Applied Biosystems[™] QuantStudio[™] 3 & 5 实时定量 PCR 仪

简明中文手册

第二部分:相对定量 (Software v1.X)



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Applied Biosystems[™] QuantStudio[™] 3 & 5实时定量PCR仪

- 双击桌面图标 , 开启QuantStudio Design & Analysis Software, 或从开始菜
 单 > All Programs > Applied Biosystems > QuantStudio Design & Analysis
 Software> QuantStudio Design & Analysis Software开启软件。
- **2.** 进入主界面后,点击 "Create New Experiment"。

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- **3.** 在 "Properties" 界面设置实验属性:
 - a. 输入实验的名称;
 - b. 选择仪器型号;
 - c. 选择仪器的 Block (加热模块) 类型;
 - d. 选择实验类型: "Comparative CT(∆∆CT)";
 - e. 选择实验试剂类型: TaqMan 探针法选择 "TaqMan Reagents", SYBR 染料法选择 "SYBR Green Reagents",其他选择 "Other";
 - f. 选择运行模式(Run mode):普通试剂选择"Standard";快速试剂可选择 "Fast"。

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Experime	nt Method	输入反应体积				Action v	D _å Save
	Volume	Cover					
	20 µL	105.0 °C		单击更	改反应温度和时间		
	Hold	i Stage	PCR	Stage /			
		95.0 °C	95.0 °C	60.0°C			
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4. 点击"Next"进入"Method"界面,设置实验的运行程序。

 4.1 (可选)设置梯度反应温度: ①单击 ♀ (Advanced Settings); ②勾选
 VeriFlex, ③然后更改Block上相应区域的反应温度,相邻区域温度差异不能超过 5℃。

	Volume	Cover			
	50 μL	105.0 °C			Include: V Pre-Po
	Pre-Read Stage	Hold Stage	PCR	Stage	Post-Read Stage
	60.0 °C	95.0 °C	95.0 °C 1.6 °C/s 00:15	60.0 °C	60.0 °C
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注:梯度反应温度设置仅限于96孔加热模块。QuantStudio 3 可设置3个梯度反应 温度;上图为QuantStudio 5示例图,可设置6个梯度反应温度。

4.2 (可选)设置暂停程序:点击① 1 图标,② 勾选Pause,③设置暂停前的反应循

环数(Pause after cycles),以及暂停后的温度(Pausing Temperature,范围:

4~99.9°C)。

Experiment Method

	Volume	Cover			
	50 µL	105.0 °C			
	Hold	Stage	PCR	Stage	
\langle	50.0 °C 1.6 °C/s 02:00	95.0 °C 1.6 °C/s 10:00	95.0 °C 1.6 °C/s 00:15	60.0 °C 1.6 °C/s 01:00 ⓒ ♀ Ⅱ	
	Step1	Step2	Step1	Step2	(I)
			40	X X	
	Volume	Cover			
	Volume 50 µL	Cover 105.0 °C			
	Volume 50 µL Holo	Cover 105.0 °C	PCF	t Stage	
	Volume 50 μL Hold 50.0 °C 1.6 °C/s 02:00 Ο Ο	Cover 105.0 °C 4 Stage 95.0 °C 1.6 °C/s 10:00 0 0 0 0 0 0 0 0 0 0 0 0	95.0 °C 1.6 °C/s 00:15	60.0 °C 1.6 °C/s 01:00	
\langle	Volume 50 μL Hold 50.0 °C 1.6 °C/s 02:00 © Φ Step1	Cover 105.0 °C 1 Stage 95.0 °C 1.6 °C/s 0 0 0 0 0 0 0 0 0 0 0 0 0	PCR 95.0 °C 1.6 °C/s 00:15 0 °C 11 0 °C 15 0 °	t Stage 1.6 °C/s 01:00 00 01:00 00:00 00:00 00:00 00:00 00:00 00:00 00:00	
\langle	Volume 50 µL Hold 50.0 °C 1.6 °C/s 02:00 © \$ Step1	Cover 105.0 °C 4 Stage 95.0 °C 1.6 °C/s 10:00 2 5 5 5 5 5 5 5 5 5 5 5 5 5	PCF 95.0 °C 1.6 °C/s 00.15 0 Pause Pause after Cycle: 10 ÷ Pausing Temperature 25.0 40	8 Stage 60.0°C 1.6°C/s 01:00 00 01:00	

5. 进入 "Plate" 界面, 点击 "Advanced Setup" ① 设置待测基因名称 (Target);

②设置样品名称(Sample)。

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5.1 在 "Targets"内点击 "Add", 添加待测基因。在 "Target Name"中编辑基因名称; "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;如果是其他形式的无荧光淬灭基团则选择"None"。

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	Target 1	FAM	NFQ-MGB		-		×

5.2 在 "Samples"内点击 "Add", 添加待测样品。在 "Sample Name" 中编辑样品名称。

-	San	nples	+ Add	Acti	Action			
		Sample Name	Comme	nts	+			
		Sample 1				×		
(m)		Sample 2				×		

5.3 编辑反应样品板:利用鼠标单选或拖拽以选择反应孔,然后勾选左侧的基因及样本,同时在"Task"选项中指定该反应孔的类型(U 代表未知样本,N 代表阴性对照)。

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5.4 点击 "Quick Setup", @设置参比荧光,如果试剂中不含 ROX 参比荧光,则改为
None; b) 设置 "Reference Sample" (对照样品), c) 设置 "Endogenous

Control"(内参基因)。

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 点击 "Next" 进入 "Run" 界面,点击 "Save" 保存文件,然后点击 "START RUN" 开始运行。

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7. 实验运行结束后,进入"Results"界面,点击右上角的"Analyze"按钮分析数据 并查看结果。

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- **8.** 结果分析
- 8.1 更改扩增曲线显示方式:单击(Show Plot Setting),在 "Graph Type"中可更改 扩增曲线的显示方式(Log 或 Linear 图)。

Results		更改扩增曲线显示方式								
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- 8.2 设置基线和阈值线:软件默认使用 "Auto" 功能自动设定基线和阈值线。
- 8.2.1 查看阈值线或基线:单击 "Show Plot Setting",选择需要查看的基因, "Show: Threshold"及 "Show: Baseline"前的选项打勾。扩增曲线图上会出现相应的基线 范围和阈值线。

Results

Results

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8.2.2 手动设置基线和阈值线:去掉 "Auto"的勾选,然后输入阈值,或用鼠标拖动阈值 线和基线进行手动调节。设置好后,点击 "Analyze"分析结果。

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8.3 选择"Gene Expression"查看基因表达柱状图。



8.4 对于 SYBR Green 实验,可以选择 "Melt Curve Plot",查看熔解曲线。 Results



8.5 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。

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9. 数据导出:在"Export"界面下根据需要导出数据。

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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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