# Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> 12K Flex 实时定量 PCR 仪

# 简明中文手册

第三部分:基因分型



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## Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> 12K Flex 实时定量 PCR 仪

双击桌面图标 QuantStudio 12K Flex Software QuantStudio 12K Flex > QuantStudio 12K Flex Software开启软件。进入主界面后 选择 "Experiment"下的"Create"。



2. 选择 "Setup" 下的 "Experiment Properties" 界面。

🐘 New Experiment 🗸	😂 Open 🛃 Save 🗸 🖆 Close 😽 Import	🗸 👧 Create Slide 📇 Print Report			
Experiment Menu	Experiment: 2014-07-18 105116	Type: Genotyping	Reagents: Ta	µMan® Reagents	?
-	How do you want to identify this expe	eriment?			
Setup	* Experiment Name: 2014-07-18 105116		Comments:		~
Experiment Properties	Barcode: User Name:				*
Define	a Mhinh block and concerning to must be	a aunanimenta			
Assign	• Which block are you using to run the	experiment			
Run Method	384-Well	Array Card	✓ 96-Well (0.2mL)	Fast 96-Well (0.1mL)	
	* What type of experiment do you wa	nt to set up?			
<u>-0</u>	Standard Curve	Relative Standard Curve	Comparative Cτ (ΔΔCτ)	Melt Curve	
Run	High Resolution Melt	✓ Genotyping	Presence/Absence		
	* Which reagents do you want to use	to detect the target sequence?			
Analysis	✓ TaqMan® Reagents	Other			
	* What properties do you want for the	e instrument run?			
Export	✓ Standard	Fast			
	Include: V Pre-PCR Read V Amplification	] Post-PCR Read			

2.1 输入实验名称 (Experiment Name)。

1.

File Edit Instrument	Analysis Tools Help
New Experiment -	📔 Open 🛃 Save 🗸 🚞 Close 🔤 Import 🗸 🛷 Create Slide 📇 Print Report
Experiment Menu	Experiment: 2014-07-18 105116 Type: Genotyping
	How do you want to identify this experiment?
Setup	* Experiment Name: 2014-07-18 105116
-	Barcode:
Properties	User Name:
Define	

#### 2.2 确认Block类型。

Which block are you using to run the experiment?										
384-Well	Array Card	✓ 96-Well (0.2mL)	Fast 96-Well (0.1mL)							

#### 2.3 选择基因分型实验类型, "Genotyping"。

What type of experiment do you was	ant to s	et up?		
Standard Curve		Relative Standard Curve	Comparative Cτ (ΔΔCτ)	Melt Curve
High Resolution Melt		Genotyping	Presence/Absence	

#### 2.4 选择试剂种类。

• Which reagents do you want to	use to detect the target sequence?		
✓ TaqMan® Reagents	SYBR® Green Reagents	Other	

#### 2.5 选择运行模式。

• What p	roperties do you want for	the instrument run?	
1	Standard	Fast	

#### 2.6 选择在定量仪器上进行预读板及扩增的过程。

• What p	properties do you want for th	e instrument run?	,	
1	Standard		Fast	
Include:	Pre-PCR Read Amplification	Post-PCR Read		

- 3. 选择 "Setup" 下的 "Define" 界面,设置位点名称和样品名称。
- 3.1 在 "SNPs"下点击 "Edit"或 "New",编辑或添加SNP检测位点。在 "SNP Assay Name"中填写待测SNP位点名称;在 "Allele1/Allele2 Name"中输入待 测位点的碱基名称; "Reporter"和 "Quencher"中选择所标记的荧光基团及淬灭 基团。对于 "Quencher"的选择,如果是MGB探针,请选择 "NFQ-MGB";如果 是TAMRA探针,请选择TAMRA;如果是其他形式的非荧光淬灭基团,请选择 "None"。

riment 15 mm	Experiment: 2014-0	7-18 105116	Type: Ge	notyping		R	teagents: Ta	qMan® Reage	ents	
Setup	SNPs 新建	SNP Assay	Dalete							
iment	SNP Assay Name	NCBI SNP Refer	Context Sequen	Allele 1	Reporter	Quencher	Allele 2	Reporter	Quencher	Colo
erties	SNP Assay 1			Allele 1	VIC	NFQ-MGB	Allele 2	FAM	NFQ-MGB	
thod	New SNP Assay     Enter a SNP assay name, th     "OK" to add the SNP assay     SNP Assay Name	nen select a SNP assay color. I to the library.	For each allele, enter an a	liele name or base(s), th	en select the repor	ter, quencher, and	i allele color. Click	*= Required		
lethod	Enter a SNP Assay Enter a SNP assay name, th "OK" to add the SNP assay SNP Assay Name Gene Symbol	nen select a SNP assay color. f to the library. :: SNP Assay 2	For each allele, enter an al Color: Gene Name:	liele name or base(s), th • Assay ID:	en select the repor	ter, quencher, and	d allele color. Click	*= Required		
ethod	Enter a SNP Assay Enter a SNP assay name, ti 'OK' to add the SNP assay SNP Assay Name Gene Symbo NCBI SNP Reference	hen select a SNP assay color. It to the library. It It It It It It It It It It It It It	For each allele, enter an al Color: Gene Name: context Sequence:	lele name or base(s), th	en select the repor	ter, quencher, and	i allele color. Click	*= Required		
Run	New SNP Assay     Enter a SNP assay name, tl     'Ok' to add the SNP assay     SNP Assay Name     Gene Symbo     NCBI SNP Reference     Allele 1 Name or Base(s     Allele 2 Name or Base(s)	hen select a SNP assay color. It to the library. It: SNP Assay 2 It: C C It: C	For each allele, enter an al Color: Gene Name: Ontext Sequence: Color: Color:		en select the repor ▼ Quen ▼ Quen	ter, quencher, and icher: NFQ-MGE icher: NFQ-MGE	f allele color. Click	*= Required		
Run	New SNP Assay Enter a SNP assay name, ti 'OK' to add the SNP assay SNP Assay Name Gene Symbo NCBI SNP Reference Allele 1 Name or Base(5 Allele 2 Name or Base(5 Comment	hen select a SNP assay color. I to the library. 2: SNP Assay 2 te 2: C 2: T 2: T	For each allele, enter an a Color: Gene Name: Context Sequence: Color: Color:	Assay ID: Assay ID: Reporter: VIC Reporter: FAM	en select the repor	ter, quencher, and cher: NFQ-MGE cher: NFQ-MGE	j allele color. Click	*= Required		
Run	New SNP Assay Enter a SNP assay name, ti 'OK' to add the SNP assay SNP Assay Name Gene Symbo NCBI SNP Reference Allele 1 Name or Base(s Allele 2 Name or Base(s Comment	en select a SNP assay color. I to the library. 2: SNP Assay 2 t: 1: C 1: C 1: T 编辑碱基种	For each allele, enter an all Color: Gene Name: Color: Color: Color:	Reporter: VIC Reporter: FAM	en select the repor ・ Quen ・ Quen は 成 、 、 、 、 、 、 、 、 、 、 、 、 、	ter, quencher, and ccher: NFQ-MGE ccher: NFQ-MGE 告基	j allele color. Click	*= Required		
Run Analysis	New SNP Assay Enter a SNP assay name, ti 'OK' to add the SNP assay SNP Assay Name Gene Symbo NCBI SNP Reference Allele 1 Name or Base(5 Allele 2 Name or Base(5 Comment	nen select a SNP assay color. I to the library. 2: SNP Assay 2 t: 2: C 1: C 1: C 5: T 编辑碱基种	For each allele, enter an all Color: Gene Name: Color: Color: Color:	ele name or base(s), th Assay ID: Reporter: VIC Reporter: FAM Same	en select the repor Quen • Quen 集荧光报 及淬灭基	ter, quencher, and cher: NFQ-MGE cher: NFQ-MGE 告基 团	i allele color. Click	*- Required		

3.2 在"Samples"下点击"New",添加待测样品。在"Sample Name"中编辑样品 名称。

Samples New Save to Library Import from Library Delete		
Sample Name	Color	
Sample 1		-
Sample 2		-
	ni anti anti anti anti anti anti anti an	

File Edit Instrument	Analysis Tools Help										
New Experiment +	🞯 Open 🛃 Save 🗸 📋 Close	e 🖳 Tmp	ort	🕶 🐼 Cre	eate Slide	💾 Print R	eport				
Experiment Menu	Experiment: 96-Well Ge	enotypir	ıg I	Example	Type: (	Genotypi	ng			Reag	ents: Ta
Setup	SNPs	T		Plate Layout	Well Table			ž			
Experiment	SNP Assay 2	U			ilis V Select	ovelis V	View Legend	F	6	7	
Properties		N			2	3	4	5	ь	1	8
Define		22		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 1	Sample 2
Assign				U SNP	U 5NP	U SNP		U SNP	U SNP		U and
Run Method			в	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12	Sample 7	Sample 8
Run			с	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 13	Sample 14
Analysis	Samples		D	Sample 19	N SNP	N 5NP	N SNP	N SNP	N SNP	Sample 19	N Sub
Export	Name       Sample 1       Sample 2       Sample 3	×	E	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 1	Sample 2

**5.** 选择 "Setup" 下的 "Run Method" 界面,编辑运行条件。

### 简明中文操作指南



**6.** 选择"Run"下的"Amplification Plot"界面,点击"Save As"保存文件,然后点击"Start Run"开始运行。

File Edit Instrument	Analysis Tools Help
New Experiment •	◎ goen. I Save - 《 Goer 保存文件 Create Side A Print Report
Concentration 1	Experiment: 96-Well Genotyping Example Type: Genotyping Reag
-	Run Status
Setur	57457454 ▼ ← 开始运行实验
	Run Status: Not Started
Run	Amplification Plot
Amplification Plot	
Temperature Plot	Show in Web V Select Web V
and the second	Amplification Plot
Kun recipio	
Notification Settings	Sen. Sen. Sen.
View Run Data	

- **7.** 实验结束后,先点击界面右上方的 "Analyze" 进行分析,然后进入 "Analysis" 下 的 "Allelic Discrimination Plot" 观察分型结果。
- 7.1 查看分型结果:选择 "Analysis" 下的 "Allelic Discrimination Plot" 查看分型结果。



7.2 查看"QC Summary"结果:反应孔可能存在异常情况时,会出现黄色三角提示, 数字1 代表有一种情况,2 代表有两种情况,以此类 推。详细信息及解决方案可以 在"Flag Details"中查看。

Experiment	t: 96-Well Genotypin 1	Type: Geno	otyping			Rea	gents	: Taq	Man®	Rea	gents	(	Analyze	Anal	ysis Set	tings
QC Summary				<	F	Plate Lay	out	Well Tab	ble							
Flag Detail	s				1	The channel	is welt	- Calar	a water 📼		View Deep	141		85	<b>M</b> 893	
Flag:	Description	Freque	Wells			Show	ar wea	• Delec	c wea Y		view Lege		_	Ba	4 62	2 <u>7</u>
PCFAIL	Positive control failed	0				1	2	3	4	5	6	7	8	9	10	11
SMCLUSTER	Small number of samples in cluster	0				1000	1000	1000	1			1	1	1	1	1
ALLELE1CR.	Allele1 high relative amplitude	0				Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample	Sample 2	Sample 3	Sample 4	Sample 5
ALLELE1CR.	Allele1 high relative noise	0			A		• •	O U	•	• 🗉	•				0.0	
ALLELE1CO.	Allele1 low Cq confidence					1		1					-			-
ALLELE2CR.	Allele2 high relative amplitude	0				-		-					1		-	
ALLELE2CR.	Allele2 high relative noise	0					Constant of	Course of	e de la compañía de	erest.	-	1	1	1	1	1
<b>ALLELE2CQ</b>	Allele2 law Cq confidence					Sample 7	Sample 8	Sample 9	sampl	Sampl	Sampl	Sample :	Sample 8	Sample 9	Sampl	Sampl
AMPNC	Amplification in negative control	0			R	• •	0	• 0	• 0	• 0	• 0	•	• 1	• 1	• 0	•
DRNMIN	Minimum Delta Rn	1										1	1997	Contraction of the		
NOAMP	No amplification	0	F						-				8		-	2
NOISE	Noise higher than others in plate	0	-			Sampl	Samol	Sampl	Sampl	Sampl	Sampl	1	1	1	1	1
PIKE	Noise spikes	0			C		0.7					Sampl	Sampl	Sampi	Sampl	Sampl
EXPFAIL	Exponential algorithm failed	0			-	- u	- u	- u	- u			• 1	•	• 1	• •	• 1
BLFAIL	Baseline algorithm failed	0														
THOLDFAIL	Thresholding algorithm failed	48	A7, A8, A9, A10,										1	<b>1</b>	-	-
CTFAIL	CT algorithm failed	0	*			Sampl						1	1	-	<b>T</b>	-
1	THE REPORT OF THE PARTY OF THE				D							Sampl	- 77	- 77	- 77	. 73
	Flag: THOLDFAIL-Thresholding	algorithm faile	d	1								<b>U</b>				
Elan	Detail: The software cannot calcul	ate a threshold	d													
riag	Details The Sonwale Califior Calcul	are a critestion	u.			1000	-	1000	1		1	1	1	1	1	1
Flagged	Wells: A7, A8, A9, A10, A11, A12,	B7, B8, B9, B	10, B11, B12, C7,			Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
	C8, C9, C10, C11, C12, D7	, D8, D9, D10	0, D11, D12, E7,		E	• 0	• •	<b>U</b>	• U	• 🗉	• 🗉				0.0	
	E8, E9, E10, E11, E12, F7,	F8, F9, F10, F	F11, F12, G7, G8,				1						-		-	
	G9, G10, G11, G12, H7, H8	, H9, H10, H1	1, H12				-		-				1		1	
	View THOLDFAIL Troublesh	ooting Inform	ation			Sample 7	Cample 0	Cample O	Campi	Samo	Samo	1	1	1	1	1
	Sect Thought at House	ocong month			-	- and	-angest o	Jampiè 9		- TT	Jamphin	Sample 7	7 Sample 8	Sample 9	Sampl	Sampl
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					G							Sampl	Sampl	Sampl	Sampl	Sampi
								-	-			• 🗉	• •	• •	• •	• 0
												1	4	4	4	
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					н	0 0				14		Sampl	- 73	- 77		
				•								<b>U</b>				
1 M/oller	Dis Drocorcod Model Dis	INCOMPANY MALE	Chill A complete Line at													

8. 数据导出:在"Export"界面下根据需要导出数据。

### 简明中文操作指南

Experiment:	96-Well Genotyping I	Exa Ty	pe: Genotyping		Reage	ents: TaqMan(	) Reagents	Ex	port
Auto Export	Format : Quant	Studio 12KFle:	< 💌	Export Data 1	Го: 🖲 One File 🔘 Se	parate Files	📝 Open file(:	s) when export	t is comple
Export File Locati	ion: D:\Applied Biosystems\/	QS 12K\exper	iments	Browse Export Fi	le Name: 96-Well Ge	enotyping Example	_QuantStudio_e	File Type: 👩	(*.txt)
Sample Setup	Raw Data Ar	本1子1坐。 nplification	Multicomponent	🖉 Results 🚽	—选择需	要导出的	数据		
Select Conte	nt			Course Marco	Comela Colleg		cup trace cal	The late	Let 7
All Fields		weil	well Position	Sample Name	Sample Color	SNP Assay Na	SNP Assay Col	Task	All
( ) Fair Floras			1 A1	Sample 1	RGB(176,23,31)	SNP Assay 1	RGB(176,23,31)	UNKNOWN	Alle
Vell			2 A2	Sample 2	RGB(176,23,31)	SNP Assay 1	RGB(176,23,31)	UNKNOWN	Alle
			3 A3	Sample 3	RGB(0,0,255)	SNP Assay 1	RGB(176,23,31)	UNKNOWN	Alle
Well Positio	in	_	4 A4	Sample 4	RGB(0,139,69)	SNP Assay 1	RGB(176,23,31)	UNKNOWN	Alle
Sample Nar	ne		5 A5	Sample 5	RGB(238,238,0)	SNP Assay 1	RGB(176,23,31)	UNKNOWN	Alle
(a) comple indi			D AD	Sample 6	KGB(139,137,112)	SNP Assay 1	RGB(1/6,23,31)	UNKNOWN	Alle
Sample Col	or		/ A/	Sample 1	KGB(1/6,23,31)	SNP Assay 2	KGB(0,0,255)	UNKNOWN	Alle
		-	8 A8	Sample 2	KGB(1/0,23,31)	SINP ASSay 2	RGB(0,0,255)	UNKNOWN	Alle
SNP Assay	Name		10 410	Sample 3	PCP(0,120,60)	SND Accay 2	PCP(0.0.255)	UNKNOWN	Alle
SNP Accav	Color		11 411	Sample 5	RGB(238,238,0)	SND Accay 2	RGB(0.0.255)	LINKNOWN	Alle
U JAF Assay	COR		12 412	Sample 5	RGR(130,137,112)	SND Accay 2	RGB(0.0.255)	UNKNOWN	Alle
Task		-	13 B1	Sample 7	RGB(238,121,66)	SNP Assay 1	RGB(176,23,31)	UNKNOWN	Alle
			14 82	Sample 8	RGB(142,56,142)	SNP Assav 1	RGB(176,23,31)	UNKNOWN	Alle
Allele1 Name	ne -	-	15 83	Sample 9	RGB(198,113,113)	SNP Assay 1	RGB(176,23,31)	UNKNOWN	Alle
Allele1 Colo	r		16 B4	Sample 10	RGB(0,245,255)	SNP Assay 1	RGB(176,23,31)	UNKNOWN	Alle
Colo Aleier Colo		-	17 85	Sample 11	RGB(238,220,130)	SNP Assay 1	RGB(176,23,31)	UNKNOWN	Alle
Allele1 Rep	orter		18 86	Sample 12	RGB(255,127,0)	SNP Assay 1	RGB(176,23,31)	UNKNOWN	Alle
			19 87	Sample 7	RGB(238,121,66)	SNP Assay 2	RGB(0.0.255)	UNKNOWN	Alle
Allele1 Oue	ncher		20.00	Camala O	000(140 50 140)	Chill Assess 2	DCD(0 0 DEE)	UNICALOULAU	Alle





## 遍布全球的技术支持服务

我们在全球 60 多个国家和地区设立了办事处,拥有 备受赞誉的技术支持团队以及现场服务工程师。您可 以在我们的官方网站上订购产品、下载技术文件,以 及寻找问题答案。也非常欢迎您通过电子邮件、电 话、以及微信平台和我们联系获取信息。





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