

# 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。



### 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?

Quantitation - Standard Curve    Quantitation - Relative Standard Curve    Quantitation - Comparative Ct ( $\Delta\Delta C_t$ )

Melt Curve    **✓ Genotyping**    Presence/Absence

Detect single nucleotide polymorphism variants of a target nucleic acid sequence in samples.

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Which reagents do you want to use to detect the target sequence?

**✓ TaqMan® Reagents**    Other

The PCR reactions contain primers designed to amplify the target sequence and a TaqMan® probe designed to detect amplification of the target sequence.

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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.

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What do you want to include in the instrument run?

Include:  Pre-PCR Read     Amplification     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) to the Selected Wells.

Create New SNP Assay    Add Saved SNP Assay    Edit ▼

Assign	SNP Assay
<input type="checkbox"/>	SNP Assay 1

Save SNP Assay  
Edit SNP Assay...  
Save Selected SNP Assay  
Delete SNP Assay

Edit SNP Assay

Make changes below, then click "OK" to save your changes to the library. Click "Reset Fields" to undo all your changes.    **\*= Required**

SNP Assay Name: rs671    Color: ■    Assay ID:

Allele 1 Name or Base(s): G    Color: ■    Reporter: VIC    Quencher: NFQ-MGB

Allele 2 Name or Base(s): A    Color: ■    Reporter: FAM    Quencher: NFQ-MGB

Comments:

Reset Fields    OK    Cancel

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

**Assign Sample to the Selected Wells.**

Add New Sample   Add Saved Sample   Save Sample   Delete Sample

Assign	Sample	Color
<input type="checkbox"/>	gDNA 1	
<input type="checkbox"/>	Sample 2	
<input checked="" type="checkbox"/>	Blood 3	

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 SNP Assay(s) to the Selected Wells.**

Create New SNP Assay   Add Saved SNP Assay   Edit

Assign	SNP Ass...	Allele 1/Allele 2 Reporter	Task
<input checked="" type="checkbox"/>	rs671	VIC/FAM	Unknown

**Assign Sample to the Selected Wells.**

Add New Sample   Add Saved Sample   Save Sample   Delete Sample

Assign	Sample	Color
<input type="checkbox"/>	gDNA 1	
<input type="checkbox"/>	Sample 2	
<input checked="" type="checkbox"/>	Blood 3	

**Select the dye to use as the passive reference.**

ROX

**View Plate Layout**   View Well Table

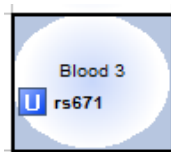
Select Wells With: - Select Item -   - Select Item -

Show in Wells   View Legend

	1	2	3	4	5	6	7	8	9
A									
B				gDNA 1 U rs...					
C				Sample 2 U rs...					
D				Blood 3 U rs...					
E									
F									
G									
H									

Wells: U 3 Unknown   N 0 Negative Control   P 0 Positive Control

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



### 1.3、Run Method 界面设置

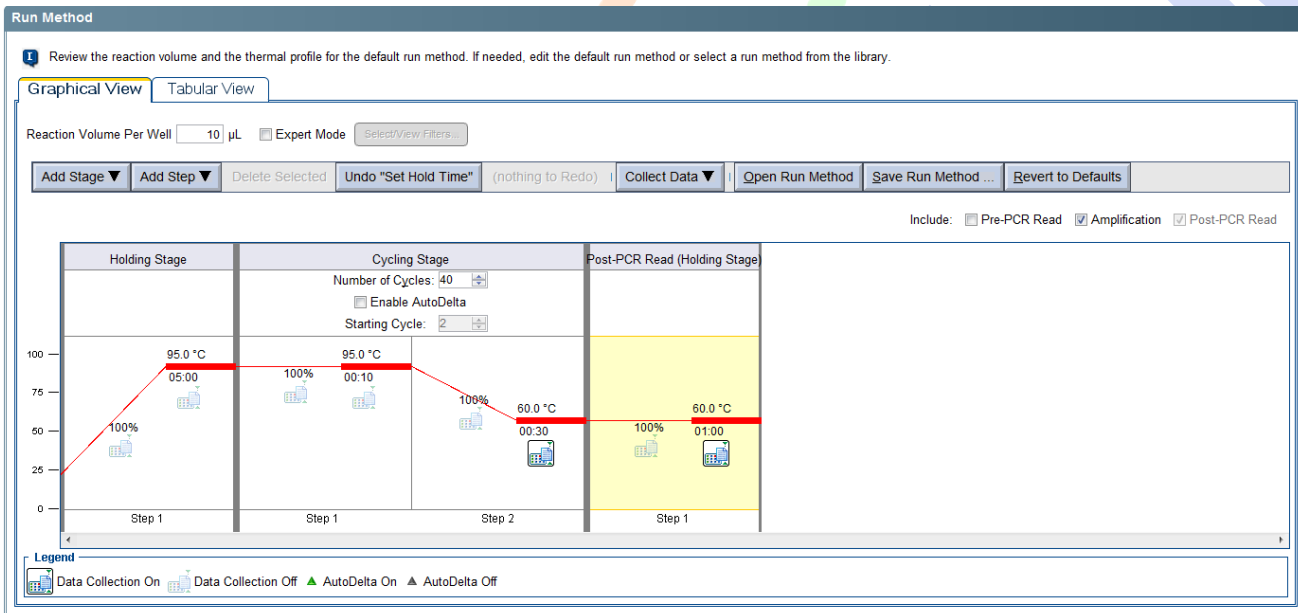
根据下图设置 Run Method 中扩增程序及荧光信号采集信息。

Reaction Volume Per  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




程序运行时长约 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-genotyper-software.html>

### 1、 Create Study

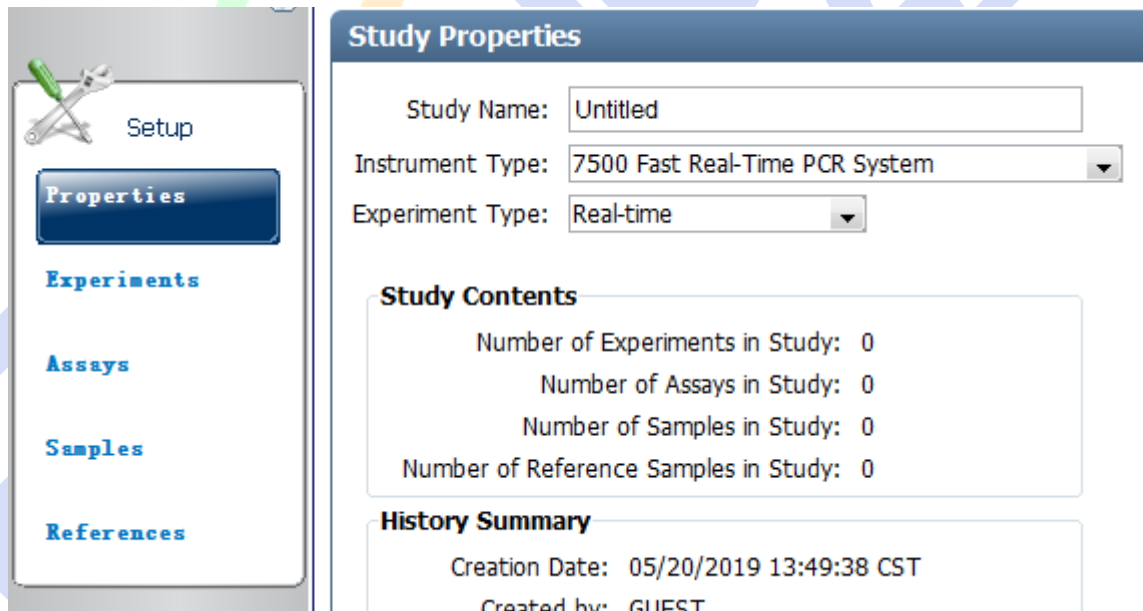
打开 TaqMan® Genotyper Software 软件，点击  创建研究方法。进入设置界面设置相关系数。

#### 1.1、 Properties 设置

Study Name: 设置人员自行确定

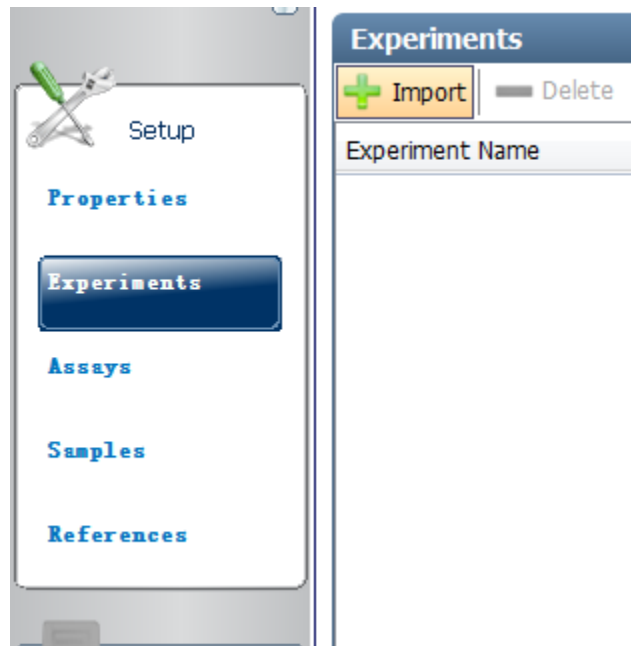
Instrument Type: 7500 Fast Real-Time PCR System

Experiment Type: Real-time



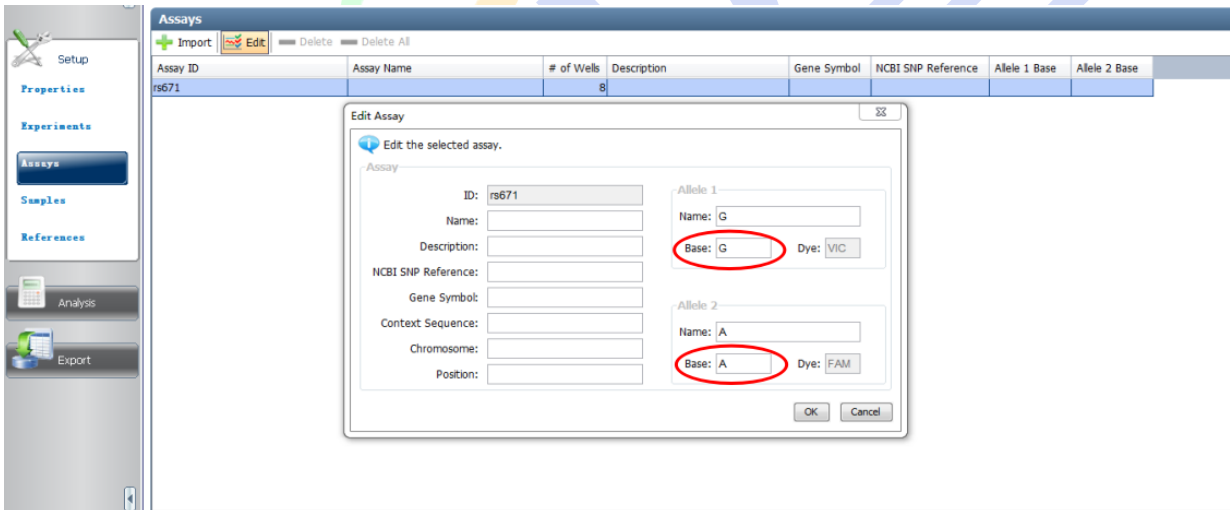
## 1.2、Experiments 设置

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



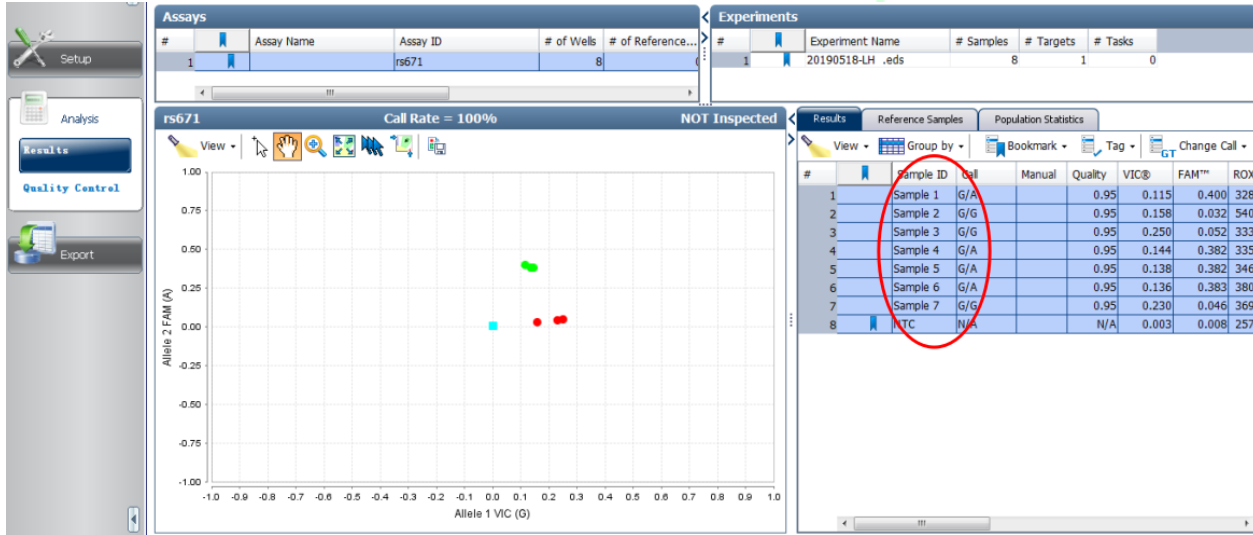
## 1.3、Assays 设置

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



## 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。



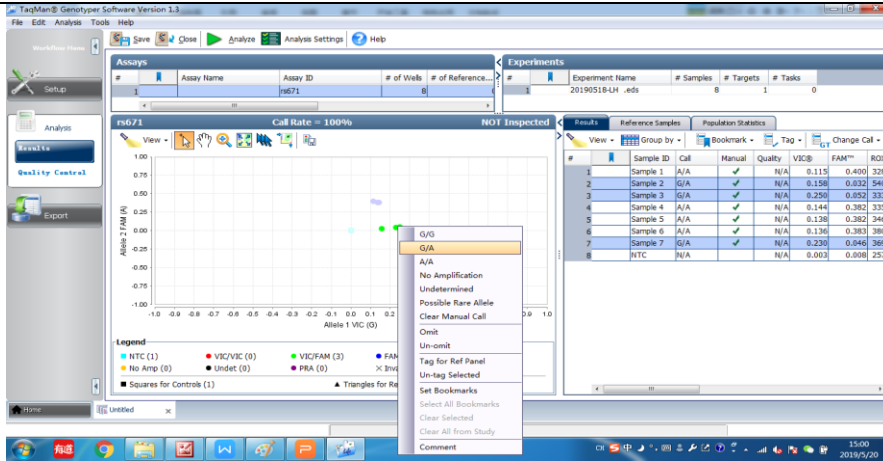
### 特别说明：

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。

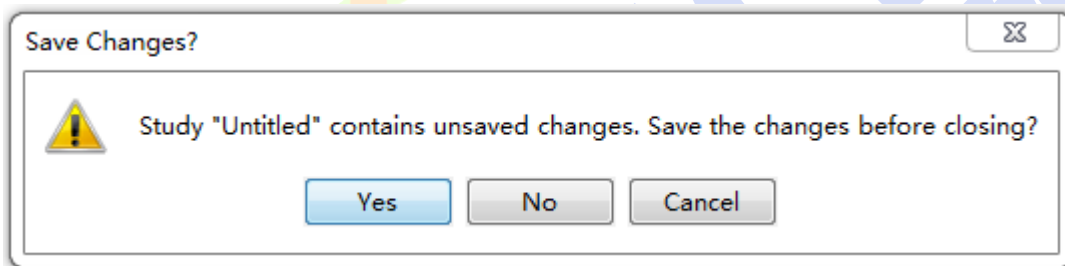
对于三种基因型不均匀分布的 SNP，特别是 90% 以上的是某单一基因型，分区界面可能只出现一种颜色区域。软件自行分析数据时，可能出现误差，需要人工校正。

### 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。



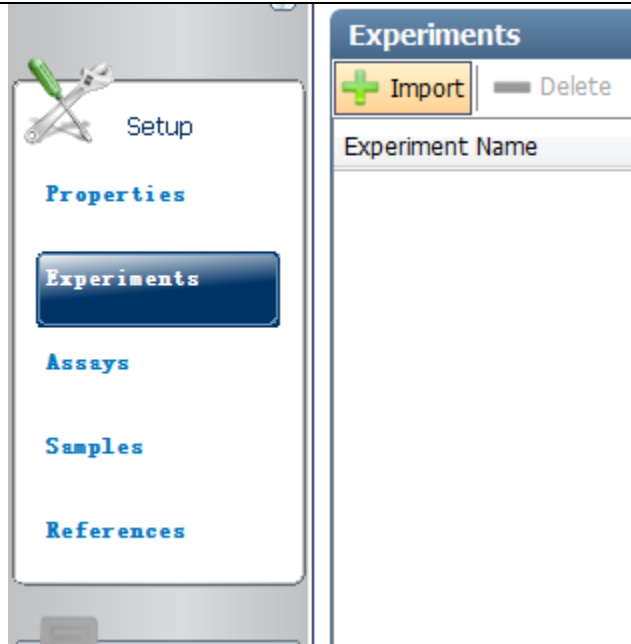
读取结果完毕后，关闭 TaqMan® Genotyper Software 软件，关闭前应点击 Yes 保存结果。该结果将保存在软件中，随时可以再次读取已分析数据。



### 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。





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