# RT-PCR

# 为什么要做 RT-PCR？

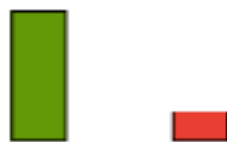
未处理 样本

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？



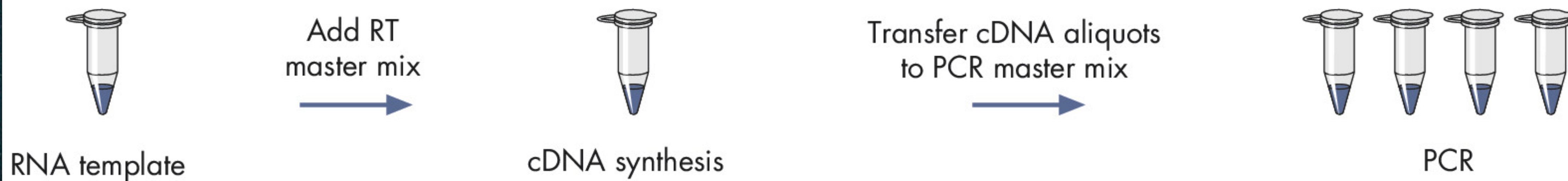
未处理 样本

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



# RT

reverse-transcription

# 目 录 CONTENTS

1 实验材料

2 实验步骤

# 实验材料

01 RNA模板：Total RNA, miRNA

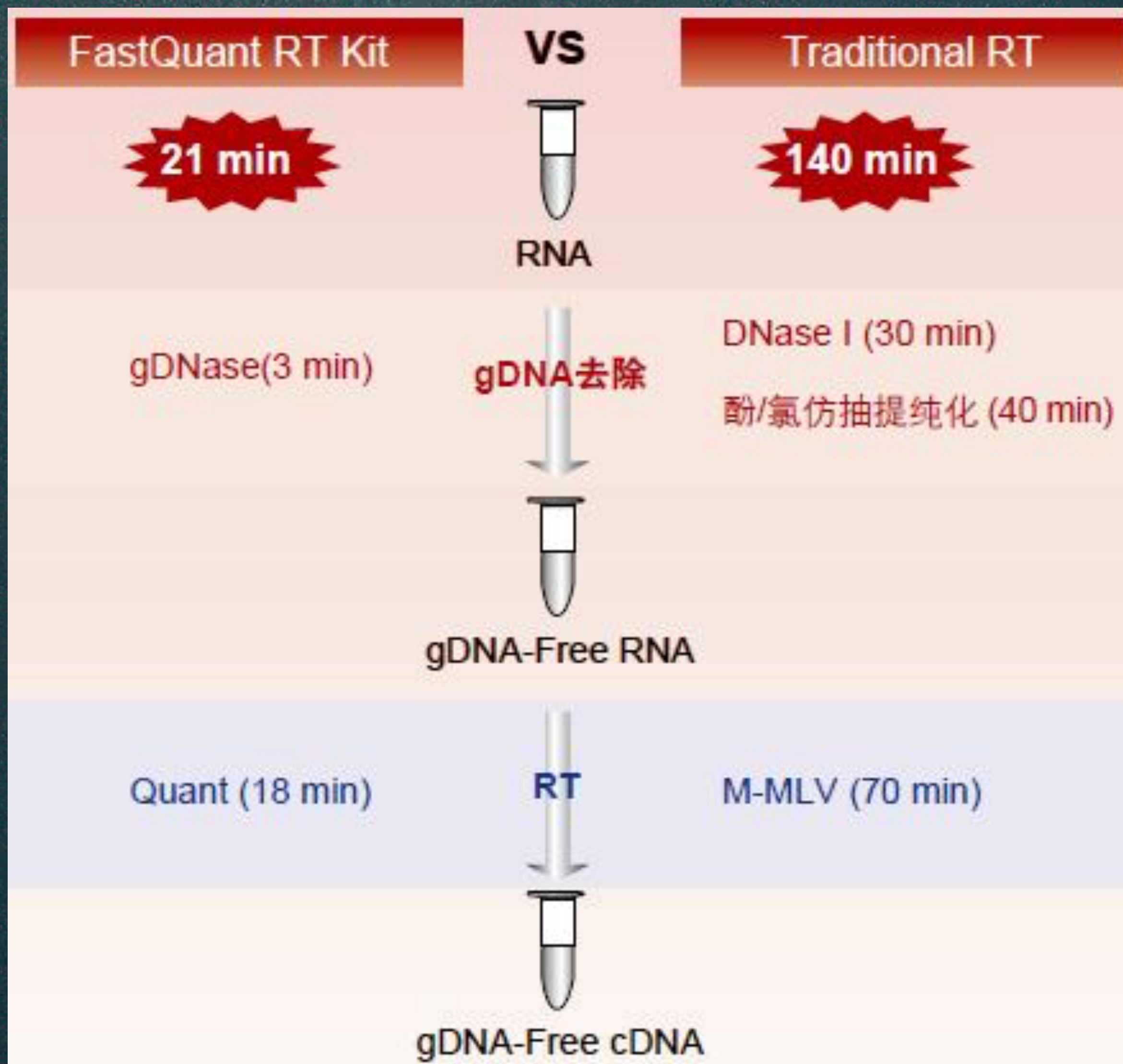
02 仪器：PCR仪

# 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-2μg。（若模板RNA的量大于2μg,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）

2. 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 dd$H_2O$ | 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 μl |
| RT Enzyme Mix | 1 μl |
| FQ-RT Primer Mix | 2 μl |
| RNase-Free dd$H_2O$ | 补足到10 μl |

# 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

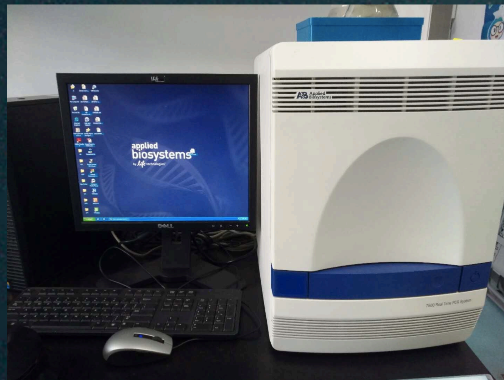
Template: cDNA (1pg -100ng),

Primer: control *vs* target (0.2 μM),

SYBR Green Mix: DNA polymerase, dNTP

02

仪器：

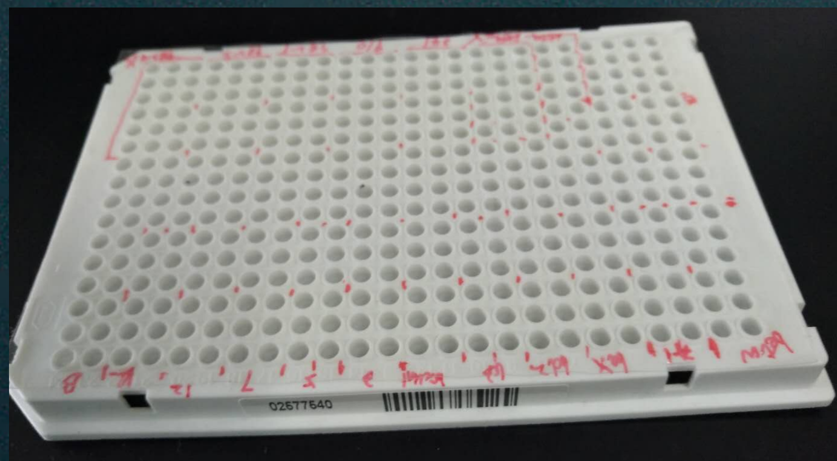



ABI 7500 Real-Time PCR Systems,

Roche LightCycler 480 II

# 实验操作

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Control<br>U 18S | Control<br>U 18S | Control<br>U 18S | Sample 1<br>U 18S | Sample 1<br>U 18S | Sample 1<br>U 18S | Sample 2<br>U 18S | Sample 2<br>U 18S | Sample 2<br>U 18S | N 18S | N 18S | N 18S |
| B | Control<br>U Plat1 | Control<br>U Plat1 | Control<br>U Plat1 | Sample 1<br>U Plat1 | Sample 1<br>U Plat1 | Sample 1<br>U Plat1 | Sample 2<br>U Plat1 | Sample 2<br>U Plat1 | Sample 2<br>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 μl 体系 | 20 μl 体系 |
| --- | --- | --- |
| 2 X PowerUp SYBR Green Master Mix | 5 μl | 10 μl |
| 正向引物和反向引物[1] | — | — |
| cDNA 模板和 $ddH_2O$[2] | — | — |
| 总体积 | 10 μl | 20 μl |

- 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 «

**Setup**

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

**Run**

**Analysis**

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: Untitled

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 $C_T$ ($\Delta\Delta C_T$)

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

Experiment Menu «

**Setup**

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

**Run**

**Analysis**

Experiment: Untitled    Type: Relative Standard Curve    Reagents: SYBR® Green 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: Untitled

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 (ΔΔCT)

Melt Curve | Genotyping | Presence/Absence

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?

TaqMan® Reagents | √ SYBR® Green Reagents | Other

The PCR reactions contain primers designed to amplify the target sequence and SYBR® Green I dye to detect double-stranded DNA.

☑ Include Melt Curve

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

√ Standard (~ 2 hours to complete a run)

Home | 20160524FLY.eds × | Untitled ×

# 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

**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 | |
| Target 2 | SYBR | None | |
| control | SYBR | None | |

**Define Samples**

Add New Sample | Add Saved Sample | Save Sample | Delete Sample

| Sample Name | Color |
|---|---|
| Sample 1 | |

# 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

**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 | |
| Target 2 | SYBR | None | |
| control | SYBR | None | |

**Define Samples**

Add New Sample | Add Saved Sample | Save Sample | Delete Sample

| Sample Name | Color |
|---|---|
| Sample 1 | |
| Sample 2 | |
| Sample 3 | |

# 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 | Qua |
|---|---|---|---|
| ☐ | Target 2 | U S N | |
| ☐ | control | U S N | |

Mixed U Unknown S Standard N Negative C

Define and Set Up Standards

**Assign sample(s) to the selected wells.**

| Assign | Sample |
|---|---|
| ☐ | Sample 2 |

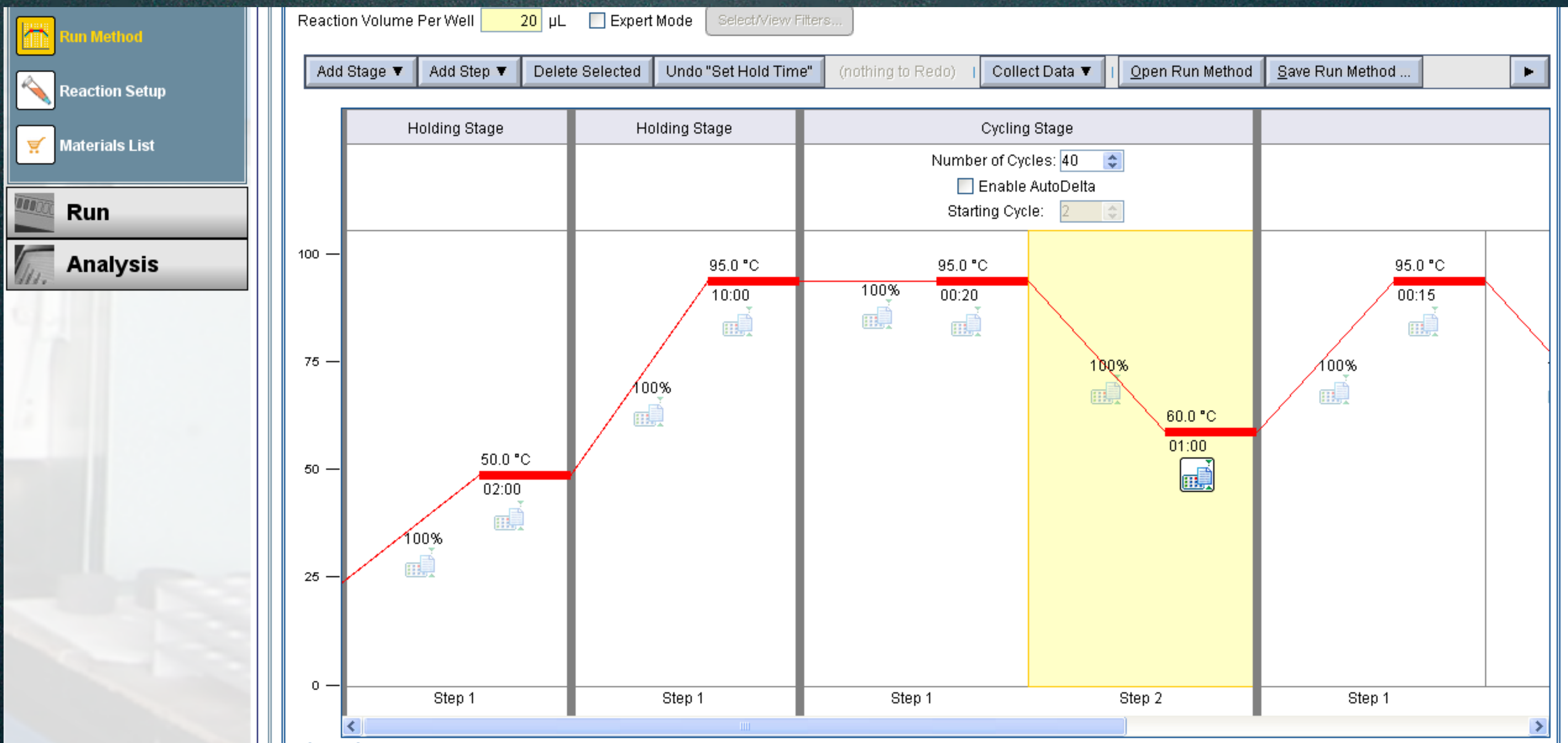
View Plate Layout | View Well Table

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

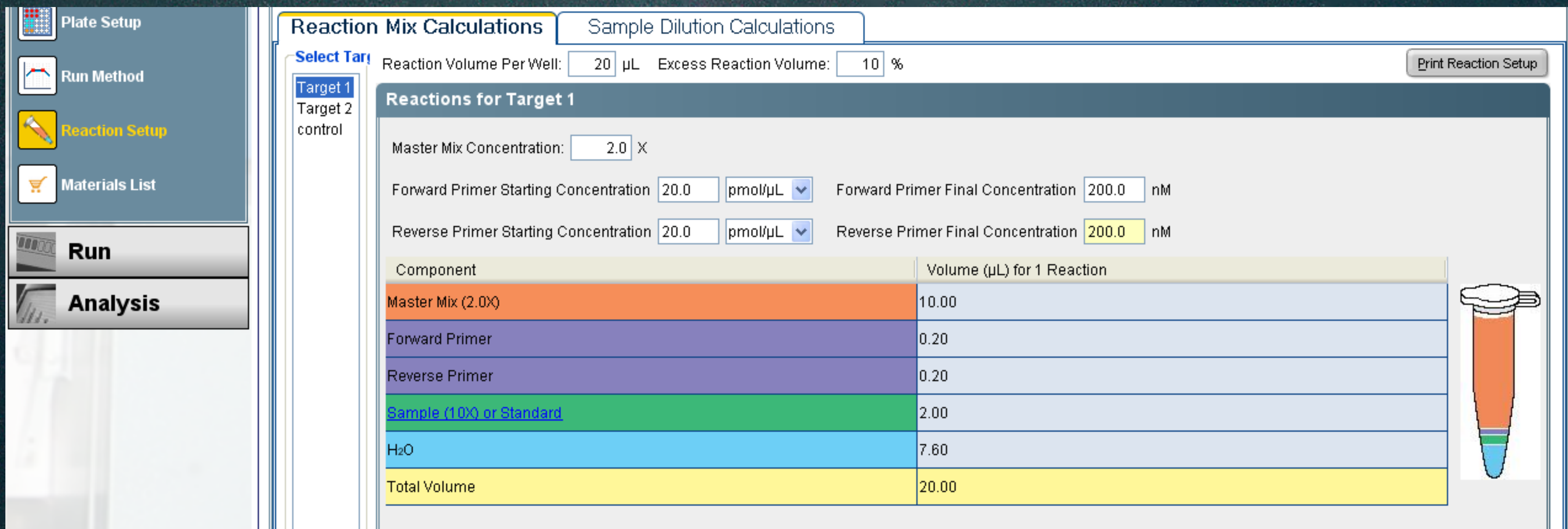
Show in Wells ▼ | View Legend

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Sample 1 U Tar... | Sample 1 U Tar... | Sample 1 U Tar... | Sample 1 U Tar... | Sample 1 U Tar... | Sample 1 U Tar... | Sample 1 U Tar... | Sample 1 U Tar... | Sample 1 U co... | Sample 1 U co... | Sample 1 U co... | Sample 1 U co... |
| B | Sample 2 U Tar... | Sample 2 U Tar... | Sample 2 U Tar... | Sample 2 U Tar... | Sample 2 U Tar... | Sample 2 U Tar... | Sample 2 U Tar... | Sample 2 U Tar... | Sample 2 U co... | Sample 2 U co... | Sample 2 U co... | Sample 2 U co... |
| C | Sample 3 U Tar... | Sample 3 U Tar... | Sample 3 U Tar... | Sample 3 U Tar... | Sample 3 U Tar... | Sample 3 U Tar... | Sample 3 U Tar... | Sample 3 U Tar... | Sample 3 U co... | Sample 3 U co... | Sample 3 U co... | Sample 3 U co... |

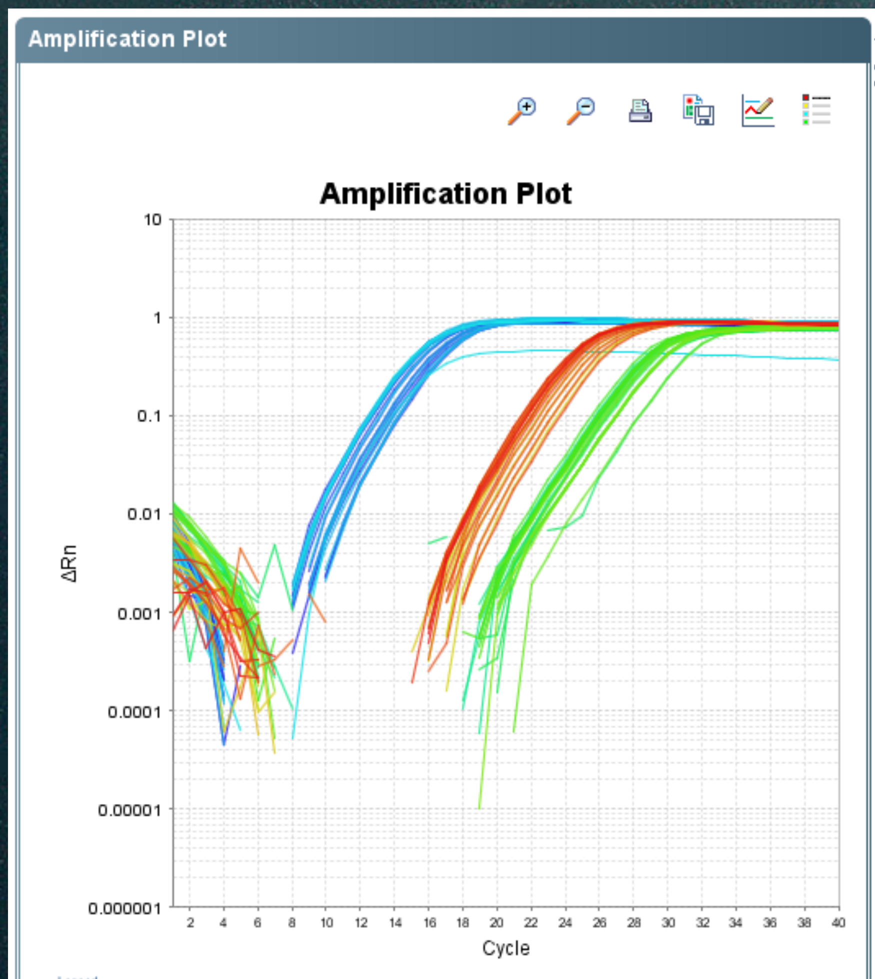
# 反应程序设置——反应方法



# 反应程序设置——反应方法

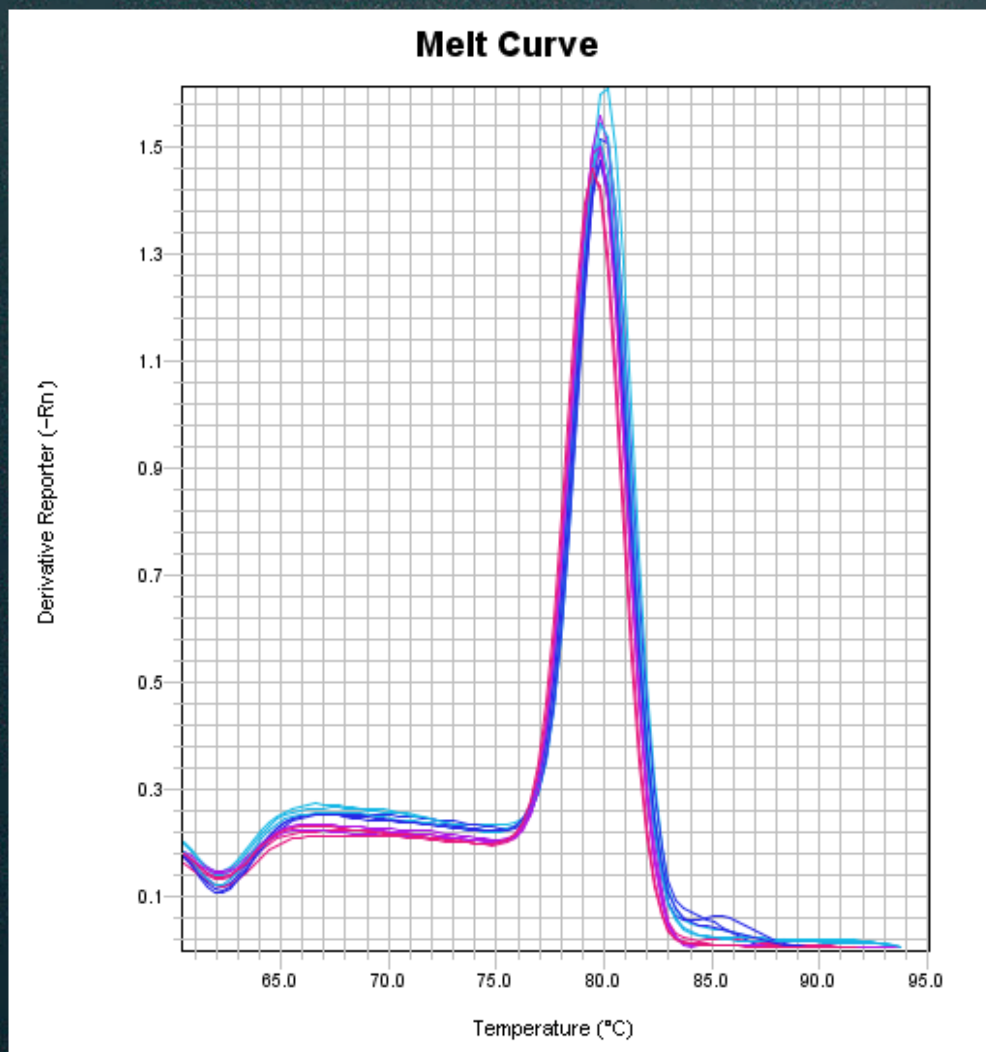


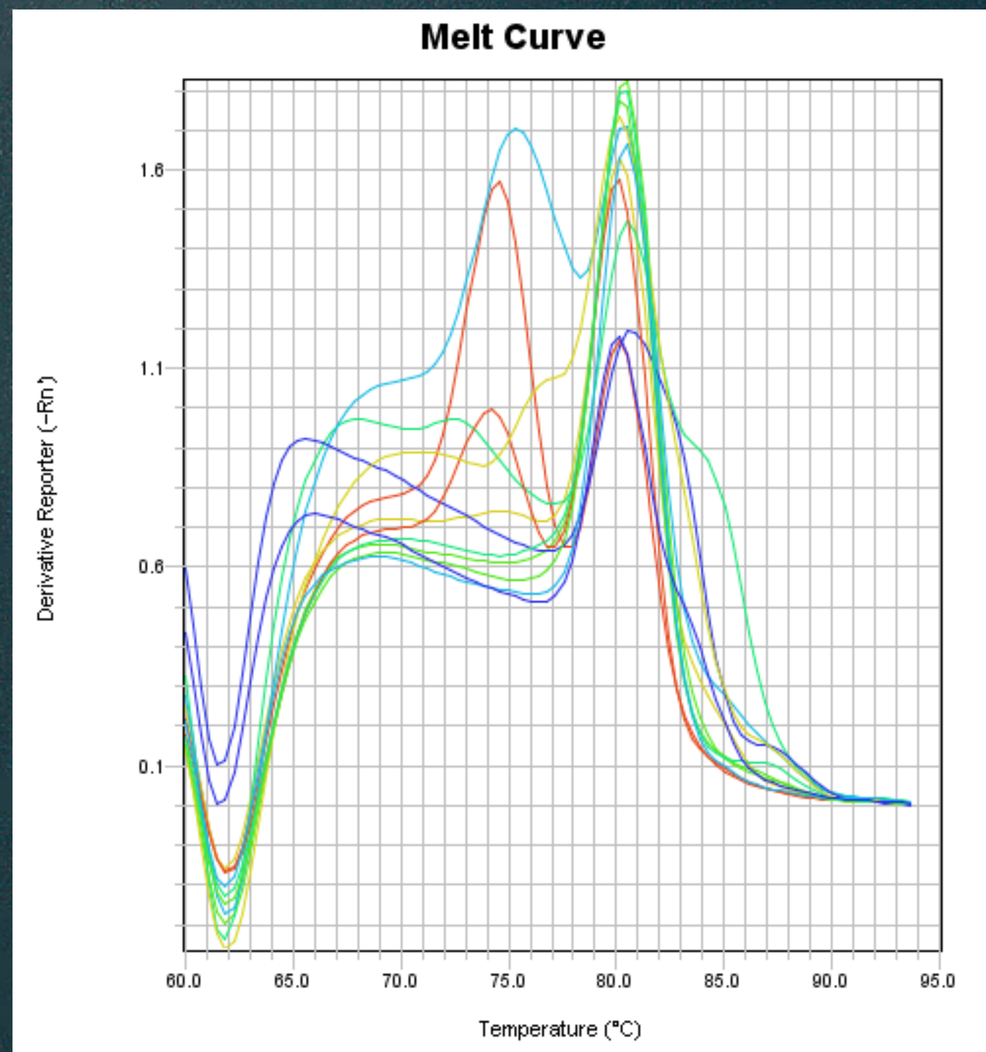
# 实验结果——扩增曲线



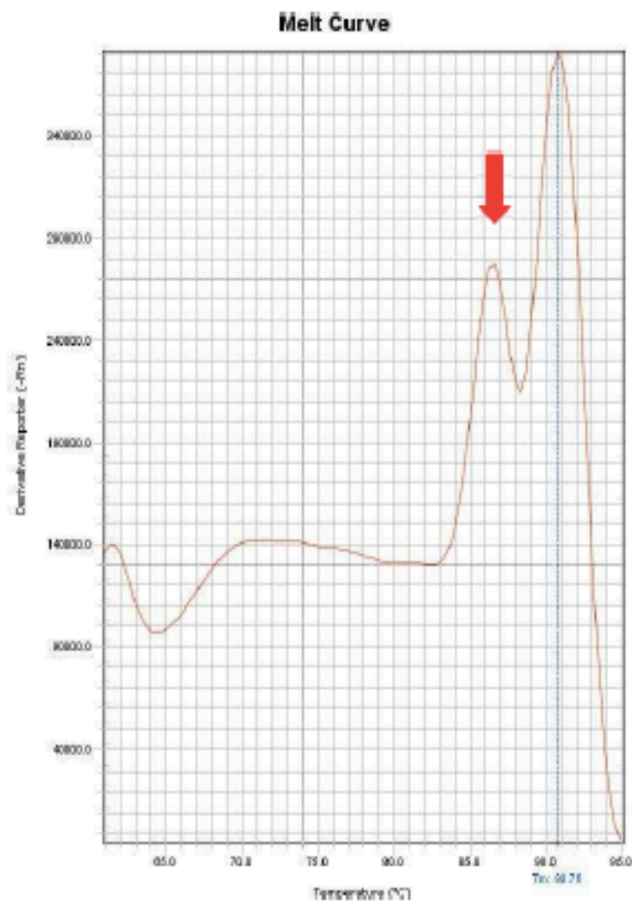

Sample A
Sample B
Log-linear phase
Threshold
Baseline
Fluorescence
0
0 10 20 30 40
Cycle number

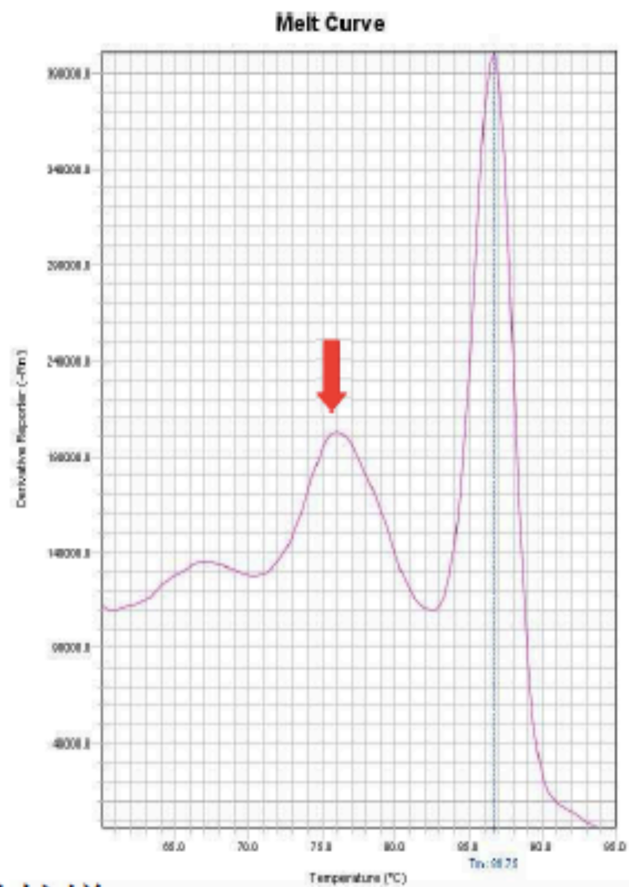
# 实验结果——溶解曲线



# 实验结果——溶解曲线




非特异扩增

引物二聚体

感谢观看