Setting-up Real-time Instruments

Conditions for use with the ABI Prism 7700

Assay and PCR Amplification Setups are described in Endpoint or Real-time Protocol with the exception that Platinum Taq (LTI) should be used instead of rTaq. A false hotstart is not possible with the ABI 7700, a hotstart enzyme is recommended .

Creating a Plate Document

Step 1: Open the Sequence Detection Systems software.

Step 2: Create a plate document with the following attributes:
Single Reporter
7700 Sequence Detector
Real Time

Configuring the FAM Dye Layer

Step 1: From the Dye Layer pop-up menu, select FAM.

Step 2: As described within the ABI 7700 Prism protocol, from the Sample Type pop-up menu, identify wells as either Unknown (UNKN), standards (STD), or no template control (NTC). For standards enter copy number and for unknowns enter sample name.

Set Thermal cycling conditions

Step 1: Click Thermal Cycler Conditions from the plate document.

Step 2: Click individual Time and Temperature text fields and enter the following settings as shown in Table 1.

Table 1: ABI 7700 PCR Program

Stage	**1**	**2**		
	Denaturation	**Repeat: 50 cycles**		
		Melt	**Anneal**	**Extend**
Step		**1**	**2**	**3**
Temperature	**95°C**	**95°C**	**55°C**	**72°C**
Time (min.)	**4:00**	**00:15**	**00:20**	**00:40**

Step 3: Set volume to 25 µl.

Step 4: Click Show Data Collect. Only collect data at 55°C.

Step 5: Click OK.

Take Rox off

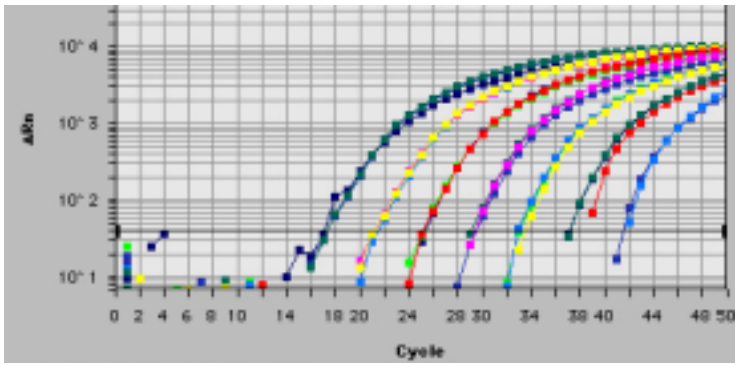
Step 1: Under Diagnostics in the Instrument menu, select the Advanced Options dialog box.
Step 2: Remove the selection for ROX.
Step 3: Click OK. Ignore the prompt to re-launch.

Identify the stage for data collection

Step 1: Under Analysis Options, select PCR stage 2 and step 2.

Note: Data collection for the Amplifluor™ System should be performed at 55°C.

A.



B.

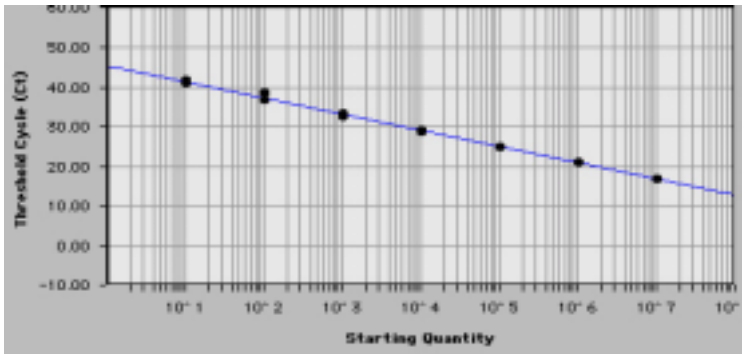


Figure 2: Real-time fluorescence measurements during PCR amplification, using the Amplifluor™ Universal System on ABI 7700. A. Threshold cycle (Ct) values were calculated from the amplification plot for 10^1 to 10^7 target copies of *bcl-2*. B. When plotted as threshold cycle (Ct) versus the input amount of target, the resulting standard curve is linear >5 orders of magnitude. The correlation coefficient (r^2) is 0.998.

Conditions for use with the Bio-Rad iCycler iQ

Assay and PCR Amplification Setups are as described in Endpoint or Real-time Protocol.

iCycler iQ set up

Step 1: Turn on and allow the host computer and detector to warm up for 30 minutes.

Step 2: From the start menu of the host computer, click on the iCycler icon. This will prompt a choice of two programs iCycler or iQConfig. Click on the iQConfig which allows masking and system calibration as stated in the iCycler manual.
Note: The same tubes and volumes that will be used in the reaction should also be used for system calibration.

Protocol Workshop Module

There are four tabs across the top of the Protocol Workshop window for editing protocols and plate setup.

Edit Protocol. Specify the thermal parameters for the protocol and select that data collection and analysis be performed at the annealing step. A yellow lens camera should appear at the data collection step (annealing step).

Edit Plate Setup. Specify the locations of samples, standards, and fluorphore (fluorescein) for each well. For standards enter copy number and unknowns enter sample name.

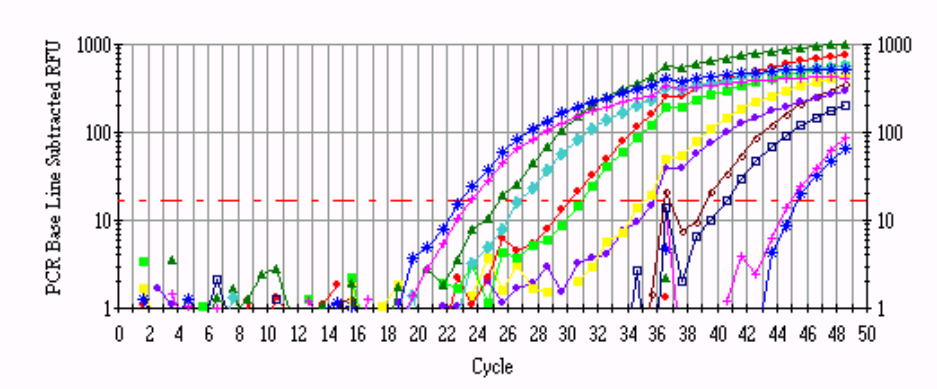
Edit Notation. Save protocols for future reference.

Edit Run Prep. Confirm the protocol file, plate setup file, reaction volume and run conditions. This is the final step before commencing PCR.

Table 2: iCycler iQ PCR Program

Stage	1	2		
	Denaturation	Repeat: 50 cycles		
		Melt	Anneal	Extend
Step		1	2	3
Temperature	95°C	95°C	55°C	72°C
Time (min.)	4:00	00:15	00:20	00:40

A.



B.

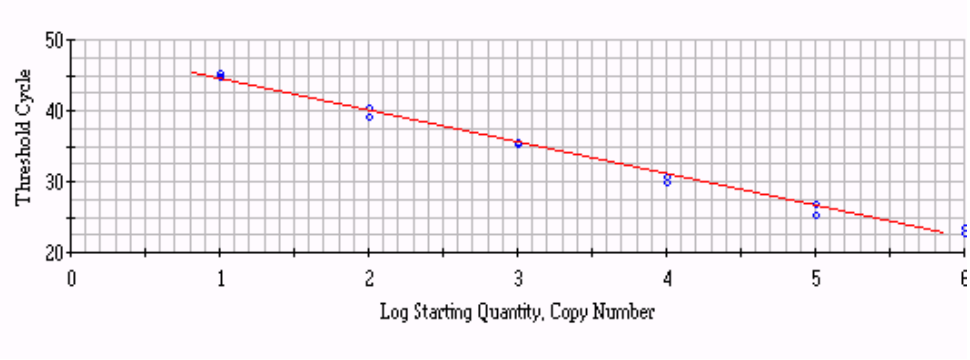


Figure 3: Real-time fluorescence measurements during PCR amplification, using the Amplifluor™ Universal System on Bio-Rad iCycler iQ. A. Threshold cycle (Ct) values were calculated from the amplification plot for 10^1 to 10^7 target copies of *bcl-2*. B. When plotted as threshold cycle (Ct) versus the input amount of target, the resulting standard curve is linear >5 orders of magnitude. The correlation coefficient (r^2) is 0.995.