Document Number: **SASoM/METHOD/083.v4**

Title: **Quality control of GeCKOv2 library for Next Generation Sequencing (NGS) using the Agilent 'Bioanalyzer' system.**

Version: **v4**

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SOP History		
Number	Date	Reason for Change
v1	05/08/2015	Original
v2	05/08/2017	Update
V3	05/08/2019	Update
V4	05/08/2021	Update

1.0 Purpose –

This SOP describes the current procedure for Quality control of GeCKOv2 library for Next Generation Sequencing (NGS) using the Agilent Bioanalyzer.

2.0 Scope –

This SOP applies to the staff in the SASoM involved in Quality control of GeCKOv2 library for NGS using the Agilent Bioanalyzer system in Laboratory 248 at the St Andrews School of Medicine (SASoM).

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 –

This protocol refers to the quantitation and sizing of AMPure-purified secondary PCR products related to GeCKOv2 library. This analysis should be performed after the QubiT (Picogreen) quantitation assay (SASoM/METHOD/079).

Equipment: Agilent 2100 Bioanalyzer System
- Chip priming station (Lab 249 cat # G2940CA)
- IKA Vortex mixer
- Agilent High Sensitivity DNA Kit (cat # 5067-4626)

For accurate determination of DNA concentration, the total DNA in each of the samples analysed must be between 100pg/µL to 10ng/µL. Dilute DNA prior to quantitation if necessary.

Each chip can analyse 11 samples at a time, therefore load 7 samples in 2 chips (total of 14 samples).

Preparing the Gel-Dye Mix

1. Allow High Sensitivity DNA dye concentrate (blue) and High Sensitivity DNA gel matrix (red) to equilibrate to room temperature for 30 min.

2. Vortex the High Sensitivity DNA dye concentrate vial (blue cap) for 10 seconds and spin down. Make sure the DMSO is completely thawed.

3. Pipette 15µl of the dye concentrate (blue cap) into a High Sensitivity DNA gel matrix vial (red). Store the dye concentrate at 4 °C in the dark again.

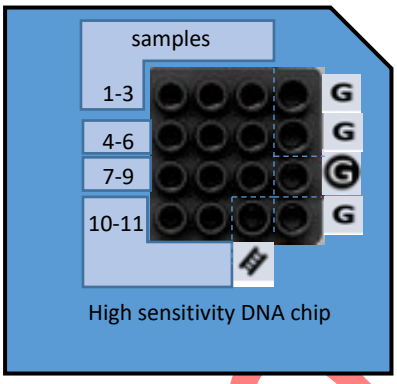
4. Vortex the tube for 10 seconds. Visually inspect proper mixing of gel and dye.

5. Transfer the complete gel-dye mix to the top receptacle of a spin filter.

6. Place the spin filter in a microcentrifuge and spin for 10 minutes at room temperature at 2240 g ± 20 % (for Eppendorf microcentrifuge, this corresponds to 6000 rpm).

Protect the gel-dye mix from light. Store the gel-dye mix at 4 °C when not in use for more than 1 hour. Use the gel-dye within 6 weeks of preparation.

Loading the Gel-Dye Mix into the High Sensitivity DNA Chip.



1. Allow the gel-dye mix to equilibrate to room temperature for 30 min before use.

2. Put a new High Sensitivity DNA chip (see above) onto the chip priming station.

3. Pipette 9.0 µl of the gel- dye mix at the bottom of the well marked G and dispense the gel-dye mix.

4. Make sure that the plunger of the syringe on the chip priming station is positioned at 1 mL and then close the chip priming station. The lock of the latch will click when the Priming Station is closed correctly. *NB: The syringe is used to force the gel-dye loaded into the well marked* G *into all the passageways inside the chip (pressurize).*

5. Press the plunger of the syringe down until it is held by the clip.

6. Wait for exactly 60 s then release the plunger with the clip release mechanism.

7. Visually inspect that the plunger moves back at least to the 0.3 ml mark.

8. Wait for 5 s, then slowly pull back the plunger to the 1 mL position.

9. Open the chip priming station and pipette 9 µL of gel-dye mix in the wells marked G *This will enable to fill the microchannel network connecting the wells with the gel-dye mix*.

Loading the Marker

Pipette 5 µL of High Sensitivity DNA marker (green cap) into the well marked with the ladder symbol and into each of the 11 sample wells.

Loading the Ladder and Samples

1. Pipette 1 µL of High Sensitivity DNA ladder (yellow) in the well marked

2. In each of the 11 sample wells, pipette 1 µL of sample (7 used wells) or 1 µL of High Sensitivity DNA marker (green cap) in the 4 remaining wells (unused wells).

3. Place the chip horizontally in the adapter of the IKA vortex mixer and make sure not to damage the buldge that fixes the chip during vortexing.

4. Vortex for 1 min at 2400 rpm.

5. Run the chip in the Agilent 2100 Bioanalyzer instrument within 5 min.

Inserting a Chip in the Agilent 2100 Bioanalyzer

1. Open the lid of the Agilent 2100 Bioanalyzer.

2. Place the chip carefully into the receptacle. The chip fits only one way.

3. Carefully close the lid. The electrodes in the cartridge fit into the wells of the chip.

4. The 2100 Expert software screen shows that you have inserted a chip and closed the lid by displaying the chip icon at the top left of the Instrument context.

Starting the Chip Run

1. In the Instrument context, select dsDNA from the Assay menu.

2. Accept the current File Prefix or modify it.
Data will be saved automatically to a file with a name using the prefix you have just entered. At this time you can also customize the file storage location and the number of samples that will be analyzed.

3. To enter sample information like sample names and comments, complete the sample name table.

4. Click the Start button in the upper right of the window to start the chip run. The incoming raw signals are displayed in the Instrument context.

5. After the chip run is finished, remove the chip from the receptacle of the bioanalyzer and dispose it according to good laboratory practices.

Cleaning Electrodes after a High Sensitivity DNA Chip Run

When the assay is complete, immediately remove the used chip from the Agilent 2100 Bioanalyzer and dispose it according to good laboratory practice. After a chip run, perform the following procedure to ensure that the electrodes are clean (no residues are left over from the previous assay).

1. Slowly fill one of the wells of the electrode cleaner with 350 µl deionized analysis-grade water.

2. Open the lid and place the electrode cleaner in the Agilent 2100 Bioanalyzer.

3. Close the lid and leave it closed for about 10 seconds.

4. Open the lid and remove the electrode cleaner.

5. Wait another 10 seconds to allow the water on the electrodes to evaporate before closing the lid.

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 the use of the Agilent Bioanalyser.

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:		
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Signature:		Date: 28/09/2021
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Position:	QA Manager	
Signature:		Date: 28/09/2021

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