Quantification of DNA using PicoGreen

Preparation of Standards

Prepare a 4 ng/ul stock solution of lambda dsDNA in TE. (eg. Add 4 ul of 100 ng/ul standard DNA into 96 ul of TE)
The standards are prepared in 8 wells of a 96 well plate in the following way.

In a new plate:
Add 100 ul 1x TE to 8 wells on a plate.
Add 100 ul lambda DNA (@4 ng/ul) to 1st well. Pipette up and down several times to mix.
Transfer 100 ul from 1st well to 2nd well. Pipette up and down several times to mix.
Repeat transferring and mixing sequentially from 2nd well to the 7th well. This will create a series of 2x dilutions.
Remove 100 ul from well 7 to give a total volume of 100 ul.
Do not transfer any DNA to the last well. This will serve as a blank control.

Well	Final Concentration	DNA Volume	Diluent Volume
1	1.0	100 of stock	100
2	0.5	100 of well 1	100
3	0.25	100 of well 2	100
4	0.125	100 of well 3	100
5	0.0625	100 of well 4	100
6	0.03125	100 of well 5	100
7	0.015625	100 of well 6	100
8	0	0	100

Set standards aside. PicoGreen will be added later.

Preparation of DNA Samples

In a new plate:
Transfer 98 ul 1x TE to each well
Add 2 ul from sample DNA

Set aside.

Preparation of PicoGreen

Prepare PicoGreen in a foil cover bottle. PicoGreen Should be protected from light.
100 ul of diluted PicoGreen is needed per sample/standard.

For each DNA plate to be quantified add:

10 ml 1x TE
50 ul PicoGreen
Mix by swirling bottle.
(An additional 1 ml TE with 5 ul PicoGreen may need to be prepared for the standards.)

Add PicoGreen to DNA samples

Add 100 1x PicoGreen to each well of the DNA/standard plate without contaminating tips.
Mix PicoGreen and DNA by pippetting up and down 5-10 times. (The same tips can be used across the plate provided that the samples are fully expelled between mixings to minimize transfer)

Incubate the plate in the dark at room temperature for 5 minutes (to allow complete binding of fluorescent dye to DNA). Cover the plate and protect from light until measurement.

Using Plate Reader to Read florescence

Turn on plate reader. Wait for the startup self-test to finish. Plate carrier may go in and out by itself. After the self-test is completed, plate carrier will stay outside.

Create new protocol:

File > New Task. Protocol > Create New. Select Calibration Protocol, enter numbers of Calibration plates and Other (sample) plates, and then click OK

Set up for standard plate

Set up reading procedure:
Expand Plate 1 > double click Procedure. Select Plate Type: Nunc 96 flat bottom generic. Click Shake to add a step for mixing samples (optional). Click Read to add a step for reading the plate. Click Full Plate and select/deselect wells with/without samples, then click OK. Click Options and Select Automatic Gain Adjustment and AutoScale, then click OK. Click OK to add the step to procedure. Drag and order the steps if needed. Click OK to finish the procedure setup for plate 1
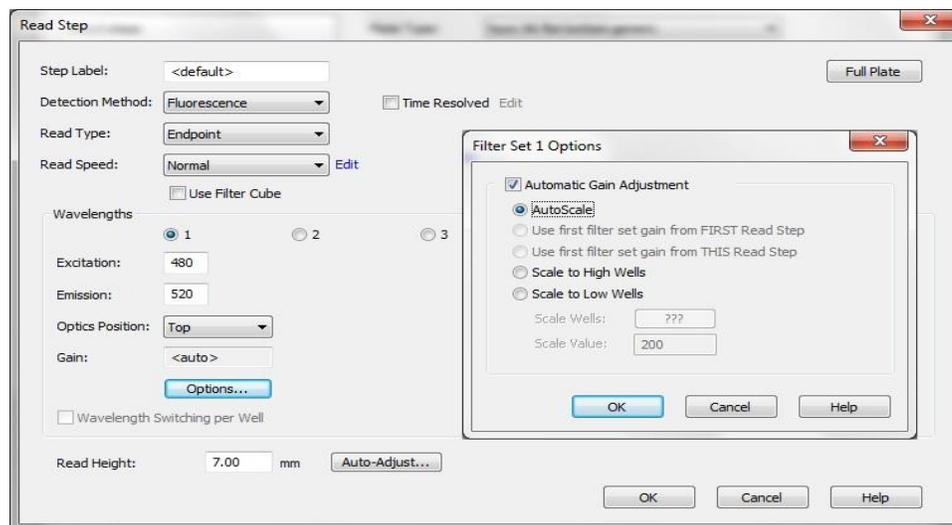
Set up plate layout:
Double click Plate Layout. Check Blanks and Standard Curves, click Next twice. Enter standard concentrations and unit. Click Finish.
Select Blank/Standard on the left and assign to the appropriate well positions. Click OK.

Set up data analysis:
Double click Data Reduction. Click Standard Curve on the left. Data In > Y Axis Data: 480,520. Curve Fit: use default settings. Data Out: use default settings. Click OK

Set up for Other Plates

Double click procedure and use the same step as Plate 1.
Double click Plate Layout. Check Samples, then click Next and Finish. Select the wells with samples.

Click OK.
Double click Data Reduction. Click custom – PLA. Select Use Curve from Calibrator Plate. Click OK.
Click File > Save to save the protocol.

Read the Plates

File > New Task > Read Now > Existing protocol, select protocol file and click Open

Select Plate 1 on the left, then click Read New button on tool bar. Put standard plate in the plate carrier.
Click OK on the screen.
Select Plate 2 on the left, then click Read New button on tool bar. Put sample plate in the plate carrier.
Click OK on the screen.
Select the next plate on the left, repeat the same steps and read all other plates.

Export Results

After reading each plate, a window with signal intensities will pop-up. You can switch to different tabs
to review different statistics in different formats.
To export the results, right click on the table, then click QuickExport. Results will be shown in the
spread sheet program.
Quick exporting another table will append the new results to the end of existing spread sheet.