



applied biosystems 7500荧光定量PCR仪

基因分型实验简易操作流程

SDS 2.0

7500定量PCR仪

基因分型实验简易操作流程

1. 双击桌面图标 ，或从 Start > All Programs > Applied Biosystems > 7500 Software > 7500 V2.0 开启软件。进入主界面后选择 Advanced Setup 。
2. 进入 Setup 下的 Experiment Properties 界面：

2.1 输入实验名称（Experiment Name）

How do you want to identify this experiment?

* Experiment Name:

Barcode (Optional):

User Name (Optional):

Comments (Optional):

2.2 确认仪器型号

Which instrument are you using to run the experiment?

☒ 7500 (96 Wells) ☐ 7500 Fast (96 Wells)

Set up, run, and analyze an experiment using a 4- or 5-color, 96-well system.

2.3 在实验类型中，选择Genotyping

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.

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

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

2.5 确认升降温速率

Which ramp speed do you want to use in the instrument run?

☒ Standard (~ 2 hours to complete a run) ☐ Fast (~ 40 minutes to complete a run)

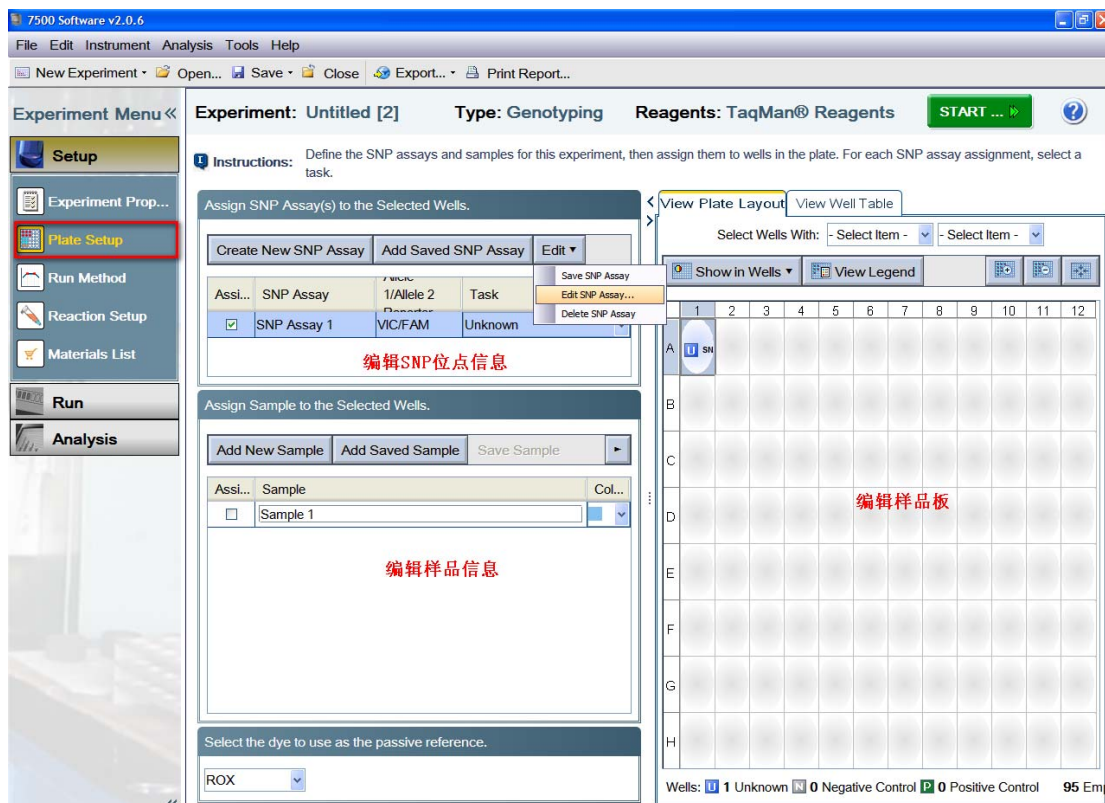
For optimal results with the standard ramp speed, Applied Biosystems recommends using standard reagents for your PCR reactions.

2.6 选择在定量仪器上进行预读板及扩增的过程

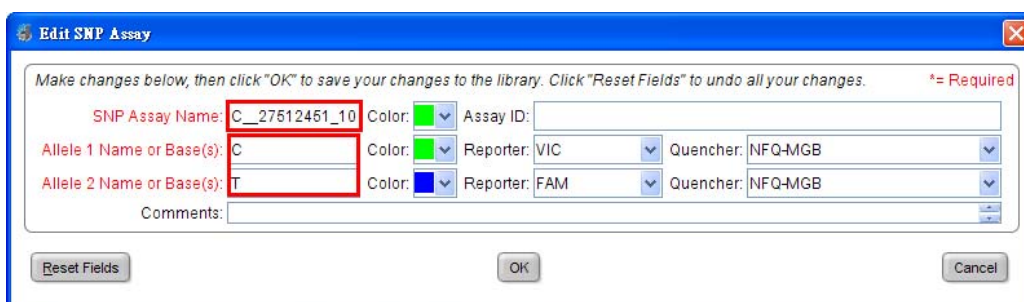
What do you want to include in the instrument run?

Include: ☒ Pre-PCR Read ☒ Amplification ☒ Post-PCR Read

3. 进入 Setup 下的 Plate Setup 界面，编辑 SNP assay 及样本：



- 3.1 在 **Assign Sample to the Selected Wells.** 界面中设置需要检测的SNP位点。选择 **Edit ▼** 下的Edit SNP assay，编辑assay的名称及碱基种类，设置Report（报告基团）和quencher（淬灭基团）。若要添加其他SNP位点，点击 **Create New SNP Assay**。



- 3.2 在 **Assign Sample to the Selected Wells.** 界面中编辑样本名称。

- 3.3 在右侧 **View Plate Layout** 界面中编辑样品板。利用鼠标单选或者

7500 Software v2.0.6

File Edit Instrument Analysis Tools Help

New Experiment Open Save Close Export Print Report...

Experiment Menu << Experiment: Genotyping Example Type: Genotyping Reagents: TaqMan® Reagents START ... ?

Instructions: Define the SNP assays and samples for this experiment, then assign them to wells in the plate. For each SNP assay assignment, select a task.

Assign SNP Assay(s) to the Selected Wells.

Create New SNP Assay Add Saved SNP Assay Edit ▾

Assi...	SNP Assay	Allele 1/Alele 2	Task
☒	SNP1	FAM/VIC	Unknown
			Unknown
			Negative Control
			Positive Control Allele 1/Alele
			Positive Control Allele 2/Alele
			Positive Control Allele 1/Alele

Assign Sample to the Selected Wells.

Add New Sample Add Saved Sample Save Sample

Assi...	Sample	Col...
☐	NTC	
☒	14474	
☐	14476	
☐	17102	
☐	17103	
☐	17104	
☐	17106	
☐	17107	

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

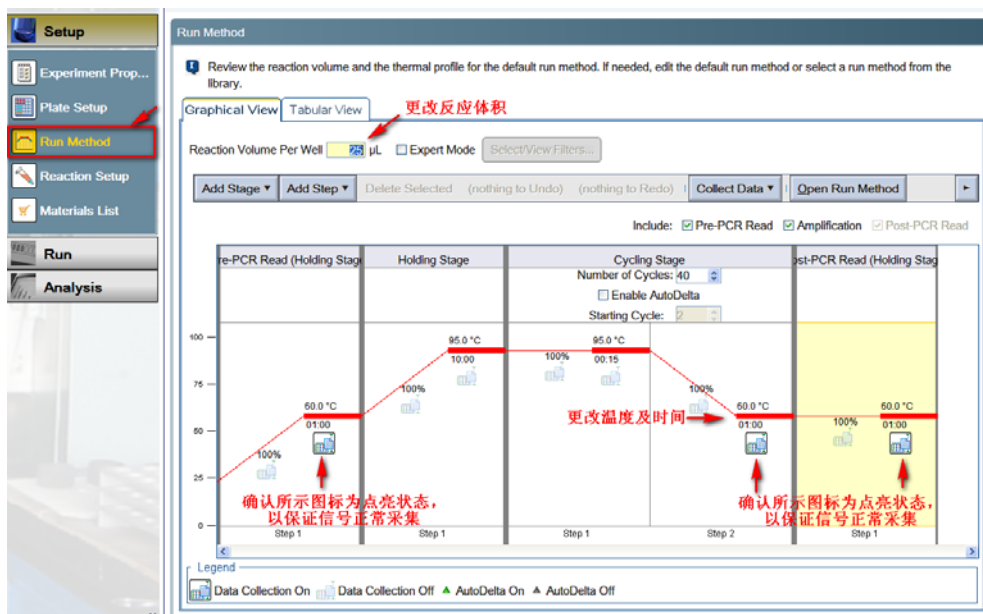
	1	2	3	4	5	6	7	8	9	10	11	12
	14476	17102	17103	17104	17106	17107	17108	10859	17109	17110		
	U	U	U	U	U	U	U	U	U	U		
	17111	17112	17113	17114	17115	17116	17117	17118	17119	17120		
	U	U	U	U	U	U	U	U	U	U		
C	NTC	17121	17122	17123	17124	17125	17126	17127	17128	17129	17130	17131
	U	U	U	U	U	U	U	U	U	U	U	U
D	NTC	17132	17136	10859	17137	17139	17140	17144	17147	17149	17148	17152
	U	U	U	U	U	U	U	U	U	U	U	U
E	NTC	17155	17188	17194	17201	17202	17203	17205	17207	17208	17209	17211
	U	U	U	U	U	U	U	U	U	U	U	U
F	NTC	17212	17213	17216	17217	17220	17221	17222	17223	17225	17226	17227
	U	U	U	U	U	U	U	U	U	U	U	U
G	NTC	17230	17231	17232	17235	17237	10859	17240	17241	17242	17245	17247
	U	U	U	U	U	U	U	U	U	U	U	U
H	NTC	17251	17252	10859	17253	17254	17255	17258	17259	17260	17261	17263
	U	U	U	U	U	U	U	U	U	U	U	U

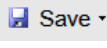
Wells: 89 Unknown 7 Negative Control 0 Positive Control 0 Empty

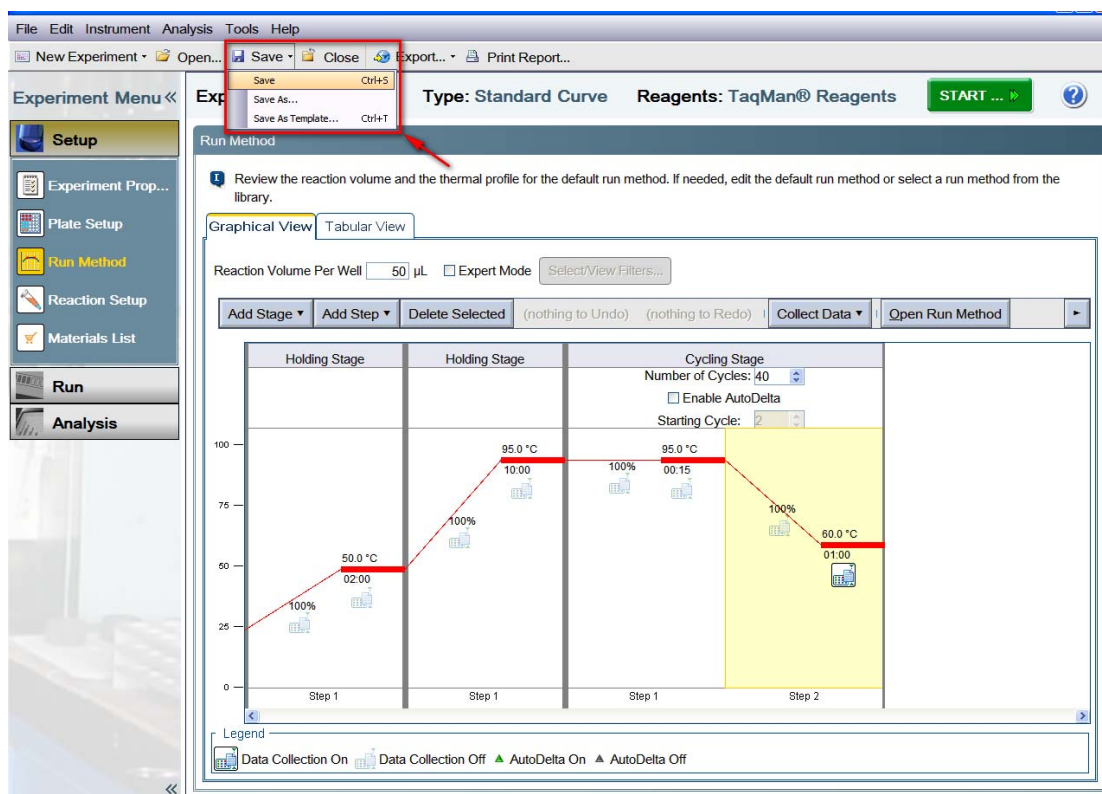
Home 120306LIUYUAN.ed... Genotyping Example.ed... Untitled ...

Disconnected

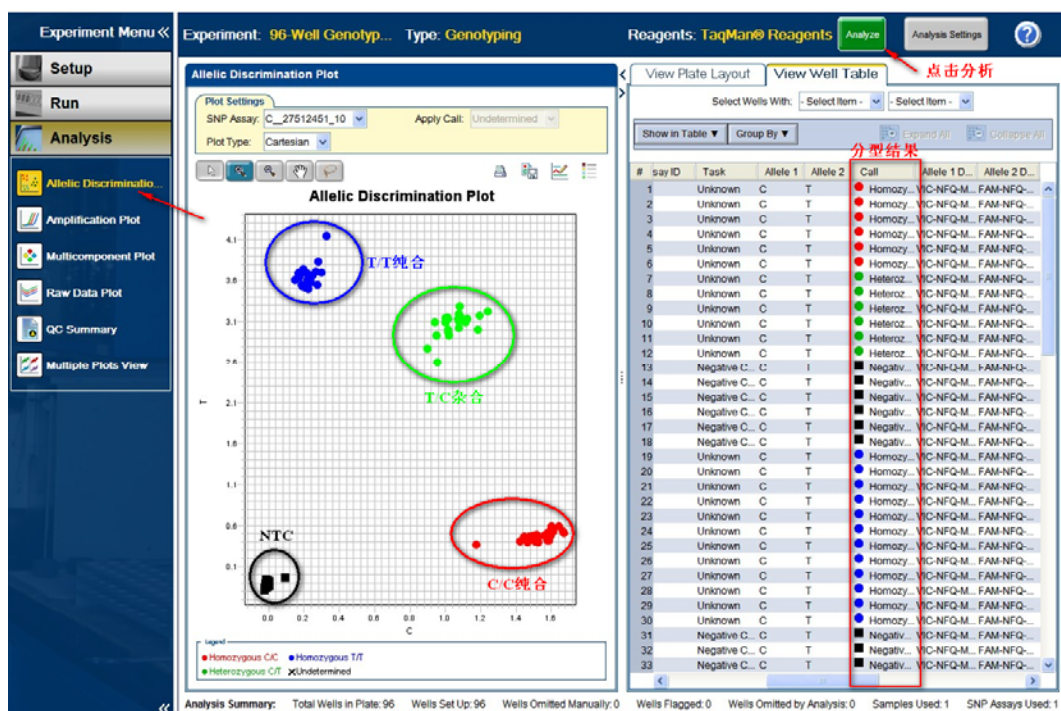
4. 进入 Run Method 界面，设定反应条件。



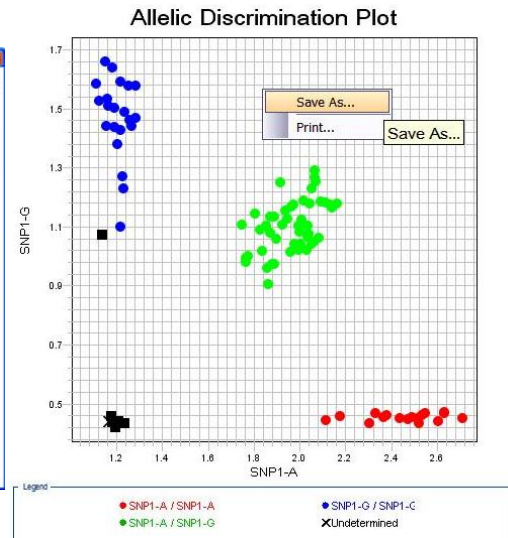
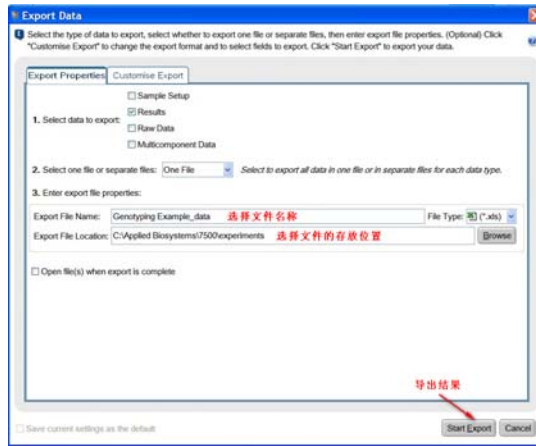
5. 点击  Save，将文件存储成 Experiment Document Single files (*.eds) 格式，按下  START RUN 开始反应。



6. 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. 分析之后的结果，可以利用菜单中的 File>Export 功能，导出 Excel 格式的结果（左图）。若想存储图片结果，可直接在图片上单击鼠标右键，选择 Save as，存成 JPEG 格式的图片（右图）。



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