Document Number:	**SASoM/METHOD/129.v1**
Title:	**DNA Extraction using the Qiagen QIAmp DNA kit.**
Version:	**v1**
Author:	**Jennifer Bre**

Effective from:	03/07/2020
Valid to:	02/07/2022

SOP History		
Number	Date	Reason for Change
v1	03/07/2020	Original

1.0 Purpose –
This SOP describes the current procedure for performing DNA extractions with the Qiagen QIAmp DNA Kit (Qiagen; #51304) in Laboratory 248/9 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved in performing DNA extractions with the Qiagen QIAmp DNA Kit (Qiagen; #51304) in the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –
All staff involved in performing DNA extractions with the Qiagen QIAmp DNA Kit (Qiagen; #51304) 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 –

DNA extraction kit (Qiagen; #51304)
Deoxyribonuclease I (Roche; #04716728001)
Phosphodiesterase I from Crotalus adamanteus venom (Sigma; P3243)
Phosphatase, Alkaline from Escherichia coli (Sigma; P4252)

Ovarian cancer cell lines: A2780, PE01
Treat with different concentrations of 1031 for 2 h and replace with drug-free media

The following protocol is adapted from Wickremsinhe 2010.

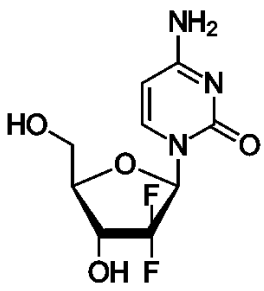
[A] DNA extraction with QIAamp DNA kit (manufacturer protocol):

1. Trypsinize cells
2. After cells have detached from the dish, collect them in medium, and transfer the cells to a 1.5ml RNAse-free microcentrifuge tube. Centrifuge for 5 min at 300 x g. Remove the supernatant completely. Note: when harvesting cells, spike control (untreated) cell pellets with 2uL of 1mM gemcitabine.
3. Resuspend cell pellet in PBS to a final volume of 200ul.
4. Add 20uL proteinase K and 10uL of RNAse A.
5. Add 200uL Buffer AL to the sample. Mix by pulse-vortexing for 15s. Incubate at 56°C for 10 min.
6. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
7. Add 200uL ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
8. Carefully apply the mixture to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
9. Add 500uL Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
10. Add 500uL Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
11. Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.
12. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube and discard the collection tube containing the filtrate. Add 30 or 50uL Buffer AE or distilled water (depending on how concentration of DNA required). Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.

[B] Hydrolysis:

- Mix 1: 11uL 10mM MgCl2; 15uL 10mM Tris-HCl (pH 6.8), 2uL deoxyribonuclease I, 2uL DW (per sample).
- Mix 2: 12.5uL 100mM Tris-HCl (pH 8.3), 3.75uL snake venom phosphodiesterase, 3.75uL bacterial alkaline phosphatase (per sample).

1. Denature DNA by heating samples at 95°C for 5 min.
2. Rapidly chill on ice for 3 min.
3. Incubate 20uL of DNA with 30uL of Hydrolysis Mix 1 at 37°C for 3 h.
4. Add 20uL of Hydrolysis Mix 2 and incubate O/N (min 16 h) at 37°C.
5. Centrifuge samples for 3 min at approx. 3200 (g or rpm?) and supernatant may be used for LC:MS:MS.
6. Include a 2% agarose gel pre/post-hydrolysis for verification before sending samples.
7. Nanodrop before/after shipping to ensure integrity of the samples.



structure of dFdC

5.0 Personal protection –

A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

6.0 Spillages –

Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard. Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training –

All staff should undergo training in this technique before performing the procedure.

8.0 Related documents –

8.1 Risk assessments
CHARM RA20156_Use of Chemotherapy drugs and their Protides.

9.0 Approval and sign off –

Author:		
Name:	Jennifer Bre	
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Management Approval:		
Name:	Peter Mullen	
Position:	Research Fellow	
Signature:		Date:
QA release by:		
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Position:	QA Manager	
Signature:		Date:

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