Document Number:	**SASoM/METHOD/077.v4**
Title:	**Genomic DNA purification using QIAGEN Midi prep kit**
Version:	**v4**
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SOP History		
Number	Date	Reason for Change
v1	02/04/2015	Original
V2	02/04/2017	Update
V3	02/04/2019	Update
V4	02/04/2021	Update

1.0 Purpose –
This SOP describes the current procedure for genomic DNA purification using the QIAGEN Midi prep kit use in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to all staff in the SASoM involved in purifying DNA using the QIAGEN Midi prep kit.

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 –
The procedure is designed for the isolation of genomic DNA using a maximum $2x10^7$ cells/ column

A) Preparation of cell pellet
1. Dissociate the cell monolayer with Trypsin or TrypLE Express (Invitrogen).
2. Wait until the cells detach from the culture vessel, then transfer the suspension to a centrifuge tube on ice.
3. Wash the culture vessel with 2 mL of ice-cold PBS and add to the centrifuge tube.
4. Recover the cells by centrifuging at 1200 rpm for 3 min at 4°C.
5. Discard the supernatant, resuspend in 4 mL of cold PBS and then re-centrifuge at 1200 rpm for 3 min at 4°C.
6. Repeat step 5.
7. Discard the supernatant and freeze the cell pellet at -80°C. Record number of cells on tube (>$6x10^7$ cells).

B) DNA purification
1. Resuspend the cells in 6ml PBS to a final concentration of 10^7 cells/ml. Use 2 ml ($2x10^7$ cells) cell suspension ($1x10^7$ cells/ml) per column – 3 columns for each cell pellet.
Transfer 2ml cell suspension in 3× 30ml centrifuge tube (Nalgene)
For each tube:
Add 1 volume (2 ml) of ice-cold Buffer C1 and then 3 volumes of ice-cold distilled water (6 ml). Mix by inverting the tube several times (10 times). Incubate for 10 min on ice.
Buffer C1 and distilled water must be equilibrated to 4°C. Keep on ice during use. Buffer C1 lyses the cells but stabilizes and preserves the nuclei.

2. Centrifuge the lysed cells at 4°C for 15 min at 3283 RPM (1300 x *g*) using a JA 25-50 rotor in the Beckman-Coulter Avanti J26-XP centrifuge (instrument room 248). Discard the supernatant.
Don't spin too fast otherwise nuclei will be disrupted. After centrifugation there should be a small nuclear pellet visible.

3. Add 1 ml of ice-cold Buffer C1 and then 3 ml of ice-cold distilled water. Resuspend the pelleted nuclei by vortexing. Centrifuge again at 4°C for 15 min at 3283rpm (1300 x *g*). Discard the supernatant.
This wash step removes all residual cell debris from the nuclear pellet.
At this point the pellet may be frozen and stored at –20°C if desired. When ready to complete the purification procedure, continue with step 4 of the protocol. The yield of DNA will be the same as for fresh samples.

4. Add 5 ml of Buffer G2, and completely resuspend the nuclei by vortexing for 10–30s at maximum speed.
Resuspend the nuclei as thoroughly as possible by vortexing. This step is critical for a good flow rate on the QIAGEN Genomic-tip.

5. Add 95µl of QIAGEN Proteinase K stock solution, and incubate at 50°C for 60 min.
Buffer G2 lyses the nuclei and denatures proteins such as nucleases, histones, and viral particles. The excess QIAGEN Protease digests the denatured proteins into smaller fragments. Buffer G2 and QIAGEN Protease, in combination, strip the genomic DNA of all bound proteins, facilitating efficient removal during purification. It is important that the lysate becomes clear at this stage. If necessary, extend the incubation time, or pellet the particulate matter by centrifugation for 10 min at 6438 rpm (5000 x g), 4°C.

6. Equilibrate a QIAGEN Genomic-tip 100/G with 4 ml of Buffer QBT, and allow the QIAGEN Genomic-tip to empty by gravity flow. Place a QIAGEN Genomic-tip over a tube using a tip holder or into a QIArack over the waste tray. Do not force out the remaining buffer.

7. Vortex the sample for 10 sec at maximum speed and apply it to the equilibrated QIAGEN Genomic-tip. Allow it to enter the resin by gravity flow.

8. Wash the QIAGEN Genomic-tip with 2 x 7.5 ml of Buffer QC. Allow Buffer QC to move through the QIAGEN Genomic-tip by gravity flow.
Two washes are sufficient to remove all contaminants in the majority of DNA preparations.

9. Place the QIAGEN Genomic-tip over a clean 30ml centrifuge tube. Elute the genomic DNA with 5 ml of Buffer QF.

10. Precipitate the DNA by adding 3.5 ml (0.7 volumes) room-temperature (15–25°C) isopropanol to the eluted DNA. Mix and centrifuge immediately at 13,000rpm in centrifuge tubes for 30 min at 4°C. Carefully remove the supernatant.

11. Wash the centrifuged DNA pellet with 2 ml, of cold 70% ethanol. Vortex briefly and centrifuge at 13,000rpm for 10 min at 4°C. Carefully remove the supernatant without disturbing the pellet. Air-dry for 5–10 min, and resuspend the DNA in ~ 100µL of 10 mM Tris·Cl, pH 8.5 or miliQ water. Leave the DNA to dissolve overnight at 4°C.
The 70% ethanol removes precipitated salt and replaces the isopropanol with the more volatile ethanol, making the DNA easier to redissolve. Overdrying the pellet will make the DNA difficult to redissolve.

12. Quantify purified DNA with QubiT fluorometer.
*Nanodrop is **NOT** sufficiently accurate for NGS related applications.*

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.

9.0 Approval and sign off –

Author:

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Position: Principal Investigator

Signature:  Date: 19/04/2021

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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