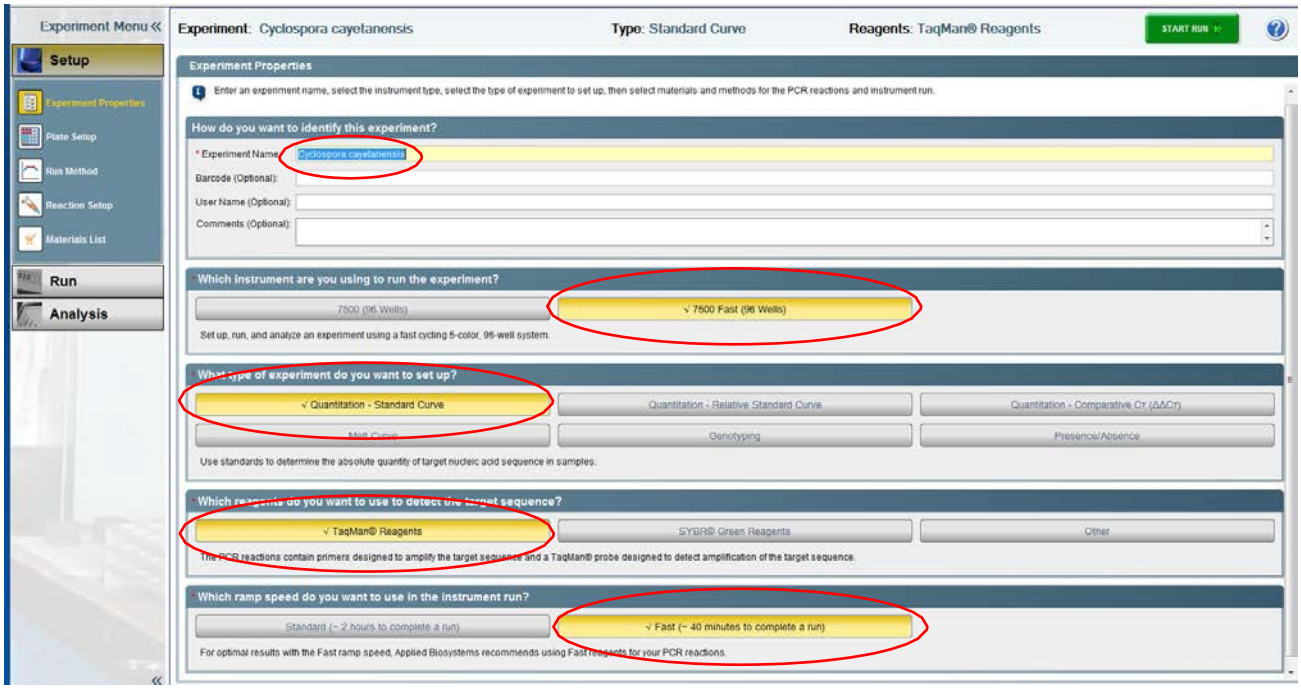
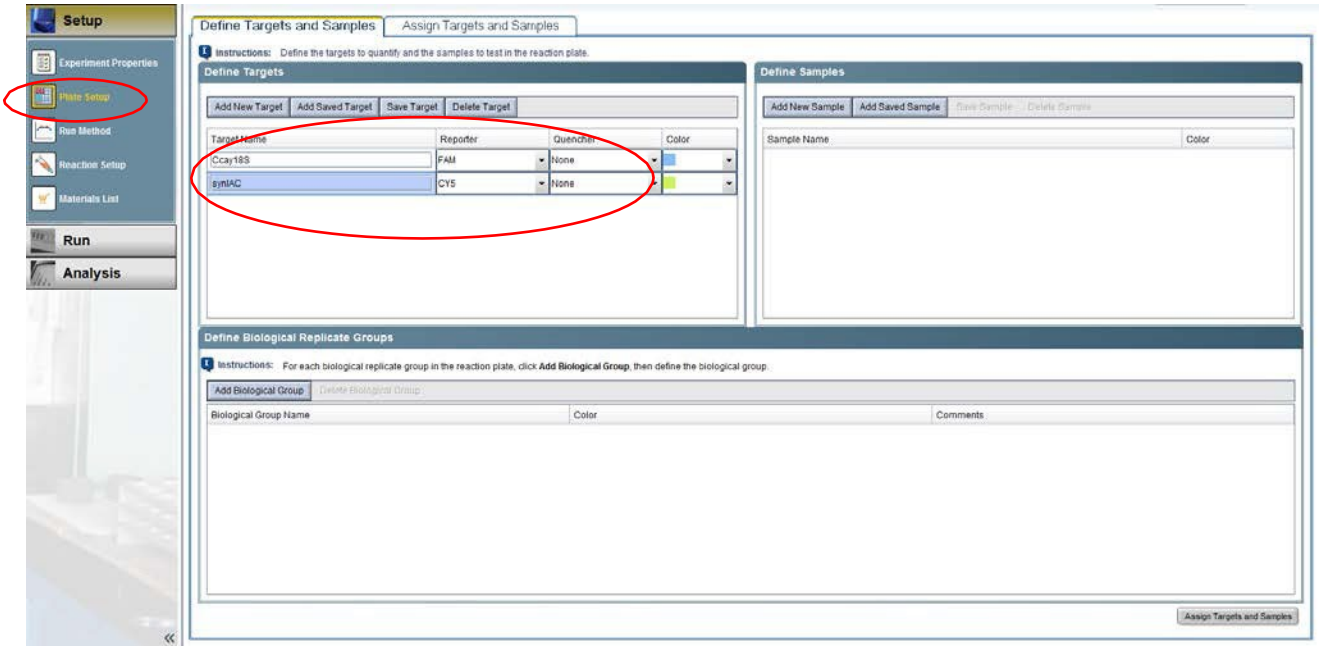
Appendix 5: ABI 7500 Fast v2.0 or 2.3 Method (1 of 8)

(A) Define a Run Template Using Software v2.0 or 2.3 on the ABI 7500 Fast Instrument:

Turn on the computer and ABI 7500 FAST Real-Time PCR system. Open the 7500 Software v2.0 or 2.3 and click "New Experiment" (Advanced Setup). Define "Experimental Properties" as shown below with "Experiment Name"→ "Cyclospora cayetanensis"

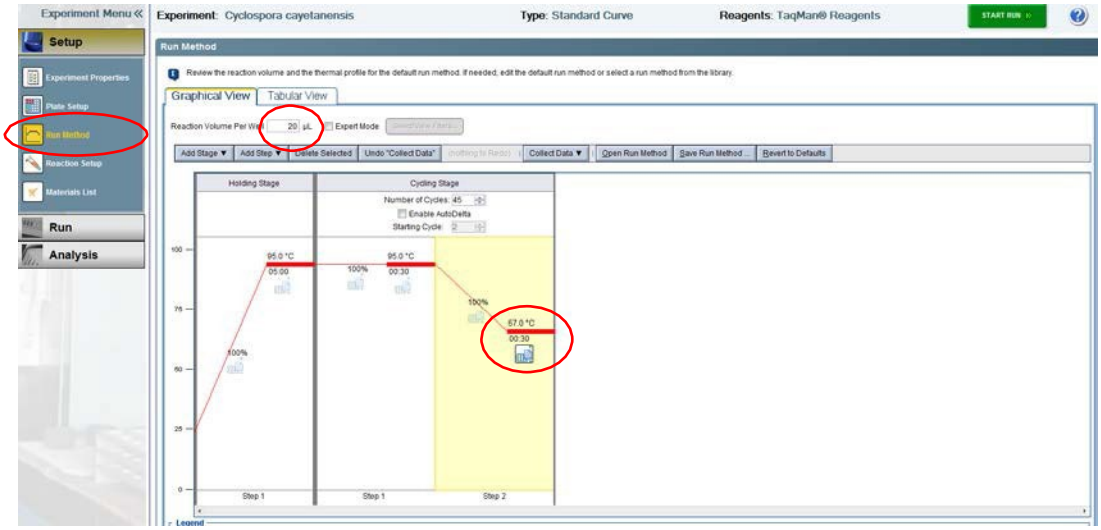


Click "Plate Setup" on left. Add targets to the "Define Targets and Samples" tab as shown below. Define the targets Ccay18S as "FAM" and synIAC as "CY5" with quencher set as "None".

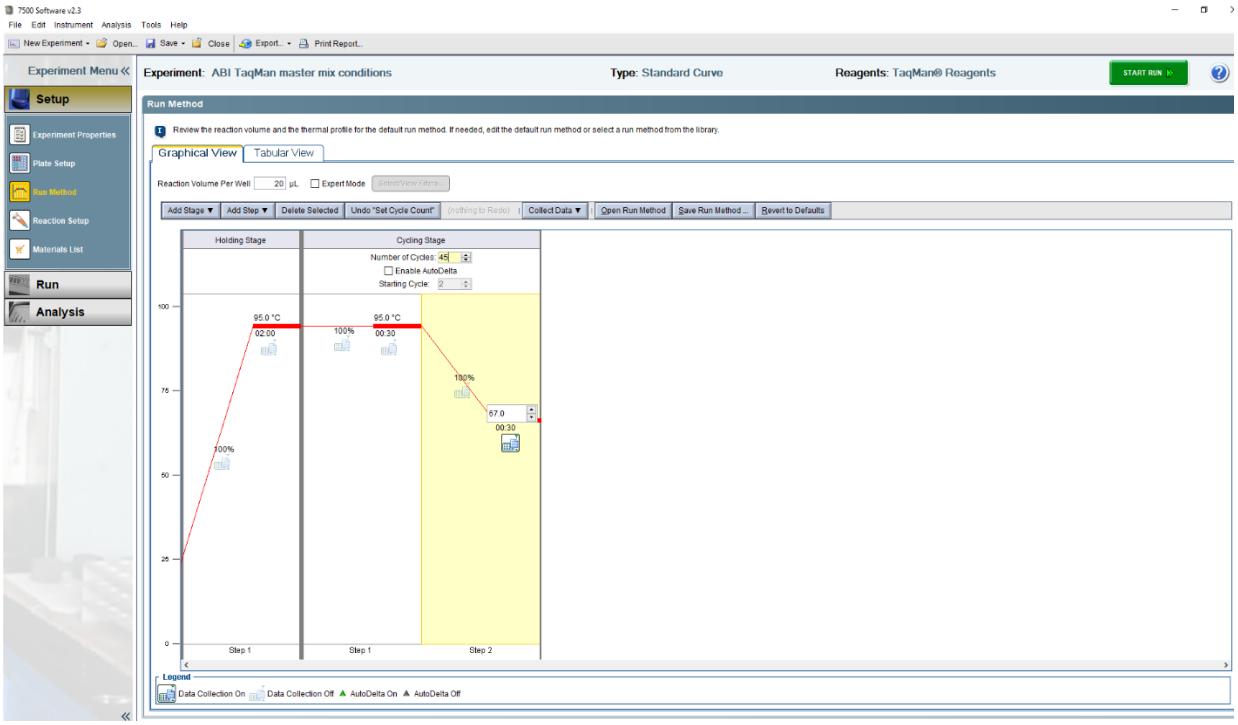


Appendix 5: ABI 7500 Fast v2.0 or 2.3 Method (2 of 8)

Click “Run Method” on left and define cycling parameters as shown below for a 20 µL reaction. Define the program with an initial step of 95°C for 5 min followed by 45 cycles of [95°C for 30 sec + 67°C for 30 sec]. Data collection should be on during the 67°C hold.

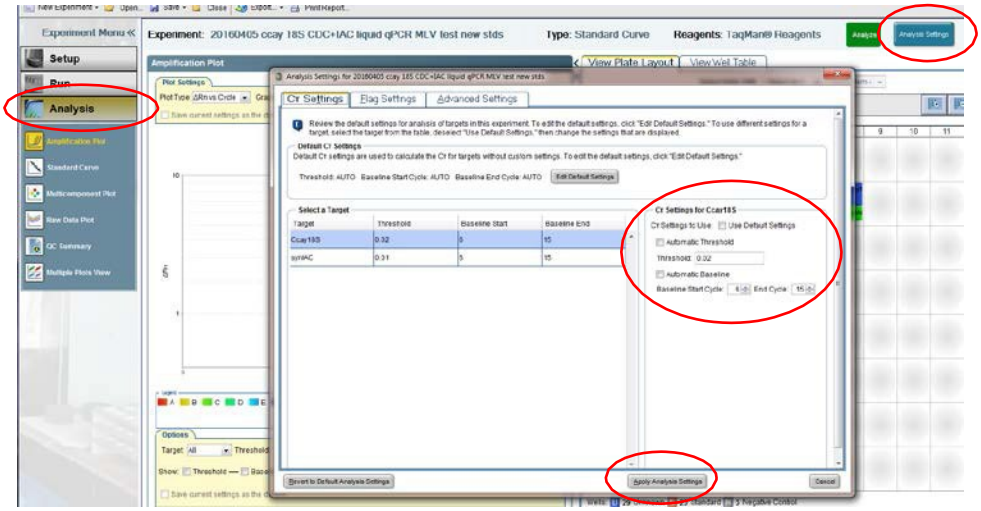


After discontinuation of Qiagen QuantiFast Multiplex PCR Kit (400), Cat No. 204654 by the manufacturer, please use TaqMan™ Fast Advanced Master Mix, ThermoFisher Scientific Applied Biosystem as: Click “Run Method” on left and define cycling parameters as shown below for a 20 µL reaction. Define the program with an initial step of 95°C for **2 min** followed by 45 cycles of [95°C for 30 sec + 67°C for 30 sec]. Data collection should be on during the 67°C hold.



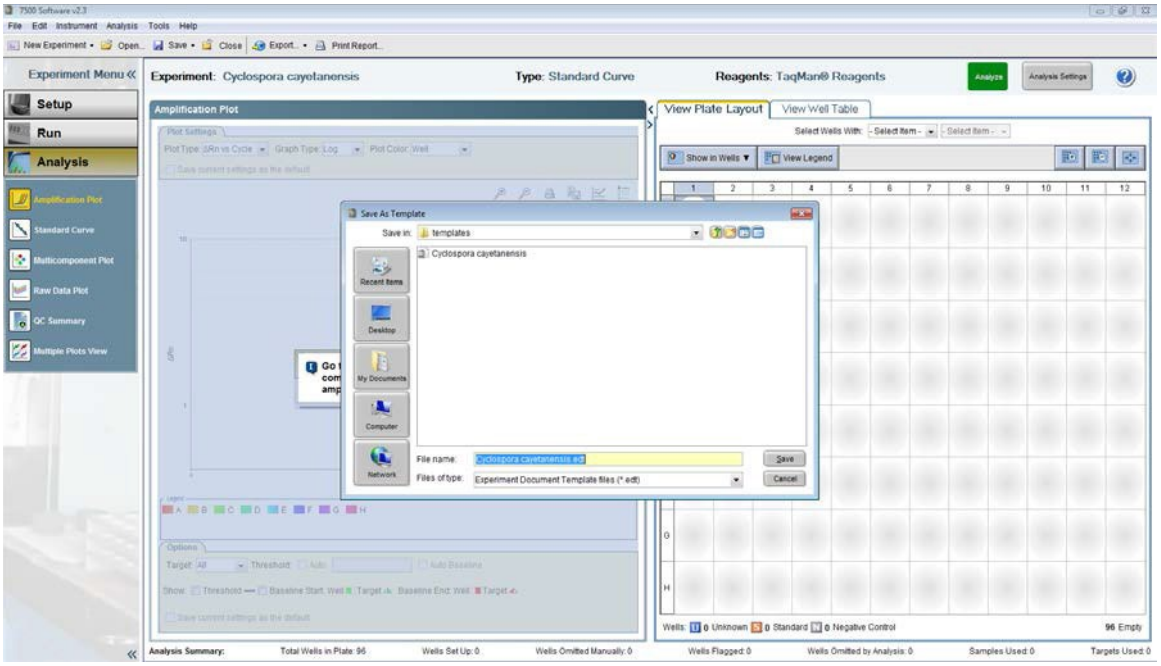
Choose "Analysis" on the left and click "Analysis Settings" in the upper right corner. Define target Ct settings in the pop up window:

1. Select the **Ccay18S** target: Turn **off:** Default Settings, Automatic Threshold, and Automatic Baseline. Set the Threshold to **0.02** and choose manual baseline Start Cycle at **6** and End Cycle at **15.**
2. Select the **synIAC** target: Turn **off:** Default Settings, Automatic Threshold, and Automatic Baseline. Set the Threshold to **0.01** and choose manual baseline Start Cycle at **6** and End Cycle at **15.**
3. Click "Apply Analysis Settings".



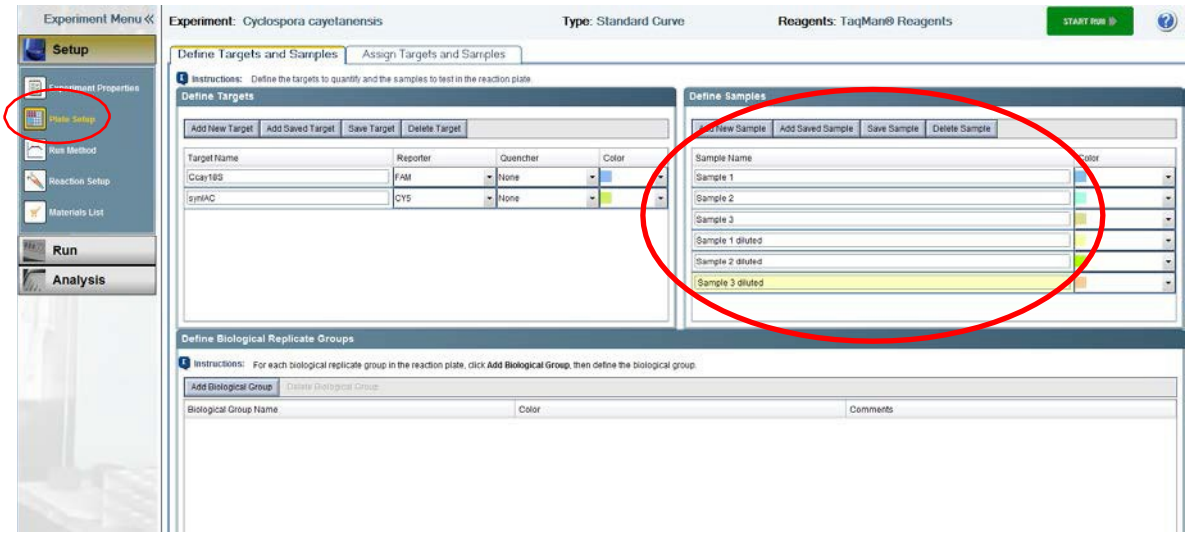
Appendix 5: ABI 7500 Fast v2.0 or 2.3 Method (3 of 8)

Click "File" → "Save as template…" → "Save"



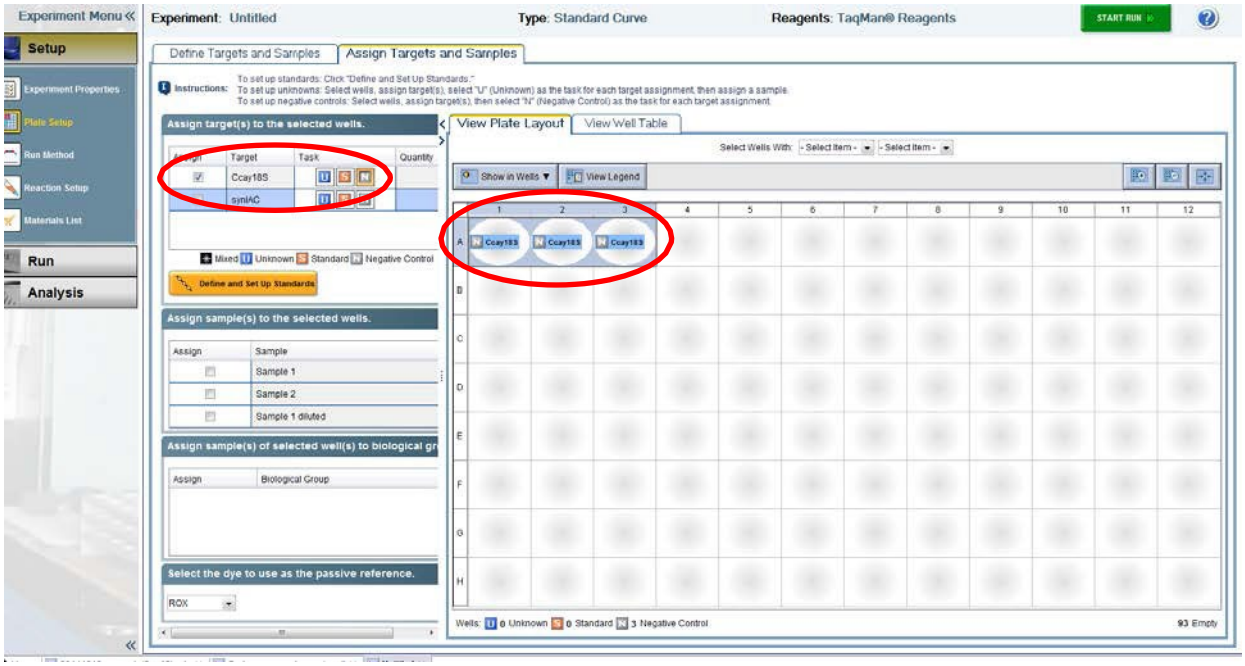
(B) Run Method Using Software v2.0 or 2.3 on the ABI 7500 Fast Instrument

Turn on the computer and ABI 7500 FAST Real-Time PCR system. Open the 7500 Software v2.0 or 2.3 and click "File"→"New Experiment"→"From Template". Choose the "Cyclospora cayetanensis.edt" template file created according to Appendix 2 instructions above. Under "Setup" on the left click "Plate Setup" and define all unknown samples or DNA extraction controls on the plate on the "Define Targets and Samples" tab by clicking "Add New Sample" until all samples are defined.

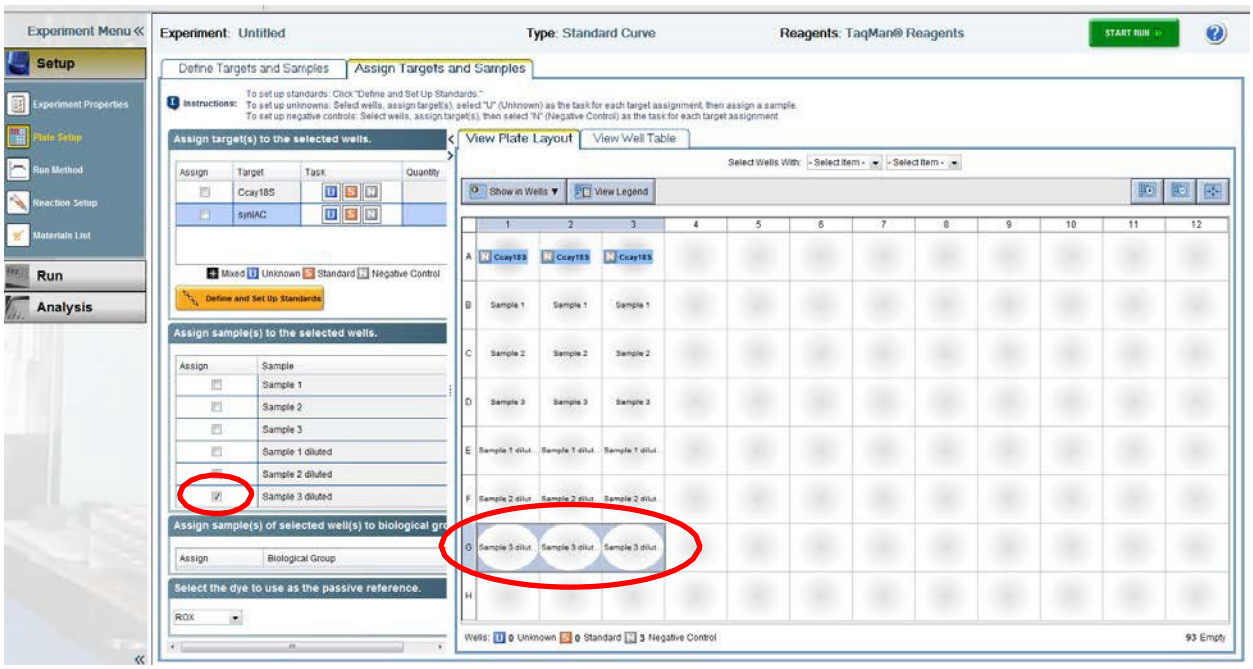


Appendix 5: ABI 7500 Fast v2.0 or 2.3 Method (4 of 8)

Click the "Assign Targets and Samples" tab to define well assignments. Define the NTC wells by selecting three wells and checking the box next to the Ccay18S target choosing "N" as task.

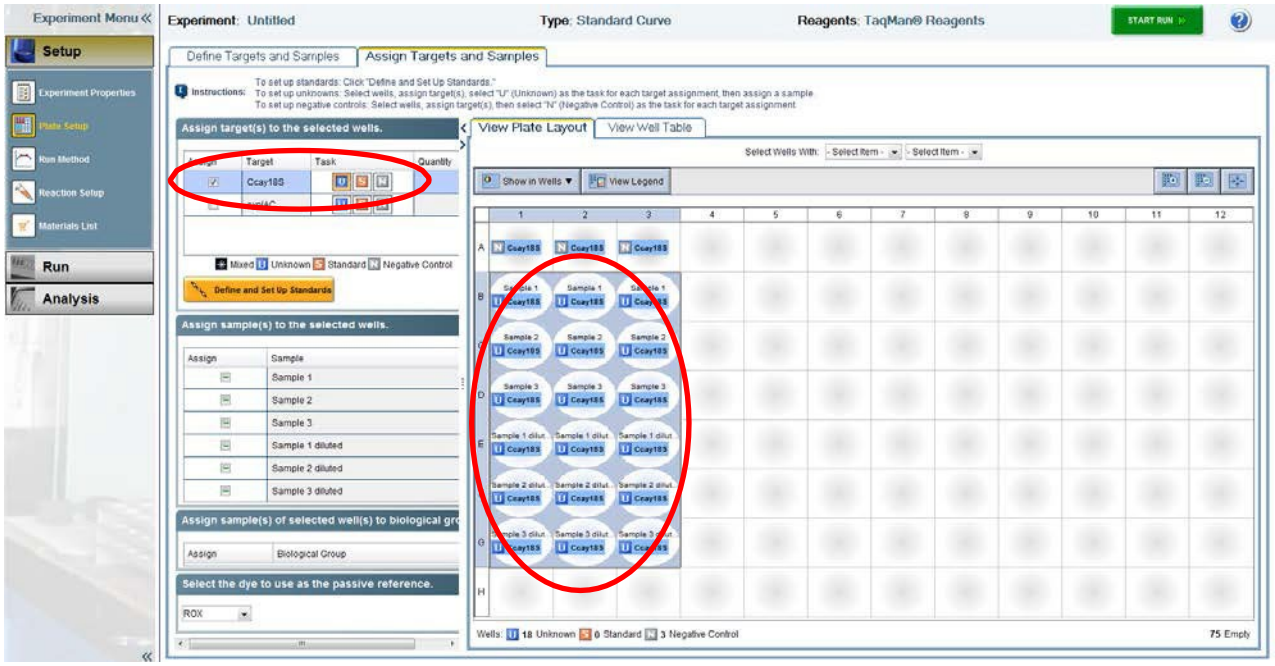


Define all unknown samples or DNA extraction controls one at a time by selecting three wells for each and checking the box next to the sample name in the "Assign sample(s) to the selected wells" panel.

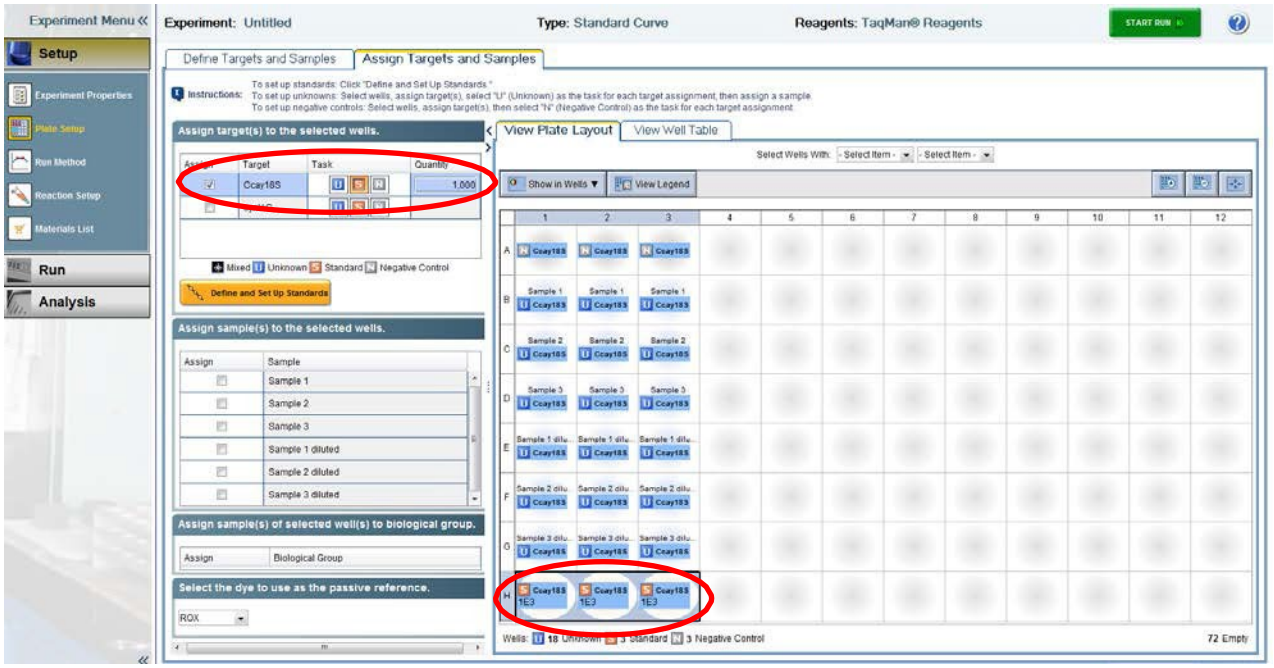


Appendix 5: ABI 7500 Fast v2.0 or 2.3 Method (5 of 8)

Then select all unknown samples or DNA extraction controls and check the box next to Ccay18S target choosing "U" as task.

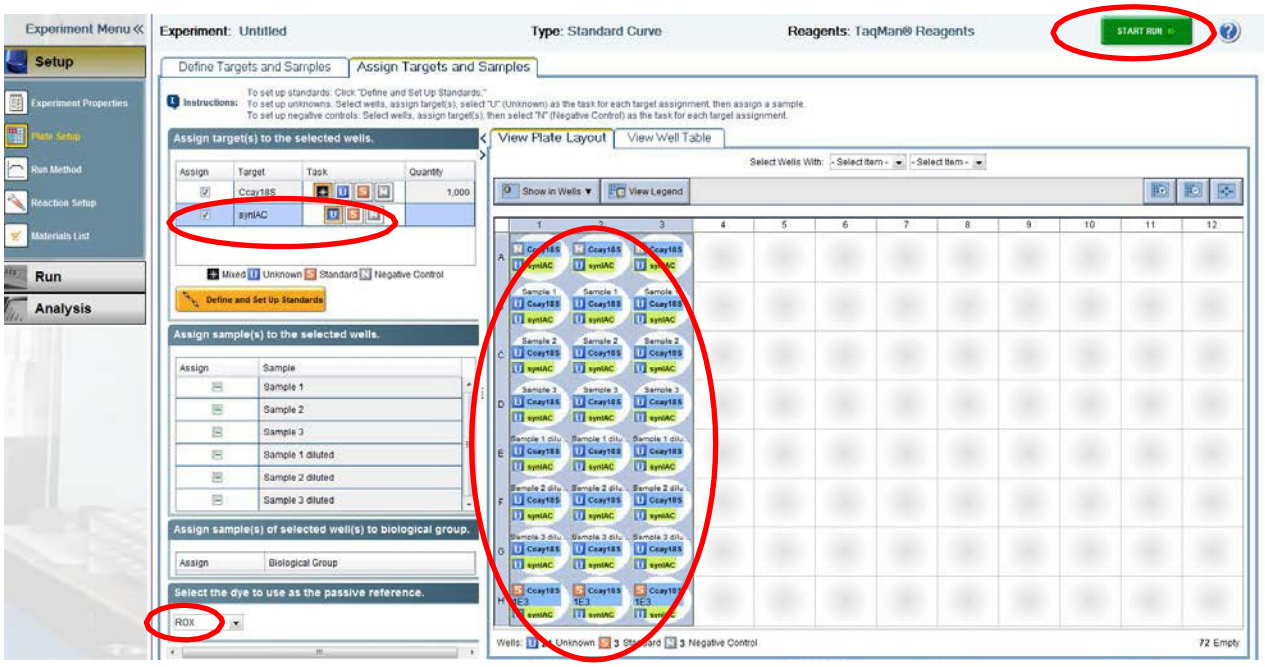


Define the Positive control (Standard) wells by selecting three wells and checking the box next to the Ccay18S target choosing "S" as task and "1000" as quantity.



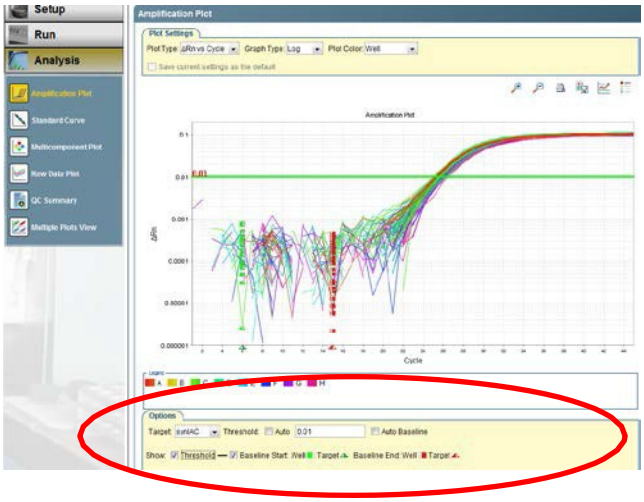
Appendix 5: ABI 7500 Fast v2.0 or 2.3 Method (6 of 8)

Assign the internal amplification control by selecting all reaction wells and checking the box next to the synIAC target choosing "U" as task. Assure that ROX is selected as a passive reference dye. "File"→"Save as"→ Experiment Document Single file (*.eds) with a unique name. Insert plate or tube strips and start the run.



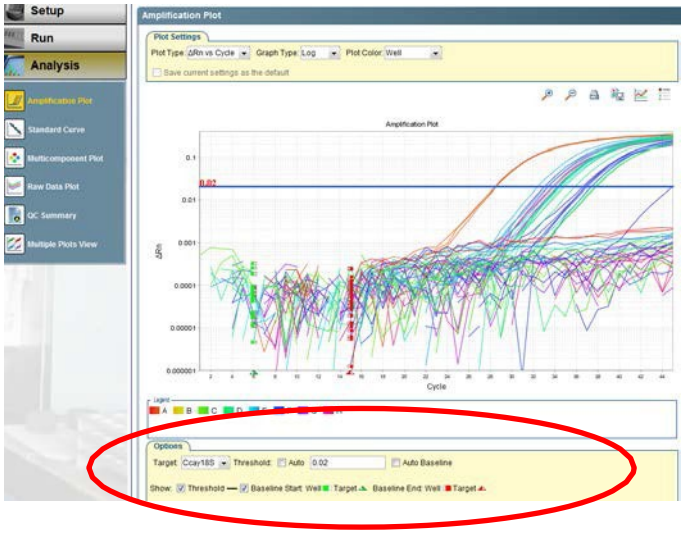
(C) Analysis Using Software v2.0 or 2.3 on the ABI 7500 Fast Instrument

When the run is complete select "Amplification Plot" under "Analysis" on the left. Ensure that all wells are selected on the "View Plate Layout" tab to the right of the amplification curves. In the options panel below the amplifications curves, select the "synIAC" target and check that the show threshold and baseline boxes are both checked. Verify threshold and baseline settings are accurate as defined in the run template above.



Appendix 5: ABI 7500 Fast v2.0 or 2.3 Method (7 of 8)

Next, select the "Ccay18S" target in the options panel and check that the show threshold and baseline boxes are checked. Verify threshold and baseline settings are accurate as defined in the run template above.



Review the amplification plots and Ct's for each target. Verify that all criteria for a valid experimental run are met as defined in the "Interpretation of Results" section of the protocol.

Then, assure that all reaction wells on the plate are selected by clicking the upper left corner of the Plate Layout. Click "Export" to open the Export Tool window.

Appendix 5: ABI 7500 Fast v2.0 or 2.3 Method (8 of 8)

On the "Export Properties" tab, select the following:

1. Select "Results" only.
2. Choose "One File"
3. Name: *use experiment name*. Location: *define a location of your choice*. File type: *choose ".xls"*

Click the "Customize Export" tab and select the following results content: Well, Sample Name, Target Name, Task, Reporter, Ct, Ct Mean, Ct SD.

Click on the "Target Name" column header to sort the table by target name. Click "Start Export". Close the export tool.