转录组学的分析与临床应用

学习框架

第一课

1. 了解基础知识框架

2. 收集相关资料，自主学习

第二课

3. 练习编程、绘图

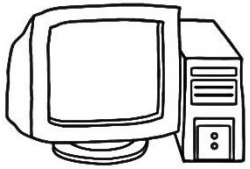
4. 将分析技能应用到研究中

5. 编写、发表生信算法、文章

生信自主学习的主要方式：读说明文档，读代码

计算机与编程

基本编程技能： R, Python (或 Perl), Linux



运算功能

01101110101101010101001

编译

汇编语言： MOV

C： int height=1；

C++： class

编译

R, Python, Perl

存储功能

01101110011001011011011

编码

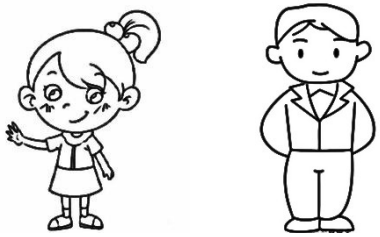
dwadawxzcvwas

xwadaw+)dwad

wwad&*%(^*(^

编码

Hello world

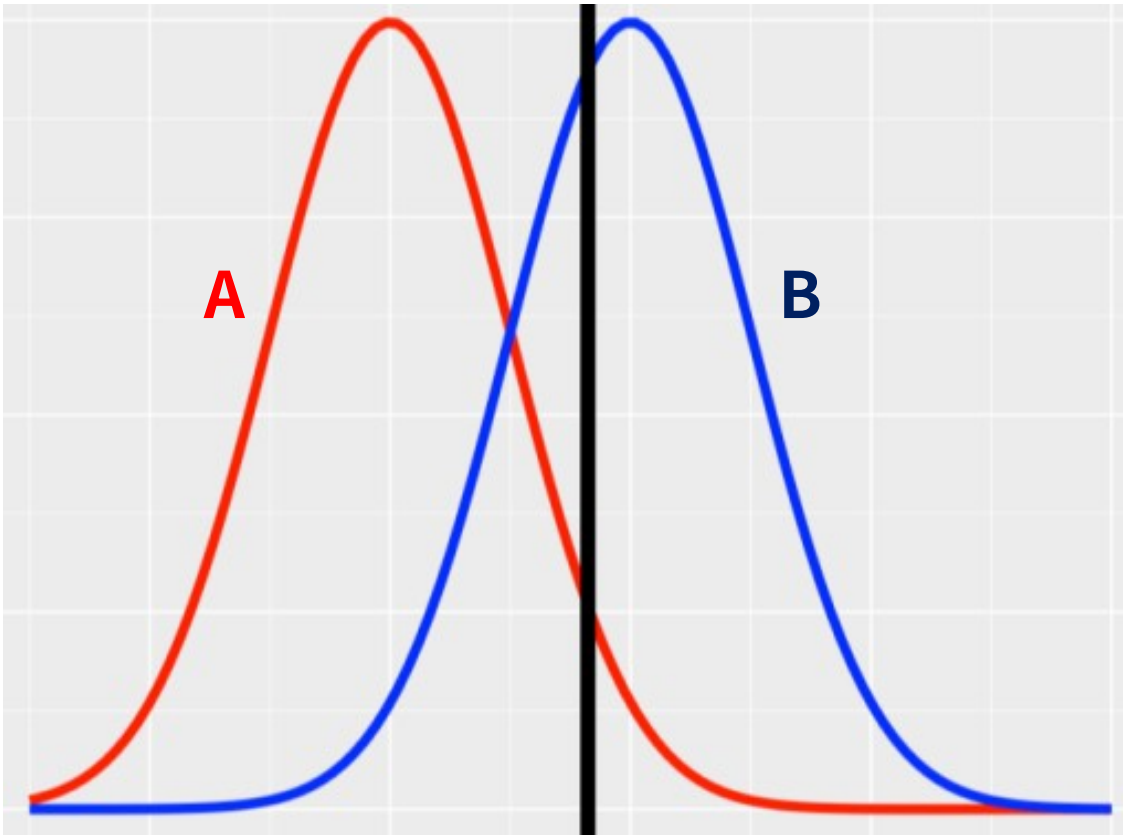


统计检验

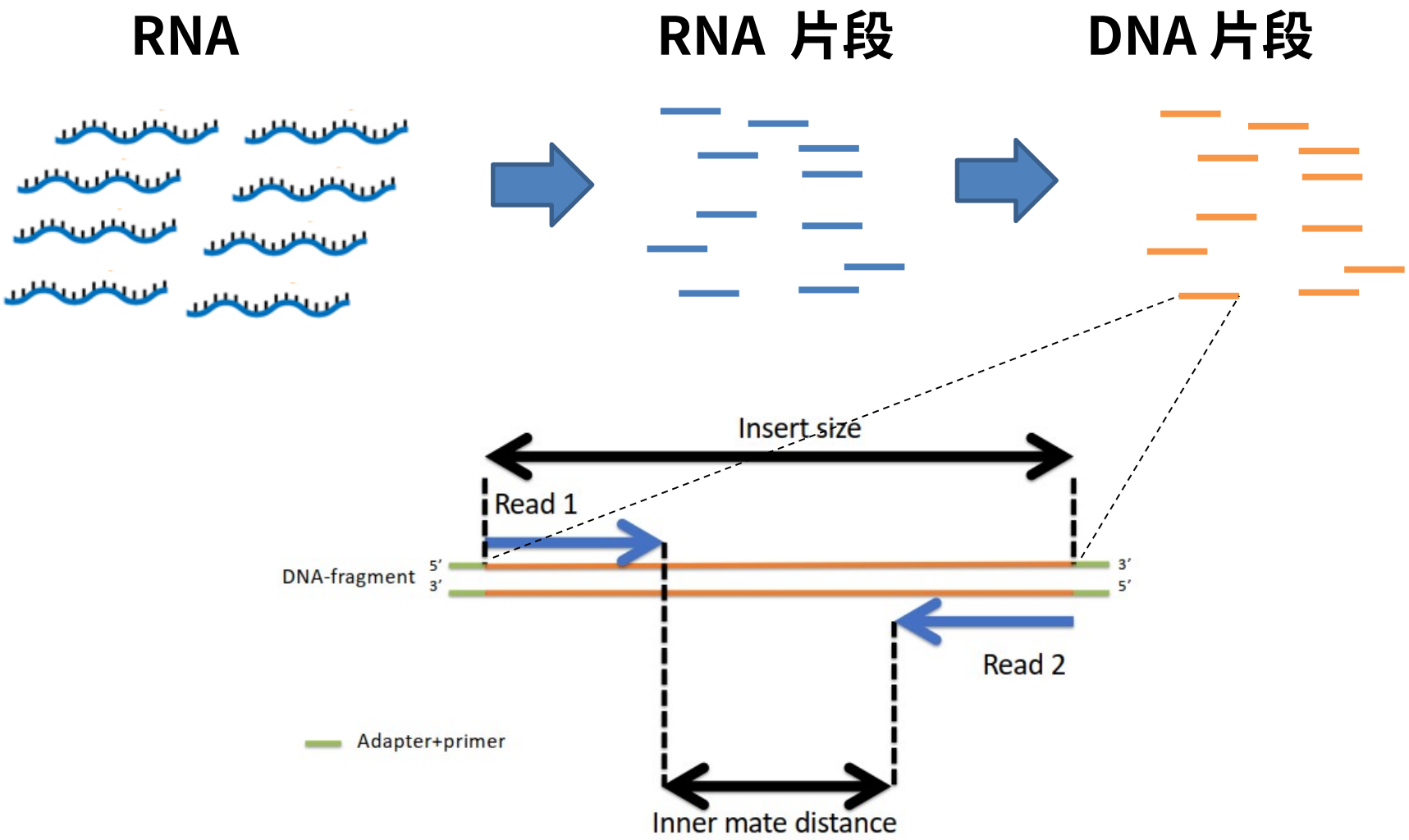
到底来自 A 还是 B？

P 值为什么要矫正?

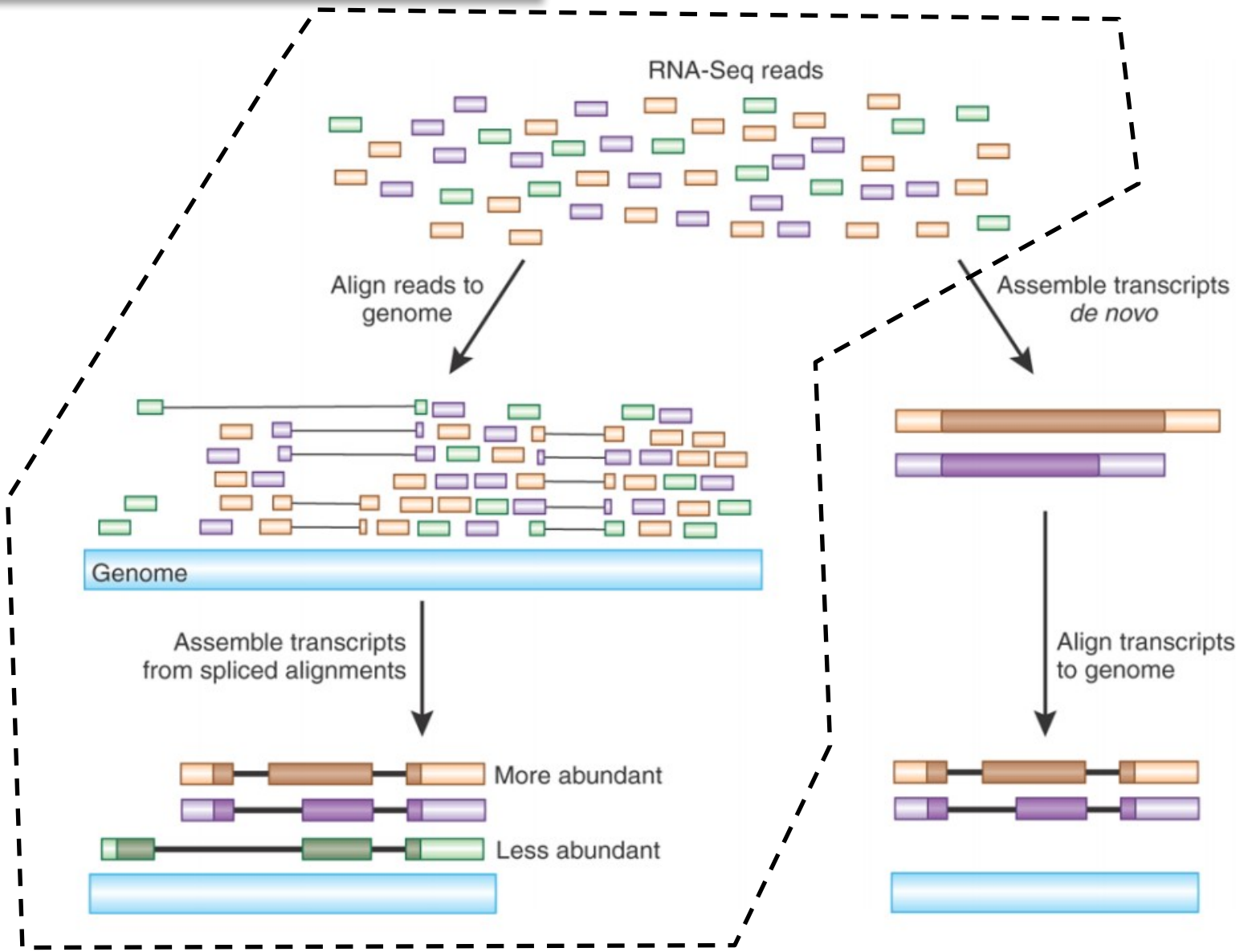
概率密度函数　　统计量



转录组测序


RNA
RNA 片段
DNA 片段
Insert size
Read 1
DNA-fragment
5′
3′
3′
5′
Read 2
Adapter+primer
Inner mate distance

转录组测序


RNA-Seq reads
Align reads to genome
Assemble transcripts de novo
Genome
Assemble transcripts from spliced alignments
Align transcripts to genome
More abundant
Less abundant

测序数据分析

FASTQ

FASTA

GTF / GFF

SAM / BAM

BED

WIG / BIGWIG / BEDGRAPH

MATRIX (EXCEL)

TXT

BWA / BOWTIE2 / HISAT2 / TOPHAT2

SRATOOLS

FASTQC

ComBat

MACS2

BEDTOOLS

FeatureCounts

SAMTOOLS

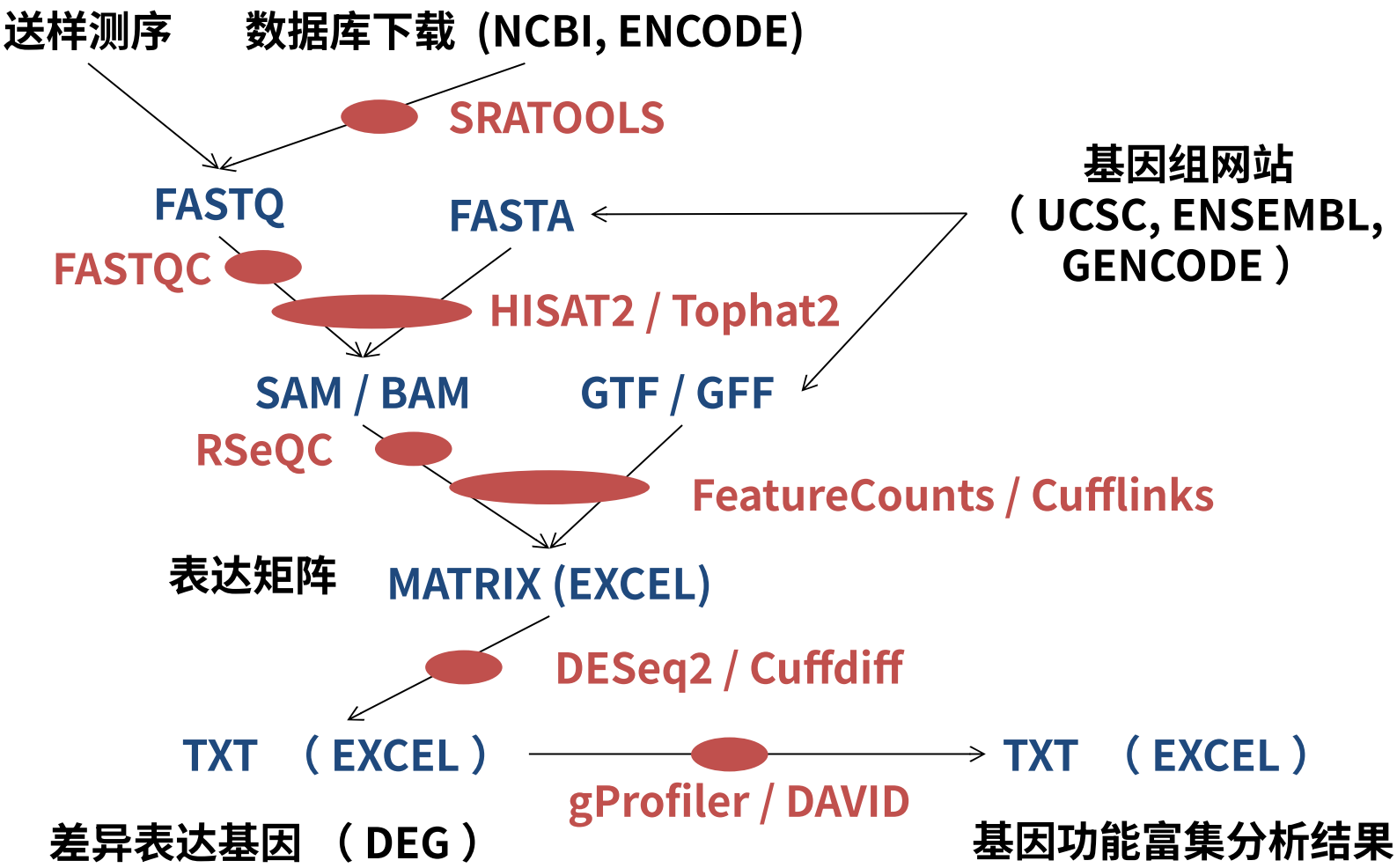
SALMON

CUFFDIFF / DESeq2

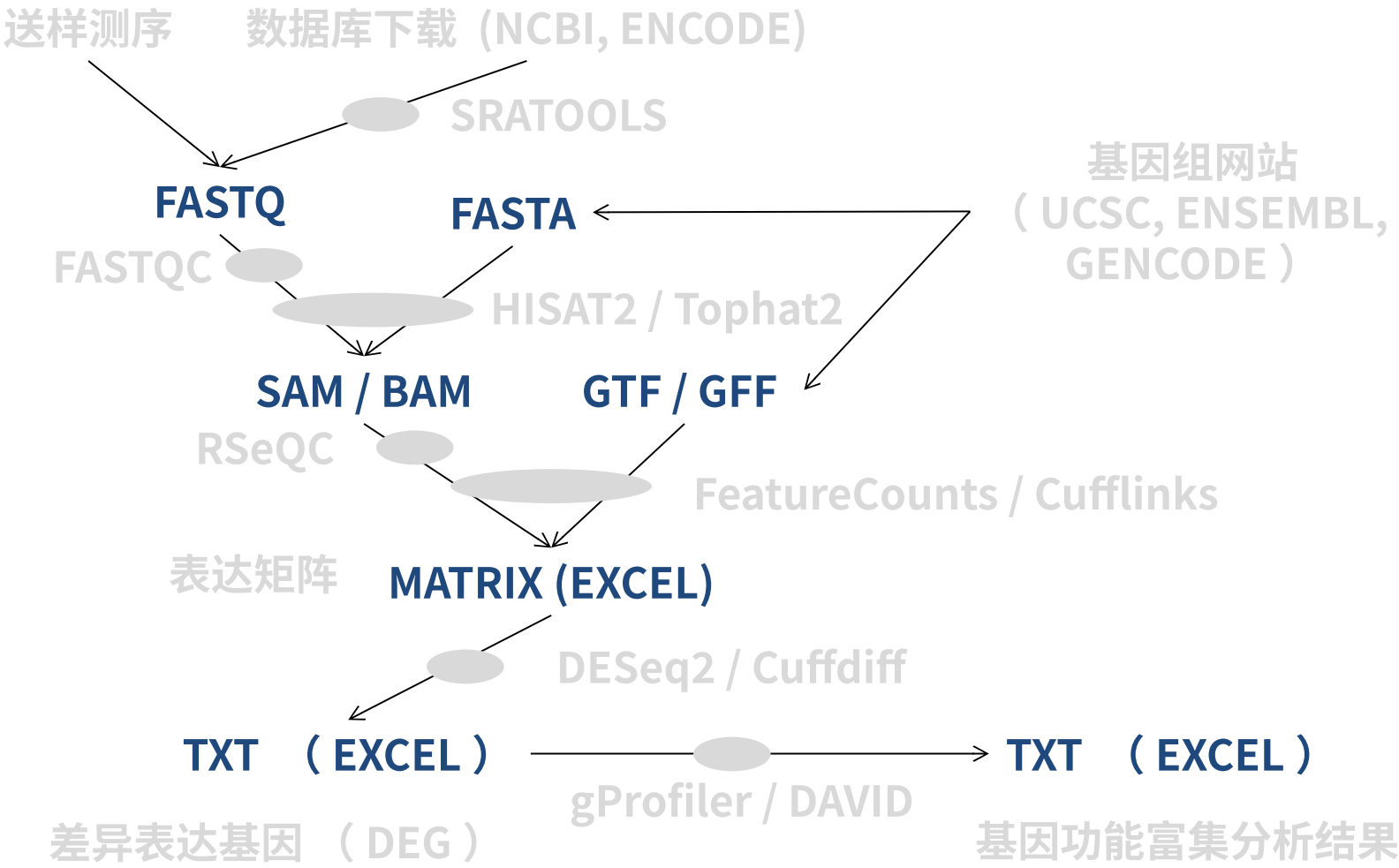
GSEA / gProfiler / DAVID / EnrichR

文件与工具

流程多变，不唯一！！！



文件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FASTQ
FASTA
基因组网站
（ UCSC, ENSEMBL,
GENCODE ）
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FeatureCounts / Cufflinks
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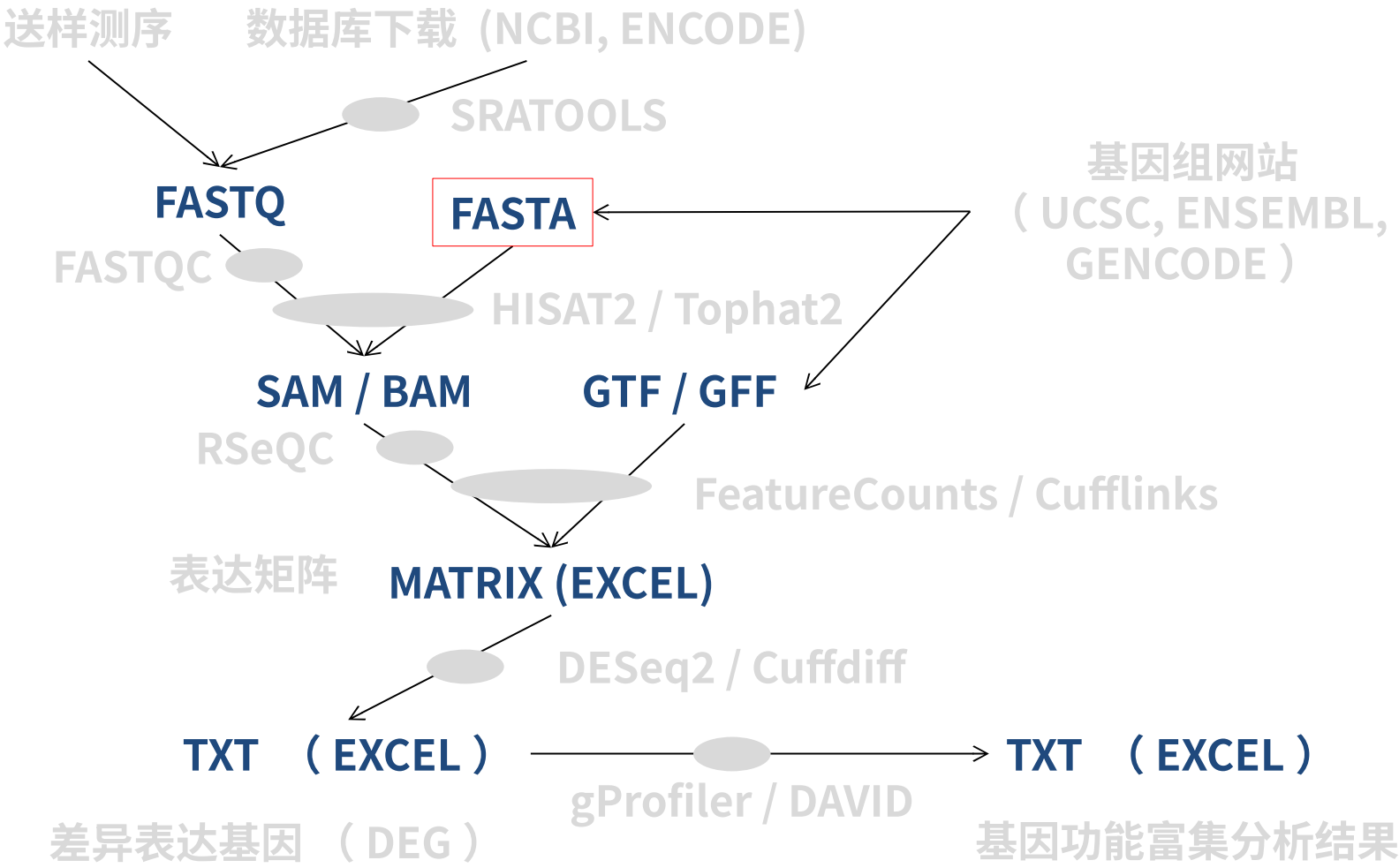
DESeq2 / Cuffdiff
TXT （ EXCEL ）
TXT （ EXCEL ）
gProfiler / DAVID
差异表达基因 （ DEG ）
基因功能富集分析结果

FASTA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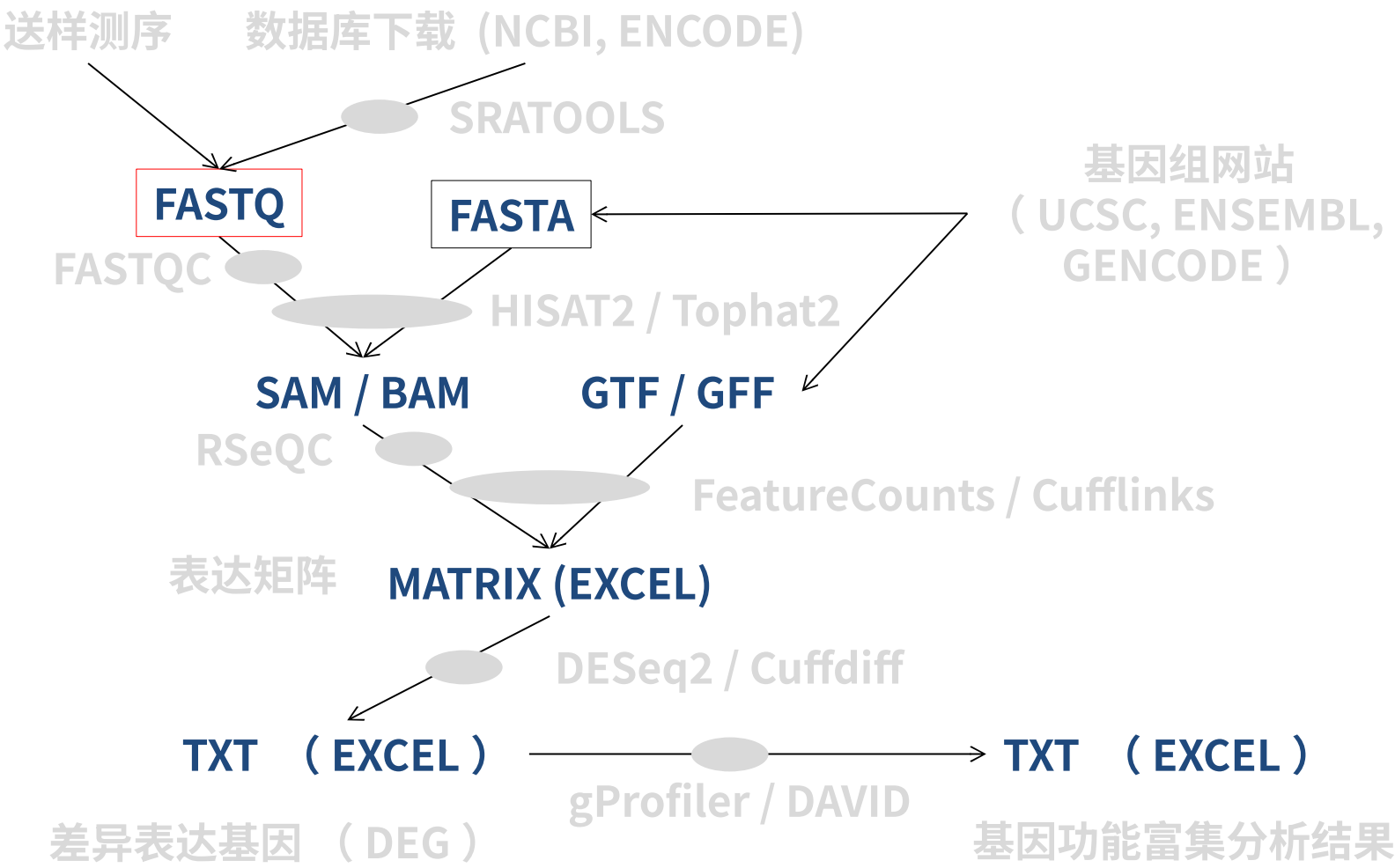
FASTA

注意染色体（或转录本）名字， 注意碱基类型大小写含义，人类基因组碱基数量 ~3GB

一定要留意基因组版本号（比如人类的 hg19 ）

```
>chrM
GATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCTCTCCATGCAT
TTGGTATTTTCGTCTGGGGGGTGTGCACGCGATAGCATTGCGAGACGCTG
                                                ...
CTATTATTTATCGCACCTACGTTCAATATTACAGGCGAACATACCTACTA
AAGTGTGTTAATTAATTAATGCTTGTAGGACATAATAATAACAATTGAAT
GTCTGCACAGCCGCTTTCCACACAGACATCATAACAAAAAATTTCCACCA
>chr1
AACCCCCCCCTCCCCCCGCTTCTGGCCACAGCACTTAAACACATCTCTGC
CAAACCCCAAAAACAAAGAACCCTAACACCAGCCTAACCAGATTTCAAAT
TTTATCTTTAGGCGGTATGCACTTTTAACAGTCACCCCCCAACTAACACA
TTATTTTCCCCTCCCACTCCCATACTACTAATCTCATCAATACAACCCCC
GCCCATCCTACCCAGCACACACACACCGCTGCTAACCCCATACCCCGAAC
                                                ...
```

FASTQ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FASTQ

```
@SRR8179797.116 116 length=151
GGCTAGTG      ...      GCCGTCT
+SRR8179797.116 116 length=151
AAAA.FFFF     ...      F.A<7)..FF7
```

每四行记录了一个读段

文件较大，一般会压缩后存储（ .gz, .sra ）

第三行可以只保留“ +”

碱基测序质量打分（ ASCII 转码）：

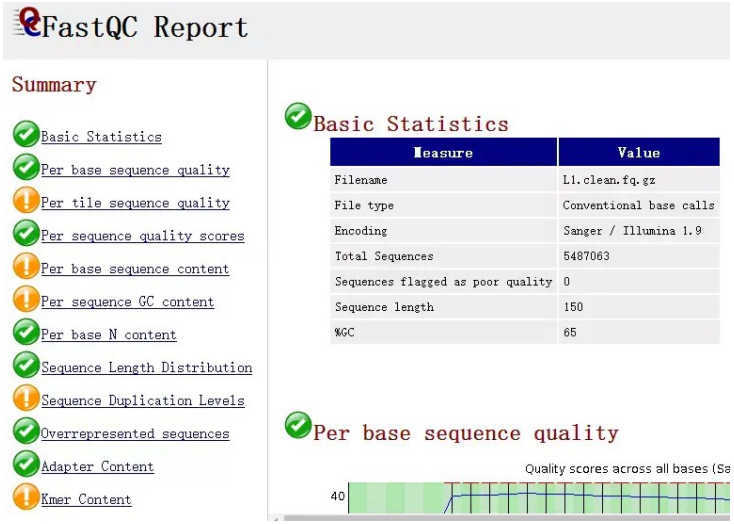
$$Q = S - N \ (N \text{ 一般是 } 33)$$

$$P = 10^{(-Q/10)}$$

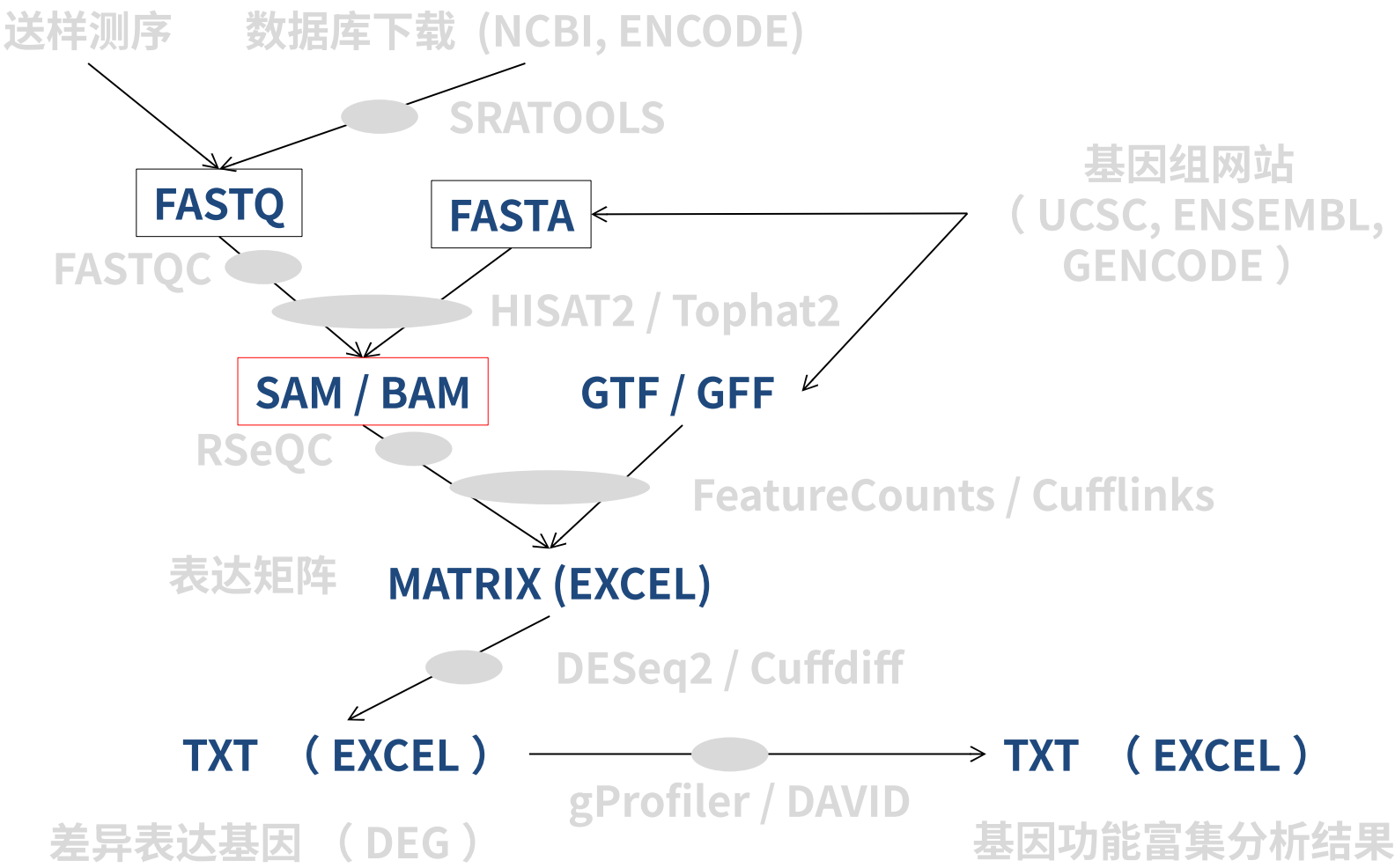
ASCII可显示字符

二进制	十进制	十六进制	图形	二进制	十进制	十六进制	图形	二进制	十进制	十六进制	图形
0010 0000	32	20	(空格)	0100 0000	64	40	@	0110 0000	96	60	`
0010 0001	33	21	!	0100 0001	65	41	A	0110 0001	97	61	a
0010 0010	34	22	"	0100 0010	66	42	B	0110 0010	98	62	b
0010 0011	35	23	#	0100 0011	67	43	C	0110 0011	99	63	c
0010 0100	36	24	$	0100 0100	68	44	D	0110 0100	100	64	d
0010 0101	37	25	%	0100 0101	69	45	E	0110 0101	101	65	e
0010 0110	38	26	&	0100 0110	70	46	F	0110 0110	102	66	f
0010 0111	39	27	'	0100 0111	71	47	G	0110 0111	103	67	g
0010 1000	40	28	(	0100 1000	72	48	H	0110 1000	104	68	h
0010 1001	41	29	)	0100 1001	73	49	I	0110 1001	105	69	i
0010 1010	42	2A	*	0100 1010	74	4A	J	0110 1010	106	6A	j
0010 1011	43	2B	+	0100 1011	75	4B	K	0110 1011	107	6B	k
0010 1100	44	2C	,	0100 1100	76	4C	L	0110 1100	108	6C	l
0010 1101	45	2D	-	0100 1101	77	4D	M	0110 1101	109	6D	m
0010 1110	46	2E	.	0100 1110	78	4E	N	0110 1110	110	6E	n
0010 1111	47	2F	/	0100 1111	79	4F	O	0110 1111	111	6F	o
0011 0000	48	30	0	0101 0000	80	50	P	0111 0000	112	70	p
0011 0001	49	31	1	0101 0001	81	51	Q	0111 0001	113	71	q
0011 0010	50	32	2	0101 0010	82	52	R	0111 0010	114	72	r

质量控制（FASTQC）



SAM / BAM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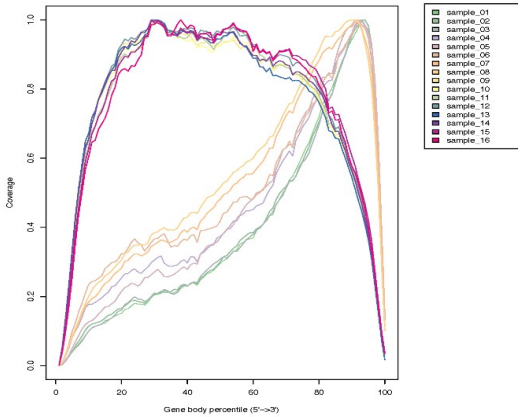
SAM / BAM

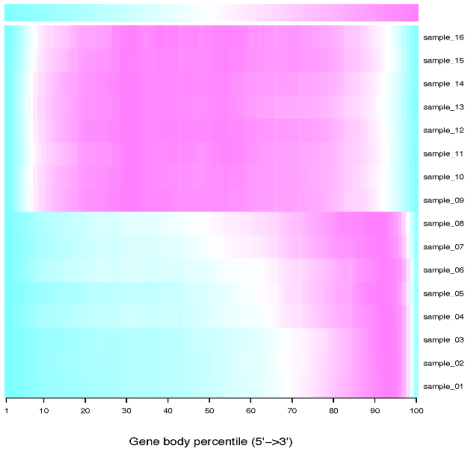
SAM 文件记录了每一个读段“如何”比对到“何处”，也保存了碱基类型和质量信息

SRR8179797.52782089 99 chrM 44 60 143M8S = 44 -159
CATGCATTTGGTATTTTCGTCTGGGGGGTGTGCACGCGATAGCATTGCGAGACGCTGGAGCCGG
AGCACCTTATGTCGCAGTATCTGTCTTTGATTCCTGCCTCATCCTATTATTTATAGCACCTACGTTCA
ATATTACAGGCAGATCGGA
AAAAAAFF.FFFFFF<FFFFFFF7FFAFFFFFFAFFFFFFAFFFFAAFFFFFF<FFFFAAFFFFFF<)FFFFF7A
<FFF<FF.FFF7FFFFFFFFF7<FFFFFF.FFF.AFF<F<FAF)FFFFF<F<<AFFAFFF7A7FF7<F.FFFFF<7
AS:i:-20 XN:i:0 XM:i:3 XO:i:0 XG:i:0 NM:i:3 MD:Z:70C35T11C24 YS:i:-18
YT:Z:CP NH:i:1

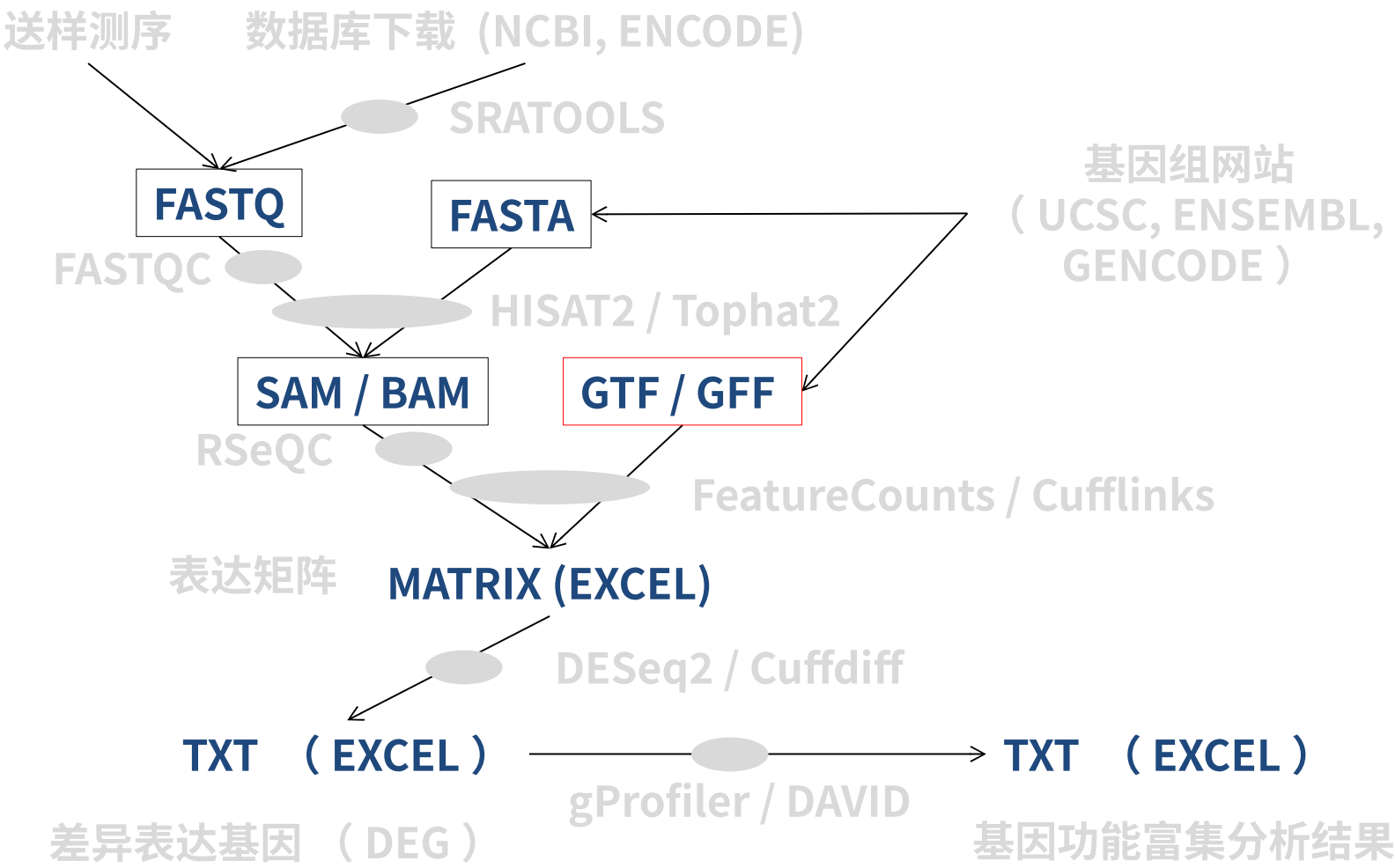
SAM 文件较大，一般使用 SAMTOOLS 将 SAM 格式转化为二进制的 BAM 格式

质量控制（QC）





GTF / GFF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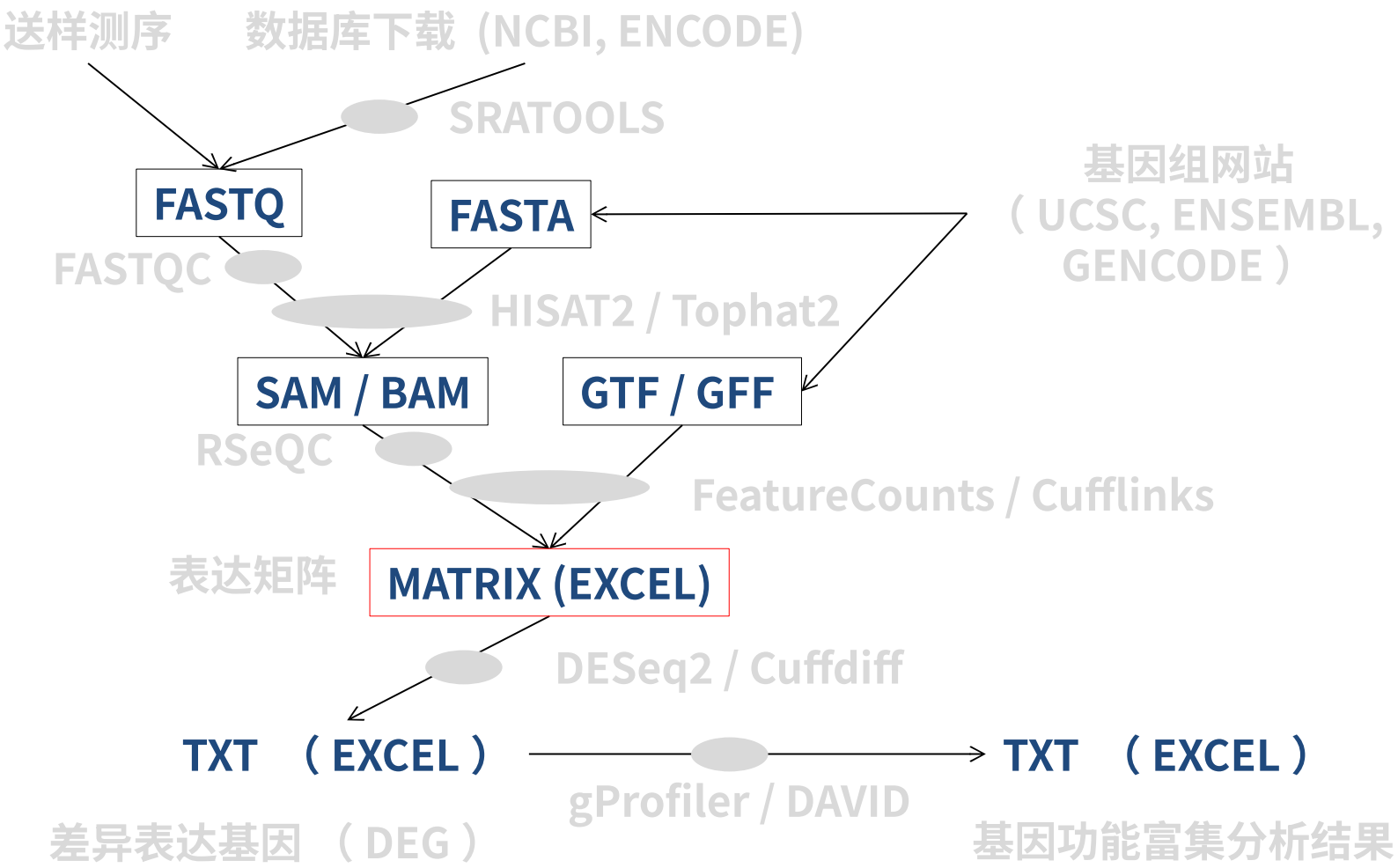
基因功能富集分析结果

GTF / GFF

GTF 文件记录了每一个基因组元件的 “性质” 和 “位置”

```
chr1    protein_coding  CDS     69091   70005   .       +
0       gene_id "ENSG00000186092"; transcript_id
"ENST00000335137"; exon_number "1"; gene_name
"OR4F5"; gene_source "ensembl_havana";
gene_biotype "protein_coding"; transcript_name
"OR4F5-001"; transcript_source "ensembl_havana";
tag "CCDS"; ccds_id "CCDS30547"; protein_id
"ENSP00000334393";
```

MATRIX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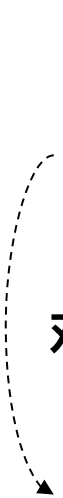
MATRIX 表达

读段个数矩阵 （ read count matrix ）

	Sample1	Sample2	Sample3	...
Gene1	5	9	1	
Gene2	353	247	271	
Gene3	3777	2364	25	
...				

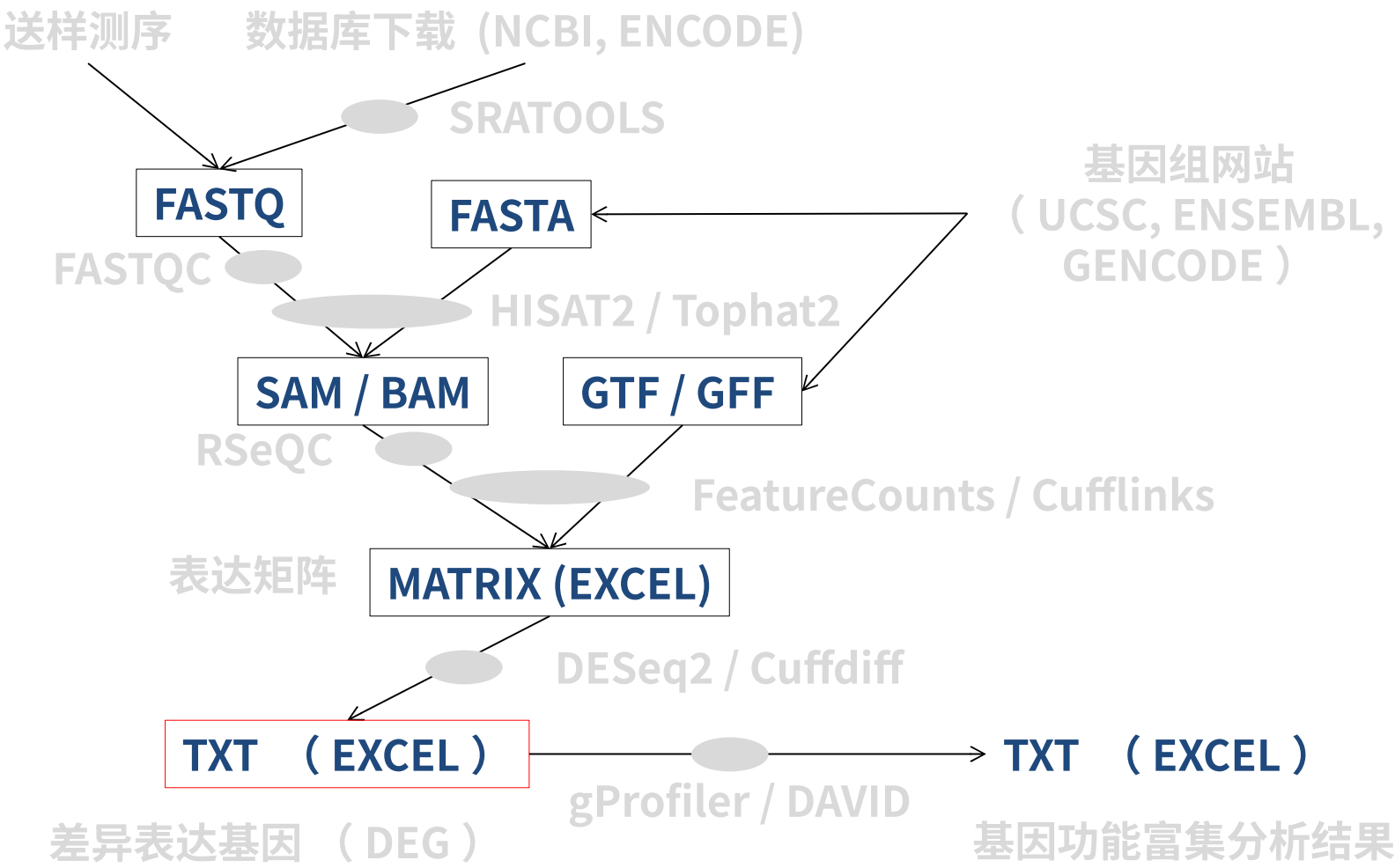


归一化 FPKM / RPKM / RPM / TPM — 不同样本同基因可比

对数变换 — 类正态，易做检验

标准化 每一行减去 均值 除以 标准差 — PCA / Heatmap

TXT 差异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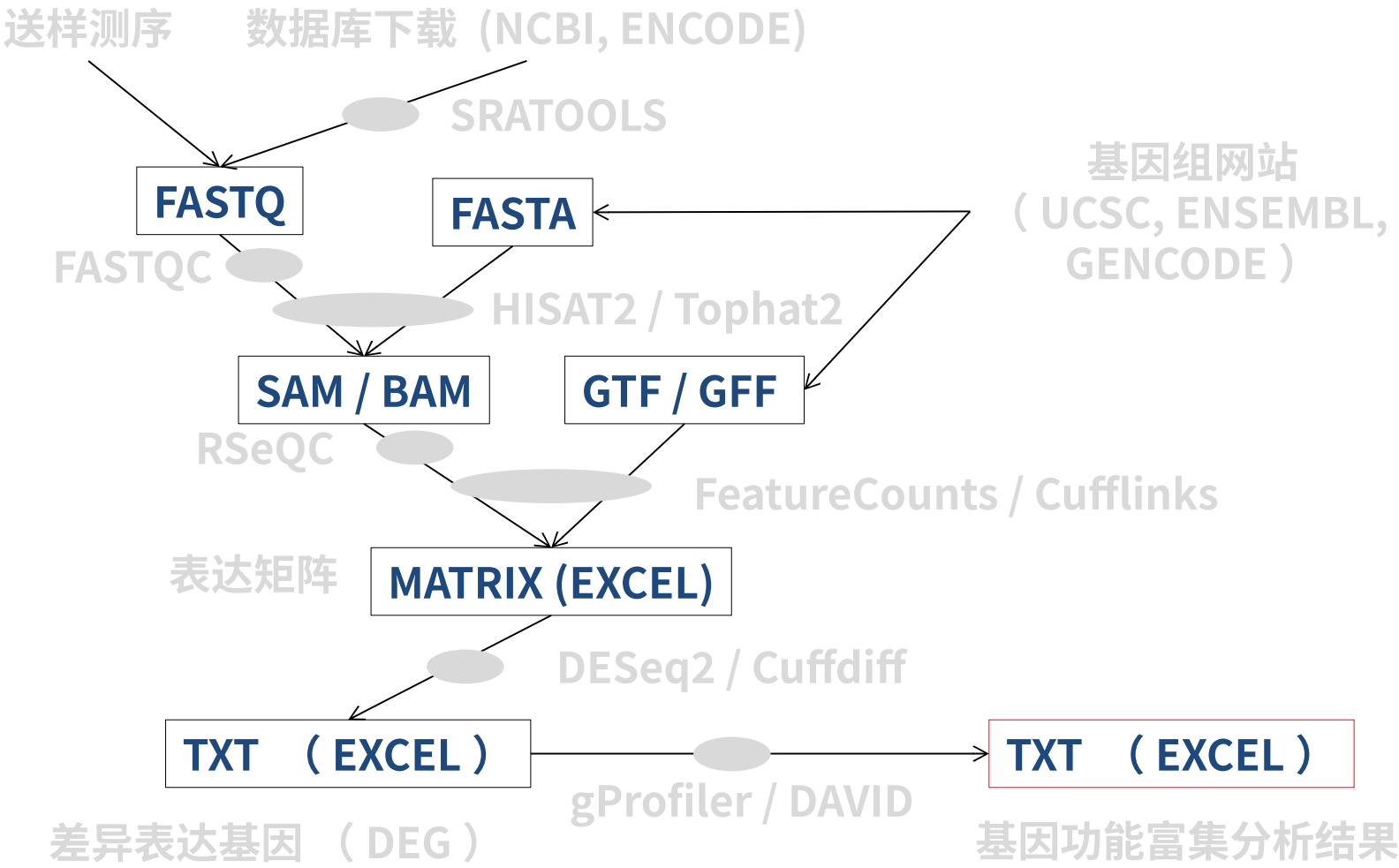
TXT 差异

一般情况下， Fold Change 和 Pvalue (Padj) 都要考虑

DESeq2 结果

	Case 1	Case 2	Ctrl 1	Ctrl 2	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Cx3cr1	13	7	363	691	309.4573221	-6.253625195	0.51093498	-12.2396	1.91E-34	2.43E-30
5830411N06Rik	1082	821	8	20	391.3886748	5.589308195	0.472684251	11.82461	2.91E-32	1.86E-28
Igkc	50	20	3819	1850	2027.636391	-7.19943295	0.611847719	-11.7667	5.79E-32	2.46E-28
Cd163l1	1147	912	17	20	427.7605469	5.216160719	0.45457985	11.47469	1.77E-30	5.63E-27
Jchain	8	8	1442	651	753.2178054	-7.844916069	0.693993672	-11.304	1.25E-29	3.19E-26
Zeb2	18	7	255	750	278.0539922	-5.789824356	0.539203416	-10.7377	6.77E-27	1.44E-23
Gzma	33	30	915	715	555.5517287	-5.402705083	0.516218347	-10.4659	1.24E-25	2.25E-22
Gzmk	10	12	204	383	175.0640247	-5.243874941	0.529835716	-9.89717	4.28E-23	6.82E-20
S1pr5	25	15	327	367	226.5920639	-4.769446278	0.509896752	-9.35375	8.46E-21	1.20E-17

TXT 富集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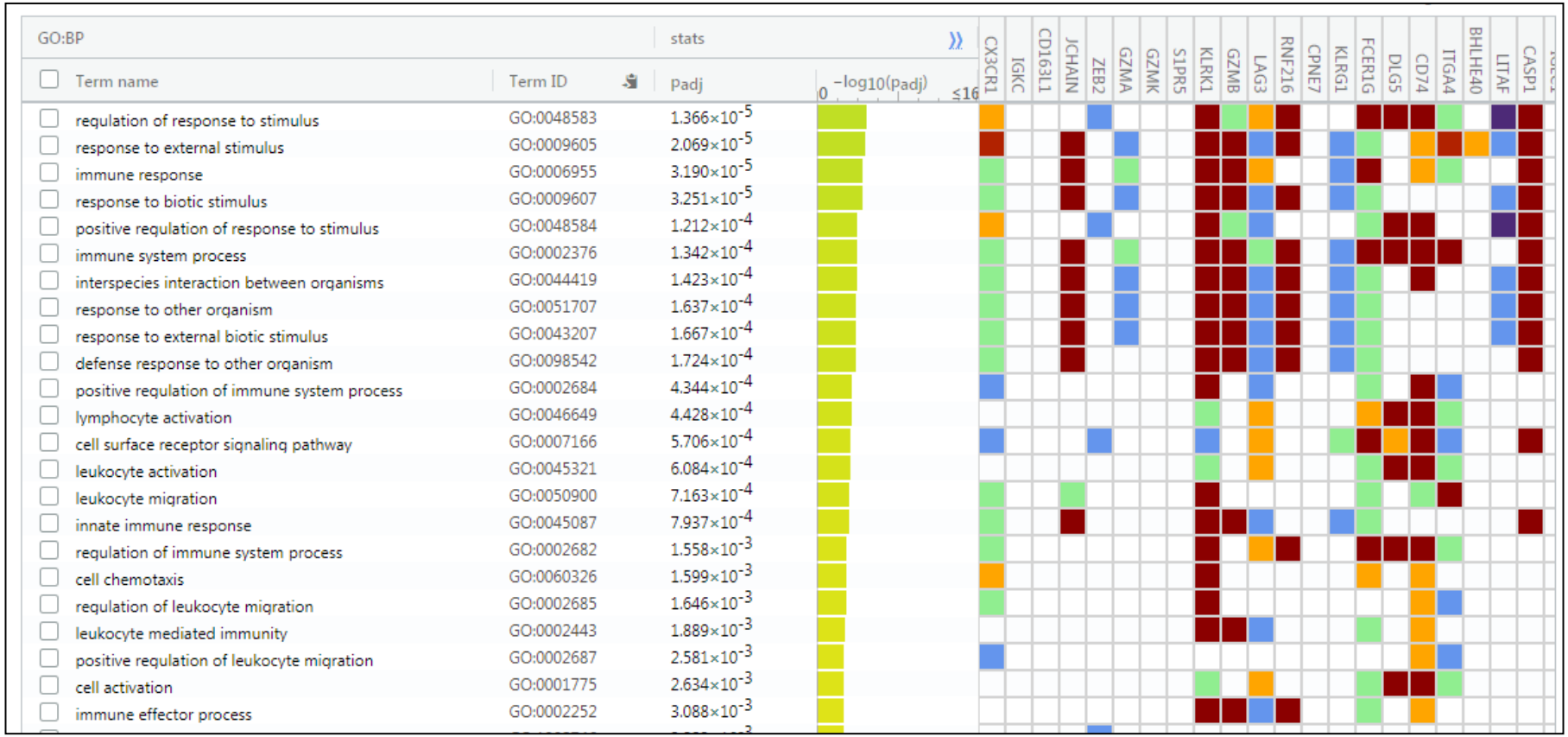
TXT 富集

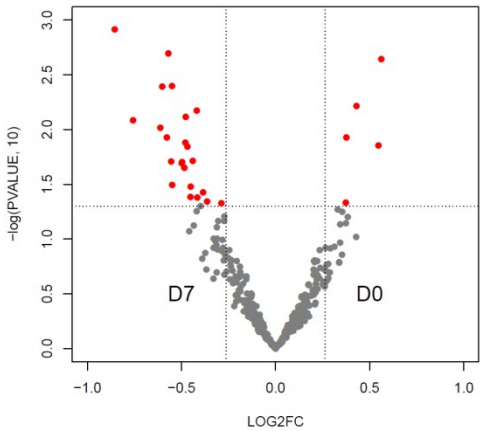
gProfiler 结果

一般情况下， 只考虑 Padj ，也可以综合考虑基因个数

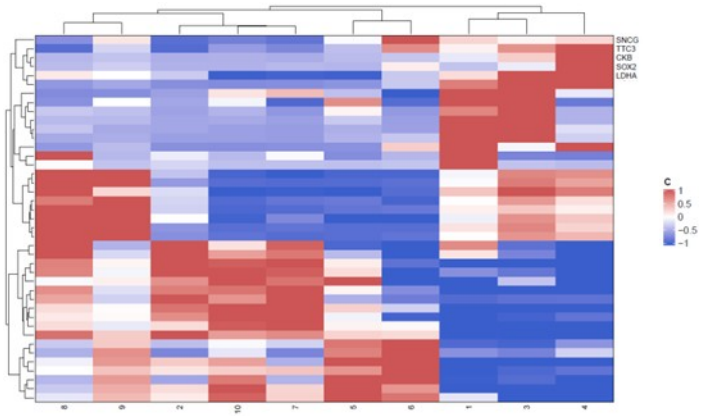


可视化

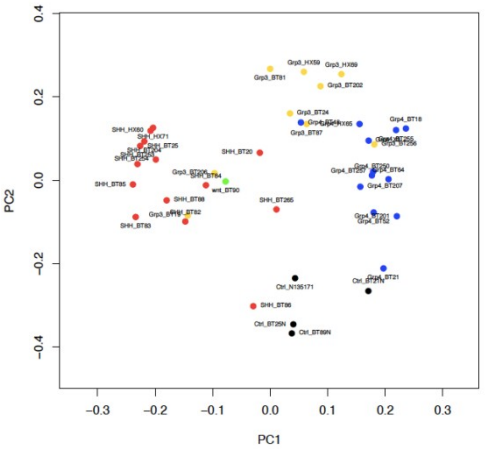
第二课内容



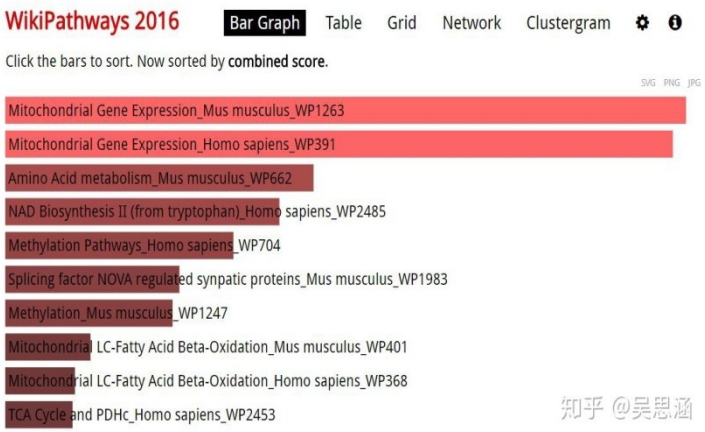
火山图



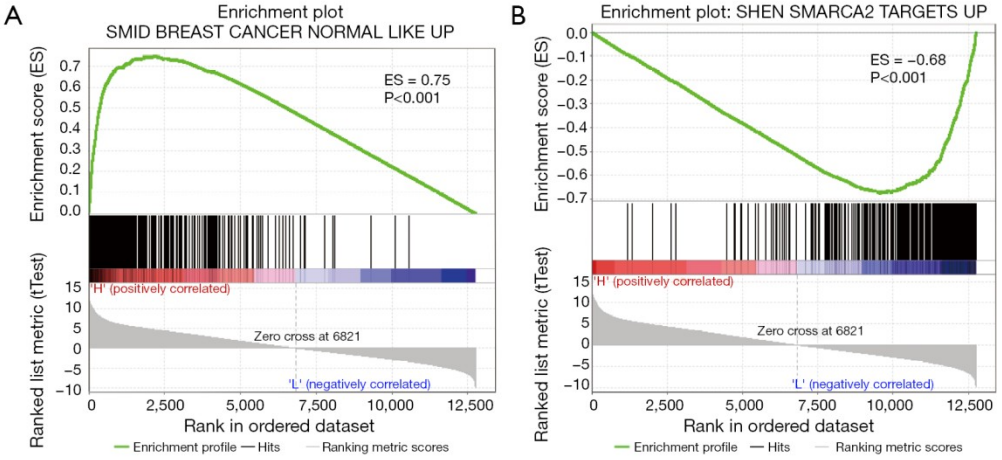
热图



PCA



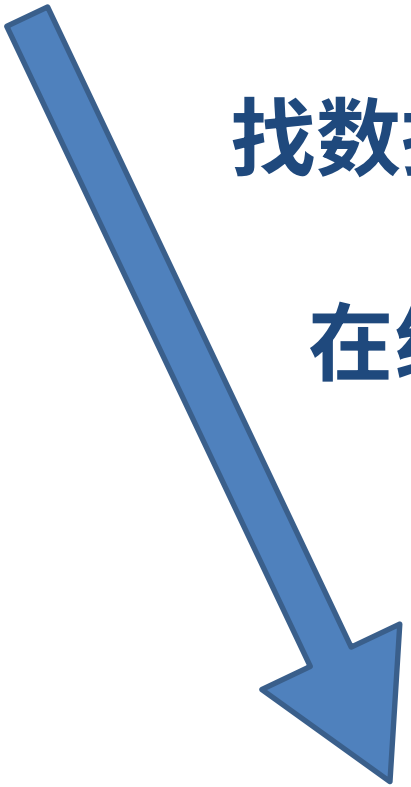
富集柱状图



GSEA

临床应用

想法 （idea）



找数据?

在线分析?

可视化?

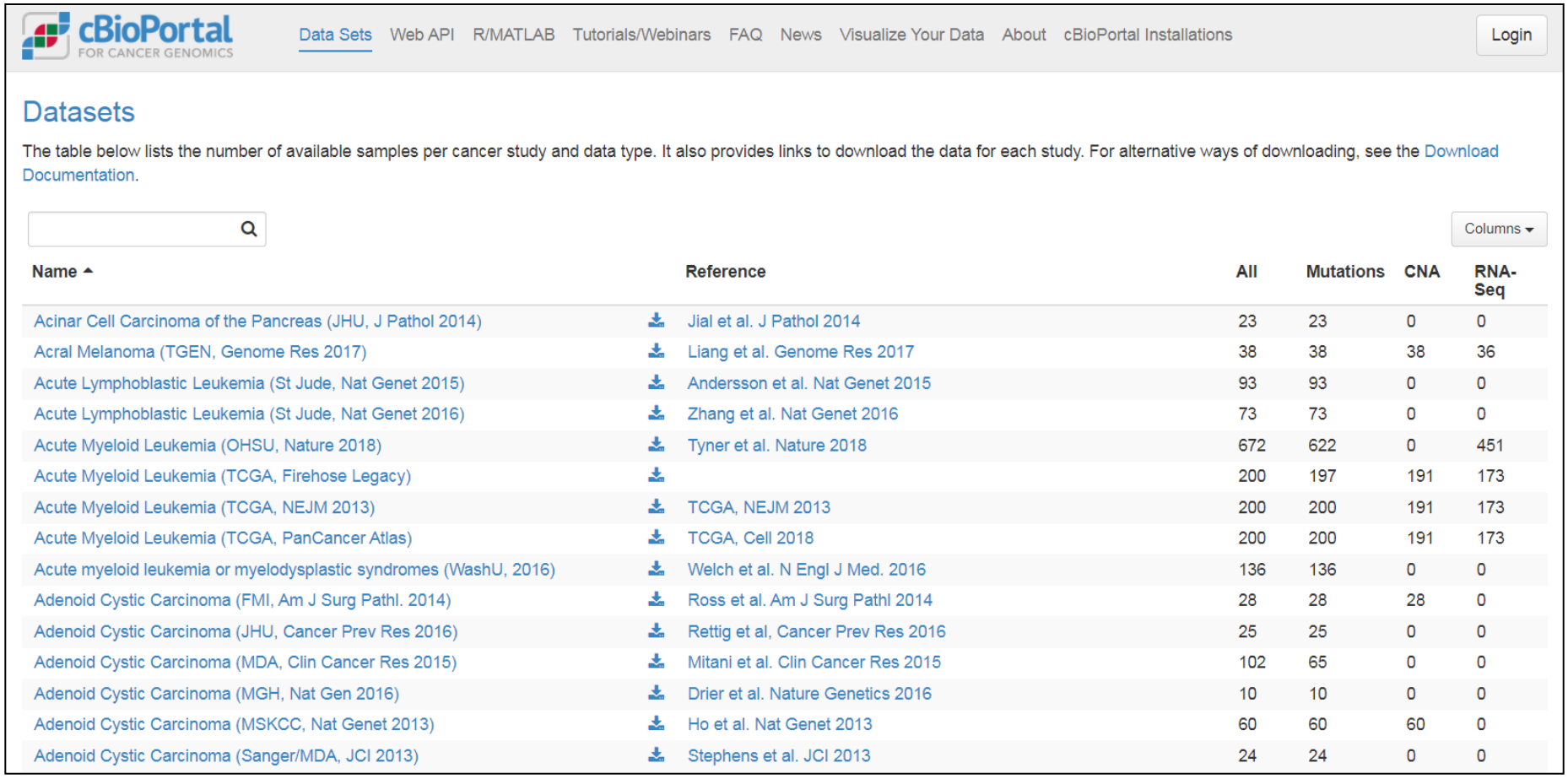
产出 （ppt， paper， funding）

临床应用 1

找数据

癌症 + 表达矩阵 + 临床信息

http://www.cbioportal.org/datasets



cBioPortal FOR CANCER GENOMICS

Data Sets | Web API | R/MATLAB | Tutorials/Webinars | FAQ | News | Visualize Your Data | About | cBioPortal Installations | Login

Datasets

The table below lists the number of available samples per cancer study and data type. It also provides links to download the data for each study. For alternative ways of downloading, see the Download Documentation.

Columns

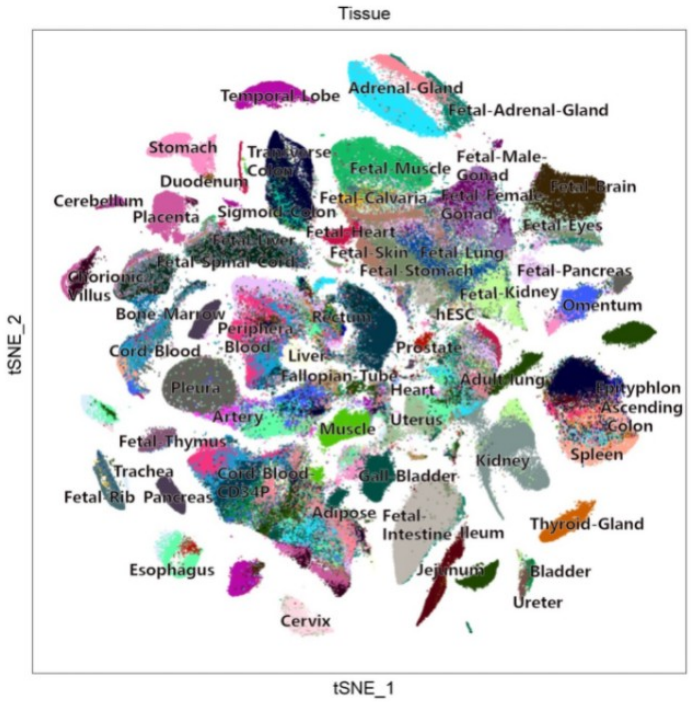
Name	Reference	All	Mutations	CNA	RNA-Seq
Acinar Cell Carcinoma of the Pancreas (JHU, J Pathol 2014)	Jial et al. J Pathol 2014	23	23	0	0
Acral Melanoma (TGEN, Genome Res 2017)	Liang et al. Genome Res 2017	38	38	38	36
Acute Lymphoblastic Leukemia (St Jude, Nat Genet 2015)	Andersson et al. Nat Genet 2015	93	93	0	0
Acute Lymphoblastic Leukemia (St Jude, Nat Genet 2016)	Zhang et al. Nat Genet 2016	73	73	0	0
Acute Myeloid Leukemia (OHSU, Nature 2018)	Tyner et al. Nature 2018	672	622	0	451
Acute Myeloid Leukemia (TCGA, Firehose Legacy)		200	197	191	173
Acute Myeloid Leukemia (TCGA, NEJM 2013)	TCGA, NEJM 2013	200	200	191	173
Acute Myeloid Leukemia (TCGA, PanCancer Atlas)	TCGA, Cell 2018	200	200	191	173
Acute myeloid leukemia or myelodysplastic syndromes (WashU, 2016)	Welch et al. N Engl J Med. 2016	136	136	0	0
Adenoid Cystic Carcinoma (FMI, Am J Surg Pathl. 2014)	Ross et al. Am J Surg Pathl 2014	28	28	28	0
Adenoid Cystic Carcinoma (JHU, Cancer Prev Res 2016)	Rettig et al, Cancer Prev Res 2016	25	25	0	0
Adenoid Cystic Carcinoma (MDA, Clin Cancer Res 2015)	Mitani et al. Clin Cancer Res 2015	102	65	0	0
Adenoid Cystic Carcinoma (MGH, Nat Gen 2016)	Drier et al. Nature Genetics 2016	10	10	0	0
Adenoid Cystic Carcinoma (MSKCC, Nat Genet 2013)	Ho et al. Nat Genet 2013	60	60	60	0
Adenoid Cystic Carcinoma (Sanger/MDA, JCI 2013)	Stephens et al. JCI 2013	24	24	0	0

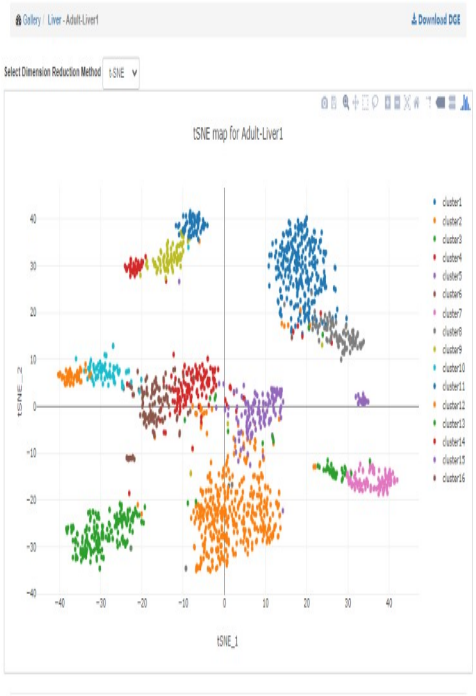
临床应用 2

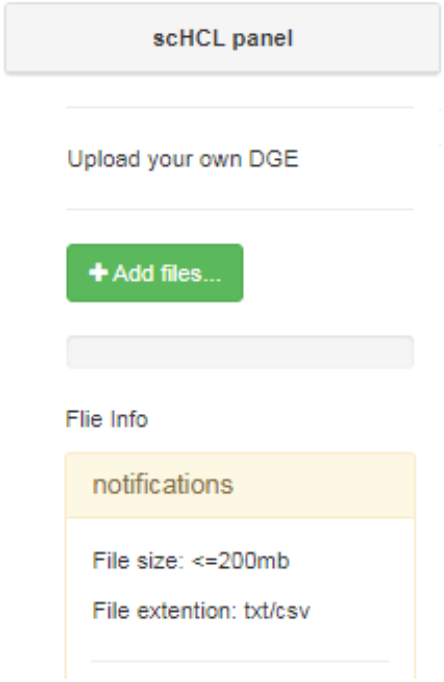
找数据

单细胞转录组数据（ HCL ）

https://db.cngb.org/HCL/index.html



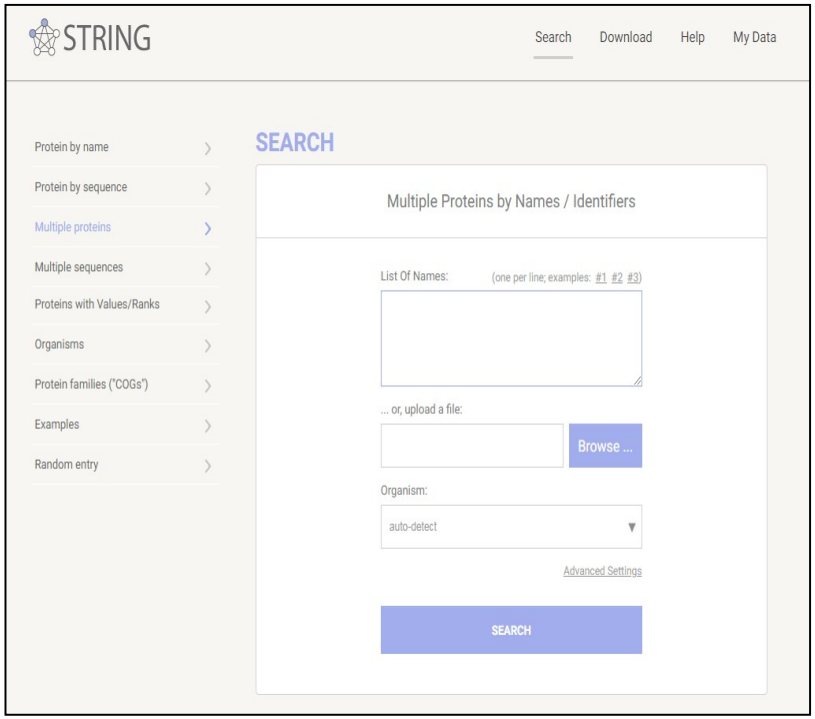


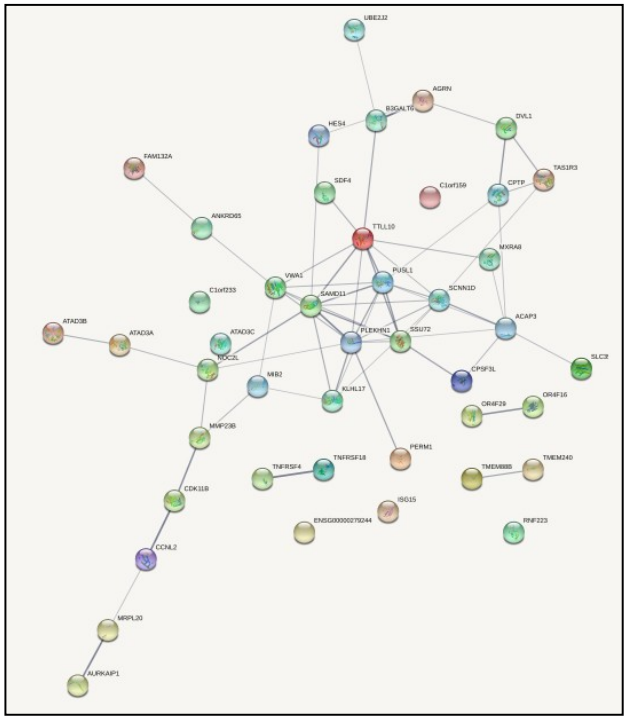


临床应用 3

在线分析

关系网络绘制 https://string-db.org/





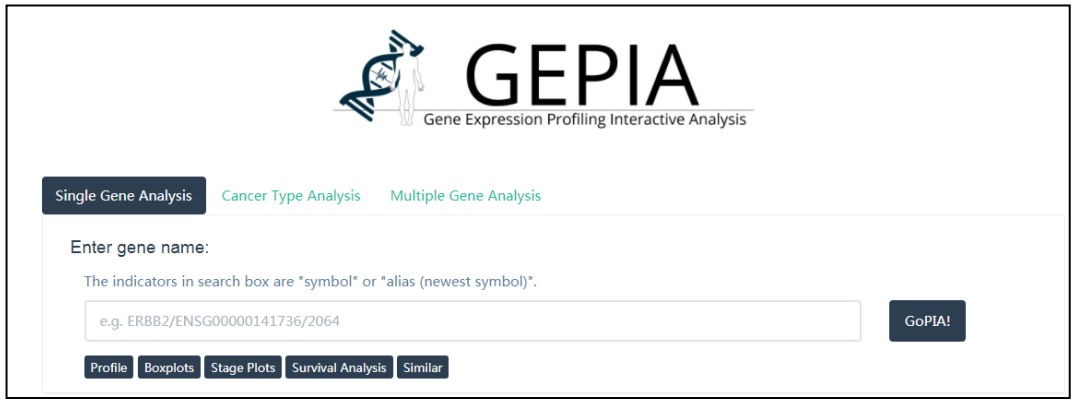
CytoScape

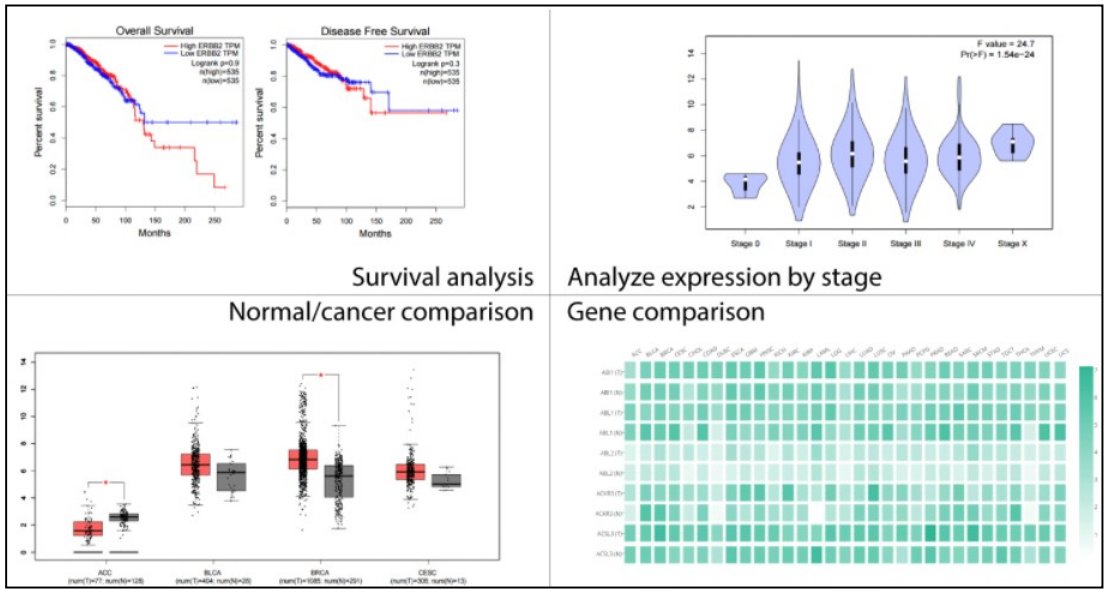
临床应用 4

在线分析

http://gepia.cancer-pku.cn/

TCGA 数据在线分析



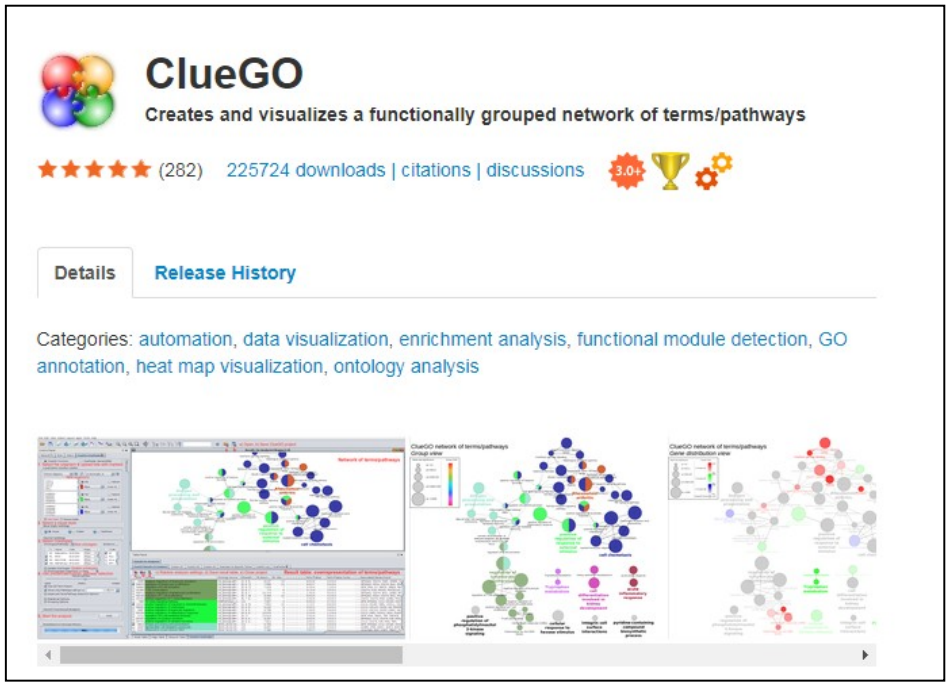


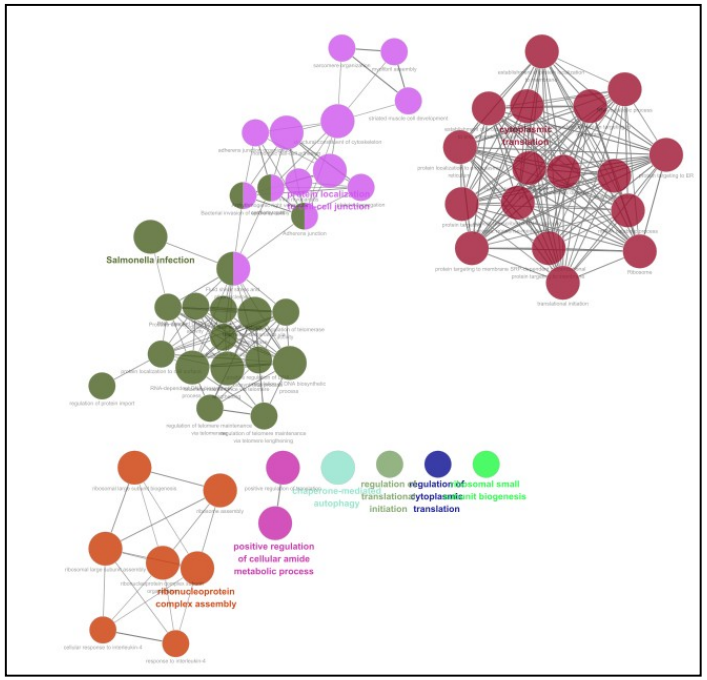
临床应用 5

可视化

富集 + 网络展示 ClueGo

https://apps.cytoscape.org/apps/cluego





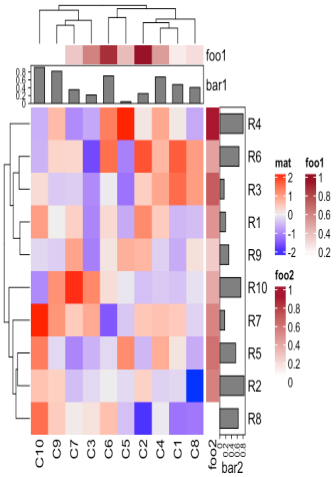
临床应用 6

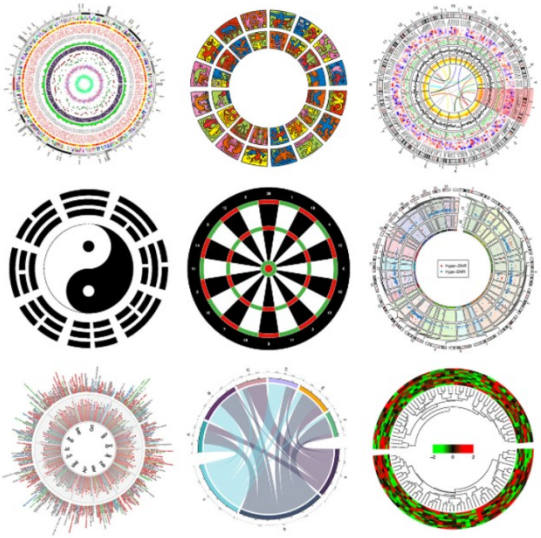
可视化

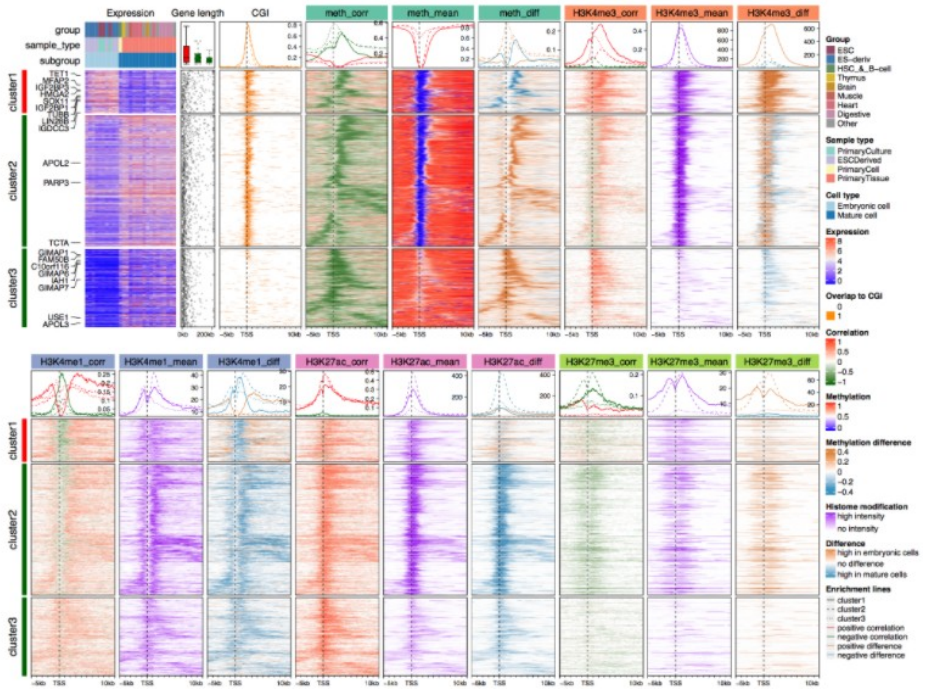
ComplexHeatmap + Circlize + EnrichedHeatmap + …

Zuguang Gu

https://github.com/jokergoo







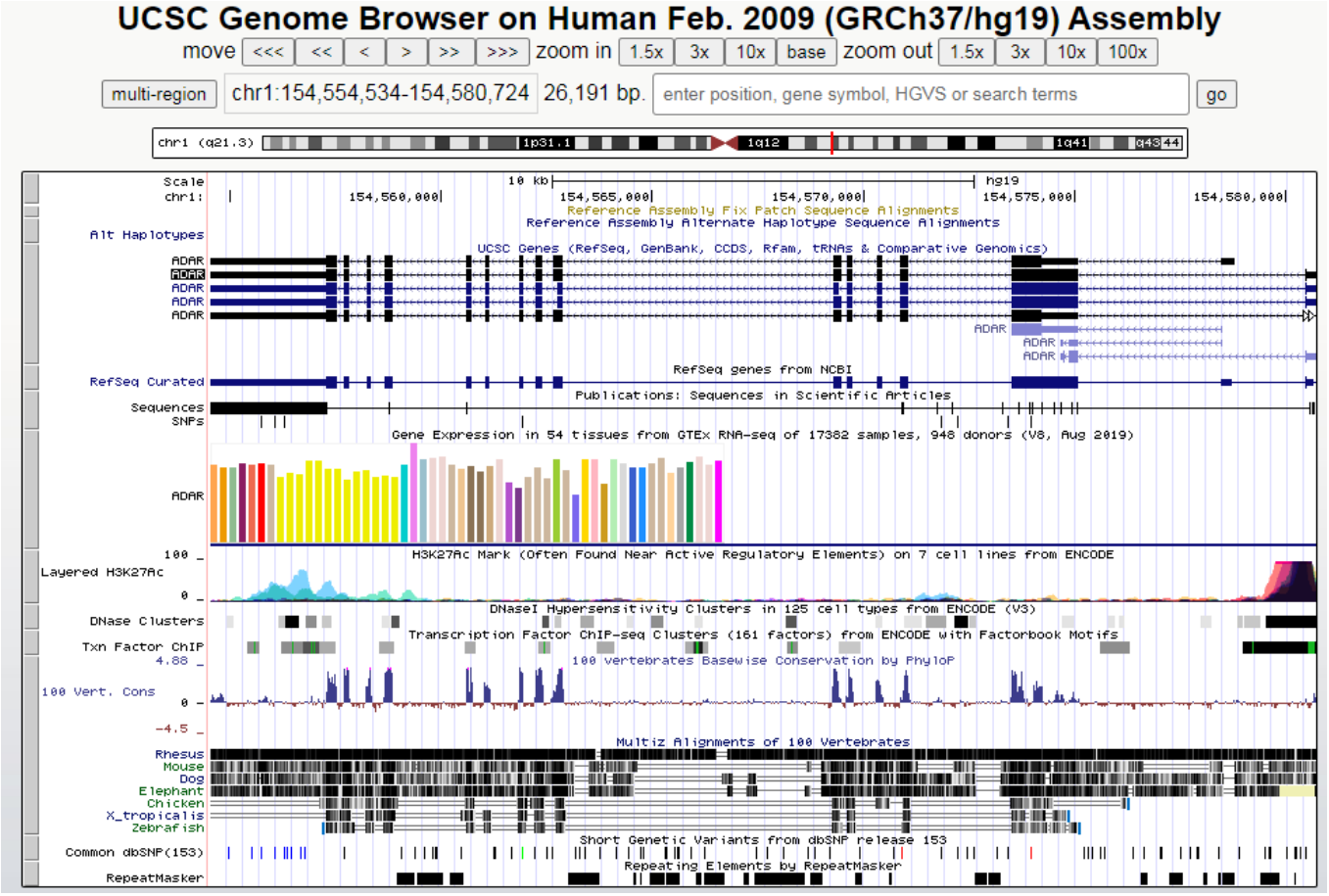
临床应用 7

可视化

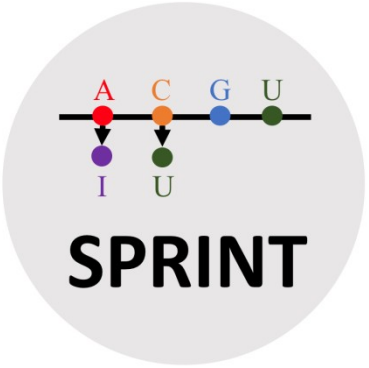
UCSC + WashU + IGV

http://genome.ucsc.edu/
http://epigenomegateway.wustl.edu/
https://software.broadinstitute.org/software/igv/

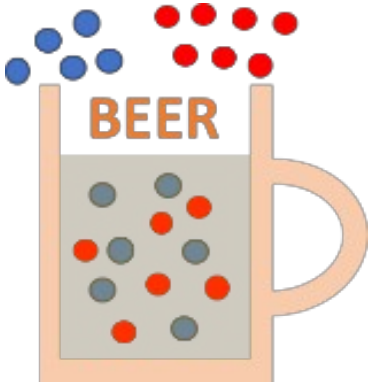
Genome Viewer



介绍研究



RNA 编辑位点鉴定

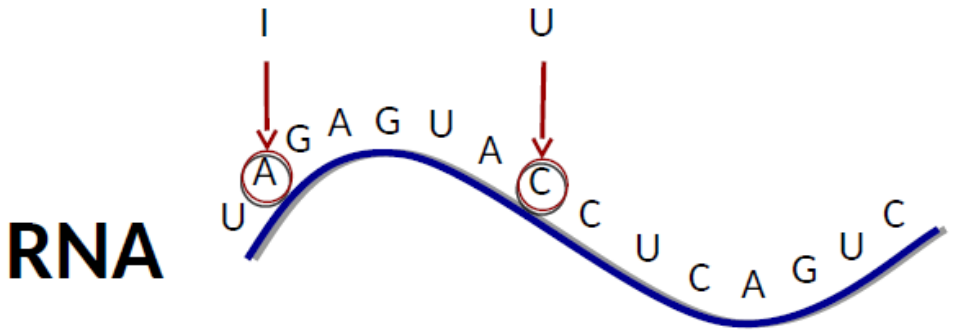


单细胞批次效应

SPRINT

RNA Editing : RNA editing generates post-transcriptional sequence alterations, primarily the modification of RNA nucleotides[1] 。

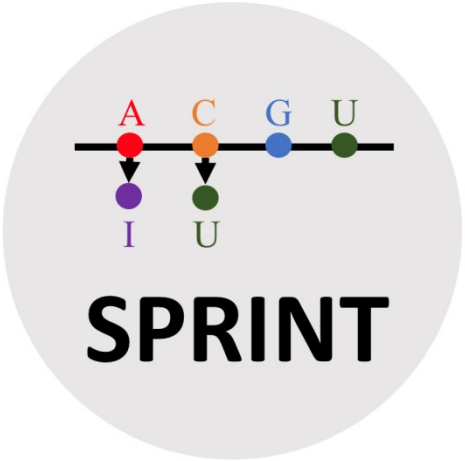
Primary types : adenosine-to-inosine (A-to-I, detected as A-to-G)[2] 、 cytosine-to-uracil (C-to-U, detected as C-to-T)[3]



1. Farajollahi, S. & Maas, S. Molecular diversity through RNA editing: a balancing act. *Trends Genet.*
2. Zipeto, M. A., Jiang, Q., Melese, E. & Jamieson, C. H. RNA rewriting, recoding, and rewiring in human disease. *Trends in molecular medicine*
3. Blanc, V. *et al.* Genome-wide identification and functional analysis of Apobec-1-mediated C-to-U RNA editing in mouse small intestine and liver. *Genome biology*

SPRINT

https://github.com/jumphone/SPRINT



输入：RNA-seq 测序数据 (.fastq)

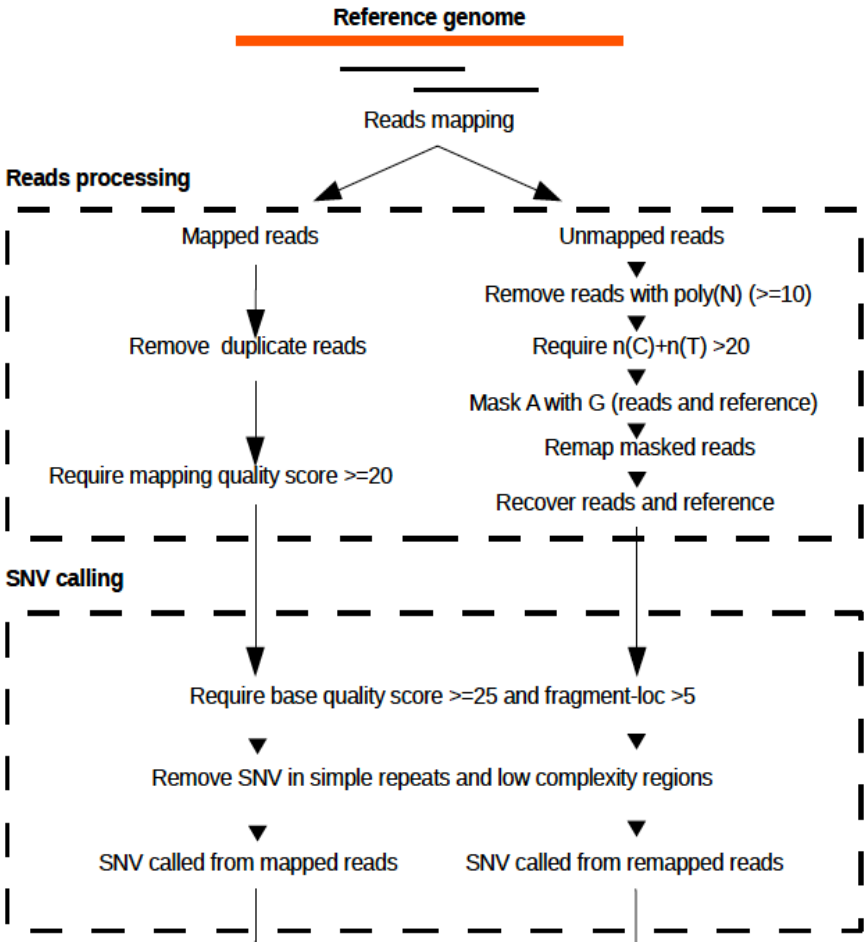
输出：RNA 编辑位点 (BED)

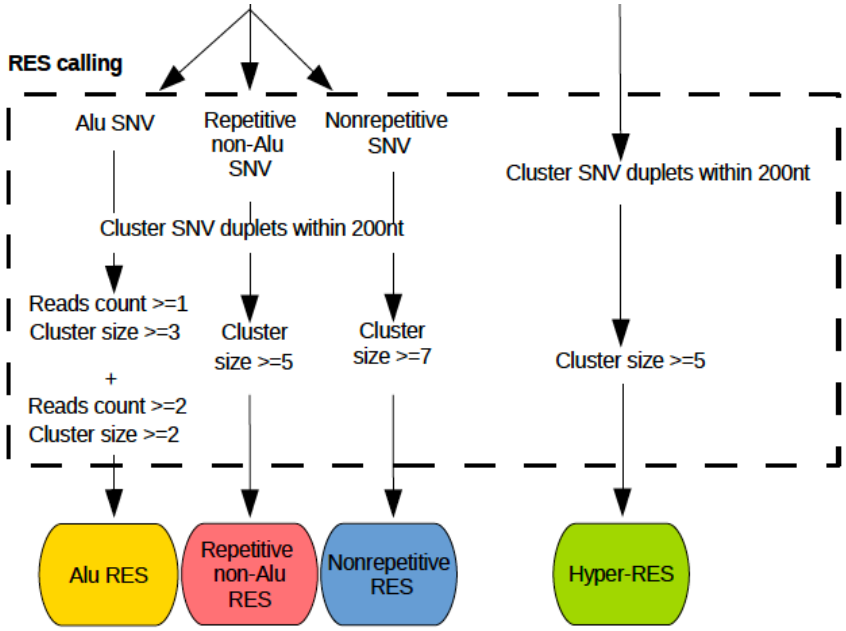
SN**P**-free **R**NA editing **I**dentification **T**oolkit

Ref. SPRINT: an SNP-free toolkit for identifying RNA editing sites, ***Bioinformatics***, 2017

SPRINT

https://github.com/jumphone/SPRINT





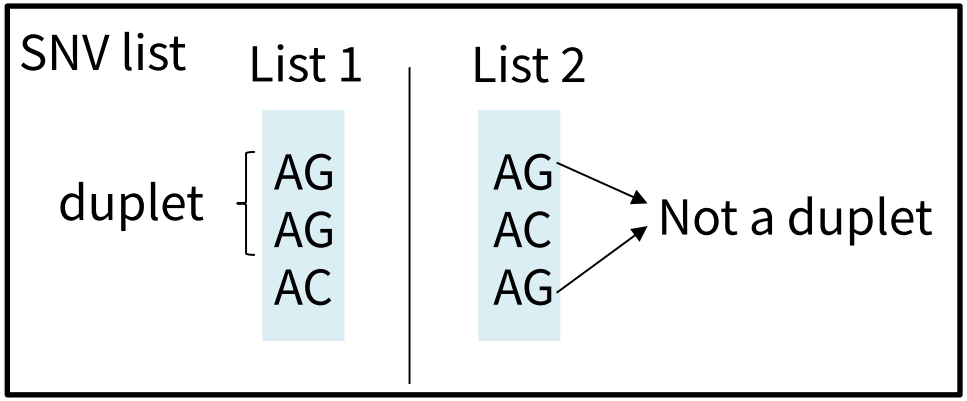
SPRINT consists of three major steps:

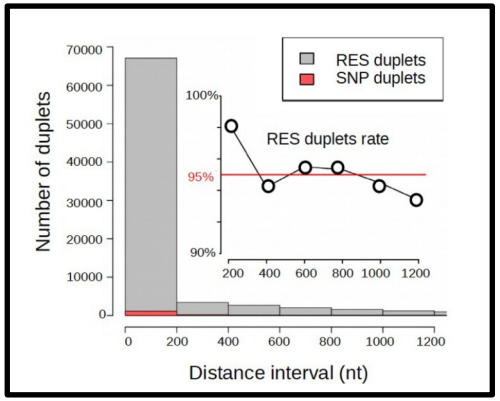
1. **Reads processing**
2. **SNV calling**
3. **RES calling**

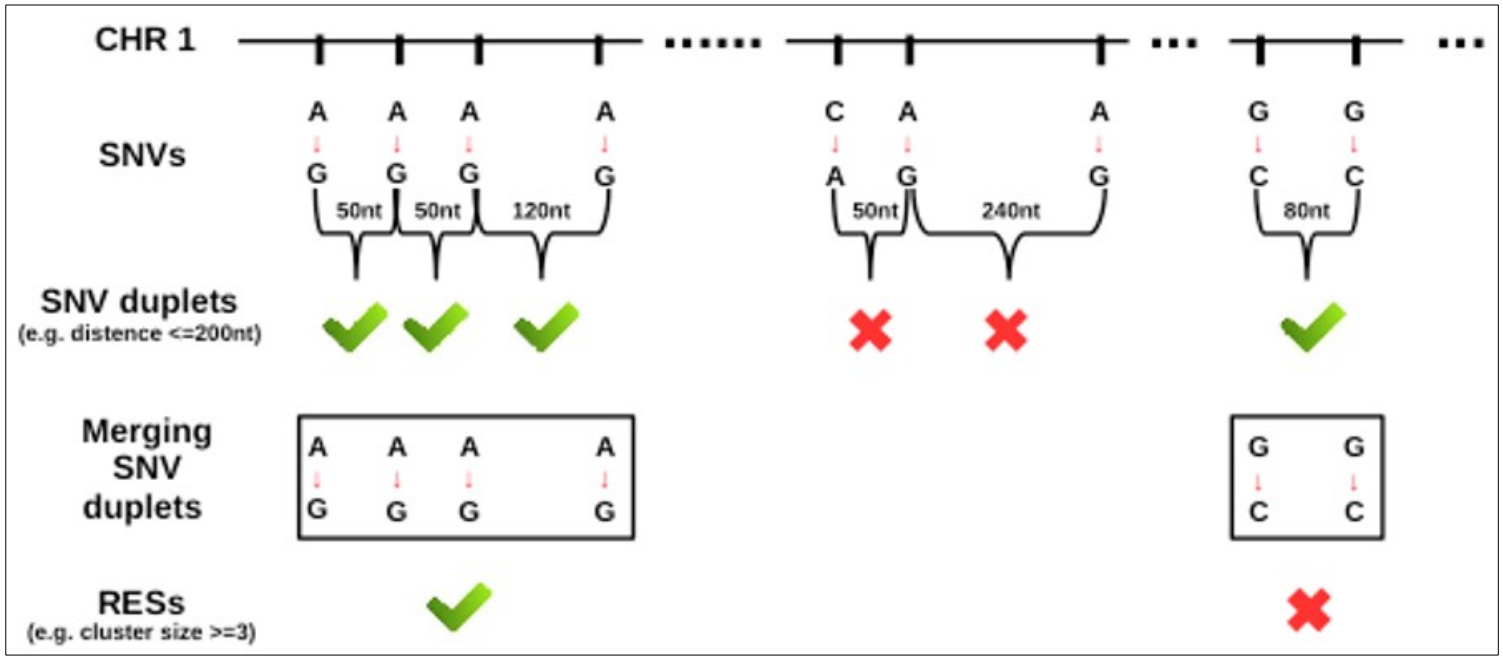
SPRINT

https://github.com/jumphone/SPRINT

SNV calling ⇨





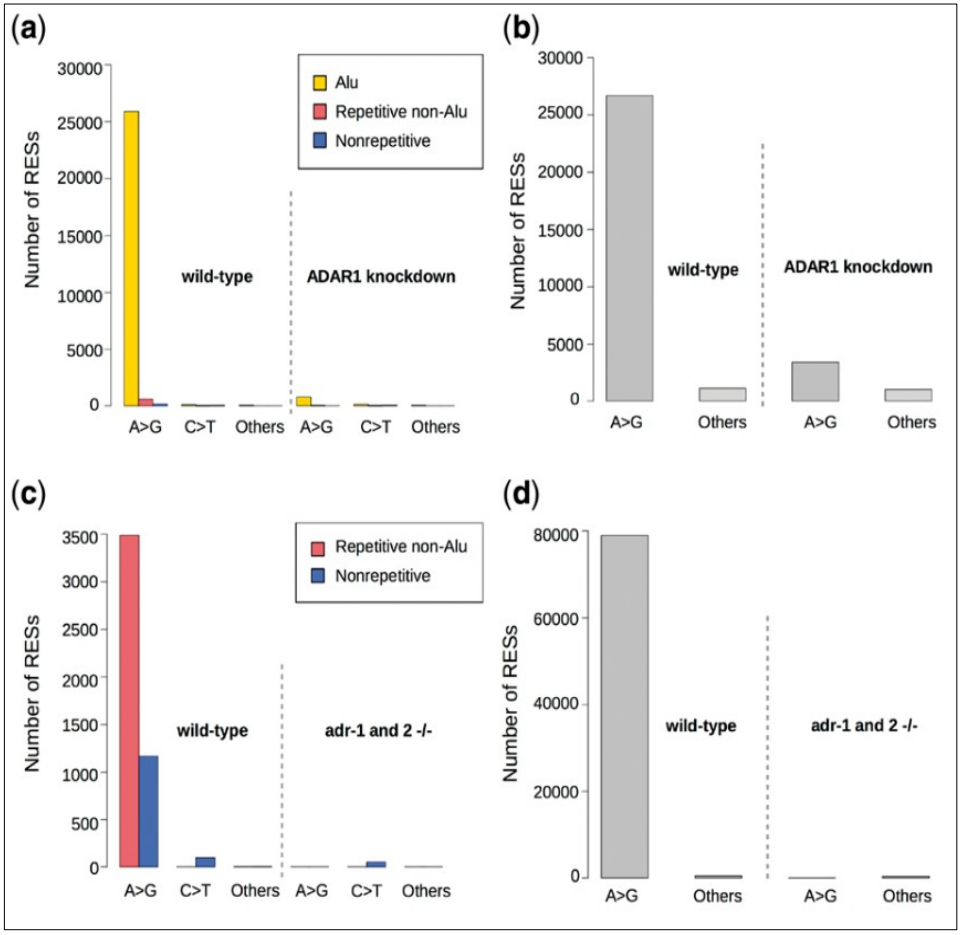


SPRINT

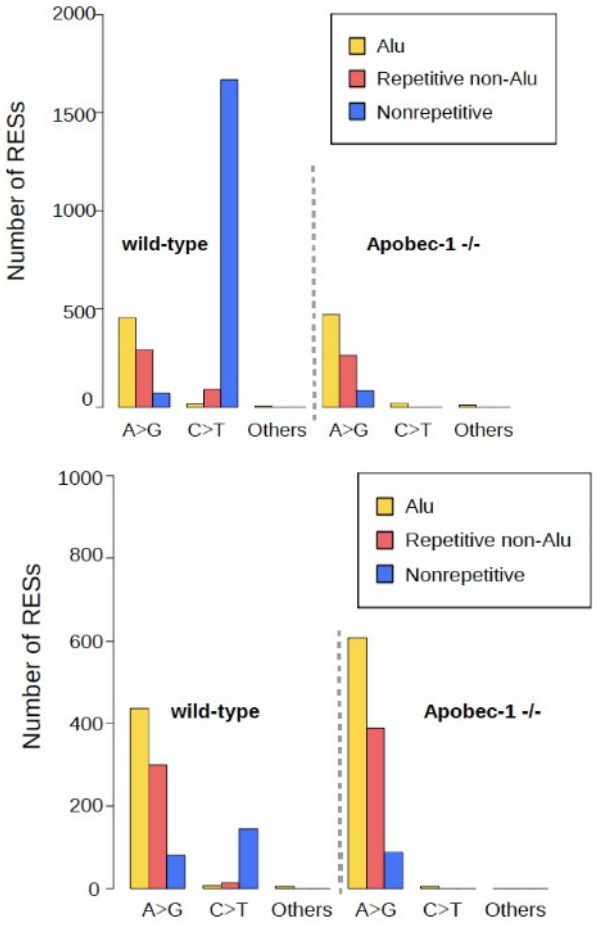
https://github.com/jumphone/SPRINT

A-to-I **C-to-U** **Mouse**

Human



C.elegans



SPRINT

https://github.com/jumphone/SPRINT

			Alu sites			Repetitive non-Alu sites				Nonrepetitive sites			
Cell lines	**Tools**	**Known SNP (%)**	**Total**	**A-to-G (%)**	**Precision (%)**	**Total**	**A-to-G (%)**	**Precision (%)**	**FDR (%)**	**Total**	**A-to-G (%)**	**Precision (%)**	**FDR (%)**
GM12878, cell	SPRINT	0	336 304	97.7	96.5	14 019	87.8	97.2	–	5407	49.8[a]	96.8	–
	Ramaswami *et al.**	100	147 029	95.8	–	2385	97.4	–	–	1451	86.6	–	–
GM12878, cytosolic	SPRINT	0	359 725	98.9	96.9	5469	96	96.8	–	2081	75.9	95.5	–
	GIREMI*	70	36 131	99	99.4	267	83.7	84.3	–	1193	82.8	73.8	–
	GIREMI*	100	39 757	99.7	–	260	88.6	–	–	1010	73.5	–	–
U87MG	SPRINT	0	48 085	99.6	96.2	988	99.5	97.1	4.5	296	87.8	91.2	0
	GIREMI	100	2152	99.8	–	114	96.5	–	9	509	88.6	–	53
	RNAEditor	100	62 979	–	–	6142	–	–	42.3	155	–	–	55.5
	REDItools (de novo)	100	628	96.5	–	238	46.2	–	80	14 949	39.7	–	100
	JACUSA (RRD)	100	2154	94.7	–	331	39	–	–	4527	20.8	–	–

SPRINT

https://github.com/jumphone/SPRINT

	Input data	Re-align & hyper-RES calling	Reads alignment assisting	SNV calling	RES calling
SPRINT	RNA-seq	√	√	√	√
GIREMI	dbSNP + RNA-seq				√
RNAEditor	dbSNP + RNA-seq		√	√	√
REDItools	dbSNP + RNA-seq or DNA-seq +RNA-seq			√	√
RED	dbSNP + RNA-seq or DNA-seq +RNA-seq				√
RES-Scanner	DNA-seq + RNA-seq		√	√	√
JACUSA	DNA-seq + RNA-seq			√	√

SPRINT

https://github.com/jumphone/SPRINT

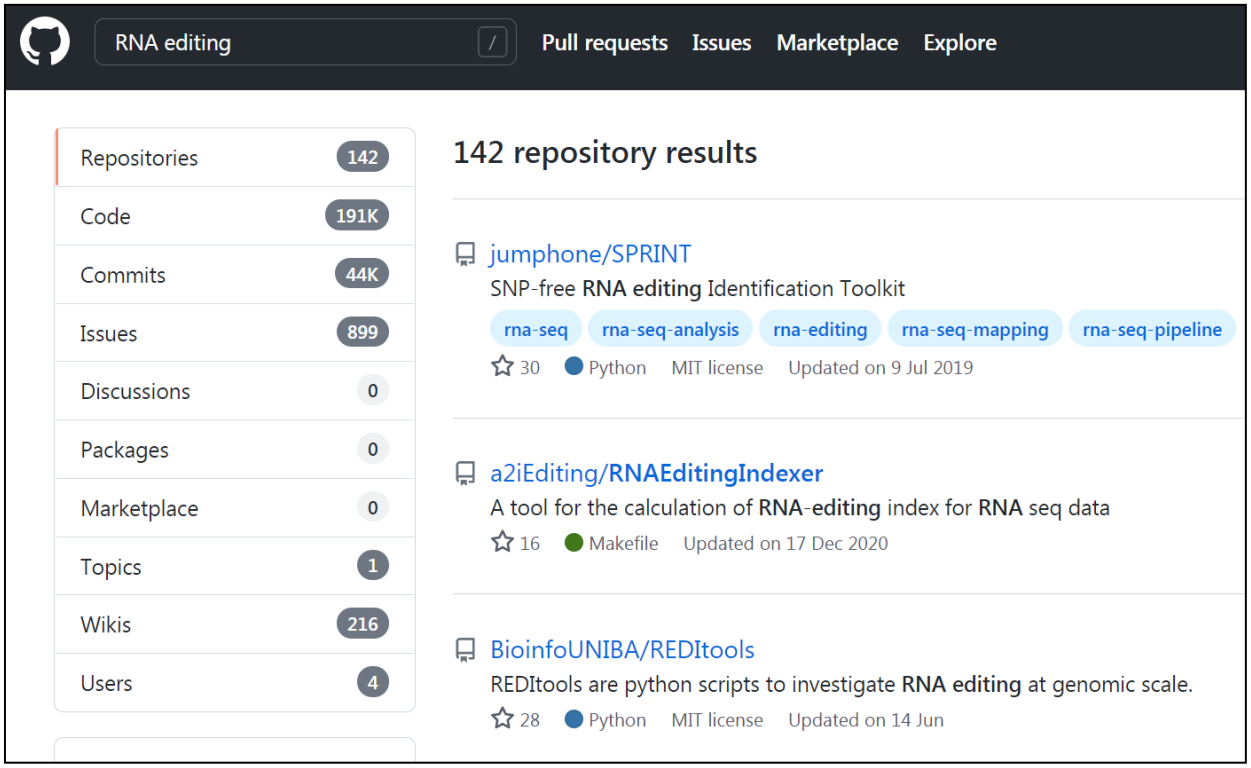
SPRINT: an SNP-free toolkit for identifying RNA editing sites.

2017 Bioinformatics | Volume: 33, Issue: 22, pp 3538-3548 | DOI: 10.1093/BIOINFORMATICS/BTX473

Feng Zhang [1], Yulan Lu [2], Sijia Yan [3], Qinghe Xing [3], Weidong Tian [3]

[1] State Key Laboratory of Genetic Engineering and Collaborative Innovation Center for Genetics and Development,
[2] The Molecular Genetic Diagnosis Center, Shanghai Key Lab of Birth Defect, Translational Medicine Research Center of Children Development and Diseases, Pediatrics Research Institute, [3] Fudan University

56 References 29 Citations*

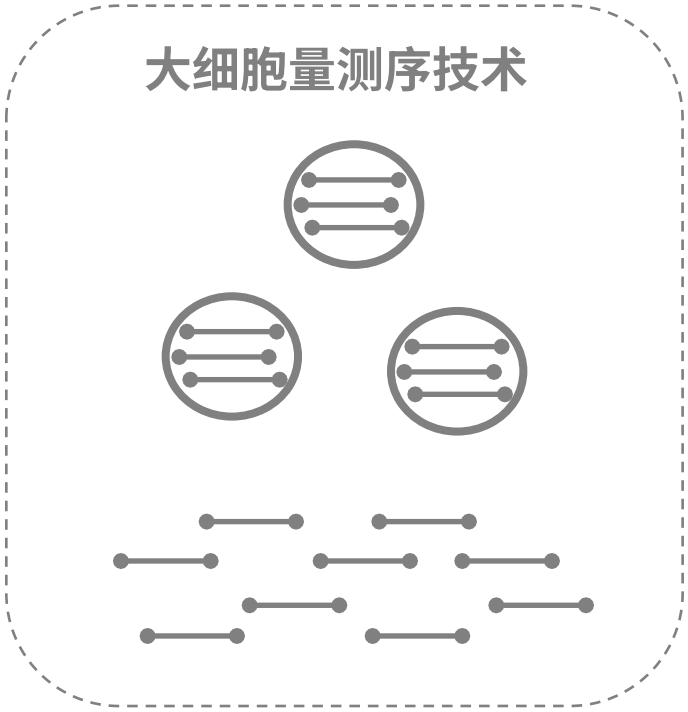


BEER

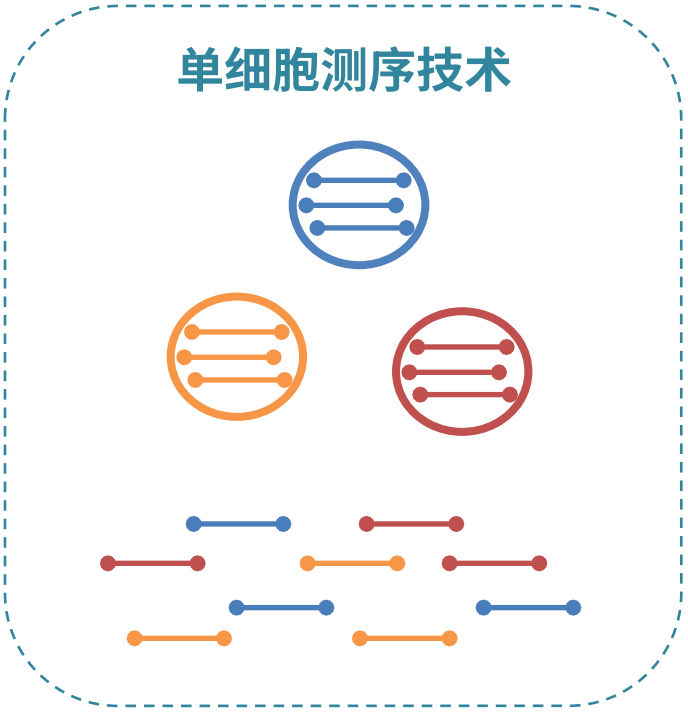
https://github.com/jumphone/BEER

单细胞测序技术是**对单个细胞的序列信息进行高通量探测的技术**。与大细胞量（bulk）测序不同，每一条测序读段都有独特的标记，用以区分来自不同细胞的测序读段。

2005 年至今



2012 年至今

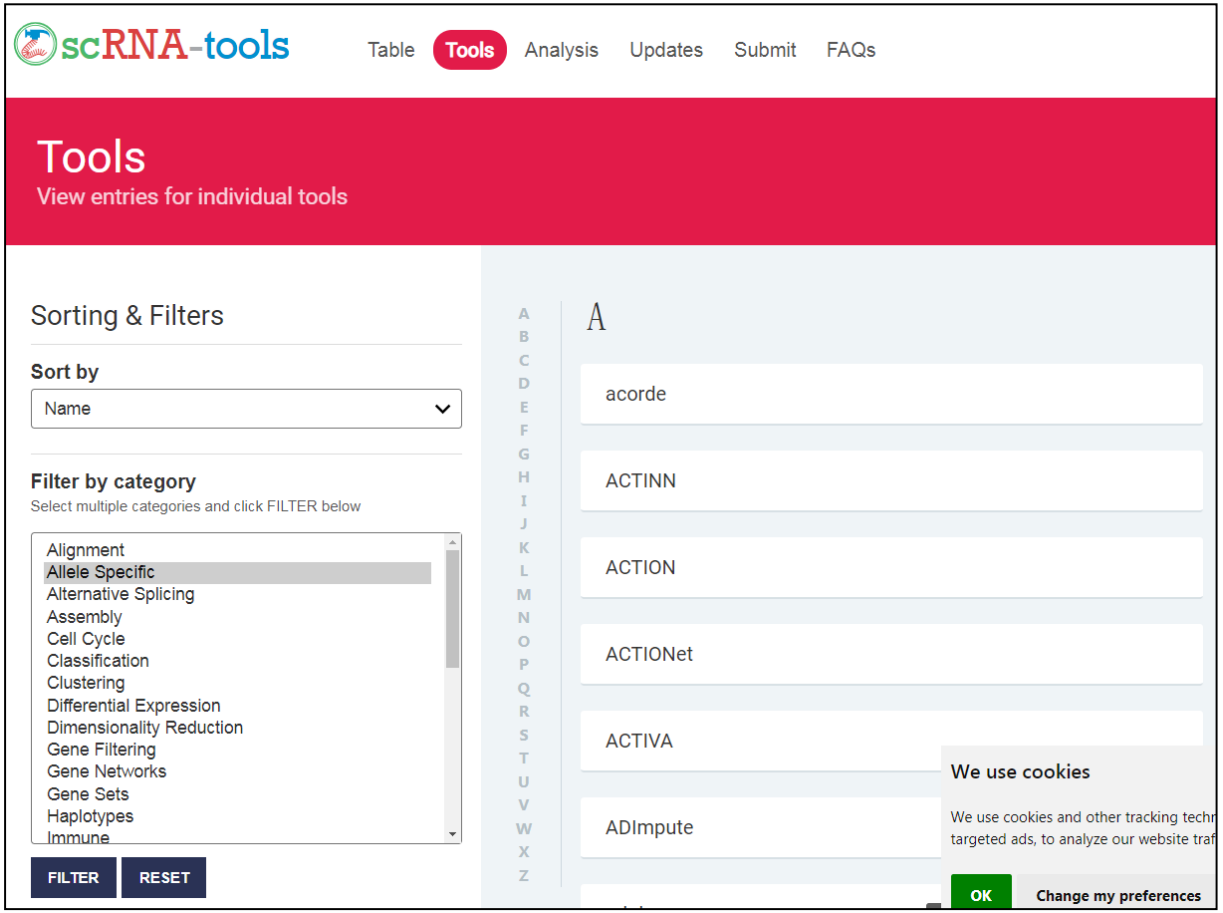


BEER

https://github.com/jumphone/BEER

单细胞分析工具非常多

https://www.scrna-tools.org/tools



BEER

降噪、降维、展示

Seurat: https://satijalab.org/seurat/

有 3 个阶段的数据是消除批次效应的突破口

数据预处理

标准化，基因数量、读段数量，线粒体基因表达，细胞周期， doublet

线性降维

	Cell1	Cell2	Cell 3	…
Gene1				
Gene2				
Gene3				
…				

线性

	Cell1	Cell2	Cell3	…
PC1				
PC2				
PC3				
…				

C1 * Gene1 + C2 * Gene2 + C3 * Gene3 + … = PC1

非线性降维（展示）

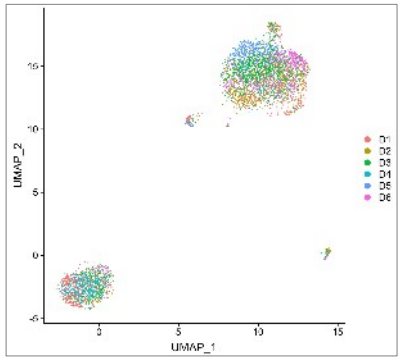
选择解释方差较大的一定数量的 PCs (top20, 50, or 100)

非线性

tSNE

UMAP

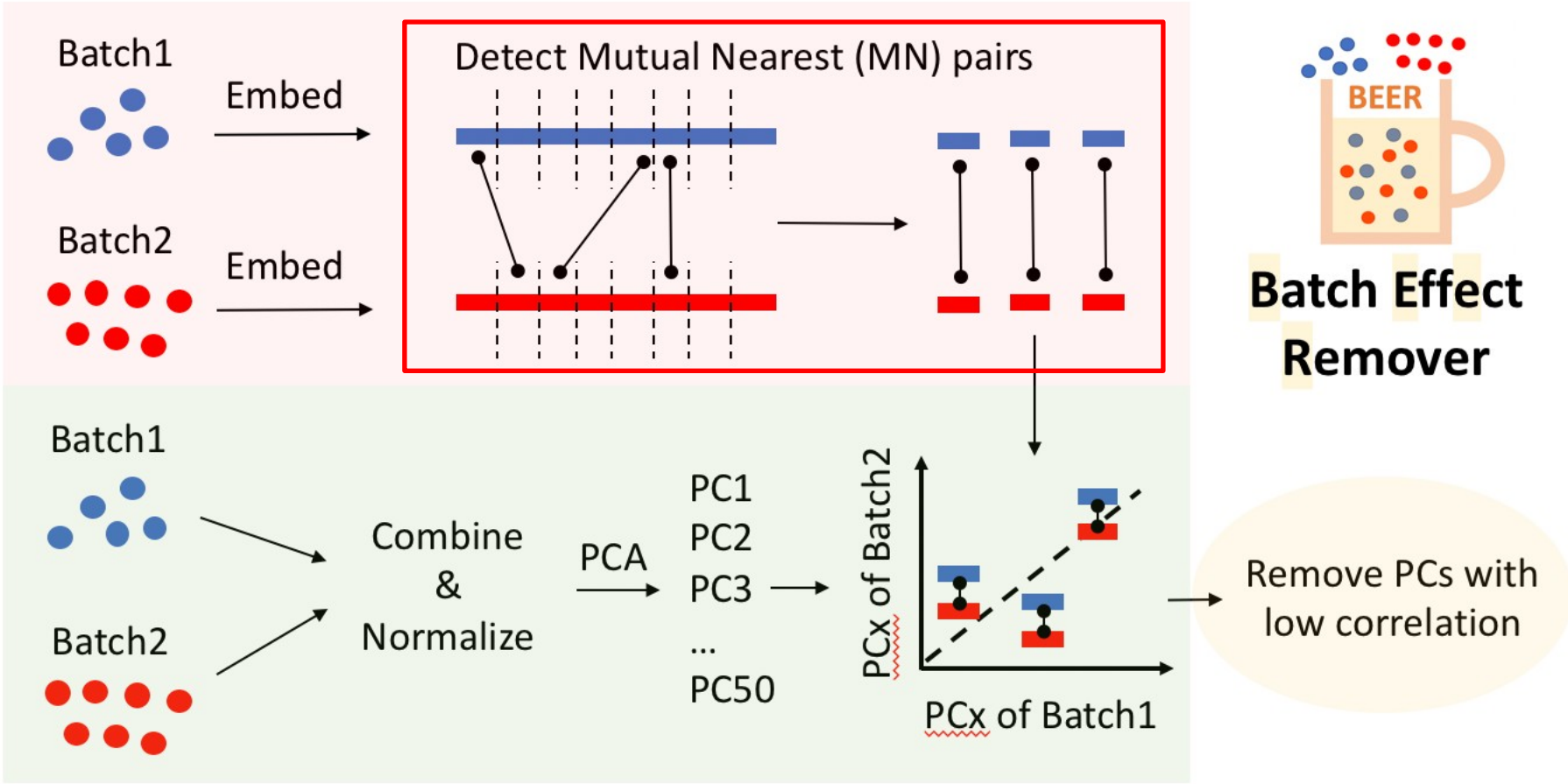
…



BEER

https://github.com/jumphone/BEER

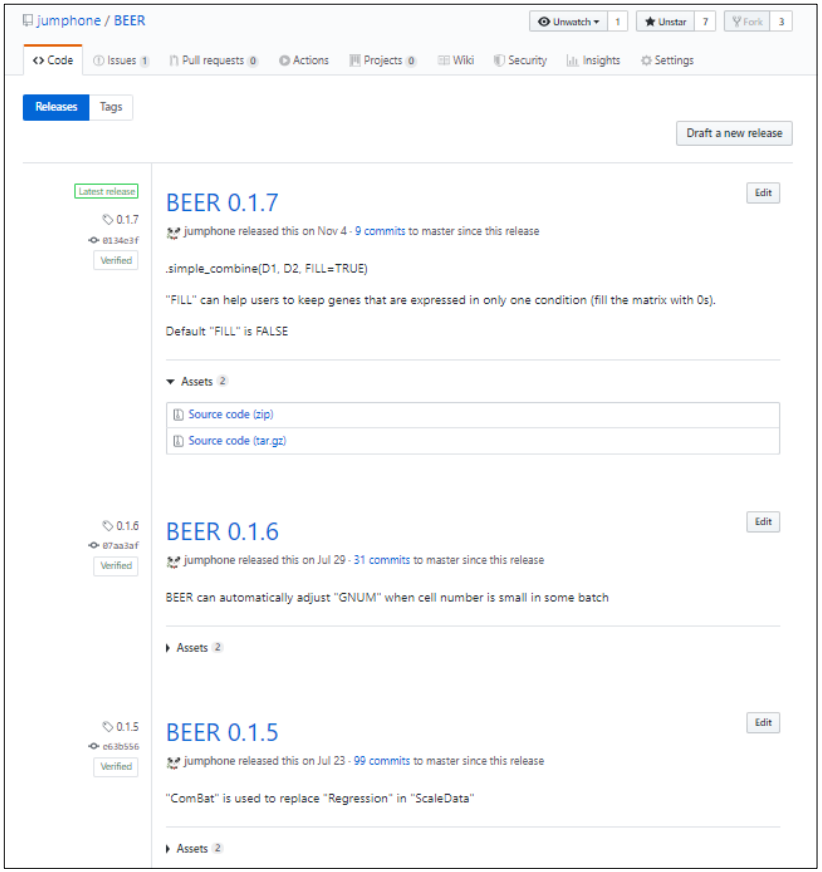
寻找互最近邻对（不同批次中相似的细胞类型）



BEER

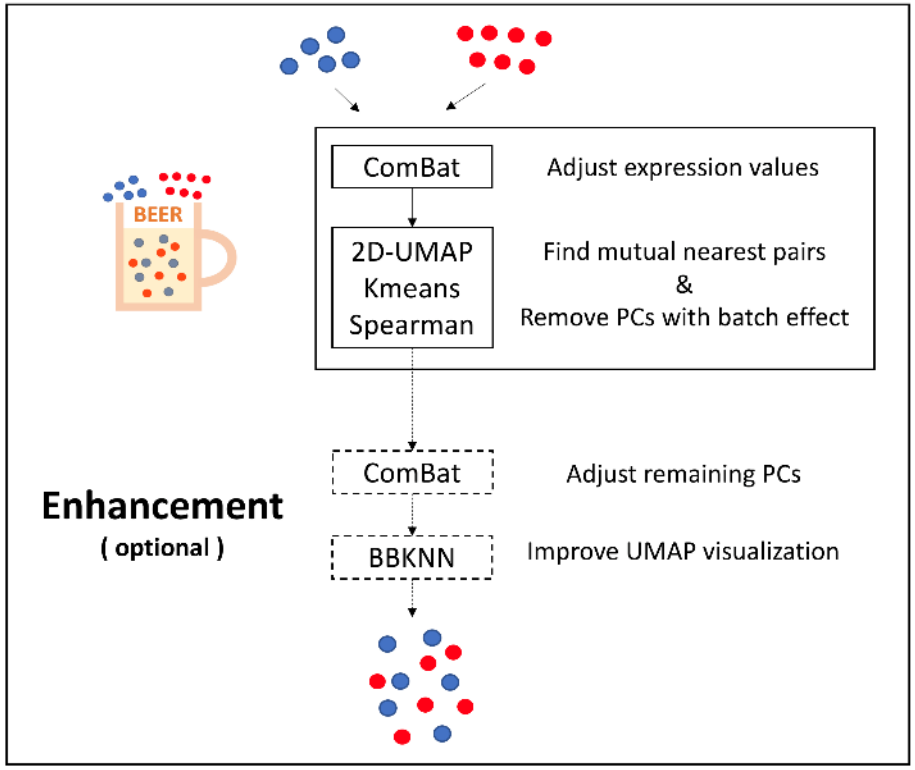
https://github.com/jumphone/BEER

Recent update



提出了加强版分析流程

（应对非线性批次效应）



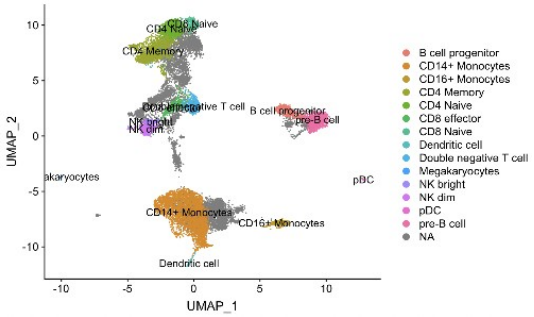
BEER

https://github.com/jumphone/BEER

合并 scATAC 与 scRNA

1. Find mutual nearest (MN) pairs
2. Remove PCs with “batch effect”
3. Use Combat & BBKNN to further adjust the output

原版 BEER



No imputation (没有使用插值【预测的值】)

No cell removing (没有删除细胞)

加强版 BEER