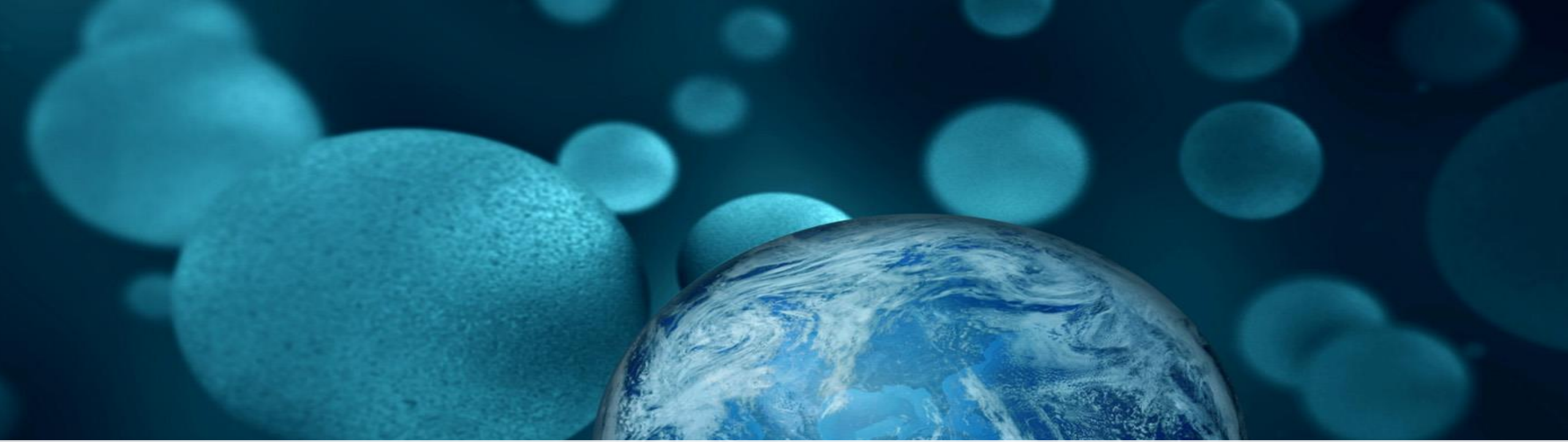
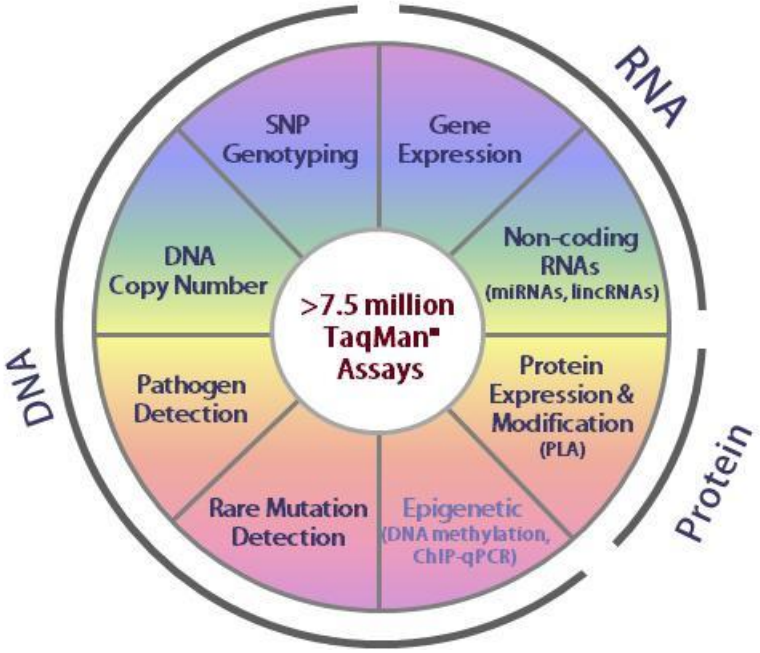


ThermoFisher SCIENTIFIC

实时荧光定量PCR技术应用

TaqMan® Assay Total Solution

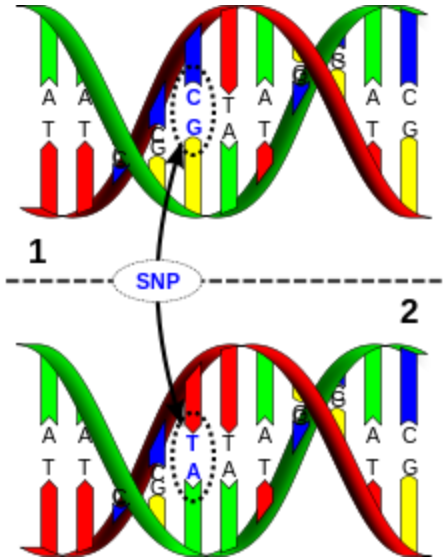
Solutions



Genotyping

为什么研究SNP

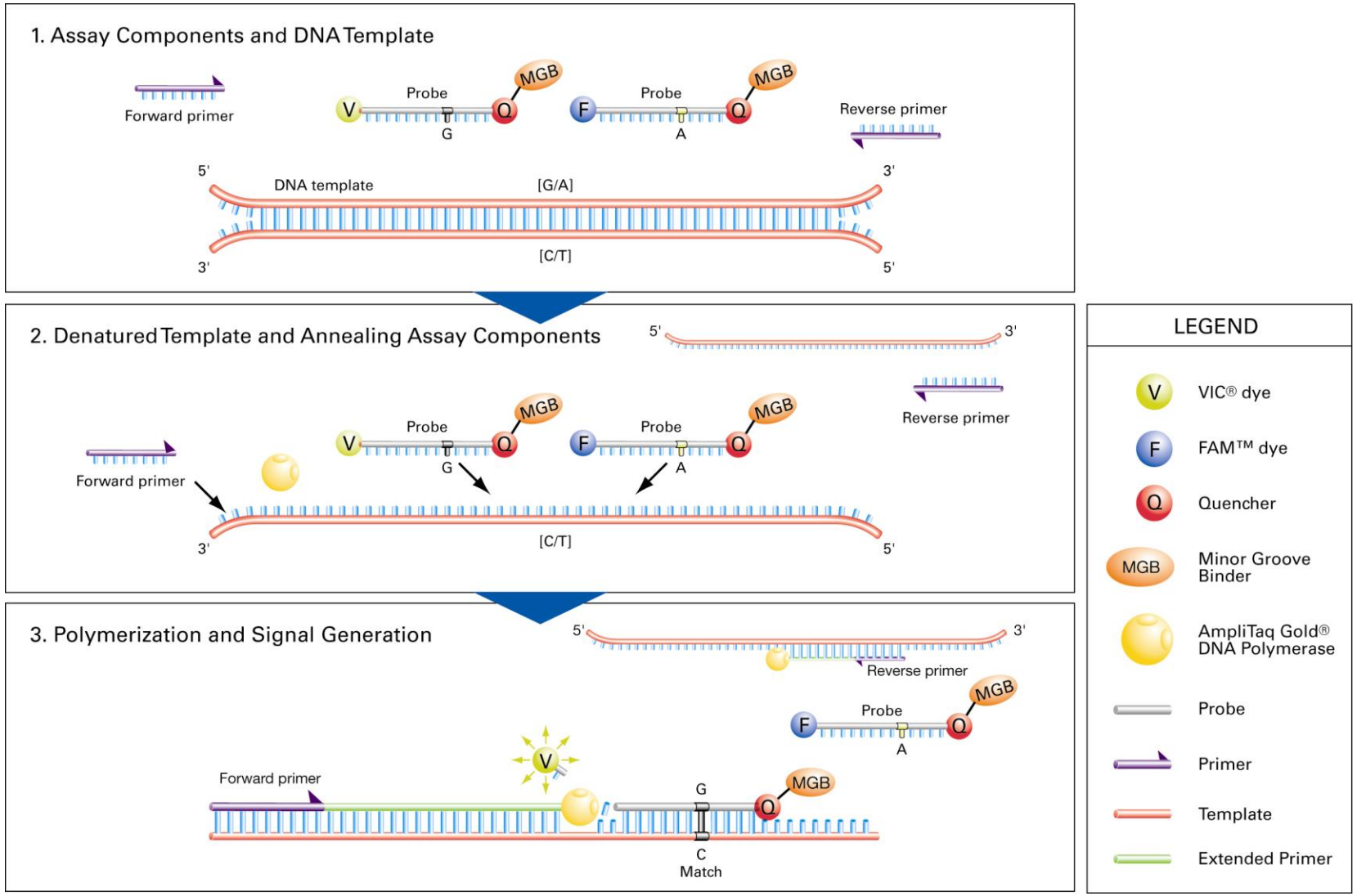
(Single Nucleotide Polymorphisms)



a change in the nucleotide present at a single base in a known DNA sequence.

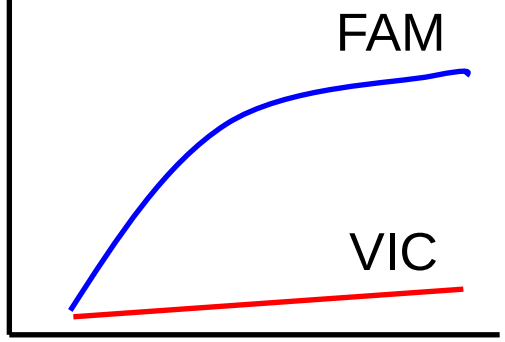
- **Diseases:**
  - Diabetes 糖尿病
  - Inflammatory bowel:Gallstones 胆结石
- **Cardiovascular conditions and lipid metabolism:**
  - Atrial fibrillation 心房颤动
  - cholesterol 胆固醇转运
- **Neuropsychiatric conditions:**
  - Amyotrophic Lateral Sclerosis 肌萎缩侧索硬化
  - Schizophrenia 精神分裂症
  - Bipolar disorder 躁郁症
- **Autoimmune and infectious diseases:**
  - Rheumatoid arthritis 类风湿性关节炎
  - Childhood asthma 儿童哮喘
- **Cancer：**
  - Prostate cancer 前列腺癌
  - Breast cancer 乳腺癌
  - Colorectal cancer 结肠直肠癌
- **Various traits:**
  - Height
  - Blue vs. green eyes

TaqMan® Genotyping Assays

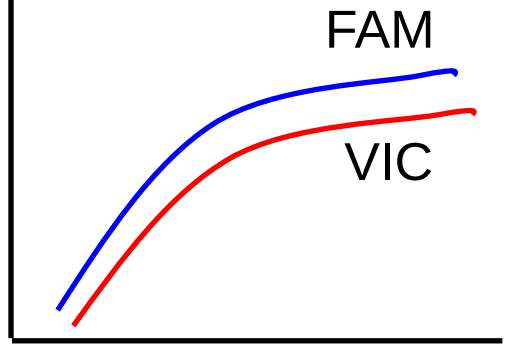


TaqMan SNP Assay检测示意图

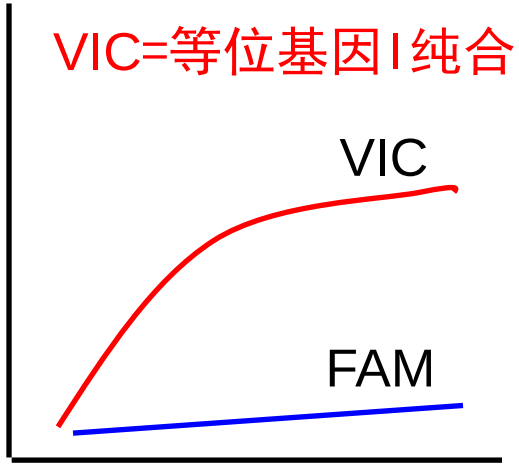
FAM=等位基因I I纯合

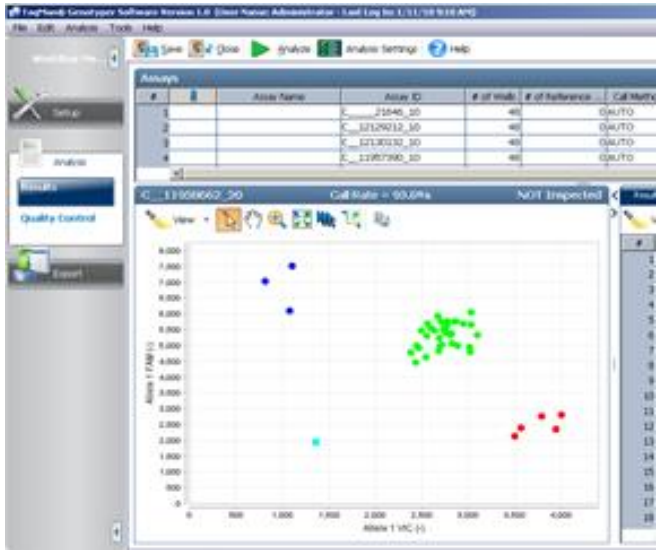


FAM+VIC=杂合子



VIC=等位基因I纯合





TaqMan® SNP基因分型分析试剂盒

- 超过4,500,000个SNP基因分型分析试剂盒可供选择，其中包含3,500,000 HapMap SNP，70,000个cSNP
- 另提供超过10,000个小鼠的SNP基因分型检测试剂盒
- SNP Browser软件可以帮助选择和订购所需检测的位点和产品
- 另提供代客设计合成服务

TaqMan® Genotyping Assays Workflow

1. Combine sample with TaqMan® SNP Genotyping Assay

- TaqMan® SNP Genotyping Assays
- TaqMan® Universal PCR Master Mix
- DNA sample

2. Perform amplification on thermal cycler

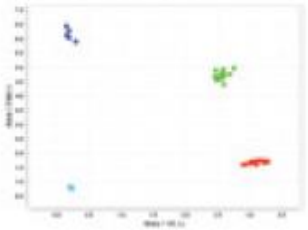
- Prepare the reaction mix
- Perform PCR

3. Read results

Real-time PCR instrument

- QS 12K, ViiA7, 7900HT, 7500, 7500 Fast, StepOne ,StepOnePlus

4. Analyze results using TaqMan® Genotyper



- TaqMan® Genotyper Software

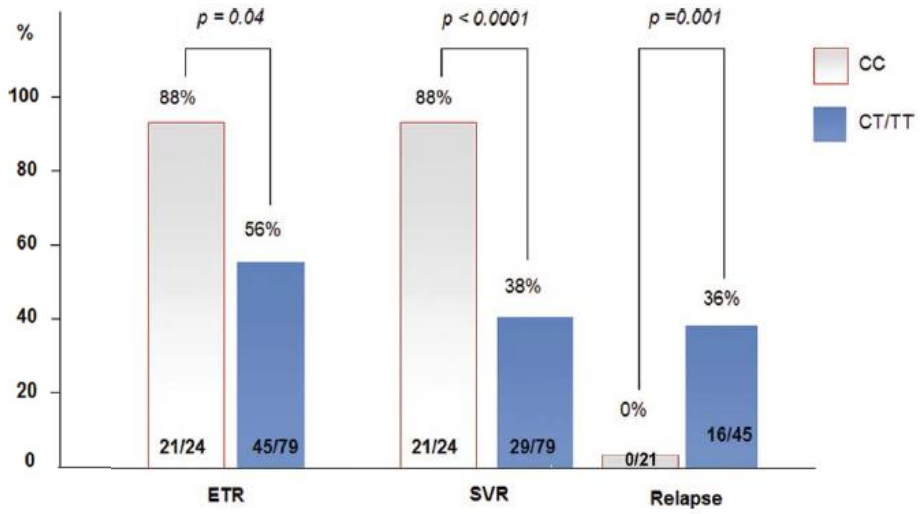
Application

VIRAL HEPATITIS

Interleukin 28B Polymorphism Predicts Pegylated Interferon Plus Ribavirin Treatment Outcome in Chronic Hepatitis C Genotype 4

Stella De Nicola,[1] Alessio Aghemo,[1] Maria Grazia Rumi,[2] Enrico Galmozzi,[1] Luca Valenti,[3] Roberta Soffredini,[1]

Patients	Overall (N=112)
rs12979860 genotypes*	
CC	24 (23%)
CT	65 (63%)
TT	14 (14%)



IL28B rs12979860 genotype is a strong predictor of treatment outcome to PegIFN plus Rbv in HCV-4 monoinfected Patients.

The incorporation of the IL28B genotype in the treatment algorithm of HCV-4 patients.

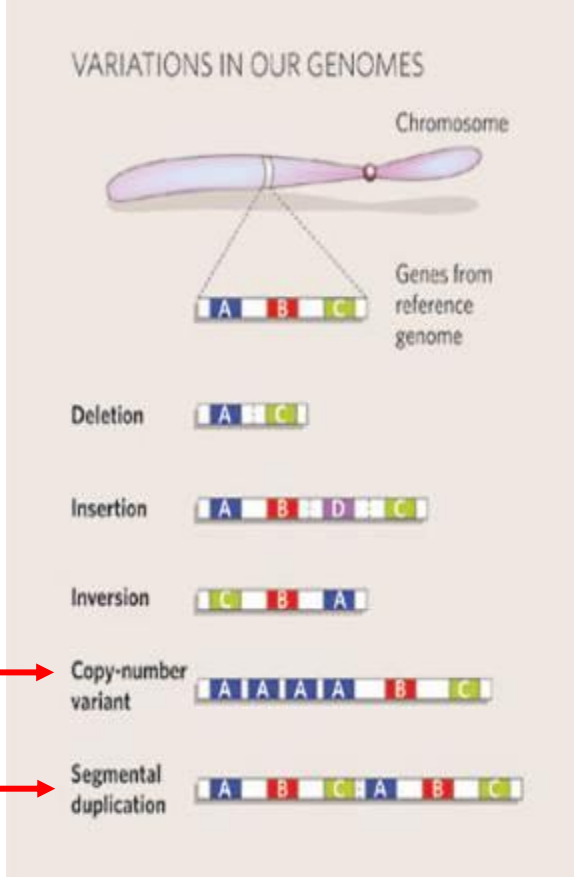
Highlight the need to consider this factor in the design and randomization of drug discovery studies

TaqMan® genotyping PCR assays
7900HT real-time PCR instrument

S. De Nicola *et al.*, *Hepatology* **55**, 336 (Feb, 2012).

Copy number variation

为什么研究CNV



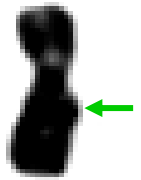
- Copy Number Variation (CNV)定义
  - 基因组发生DNA片段缺失、重复、插入、倒位
  - 片段范围：50bp-1mb. Most in 1-10kb
  - CNV分布于人类基因组

    ~ > 290, 000 identified CNVs

- CNV的重要性：与多种疾病表型紧密相关
  - HIV/AIDS susceptibility (CCL3L1)
  - Rheumatoid Arthritis风湿性关节炎
  - Neuropathies 神经系统疾病(PMP22)
  - Type I diabetes
  - Lupus 红斑狼疮-免疫系统疾病(FCGR3B & C4)
  - Crohn'sdisease节段性回肠炎(DEFB4)
  - Cancer
  - Drug Metabolism (e.g. CYP2D6)

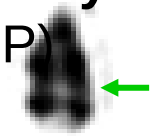
Copy number variation（CNV）
by Relative Quantitation (an example)

Test assay
(FAM, YIPF6)



Chr X

Reference assay
(VIC, RNase P)



Chr 14

Test
Sample (♂)

Ct (FAM) = 28.5

Ct (VIC) = 27.0

Calibrator
Sample (♀)

Ct (FAM) = 27.5

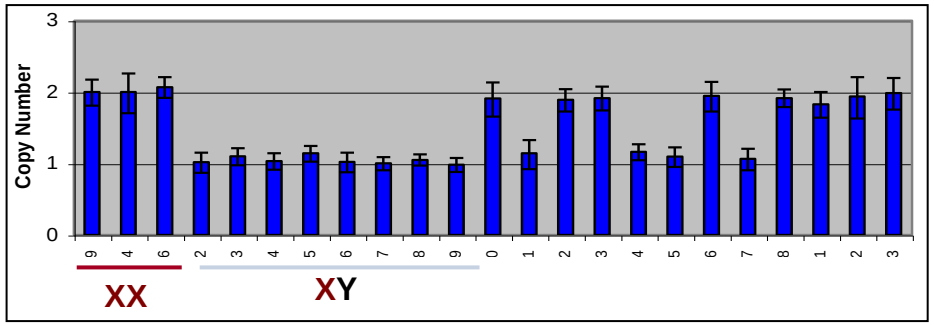
Ct (VIC) = 27.0

ΔCt = 1.5

ΔCt = 0.5

ΔΔCt = 1

Copy number
$2^{-\Delta\Delta Ct} \times 2 = 1$



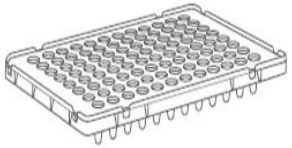
TaqMan® Copy Number Assays

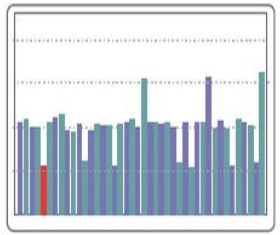
- Human: 1,600,000 assays
- Mouse: 180,000 assays

Easy to use, robust, and accurate

TaqMan® Copy Number Assay Workflow

1. Prepare Sample
2. Combine and load plate
3. Run on real-time PCR instrument
4. Analyze with CopyCaller™ Software





- TaqMan® Copy Number Assay (Test Assay)
- TaqMan® Copy Number Reference Assay
- TaqMan® Master Mix
- gDNA

- Prepare the reaction mix
- Add the reaction mix to the prepared DNA reaction plate
- Perform PCR

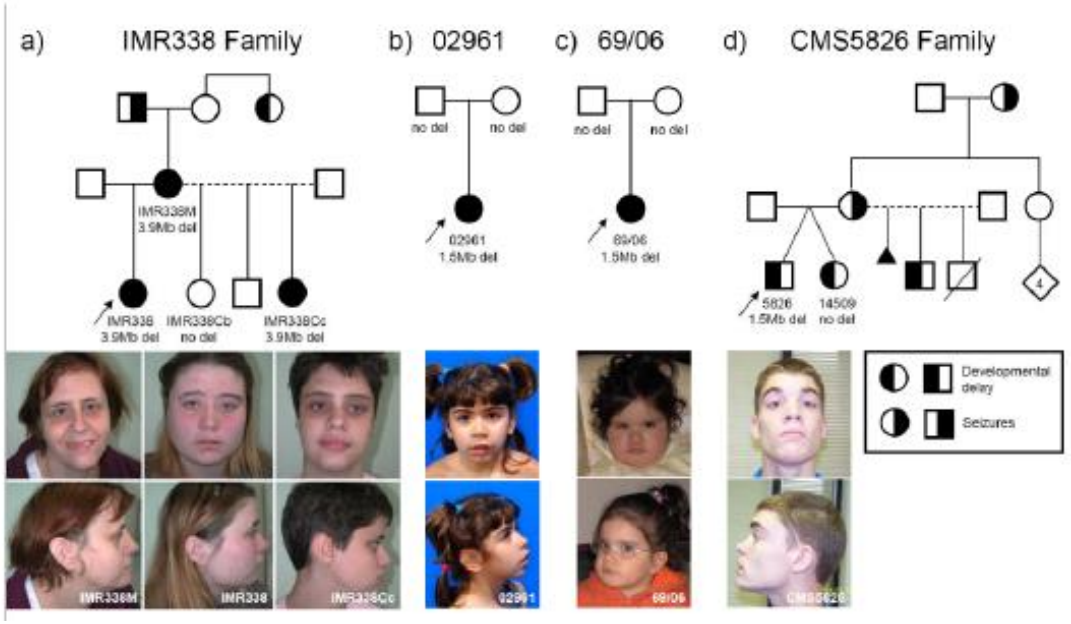
- QS 12K, ViiA7, 7900HT, 7500, 7500 Fast, StepOne ,StepOnePlus

- CopyCaller® Software

Application

Published as: *Nat Genet*. 2008 March ; 40(3): 322–328.

A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures



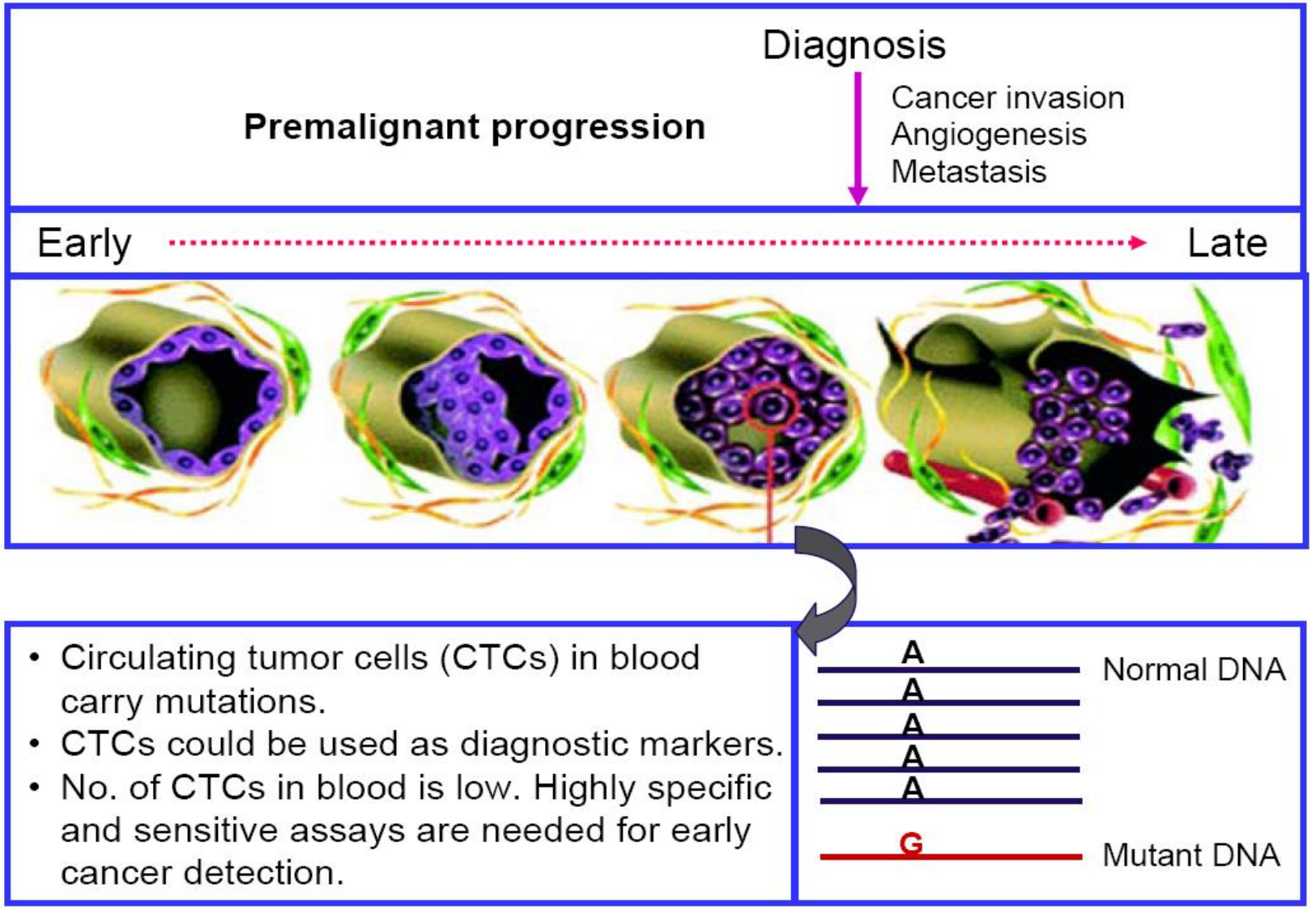
This recurrent 1.5 Mb deletion contains six genes, including a candidate gene for epilepsy (CHRNA7) that is likely responsible for the observed seizure phenotype

TaqMan® Copy Number Assay
Applied Biosystems 7900HT SDS instrument

A. J. Sharp et al., Nat Genet 40, 322 (Mar, 2008).

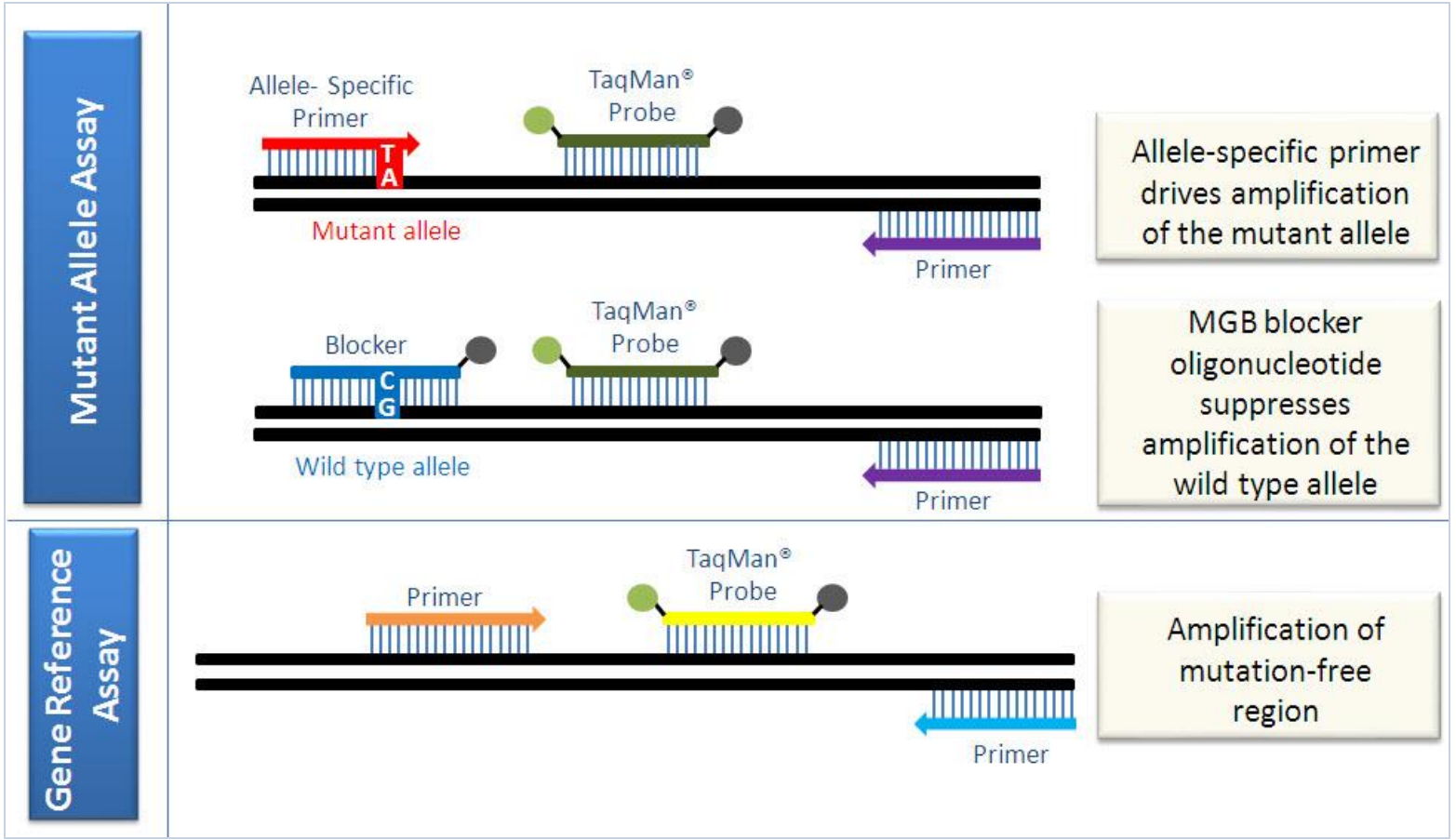
Rare Mutation Detection

如何在早期发现肿瘤？


Diagnosis
Premalignant progression
Cancer invasion
Angiogenesis
Metastasis
Early
Late
• Circulating tumor cells (CTCs) in blood carry mutations.
• CTCs could be used as diagnostic markers.
• No. of CTCs in blood is low. Highly specific and sensitive assays are needed for early cancer detection.
A
A
A
A
A
G
Normal DNA
Mutant DNA

castPCR™:Competitive Allele-Specific TaqMan® PCR

- 只有突变等位基因（ mutant allele ）可以进行扩增
- 野生型等位基因扩增被Blocker抑制



稀有突变检测试剂盒

TaqMan® Mutation Detection Assays (TMDA)

- 产品组合：
  - 46个癌症基因上的778个关键突变位点
  - Wild-type assays for only the subset of mutation targets
  - Internal Positive Control Reagents (IPC kit)
  - Mutation Detector™ Software

- 适用于FFPE组织，新鲜、冰冻组织和培养细胞等抽提得到的基因组DNA

Gene Name		
ABL1	FGFR3	NRAS
AKT1	FLT3	PDGFRA
ALK	GNAS	PIK3CA
APC	HNF1A	PTEN
ATM	HRAS	PTPN11
BRAF	IDH1	RB1
CDH1	JAK2	RET
CDKN2A	JAK3	SMAD4
CSF1R	KDR	SMARCB1
CTNNB1	KIT	SMO
EGFR	KRAS	SRC
ERBB2	MET	STK11
ERBB4	MLH1	TP53
FBXW7	MPL	VHL
FGFR1	NOTCH1	
FGFR2	NPM1	

TaqMan® Mutation Detection Assays (TMDA)特点

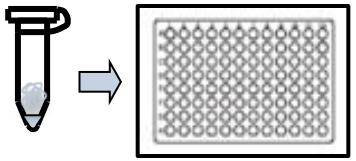
- **高灵敏度（High Sensitivity）**
  - 可以检测0. 1%突变基因
  - 在不同细胞系中，具有相同的灵敏度
- **高特异性（High Specificity）**
  - 在稀有突变检测应用中，与standard allele specific PCR和TaqMan® SNP assays相比具有更高的特异性
- **简单操作步骤（Simple Workflow）**
  - 整个工作流程 <3 hrs

TaqMan® Mutation Detection Assay /cast-PCR™ Workflow

1. Select and Order Assays

- Target assays
- Gene reference assays
- Optional IPC reagents
- TaqMan® Genotyping Master Mix

2. Prepare gDNA and reaction mix



- Prepare genomic DNA Sample
- Add Mutation Detection Assay
- Add Genotyping Master mix
- (optional: IPC)

3. Run by real-time PCR

- QS 12K, ViiA7, 7900HT, 7500, 7500 Fast, StepOnePlus

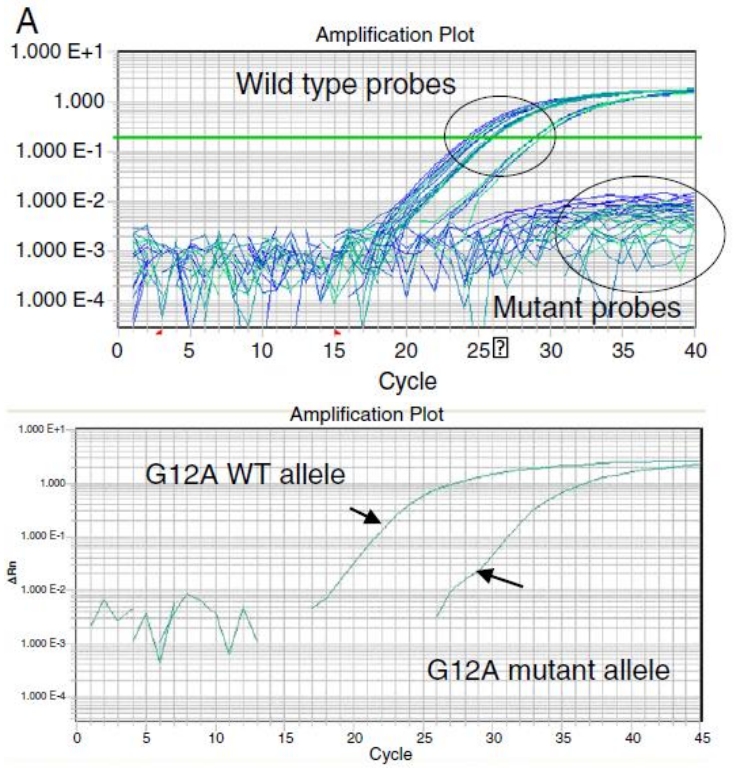
4. Detect & Quantify mutations

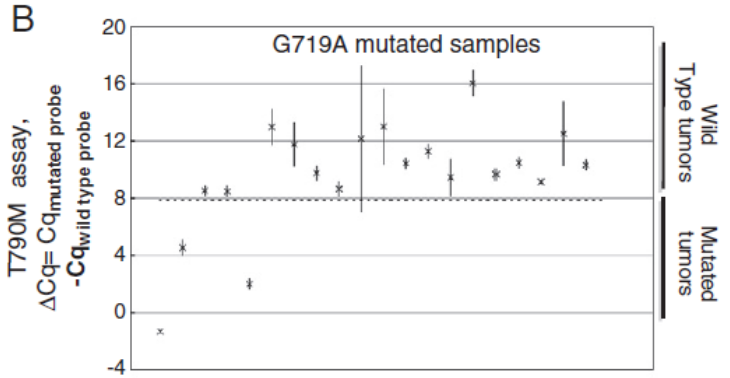
- Mutation Detector™ Software

Application

Competitive allele specific TaqMan PCR for *KRAS*, *BRAF* and *EGFR* mutation detection in clinical formalin fixed paraffin embedded samples

Audrey Didelot [a,b], Delphine Le Corre [a,b], Armelle Luscan [c], Aurélie Cazes [b,c], Karine Pallier [a], Jean-François Emile [d], Pierre Laurent-Puig [a,b,c], Hélène Blons [a,b,c,*]





"less laborious and requires less stringent precautions to prevent crossover"
"an important technology to consider in the field of mutation detection for clinical purpose "

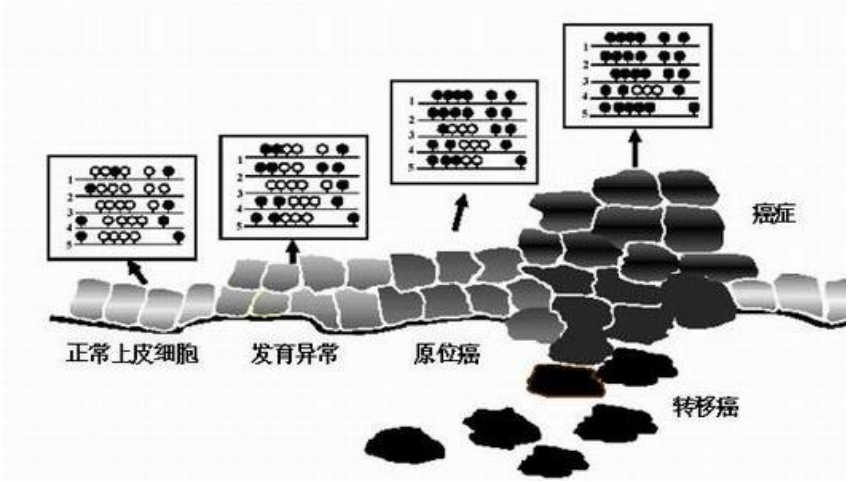
TaqMan® Mutation Detection assay
TaqMan® genotyping master mix
Applied Biosystems Prism 7900 HT sequence detection system

A. Didelot et al., Experimental and molecular pathology 92, 275 (Jun, 2012).

Percentage of Methylation

为什么研究甲基化

CpG岛甲基化与癌症



肿瘤的特征之一是不平衡的甲基化。

肿瘤的DNA甲基化改变表现为基因组整体甲基化水平降低和CpG岛局部甲基化水平异常增高。导致基因组的不稳定。

肿瘤中异常的表观遗传学改变

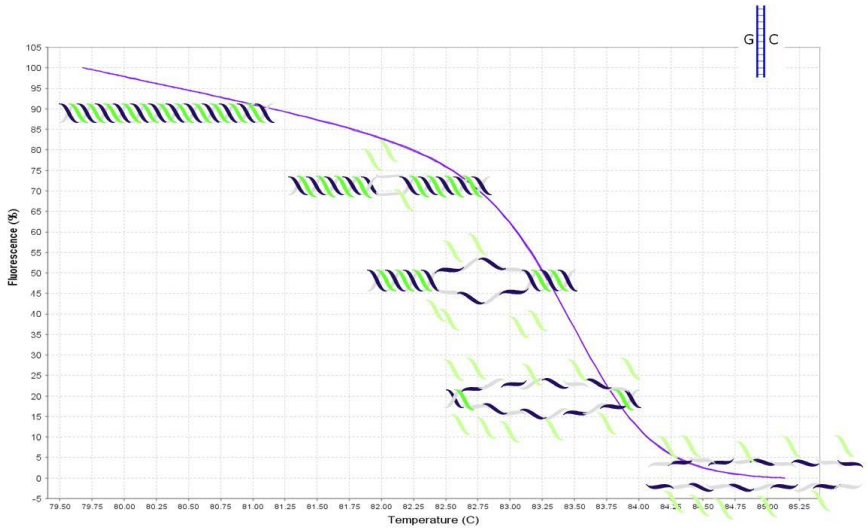
表1　肿瘤中异常的表观遗传学改变

肿瘤类型	表观遗传学改变
结肠癌	CpG岛高甲基化(hMLH1、$p16^{INK4a}$、RARB2、SFRP1和WRN)；miRNAs高甲基化(miR-124a)；基因组范围内的低甲基化；IGF2印记作用丢失；组蛋白修饰突变(EP300和HDAC2)；单乙酰化和组蛋白H4环丙烷形式的降低
乳腺癌	CpG岛高甲基化(BRCA1、E-cadgerin、TMS1及雌激素受体)；基因组范围内的低甲基化
肺癌	CpG岛高甲基化($p16^{INK4a}$、DAPK1和RASSF1A)；基因组范围内的低甲基化；CBP基因组及染色体重组因子BRG1的缺失
神经胶质瘤	CpG岛高甲基化(DNA修复酶MGMT、EMP3和THBS1)
白血病	CpG岛高甲基化($p15^{INK4b}$、EXT1和ID4)，组蛋白修饰易位(CBP、MOZ、MORF、MLL1、MLL3和 NSD1)
淋巴瘤	CpG岛高甲基化($p16^{INK4a}$、p73和DNA修复酶MGMT)；单乙酰化和组蛋白H4环丙烷形式的降低
膀胱癌	CpG岛高甲基化($p16^{INK4a}$和TPEF/HPP1)；miRNAs高甲基化(miR-127)；基因组范围内的低甲基化
肾癌	CpG岛高甲基化(VHL)；基因组范围内的低甲基化；IGF2印记作用丢失；
前列腺癌	CpG岛高甲基化(GSTP1)；polycomb组蛋白甲基转移酶EZH2基因扩增；组蛋白H3和H4异常修饰
食管癌	CpG岛高甲基化($p16^{INK4a}$和$p14^{ARF}$)；组蛋白去甲基基因JMJD2C/GASC1扩增
胃癌	CpG岛高甲基化(hMLH1和$p14^{ARF}$)
肝癌	CpG岛高甲基化(SOCS1和GSTP1)；基因组范围内的低甲基化；
卵巢癌	CpG岛高甲基化(BRCA1)

High Resolution Melting (HRM)

一种利用熔解曲线对DNA 进行分析的方法，
利用不同PCR产物，Tm值不同的原理进行分析.

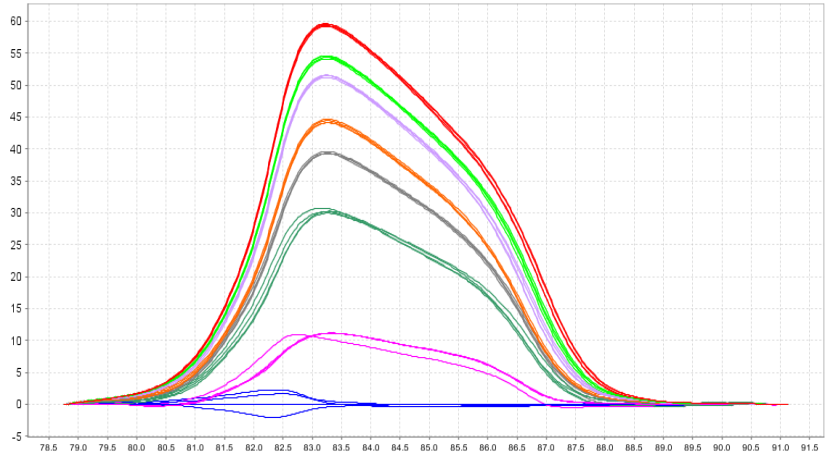
HRM Melt Curve Dynamics



Tm值：DNA的双螺旋结构打开一半时的温度

不同甲基化程度产生不同熔解曲线，通过不同Tm值进行甲基化程度的区分

Reading a High Resolution Melt Curve



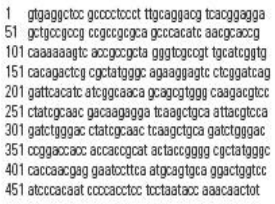
High Resolution Melting (HRM) Reagents

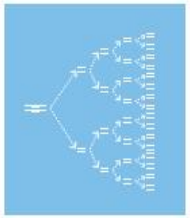
➢Specially formulated for superior performance and full integration into the AB HRM Workflow

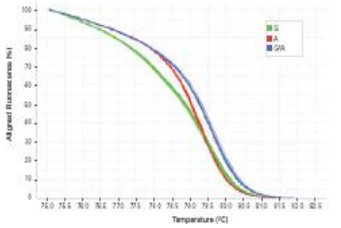
- MeltDoctor™ HRM Master Mix
- MeltDoctor™ HRM Reagent Kit
- MeltDoctor™ HRM Dye
- MeltDoctor™ HRM Calibration Plates
  - 384-well
  - 96-well
- MeltDoctor™ HRM Positive Control Kit

HRM Experiments Workflow

1. Instrument Calibration → **2. PCR Design & Optimization** → **3. PCR** → **4. Sample Melt** → **5. Data Analyze**

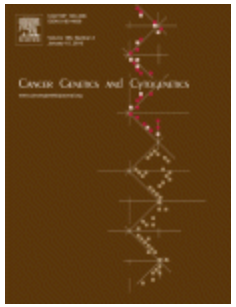






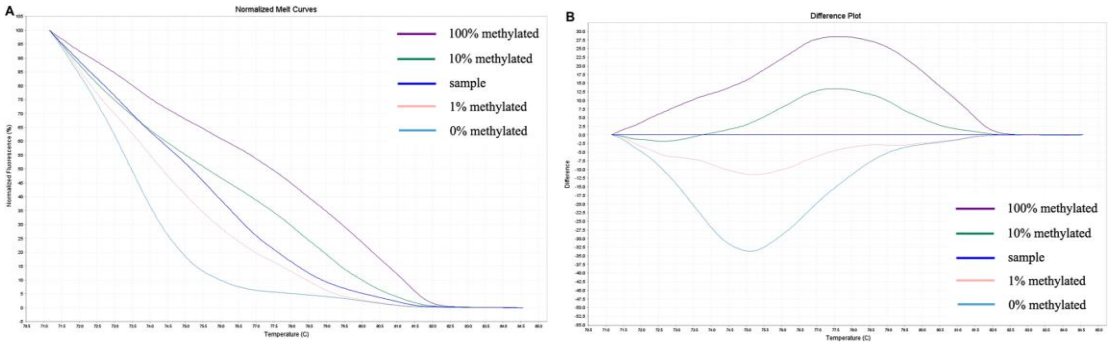
1. Instrument Calibration	2. PCR Design & Optimization	3. PCR	4. Sample Melt	5. Data Analyze
MeltDoctor™ HRM Calibration Plate StepOne™, StepOnePlus™, 7500 Fast, 7900HT Fast, or ViiA™ 7 Real-Time PCR System * Only required once every 6 months	Primer Express® Software	MeltDoctor™ HRM Master Mix MeltDoctor™ HRM Reagent Kit MeltDoctor™ HRM Positive Control Kit StepOne™, StepOnePlus™, 7900HT Fast, 7500 Fast, or ViiA™ 7 Real-Time PCR System	MeltDoctor™ HRM Master Mix MeltDoctor™ HRM Reagent Kit MeltDoctor™ HRM Positive Control Kit	High Resolution Melt Software v2.0—for use with 7900HT Fast System High Resolution Melt Software v3.0—for use with 7500 Fast, StepOne™, and StepOnePlus™ Systems ViiA™ 7 Software HRM Module—for use with ViiA™ 7 Real-Time PCR System

Applilcation



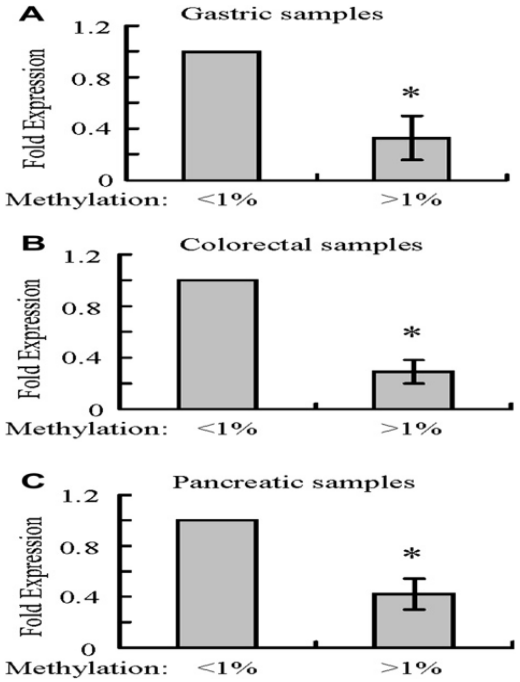
High-resolution melting analysis of *ADAMTS9* methylation levels in gastric, colorectal, and pancreatic cancers

Chao Zhang[a,1], Yong Shao[a,b,1], Wei Zhang[c], Qi Wu[a], Hong Yang[a,d], Qili Zhong[b], Jie Zhang[b], Ming Guan[e], Bo Yu[a,b,2], Jun Wan[a,f,2,*]



Methylation levels of *ADAMTS9* in cancer samples and normal tissues

Sample type	Total, no.	Methylation, no. 0%	0–1%	1–10%	10–100%	*P*-value[a]
Gastric cancer						
Cancer samples	100	41	20	26	13	<0.001
Normal tissues	100	81	17	2	0	
Colorectal cancer						
Cancer samples	100	17	29	31	23	<0.001
Normal tissues	100	67	32	1	0	
Pancreatic cancer						
Cancer samples	70	28	9	21	12	<0.001
Normal tissues	70	61	8	1	0	



High-resolution DNA melting (HRM) analysis

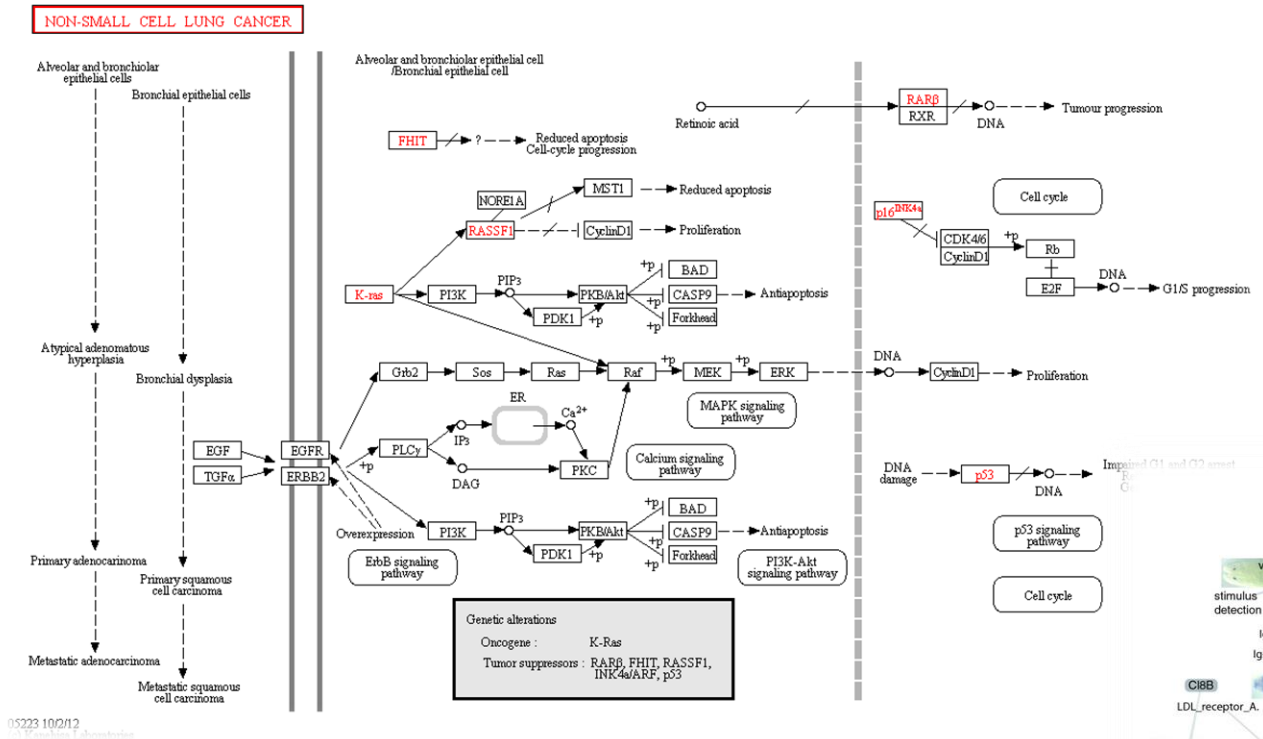
7500 FastReal-Time PCR System

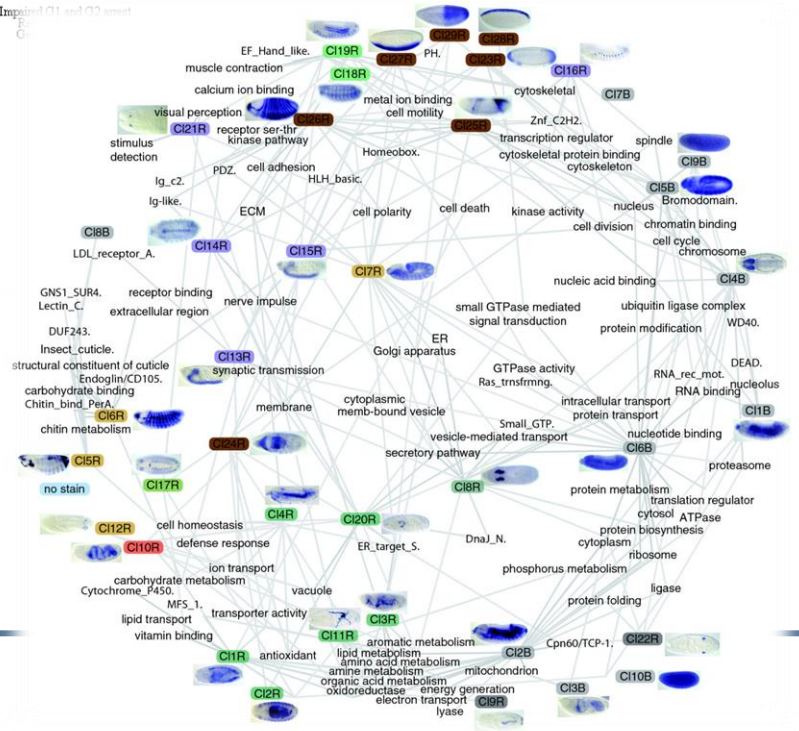
High-resolution melting v 2.0 software

Cancer Genetics and Cytogenetics 196 (2010)

Gene Expression

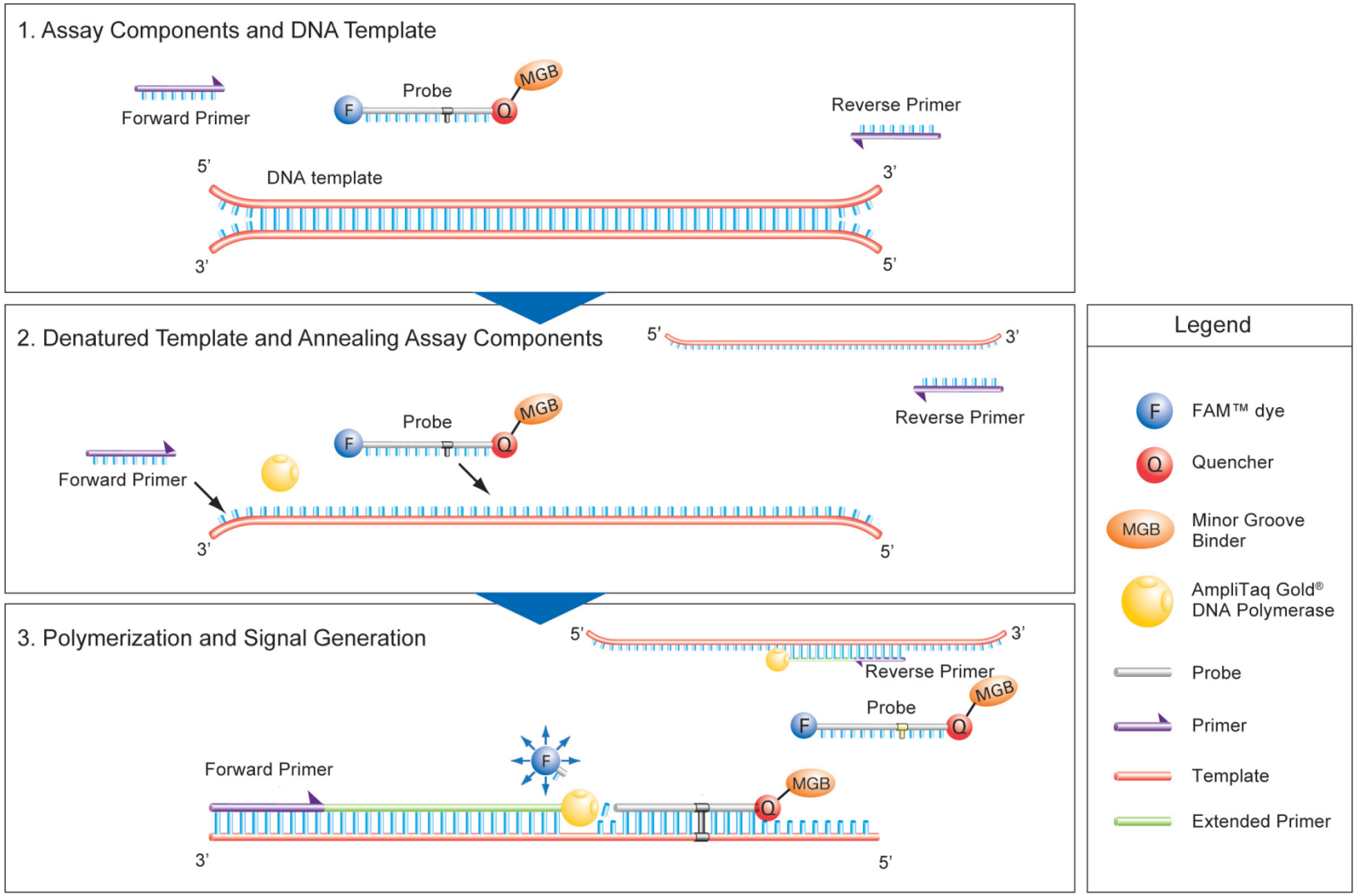
从单基因到网络调控





From single to network

TaqMan® Gene Expression Assay



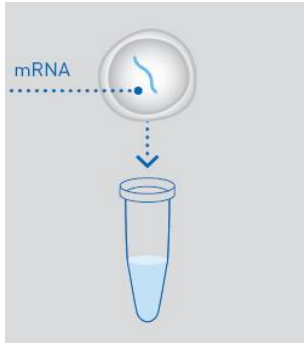
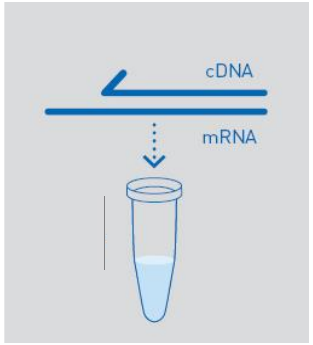
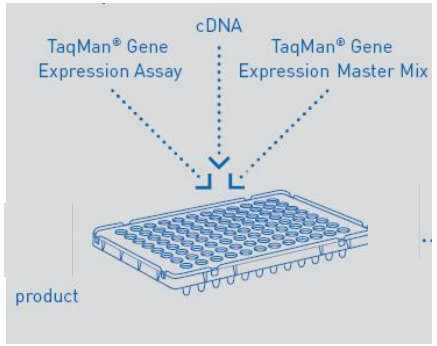
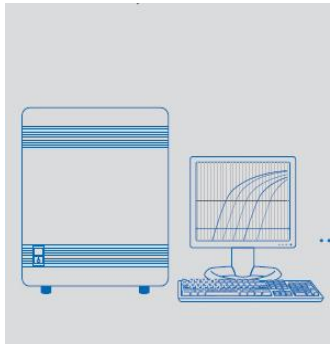
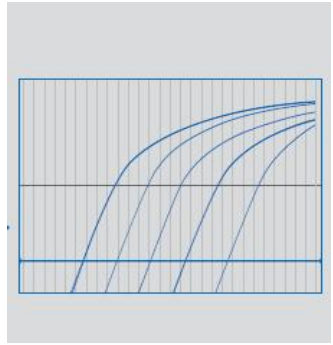
TaqMan® Gene Expression Assays

- 提供超过130万个分析试剂盒
- 涵盖人、大鼠、小鼠、犬、果蝇、拟南芥、线虫、恒河猴、猪、鸡、牛、兔和斑马鱼等29个物种
- PCR引物和TaqMan® 探针，无需优化，即可直接使用；
- 提供用户订制服务；

Species	Number of assays	Gene coverage (%)*
Human (*H. sapiens*)	212,207	99.7
Mouse (*M. musculus*)	185,566	99.6
Rat (*R. norvegicus*)	151,955	86.7
Rice (*O. sativa*)	99,822	95.6
Arabidopsis (*A. thaliana*)	97,879	94.4
Nematode (*C. elegans*)	92,687	96.2
Cow (*B. taurus*)	67,104	82.5
Rhesus monkey (*M. mulatta*)	66,480	53.9
Zebrafish (*D. rerio*)	63,712	80.9
Frog (*X. tropicalis*)	56,764	88.8
Dog (*C. familiaris*)	55,202	64.3
Chicken (*G. gallus*)	48,432	93.0
Fruit fly (*D. melanogaster*)	41,607	95.3
Sweet corn (*Z. mays*)	38,446	59.6
Pig (*S. scrofa*)	9,868	90.5
Fission yeast (*S. pombe*)	6,538	94.6
Rabbit (*O. cuniculus*)	5,924	81.0
Baker's yeast (*S. cerevisiae*)	5,525	93.8
Soybean (*G. max*)	3,456	41.1**
Horse (*E. caballus*)	2,970	76.3
Guinea pig (*C. porcellus*)	2,037	72.4%
Grape (*V. vinifera*)	965	60.1%**
Wheat (*T. aestivum*)	760	47.1%**
Summary	**1,315,906**	**80.32%, 23 species**

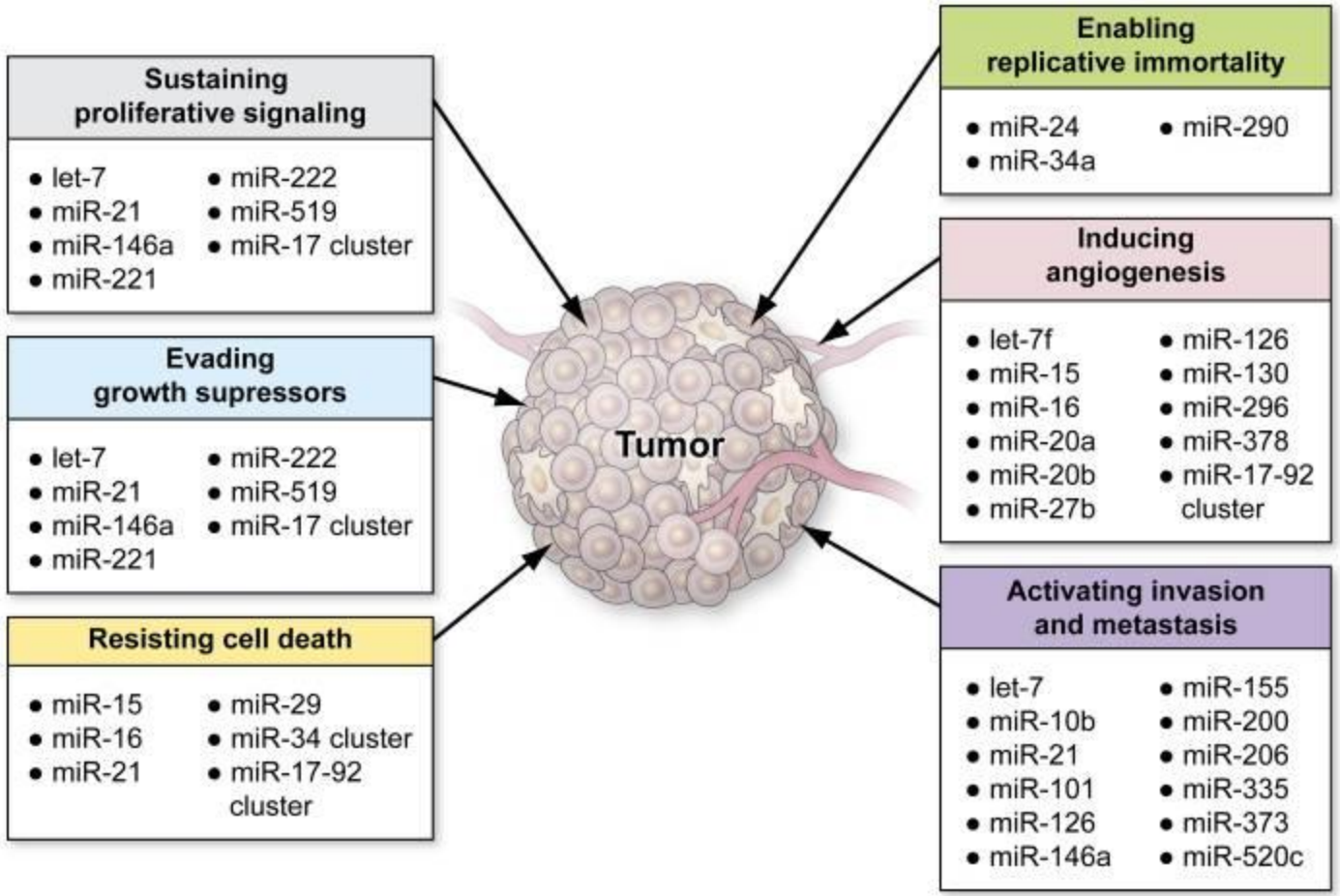
*Percent coverage refers to genes in the RefSeq database.
**These species are not represented in the RefSeq database.

TaqMan® Gene Expression Assay Workflow

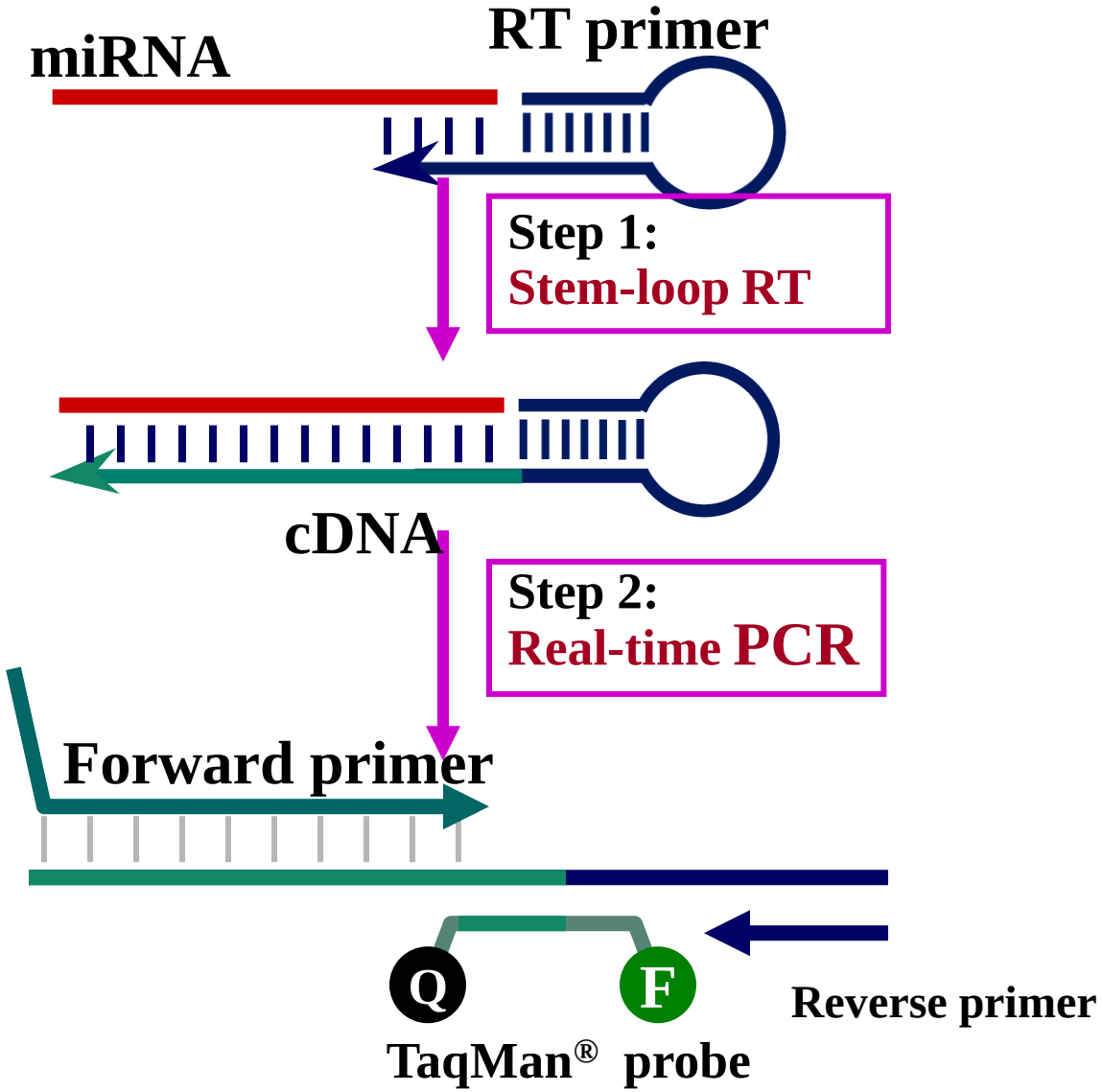
1. Extract RNA	2. Reverse-transcribe RNA	3. Set up singleplex TaqMan® Assay reactions	4. Perform real-time PCR amplification	5. Analyze data
				
• Prepare RNA	• Prepare cDNA • Add TaqMan® Gene Expression Assay •Gene reference assays	•8-Tube Strip •96-well •96-well fast •384-well •TLDA •OpenArray	• QS 12K, ViiA7, 7900HT, 7500, 7500 Fast, StepOnePlus	• SDS •RQ Manager

microRNA profiling/expression

为什么研究 microRNA



TaqMan® MicroRNA Assays



成分：

1. RT primer
2. Forward primer
3. Reverse primer
4. TaqMan® probe

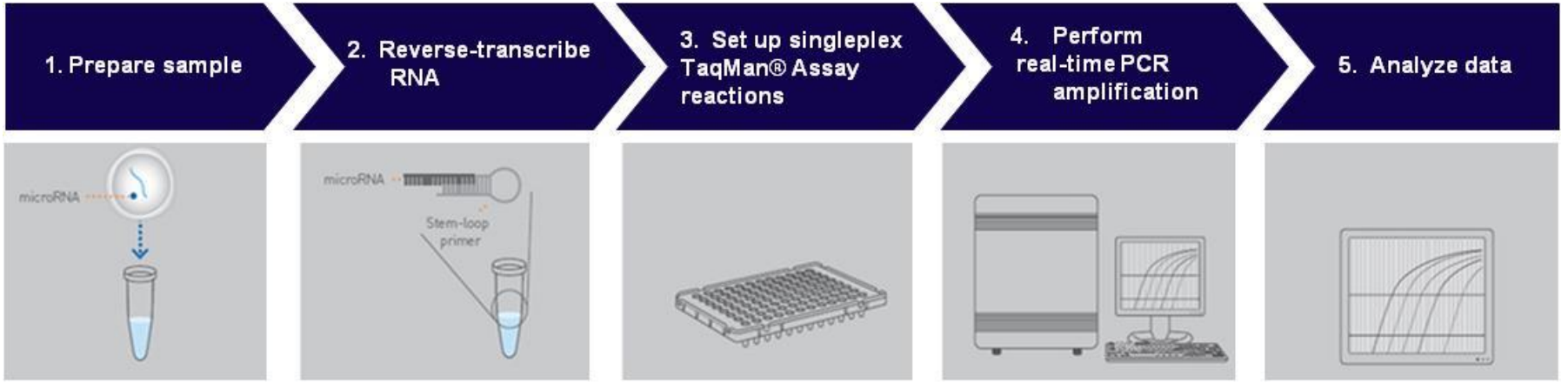
优点：

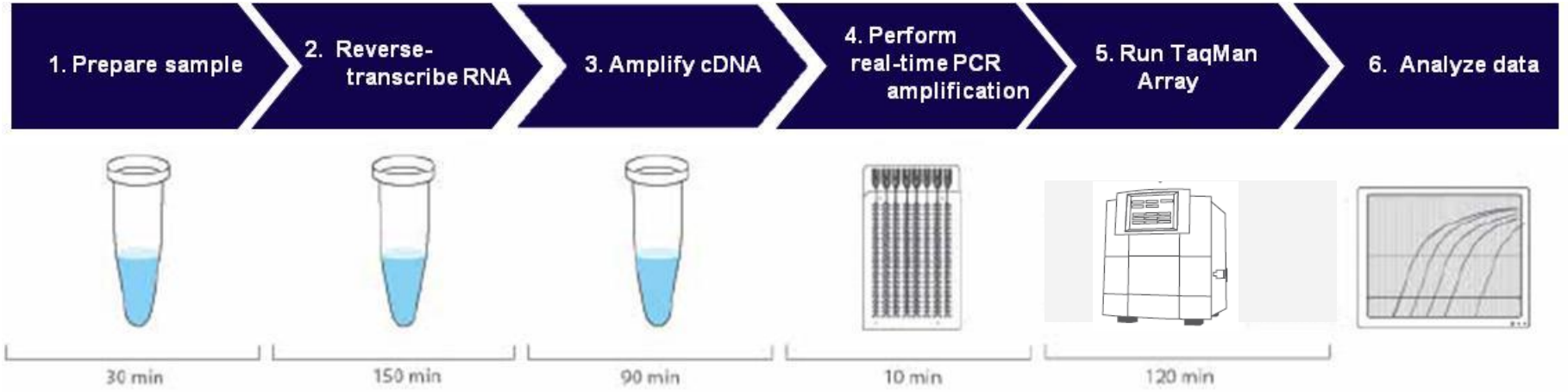
1. 高特异性，能区分前体和成熟miRNA；
2. 高灵敏度， 1-10ng样本；
3. 精确度高，动态范围宽，不同拷贝都能检测；
4. 快速-- 3小时。

TaqMan® microRNA Assays

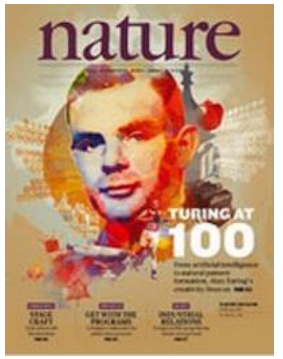
- Individual Assays
  - 1710 Human
    - ~18 Endogenous controls
  - 868 mouse
    - ~Plus 10 Endogenous controls
  - 370 Rat
  - 337 *C. elegans*
  - 184 *Arabidopsis*
  - 371 *Drosophila*
  - total 12,000

TaqMan® microRNA Assay Workflow





Application



LETTER

doi:10.1038/nature10834

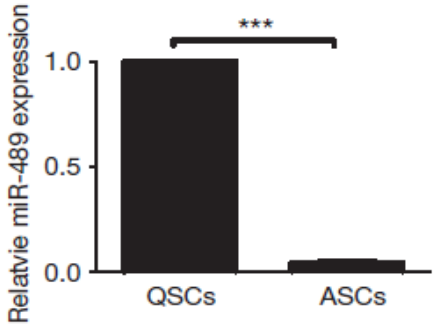
Maintenance of muscle stem-cell quiescence by microRNA-489

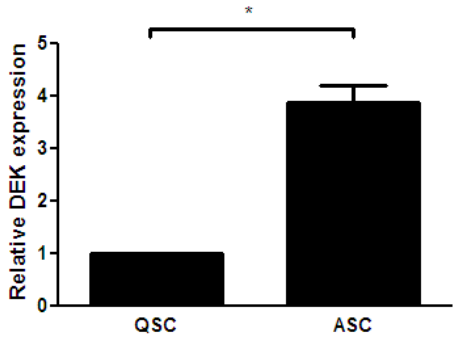
Tom H. Cheung[1,2], Navaline L. Quach[1,2], Gregory W. Charville[1,2,3], Ling Liu[1,2], Lidia Park[1,2], Abdolhossein Edalati[1,2], Bryan Yoo[1,2], Phuong Hoang[1,2] & Thomas A. Rando[1,2,4,5]

a

MiRNA name	Average fold change compared to 0 h: 36 h	60 h	84 h
miR-433			
miR-423-5p			
miR-329			
miR-673-3p			
miR-543			
miR-127			
miR-667			
miR-215			
miR-188-5p			
miR-328			
miR-673-3p			
miR-685			
miR-489			
miR-676			
miR-370			
miR-495			
miR-708			
miR-434-3p			
miR-23b			
miR-382			
miR-134			
miR-337-5p			

> 2-fold decrease
> 50-fold decrease
> 1,000-fold decrease





The finding that Dek is a key target of miR-489 in maintaining quiescence provides insight into the molecular pathways that regulate the quiescent state

TaqMan® miRNA microarrays

TaqMan® miRNA assays

TaqMan® mRNA assays

7900HT Fast Real-Time PCR System

T. H. Cheung et al., nature 482, 524 (Feb 23, 2012).

Long Non-coding RNA profiling/expression

为什么研究 non-coding RNA

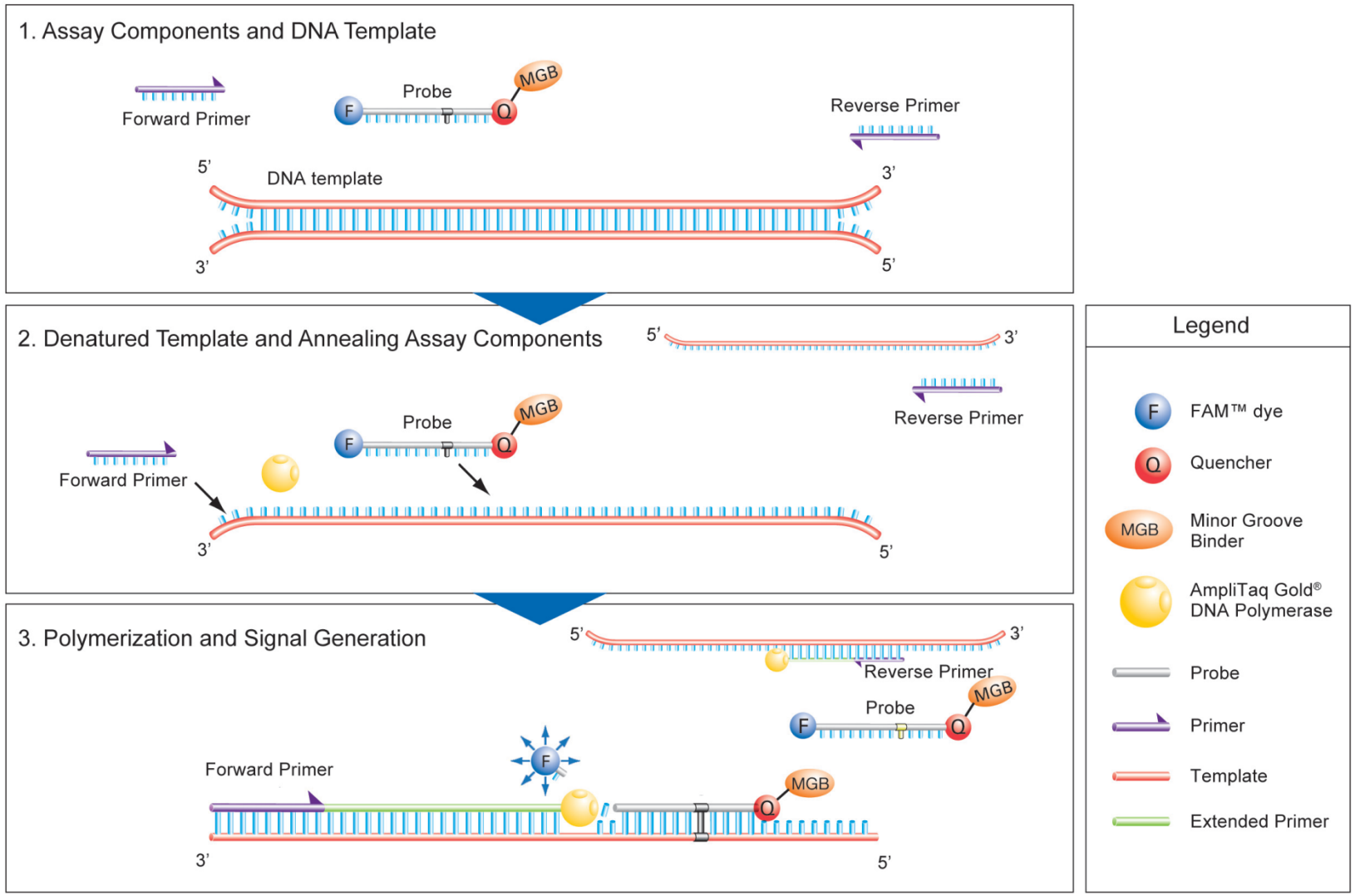
➢Cancer type:

- •colon
- •Breast
- •Lung
- •Epithelial tumours
- •Leukaemia

➢non-tumoural Disease:

- •Atheromatosis and atherosclerosis 动脉粥样硬化
- •Transient neonatal diabetes mellitus 新生儿暂时性糖尿病
- •Beckwith–Wiedeman syndrome 突脐、巨舌、巨体综合征
- •Silver–Russell syndrome 纤维性骨营养不良综合征
- •McCune–Albright syndrome 多发性骨纤维发育不良伴性早熟综合征
- •Alzheimer’s disease 阿尔茨海默病

TaqMan® Non-coding Assay



TaqMan® Non-coding Assays

- Human 14,074
- Mouse 10,441
- Rat 47

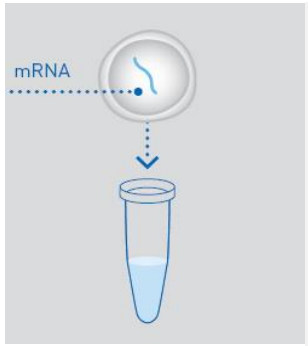
- Total 24,562

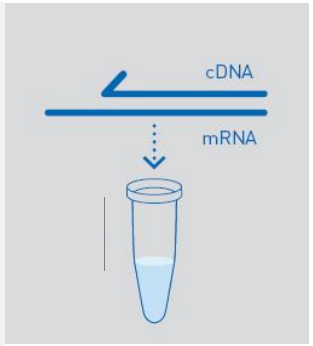
- Customized assays

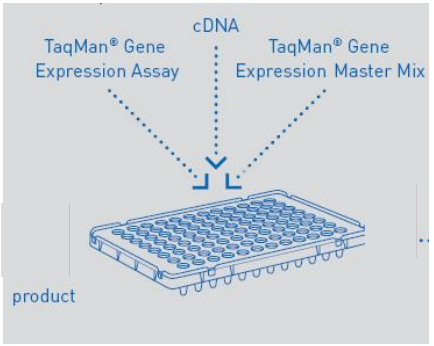
Species	# Assays
Human	14,074
Mouse	10,441
Rat	47
Total	**24,562**

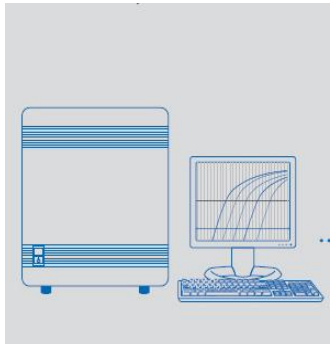
TaqMan® Non-coding Assay Workflow

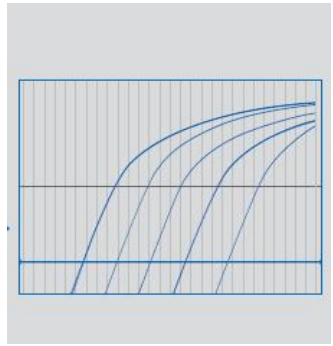
1. Extract RNA → **2. Reverse-transcribe RNA** → **3. Set up singleplex TaqMan® Assay reactions** → **4. Perform real-time PCR amplification** → **5. Analyze data**











- Prepare RNA

- Prepare cDNA
- Add TaqMan® Gene Expression Assay
- Gene reference assays
- Add GE Master Mix

- 8-Tube Strip
- 96-well
- 96-well fast
- 384-well
- TLDA®
- OpenArray®

- QS 12K, ViiA7, 7900HT, 7500, 7500 Fast, StepOnePlus

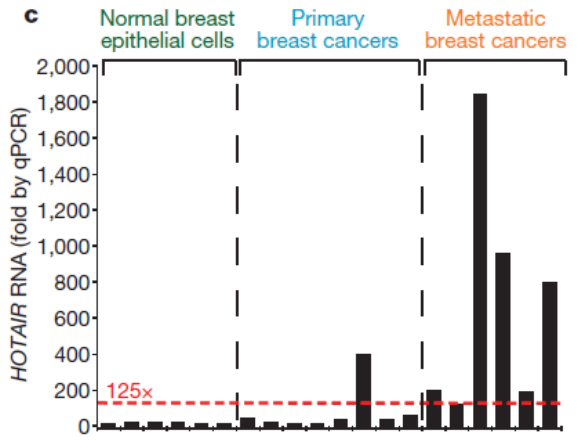
- SDS
- RQ Manager

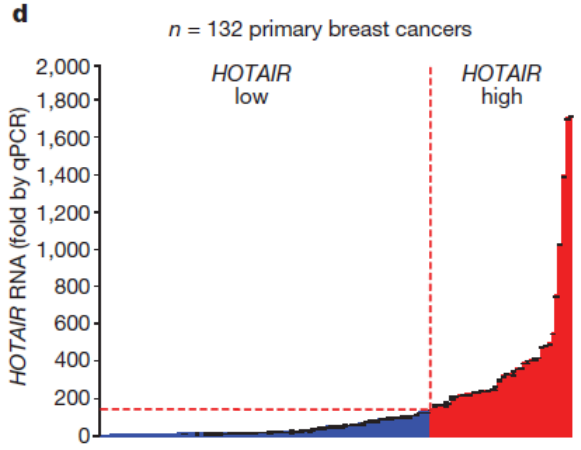
Application

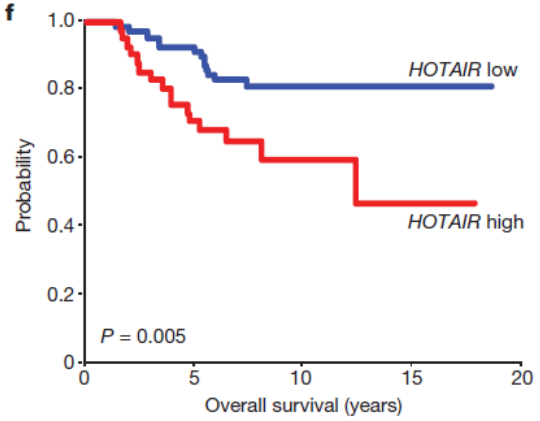
Vol 464|15 April 2010|doi:10.1038/nature08975 nature

LETTERS

Long non-coding RNA *HOTAIR* reprograms chromatin state to promote cancer metastasis







TaqMan® assays and preamplification pool

Non-coding TaqMan® assays

TaqMan® PreAmp Master Mix Kit

7900HT Fast Real-Time PCR System

These findings indicate that lincRNAs have active roles in modulating the cancer epigenome and may be important targets for cancer diagnosis and therapy.

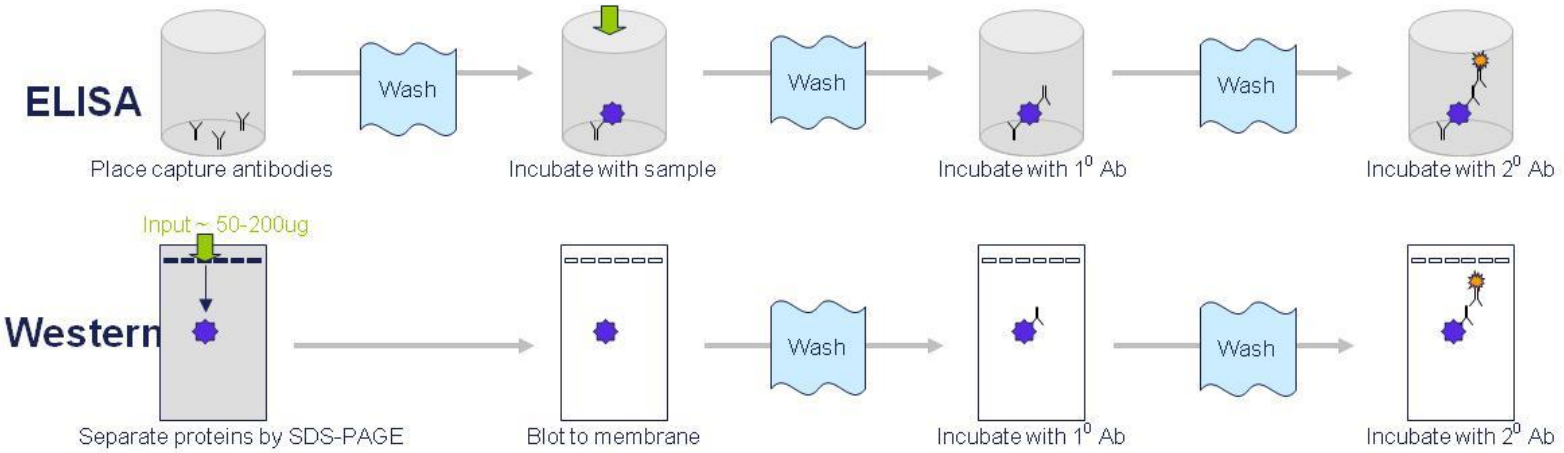
R. A. Gupta *et al.*, *nature* **464**, 1071 (Apr 15, 2010).

Protein expression

蛋白表达分析

➢ 基于抗体方法

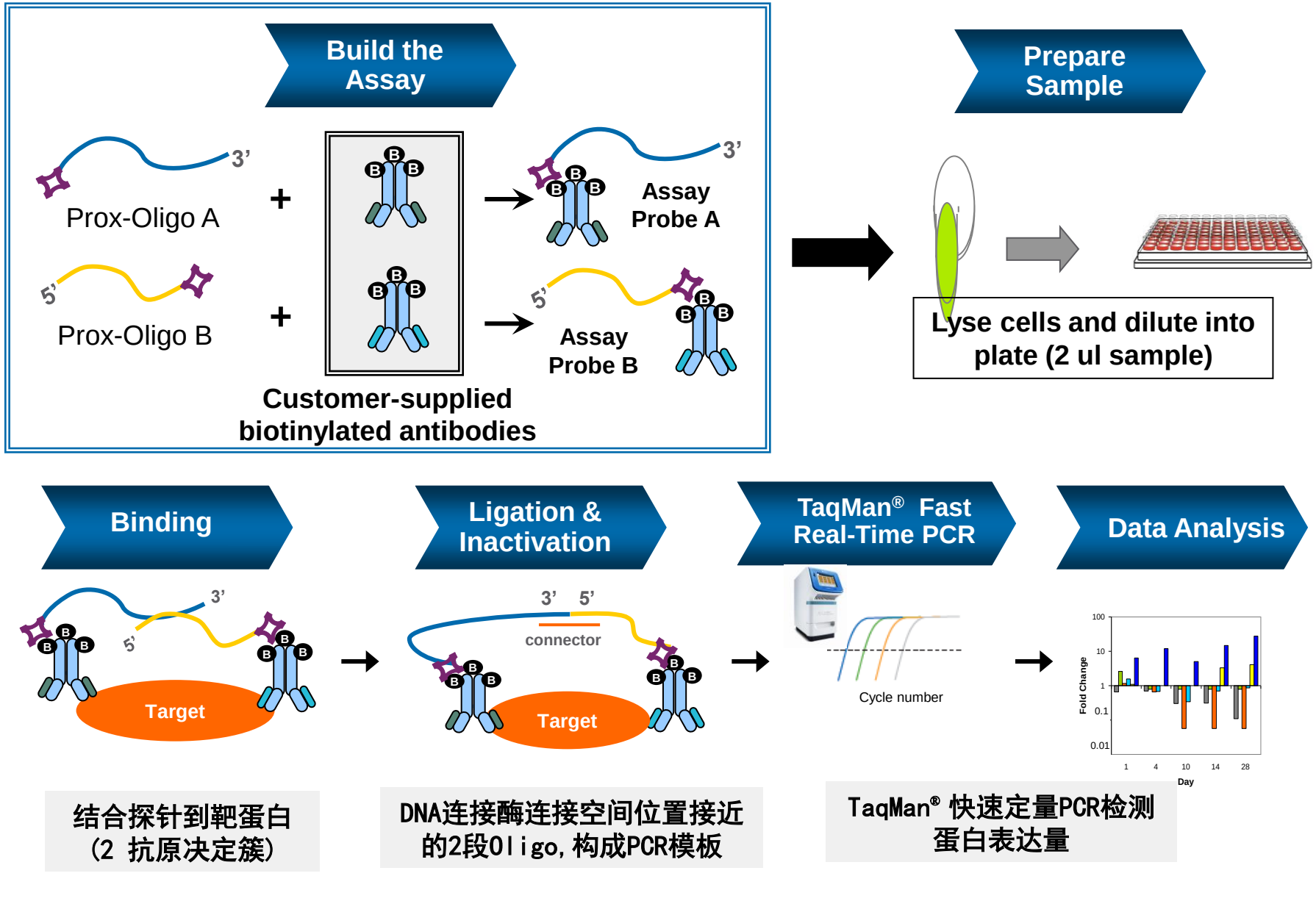
- ELISA
- Western



➢ 局限性

- 较大的样品量 (10^5 cells or 1 ug and up)
- 由单个抗体的特异性决定反应的特异性
- 需要通过多步洗涤，降低背景信号

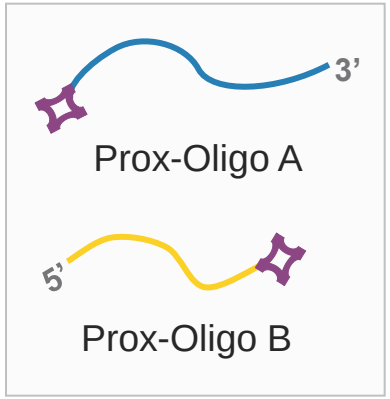
TaqMan® Protein Assays



开放式TaqMan® 蛋白表达试剂盒

Open Kit Components

- **Oligo Probe Kit**



- **Buffer Kit**
  - **Lysate Dilution**
  - **Antibody Dilution**
  - **Assay Probe Dilution**
  - **Assay Probe Storage**

Enough to make 40 x 96-well plates of Assay Probes

TaqMan® Protein Assay 优势

➢**微量样品蛋白定量**

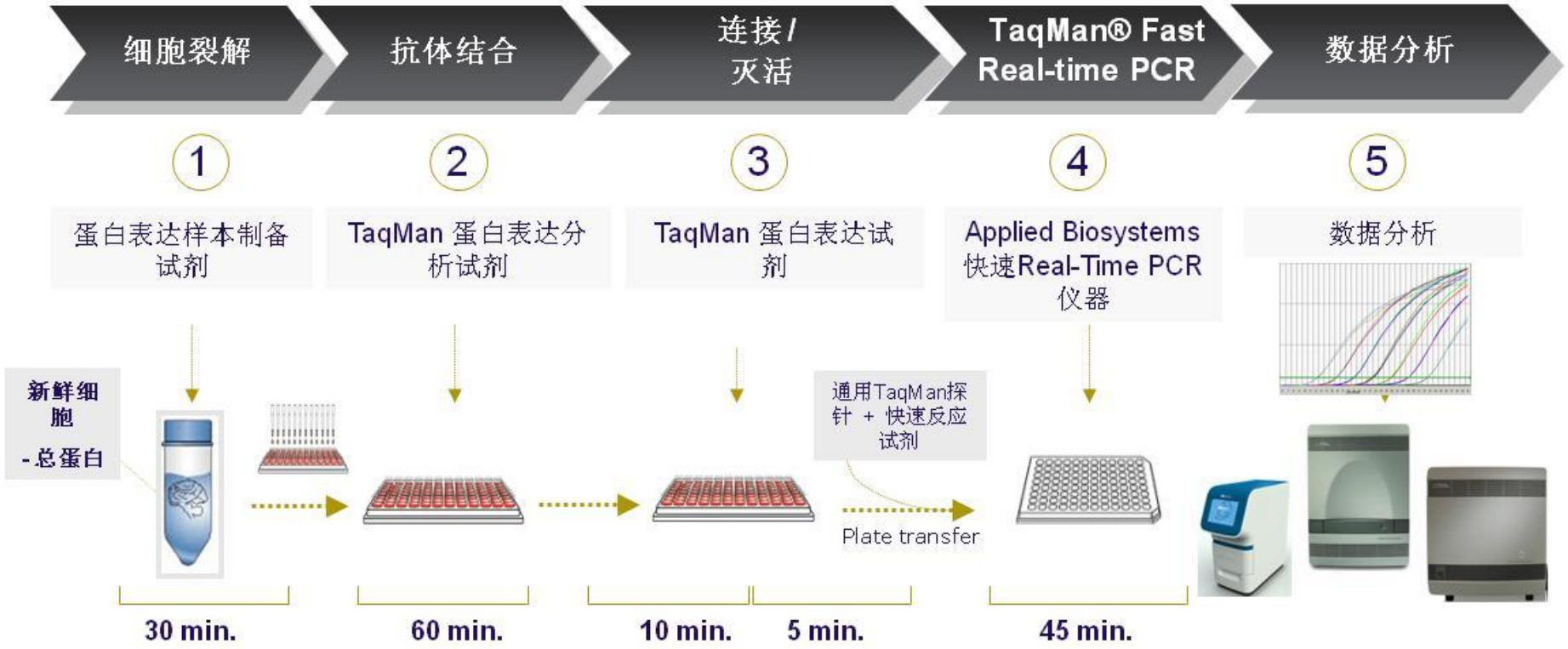
- 培养细胞样品：10-500 cells
- 组织裂解物： 1-1000 ng total protein

➢**检测细胞或组织样本里的蛋白质**

- 可以是细胞，组织，甚至FFPE和冰冻切片
- 无需蛋白纯化步骤

➢**结合抗体检测的准确性**
和荧光定量PCR的灵敏度和精确度

TaqMan® Protein Assay Workflow



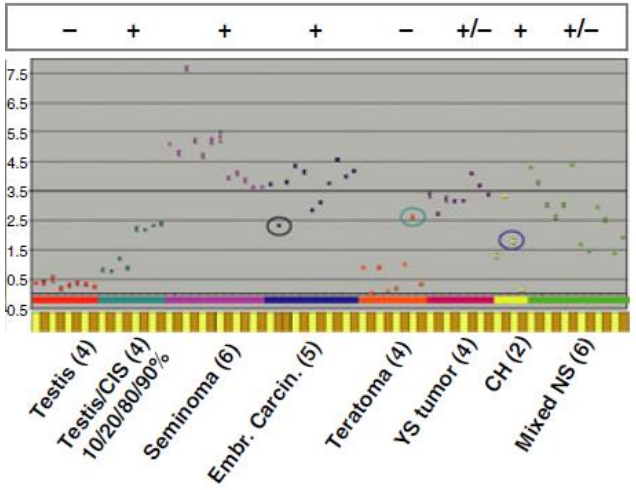
	Total time
Sample to result	~3.5 hr

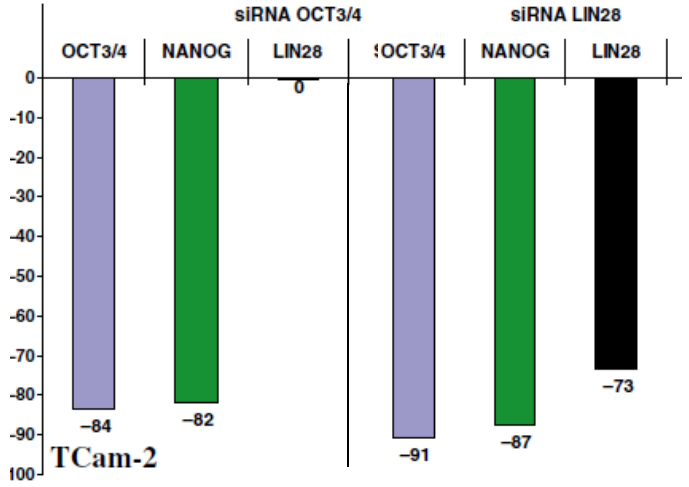
Application

International Journal of Andrology
E·A·A
international journal of andrology ISSN 0105-6263

ORIGINAL ARTICLE

Expression and interdependencies of pluripotency factors LIN28, OCT3/4, NANOG and SOX2 in human testicular germ cells and tumours of the testis





LIN28 functions upstream of OCT3/4 (and NANOG) in pluripotent stem and germ cells

TaqMan® Protein Assay

Protein Expression Sample Prep Kit

7900HT Fast Real-Time PCR System

ProteinAssist software

TaqMan® Protein Assay is a quantitative PCR-method for protein detection and is highly important implementable approach for protein detection and quantification suitable for routine practice.

A. J. Gillis *et al.*, *Int J Androl* **34**, e160 (Aug, 2011).

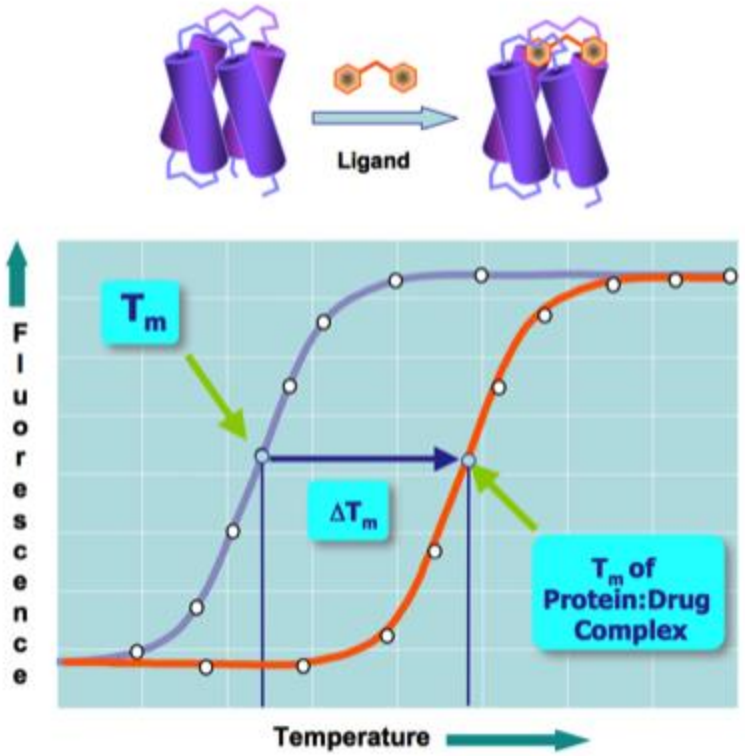
Protein Stability & Ligand Screening

用于新药物开发的配体结合研究

小分子配体同靶蛋白的亲和力是其成药性的关键指标之一

组合化学认为通过基团随机组合合成能得到各种各样的化合物，这些化合物构成组合库(combinatorial library)；对于选定的靶蛋白，只要所用组合库中候选化合物的结构多样性足够丰富，总能从中筛选到所需药物

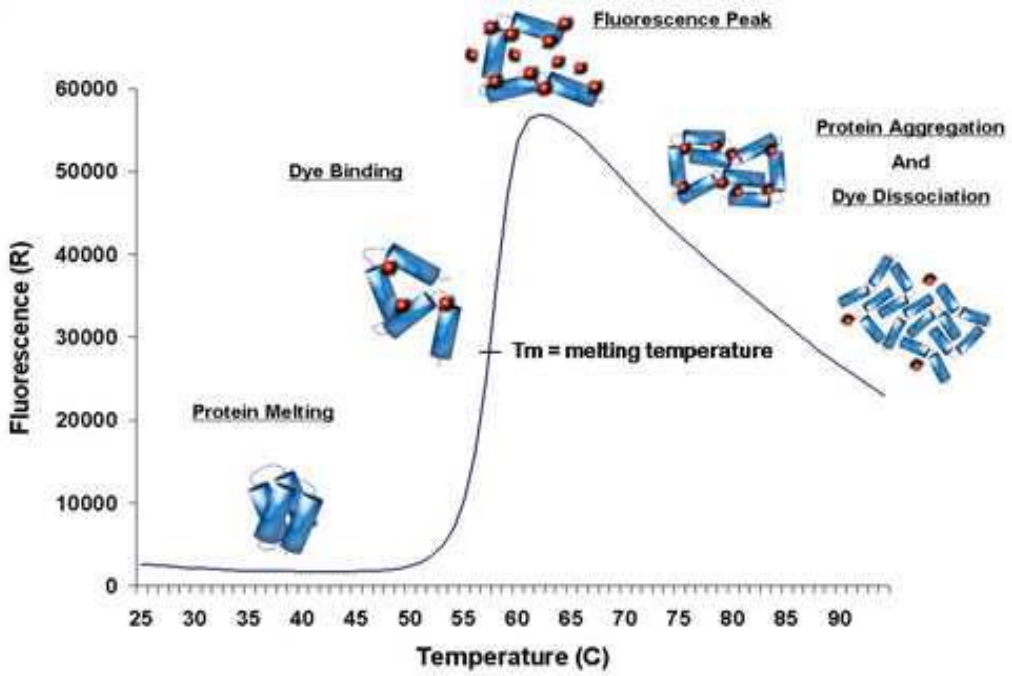
制备和测定组合库中候选配体的亲和力是发现配体类小分子药物的关键环节，而制备和筛选的成本与效率成为发现小分子配体药物的难题。



Protein Thermal Shift™ 的技术原理

➢Theory behind the application:

- 正常蛋白折叠，将疏水基团包裹在内
- 加热时，蛋白折叠打开，疏水基团暴露
- 在蛋白发生凝集之前，Protein Thermal Shift™ 染料结合到暴露的疏水基团上

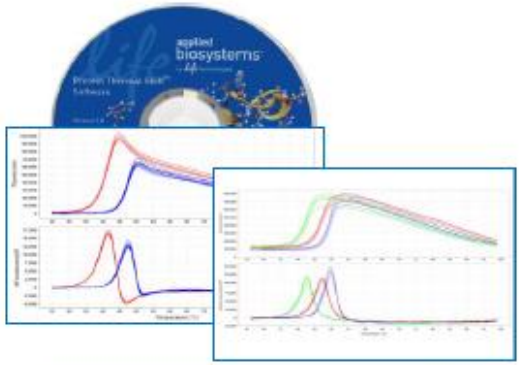


➢How to calculate

- 通过熔解曲线计算蛋白的Tm值
- Tm值的改变就表示蛋白稳定性的改变
- Tm值越大，蛋白越稳定

Protein Thermal Shift™ kit

Protein Thermal Shift™ Starter and Dye Kits



Protein Thermal Shift™ Analysis Software

- **Protein Thermal Shift™ Dye Kit**
  - Protein Thermal Shift™ Dye
  - Protein Thermal Shift™ Buffer

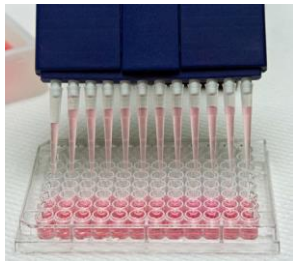
- **Protein Thermal Shift™ Starter Kit**
  - Protein Thermal Shift™ Dye
  - Protein Thermal Shift™ Buffer
  - Protein Thermal Shift™ Control Ligand
  - Protein Thermal Shift™ Control Protein

Protein Thermal Shift™ kit 优势

- 每个反应只需要不到1ug的蛋白
- 不需要预先了解蛋白或者配体的结构
- 不需要太多的优化工作
- 可以进行大通量筛选实验

TaqMan® Protein Thermal Shift™ Workflow

1. Mix protein, buffer, ligand (if applicable), and PTS dye

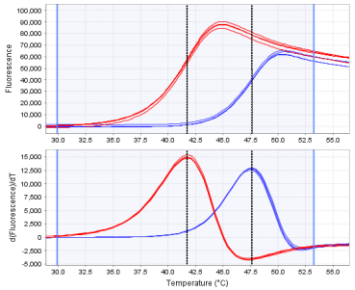


- Protein Thermal Shift™ Dye Kit

2. Run melt reaction on qPCR system

- · ViiA™ 7 Real-Time PCR System
- · 7500 Fast Real-Time PCR System
- · 7500 Real-Time PCR System
- · StepOne™ Real-Time PCR System
- · StepOnePlus™ Real-Time PCR System

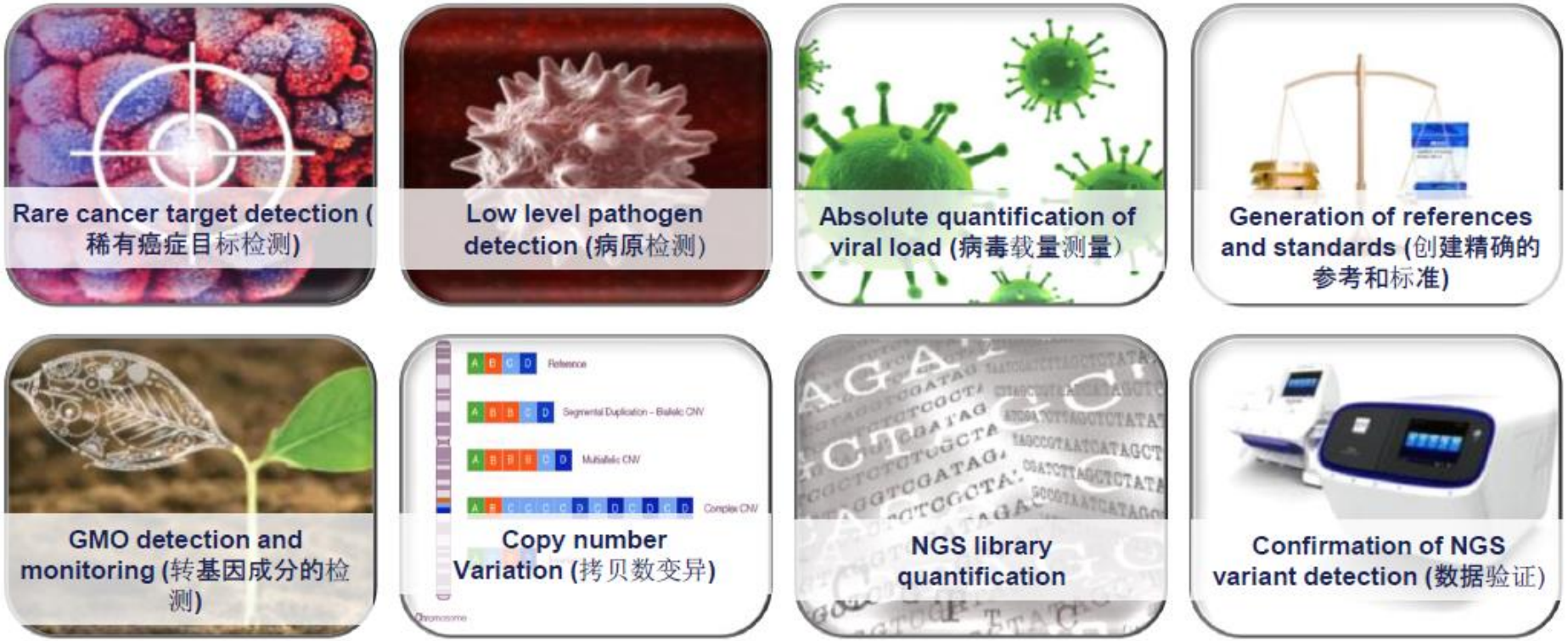
3. Analyze with data analysis software



- Protein Thermal Shift™ Software

Digital PCR

Application



精确，灵敏，快速，高通量

什么是 Digital PCR

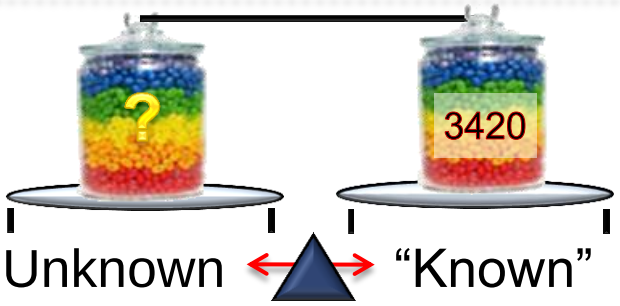
数字PCR是一种对单个核酸分子进行PCR扩增的技术

不需要参照标准曲线，对核酸进行绝对定量

How many beans in the jar?

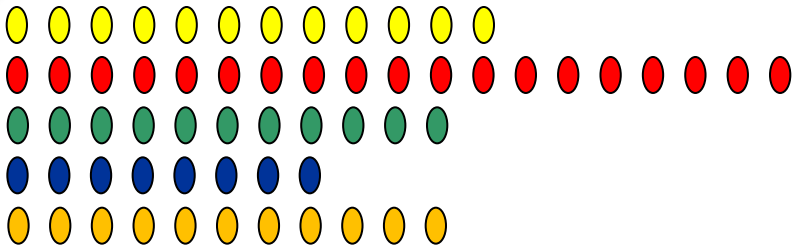
Analog

Answer: same # as in reference jar



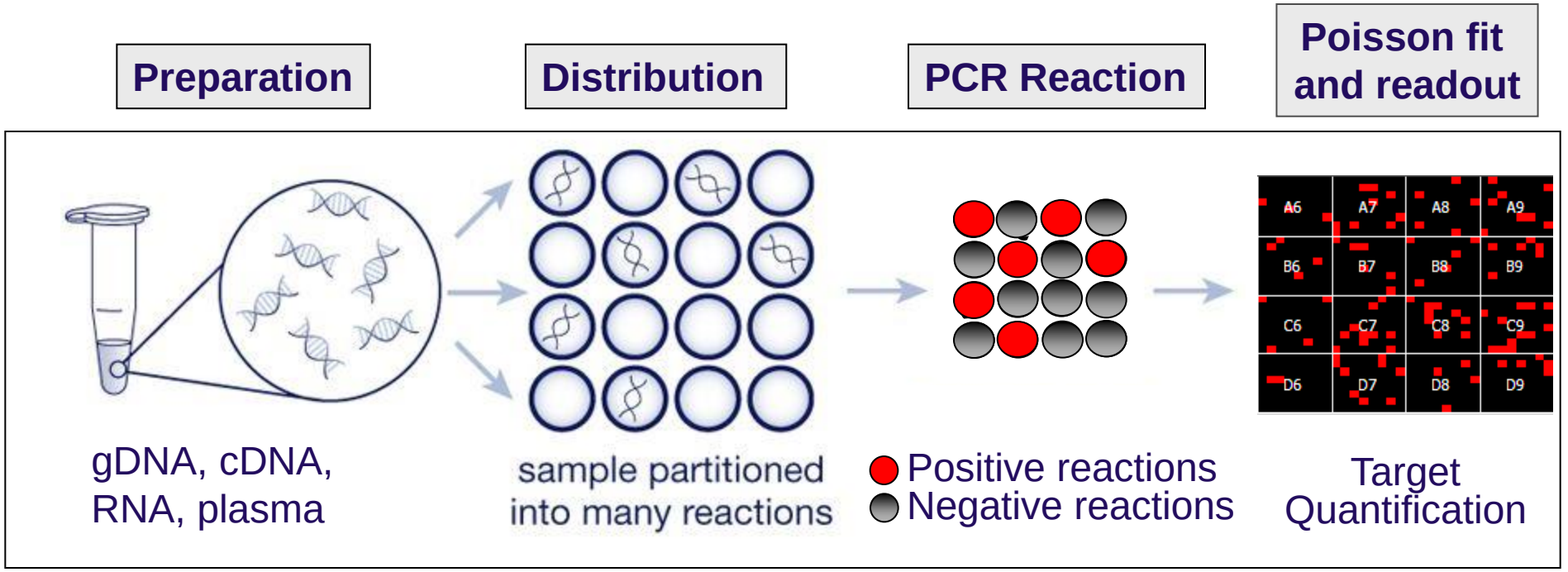
Digital

Answer: 672 yellow, 912 red…



Digital PCR(数字PCR)

将样品稀释后分配到众多反应孔，使每个反应孔里的样品平均含量小于一个拷贝。所有反应孔荧光PCR扩增，然后计算PCR结果有/无的数量，最后通过统计学公式就可以得出初始样品的精确拷贝数。



Use the number of **positive** and **negative** PCR reactions to count the number of target molecules.

QuantStudio™ 3D 数字 PCR 系统

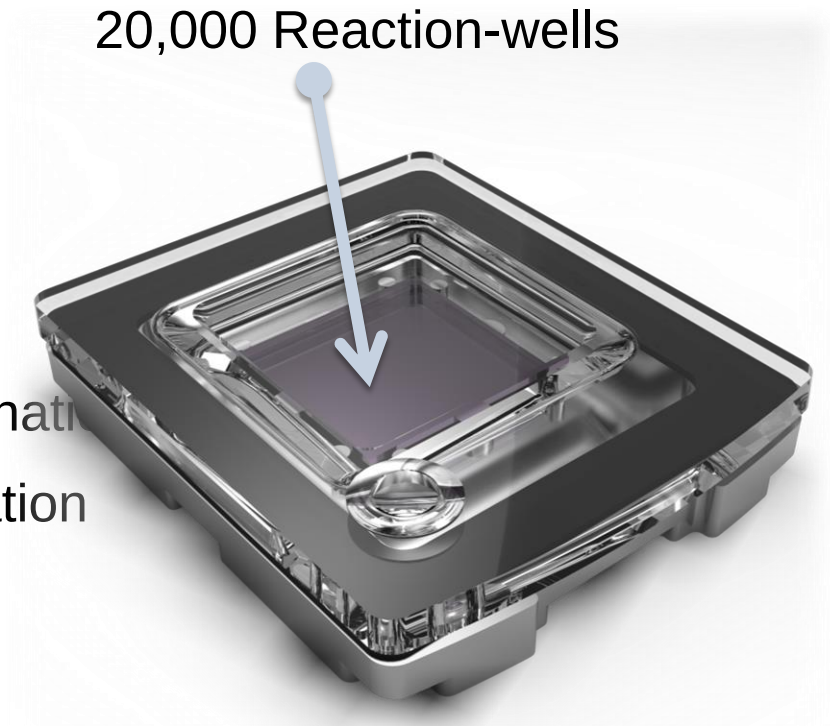
简单实验流程
2小时的实验时间，手动操作只需要几分钟

可扩展的架构
让您选择不同的芯片，以最少的投资去进行您的研究

多色荧光系统
FAM/VIC/ROX

QuantStudio™ 3D Digital PCR 20K Chip

- 20,000 reaction wells per chip
- Minimal sample loss
- One sample per chip
- Simple and consistent loading
- Sealed consumable minimizes contaminati
- Each chip identified by unique identification number



- Fixed reaction volume minimizes upfront sample manipulation

Digital PCR应用

应用研究	使用领域	需求
绝对定量	NGS	emulsion PCR前，测序模板文库精确定量
	标准品标定	核酸标准品精确定量
	单细胞研究	单细胞基因表达精确定量
相对定量	CNV分析，染色体非整倍体研究	拷贝数少量变异精确检测（10%~50%）
稀有等位基因检测	产前诊断	母亲血清中微量胎儿DNA精确分析
	Cancer 研究	在野生型DNA含量>90%背景下，精确检测稀有突变（0.1%~10%)
	病毒检测	微量病毒载量精确检测
	GMO 检测	食物中的转基因成分精确定量