

STANDARD OPERATING PROCEDURE (SOP) FOR PICOGREEN DNA QUANTIFICATION (MANUAL)

I. SCOPE AND PURPOSE

PicoGreen is an ultra-sensitive fluorescent nucleic acid stain used for quantifying double-stranded DNA (dsDNA). Unlike many other DNA quantitation methods, PicoGreen is minimally affected by the presence of lipid, protein, carbohydrates and other cellular components, making it an ideal method to quantify dsDNA in cell or tissue lysates or other unfractionated matrices.

This SOP establishes a procedure for determining the concentration of double-stranded DNA in a sample and applies to all laboratory personnel. Any deviation from this SOP will be noted on the PicoGreen assay worksheet; the number of the samples affected by the deviation will be noted as well.

II. PROCEDURE

A. Safety Procedures

1. Wear personal protective equipment (PPE), which includes lab coats and gloves.
2. Picogreen is a potential carcinogen. It is dissolved in DMSO, which is known to facilitate movement of organic molecules through the skin. Wear nitrile gloves. Do not wear latex gloves.
3. Wash hands after handling PicoGreen.

B. Quality Control

1. All new lots of reagents are tested in parallel with the one in current use before being put into use. Results are recorded in QC log. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be used interchangeably between kit lot numbers.
2. Positive and negative controls are run with each assay for quality control. If either control fails (positive is outside of the acceptable range or negative has a concentration higher than "0"), the results will not be used and the entire assay will be repeated.
3. Samples which are within the acceptable DNA concentration range, but have a CV of greater than 0.1 between duplicate readings will be repeated using the same prepared dilution.
4. Worksheets are used to prepare each assay. Lot numbers, concentrations and expiration dates of reagents used are recorded where applicable. Unusual observations in set up of assays are also noted on these worksheets. All control samples are tested at the same time, in an identical manner and by the same technologist as the samples included with each assay group.

C. Reagents, Equipment, And Supplies

1. Single channel pipettes
2. Multi-channel pipettes (only to be used where noted)
3. Pipet-Aid

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4. Pipette tips, assorted sizes
5. Serological pipettes, assorted sizes
6. Sample to be analyzed
7. Picogreen assay kit (Invitrogen, P7589)
 - a. Picogreen Reagent
 - b. Tris-EDTA Buffer (10 mM tris, 1 mM EDTA), pH8.0, 20X
 - c. 100 ng/ μ L of Lambda (λ) DNA, sheared by intensive vortexing
8. 50 mL conical tube
9. 1.5 mL micro-centrifuge tubes
10. Water nuclease-free (Sigma, W4502-6X1L)
11. FilterMax F5 Multi-Mode Microplate Reader (Molecular Devices)
12. Excitation Filter – EXP1 F535/25, F485/20, F485/20, F370/TRF
13. Emission Filter – EMP1 F595/35, F535/25, F535/25, F535/25, F625/35
14. Grenier Bio-one black flat bottom 96 well plate (Bio-Express, T-3025-16)
15. Foil seal (BioRad, MSF-1001)
16. ABgene 96-well PCR Plate, Non-Skirted (Fisher, AB-0600L)
17. Reservoirs, Sterile, 25 mL with divider (Diversified Biotech, RESE-2000)
18. CentriVap DNA Concentrator (Labconco, or equivalent)
19. Vortex Mixer
20. Minifuge Centrifuge
21. Centrifuge for spinning 96-well plates

D. Dilution of Reagents

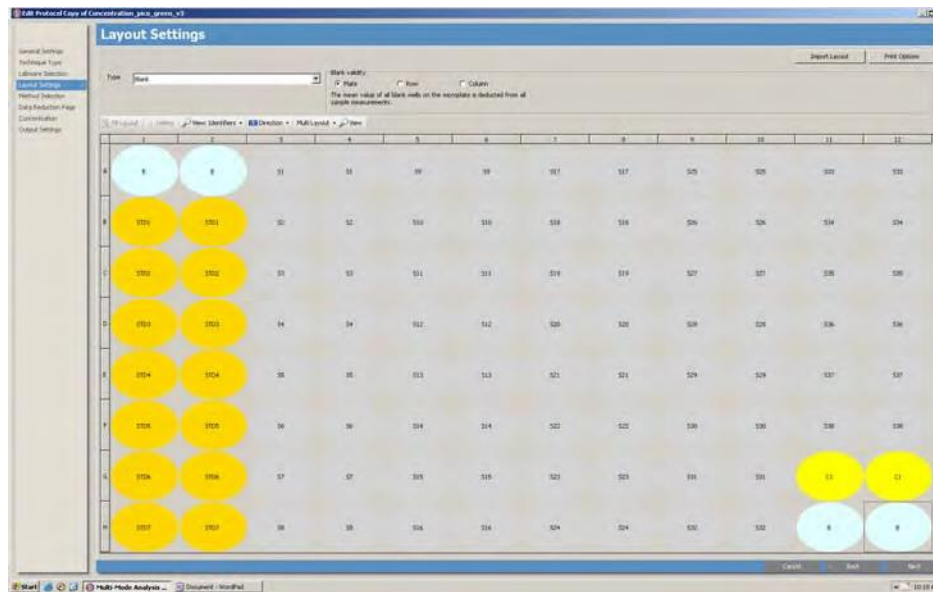
1. Preparing 1X TE from 20X TE Stock:
 - a. To make a 50 mL aliquot: add 2.5 mL 20X TE to 47.5 mL sterile water.
 - b. To make 1 Liter: add 50 mL of 20X TE to 950 mL of sterile water.
 - c. 1X TE can be stored at room temperature and expires one year from date prepared. The container should be labeled with the Stock 20X TE lot number, date prepared, date of expiration, applicable hazards warnings, and preparer initials.
2. Dilute the PicoGreen reagent 1:200 by adding 5 μ L of stock dye per 1 mL 1X TE. PicoGreen is a light sensitive dye and should be protected from light when possible. Keep this reagent in the dark until ready for use.
 - a. Each well used will need 100 μ L of the diluted PicoGreen reagent; prepare enough 1:200 PicoGreen for number of samples to be analyzed (including controls and calibration standards).
 - b. The PicoGreen reagent is stored refrigerated and expires one year from date opened.
3. 100 ng/mL DNA standard is stored refrigerated and expires one year from date opened.

III. PROCEDURE STEPWISE

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A. Preparation of Samples

- Each sample is run in duplicate (two separate dilutions, both run once on the same plate). For example, sample #1 (S1) will be run in wells A3 and A4, sample 2 (S2) in wells B3 and B4, and so on throughout the plate up to 38 samples per plate. A duplicate reading of the control DNA used to make the standard curve is run in wells G11 and G12. This control comes with the kit at a concentration of 100 ng/μL. If the average value of these duplicates falls outside 15% of that (85-115 ng/μL) the plate must be repeated. A negative control is also read in wells H11 and H12, where no control DNA or sample is added. See the example below.



- All samples to be run will be filled in on a Sample PicoGreen Worksheet (Located: N:\Molecular Genetics\BCR\Picogreen\CCG BCR\Blank worksheet for Fridge, example shown below). All sample IDs and volumes must be documented before beginning.

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	A	B	C	D	E	F	G	H	I	J	K	L	M
1	BCR PicoGreen:												
2	Date:												
3	Initials:												
4		1	2	3	4	5	6	7	8	9	10	11	12
5		Standard Curve		Sample	Vol	Sample	Vol	Sample	Vol	Sample	Vol	Sample	Vol
6	A	STD Blank	STD Blank										
7	B	STD 15.625 ng/ul	STD 15.625 ng/ul										
8	C	STD 31.25 ng/ul	STD 31.25 ng/ul										
9	D	STD 62.5 ng/ul	STD 62.5 ng/ul										
10	E	STD 125 ng/ul	STD 125 ng/ul										
11	F	STD 250 ng/ul	STD 250 ng/ul										
12	G	STD 500 ng/ul	STD 500 ng/ul								control	control	
13	H	STD 1000 ng/ul	STD 1000 ng/ul								0	0	
14													
15				Reagent		Lot #		Exp date					<input type="text"/>
16				1XTE									Manage
17				DNA Standard (100ng/ul)									Quantity
18				PicoGreen Reagent									
19													

3. Make a DNA dilution plate according to the prepared plate layout using a 96-well PCR plate. Add 49 μL of 1X TE and 1 μL of stock sample DNA or control DNA for each dilution as appropriate. Control DNA is placed in positions G11 and G12. Pipet all dilutions up and down to mix.
4. Cover the plate with a foil seal, vortex and spin down.

B. Preparation of Standards

1. The first two columns of each plate are used to make a serial dilution in duplicate of the standard curve. Add 100 μL 1X TE into all wells of columns 1 and 2 (A1 through H1 and A2 through H2) of a black flat bottom Greiner plate (PicoGreen plate).
2. Add an additional 96 μL 1X TE into wells H1 and H2 of the same plate.
3. Pipette 4 μL of standard DNA (100 ng/ μL) into wells H1 and H2 and mix by pipetting. Wells H1 and H2 will be the highest points of the standard curve.
4. Serial dilutions are then prepared by transferring 100 μL of standard beginning from well H1 to G1, and H2 to G2 using a multichannel pipet. Pipet up and down to mix.
5. Continue the serial dilution up the plate transferring 100 μL from G1 to F1, F1 to E1, E1 to D1, D1 to C1 and C1 to B1 (same steps with column 2), mixing by pipetting in between each transfer. At this point take the extra 100 μL from wells B1 and B2 and discard, leaving A1 and A2 as blanks. Now all wells in the first two columns of the PicoDNA plate should have a volume of 100 μL .
6. This set of dilutions will give you a standard curve from 0-1,000 pg/ μL . Adding the subsequent dilutions made to the samples and plates, another 1:1,000 dilution (1:50 dilution for the stock DNA plate, 1:10 dilution when that stock DNA plate is added to

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the pico plate, and an additional 1:2 dilution when the pico green is added to the plate) is applied and adjusts the standard curve to read from 0-1000 ng/μL.

- a. H1/H2 – 1,000 ng/μL
- b. G1/G2 – 500 ng/μL
- c. F1/F2 – 250 ng/μL
- d. E1/E2 – 125 ng/μL
- e. D1/D2 – 62.5 ng/μL
- f. C1/C2 – 31.25 ng/μL
- g. B1/B2 – 15.625 ng/μL
- a. A1/A2 – blank

NOTE: The software used to analyze the data is programmed with the necessary factors to correct for concentration and dilutions applied to samples/controls on the bench.

C. Addition of Diluted DNA samples to prepared Black flat-bottom 96-well “PicoGreen” Plate

1. To the black flat bottom PicoGreen plate (containing prepared standard serial dilutions), add 90 μL of 1X TE to all wells that will have samples or controls.
2. Add 10 μL of the diluted DNA from the 96-well PCR plate to the black flat bottom PicoGreen plate according to the plate layout. Pipet up and down to mix.
3. Add 100 μL of the prepared PicoGreen reagent to all samples, controls, and standards. Pipet up and down to mix, avoiding the addition of bubbles.
4. Immediately proceed to Section D (assay is time sensitive once PicoGreen has been combined with DNA samples).

D. Load samples into the Fluorimeter plate

NOTE: the FilterMax should be turned on and the software opened prior to adding PicoGreen to samples so the incubation time of the PicoGreen plate can be controlled.

1. Preparing FilterMax 5 Software:
 - a. Samples will be measured using the excitation at 485 nm and the emission at 535 nm with the Molecular Devices FilterMax F5 Fluorimeter (see SOP MGL-EQP-31).
 - b. Turn on the power to the FilterMax F5.
 - c. Once the FilterMax F5 has completely powered on, double-click on the Multi-Mode Analysis Software.
 - d. Select the protocol “Concentration_pico_green_v3.”
 - e. Click Run.
2. Click “Eject Plate Carrier.”
3. Load prepared PicoGreen plate (step C.4) onto the FilterMax F5 to be read with position A1 in the upper right hand corner.
4. Click “Close Plate Carrier.”

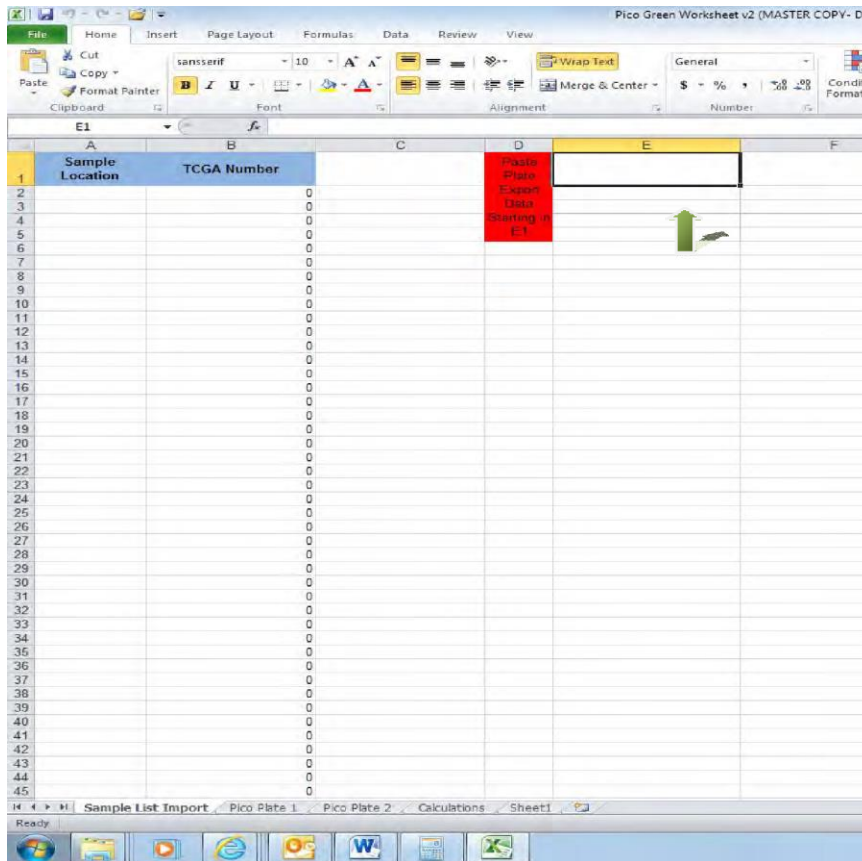
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5. Click “Run.” The program is designed to incubate the samples for 5 minutes before reading. (If the plate has already incubated for the appropriate time, this step can be skipped by clicking the next/skip step button in the software.)
6. Once the program is completed, record the “Correlation” and “R²” values on the worksheet. Verify that the standard curve fits a linear model. If not, the entire plate will need to be repeated.
7. Four files will appear after the run. Save the file labeled “Sheet 2” to a flash drive, naming with the date and the number of the run for that day (i.e. 031415_1). This file includes the concentration data for each sample. Use the flash drive to open the data file on a networked computer. Save a copy of the raw data in the file folder LabShared\Molecular Genetics\BCR\Picogreen\RAW PICO Data.

E. Data Analysis

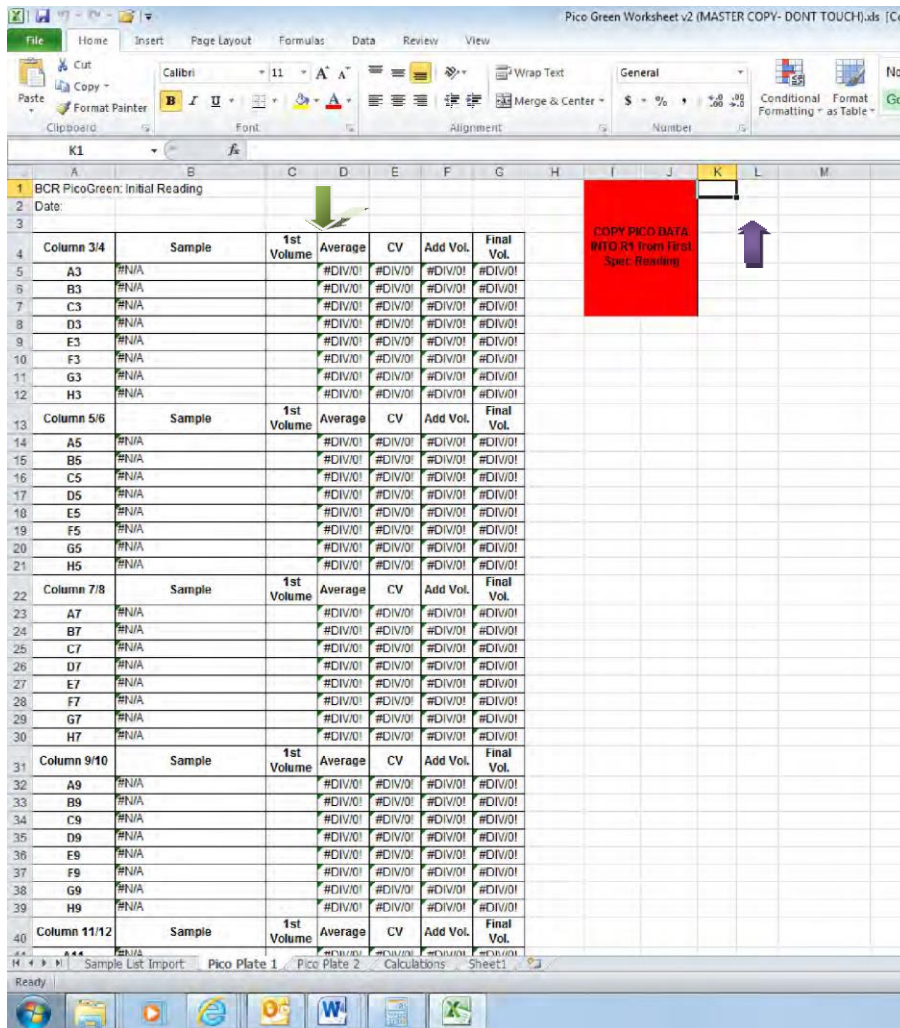
1. A spreadsheet has been created that will calculate the average concentration values, and normalization volume needed for each sample. This can be found on the Molecular Genetics Shared Drive at N:\Molecular Genetics\BCR\Picogreen\CCG BCR\PICO GREEN WORKSHEET v2.xls.
2. Log into LabVantage.
3. Under Sample Management, click on Lab Operations.
4. Under the SearchQuery, click on SamplesByBoxSorted.
5. Type in the box number of the PicoGreen plate.
6. Select all the samples and place them into a new folder.
7. Select all the samples and click on “View as Excel” on the far left side. Click Open.
8. Copy all data and paste into E1 (as instructed by the template) on the Sample List Import tab.

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9. The sample information is automatically transferred to the next tab in the spreadsheet. Confirm that all sample ID's were assigned to the correct position on the "Pico Plate 1" tab and that these positions correspond to the position of the physical tubes in the plate. Enter the beginning volume for each sample in the appropriate cell in column C of "Pico Plate 1" tab using the Sample PicoGreen Worksheet.

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10. Copy the raw data saved in Step D7 and paste into cell K1 (as instructed by the template) on the Pico Plate 1 tab (example shown above). The template also includes a second tab (Pico Plate 2) that will allow documentation for the second round of PicoGreen results after normalizing.
11. If samples are outside the standard curve (concentration greater than 1,000 ng/ μL) they will need to be diluted and run again to obtain an accurate concentration.
12. This worksheet uses the average concentration value from the duplicate dilutions for each sample to normalize the sample to 160 ng/ $\mu\text{L} \pm 15$ ng/ μL using the following calculation:

$$((\text{current concentration}/\text{desired concentration}) \times \text{current volume}) - \text{current volume}$$

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- a. This calculation will give the amount of diluent (0.1X TE) to be added for samples that are ≥ 175 ng/ μ L, or the target volume for concentrating samples that are ≤ 145 ng/ μ L.
 - b. Concentration ranges may vary between projects, and as such the target concentration and range used for calculations can be adjusted if required.
13. After adjusting the volume to normalize the sample, record the adjusted volume. Repeat this procedure to confirm that the target concentration has been achieved.
14. A printed copy of all assay data sheets is kept in the most recent Picogreen Data Binder in the lab. An electronic copy is kept in the Molecular Genetics Share Drive under N:\Molecular Genetics\BCR\Picogreen\CCG BCR named with the run date and the plate number(s) included.

IV. REFERENCES -None

V. COMPREHENSIVE REVISION HISTORY

A. Changes made to Version # 3 Effective Date 7/28/2015

- a. Updated formatting
- b. Updated disclaimer
- c. Removed TCGA reference
- d. Updated location of Pico Green Worksheet
- e. Added reagent preparation of 1 liter of 1X TE
- f. Added description on creating the Pico Green Worksheet in the data analysis section.
- g. Updated loading the plate into fluorimeter to include instruction on using the software.

B. Changes made to Version # 2 Effective Date _____

1. New Format Used
2. Minor word changes made throughout
3. In section II.F.1.
 - a. Number of separate dilutions each sample is run was changed from triplicate to duplicate
 - b. Average value is now used to determine repeat rather than median value
4. Section II.F.2.: An excel file directly from LabVantage is used to fill in the names of the samples run on the pico plate.
5. Section II.I.3: Concentration ranges were changed.

C. Version #1, Effective Date 10/2/2012 - New

Effective Date: 7/28/2015

Biospecimen Core Resource



**M017
Version 3**

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Signatures

Approved By:

Signature on file

Date:

Date on file

**Julie Gastier-Foster, PhD, FACMG
Principal Investigator**