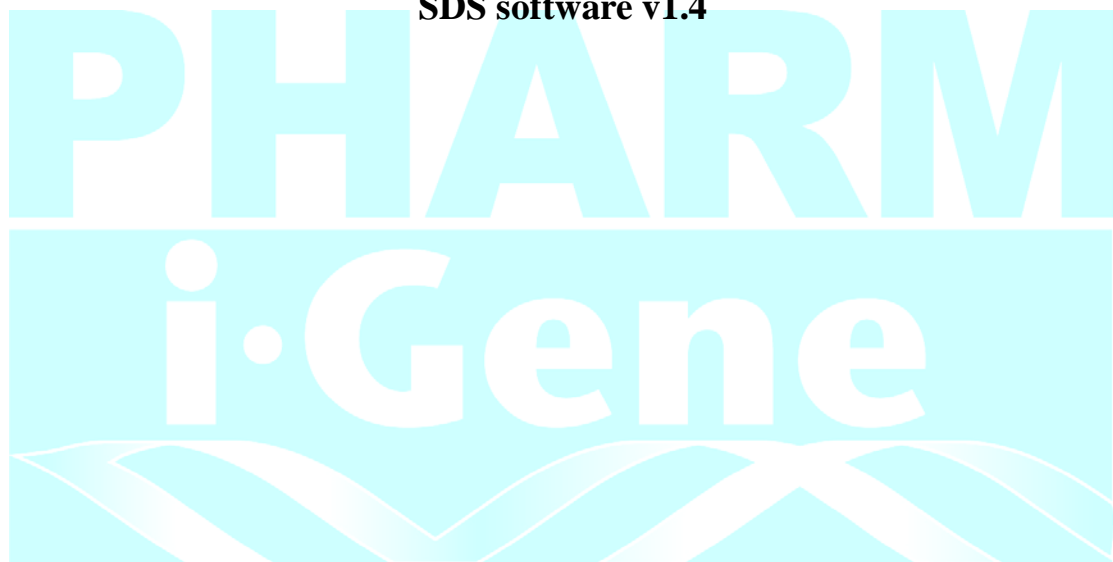


Instruction of

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

Applied Biosystems 7500 Real-Time PCR System

SDS software v1.4



Contents

Warfarin Template files Setup(AD(.sdt) 、AQ(.sdt))

1 Warfarin template-AD file (*.sdt) Setup

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- 1.2 Define AD Document
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- 1.4 Edit “Markers” Information
- 1.5 Edit AD Thermal Cycler Protocol
- 1.6 Save as Template File (*.sdt)

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- 2.1 Define AQ Document
- 2.2 Select Detectors
- 2.3 Edit AQ Thermal Cycler Protocol
- 2.4 Save as a Template File (*.sdt)

New Experiment Files Setup (AD(*.sds) 、AQ(*.sds))

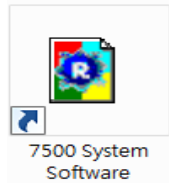
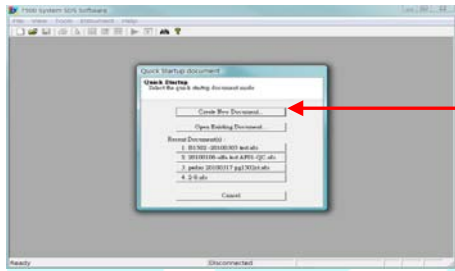
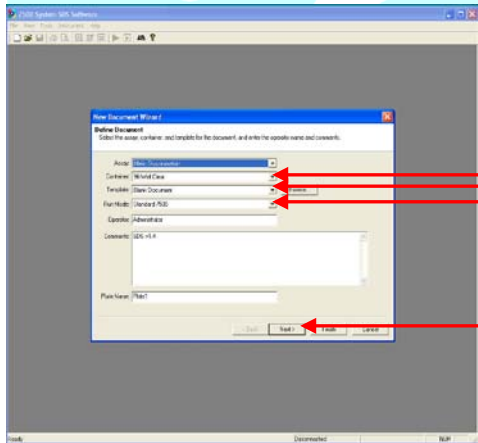
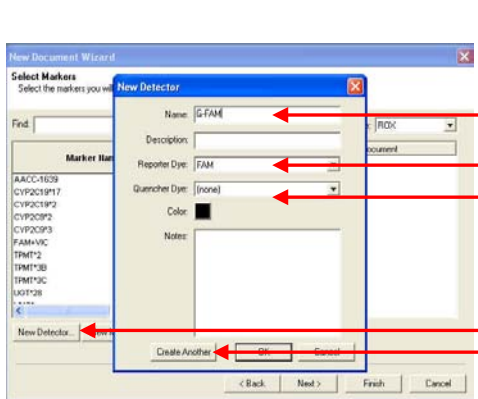
3 Run Warfarin files

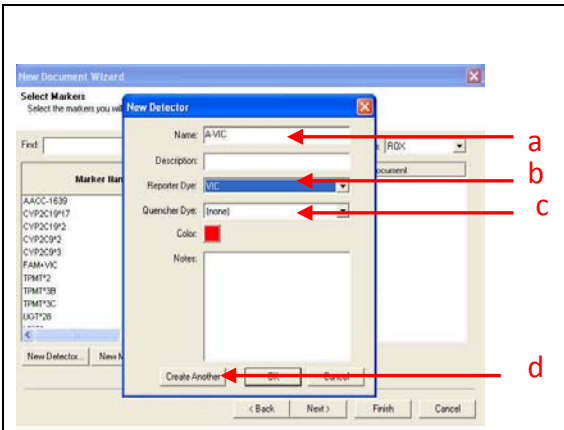
- 3.1 Run Warfarin – AD file (Pre-Read)
- 3.2 Run Warfarin –AQ file (Amplification)
- 3.3 Run Warfarin – AD file (Post-Read)

Data Analysis

- 3.4 Data Analysis in AD File

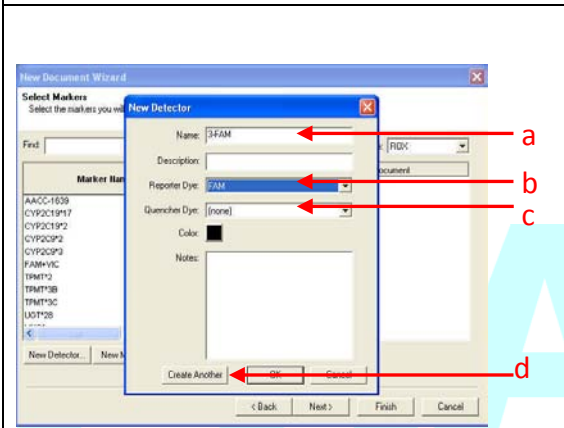
Warfarin Template files Setup (AD(.sdt) 、AQ(.sdt) files)

 <p>7500 System Software</p>	<p>For the first time setup, we suggest that you can edit two template files, one is Warfarin template-AD file and another is Warfarin template-AQ file. After you finished this template setup procedure, you can use these two files directly without resetting again.</p> <p>1. Warfarin template-AD file Setup</p> <p>1.1 Open a new document</p> <p>1.1.1 Open ABI 7500 System Software</p>
	<p>1.1.2 Select “Create New Document.” (a)</p>
	<p>Edit Warfarin template AD file</p> <p>1.2 Define AD Document</p> <p>1.2.1 Assay: “Allelic Discrimination” (a)</p> <p>1.2.2 Container: “96- Well Clear”(b)</p> <p>1.2.3 Template: “Blank Document”</p> <p>1.2.4 Run Mode: “Standard 7500”(c)</p> <p>1.2.5 Press ”Next”(d)</p>
	<p>1.3 Detectors Setup</p> <p>1.3.1 Press “New Detector” (a), and “New Document Wizard” window will appear.</p> <p>1.3.2 Edit Name : G-FAM(b), Reporter Dye : FAM(c), Quencher Dye : (none)(d)</p> <p>1.3.3 Press “Create Another..” (e) (Create another Detector)</p>



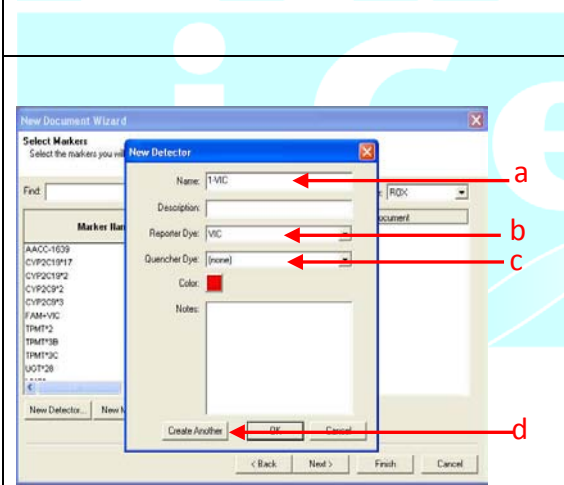
1.3.4 Edit Name : **A-VIC(a)** ; Reporter Dye : **VIC(b)** ; Quencher Dye : **(none)(c)**. (The above two Detectors are prepared for PG1639 Marker.)

1.3.5 Press **“Create Another..”(d)** (Create another Detector)



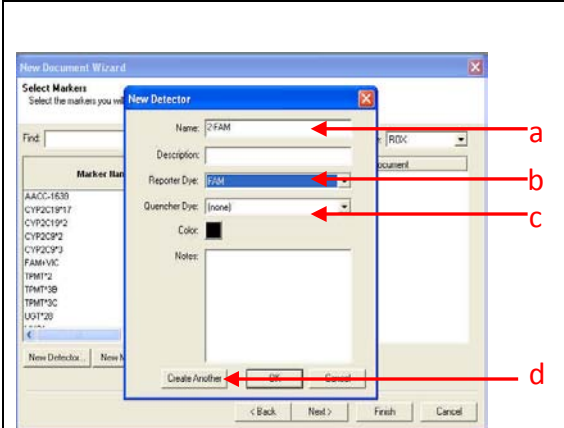
1.3.6 Edit Name : **3-FAM(a)** ; Reporter Dye **(b)** : **FAM** ; Quencher Dye : **(none) (c)**

1.3.7 Press **”Create Another”(d)** (Create another Detector)



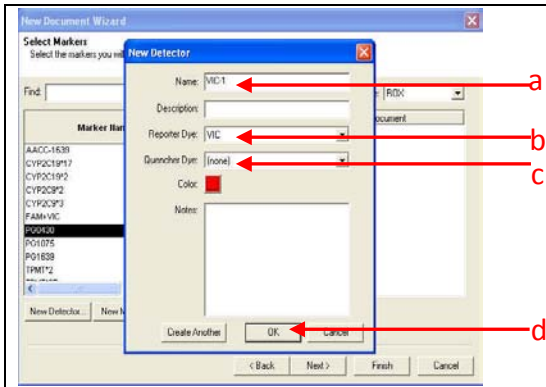
1.3.8 Edit Name: **1-VIC (a)** ; Reporter Dye : **VIC(b)** ; Quencher Dye : **(none)(c)** (The above two Detectors are prepared for PG1075 Marker.)

1.3.9 Press **“Create Another.”(d)** (Create another Detector)

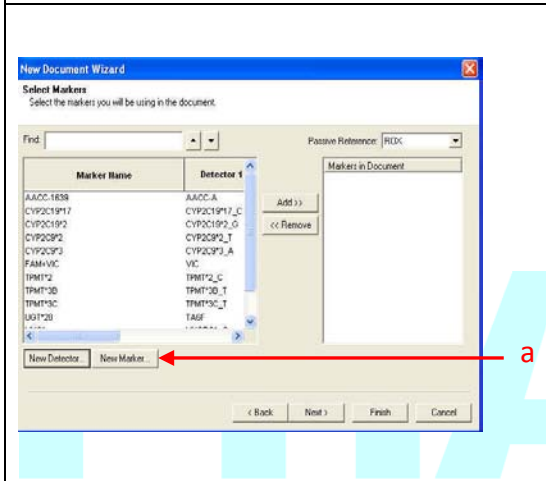


1.3.10 Edit Name : **2-FAM(a)** ; Reporter Dye : **FAM(b)** ; Quencher Dye : **(none)(c)**

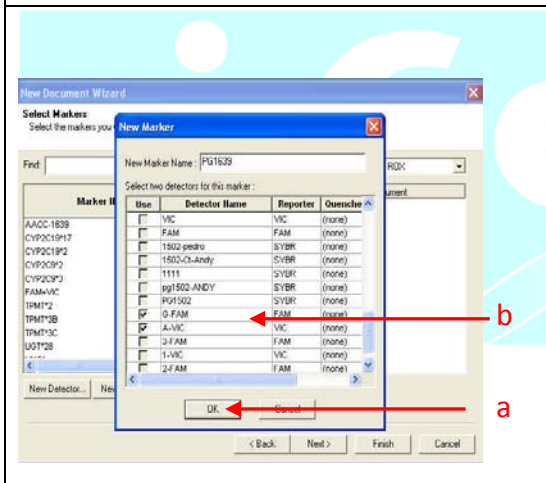
1.3.11 Press **“Create Another”(d)** (Create another Detector)



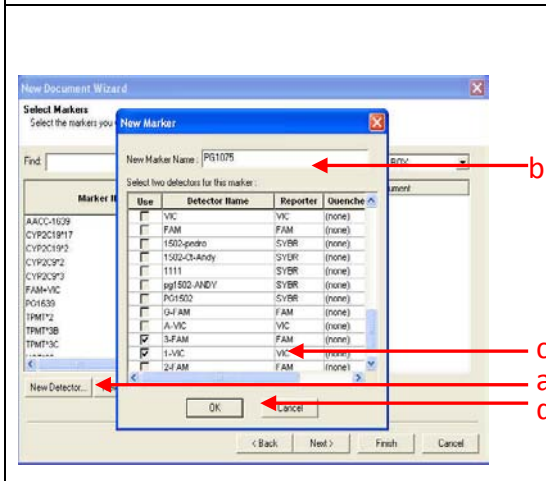
1.3.12 Edit Name : **VIC-1(a)**(cannot key in same “1-VIC” name) ;
Reporter Dye : **VIC(b)** ;
Quencher Dye : **(none)(c)** ,
(The above two Detectors are prepared for PG0430 Marker)
1.3.13 Press ”OK”(d)



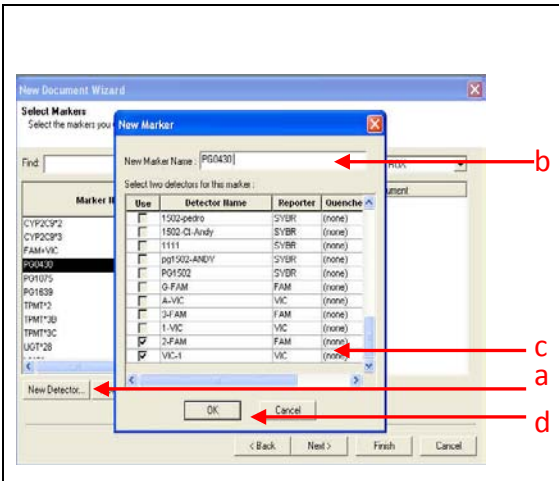
1.4 Edit “Markers” Information
1.4.1 Press “New Marker”(a)..., a setting window of New Marker will appear



1.4.2 Edit New Marker Name :
PG1639
1.4.3 Select two detectors for this marker: select **G-FAM** and **A-VIC**.
1.4.5 Press ”OK”



1.4.6 Press ”New Marker...,”(a)
1.4.7 Edit New Marker Name :
PG1075(b)
1.4.8 Select two detectors for this marker : select **3-FAM** and **1-VIC (c)**
1.4.9 Press ”OK”(d)

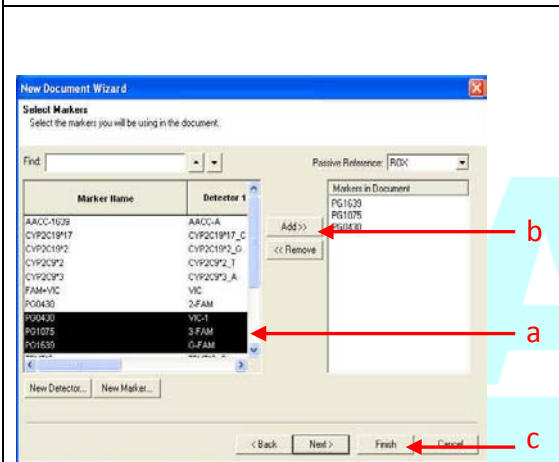


1.4.10 Press "New Marker..." (a)

1.4.11 Edit New Marker Name :
PG0430 (b)

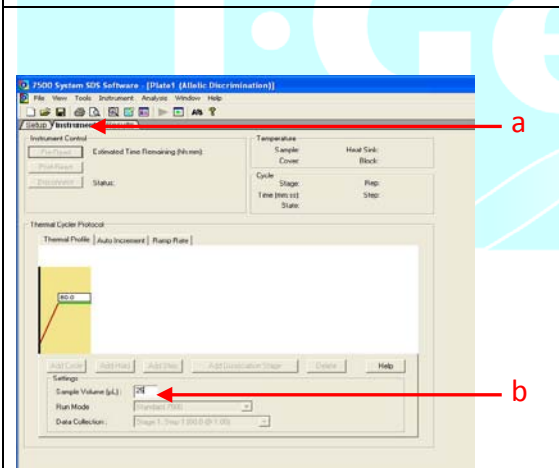
1.4.12 Select two detectors for this
marker : **2-FAM** and **VIC-1**(c)

1.4.13 Press "OK"(d)



1.4.14 Select 3 markers(a) and
press "Add"(b) button to add
these makers into "Markers in
Document"

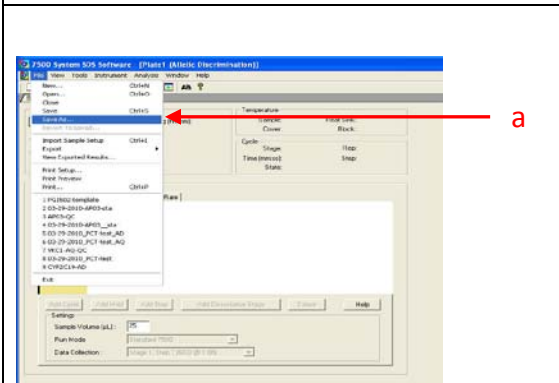
1.4.15 Press "Finish"(c) to end this
setting



1.5 Edit AD Thermal Cycler Protocol

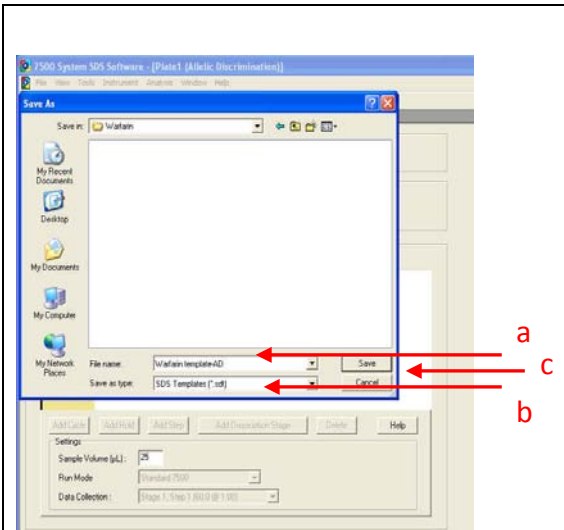
1.5.1 Select "Instrument" tab (a)

1.5.2 Edit Sample Volume (uL) : **25** (b)

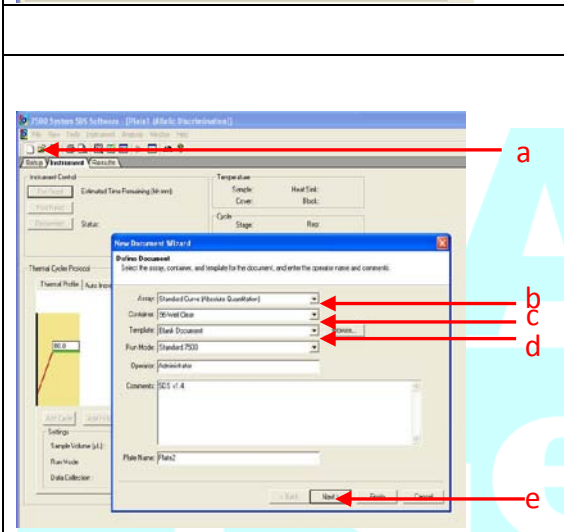


1.6 Save as Template File(*.sdt)

1.6.1 Select "File""Save as..."(a)



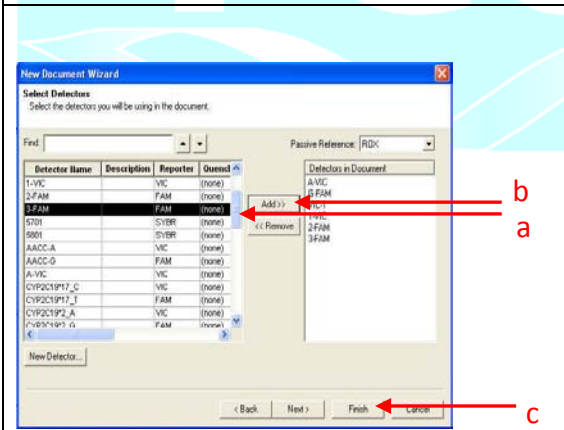
- 1.6.2 Edit File name as “Warfarin-
template-AD”(a)
- 1.6.3 Save type as “SDS Templates
(* .sdt)”(b)
- 1.6.4 Press “Save”(c)



2 · Warfarin template-AQ file setup

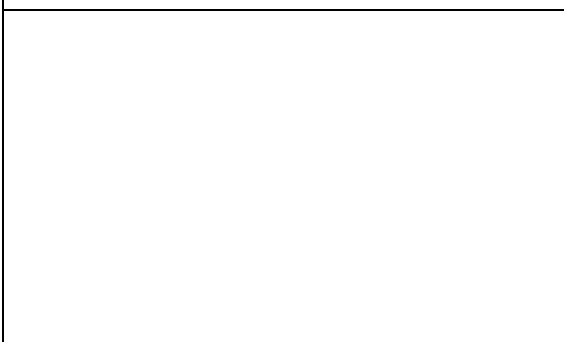
2.1 Define AQ Document

- 2.1.1 Open a new document(a)
- 2.1.2 Assay : **Standard Curve
(Absolute Quantitation)** (b)
- 2.1.3 Container: **96- Well Clear**(c)
- 2.1.4 Template: **Blank Document**
- 2.1.5 Run Mode : **Standard 7500**(d)
- 2.1.6 Press ”Next” (e)



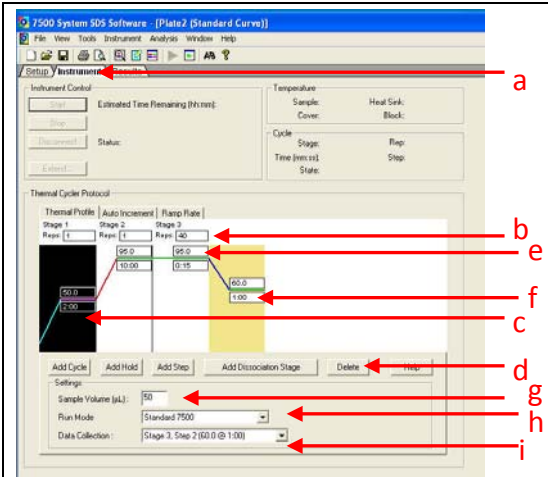
2.2 Select Detectors

- 2.2.1 Select **A-VIC, G-FAM, 3-FAM,
1-VIC, 2-FAM, VIC-1**(a)
- 2.2.2 Press “Add>>” to add Detectors
into “**Markers in Document**”
window(b)
- 2.2.3 Press ”Finish”(c)

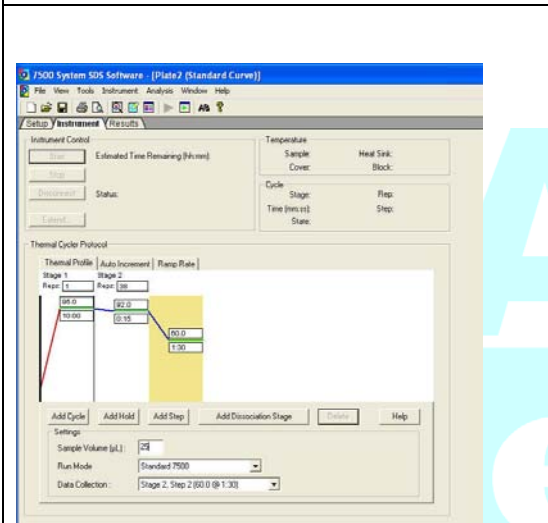


2.3 Edit AQ Thermal Cycler Protocol

- 2.3.1 Select ”**Instrument**” tab(a)
- 2.3.2 Edit Reps : **38**(b)
- 2.3.3 Select **Stage1(50°C)** (c) and
press ”**Delete**” to erase this
step(d)

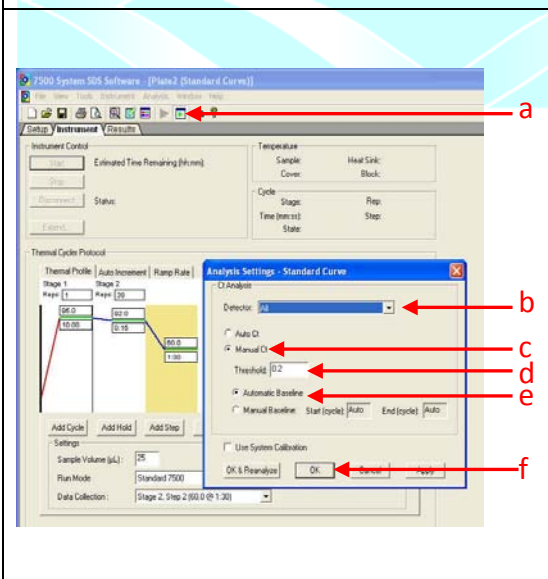


- 2.3.4 Edit this step as **92°C**(e)
- 2.3.5 Edit time as **1:30**(f) at this step
- 2.3.6 Sample Volume (uL) : **25uL**(g) ;
- Run Mode : **Standard 7500**(h) ;
- Data Collection : **Stage2, Step2(60.0@1:00)**(i)



Please follow protocol of package insert described

	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



2.3.7 Select “**Analysis Settings**”

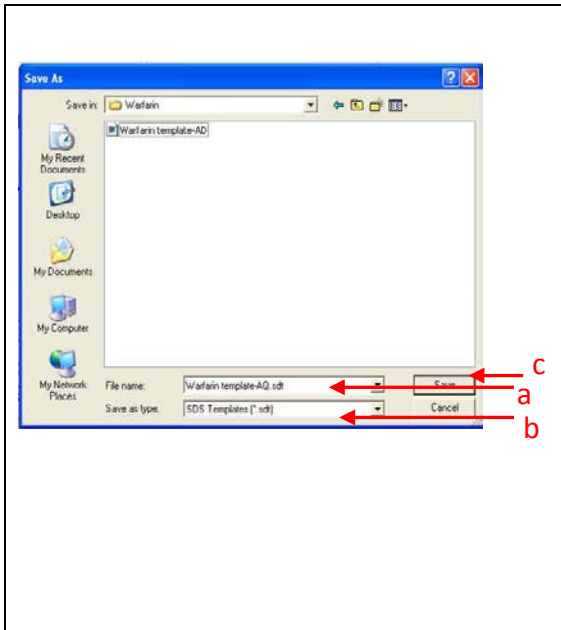
(a). A window of “**Analysis Setting -Standard Curve**” window will appear.

- 2.3.8 Detector : **All**(b)
- 2.3.9 Select **Manual Ct**(c).

Threshold : 0.2(d)

2.3.10 select “**Automatic Baseline**”(e)

2.3.11 Press ”**OK**”(f)



2.4 Save asTemplate File (*.sdt)

2.4.1 Select "File" and select "Save as..."

2.4.2 Edit File name as "Warfarin-template-AQ"(a)


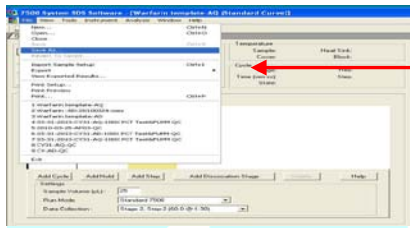
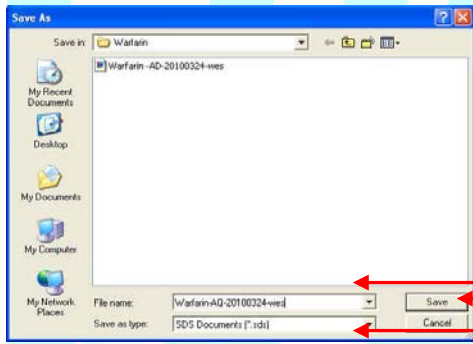
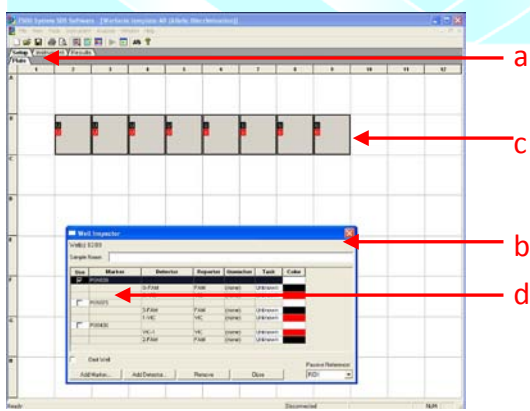
2.4.3 Save type as "SDS Templates (*.sdt)"(b)

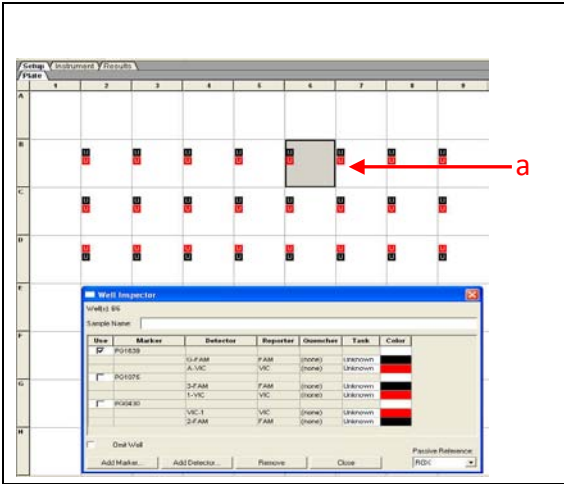
2.4.4 Press "Save"(c)

2.4.5 Close all file

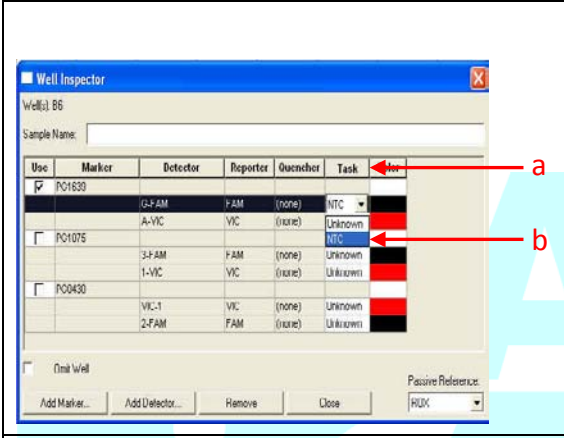
PHARM
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New Experiment Files Setup (AD(*.sds) \ AQ(*.sds))

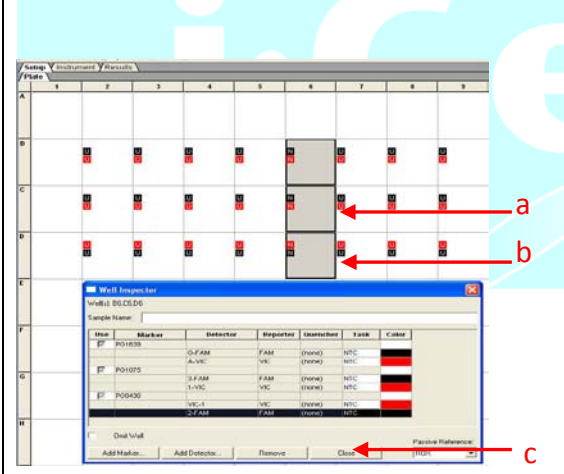
 <p>Warfarin template-AD</p>	<p>(You have to progress on three steps for each new warfarin experiment without resetting template files. The first is AD file (Allelic Discrimination)- Pre-read, the Second is AQ(Absolute Quantitation) – Amplification, and the third is AD(Allelic Discrimination)- Post-read.</p> <p>3. Run Warfarin files</p>
 <p>a</p>	<p>3.1 Run Warfarin- AD file (PreRead)</p> <p>3.1.1 Open Warfarin template- AD file</p> <p>3.1.2 Save as a new file</p> <p>3.1.3 Select “File” and select “Save as..”</p> <p>(a)</p>
 <p>a b c</p>	<p>3.1.4 Edit File Name : Warfarin-AD-20100324-wes(a)(Warfarin-AD-year, month, day-Operator)</p> <p>3.1.5 Save type : SDS Documents (*.sds) (b)</p> <p>3.1.6 Press “Save”(c)</p>
 <p>a b c d</p>	<p>Edit Plate Information</p> <p>3.1.7 Select ”Setup” tab and ” Plate”(a) sub tab</p> <p>3.1.8 A new ”Well Inspector” window will appear when you double click by mouse on any position of 96 wells window(b)</p> <p>3.1.9 Select particular Wells (Ex. PG1639 on B2~B9)(c)</p> <p>3.1.10 Select Marker : PG1639 (d)</p> <p>3.1.11 Repeat steps 3.1.8 to 3.1.9 and Continue connecting between Wells and PG1075, PG0430 Marker)</p>



3.1.12 Select each **NTC well** (Ex. B6 Well is the location of PG1639's NTC)(a)

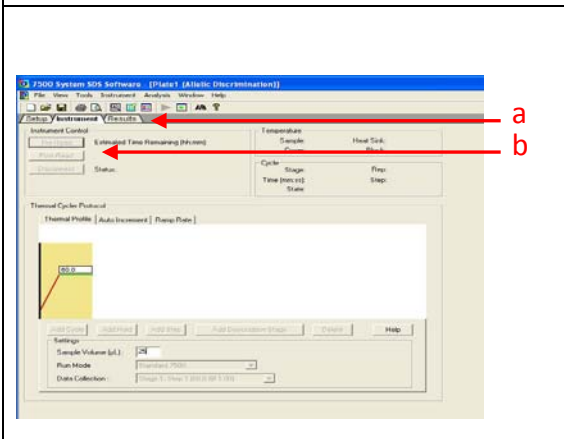


3.1.13 Click **“unknown”** which is under **”Task”** (a) column of **PG1639 marker** and select **”NTC”**(b)



3.1.14 Continue marking each NTC Well with its own marker like steps 3.1.12 to 3.1.13 (Ex. C6 Well(a) is PG1075's NTC and D6 Well(b) is PG0430's NTC) .

3.1.15 Select **“Close”** (c)



Run” Pre-Read” (put plate into machine)

3.1.16 Select **“Instrument”**(a)

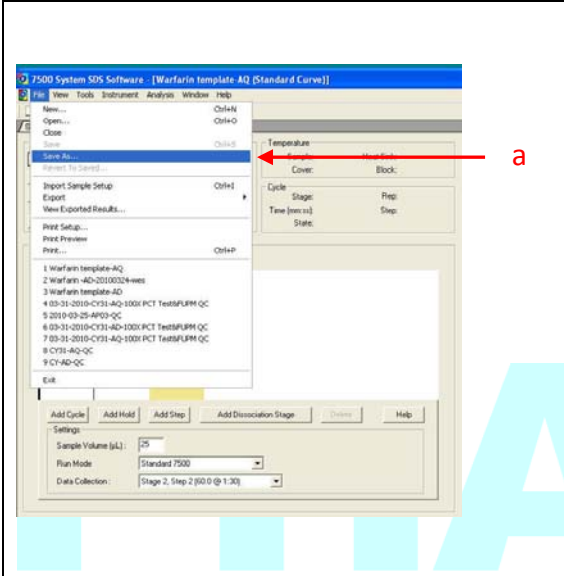
3.1.17 Press **“PreRead”** button which is under **Instrument Control** region (b) (The run needs about **1 min**)

3.1.18 If you cannot press Pre-Read button, please save this file again and reopen it.

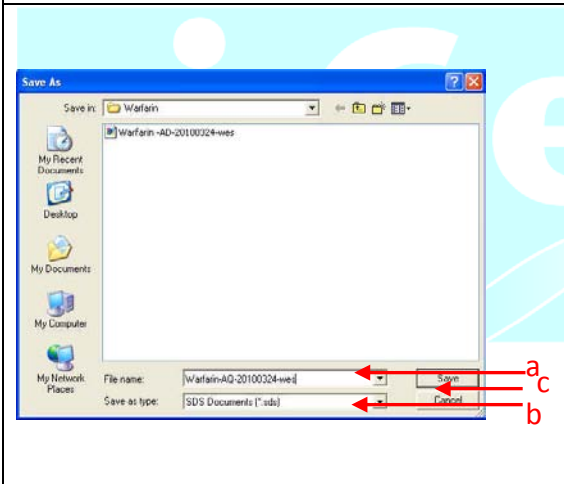
3.1.19 After the run is finished, please close the file.



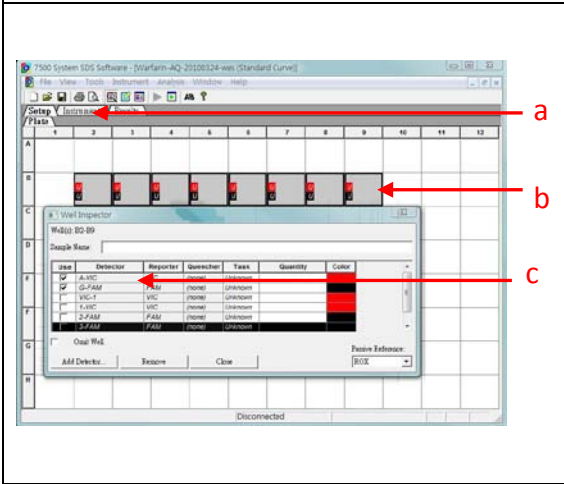
3.2 Run Warfarin AQ file
3.2.1 Open Warfarin template –AQ file



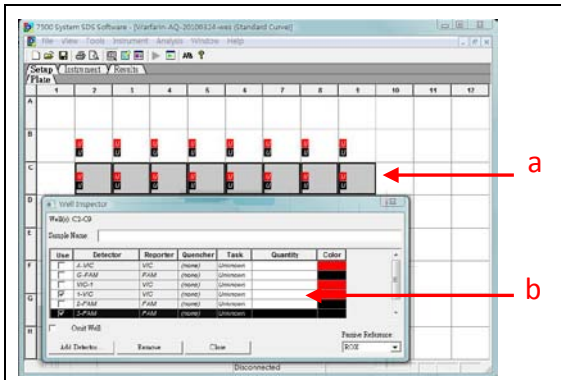
3.2.2 Select “**File**” and then select “**Save as...**”



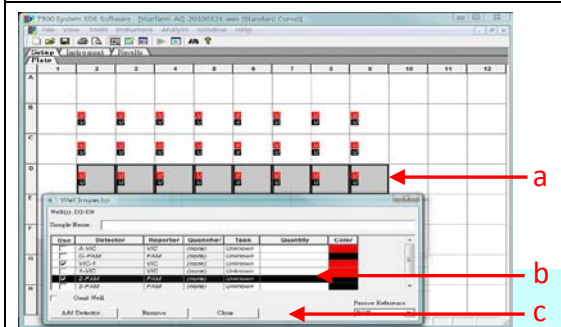
3.2.3 Edit File Name : **Warfarin-AQ-20100324-wes**
3.2.4 Save type : **SDS Documents (*.sds)**
3.2.5 Press “**Save**”



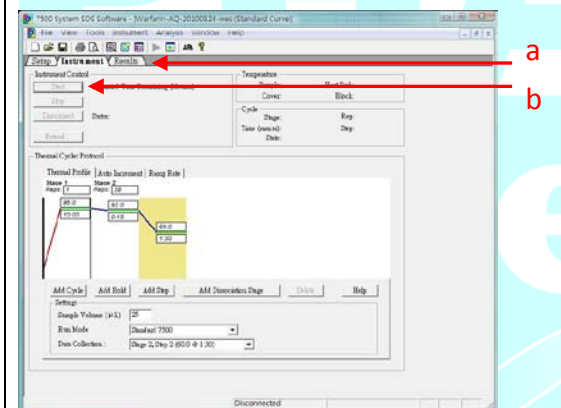
3.2.6 Select “**Setup**” tab and “**Plate**”(a) sub tab
3.2.7 Connect the information between Wells and Detectors(Ex. **PG1639’s wells (B2~B9(b))** which have to connect(select) to both **G-FAM and A-VIC (c)**)



3.2.8 Connect next wells' information
(Ex. **PG1075's wells (C2-C9)**
(a) Which have to connect (select)
to both **3-FAM** and **1-VIC** (b)



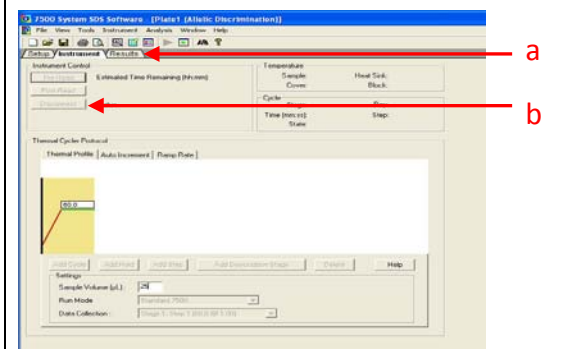
3.2.9 Connect next wells' information
(Ex. **PG0430's wells (D2-D9)**(a)
which have to connect(select) to
both **2-FAM** and **VIC-1**(b)
3.2.10 When you finished, please
Press "Close"(c)



3.2.11 Select "**Instrument**"(a)
3.2.12 Press "**Start**" button(b) which is
under "**Instrument Control**"
Region (The run needs about **80 min**)
3.2.13 If you cannot press "**Start**"
button, please save this file
again and reopen it.
3.2.14 After the run is finished, please
close this file.



3.3 Run Warfarin AD file (Post-Read)



3.3.1 Open Warfarin -AD- 201003
24 -wes and select "**Instrument**"
tab(a)
3.3.2 Press "**Post-Read**" (b) button
which is under "**Instrument
Control**" region.
(The run time needs about 1min)
3.3.3 When you finished, please close
this file.

7500 System SDS Software - [03:29-2010_PCT-test_AD (Allelic Disc

File View Tools Instrument Analysis Window Help

Setup Instrument Results

Plate

1 2 3 4 5

A

B

C

D

Analysis Settings - Allelic Discrimination Assay

Data Analysis:

Analyze post-read data only

Allele Calling:

Keep Manual Calls from previous Analysis

Marker: ALL Markers


Automatic Allele Calling

Quality Value: 90.0000 %

Two cluster Calling On

OK & Reanalyze OK Cancel Apply

3.3.4 Select "Analysis Settings

 (a) and a "Analysis Setting

- Allelic Discrimination Assay"


window will appear.

3.3.5 Select "Automatic Allele

Calling" (b) only.

3.3.6 Quality : 90% (c)

3.3.7 Press "OK" (d)

3.3.8 Press "Analyze"  (e) to

analyze data

3.3.9 When you finished, please save

this file again.

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Data Analysis in Warfarin AD file

Allelic Discrimination Plot for PG1639. The plot shows Allele Y (VIC) vs Allele X (FAM). Data points are labeled with genotypes: G/G (VIC/FAM), G/A (VIC/FAM), A/A (VIC/FAM), NTC (VIC/FAM), and Undetermined (VIC/FAM). Red arrows point to the 'Results' tab (a), the 'Allelic Discrimination' plot (b), and the 'PG1639' marker selection (c). Below the plot is a grid showing the relative spot positions for each genotype across 12 wells.

3.4 Data Analysis -AD File

3.4.1 Open **Warfarin -AD-20100324** – wes file and then select **”Results”** tab (a)

3.4.2 Make sure the setting of steps 3.3.3 to 3.3.7 are correct

3.4.3 Select **”Allelic Discrimination”** (b)

3.4.4 Select Marker : **PG1639** (c)

3.4.5 If you pick up a PG1639’s well (d), the relative spot will appear on Allelic Discrimination Plot. (Ex. NTC ; G type ; A type ; Both(A/G) or undetermined- (Fail))

Allelic Discrimination Plot for PG0430. The plot shows Allele Y (VIC) vs Allele X (FAM). Data points are labeled with genotypes: G/G (VIC/FAM), G/A (VIC/FAM), A/A (VIC/FAM), NTC (VIC/FAM), and Undetermined (VIC/FAM). Red arrows point to the 'Results' tab (a), the 'Allelic Discrimination' plot (c), and the 'PG0430' marker selection (b). Below the plot is a grid showing the relative spot positions for each genotype across 12 wells.

3.4.6 Select Marker : **PG0430** (a)

3.4.7 If you pick up a PG0430’s well, the relative spot will appear on Allelic Discrimination Plot. (Ex. NTC; 2 type; 1 type; Both (2/1) or undetermined-Fail).

3.4.8 you can analyze PG1075 by the same procedure.

Interpretation table

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

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