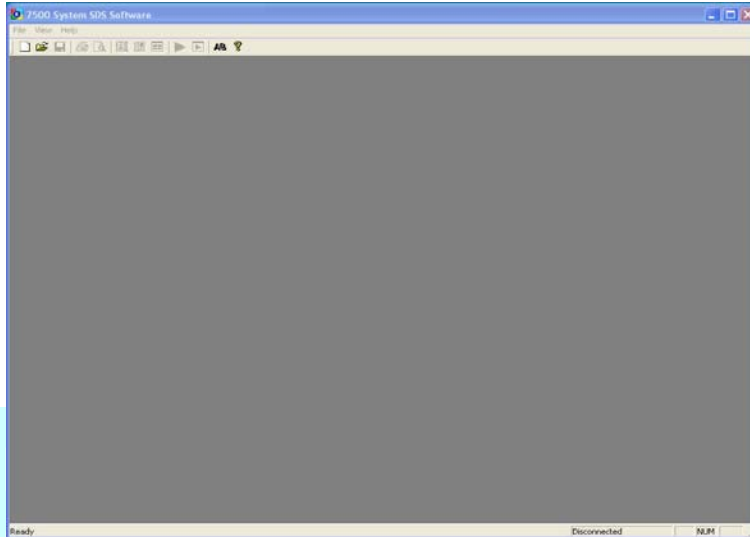
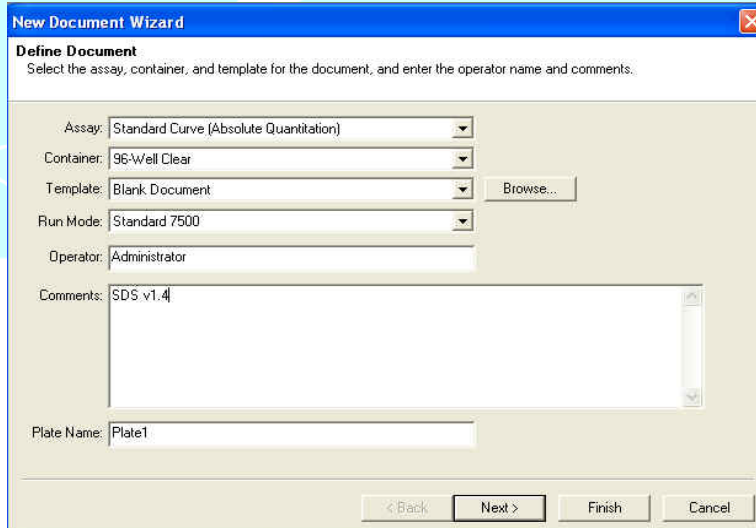


# Instruction of PG5801 DNA Detection Kit for Applied Biosystems7500 Real-Time PCR System

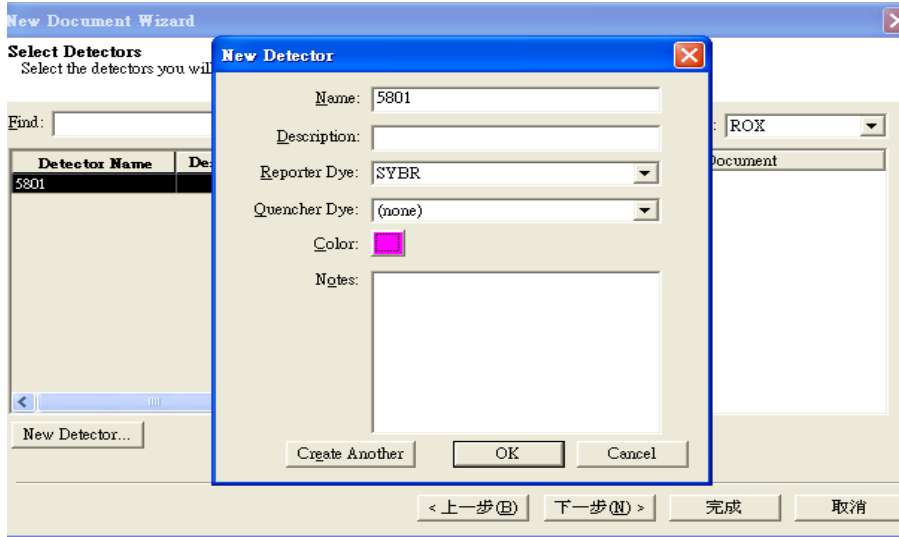
- 1 Open the ABI 7500 System SDS Software on the desktop of the computer that is connected to the ABI 7500 system.



- 2 Select File > New, the New Document Wizard dialog box will appear.
- 3 Select Assay > Absolute Quantification (Standard Curve).



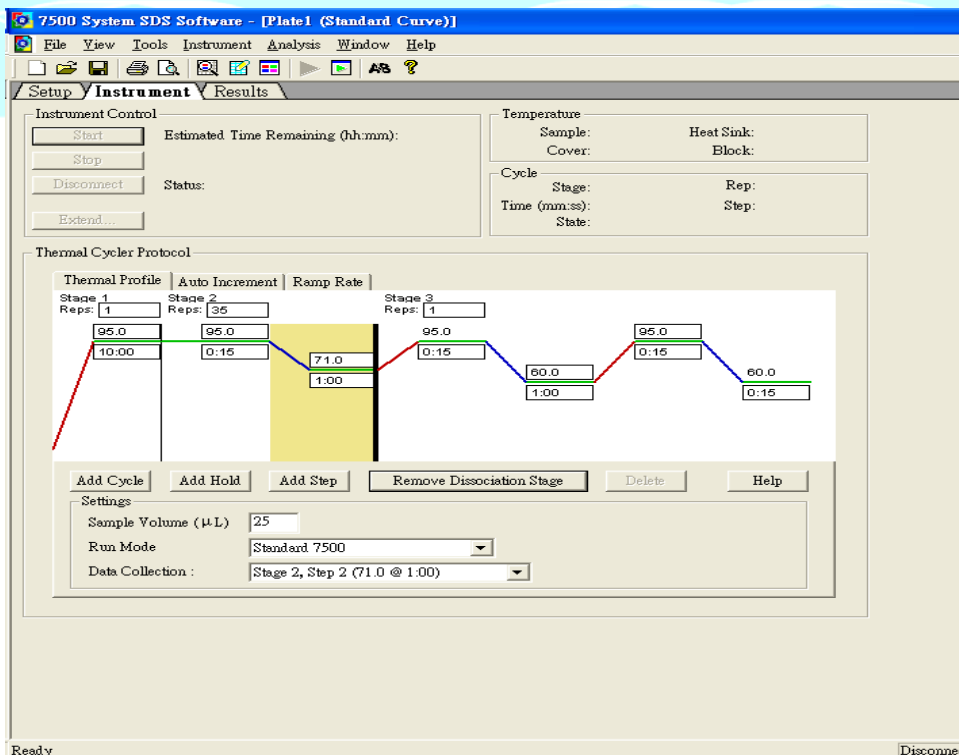
- 4 Select SYBR for the reporter dye and add it to the Detectors in Document box
  - 4.1 Name: 5801 ◦
  - 4.2 Reporter Dye: SYBR ◦
  - 4.3 Quencher Dye: none ◦
  - 4.4 Press "OK"



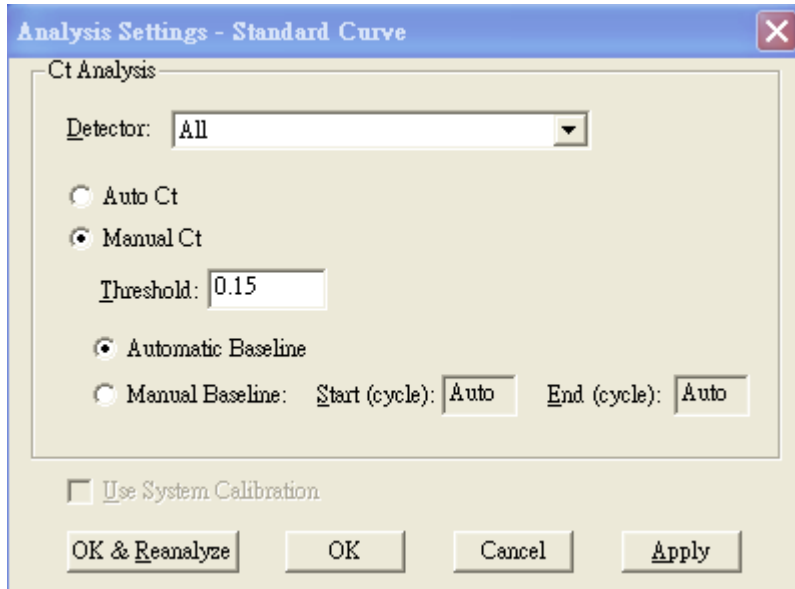
- 5 Select the Instrument tab. Set PCR amplification program and add dissociation curve stage as below. Edit 25 uL for Sample Volume and select Stage 2, Step 2 (71.0@1:00) for Data Collection

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

\*Add Dissociation stage



- 6 Select the Instrument tab. click Start to begin the PCR run.
- 7 When the PCR run is complete, a small dialog box stating “The run completed successfully” will appear on the screen. Click OK, this will close the box.
- 8 Click the Result tab and choose the Amplification Plot page.
- 9 Select Analysis> Analysis Settings. Select the “Manual Ct” and set the Threshold at 0.15. And “Automatic Baseline” should be selected then click “OK & Reanalyze”.



- 10 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  $\Delta Ct$  value is equal to or less than 7, the result should be identified as "HLA-B\*5801 allele positive". Whereas, the  $\Delta Ct$  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.

IC Ct ≤ 27	Ct <sub>Genotype Detection Mix</sub> ≤ 35	$\Delta Ct \leq 7$	HLA-B*5801 positive
		$\Delta Ct > 7$	HLA-B*5801 negative
	Ct <sub>Genotype Detection Mix</sub> > 35 (undetermined)		
IC Ct > 27	PCR inhibitors may be present in specimen		Retest
	Inappropriate gDNA quantity		

( IC: internal control)