



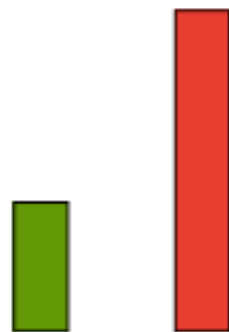
分子流行病学实验

RT-PCR

为什么要做 RT-PCR ?



未处理 样本



处理后 样本

目的基因: **Plat1**

问题: 不同处理组间, **Plat1**基因表达量的差异?

为什么要做 RT-PCR ?



未处理 样本



处理后 样本

目的基因: Plat1

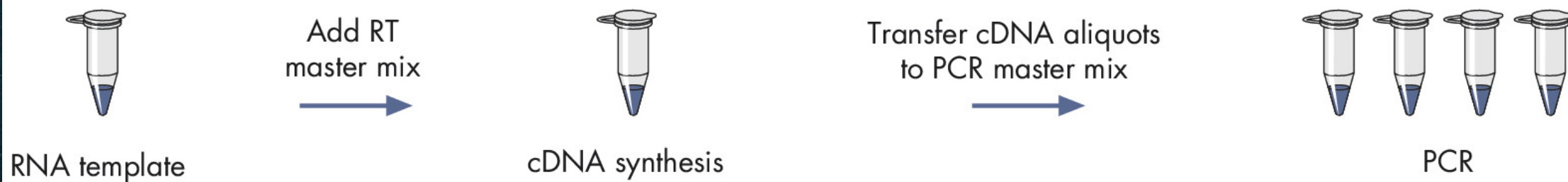
问题: 不同处理组间, Plat1 基因表达量的差异?

一步法 / 两步法 RT-PCR

A



B





RT

reverse-transcription

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1 实验材料

2 实验步骤

实验材料

01

RNA模板：Total RNA, miRNA

02

仪器：PCR仪

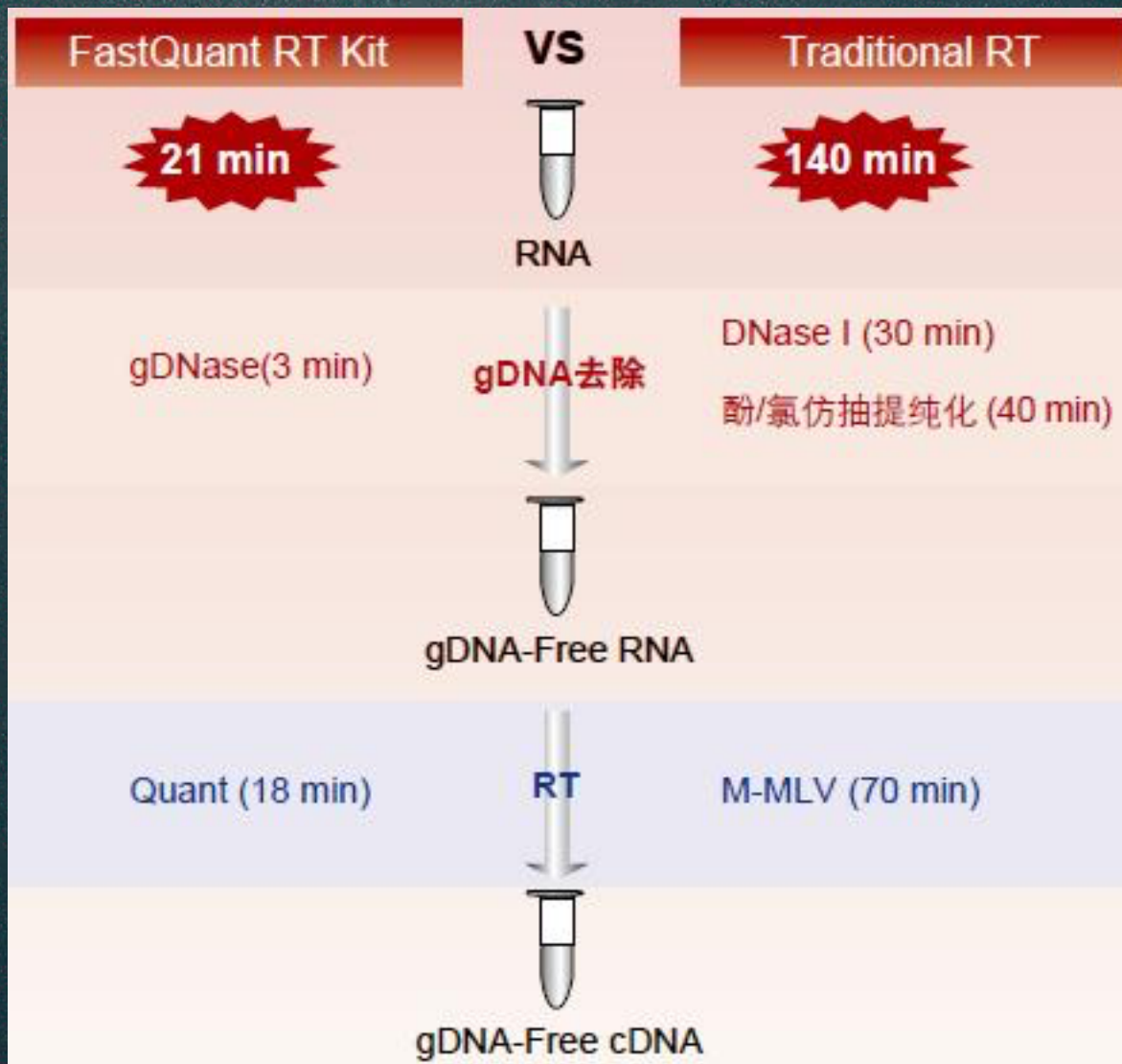


对RNA 模板的要求

逆转录酶以RNA为模板合成第一链cDNA，模板RNA的质量和数量直接影响逆转录的结果。

1. **模板的完整性**：模板RNA的完整性对逆转录非常重要,若RNA模板中含有RNase酶将降解模板RNA,最后导致cDNA产物的量少甚至无cDNA产物。
2. **模板的纯度**：若RNA模板中含有蛋白、盐离子、EDTA、乙醇、酚等杂质,将影响逆转录酶的活性,最后影响逆转录结果。
3. **模板量**：一般模板RNA量为50ng-2 μ g。（若模板RNA的量大于2 μ g,则需按比例扩大反应体系。）

实验操作（简易流程）



实验步骤（在冰上操作）

1. 将模板RNA在冰上解冻;5×gDNA Buffer、FQ-RT Primer Mix、10×Fast RT Buffer、RNase-Free ddH₂O在室温(15-25°C)解冻,解冻后迅速置于冰上。使用前将每种溶液 涡旋振荡混匀,简短离心以收集残留在管壁的液体。

实验步骤（在冰上操作）

- 按照表1的基因组DNA的去除体系配制混合液,彻底混匀。简短离心,并置于42°C,孵育 3 min。然后置于冰上放置。

表1 gDNA去除反应体系

组成成分	使用量
5×gDNA Buffer	2 μl
Total RNA	-
RNase-Free ddH ₂ O	补足到10 μl

实验步骤（在冰上操作）

3. 按照表2的反转录反应体系配制混合液。

表2 反转录反应体系

组成成分	使用量
10×Fast RT Buffer	2 μ l
RT Enzyme Mix	1 μ l
FQ-RT Primer Mix	2 μ l
RNase-Free ddH ₂ O	补足到10 μ l

实验步骤（在冰上操作）

4. 将反转录反应中的Mix, 加到gDNA去除步骤的反应液中, 充分混匀。
5. 42°C, 孵育15 min。
6. 95°C, 孵育3 min之后放于冰上, 得到的cDNA可用于后续实验, 或低温保存。

RT-PCR —— 注意事项

在进行RT-PCR时，必须仔细的选择逆转录的酶和引物。引物要能逆转录所有感兴趣的，并且逆转录酶所得到的cDNA的量必须能精确的反映原始RNA量，以便能精确定量检测。除此之外，逆转录反应的成分对于后续的real-time PCR反应的影响要达到最小。



qPCR

quantitative PCR

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1 实验材料

2 实验步骤

3 实验结果

实验材料

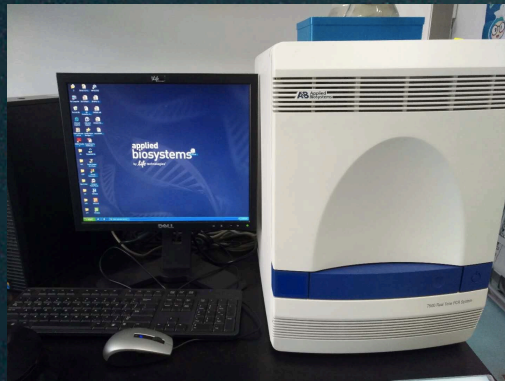
01

Template: cDNA (1pg -100ng),
Primer: control vs target (0.2 μ M),
SYBR Green Mix: DNA polymerase, dNTP

02

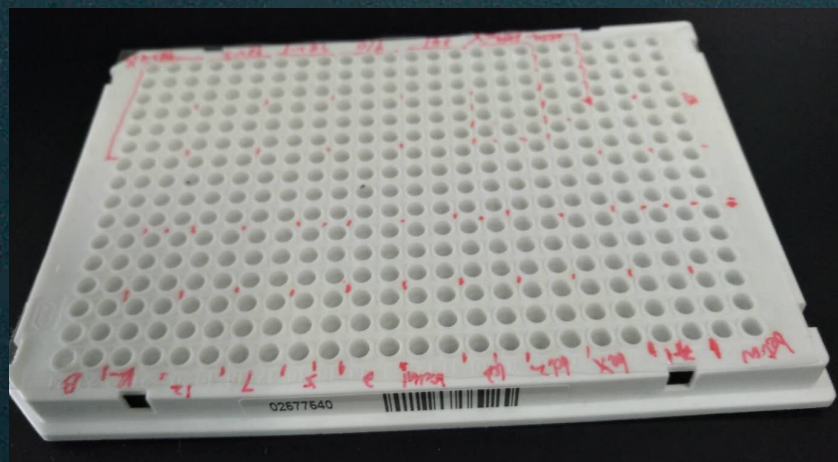
仪器：

ABI 7500 Real-Time PCR Systems,
Roche LightCycler 480 II



实验操作

	1	2	3	4	5	6	7	8	9	10	11	12
A	Control U 183	Control U 183	Control U 183	Sample 1 U 183	Sample 1 U 183	Sample 1 U 183	Sample 2 U 183	Sample 2 U 183	Sample 2 U 183	N 183	N 183	N 183
B	Control U Plat1	Control U Plat1	Control U Plat1	Sample 1 U Plat1	Sample 1 U Plat1	Sample 1 U Plat1	Sample 2 U Plat1	Sample 2 U Plat1	Sample 2 U Plat1	N Plat1	N Plat1	N Plat1



实验步骤（在冰上操作）

1. 按照表3配制PCR反应体系。(注：配制多个反应孔时，请为各组分预留10%的余量，以免移液损失)

组成成分	10 μ l 体系	20 μ l 体系
2 X PowerUp SYBR Green Master Mix	5 μ l	10 μ l
正向引物和反向引物 ^[1]	—	—
cDNA 模板和 ddH ₂ O ^[2]	—	—
总体积	10 μ l	20 μ l

- 正、反向引物的终浓度为200 nM
- 每个反应孔使用cDNA 1-10ng

实验步骤（在冰上操作）

2. 反应体系配好后，盖上反应盖，充分涡旋混匀，离心。
3. 将反应液分装到每个反应孔中。封上贴膜，离心，避免产生气泡。
4. 运行qPCR反应程序。

实验步骤 (反应程序设置)

7500 Software v2.0.6

File Edit Instrument Analysis Tools Help

New Experiment Open... Save Close Export... Print Report...

Experiment Menu << Experiment: Untitled Type: Standard Curve Reagents: TaqMan® Reagents START RUN ?

Experiment Properties

Enter an experiment name, select the instrument type, select the type of experiment to set up, then select materials and methods for the PCR reactions and instrument run.

How do you want to identify this experiment?

* Experiment Name:

Barcode (Optional):

User Name (Optional):

Comments (Optional):

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.

What type of experiment do you want to set up?

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

Melt Curve Genotyping Presence/Absence

Use standards to determine the absolute quantity of target nucleic acid sequence in samples.

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.

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.

反应程序设置——实验特性

The screenshot displays a software interface for setting up a PCR experiment. The top bar shows the experiment name as 'Untitled', the type as 'Relative Standard Curve', and the reagents as 'SYBR® Green Reagents'. A green 'START RUN' button is visible on the right. A left-hand navigation menu includes sections for 'Setup', 'Run', and 'Analysis', with 'Experiment Properties' selected under 'Setup'. The main area is titled 'Experiment Properties' and contains several sections for configuration:

- Experiment Properties:** A text box for the experiment name, currently set to 'Untitled'. Below it are optional fields for 'Barcode', 'User Name', and 'Comments'.
- Which instrument are you using to run the experiment?:** Two buttons are shown: '7500 (96 Wells)' (selected) and '7500 Fast (96 Wells)'. A note below states: 'Set up, run, and analyze an experiment using a 4- or 5-color, 96-well system.'
- What type of experiment do you want to set up?:** Six buttons are shown: 'Quantitation - Standard Curve', 'Quantitation - Relative Standard Curve' (selected), 'Quantitation - Comparative Ct ($\Delta\Delta C_t$)', 'Melt Curve', 'Genotyping', and 'Presence/Absence'. A note below states: 'Use standards, a reference sample, and an endogenous control to determine the relative quantity of target nucleic acid sequence in samples.'
- Which reagents do you want to use to detect the target sequence?:** Three buttons are shown: 'TaqMan® Reagents', 'SYBR® Green Reagents' (selected), and 'Other'. A note below states: 'The PCR reactions contain primers designed to amplify the target sequence and SYBR® Green I dye to detect double-stranded DNA.' A checkbox for 'Include Melt Curve' is checked.
- Which ramp speed do you want to use in the instrument run?:** One button is shown: 'Standard (~ 2 hours to complete a run)' (selected).

The bottom of the interface shows a taskbar with a home icon, a browser window titled '20160524FLY.eda x', and an 'Untitled' window.

反应程序设置——Target/Sample

Setup

- Experiment Properties
- Plate Setup**
- Run Method
- Reaction Setup
- Materials List

Run

Analysis

Define Targets and Samples Assign Targets and Samples

I Instructions: Define the targets to quantify and the samples to test in the reaction plate.

Define Targets

Add New Target Add Saved Target Save Target Delete Target

Target Name	Reporter	Quencher	Color
Target 1	SYBR	None	Blue
Target 2	SYBR	None	Purple
control	SYBR	None	Yellow

Define Samples

Add New Sample Add Saved Sample Save Sample Delete Sample

Sample Name	Color
Sample 1	Blue

反应程序设置——Target/Sample

Setup

- Experiment Properties
- Plate Setup**
- Run Method
- Reaction Setup
- Materials List

Run

Analysis

Define Targets and Samples

Assign Targets and Samples

I Instructions: Define the targets to quantify and the samples to test in the reaction plate.

Define Targets

Add New Target Add Saved Target Save Target Delete Target

Target Name	Reporter	Quencher	Color
Target 1	SYBR	None	Blue
Target 2	SYBR	None	Purple
control	SYBR	None	Yellow

Define Samples

Add New Sample Add Saved Sample Save Sample Delete Sample

Sample Name	Color
Sample 1	Blue
Sample 2	Green
Sample 3	Yellow

反应程序设置——Plate

Setup

- Experiment Properties
- Plate Setup**
- Run Method
- Reaction Setup
- Materials List

Run

Analysis

Define Targets and Samples

Assign Targets and Samples

Instructions:
To set up standards: click "Define and Set Up Standards."
To set up unknowns: select wells, assign target(s), select "Unknown (double-click U icon)" as the task for each target assignment, then assign a sample.
To set up negative controls: select wells, assign target(s), then select "Negative Control (double-click N icon)" as the task for each target assignment.

Assign target(s) to the selected wells.

Assign	Target	Task	Qual
<input type="checkbox"/>	Target 1		
<input type="checkbox"/>	Target 2		
<input type="checkbox"/>	control		

* Mixed Unknown Standard Negative Control

Define and Set Up Standards

Assign sample(s) to the selected wells.

Assign	Sample
<input type="checkbox"/>	Sample 2

View Plate Layout

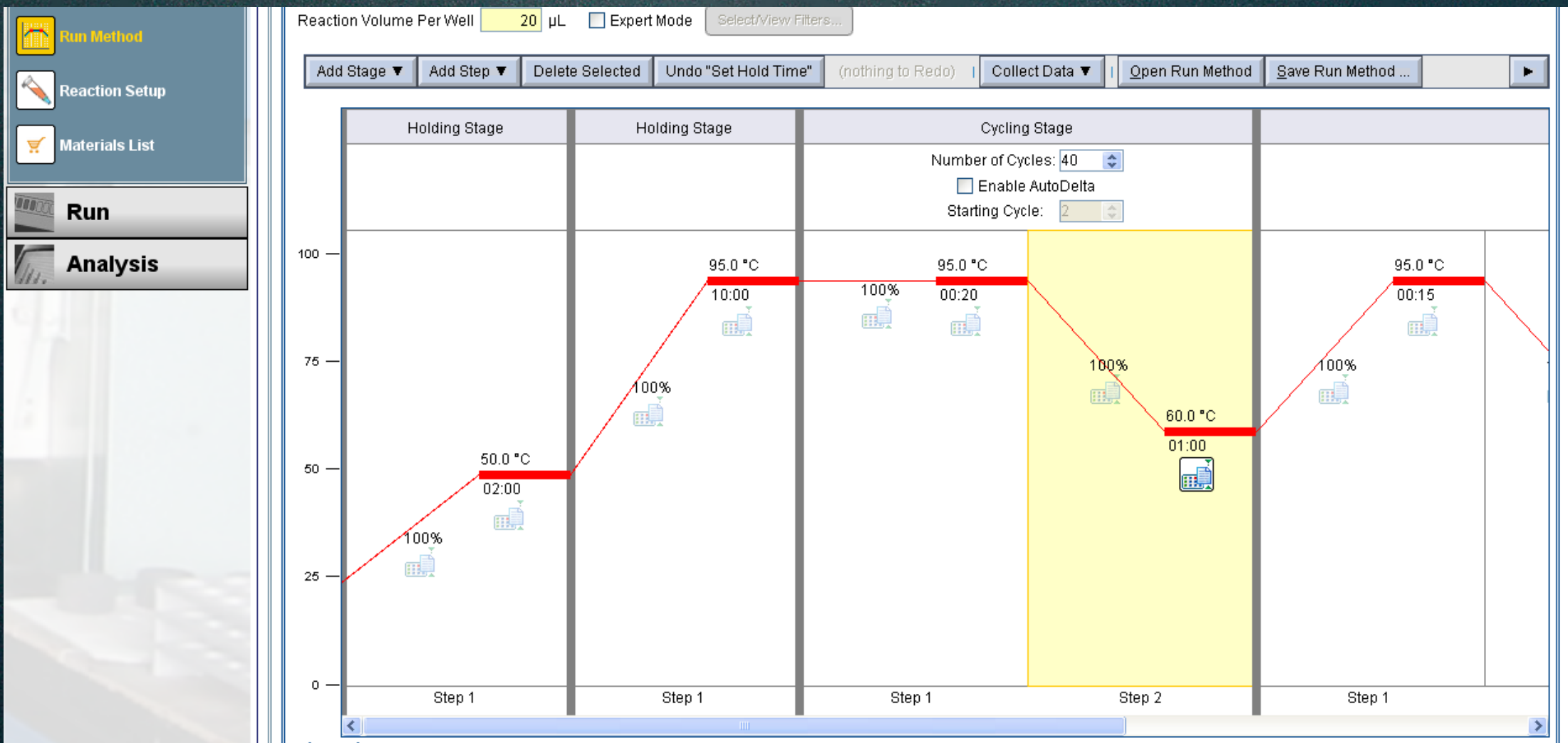
View Well Table

Select Wells With:

Show in Wells View Legend

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1 	Sample 1 	Sample 1 	Sample 1 	Sample 1 	Sample 1 	Sample 1 	Sample 1 	Sample 1 	Sample 1 	Sample 1 	Sample 1
B	Sample 2 	Sample 2 	Sample 2 	Sample 2 	Sample 2 	Sample 2 	Sample 2 	Sample 2 	Sample 2 	Sample 2 	Sample 2 	Sample 2
C	Sample 3 	Sample 3 	Sample 3 	Sample 3 	Sample 3 	Sample 3 	Sample 3 	Sample 3 	Sample 3 	Sample 3 	Sample 3 	Sample 3

反应程序设置——反应方法



反应程序设置——反应方法

Plate Setup
Run Method
Reaction Setup
Materials List

Run
Analysis

Reaction Mix Calculations | Sample Dilution Calculations

Select Target: Target 1 | Reaction Volume Per Well: 20 μL | Excess Reaction Volume: 10 % | [Print Reaction Setup](#)

Target 2 control

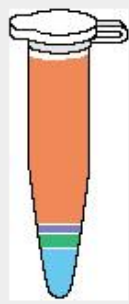
Reactions for Target 1

Master Mix Concentration: 2.0 X

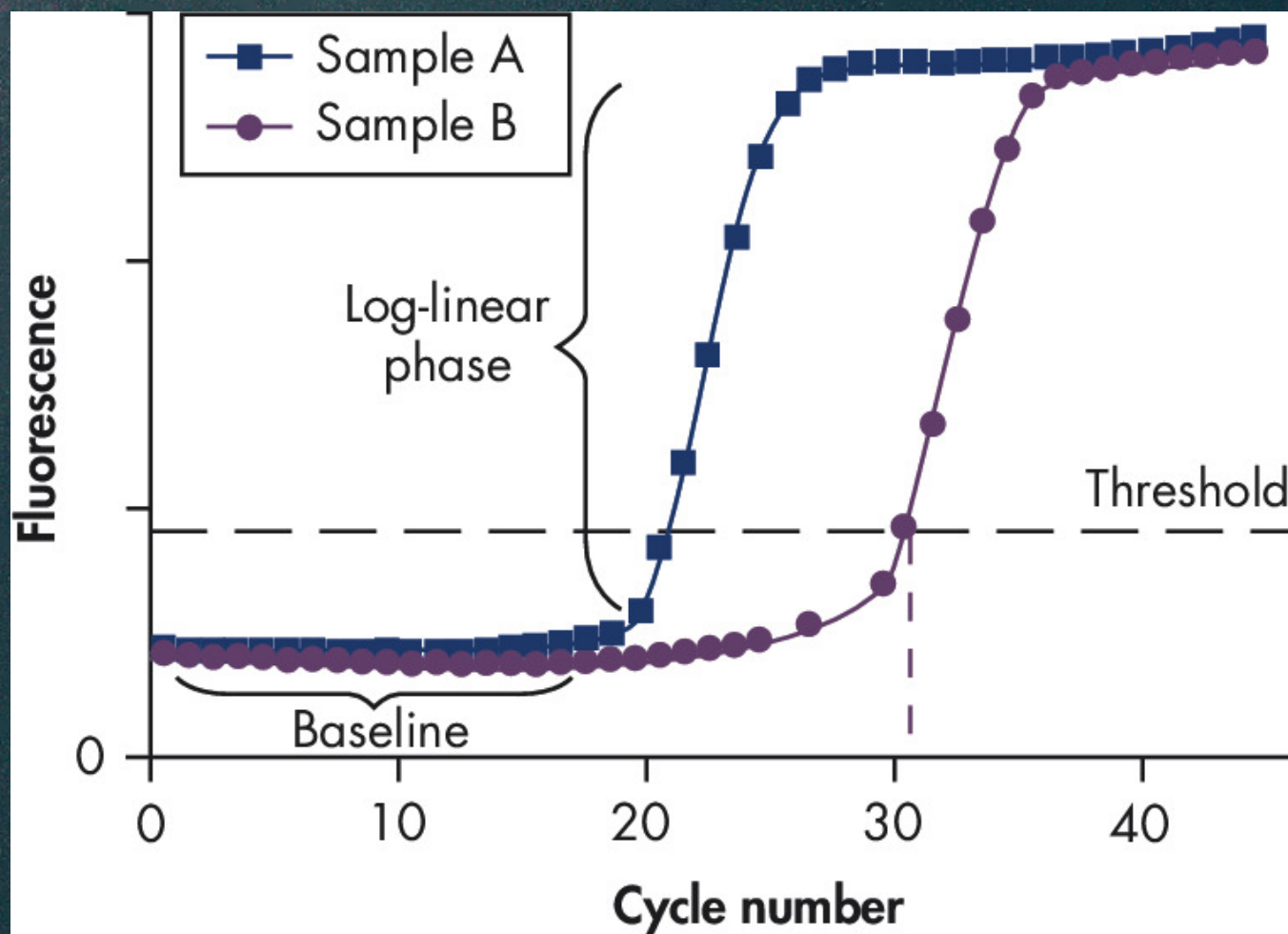
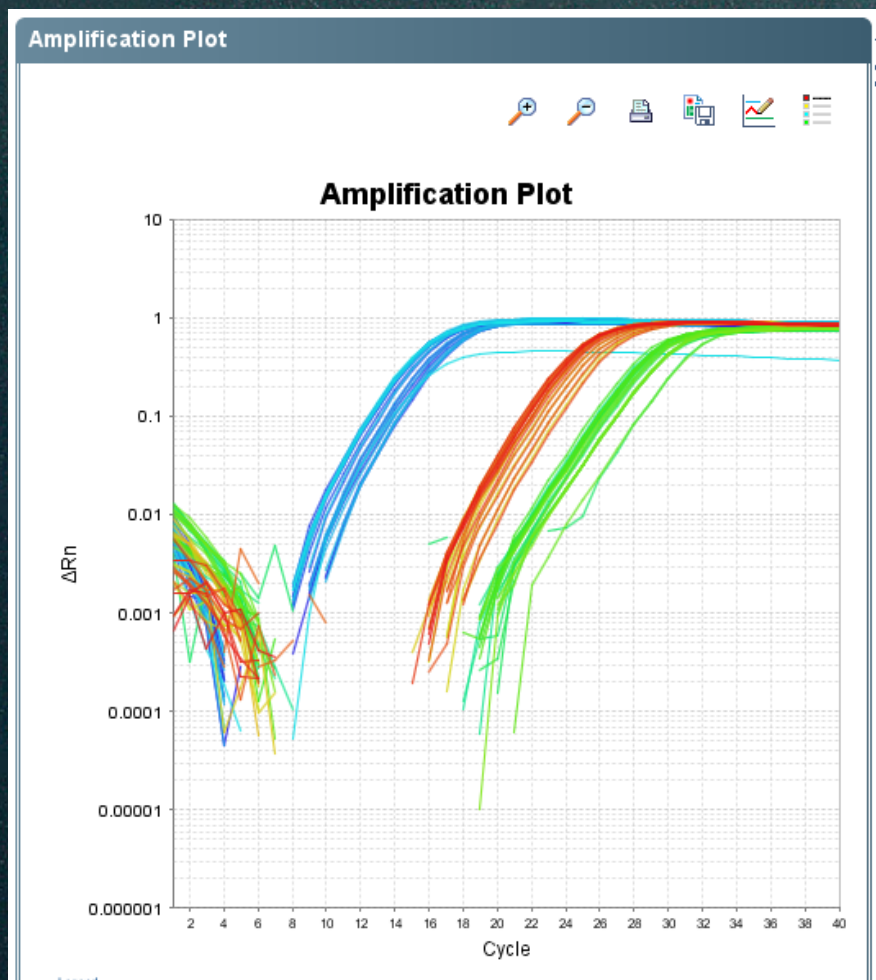
Forward Primer Starting Concentration: 20.0 pmol/ μL | Forward Primer Final Concentration: 200.0 nM

Reverse Primer Starting Concentration: 20.0 pmol/ μL | Reverse Primer Final Concentration: 200.0 nM

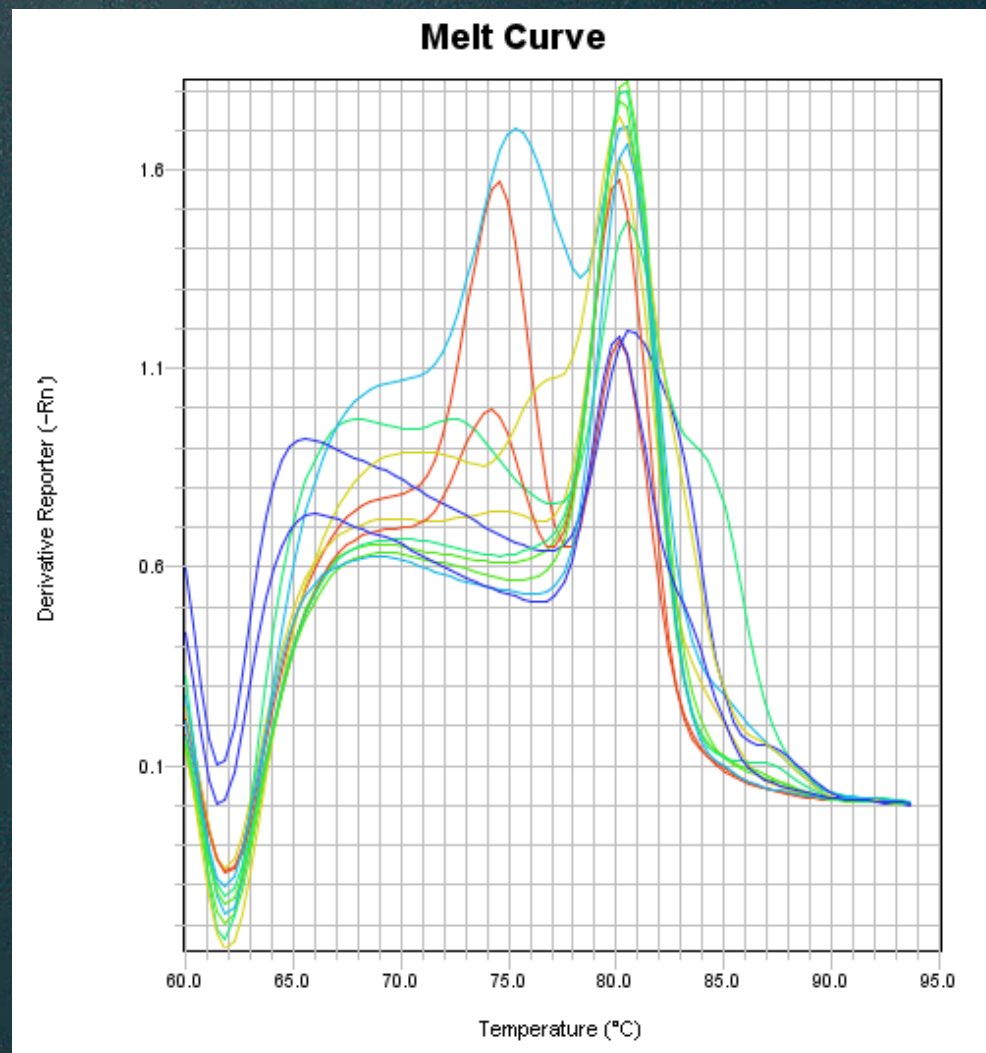
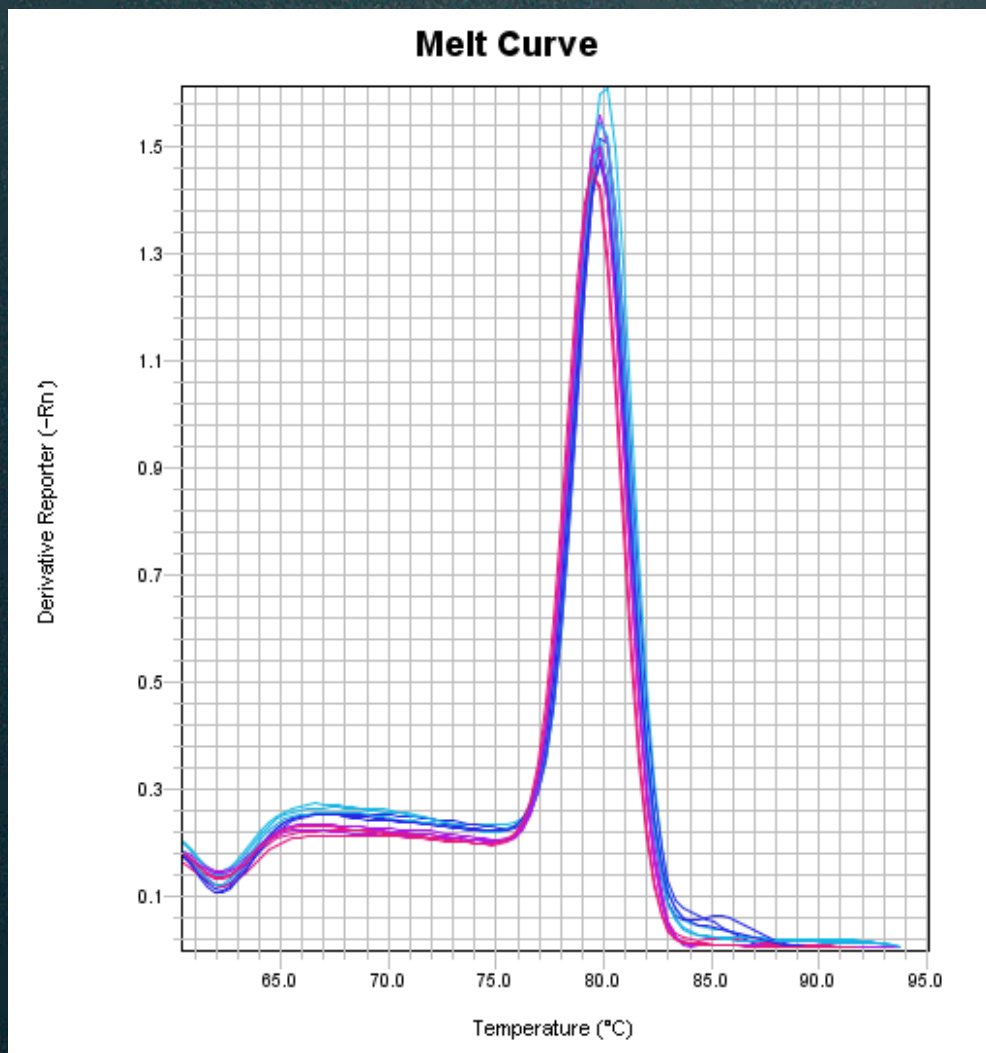
Component	Volume (μL) for 1 Reaction
Master Mix (2.0X)	10.00
Forward Primer	0.20
Reverse Primer	0.20
Sample (10X) or Standard	2.00
H ₂ O	7.60
Total Volume	20.00



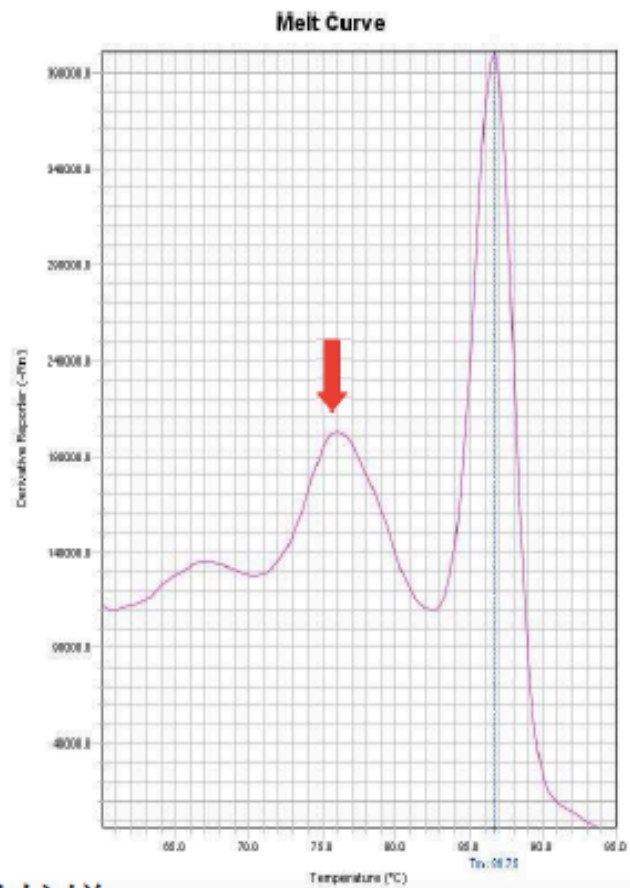
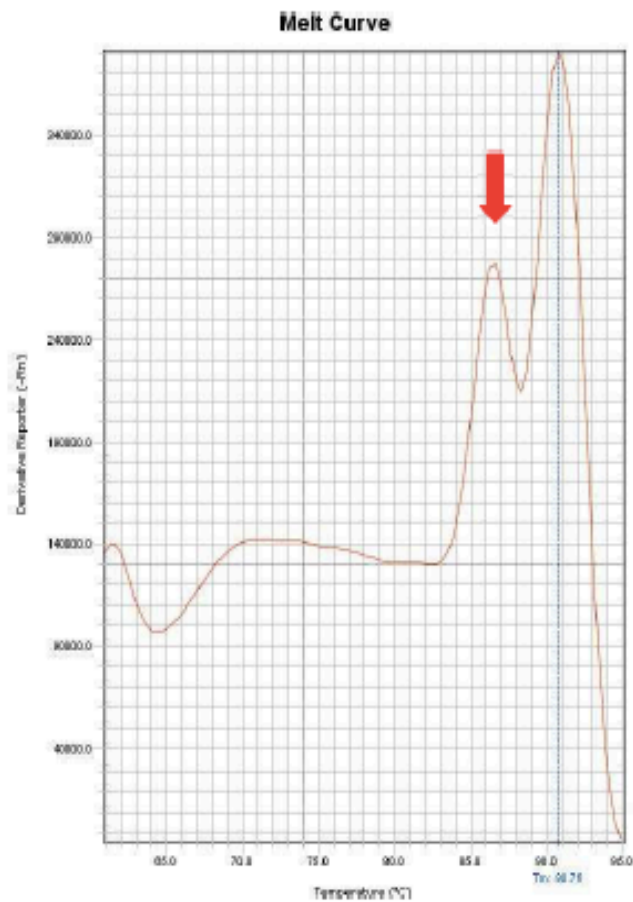
实验结果——扩增曲线



实验结果——溶解曲线



实验结果——溶解曲线



非特异扩增
引物二聚体



感谢观看