

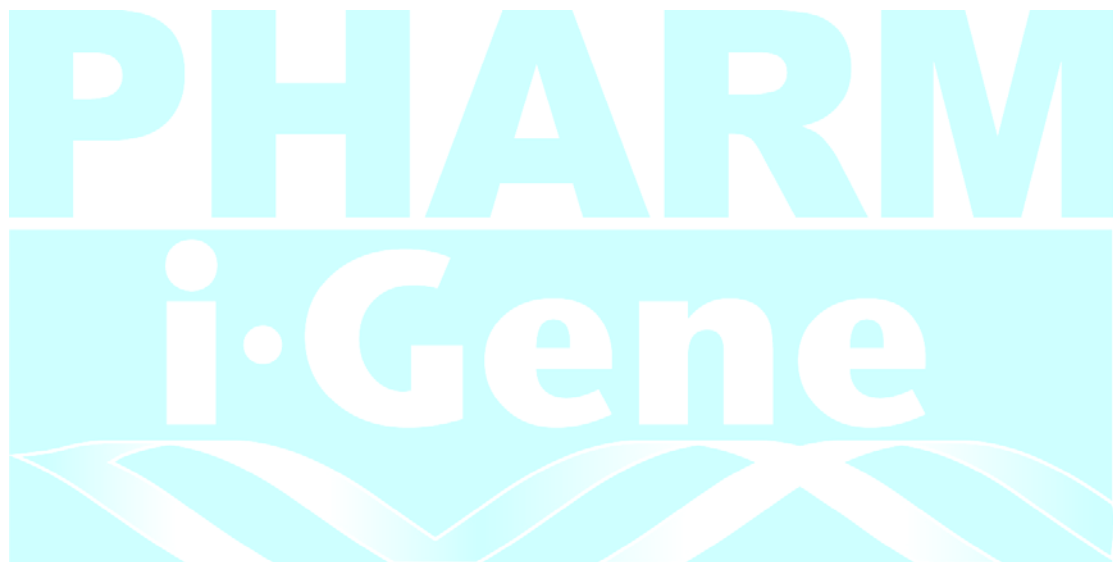
Instruction of

**PG1639 Detection Kit,
PG1075 Detection Kit,
PG0430 Detection Kit**

For

Applied Biosystems Real-Time PCR System

Software v2.0



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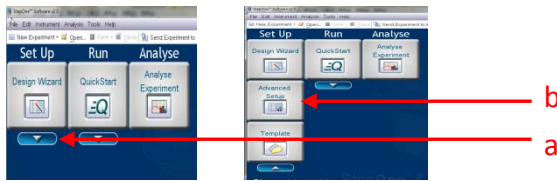
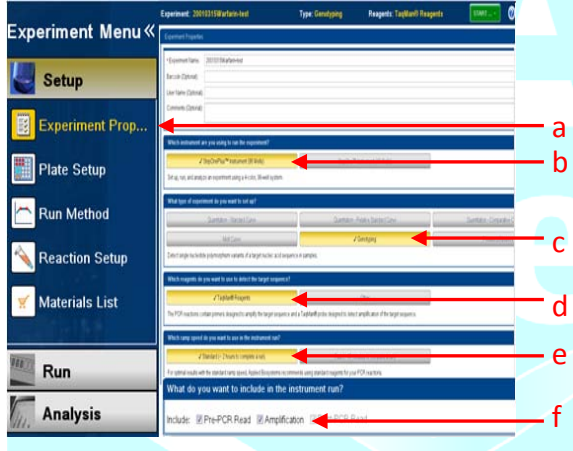
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1. Warfarin Template Setup

 <p style="text-align: center;">Fig1</p>	<p>1.1 Open a New File (Advanced Setup)</p> <p>1.1.1 Click software v2.0 icon (a) on the screen of notebook and it is connected to the ABI Real-Time PCR System.</p>
 <p>Fig2A Fig2B</p>	<p>1.1.2 The new screen is as Fig 2 A & B</p> <p>1.1.3. Click the Drop Down Menu (a) under the “Design Wizard” icon in the “Set Up” column</p> <p>1.1.4 The new screen is as Fig 2 B. Click the “Advanced Setup” icon (b).</p>
 <p>Fig3</p>	<p>1.2 Experiment profiles setup</p> <p>1.2.1 The new screen is as Fig 3</p> <p>1.2.2 Click the “Experiment Profiles”(a)</p> <p>1.2.3 Select the “Step One Plus™ Instrument 96 wells” or “Step One Plus™ Instrument 48 wells” icon (Optional)(b)</p> <p>1.2.4 Select “Genotyping” icon (c)</p> <p>1.2.5 Select “TaqMan® Reagents” icon(d)</p> <p>1.2.6 Select “Standard (~ 2 hours to complete a run)”(e)</p> <p>1.2.7 Mark the “<input type="checkbox"/> Pre-PCR” and “<input type="checkbox"/> Amplification” (f)</p>
	<p>1.3 Create Assay Menu</p> <p>1.3.1 Click the “Plate Setup”(a) on the left column and the new screen is as Fig 4</p> <p>1.3.2 Click “Create New..” button (b)</p> <p>1.3.3 Edit “SNP AssayName”(c) as <u>PG1639</u>(Kit’s Abbr. name)</p> <p>1.3.4 Edit “Allele 1 Name or Base(s)” as <u>G-FAM</u> ; Select “Reporter” as <u>FAM</u> and “Quencher” as <u>None</u> (d).</p> <p>1.3.5 Edit “ Allele 2 Name or Base(s)” as <u>A-VIC</u> ; Select “Reporter” as <u>VIC</u> and</p>

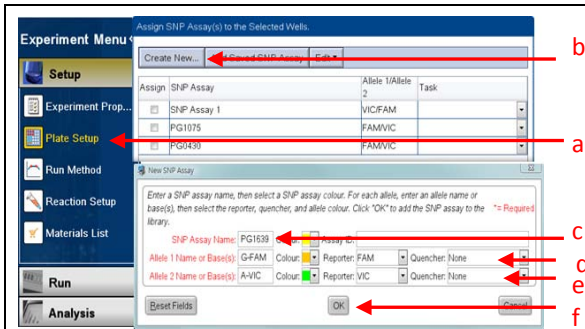


Fig 4

“Quencher” as None (e).

1.3.6 Click “OK”: to end this setting.(f)

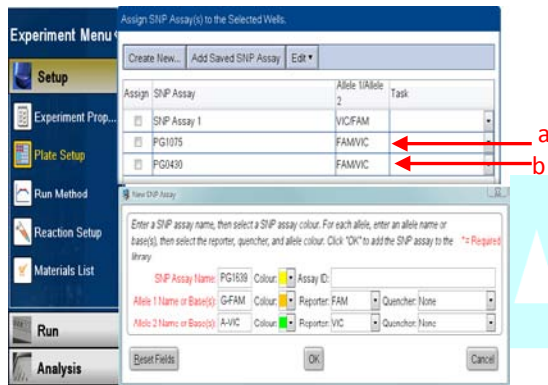


Fig 5

1.3.7 Repeat step 1.3.2 to 1.3.6 to edit other two settings for PG 1075 & PG 0430.(Fig5)

(ex.1: Edit SNP Name as PG1075. “Allele 1 Name or Base(s)” as 3-FAM ; Select “Reporter” as FAM and “Quencher” as None . Edit “ Allele 2 Name or Base(s)” as 1-VIC ; Select “Reporter” as VIC and “Quencher” as None (a).Click “OK” to end this setting.

(ex.2: Edit SNP Name as PG0430. “Allele 1 Name or Base(s)” as 2-FAM ; Select “Reporter” as FAM and “Quencher” as None . Edit “ Allele 2 Name or Base(s)” as 1-VIC ; Select “Reporter” as VIC and “Quencher” as None (b).Click “OK” to end this setting.

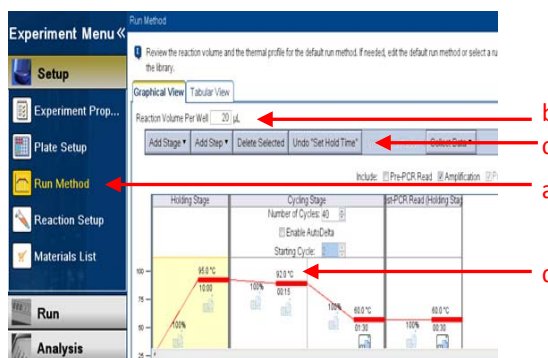


Fig 6

1.4 Edit Run Method

1.4.1 Click “Run Method” (a) on left column and the new screen is as Fig 6.

1.4.2 Edit Reaction Volume as 25ul (b).Follow the Package Inserts of each kits to edit PCR program by these buttons (c)

1.4.3 Edit individual setting (d) with below table. Please follow step 1.4.4.

	Time	Temp.	Cycle	Description
1. DNA polymerase hot-start step	10 min	95°C	1	Hot-start DNA polymerase is activated by this step.
2. Amplification cycle			38	
(i) Denaturation	15 secs	92°C		
(ii) Annealing /Extension	1 min 30 secs	60°C		Fluorescence signal is collected in this step in each cycle

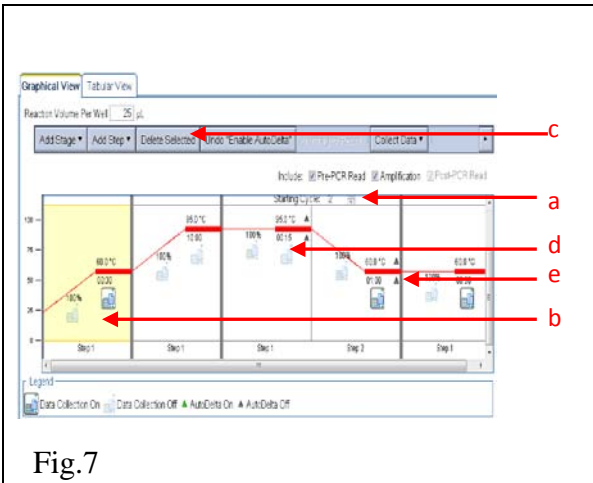


Fig.7

- 1.4.4 Add 38 cycles on(a)
- 1.4.5 Select the needless region (b) and click “Delete Selected(c) to delete this region.
- 1.4.6 Edit each setting time ((d) = 92°C) and time ((e) = 1:30).

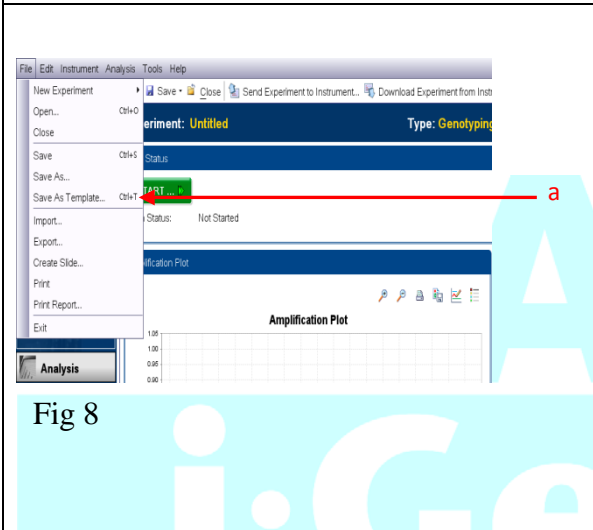
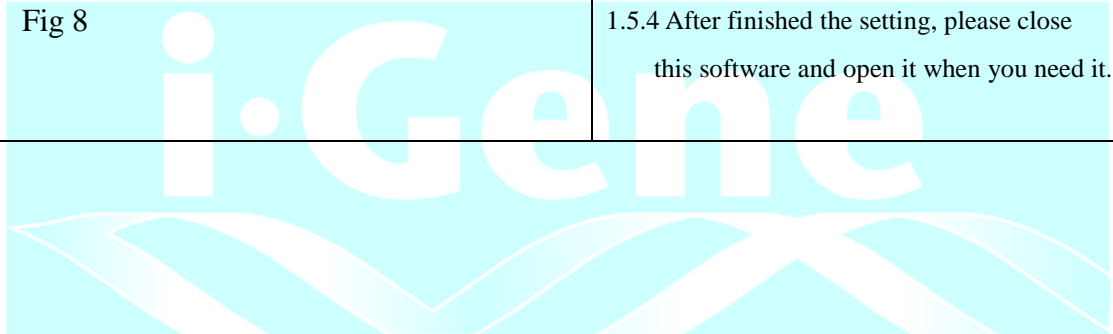
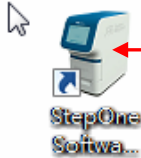
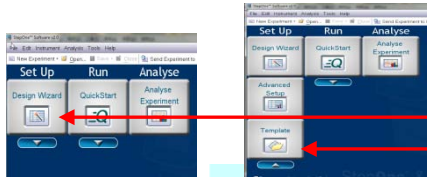
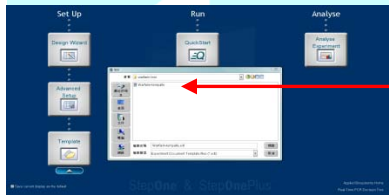
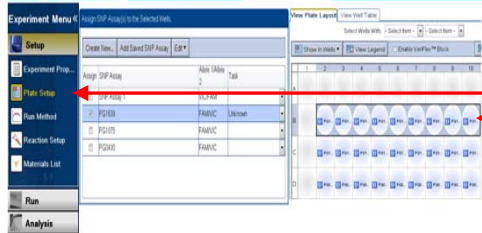


Fig 8

- 1.5 Save it as Template
- 1.5.1 After all the setting in Fig 7, click the “File” on the upper-left. The new column will be shown as Fig 8.
- 1.5.2 Click the “Save as Template...” which is under “File” (a)
- 1.5.3 File name could be named as “Warfarin Template.edt”.
- 1.5.4 After finished the setting, please close this software and open it when you need it.



2. Start a new experiment

 <p style="text-align: center;">F</p>	<p>2.1 Open Template File (Warfarin Template.edt)</p> <p>2.1.1 Click the StepOne software icon (a) on the screen of notebook and it is connected to the ABI StepOne (plus) Real-Time PCR System.</p>
 <p style="text-align: center;">Fig9A Fig9B</p>	<p>2.1.2 The new screen is as Fig 9 A &B.</p> <p>2.1.3. Click the Drop Down Menu (a) under the “Design Wizard” icon in the “Set Up” column.</p> <p>2.1.4 The new screen is as Fig 9 B. Click the “Template” icon (b).</p>
 <p style="text-align: center;">Fig10</p>	<p>2.1.5 The new dialog box will be shown as Fig 10. (a)</p> <p>2.1.6 Select “Warfarin Template.edt” file (a) and open it.</p>
 <p style="text-align: center;">Fig11</p>	<p>2.2 Edit Plate information</p> <p>2.2.1 The new screen will be shown as Fig 11.</p> <p>2.2.2 Select “Plate Setup” (a) which is under “Setup” column on the left of the screen.</p> <p>2.2.2 Draw specific reaction wells and mark its own SNP name. (b) (ex. PG1639 wells are selected from B2 to B7 as the same as samples location on the plate. Make a mark on the “assign” of PG1639.)(c)</p> <p>2.2.3 follow the 2.2.2 to set the PG 1075 & PG 0430 wells’s relative information on the same screen.</p>
	<p>2.3 Define NTC(no template control) wells (Fig 12)</p> <p>2.3.1 Select the NTC well of each kit, and mark it with “Negative Control” (b) of</p>

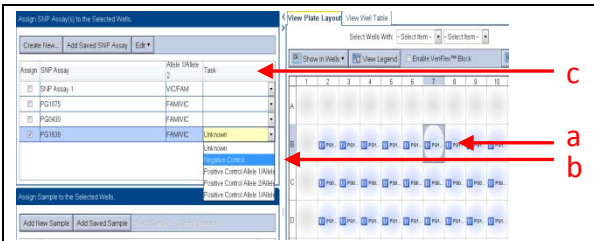


Fig12

each SNP name which is under Task column (c). (ex. PG1639 negative control is select as B7 well. . Make a mark on the “assign” of PG1639 and select “negative control” on the “Task” column.)(c)

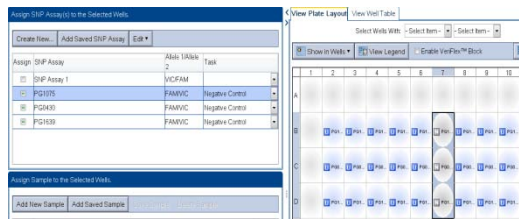


Fig.13

2.3.2 Select other NTC wells of different kits and mark them with each “Negative Control” task. Fig13
(ex. follow the 2.2.4 to set the PG 1075 & PG 0430 negative control well relative information on the same screen.)

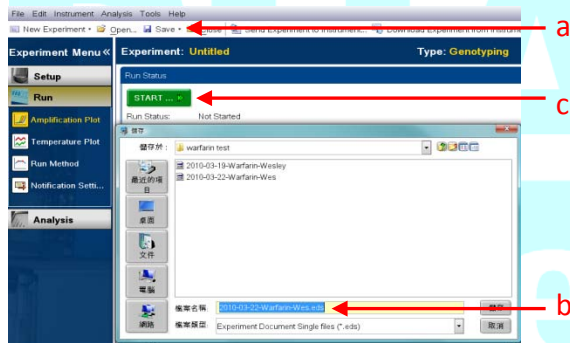


Fig.14

2.3.3 Save as new experiment file(a) Fig.14

(Ps: usual we put “year month date Warfarin” ex.20010315-Warfarin)(b)

2.3.4 Check all the program setting on the “Run Method” and “Plate Setup” on the “Set Up” column on the left of screen again.

2.3.5 Click the “RUN” icon and select “STARTS...” to start this run. (c)

3. Data Analysis

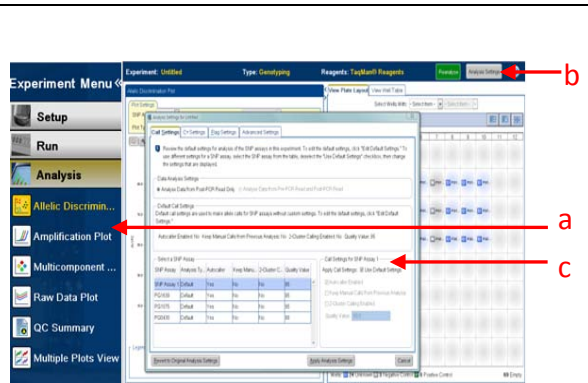


Fig.15

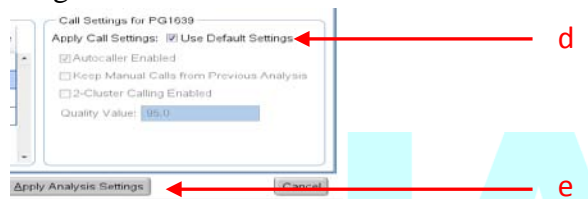


Fig.16

3.1 Allelic Discrimination Analysis

3.1.1 Click the “Analysis” Icon on the left column on the screen and select” Allelic Discrimination “which Is under “Analysis”(a) Fig.15

3.1.2 Click “Analysis Settings” button (b)

3.1.3 Mark “ Use Default setting”(c)(d)

3.1.6 Click “Apply Analysis Settings” (e)

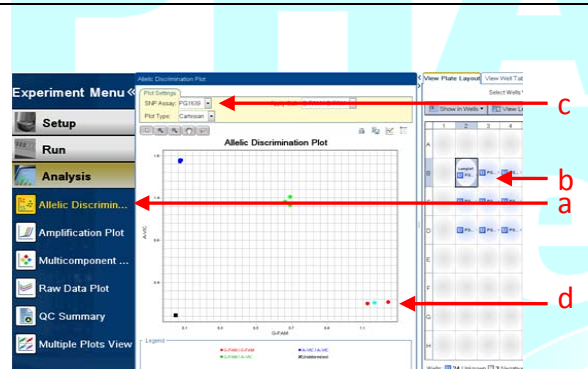


Fig.17

Under “Allelic Discrimination” of “Analysis” column. (a) Fig.17

3.1.7 Select a well (sample) (b) and choose its “SNP Assay” as its original setting. (c). (ex. PG 1639 well choice “SNP Assay” as PG 1639. A sample relative light spot (d) will appear on Discrimination Plot.

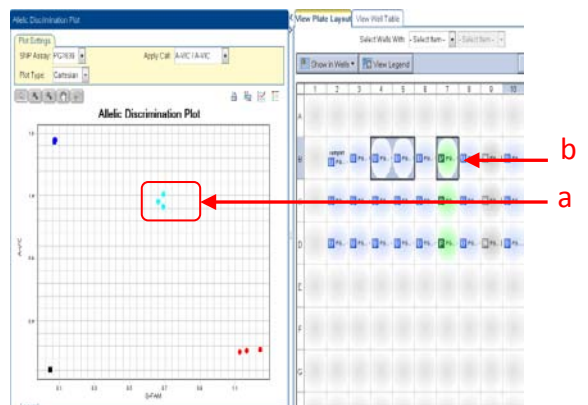


Fig.18

3.1.8 You can also select a set of spots (a) on Allelic Discrimination Plot; the relative wells (b) will be mark in Fig.18.

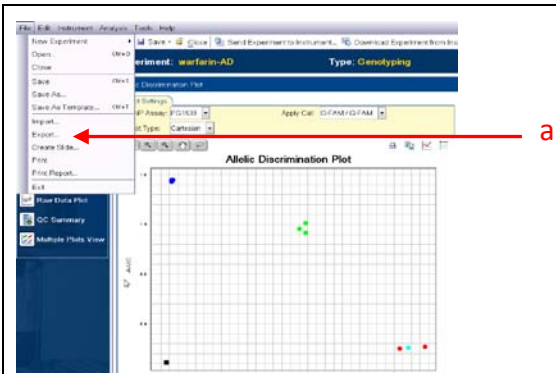


Fig. 19

3.2 Export data as Excel

3.2.1 Click the “Export...” which is under “File” (a)

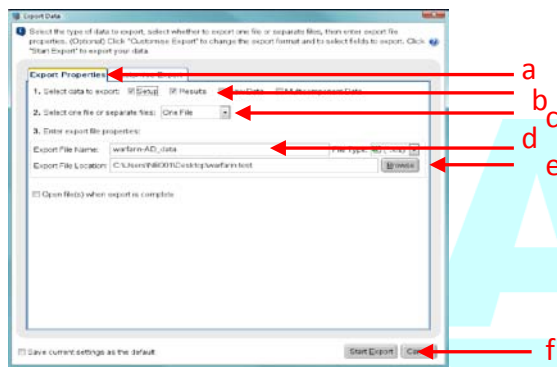


Fig. 20

3.2.2 Select “Export Properties”(a) Fig.20

3.2.3 Mark “ Results”(b)

3.2.4 Select “One file”(c)

3.2.5 Edit” Export File Name” as “year month date Warfarin”
ex.20010315-Warfarin) (d)

3.2.6 Click the “Browse” icon of the “Export File Location” to save your data .(e)

3.2.7 Click “Start Export” to export data. (f)



Fig. 21

3.2.8 Click”Close Export Tool”. (Fig.21)

Well	Sample Name	SNP Assay Name	Task	Call
B2		PG1639	UNKNOWN	Homozygous 1/1
B3		PG1639	UNKNOWN	Homozygous 2/2
B4		PG1639	UNKNOWN	Heterozygous 1/2
B5		PG1639	UNKNOWN	Heterozygous 1/2
B6		PG1639	UNKNOWN	Homozygous 1/1
B7		PG1639	UNKNOWN	Heterozygous 1/2
B8		PG1639	UNKNOWN	Homozygous 2/2
B9		PG1639	NTC	Negative Control (NC)
B10		PG1639	UNKNOWN	Homozygous 1/1
B11		PG1639	UNKNOWN	Homozygous 2/2
B12				
C1				
C2		PG1075	UNKNOWN	Heterozygous 1/2
C3		PG1075	UNKNOWN	Homozygous 2/2
C4		PG1075	UNKNOWN	Homozygous 1/1
C5		PG1075	UNKNOWN	Homozygous 2/2
C6		PG1075	UNKNOWN	Homozygous 2/2
C7		PG1075	UNKNOWN	Heterozygous 1/2
C8		PG1075	UNKNOWN	Homozygous 1/1
C9		PG1075	NTC	Negative Control (NC)
C10		PG1075	UNKNOWN	Heterozygous 1/2
C11		PG1075	UNKNOWN	Homozygous 2/2

Fig. 22

3.3 Data Interpretation

3.3.1 Open the export data, Excell data, and you can organize the raw data through interpret “Call” column data. (Fig.22)

3.3.2 Interpretation table

Alleles	PG1639	PG1075	PG0430	Labeled
1/1	G/G	C/C (*3/*3)	T/T (*2/*2)	FAM
1/2	G/A	C/A (*3/*1)	T/C (*2/*1)	FAM/VIC
2/2	A/A	A/A (*1/*1)	C/C (*1/*1)	VIC

4. When the experiment is finished, please turn off machine, software and computer.