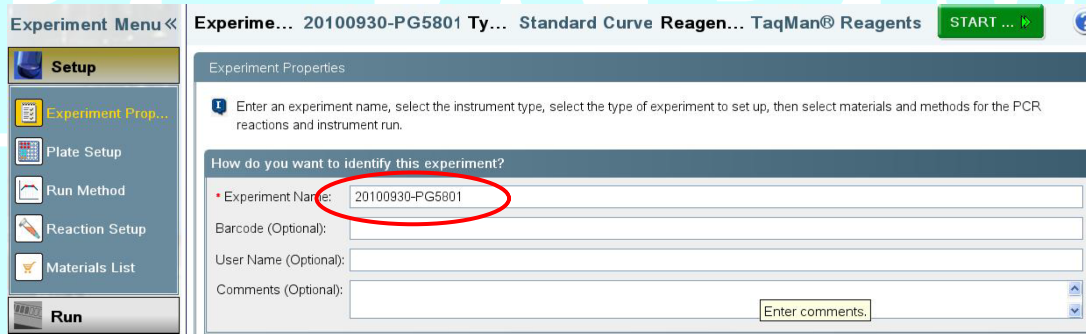
Instruction of PG5801 DNA Detection Kit for Applied Biosystems7500 Real-Time PCR System

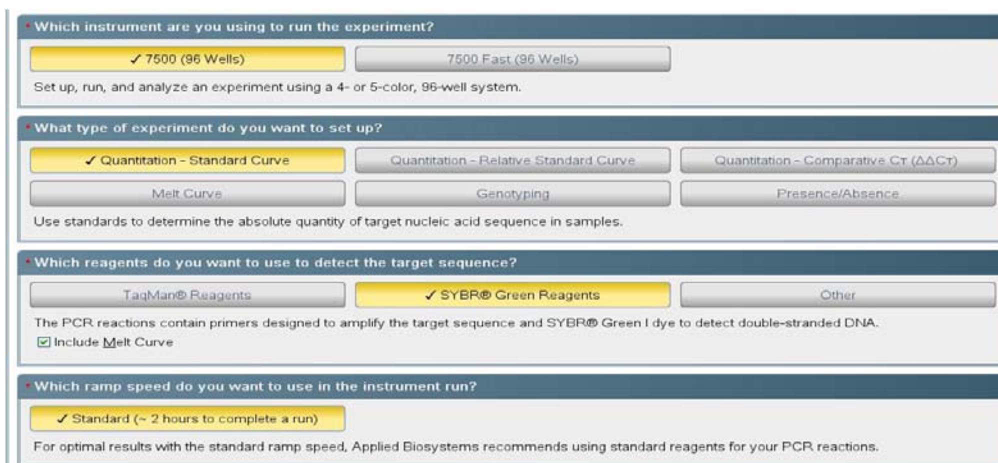
1 Open the ABI 7500 System SDS Software on the desktop of the computer that is connected to the ABI 7500 system.

2 **Select "Advanced Setup".** Enter Experiment Name : **PG5801 Template.**



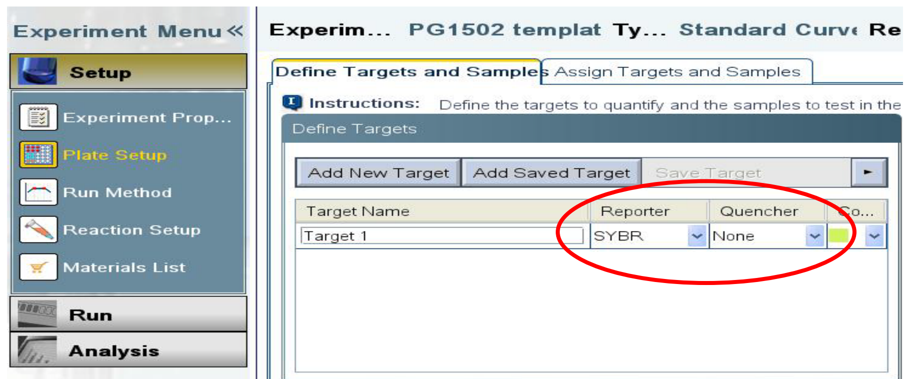
3 **Edit Experiment Prosperities**

Select **7500 (96 Wells)**, **Quantitation- Standard Curve**, **SYBR® Green Reagents**, **Standard (~2 hours to complete a run)**



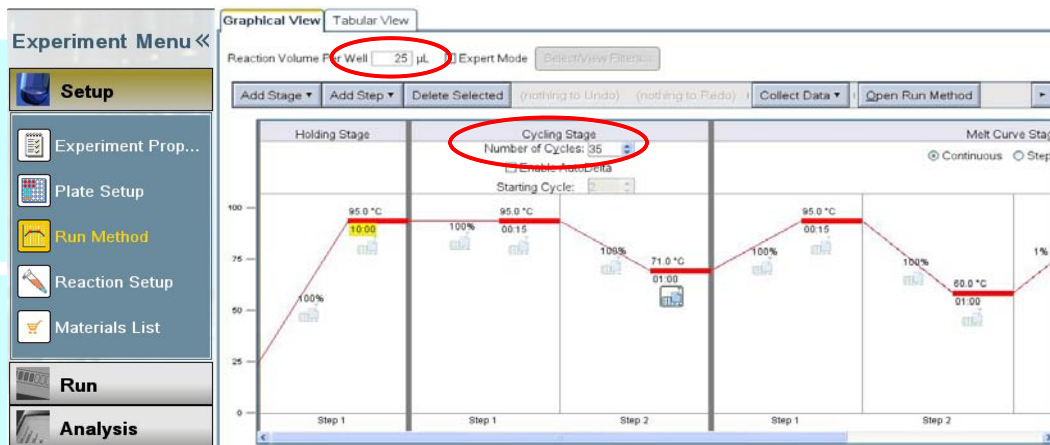
4 **Define Target**

Select **SYBR** in Reporter column, Select **None** in Quencher column.



5 Edit the reaction volume and the thermal profile for the run method

Edit the reaction volume as **25** (μl), Number of Cycling as **35**. Edit the thermal profile as package insert described.

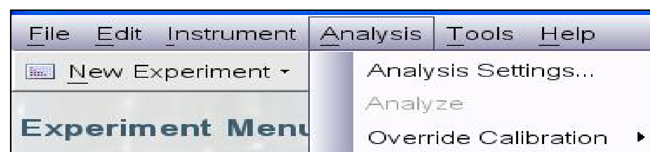


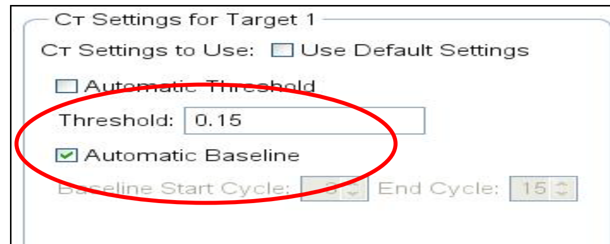
	Time	Temperature	Cycle number	Additional comments
Initial PCR activation step:	10 min	95℃	1	Hot Start DNA Polymerase is activated by this heating step.
2-step cycling:			35	
Denaturation:	15 sec	95℃		
Annealing/ Extension:	1 min	71℃		Data will collected at this step of every cycle

*Add Dissociation stage

6 Edit analysis setting

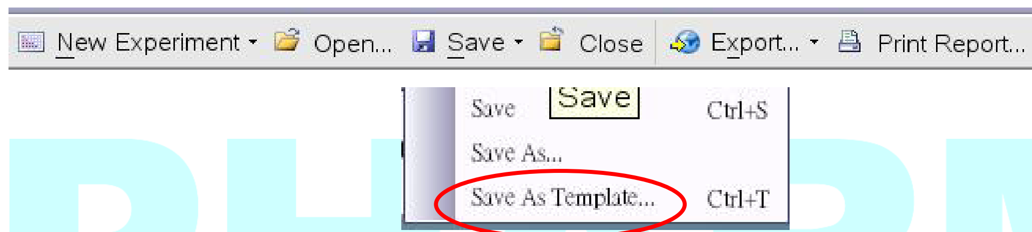
Edit Threshold as **0.15** and select **auto baseline**. Click **save changes**.





7 Save as a template document

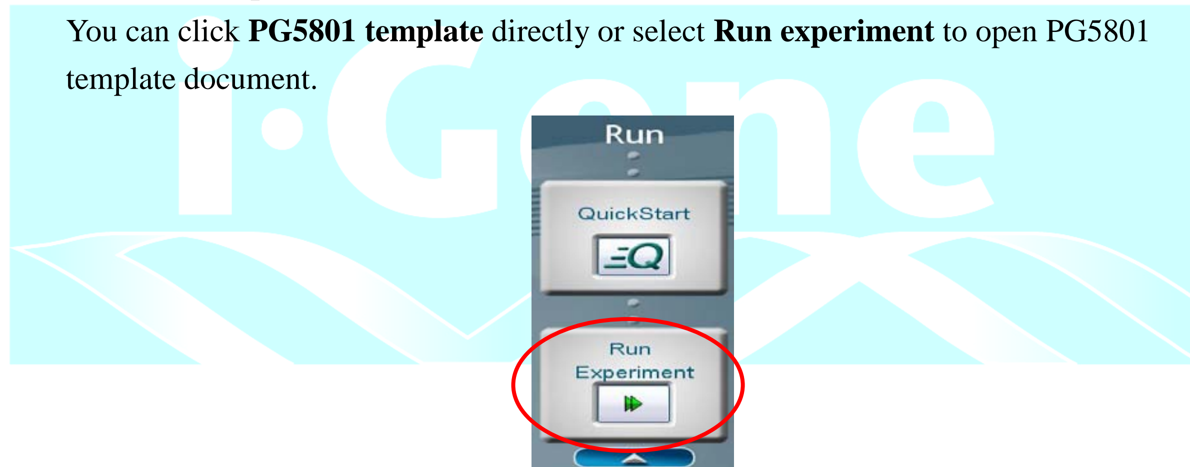
Template document name can be "**PG5801 template. edt**". If you run a new experiment next time, just need to open this template document directly.



PHARM i-Gene

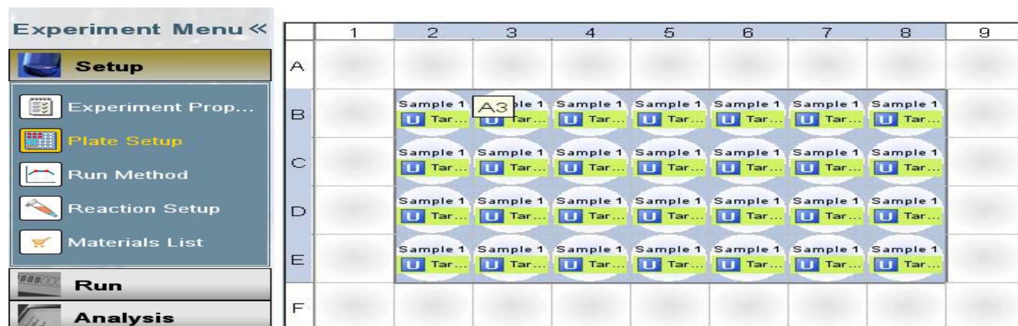
8 Run a new experiment

You can click **PG5801 template** directly or select **Run experiment** to open PG5801 template document.



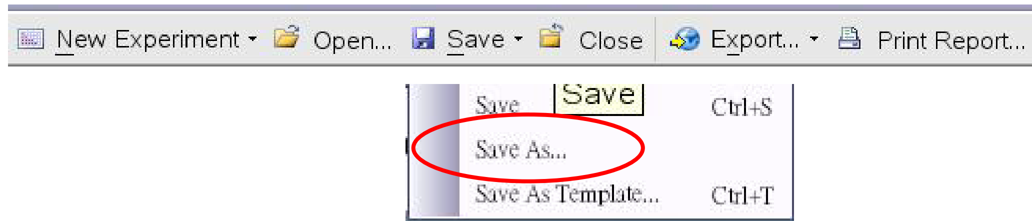
9 Define plate information

Select reaction wells and assign target 1(SYBR) and sample 1 to them.



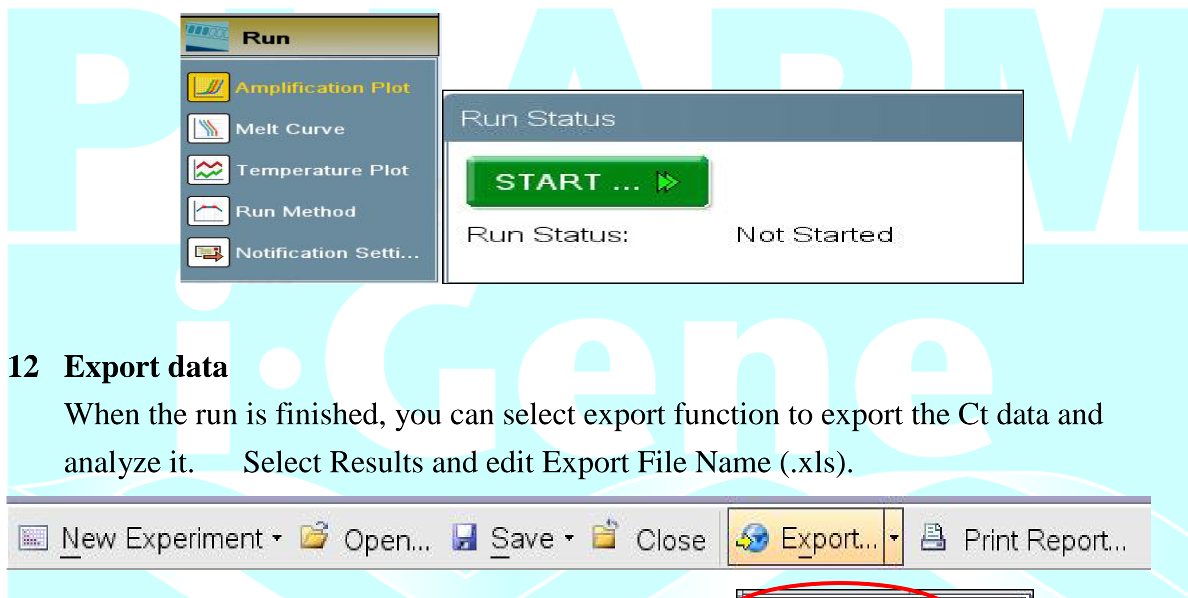
10 Save it as experiment document(.eds)

When you finished all settings, save this document as a new experiment formate (eds.) for a common experiment document.



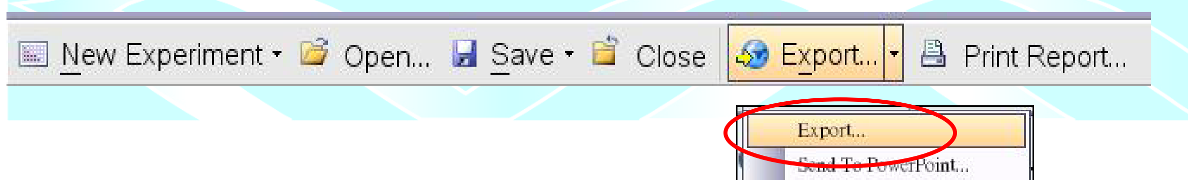
11 Start the run

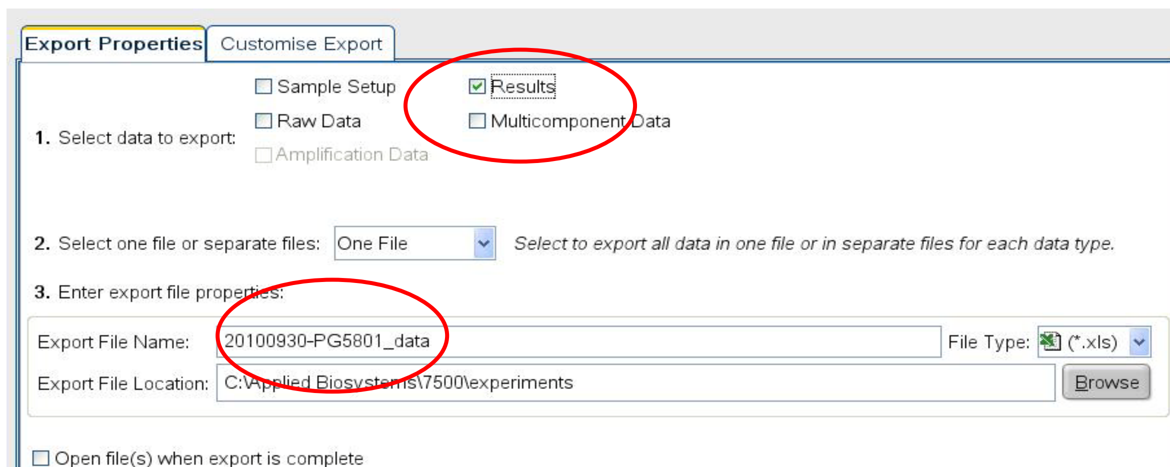
After you put the sample reaction plate or strips into PCR machine, click “Start” symbol to start this run.



12 Export data

When the run is finished, you can select export function to export the Ct data and analyze it. Select Results and edit Export File Name (.xls).





13 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.

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

(IC: internal control)