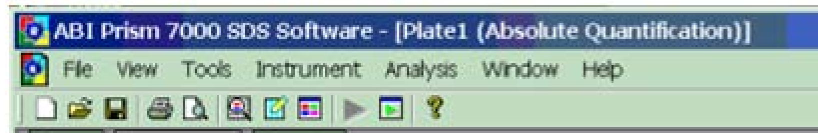
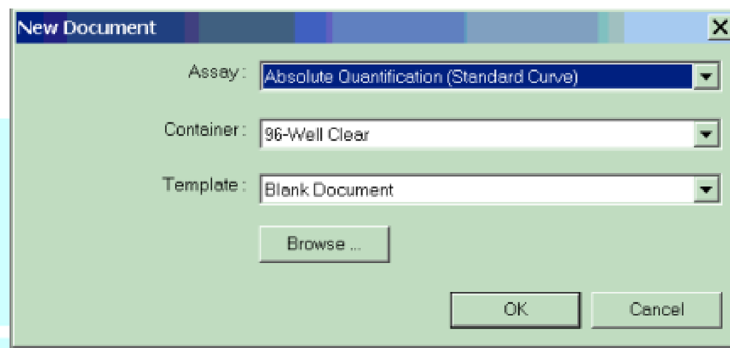
Instruction of PG5801 DNA Detection Kit for Applied Biosystems7000 Real-Time PCR System

1 Open the ABI 7000 System SDS Software on the desktop of the computer that is connected to the ABI 7000 system.



2 **New document > Select "File" and choose** "**New**"

3 **Select Assay >** "**Absolute Quantification (Standard Curve)**".



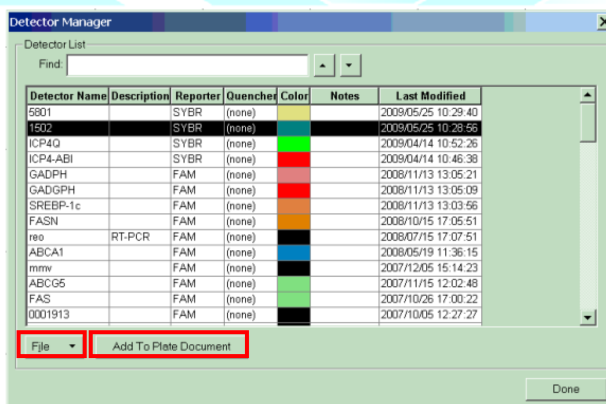
4 **Define 5801 Detector >**

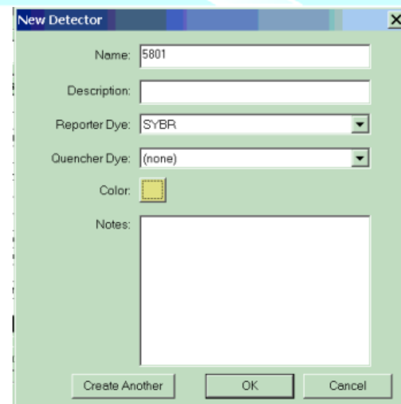
4.1 Select **"Tools"** tab and then choose "**Detector Manager**".

4.2 Select "**File**" and then click "**New**" to edit a New Detector.

4.3 Edit "**Name: 5801**", "**Reporter Dye: SYBR**", "**Quencher Dye: none**".

4.4 Choose "**5801 Detector**" and then select "**Add To Plate Document"** to add **"5801"** detector into the new document.

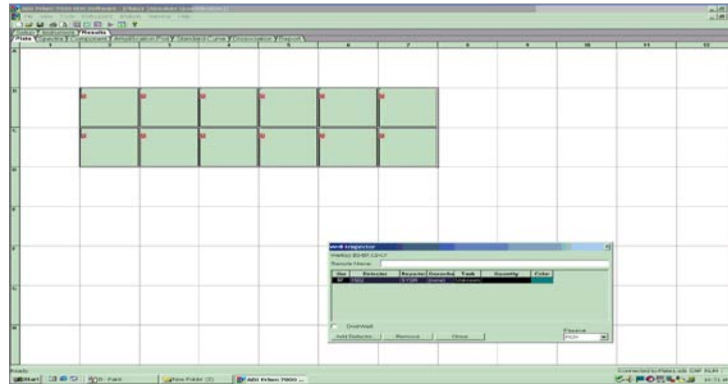


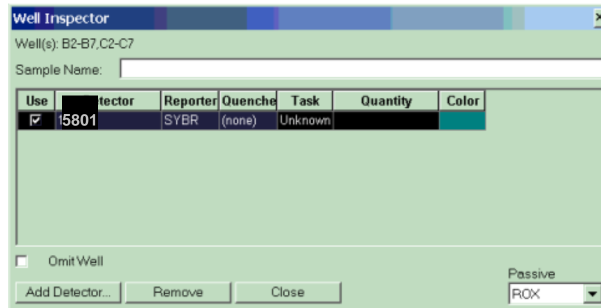


5 **Define plate document >**

5.1 **Select wells** for 5801 detection

5.2 Click right button of mouse to select "**Well Inspector**" and then choose "**5801**" as the detector。 Keep passive box as "**Rox**".



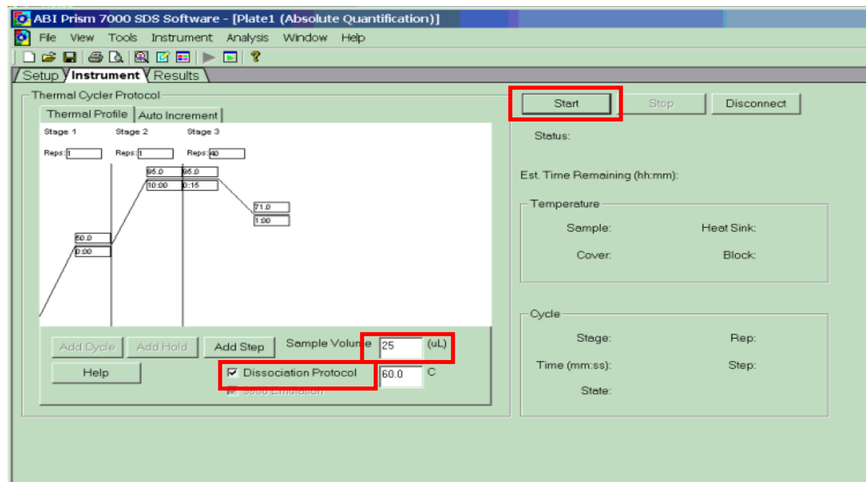


6 Set up 5801 program >

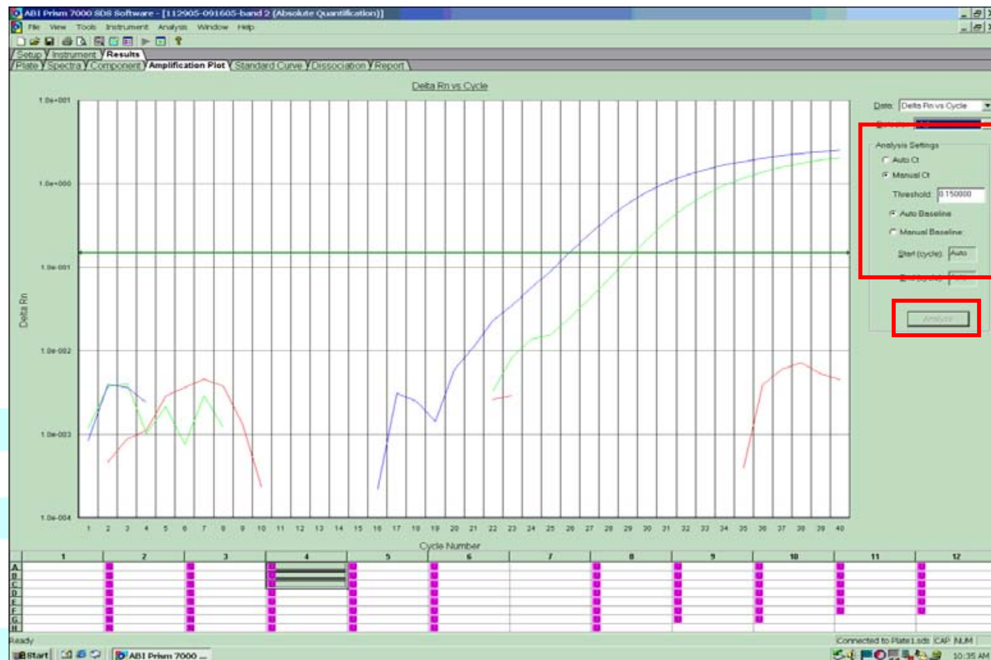
Select the "**Instrument**" tab. Edit PCR amplification program as below. "**Sample volume** is 25 ul". Select "**Step 2 (71.0@1:00)**" for Data Collection. Click "**Start**" to begin the PCR run.

	Time	Temperature	Cycle number	Additional comments
Initial PCR activation step:	10 min	95℃	1	Hot Start DNA Polymerase is activated by this heating step.
2-step cycling:			**35**	
Denaturation:	15 sec	95℃		
Annealing/ Extension:	1 min	71℃		Data will collected at this step of every cycle

*__Add Dissociation stage__



7 When the PCR run is complete, click the "**Result**" tab and choose the "**Amplification Plot**" page. Select the "**Manual Ct**", "**Automatic Baseline"** and then set the "**Threshold**" at **0.15**. Click "**Analyze**" to run these settings. Select "**Report**" tab to check Ct value.



8 Data analysis >

Two Ct values for each sample will be obtained, one from Genotype Detection Mix and the other from Internal Control Detection Mix. The differences of Ct values are calculated according to the equation shown below.

$$\Delta Ct = Ct_{\text{Genotype Detection mix}} - Ct_{\text{Internal control detection mix}}$$

When the Ct value of internal control is equal to or less than 27, and the ΔCt value is equal to or less than 7, the result should be identified as "HLA-B*5801 allele positive". Whereas, the ΔCt value is greater than 7, the result should be identified as "HLA-B*5801 allele negative". When the Ct value of internal control is greater than 27, the PCR inhibition should be suspected and repeating the test is highly recommended.

<table>
<tr><td rowspan="3">IC Ct ≤ 27</td><td rowspan="2">$Ct_{\text{Genotype Detection Mix}}$ ≤ 35</td><td>ΔCt ≤ 7</td><td>HLA-B*5801 positive</td></tr>
<tr><td>ΔCt > 7</td><td>HLA-B*5801 negative</td></tr>
<tr><td colspan="2">$Ct_{\text{Genotype Detection Mix}}$ > 35 (undetermined)</td><td>HLA-B*5801 negative</td></tr>
<tr><td rowspan="2">IC Ct > 27</td><td colspan="2">PCR inhibitors may be present in specimen</td><td rowspan="2">Retest</td></tr>
<tr><td colspan="2">Inappropriate gDNA quantity</td></tr>
</table>

(IC: internal control)