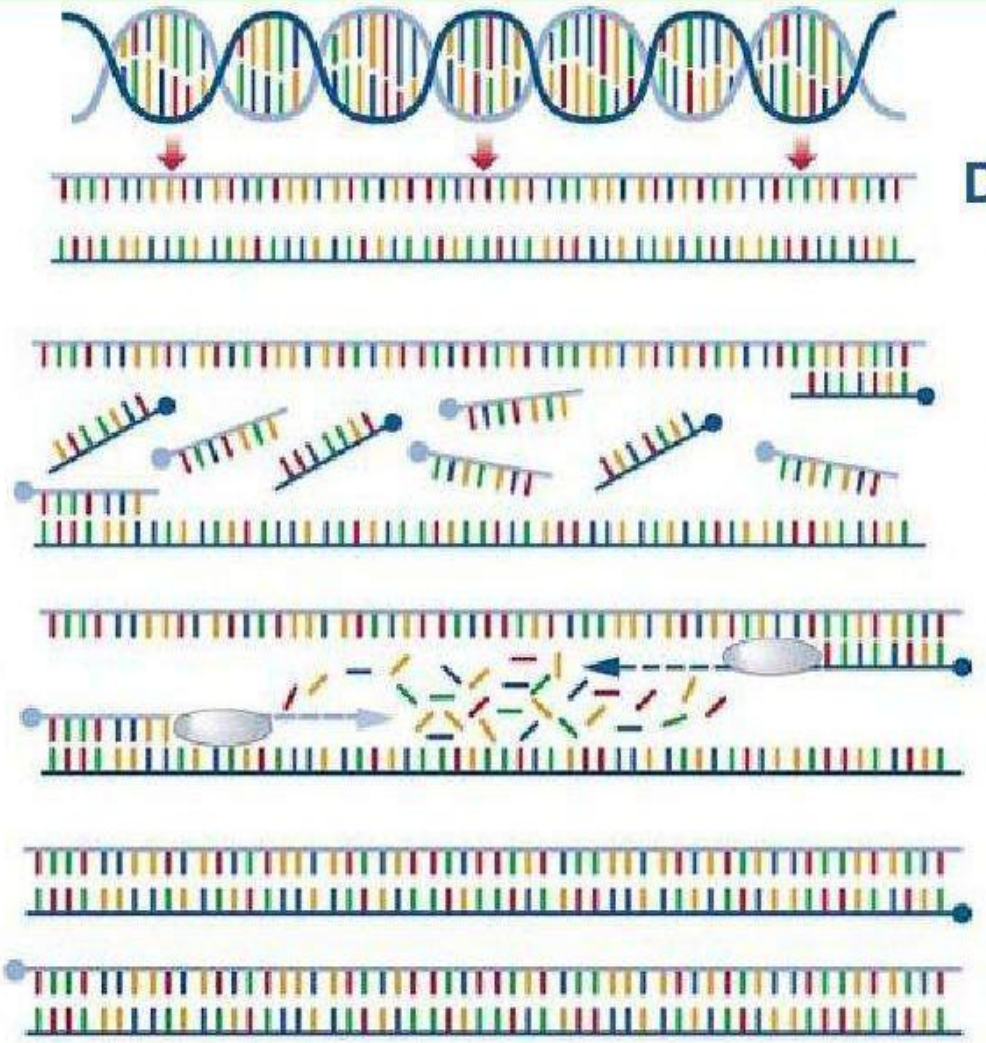
复习-PCR的过程

基本要素：

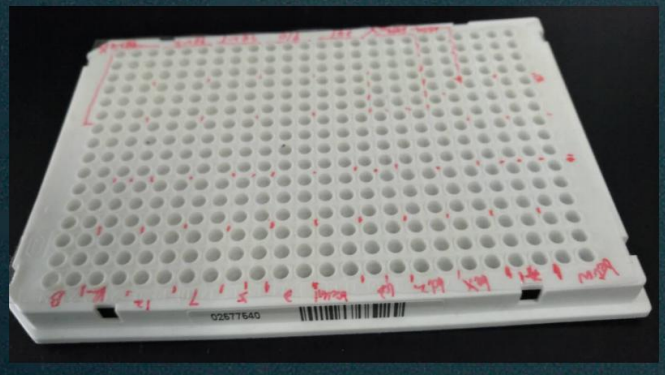
- Template
- Primers
- DNA polymerase
- dNTP, N=A,G,C or T

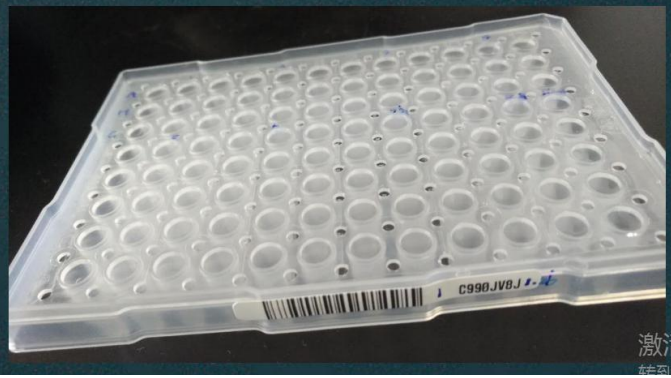


PCR视频（3分24秒视频）

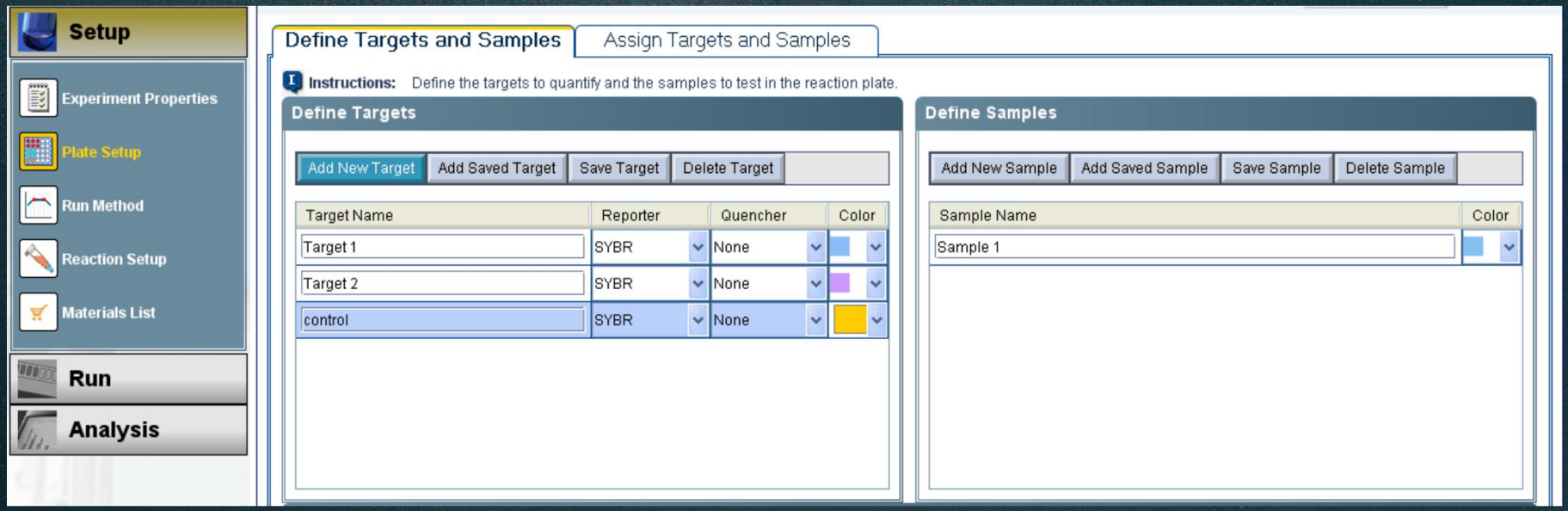
实验操作

	1	2	3	4	5	6	7	8	9	10	11	12
A	Control U 16S	Control U 16S	Control U 16S	Sample 1 U 16S	Sample 1 U 16S	Sample 1 U 16S	Sample 2 U 16S	Sample 2 U 16S	Sample 2 U 16S	N 16S	N 16S	N 16S
B	Control U Plat1	Control U Plat1	Control U Plat1	Sample 1 U Plat1	Sample 1 U Plat1	Sample 1 U Plat1	Sample 2 U Plat1	Sample 2 U Plat1	Sample 2 U Plat1	N Plat1	N Plat1	N Plat1





反应程序设置——Target/Sample



第1组

	Target gene	Actin
Con	24.14845085	16.55241203
Con	23.78612328	16.76924133
Con	23.56947327	15.59859276
Treatment	26.34865952	15.333601
Treatment	26.32477951	16.53817177
Treatment	26.1138134	16.70038795

第2组

	Target gene	Actin
Con	19.16145897	15.9144907
Con	19.13906479	16.08562279
Con	19.13013077	15.98913956
Treatment	19.51882935	15.95613289
Treatment	19.5342617	15.74811745
Treatment	19.53890991	15.76190281

第3组

	Target gene	Actin
Con	24.9741745	12.30350971
Con	25.81702423	12.36810875
Con	25.81064415	12.47786903
Treatment	25.22530365	12.42676926
Treatment	25.38946533	12.80829716
Treatment	25.29281235	12.72102833

第4组

	Target gene	Actin
Con	23.82953453	12.18450928
Con	23.76161957	11.94463253
Con	23.57886314	12.13293934
Treatment	24.17607498	11.85026455
Treatment	23.97296524	11.77577782
Treatment	23.99043465	11.61434174

第5组

	Target gene	Actin
Con	19.7154541	15.53760338
Con	19.71245193	15.53926373
Con	19.85190201	15.6465416
Treatment	20.22059059	15.65452385
Treatment	20.37389755	15.76074982
Treatment	20.17551422	15.94728374

第6组

	Target gene	Actin
Con	20.22059059	16.48932266
Con	20.37389755	16.64192963
Con	20.17551422	16.39921761
Treatment	26.68830872	14.3519001
Treatment	26.61764908	14.52338982
Treatment	26.59664536	14.97670746

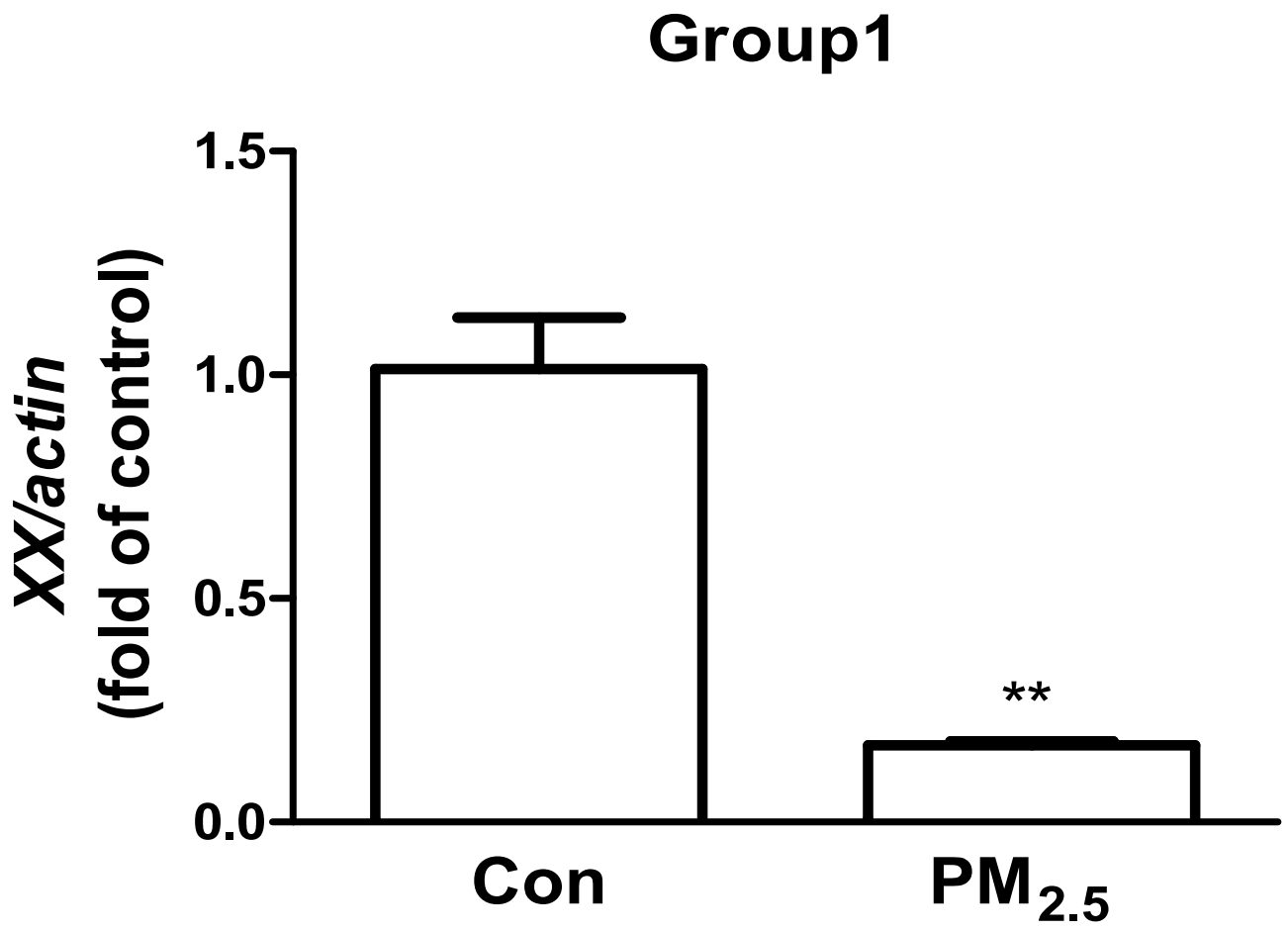
复习-数据计算公式

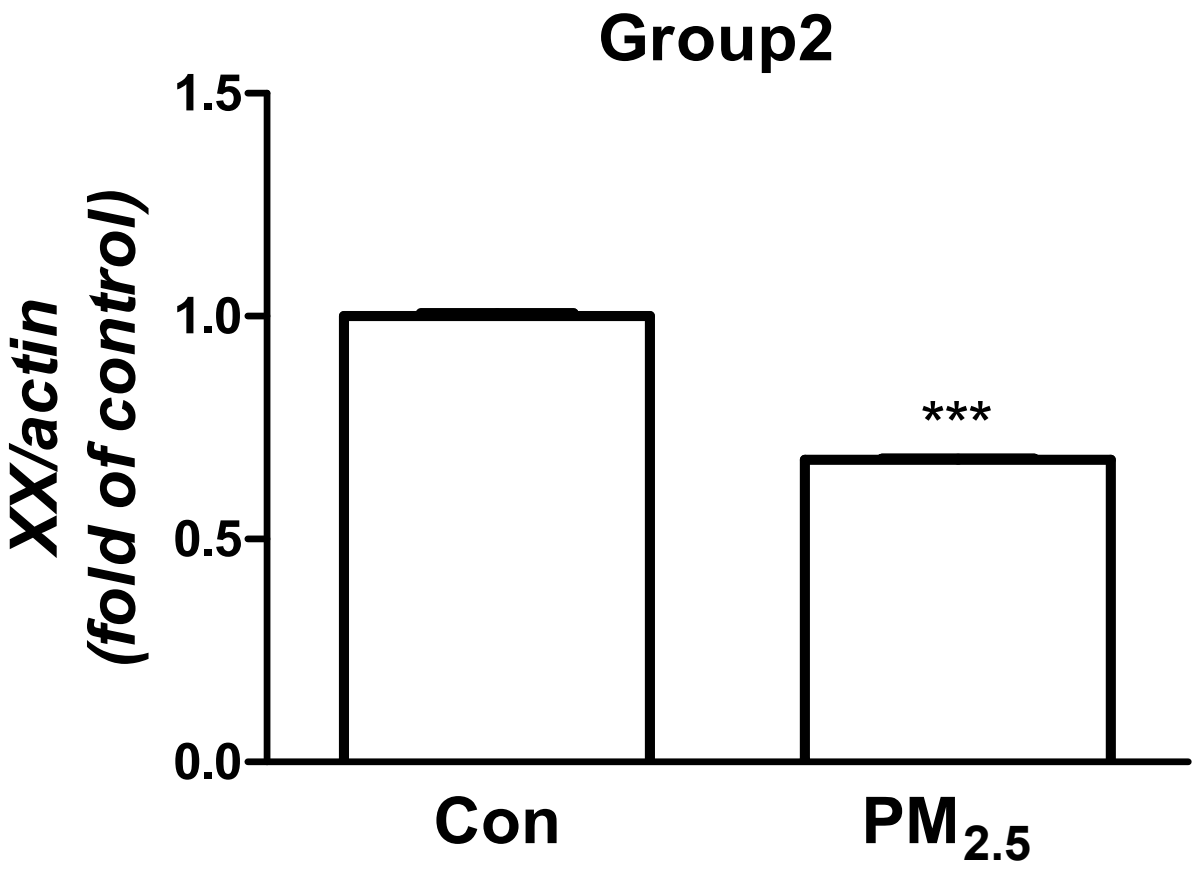
- **相对定量：$2^{-\triangle\triangle ct}$**

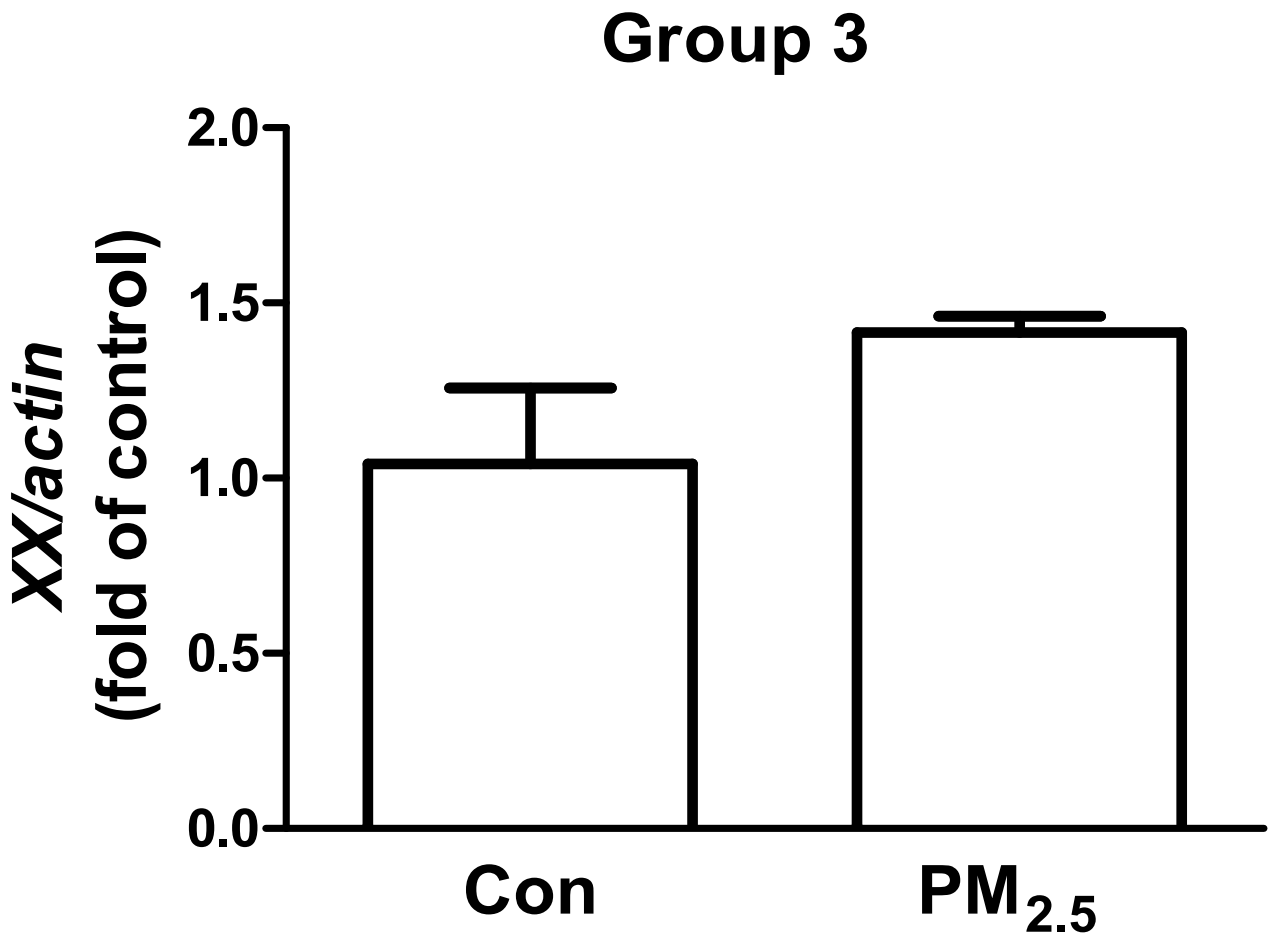
$\triangle\triangle ct$=实验组（目的ct-内参ct）-对照组（目的ct-内参ct）

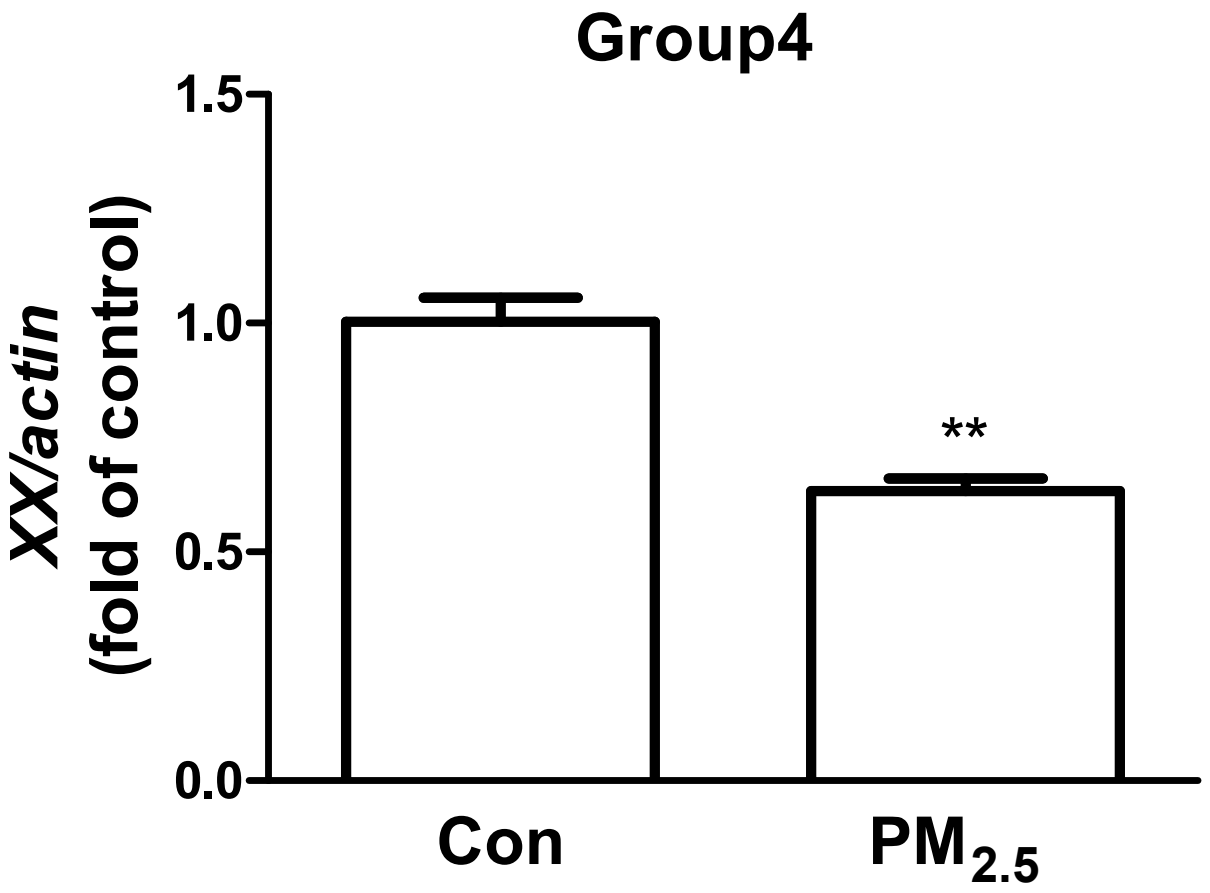
	Target	Average	GAPDH	Average	Target-GAPDH	Average T-G	△△ct	Ratio	Average of Ratio
Con	24.14845085	23.83468246	16.55241203	16.30674871	7.841702143	7.527933757	-0.313768387	0.80453752	1.013526452
	23.78612328		16.76924133		7.479374568		0.048559189	1.03423153	
	23.56947327		15.59859276		7.262724559		0.265209198	1.20181031	
group1	26.34865952	26.26241748	15.333601	16.19072024	10.15793928	10.07169724	-2.630005519	0.16154349	0.171961496
	26.32477951		16.53817177		10.13405927		-2.606125514	0.16423966	
	26.1138134		16.70038795		9.92309316		-2.395159403	0.19010134	
Con	19.16145897	19.14355151	15.9144907	15.99641768	3.165041288	3.147133827	-0.017907461	0.98766421	1.000041654
	19.13906479		16.08562279		3.142647107		0.00448672	1.0031148	
	19.13013077		15.98913956		3.133713086		0.013420741	1.00934595	
group2	19.51882935	19.53066699	15.95613289	15.82205105	3.696778297	3.708615939	-0.54964447	0.68318847	0.677617692
	19.5342617		15.74811745		3.712210655		-0.565076828	0.67591942	
	19.53890991		15.76190281		3.716858864		-0.569725037	0.67374519	
Con	24.9741745	25.53394763	12.30350971	12.3831625	12.591012	13.15078513	0.559773127	1.4740374	1.040450993
	25.81702423		12.36810875		13.43386173		-0.283076604	0.82183655	
	25.81064415		12.47786903		13.42748165		-0.276696523	0.82547903	
group3	25.22530365	25.30252711	12.42676926	12.65203158	12.57327207	12.65049553	0.577513059	1.49227462	1.41603502
	25.38946533		12.80829716		12.73743375		0.413351377	1.33177594	
	25.29281235		12.72102833		12.64078077		0.510004361	1.4240545	
Con	23.82953453	23.72333908	12.18450928	12.08736038	11.74217415	11.6359787	-0.10619545	0.9290348	1.002726264
	23.76161957		11.94463253		11.67425919		-0.038280487	0.97381492	
	23.57886314		12.13293934		11.49150276		0.144475937	1.10532907	
group4	24.17607498	24.04649162	11.85026455	11.7467947	12.42928028	12.29969692	-0.793301582	0.57702207	0.632512117
	23.97296524		11.77577782		12.22617054		-0.590191841	0.66425457	
	23.99043465		11.61434174		12.24363995		-0.607661247	0.6562597	
Con	19.7154541	19.75993601	15.53760338	15.57446957	4.140984535	4.185466448	0.044481913	1.03131276	1.001005722
	19.71245193		15.53926373		4.137982368		0.04748408	1.0334611	
	19.85190201		15.6465416		4.277432442		-0.091965993	0.93824331	
group5	20.22059059	20.25666746	15.65452385	15.78751914	4.433071454	4.469148318	-0.247605006	0.84229353	0.822899659
	20.37389755		15.76074982		4.586378415		-0.400911967	0.75737937	
	20.17551422		15.94728374		4.387995084		-0.202528636	0.86902607	
Con	20.22059059	20.25666746	16.48932266	16.51015663	3.71043396	3.746510824	0.036076864	1.02532186	1.001713748
	20.37389755		16.64192963		3.863740921		-0.117230097	0.92195606	
	20.17551422		16.39921761		3.66535759		0.081153234	1.05786332	
group6	26.68830872	26.63420105	14.3519001	14.61733246	12.07097626	12.01686859	-8.324465434	0.00311951	0.003239916
	26.61764908		14.52338982		12.00031662		-8.253805796	0.0032761	

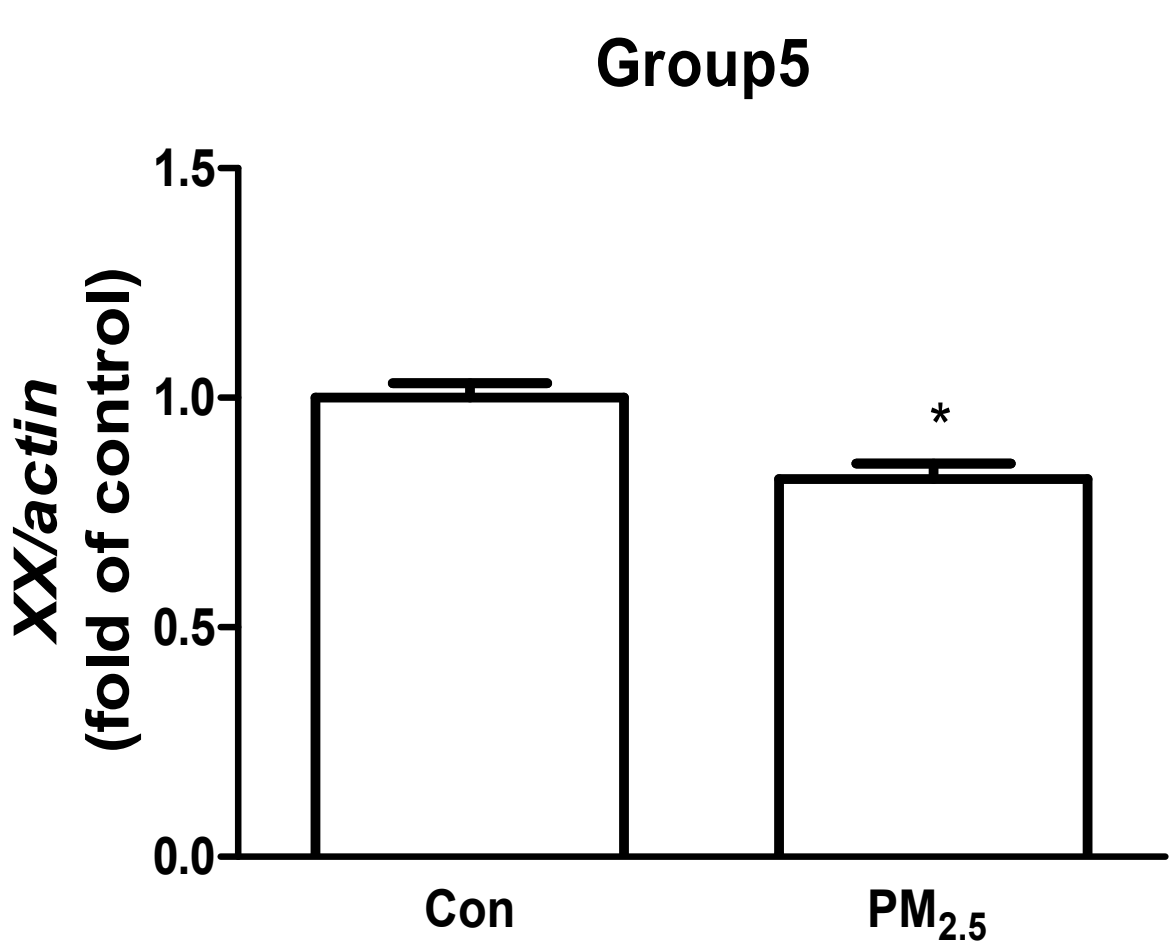
数据结果

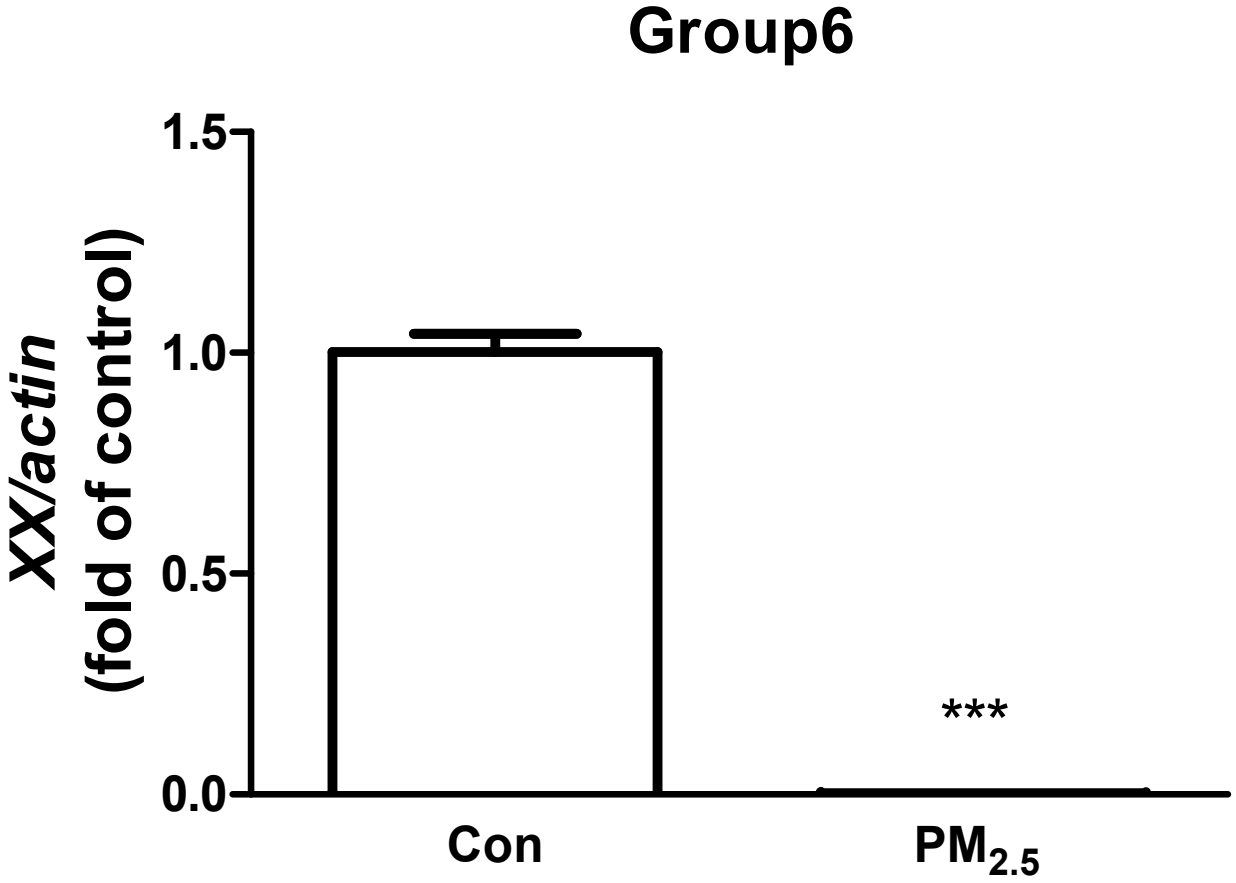



Group2
1.5
1.0
0.5
0.0
XX/actin
(fold of control)
***
Con
PM2.5


Group 3
2.0
1.5
1.0
0.5
0.0
XX/actin
(fold of control)
Con
PM2.5


Group4
1.5
1.0
0.5
0.0
XX/actin
(fold of control)
**
Con
PM2.5


Group5
1.5
1.0
0.5
0.0
XX/actin
(fold of control)
*
Con
PM2.5


Group6
1.5
1.0
0.5
0.0
XX/actin
(fold of control)
***
Con
PM2.5

分子流行病学实验课

-SNP、甲基化、Western blotting

邓晓蓓

2018.6.13

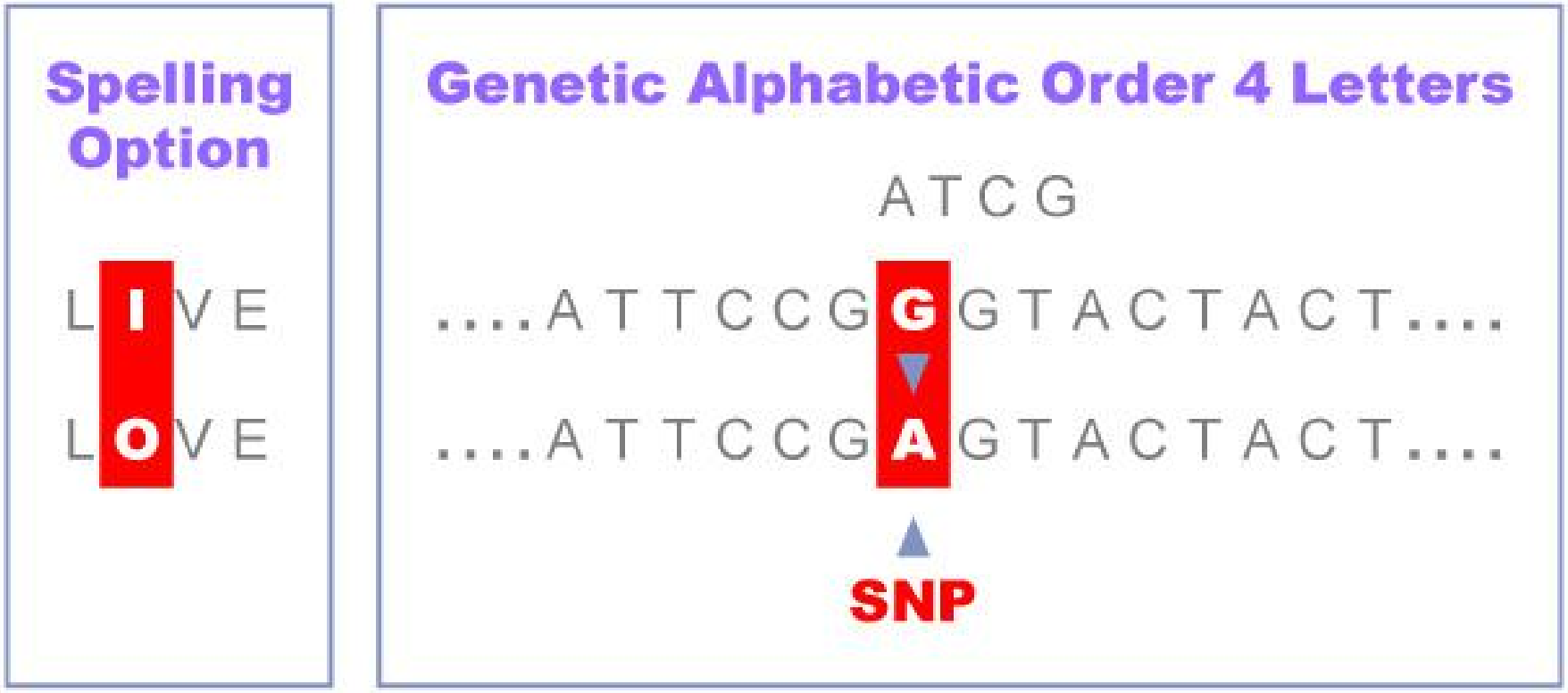
易感性基因标志物：

1. 人类基因组多态性（SNPs）

2. 基因组表遗传修饰（甲基化，乙酰化）

What is SNP?

A mutation in the DNA sequence with a frequency of >1%

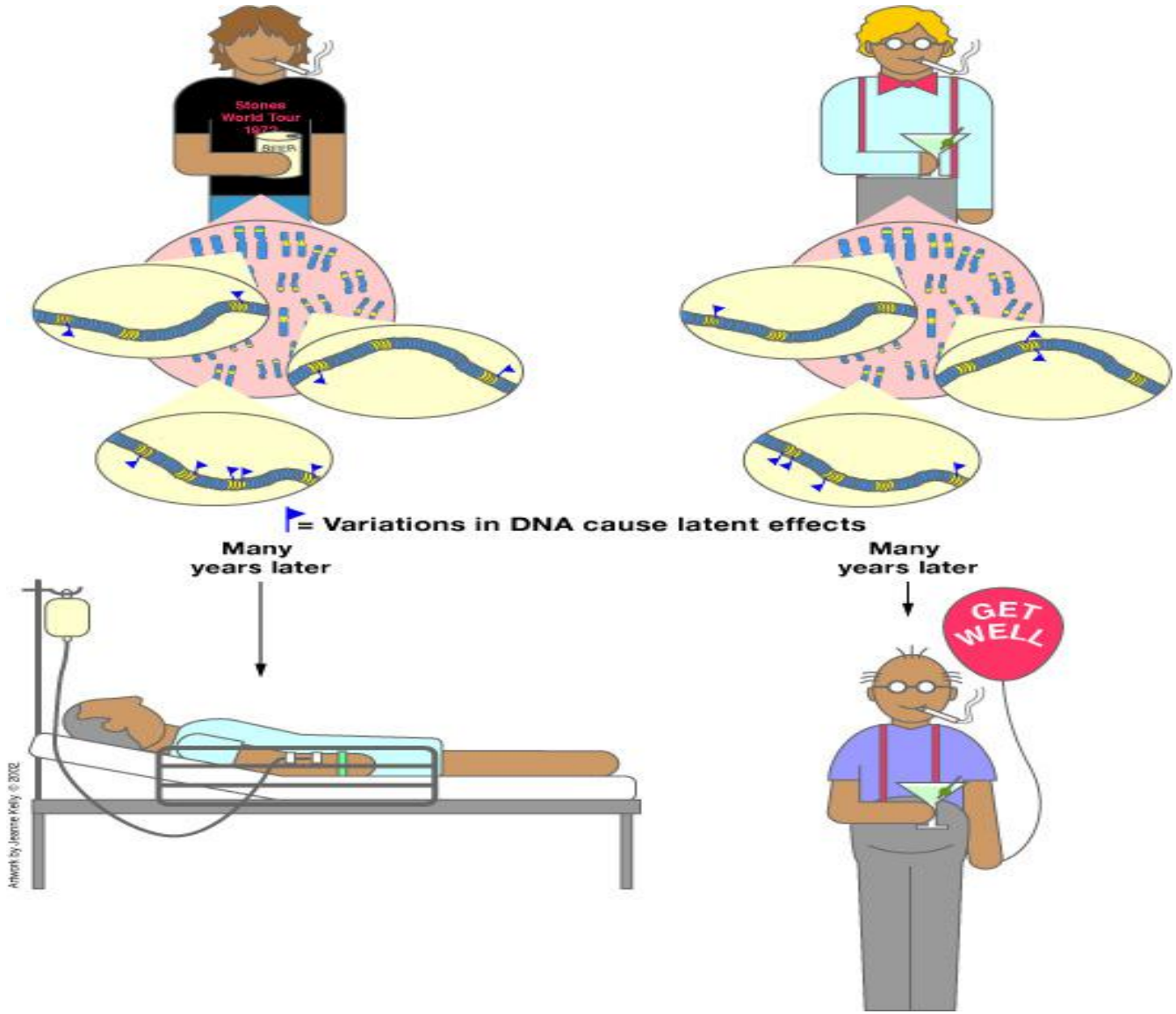


单核苷酸多态
(Single nucleotide polymorphism, SNP)

- SNP是指在人群中出现的频率≥1%的先天突变。SNP存在于整个人类基因组中，可能每100~300bp就存在一个SNP，估计总数在数百万个。

- 人类基因组计划研究结果表明，不同个体的基因99.9%是一样的，但在序列上有极小(0.1%)的遗传差异，其中主要是 SNP。

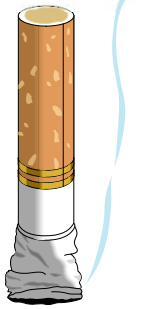
- 研究表明，正是这0.1%的差异授予你我他特有的表型；这个小小的差异还与个体对疾病(包括肿瘤)的易感性、对药物的反应性差别有密切关系。


Stones
World Tour
BEER
= Variations in DNA cause latent effects
Many
years later
Many
years later
GET
WELL
Artwork by Jeanne Kelly © 2002

研究问题 1:

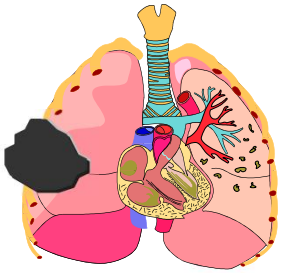
Why only < 20% smokers develop lung cancer?

Exposure

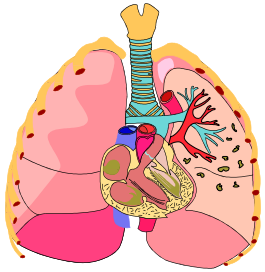


Genetic susceptibility?

SNPs?



Cancer



Cancer-free

CIR by 70 yrs

M: 15.9%

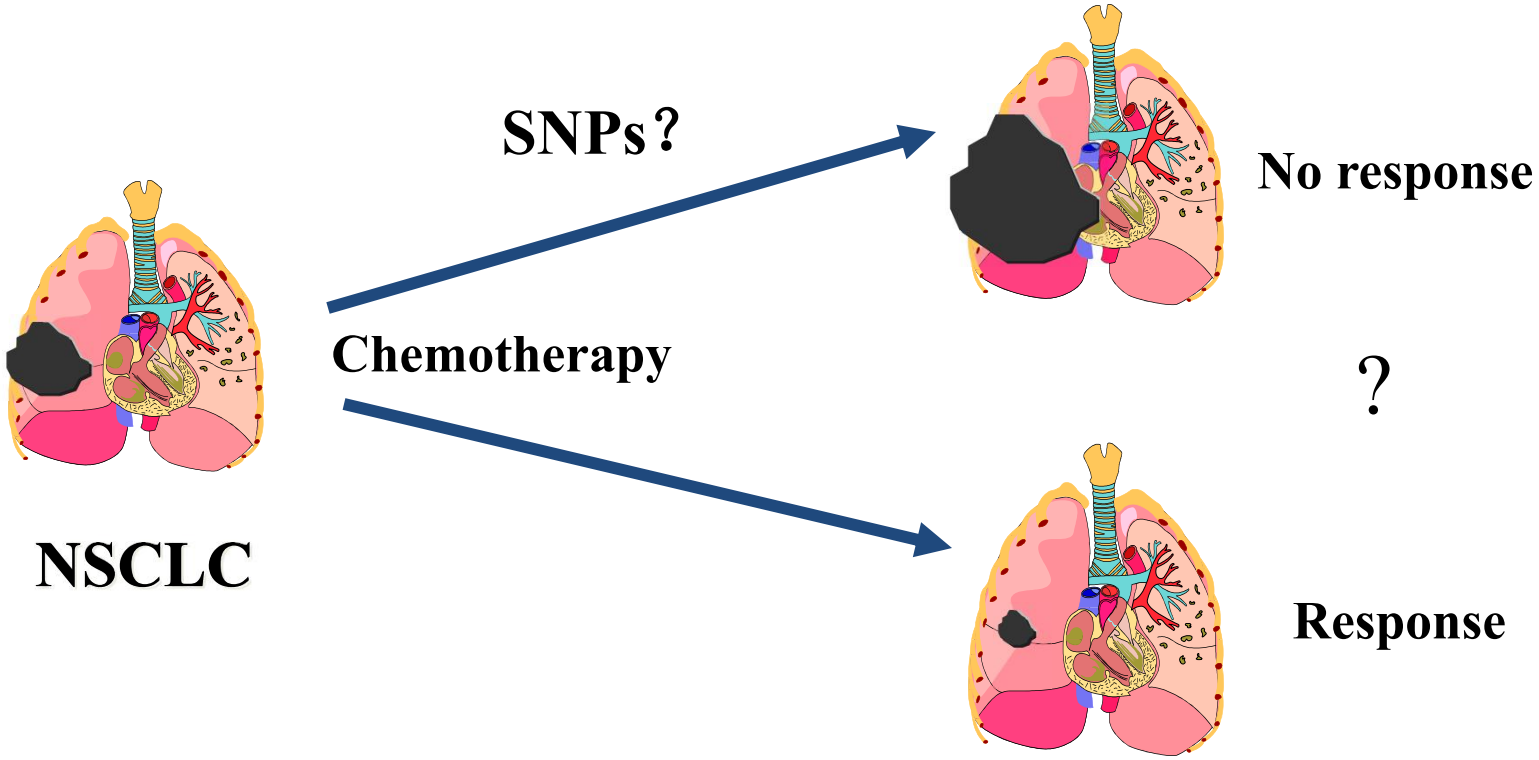
F: 9.5%

(Peto, BMJ, 2000)

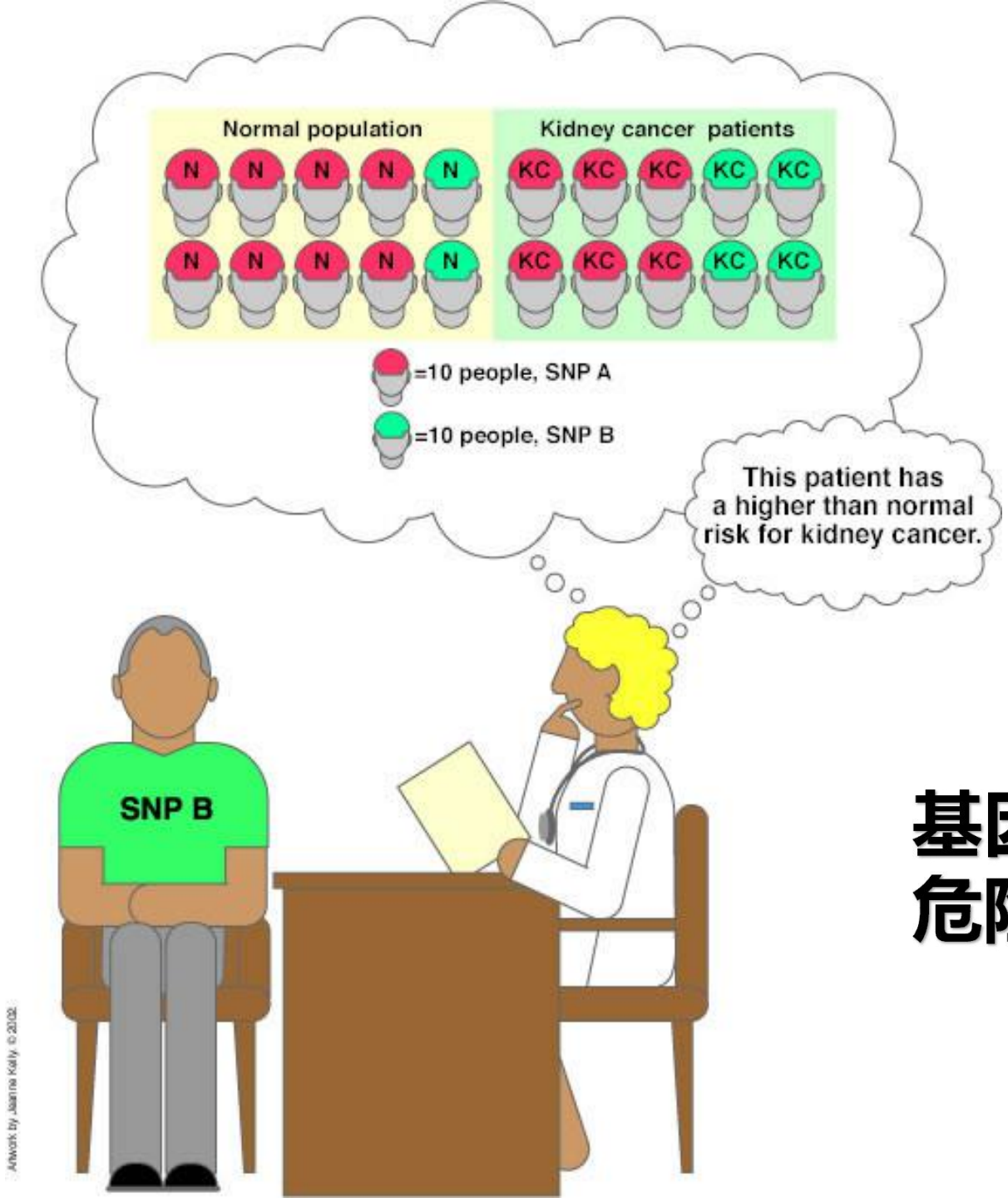
Screening, prevention

研究问题 2:

Why some lung cancer patients have no response to chemotherapy?



SNPs & Chemosensitivity


Normal population
Kidney cancer patients
N
KC
=10 people, SNP A
=10 people, SNP B
This patient has a higher than normal risk for kidney cancer.
SNP B
Artwork by Jeanne Kelly © 2002

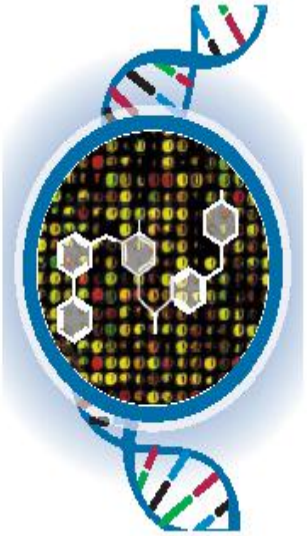
基因检测-危险度评价

Human Genome Project

Biology

SNPs

Response



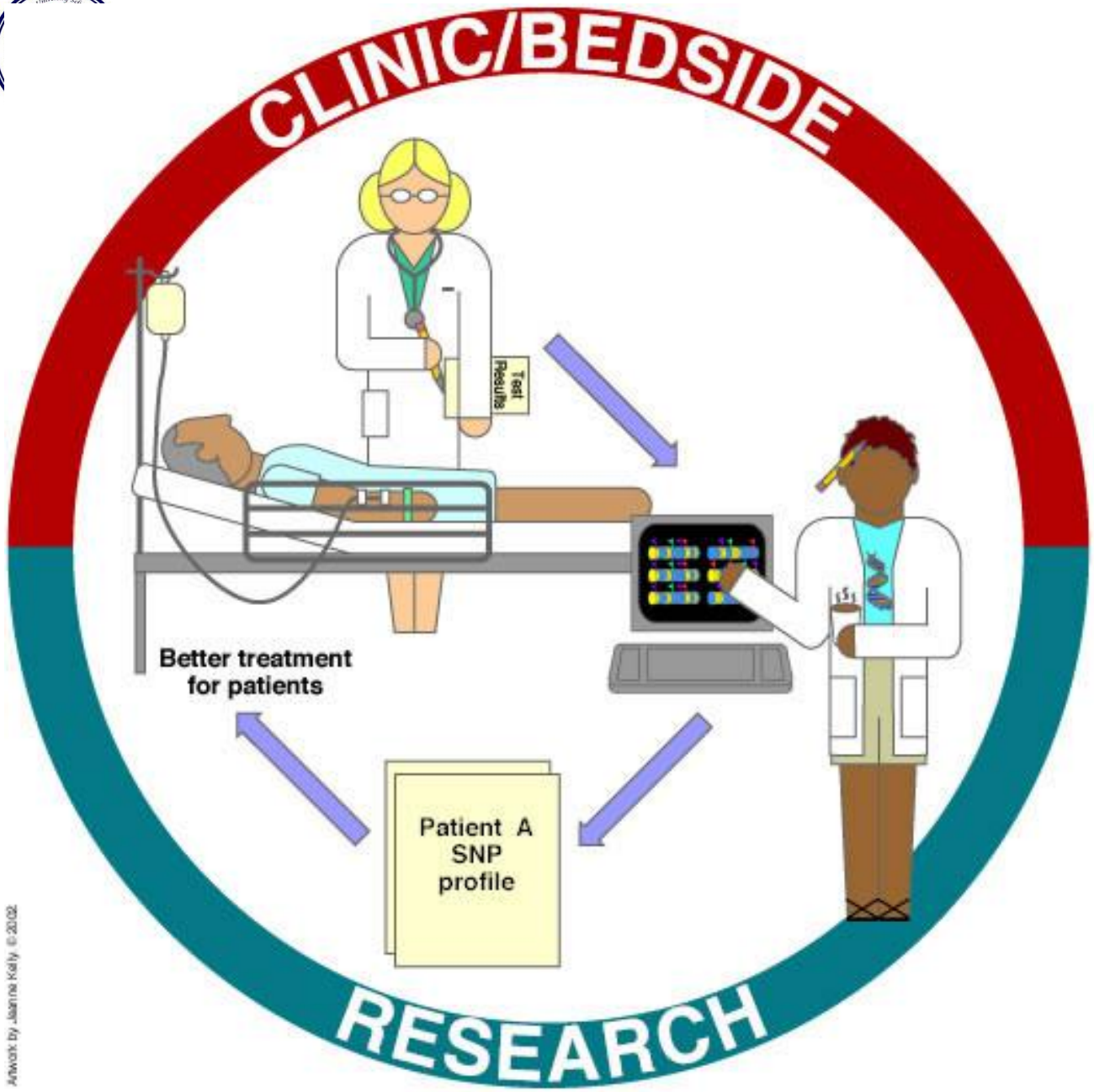
Disease

Prevention

SNPS=Single Nucleotide Polymorphisms

人类基因组计划

Collins et al., Nature, 2003


CLINIC/BEDSIDE
Test Results
Better treatment for patients
Patient A SNP profile
RESEARCH
Artwork by Jeanne Kelly ©2002

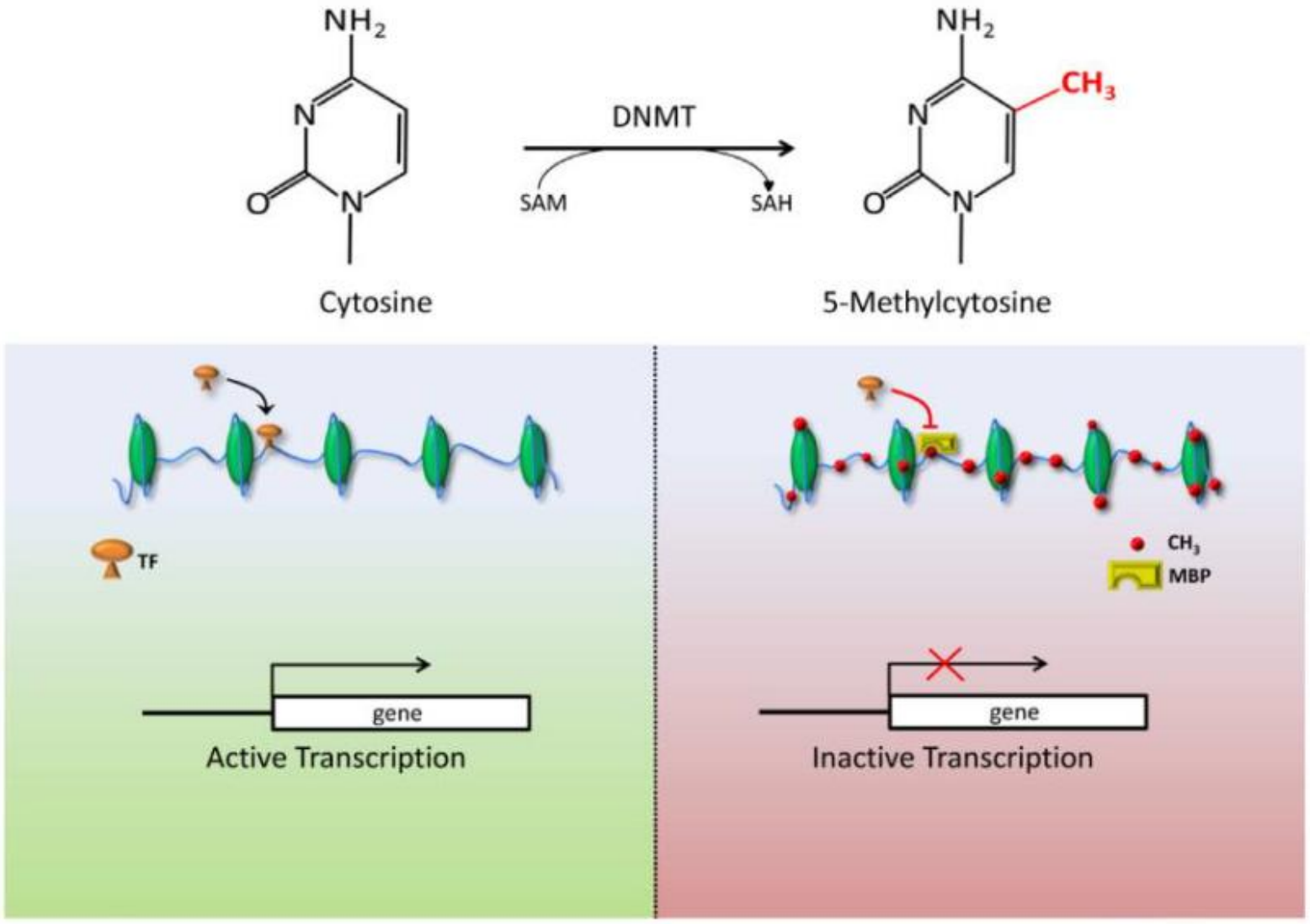
从科学研究→临床实践


USE ONLY WITH "G"
AT Chr17: 36, 667, 738

DNA甲基化

DNA甲基化

- DNA甲基化：在**DNA甲基化转移酶**的作用下将甲基选择性地添加到**胞嘧啶**上形成**5-甲基胞嘧啶**的过程；
- 刚被发现时被定义为第五种碱基，实际上它是一种重要的表观遗传学标记；
- **在调控基因表达、维持染色质结构、基因印记、X染色体失活以及胚胎发育等生物学过程中发挥着重大的作用。**
- 它就像DNA的一顶最神奇的“帽子”。



DNA甲基化修饰和对基因表达的转录调控作用

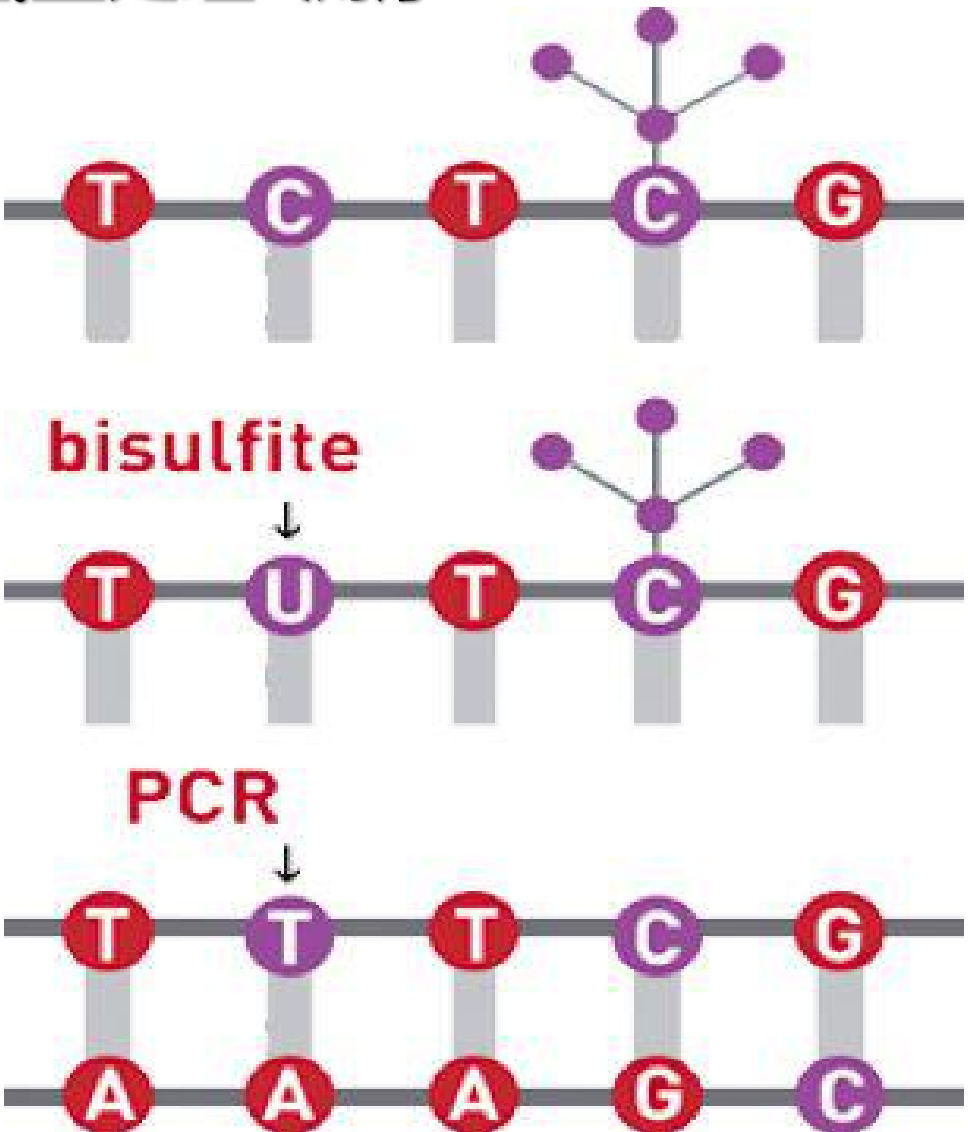
检测方法(1)

1) 甲基化特异性的PCR(Methylation-specific PCR，MSP)

- **亚硫酸氢盐**处理基因组DNA，所有**未发生甲基化的胞嘧啶被转化为尿嘧啶**，而**甲基化的胞嘧啶不变**；
- 随后设计**针对甲基化和非甲基化序列的引物进行PCR。**
- 通过电泳检测MSP扩增产物，如果用针对处理后甲基化DNA链的引物能得到扩增片段，则说明该位点存在甲基化；反之，说明被检测的位点不存在甲基化。
- 定性研究。

检测方法(2)

2）亚硫酸氢盐处理+测序



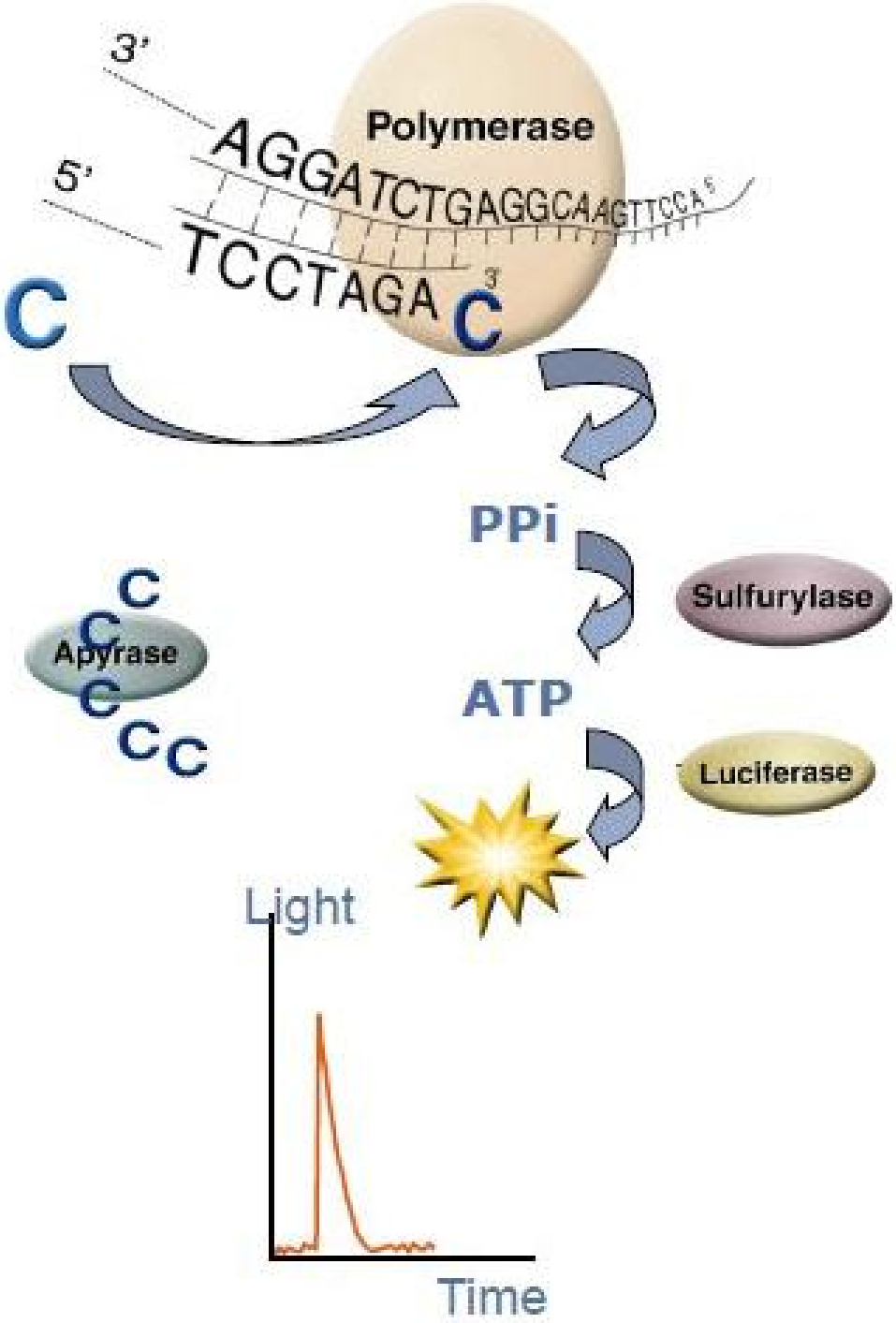
检测方法(3)

3）焦磷酸测序(Pyrosequencing)

- 焦磷酸测序技术作为一种新的序列分析技术，能够快速地**检测甲基化的频率**，对样品中的**甲基化位点进行定性及定量检测**，为甲基化研究提供了新的途径。
- 通过准确定量单个连续的**CpG 位点上的甲基化频率**，能够检测并定量甲基化水平上的细微改变。
- **在序列延伸过程中，根据C和T的掺入量来定量确定单个位点的C-T 比例**。因此，不同位点的甲基化变异就能被准确检测。
- 由于焦磷酸测序提供了真实的序列数据，**甲基化状态也就以序列形式**呈现。

检测方法(4)

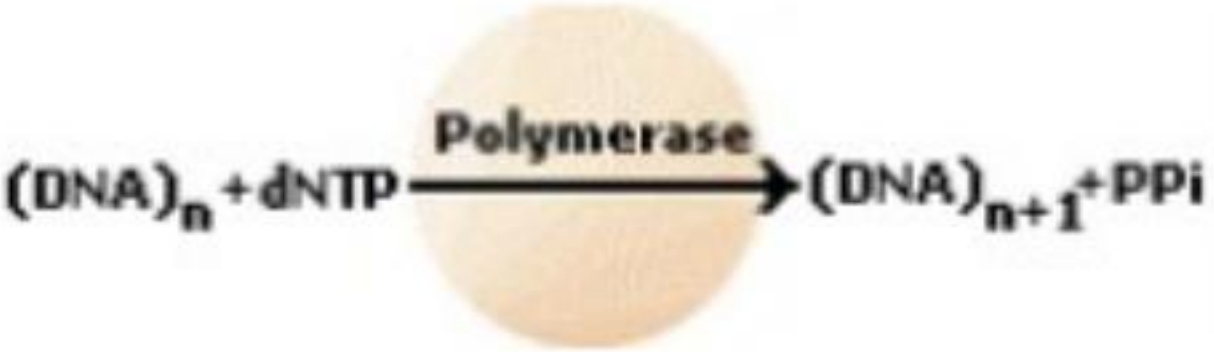
- 联合亚硫酸氢钠的限制性内切酶分析法
- 荧光定量法(Methylight)
- 甲基化敏感性高分辨率熔解曲线分析
- 基于芯片的甲基化图谱分析
- 高通量测序
- 飞行质谱



Qiagen Q96 ID

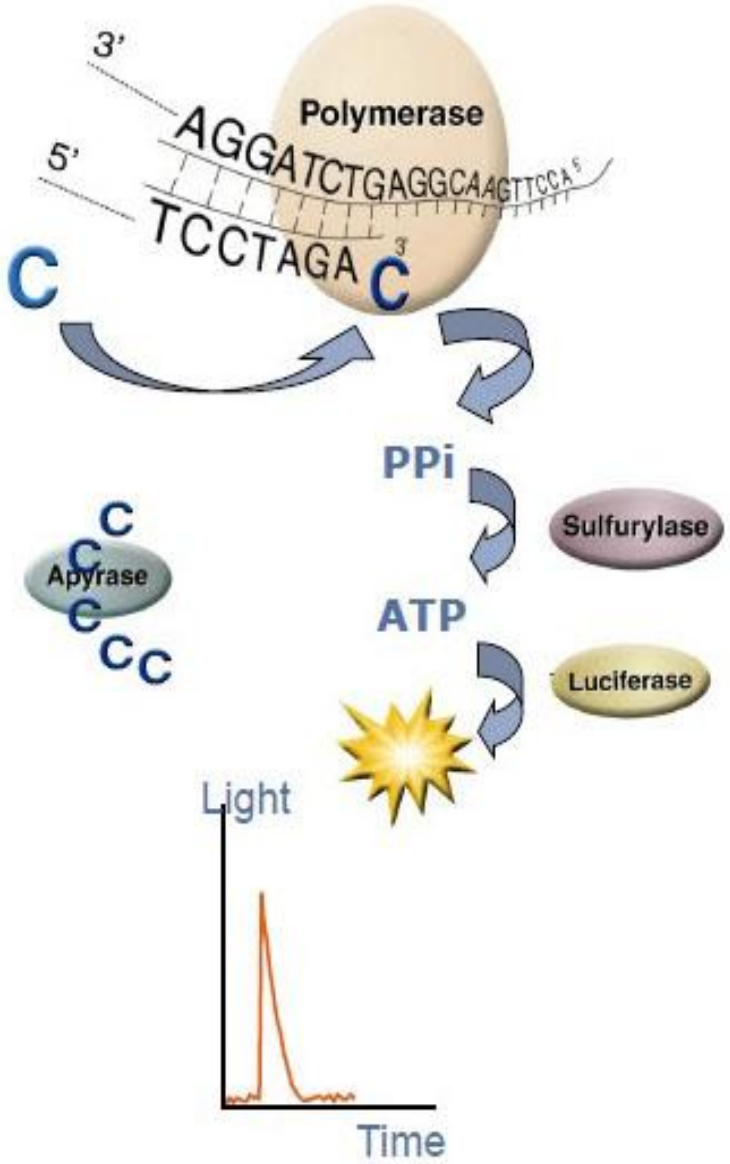
焦磷酸测序的技术原理（1）

- 焦磷酸测序技术是由4种酶（**DNA 聚合酶、ATP硫酸化酶、荧光素酶和三磷酸腺苷双磷酸酶**）催化的同一反应体系中的酶级联化学发光反应。具体过程如下：
  - 仪器向待检测样品中**每次只加入一个核苷酸碱基(dNTP)**；
  - 互补的碱基结合在模板上时，会释放出**焦磷酸基(PPi)** ；



焦磷酸测序的技术原理（2）

- PPi在**ATP硫酸化酶(ATP Sulfurylase)作用**下，**合成ATP**；
- ATP作为能量分子，驱动荧光素在**荧光素酶**的催化下转化为氧化**荧光素**，并发出与**ATP能量成正比的荧光信号**；
- 信号被CCD数码相机捕捉，形成峰形PyrogramTM；
- 未与模板结合的碱基将被**三磷酸腺苷双磷酸酶(Apyrase)降解，**加入下一种dNTP。

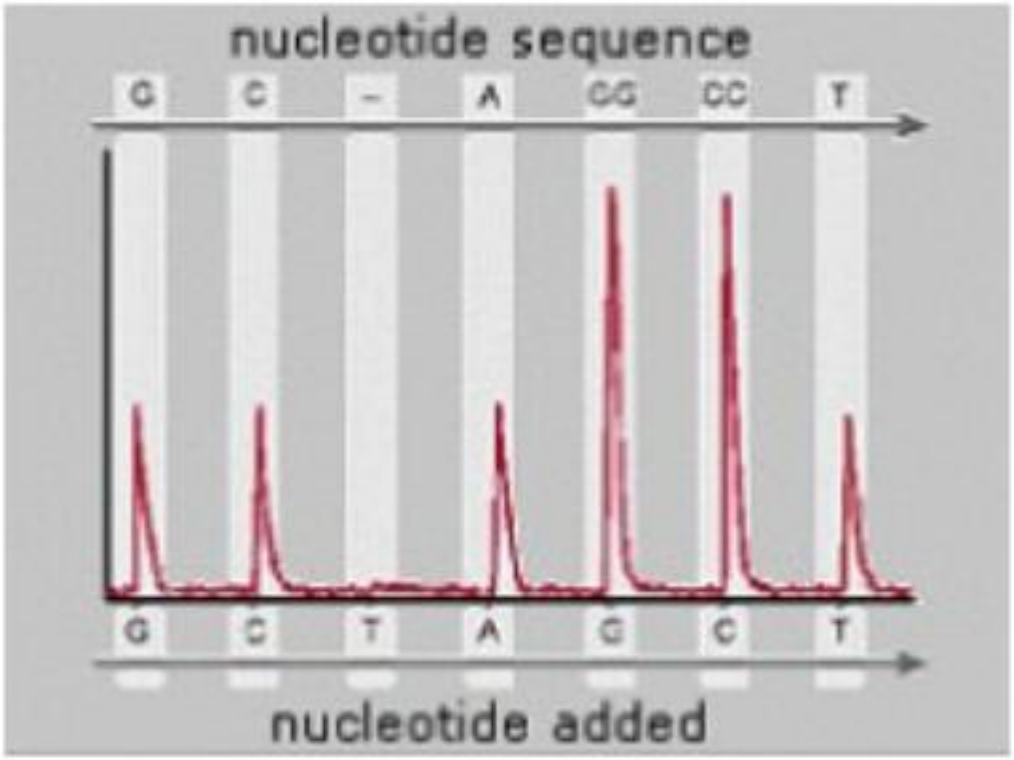


焦磷酸测序的技术原理（3）

- 未与模板结合的碱基将被三磷酸腺苷双磷酸酶(Apyrase)降解，加入下一种dNTP。

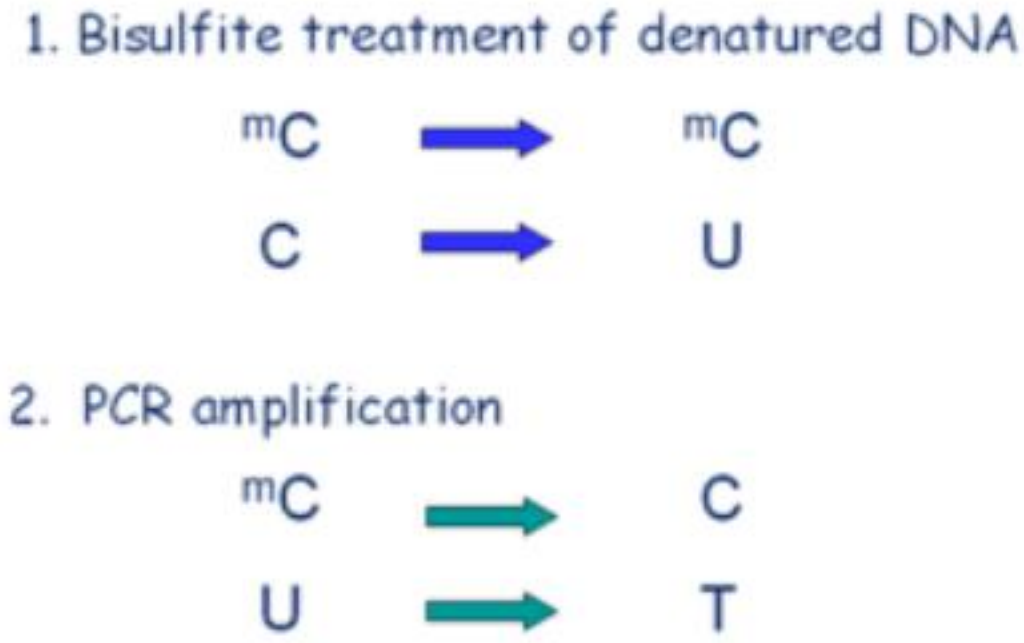
焦磷酸测序的技术原理（4）

- 加入另一种dNTP，使第2-4步反应重复进行，根据获得的峰值图即可读取准确的DNA序列信息。



焦磷酸测序的技术原理（5）

- 在重硫酸盐的作用下，所有**未甲基化的胞嘧啶**发生**脱氨基反应转变成了尿嘧啶**，但是**5‘-甲基胞嘧啶**不发生转变。

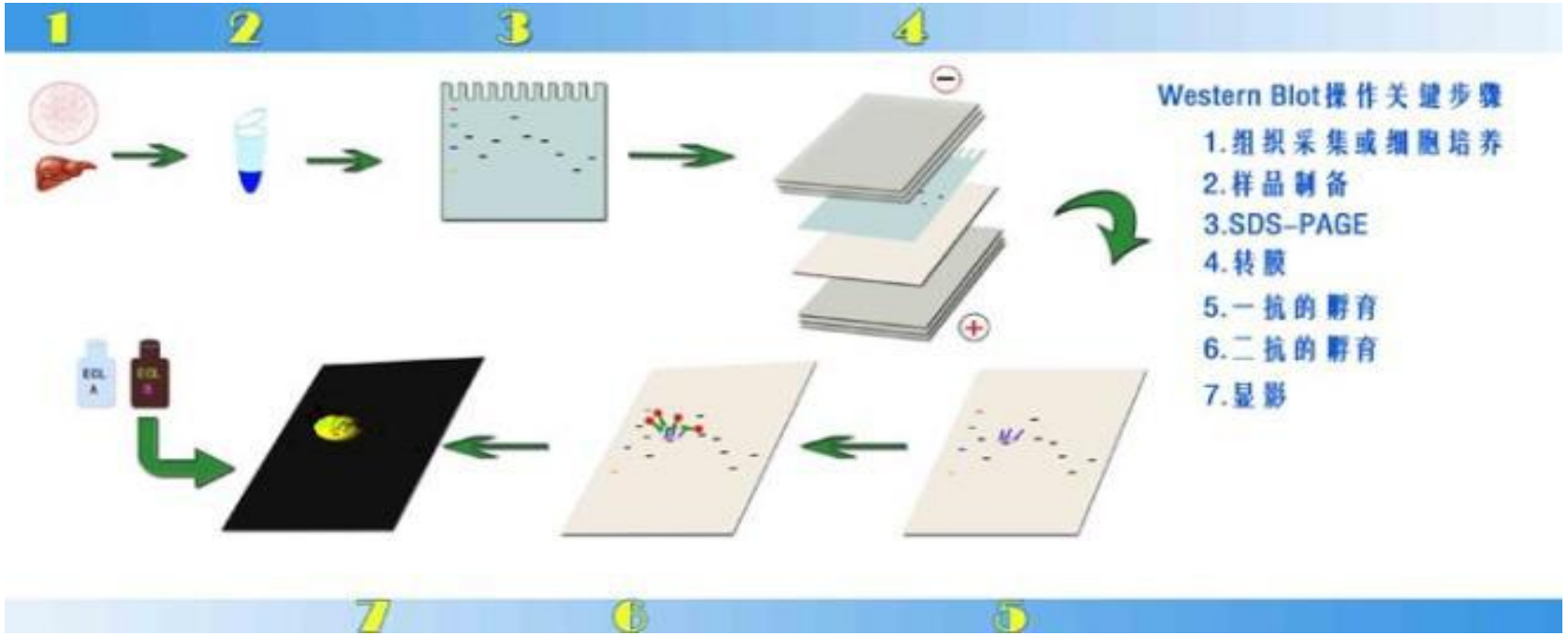


免疫印迹技术

-Western blotting

免疫印迹技术-western blotting

● **基本原理**：将电泳分离后的**细胞或组织总蛋白质**从凝胶转移到**固相支持物NC膜或PVDF膜**上，然后用**特异性抗体检测某特定抗原的一种蛋白质检测技术**，现已广泛应用于基因在蛋白水平的表达研究、抗体活性检测和疾病早期诊断等多个方面。



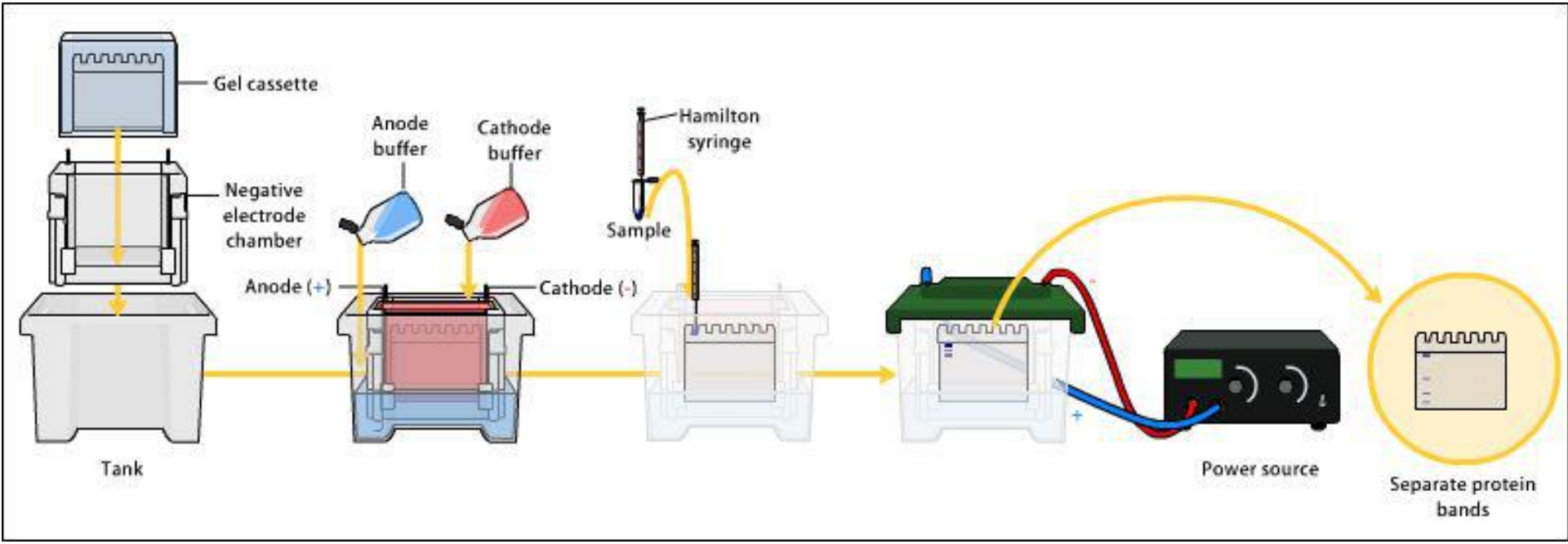
WB-操作过程

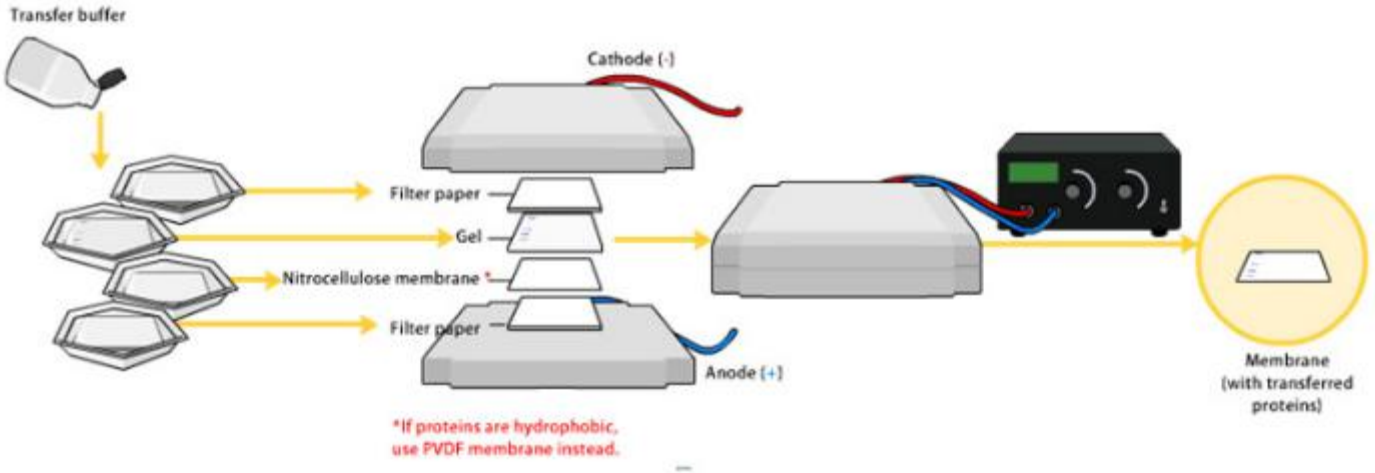

组织/细胞
重组蛋白
ECL
DAB
蛋白提取(表达)
蛋白
SDS-PAGE/转膜
检测信号


蛋白印迹流程
电泳
印迹
标记
检测
• 分子量标准
• 电泳系统
• 电源
• 电转移系统
• 印迹膜
• 二抗
• ECL检测试剂盒
• 底片
• 压片盒

SDS-聚丙烯酰胺凝胶电泳

● **基本原理**：聚丙烯酰胺凝胶为网状结构，具有分子筛效应。SDS-PAGE仅根据蛋白质亚基分子量的不同就可以分开蛋白质。

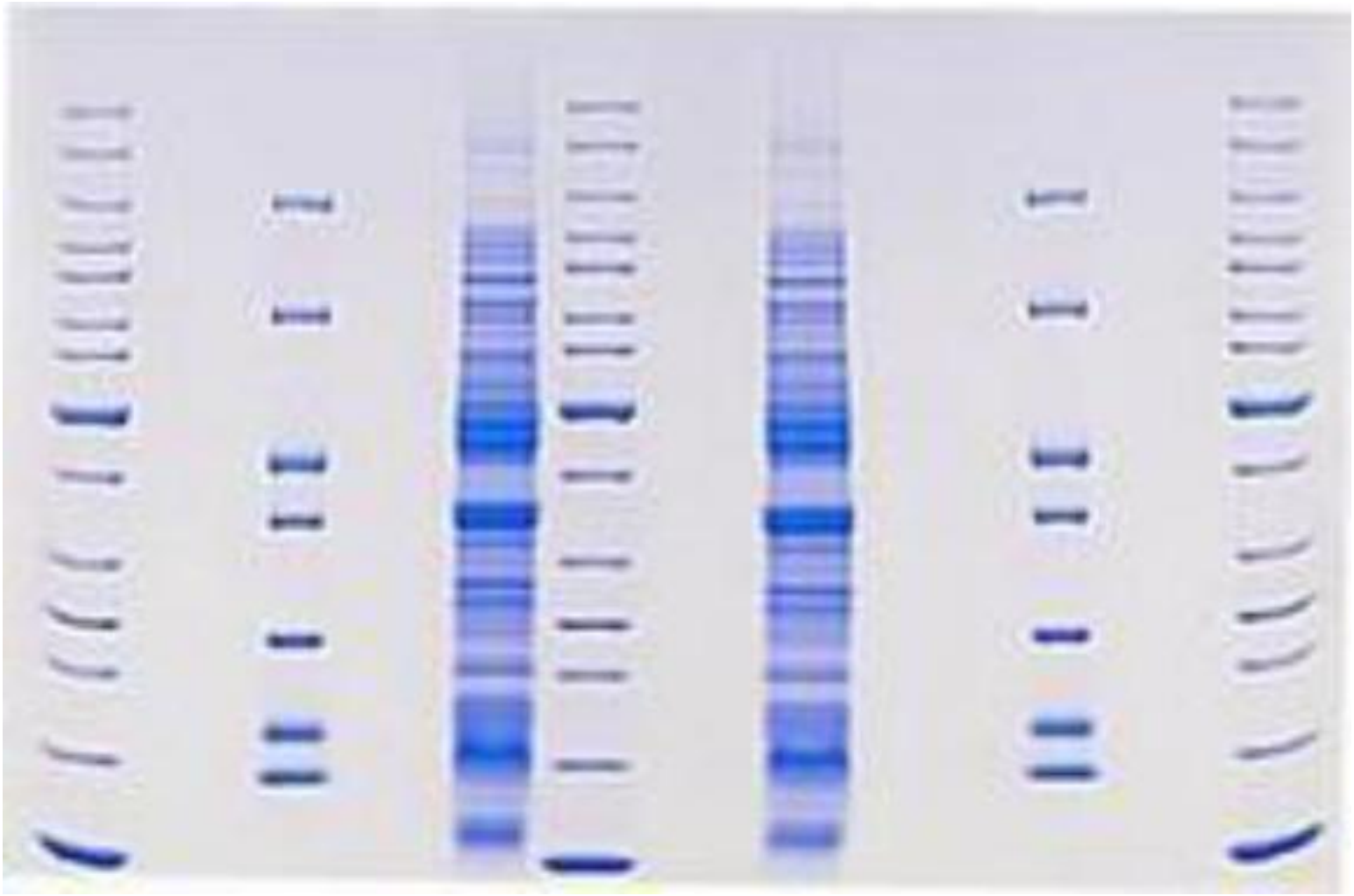



Transfer buffer
Cathode (-)
Filter paper
Gel
Nitrocellulose membrane *
Filter paper
Anode (+)
*If proteins are hydrophobic,
use PVDF membrane instead.
Membrane
(with transferred
proteins)

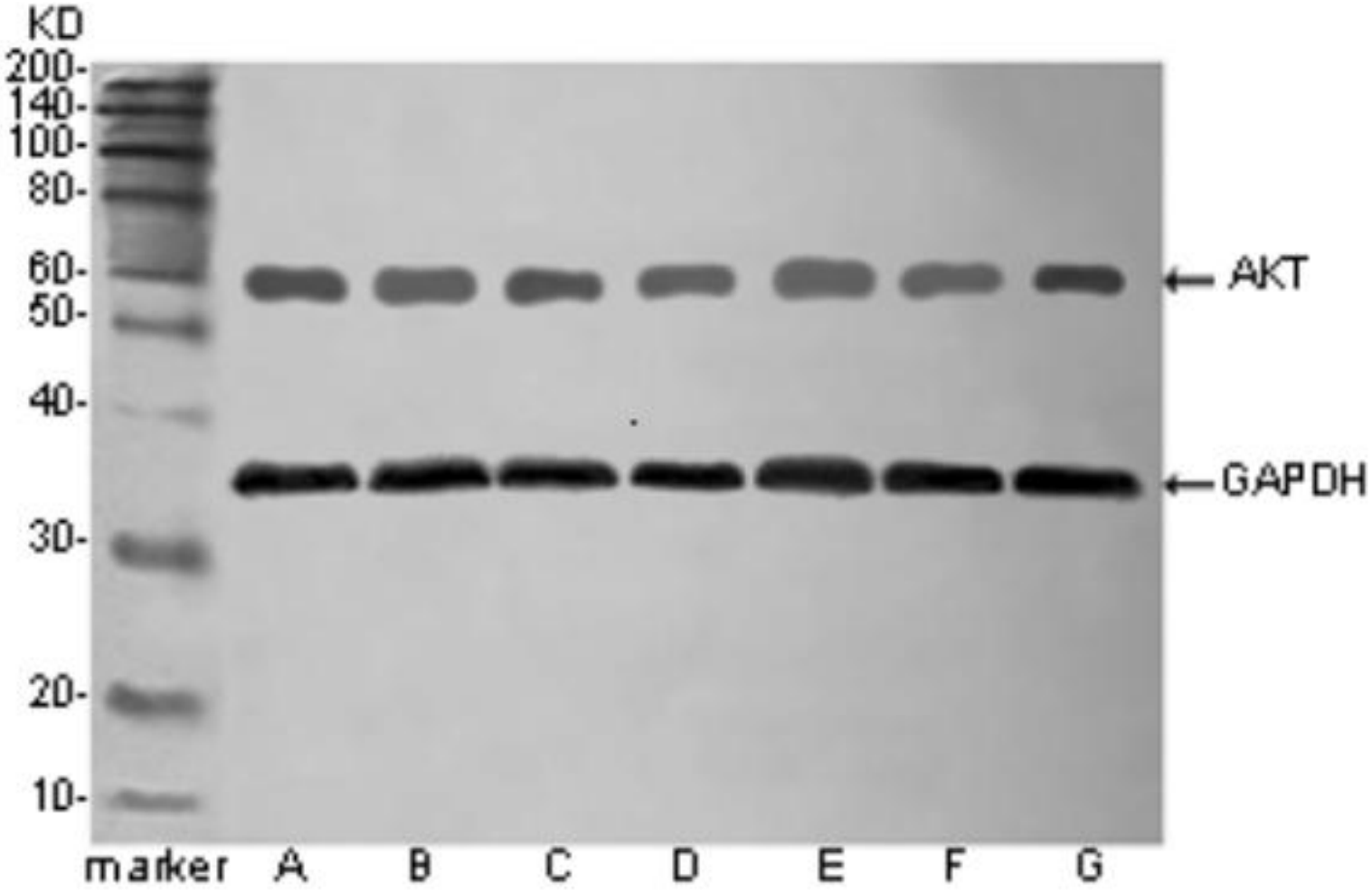
SDS-聚丙烯酰胺凝胶制备原理

- 聚丙烯酰胺凝胶作为支持介质的一种常用电泳技术。
- 聚丙烯酰胺凝胶由**单体丙烯酰胺**和**甲叉双丙烯酰胺**聚合而成，聚合过程由自由基催化完成。
- 化学聚合以**过硫酸铵（APS）为催化剂**，以**四甲基乙二胺（TEMED）为加速剂**。
- 在聚合过程中，TEMED催化过硫酸铵产生自由基，后者引发丙烯酰胺单体聚合，同时甲叉双丙烯酰胺与丙烯酰胺链间产生甲叉键交联，从而形成三维网状结构。

SDS-聚丙烯酰胺凝胶

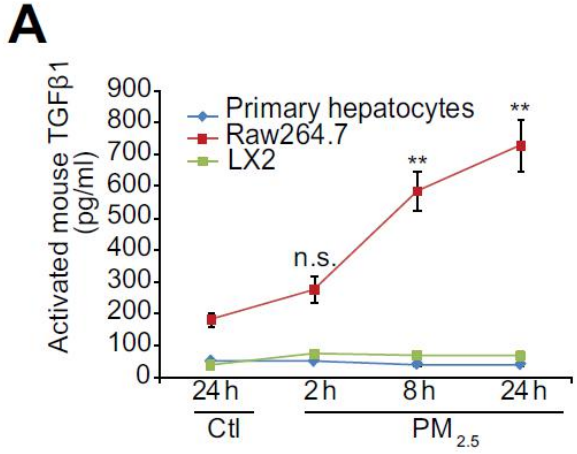


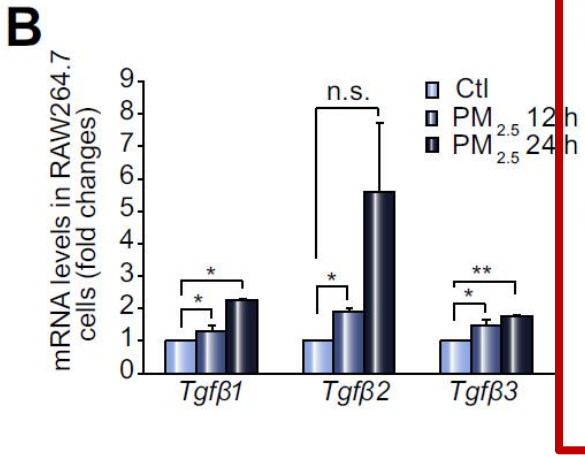
曝光结果

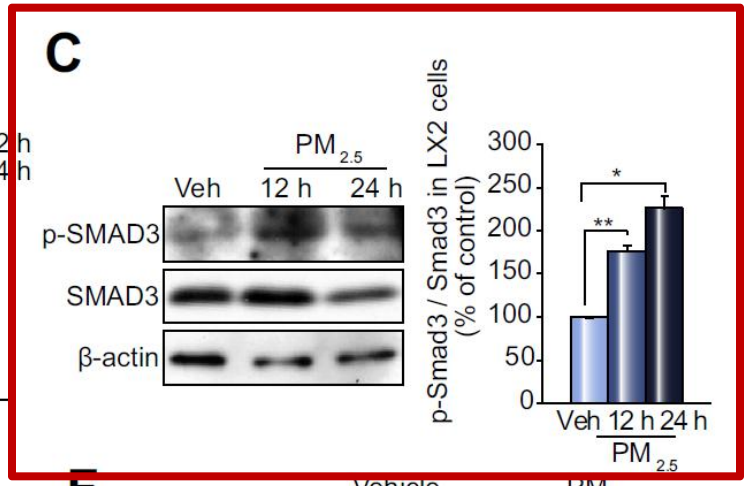


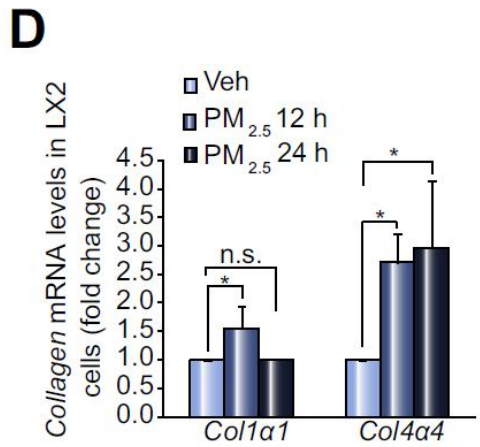
WB视频约4分钟

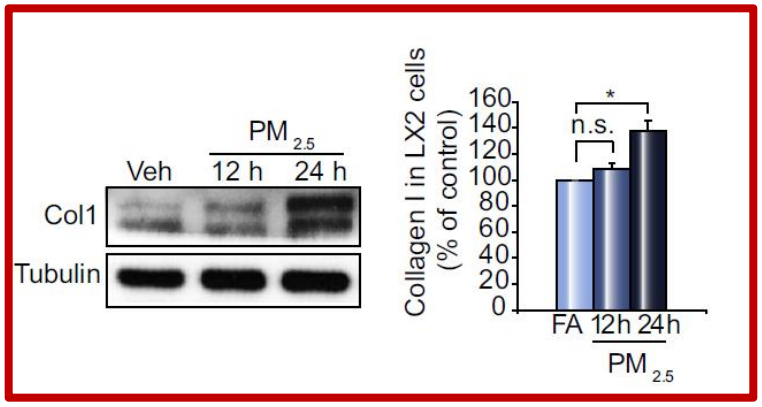
举例—$PM_{2.5}$导致巨噬细胞TGF-β信号通路激活，肝脏细胞中胶原水平升高

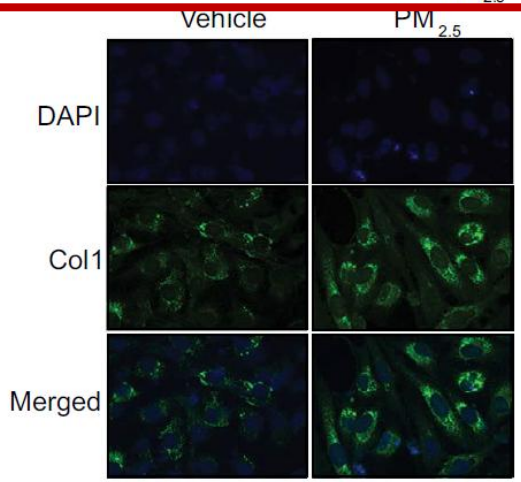












- LX-2, a human HSC cell line; **p-SMAD3**: a key mediator of TGFb-triggered fibrotic response.

结果分析

Image J软件