

# ABI 7500 实验上机设置及数据分析

## 实验上机前设置

1、进入 Real-Time PCR Software 软件 Setup 界面,完成以下实验前设置。

Experiment Menu «
Setup
Experiment Properties
Plate Setup
Run Method
Reaction Setup
Materials List

#### 1.1、Experiment Properties 设置

按照下图所示选择: Genotyping →Taqman®Reagents →Standard →Include: Pre-PCR、Read

Amplification, Post-PCR Read



• What type of experiment do you want to set up?			1							
Quantitation - Standard Curve	Quantitation - Relative Standard Curve	Quantitation - Comparative Cr (ΔΔCr )								
Melt Curve	√ Genotyping	Presence/Absence								
Detect single nucleotide polymorphism variants of a target nucleic acid seq	uence in samples.									
• Which reagents do you want to use to detect the target seque	nce?									
√ TaqMan® Reagents Other										
The PCR reactions contain primers designed to amplify the target sequence	e and a TaqMan® probe designed to detect amplification of the target sequer	nce.								
• Which ramp speed do you want to use in the instrument run?										
√ Standard (~ 2 hours to complete a run)										
For optimal results with the standard ramp speed, Applied Biosystems recommends using standard reagents for your PCR reactions.										
What do you want to include in the instrument run?										
Include: Vere-PCR Read Vert Amplification Vert Post-PCR Read										

### 1.2、Plate Setup 设置

按照下图所示设置 Assign SNP Assay(s) to the Selected Wells 中探针荧光基团标记内容

Assign SNP Assay(s	s) to the Selected Wells.							
-		4						
Create New SNP As	ssay Add Saved SNP Assa	/ Ed	lit 🔻					
		_	Save SN	NP Assay				
Assign SNP Assay			Edit SN	P Assav	Soloc	tod SNP Assa	,	
SNP Assav 1			Delete 9	SNP Assay	Selec	LEU ONF ASSA	/	-
Fdit SNP Assav								23
Make changes below, then	click "OK" to save your changes to the	brary. C	lick "Reset	Fields" to und	o all yo	ur changes.	*= Req	luired
SNP Assay Name:	rs671	Color:	<ul> <li>Assay</li> </ul>	ID:				
Allele 1 Name or Base(s):	G	Color:	<ul> <li>Report</li> </ul>	ter: VIC	•	Quencher: NFQ-N	GB	•
Allele 2 Name or Base(s):	A	Color:	<ul> <li>Report</li> </ul>	ter: FAM	•	Quencher: NFQ-N	GB	•
Comments:								
Reset Fields	(	ок					Ca	ncel

按照下图所示设置 Assign Sample to the Selected Wells 中样本信息



ssign \$	Sample to	the Selected Wel	ls.			
Add Ne	w Sample	Add Saved Sample	Save Sample	Delete Sample		
Assign	Sample				Color	
	gDNA 1				•	
	Sample 2	!			•	
	Blood 3				•	
	1					

按照下图所示设置 Select the dye to use as the passive reference 中 ROX 信息

Select the dye to use as the passive reference.	
ROX •	

根据下图设置 View Plate Layout 中样本面板的信息。根据实际实验内容和八联排 PCR 管或 96 孔

板位置设置。设置方法:在96孔面板界面左键单击选中需要设置孔,在SNP Assay name 和 Sample name 对应小方框中左键单击选中。

Assign 9	SNP Assay(s	) to the Selec	ted Wells	i.			<		/iew Pl	ate Lay	yout	View V	Vell Ta	ble				
Create I	New SNP Assa	y Add Saved	SNP Assay	Edit 🔻	•		>				\$	Select Wells	With:	- Select Ite	em - 💌	- Select	ltem - 🔻	•]
	/	Viele 1/Allele 2							Show	v in Wells	•	📘 View Le	gend					
Assign	SNP Ass F	Reporter	Task			~			1	2	3	4	5	6	7	8	9	Τ
	13071		Onknown			Ŧ		A										
Assign S	Sample to th	e Selected V	/ells.					в				gDNA 1						T
Add Nev	w Sample 🛛 A	dd Saved Sampl	e Save S	ample	Delete Samp	le	:	С				Sample 2						
Assign	Sample				Colo	r		D				Blood 3						T
	gDNA 1					•		E				0 13						T
	Sample 2					•												+
	Blood 3					•		F										
								G										
Select th	he dye to us	e as the pas	sive refer	ence.				н										
ROX	•							w	ells: 🚺 :	3 Unknow	/n <b>N 0</b>	Negative C	ontrol	P 0 Posit	tive Contr	ol		_

Well 信息必须包含 SNP Assay name 和 Sample name。



弗元(上海)生物科技有限公司



1.3、Run Method 界面设置

根据下图设置 Run Method 中扩增程序及荧光信号采集信息。

Reaction Volume Per 10 Well

Holding Stage: 95.0°C 10:00min

Cycling Stage: 95.0°C 00:10min 60.0°C 00:30min (Collect Data) Number of Cycles 40

Post-PCR Read : 60.0°C 01:00min (Collect Data)



1.4、Reaction Setup 和 Materials List 无需设置。

1.5、程序运行

所有设置完成后,按照面板设置信息放入需要扩增分析的八联管或96孔PCR板,点击START

RUN 运行程序

Experiment: Untitled	Type: Ge	notyping Reagen	ts: TaqMan® Reagents	START RUN 📡
程序运行印	寸长约1H,程序运行结	束后,数据自动保存。	)	



## 数据分析

为更准确、直观的分析基因分型结果,需将7500运行的 eds 数据文件导入 TaqMan® Genotyper Software 软件中进行进一步分析。该软件的官方下载地址为

https://www.thermofisher.com/cn/zh/home/technical-resources/software-downloads/taqman-genotypersoftware.html

1、 Create Study

打开 TaqMan® Genotyper Software 软件,点击 Create Study 创建研究方法。进入设置界面设置相

关系数。

1.1、Properties 设置

Study Name: 设置人员自行确定

Instrument Type: 7500 Fast Real-Time PCR System

Experiment Type: Real-time

	Study Properties
Setup Properties	Study Name:       Untitled         Instrument Type:       7500 Fast Real-Time PCR System         Experiment Type:       Real-time
Experiments	Study Contents
	Number of Experiments in Study: 0
Assays	Number of Assays in Study: 0
61	Number of Samples in Study: 0
Sampies	Number of Reference Samples in Study: 0
References	History Summary
	Creation Date: 05/20/2019 13:49:38 CST
	Created by: GUEST



1.2、Experiments 设置

在 Experiments 设置界面内, 点击 Import 导入需要分析的 7500 运行 eds 数据



1.3、Assays 设置

在 Assays 设置界面点击 Edit 设置 Assay 探针信息。特别注意: Base 不得漏填

	Assays								
	🕂 Import 🚟 Edit 🛑 Delete 🛛	Delete All							
Jetup Setup	Assay ID	Assay Name	# of Wells	Description	Gene Symbol	NCBI SNP Reference	Allele 1 Base	Allele 2 Base	
Properties	rs671		8						
Experiments		Edit Assay				22			
Asseys		Edit the selected assay. Assay							
Sumples		ID: rs671		Allele 1					
References		Name: Description:		Base: G	Dye: VIC				
		NCBI SNP Reference:							
Analysis		Gene Symbol:		Allele 2		_			
<b>(</b> )		Context Sequence:		Name: A					
Export		Chromosome:		Base: A	Dve: FAM				
		Position:							
					OK Can	cel			
		(							
3									



2、结果读取

正确完成上述所有设置后,点击 Analysis 进入结果读界面,自动生成基因分型结果。Sample ID 右栏的 Call 即为基因分型结果。



特别说明:

对于三种基因型均匀分布的 SNP, 分区界面应呈现明显的蓝、绿、红三个区域。蓝色代表 FAM 标记的纯合基因型,绿色代表杂合型,红色代表 VIC 标记的纯合基因型。

对于三种基因型不均匀分布的 SNP,特别是 90% 以上的是某单一基因型,分区界面可能只出现 一种颜色区域。软件自行分析数据时,可能出现误差,需要人工校正。

校正方法:

进入 Analysis 界面, 左键选中需要调整的点, 右键单击, 根据基因型频率, 选择样本实际基因

型。



	Assays				<	Ехр	erimen	ts								
	# Assay Name	Assay ID	# of	f Wells	# of Reference >	=		E	periment Na	me	# Sample:	a # Targe	rts # Ta	asks		
Setup	1	rs671		8	s (		1	20	190518-LH .	eds		8	1	0		
	•	1			÷											
Anaberiz	rs671	Call Rate = 100%			NOT	Insp	ected	< 🗖	esults R	eference Sampl	ns Po	pulation Stat	istics			
nnaysis	🔪 View - [ 🔊 🖑 🍳	🔀 🐘 🔟 🐁						> 💊	View •	Group by	- 6	Bookmark ·	- E, T	ag - 🗐	Change C	al •
•	1.00									Sample ID	Cal	Manual	Quality	VIC®	FAM**	RO
r Control	0.75								1	Sample 1	A/A	1	N/A	0.115	0.400	32
	0.70								2	Sample 2	G/A	1	N/A	0.158	0.032	54
	0.50								3	Sample 3	G/A	1	N/A	0.250	0.052	33
Course 1	€ 0.25								4	Sample 4	A/A	1	N/A	0.144	0.382	33
apurt	N.			_		_			5	Sample 5	A/A	1	N/A	0.138	0.382	: 34
	0.00			6	/G				6	Sample 6	A/A	1	N/A	0.136	0.383	. 38
	@ -0.25 ·			G	i/A			:	7	Sample 7	G/A	~	N/A	0.230	0.046	30
	0.50			A	/A			-	0	and a	N/A		ny/	0.003	0.000	20
				N	lo Amplification											
	-0.75 -			U	Indetermined											
	-1.00			P	ossible Rare Allele											
	-1.0 -0.9 -0.8 -0.7 -0	8 -0.5 -0.4 -0.3 -0.2 -0.1 0.0	0.1 0.2	c	lear Manual Call		.9 1.0									
		Allele 1 V	1C (G)	ō	mit											
	Legend			U	In-omit											
	NTC (1) • VIC/V	/IC (0) • VIC/FAM (3)	EAN	T.	ag for Ref Papel											
	<ul> <li>No Amp (0)</li> <li>Under</li> </ul>	t (0) • PRA (0)	× Invi		In-tag Selected											
4	<ul> <li>Squares for Controls (1)</li> </ul>	🔺 Tria	ngles for Re		at Baselemanks				1							
<u> </u>	6			8	et Bookmarks										_	_
46	Untitled ×				elect All Bookmarks											
					lear selected											

读取结果完毕后,关闭 TaqMan<sup>®</sup> Genotyper Software 软件,关闭前应点击 Yes 保存结果。该结果将保存在软件中,随时可以再次读取已分析数据。

Save Ch	anges?
	Study "Untitled" contains unsaved changes. Save the changes before closing?
	Yes No Cancel

3、再次进行类似实验操作

再次进行类似实验,如果继续分析同一个 SNP 基因型,只需点击 Experiments 界面内的 Import 导入 7500 运行 eds 数据即可,其他设置不需要重复设置。



