Lu, Fuchou Tang, Jie Qiao, and X. Sunney Xie

PNAS December 29, 2015 112 (52) 15964-15969; published ahead of print December 28, 2015
https://doi.org/10.1073/pnas.1523297113

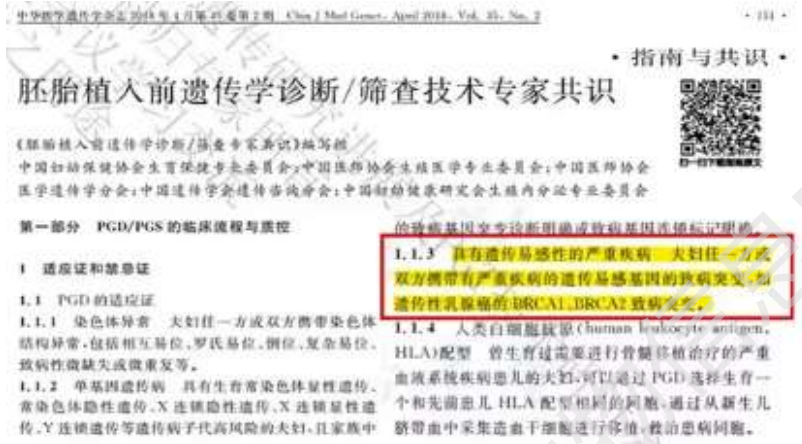
Contributed by X. Sunney Xie, November 25, 2015 (sent for review November 21, 2015; reviewed by John Rasko and

做法：将扩增产物分为3个部分，分别进行CNV检测、致病位点检测以及SNP分析。此方法测序测序数据量较少（1M reads），成本较低、所以临床应用多，目前主要是这种做法



单基因突变、染色体异常、连锁分析的同时检测

遗传性肿瘤辅助生殖遗传学检测



·指南与共识·

胚胎植入前遗传学诊断/筛查技术专家共识

第一部分 PGD/PGS的临床流程与质控

1 适应证和禁忌证

1.1 PGD的适应证

1.1.3 具有遗传易感性的严重疾病 夫妇任一方或双方携带有严重疾病的遗传易感基因的致病突变，如遗传性乳腺癌的BRCA1、BRCA2致病突变。

遗传性肿瘤PGD能帮助患癌夫妇或患癌高风险夫妇筛选出不携带遗传性肿瘤易感基因致病突变的胚胎，阻断遗传性肿瘤的遗传，降低后代患癌风险。

遗传性肿瘤综合征与癌症风险

遗传性肿瘤综合征	基因	癌症风险						
		乳腺癌	卵巢癌	子宫内膜癌	结直肠癌	胃癌	胰腺癌	其他癌肿
遗传性乳腺癌卵巢癌综合征	BRCA1	√	√				√	√
	BRCA2	√	√				√	√
李法美尼综合征	TP53	√	√	√	√	√	√	√
林奇综合征（Lynch）	MLH1		√	√	√	√	√	√
	MSH2		√	√	√	√	√	√
	MSH6		√	√	√	√	√	√
	PMS2		√	√	√	√	√	√
	EPCAM		√	√	√	√	√	√
家族性腺瘤性息肉病（FAP）	APC				√	√		√
MUTYH相关性息肉病	MUTYH				√			√
PTEN错构瘤综合征	PTEN	√		√	√			√
黑斑息肉综合征	STK11	√	√	√	√	√	√	√
遗传性弥漫型胃癌	CDH1	√		√		√		
幼年性息肉综合征	BMPR1A				√	√	√	
	SMAD4				√	√	√	

北医三院进入PGD周期遗传性肿瘤种类

疾病名称	致病基因
遗传性多发性骨软骨瘤	EXT1
遗传性多发性骨软骨瘤	EXT2
神经纤维瘤I型	NF1
神经纤维瘤II型	NF2
黑斑息肉综合症	STK11
家族性甲状腺髓样癌综合征	RET
范可尼贫血	FANCA
痣样基底细胞癌综合征	PTCH1
结节性硬化症	TSC2
Birt-Hogg-Dubé综合征	FLCN

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