

Setting Up a Multiplex (two probe) Real Time 5' Nuclease Reaction on the ABI PRISM® 7700 Sequence Detection System

Overview

The goal of this tutorial is to demonstrate how to correctly label a plate set-up for a multiplex reaction (two reporter dyes in one tube) on an ABI PRISM® 7700 Sequence Detection System (SDS).

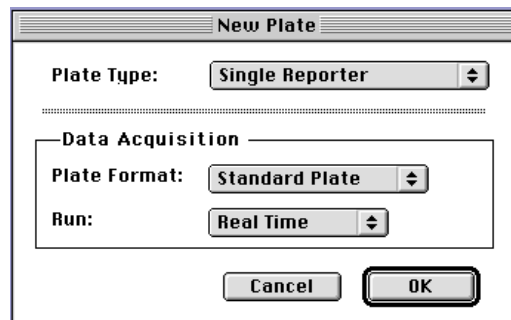
For this example we will use a TaqMan® FAM™ dye-labeled probe and a TaqMan® VIC® dye-labeled probe.

*Throughout this tutorial, words in **bold** type are actual menu options in the ABI PRISM® 7700 SDS software.*

Please Note: If you are not familiar with setting up the ABI PRISM® 7700 SDS software, please refer to the tutorial [Setting-up Singleplex \(single probe\) Real Time 5' Nuclease Reactions on the ABI PRISM® 7700 Sequence Detection System](#) before proceeding. The Singleplex tutorial covers certain basic setup and operation steps that will not be covered in this tutorial.

Launching ABI PRISM® 7700 SDS Software

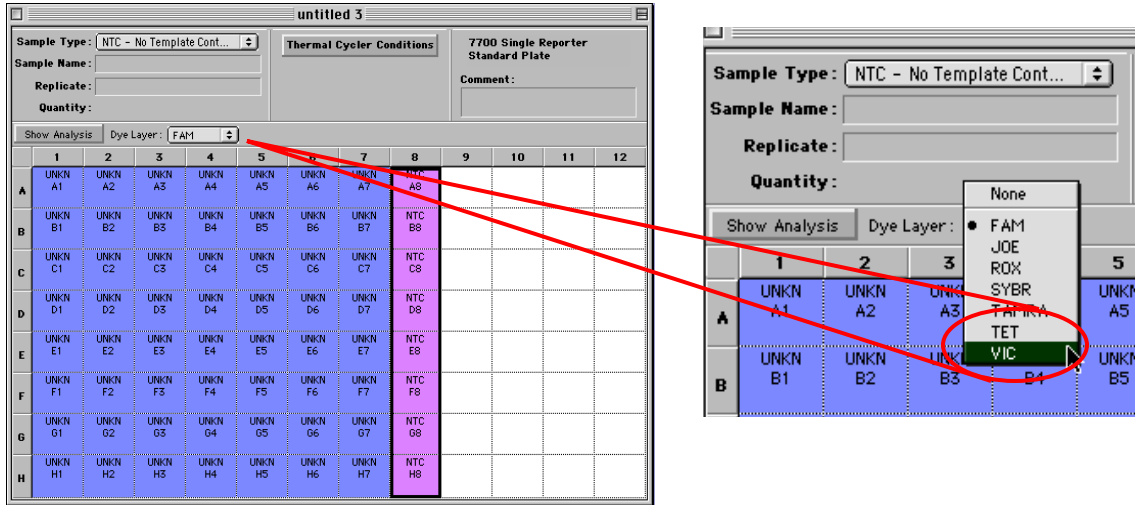
Launch the ABI PRISM® 7700 SDS software. For a real-time run, the **Plate Type** is Single Reporter, **Plate Format** is Standard Plate, and **Run** is Real Time.



Labeling the Dye Layers

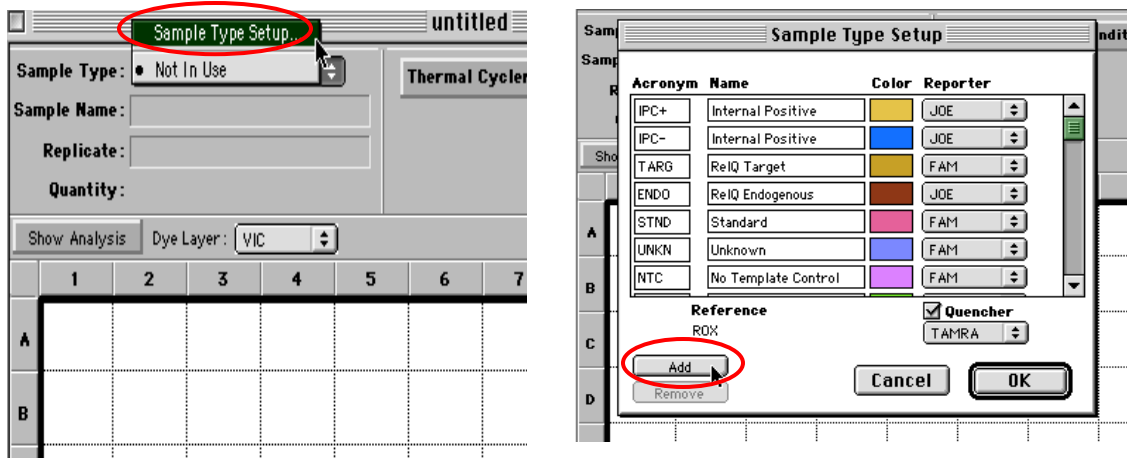
The SDS software is set up so that FAM™ is always the default dye layer. Set up the FAM™ dye layer first, labeling all of the wells that contain the TaqMan® FAM™ dye-labeled probe.

Then change to the VIC® dye layer using the **Dye Layer** drop-down menu, which is just to the right of the **Show Analysis** button on the plate set-up.



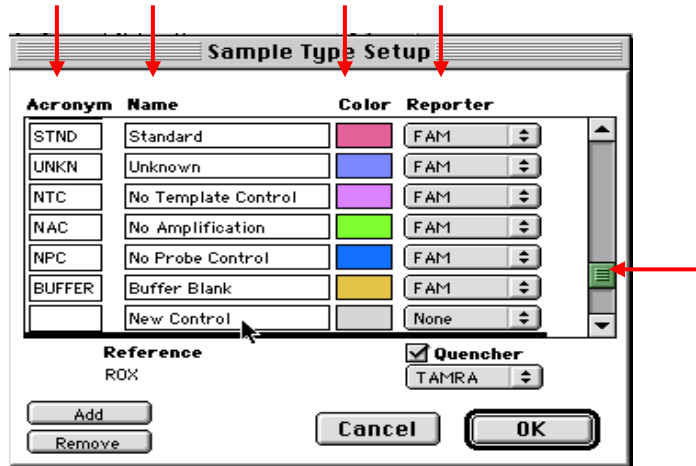
Sample Type Setup

In order to set up the VIC® dye layer, you will have to generate some new **Sample Type** names. To do this, go to **Sample Type Setup...** which is found in the drop down menu next to **Sample Type**, at the top left of the setup window.



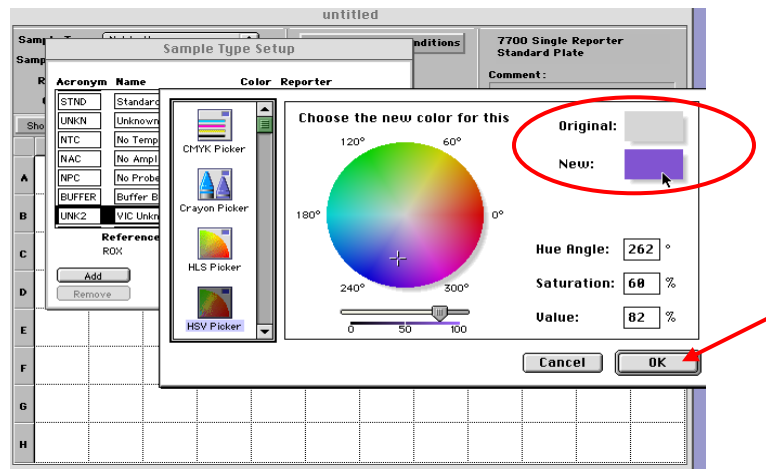
Click the **ADD** button on the bottom left corner of this window. Scroll down within the

window to the newly created row in which you can assign the *Acronym*, *Name* (Sample Type), *Color*, and *Reporter*.

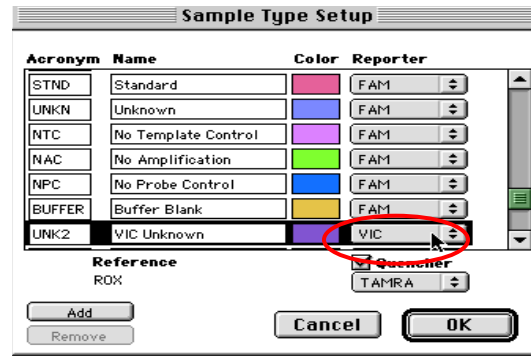
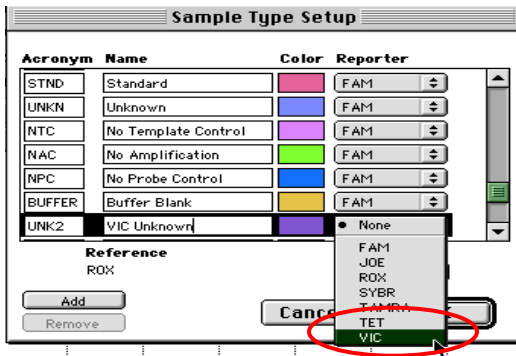


When choosing an *Acronym*, do not use any of the existing acronyms displayed in the list in this window. For example, if you want to add an unknown in the VIC[®] dye layer, do not use the acronym UNKN, because this is already used in the FAM[™] dye layer. Instead choose something different like UK or UNK2. Then choose a name for the sample.

The *Color* does not necessarily need to be changed. The default *Color* in the **Sample Type Setup** is gray. If you would like to change it, double-click on the gray *Color* box. This will open up a window in which you can pick a color. Simply click anywhere in the color circle, and the *New Color* will be shown in the top right corner of the window. Once you have changed the color, click the **OK** button.



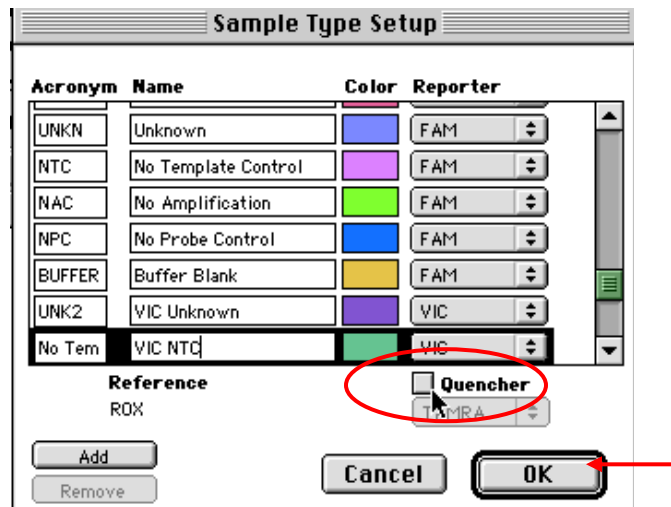
The last column is *Reporter*. Change the **None** to **VIC**.



Repeat this for as many new sample types as you need to create. The new sample types will now appear in the **Sample Type** menu of the VIC[®] dye layer and be available for you to finish labeling your plate.

Changing the Quencher Setting

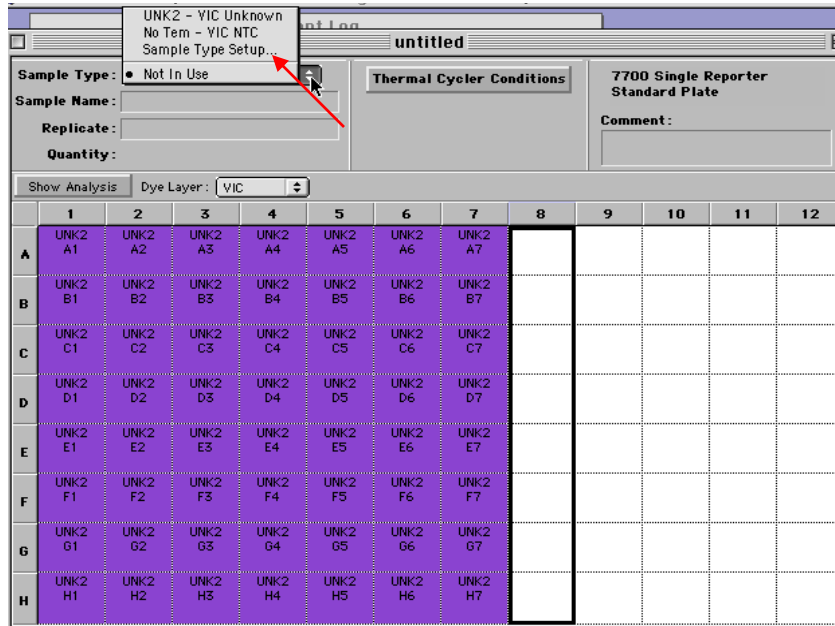
If you are using at least one TaqMan[®] TAMRA[™] dye-labeled probe in the multiplex reaction, do not change the **Quencher** setting. If you are using TaqMan[®] MGB probes you will need to change the **Quencher** setting. Since a TaqMan[®] MGB probe has a non-fluorescent quencher, simply uncheck the **Quencher** box. Then click **OK**.



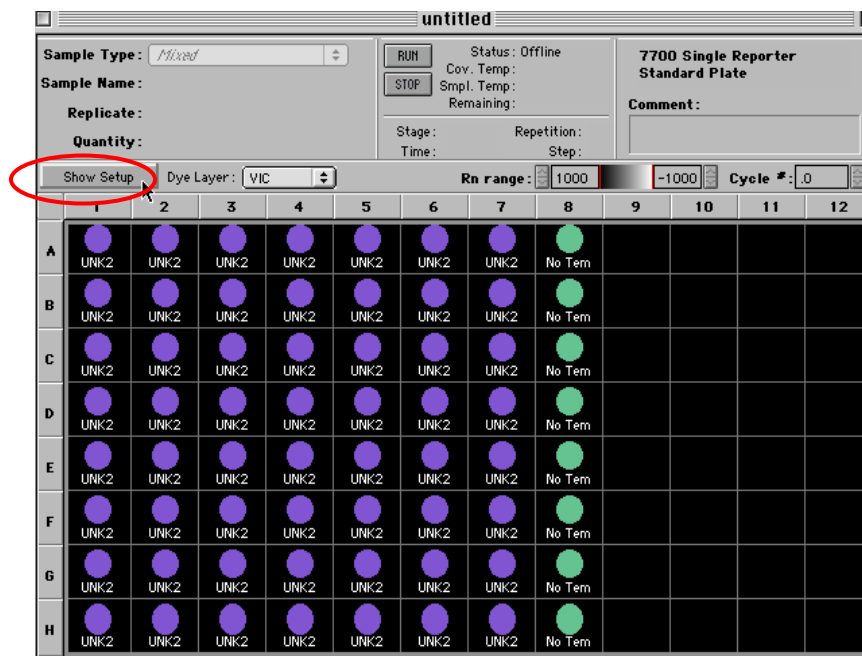
Note: It is possible to run TaqMan[®] TAMRA[™] and TaqMan[®] MGB probes in multiplex reactions. If you are running a TaqMan[®] TAMRA[™] and a TaqMan[®] MGB probe in the same wells, leave the Quencher set to TAMRA[™], and analyze as usual.

Labeling the VIC[®] Dye Layer

Label the VIC[®] dye layer using the new **Sample Types** that you have created.

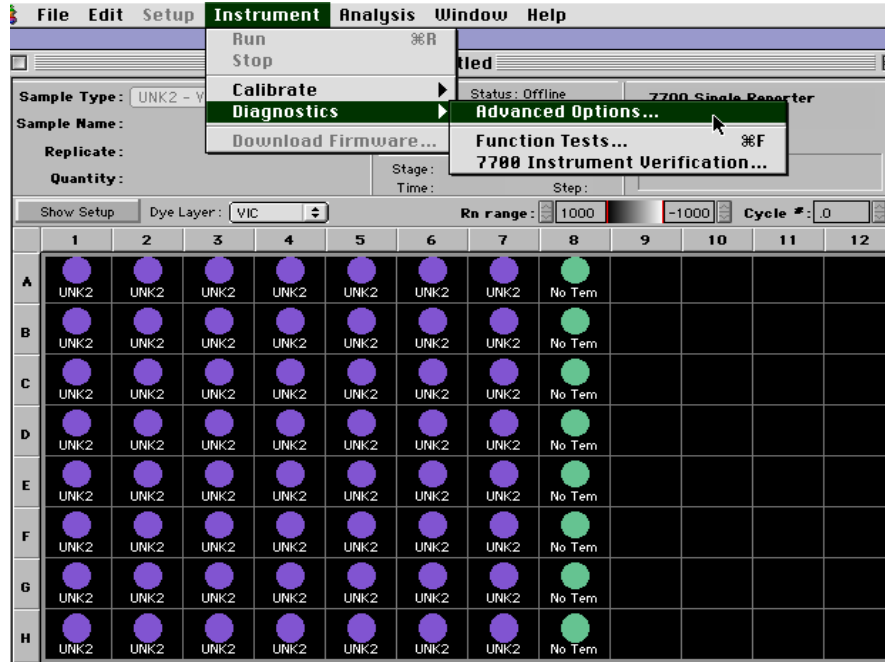


Once both layers are set up, click the **Show Analysis** button so that it now reads **Show Setup**.

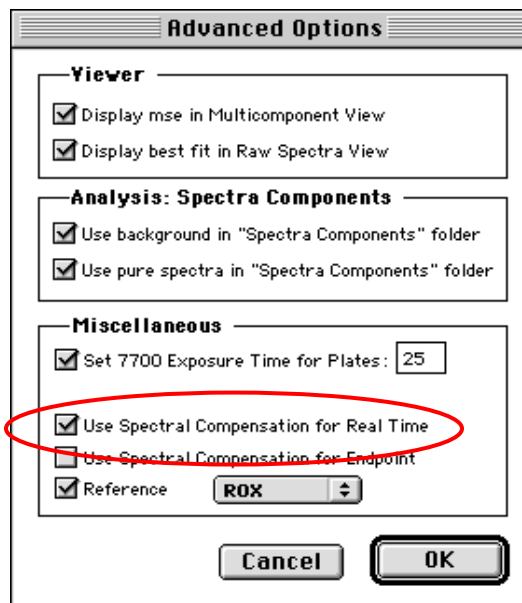


Checking Instrument Settings

Under the **Instrument** menu, at the top of your screen, go down to **Diagnostics > Advanced Options...**



Make sure that, in the **Miscellaneous** box, you have checked **Use Spectral Compensation for Real Time**.



Click **OK**. You will receive the following message:



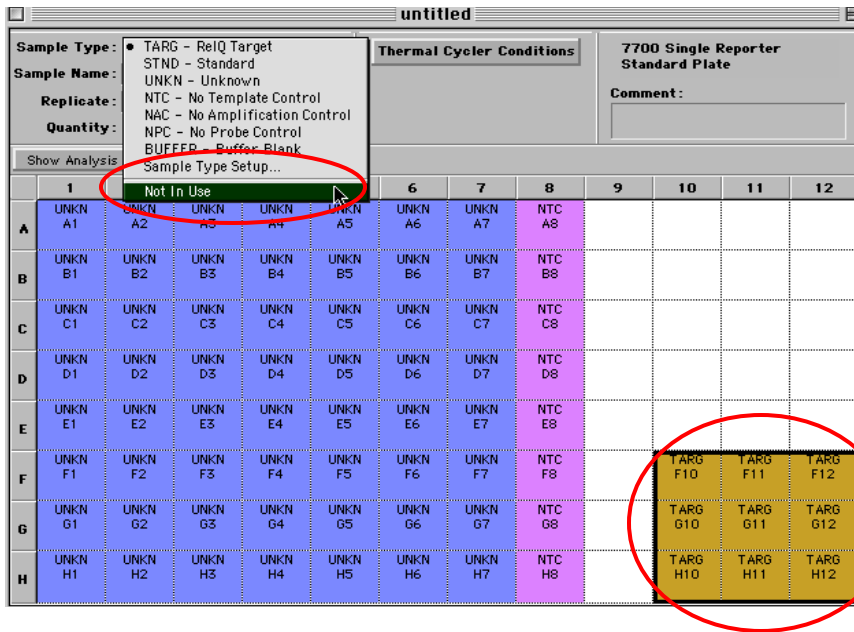
It is not necessary to re-launch the application. Simply click **OK** and start your run.

Data Analysis of Singleplex and Multiplex Samples on the Same Plate

When running singleplex and multiplexed samples on the same plate, you need to make some software adjustments for both sample types to be effectively analyzed.

The Spectral Compensation option should only be checked when analyzing multiplex samples. The Spectral Compensation settings are unique for the analysis of two reporter dyes in one well. While the multiplex algorithm (Spectral Compensation) enhances the analysis of a multiplex reaction, the advantages are not realized in analyzing a singleplex reaction, and therefore should not be used for this. If both singleplex and multiplex samples are run on the same plate, you will need to take one of these sets out of use, post run, for analysis of the other. For example, one could first take the singleplex samples out of use and analyze the multiplex samples (using Spectral Compensation). After analysis and exporting of the results you would reverse this process and take the multiplex samples out of use and analyze the singleplex samples (without Spectral Compensation).

The example below will illustrate this process. Here the gold-labeled wells are singleplex reactions. To take them out of use, highlight the desired wells, go to the **Sample Type** Menu, and choose **Not In Use**. Since the rest of the wells are multiplex, they would then be analyzed with **Use Spectral Compensation for Real Time** checked. You may wish to save this setup as a separate file using the **Save As** command. To analyze the singleplex samples you would put the singleplex reaction wells back in use, and put all multiplex samples **Not in Use**. Analyze the singleplex wells (with **Use Spectral Compensation for Real Time** unchecked). You may want to save this configuration as a separate plate for future analysis.



To analyze the results of both singleplex and multiplex samples in a secondary software, such as Microsoft® Excel, you would select **File > Export > Results**. This will export the results (including all dye layers, Ct values, sample names, etc.) in a tab-delimited format for additional downstream analysis.

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