Document Number: SASoM/METHOD/079.v4

Title: DNA quantification using QubiT fluorometer 2.0 for GeCKO library

Version: v4

Author: Paul Reynolds

Effective from:	19/04/2021
Valid to:	19/04/2023

SOP History		
Number	Date	Reason for Change
v1	02/04/2015	Original
V2	02/04/2017	Update
V3	02/04/2019	Update
V4	19/04/2021	Update

1.0 Purpose –

This SOP describes the current procedure for DNA quantification using Qubit fluorometer 2.0 for GeCKO library genetic screen in Laboratory 248 and 249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff in the SASoM involved in DNA quantification for loss of function genetic screen using GeCKO library.

3.0 Responsibilities –

All staff are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

Use Qubit® dsDNA BR Assay Kit (Invitrogen)

Catalog no. Q32850

For 14 samples and 2 standards:
Prepare 16 (0.5 ml) QubiT assay tubes (Cat. no. Q32856 or ask to Scott Millar in Lab 248), and label lids.

Dilute dsDNA BR reagent in dsDNA BR buffer to 1/200 in a plastic tube = Working solution (WS)
~200 µl total solution are needed per tube therefore prepare for 18 tubes 3.6 ml Working Solution: = 3582 µl buffer + 18 µl dsDNA BR reagent.

Put 190µl of Working Solution into both 'standards' tubes. To each one add 10 µl of the appropriate standard DNA and mix by vortexing for 2-3s.

Put 198 µl of Working Solution into the 14 remaining tubes. Add 2 µl of appropriate sample and vortex for 2-3s (avoid bubbles!).

Incubate all 16 tubes at room temperature for 2 min.

On the QubiT fluorometer (Lab 249):
Press 'DNA' and then select assay type: dsDNA Broad Range.

The Standard screen is then automatically displayed: press 'Yes' to run a new calibration.

Insert tube 'Standard 1' into the fluorometer, close the lid and press Read (~3sec).

Remove 'Standard 1' tube.

Insert the tube containing 'Standard 2' and repeat the previous step.

Remove 'Standard 2' tube.

Insert sample tubes one by one in the fluorometer, close the lid and press Read --->

Measurement will display on the screen (the value is the concentration of the diluted sample in the working solution in ng/ml).

Click 'Read next sample' between each sample (data are automatically saved in the QubiT fluorometer: Data).

Calculate sample concentration= QF value × (200/ x)

QF value = value given by the fluorometer
x= vol of sample added to the assay tube (2 µl)

Original sample concentration can also be calculated directly by QubiT fluorometer

CALCULATING THE ORIGINAL SAMPLE CONCENTRATION

The Dilution Calculator feature of the Qubit® 2.0 Fluorometer calculates the concentration of your original sample based on the volume of sample you have added to the assay tube.

Dilution Calculator

1. To calculate the concentration of your original sample, press Calculate Stock Conc. The Dilution Calculator Screen containing the volume roller wheel is displayed.
2. Using the volume roller wheel, select the volume of your original sample that you have added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.
3. To change the units in which the original sample concentration is displayed, press ng/mL. A pop-up window showing the current unit selection (as indicated by an adjacent red dash) opens.
4. Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up. The Qubit® 2.0 Fluorometer automatically converts the units to your selection when the unit selection pop-up window is closed. Note: The unit button next to your sample concentration reflects the change in the units (e.g., if you change the unit to pg/µL, the button displays pg/µL).
5. To save the data from your calculation, see 'Saving the Calculation' to the Qubit® 2.0 Fluorometer, next page.
6. To exit the Dilution Calculator Screen, press any navigator button on the bottom of the screen or Read Next Sample. Note: When you navigate away from the Dilution Calculator Screen, the Qubit® 2.0 Fluorometer saves the last values for the sample volume and the units in the Dilution Calculator Screen only. Returning to the Dilution Calculator Screen displays these last selected values.

Saving the Calculation to the Qubit® 2.0 Fluorometer

1. To save the data from your calculation to the Qubit® 2.0 Fluorometer, press 'Save' on the Dilution Calculator Screen. The last calculated value of your measurement is saved in the .CSV file and tagged with a time and date stamp.
2. To review, rename, and save the data to the Qubit® 2.0 USB drive, see Data Handling, next page.

DATA HANDLING

The Qubit® 2.0 Fluorometer presents comprehensive data with graphic reports, and it allows saving of data as a .CSV (comma separated value) file for sample comparisons. Each measurement data point in the .CSV file is numbered and exhibits a time and date stamp.

Reviewing the Data

1. To review the data saved on the Qubit® 2.0 Fluorometer, press 'Data' on any screen. The Data Screen displays the data in a spreadsheet format with additional data columns hidden to the right. The most recent data point is displayed in the first line of the spreadsheet.
2. Drag the scrollbars with a finger to move continuously across the spreadsheet to access the data columns hidden to the right.

Renaming Data Files

1. Select the data file to rename by touching the corresponding line of the spreadsheet on the Data Screen. The selected line will be highlighted. If no lines are selected, you are prompted to select a line or multiple lines to rename. Note: To select multiple files, highlight each line by-one-one or press the column headers to highlight all files. To deselect all rows, simply press the column header again.

2. Press 'Rename' to enter a name for the selected line. Alphabet Keys Screen is displayed. To switch to the Numeric Keys Screen, press 123.

3. Enter the file name using the keypad buttons displayed on the Save menu. The maximum number of characters you can enter is 20. Note: If you have selected to rename multiple lines at once, files will be differentiated by numbers at the end of the name. The numbers will appear in ascending order, with the most recent sample starting with 1 (e.g., SAMPLE_1, SAMPLE_2, etc.).
4. Press OK to save the name you have entered as the file name and to go back to the Data Screen.

Saving the Data to the Qubit® 2.0 USB Drive

The Qubit® 2.0 Fluorometer is designed for stand-alone use; it does not require the use of an external computer. However, to archive data and generate reports, you may transfer the numeric data stored in the .CSV file to your computer using the USB drive and import the file into any spreadsheet program. To archive your data:

1. Insert the Qubit® 2.0 USB drive into the USB port.
2. Press Data to access the Data Screen. Note: A green dot on the USB icon indicates that the instrument recognizes the USB drive; a red dot indicates that the USB drive is not inserted into the USB port or that the instrument does not recognize the USB drive. Continued on next page 28
3. Select the data file to save by touching the corresponding line of the spreadsheet on the Data Screen. The selected line is highlighted. Note: By default, the entire .CSV file will be saved to the USB drive if no lines are selected.
4. Save your data on the USB drive by pressing the USB icon. The numeric data is automatically saved as a .CSV file that can be opened with any spreadsheet program.
5. To delete all data from the .CSV file and start with a blank file, press the Clear Data button.
6. Transfer the Qubit® 2.0 USB drive to the USB port on your PC. You may open the .CSV file using a spreadsheet program.

5.0 Personal protection -
A Howie laboratory coat and lab gloves must be worn at all times.

6.0 Spillages -
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

7.0 Training -
All users must be fully trained in cell culture. All users must be fully trained in the handling of virus cultures.

8.0 Related documents –

8.1 Risk assessments:
RA/BIOL/001 - Handling of bacteria

RA/BIOL/004 - Tissue culture

RA/GM/002 - Viral-mediated gene delivery into mammalian cells

8.2 SOPs:
SASoM/METHOD/075 - GeCKO lentiCRISPRv2 library DNA amplification in Lucigen bacteria.

SASoM/METHOD/077 - GeCKO genomic DNA purification Qiagen Midi kit.

SASoM/METHOD/078 - Transduction of GECKO Library in MiaPaCa2 cells.

SASoM/METHOD/079-DNA quantification using QubiT fluorometer 2.0 for GeCKO library.

SASoM/METHOD/080- PCR for GeCKOv2 library preparation for Next generation sequencing.

SASoM/METHOD/081-sgRNA Target Guide Sequence Cloning into pLentiCRISPRv2.

SASoM/METHOD/082-AMPure PCR purification for GeCKOv2 NGS.

SASoM/METHOD/083-Quality control of GeCKOv2 for NGS using Agilent Bioanalyzer system.

9.0 Approval and sign off –

Author:

Name: Paul Reynolds

Position: Principal Investigator

Signature:  Date: 19/04/2021

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 19/04/2021

QA release by:

Name: Peter Mullen

Position: QA Manager

Signature:  Date: 19/04/2021

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE