MWAC/UNSW Biospecimen Services

Standard Operating Procedure
Extracting DNA from Saliva

Effective date: 17th May 2023
Review date: 17th May 2026

1. Purpose

The purpose of this document is to describe the procedure for extracting DNA from saliva.

2. Scope

This SOP describes how to extract DNA from saliva using a Genotek DNA OG-500 kit. Methods for manual extraction and extraction using the QIAsymphony SP are included in this SOP. The QIAsymphony method utilises the QIAsymphony DSP DNA Midi Kit (QIAGEN® catalogue number: 937255 or 937236) and the protocol Oragene, ID372, V2.

3. Roles & Responsibilities

The SOP applies to all Biospecimen Services personnel responsible for extracting DNA from saliva.

Personnel	Responsibility
Technician	• To ensure that the procedure outlined in this standard operating procedure is closely adhered to, as any changes in the methodology will jeopardise the validity of the results. • To be trained in Good Laboratory Practice and be inducted into the PC2 facility. • Carry out accurate pipetting and avoid cross-contamination of specimens.

4. Materials & Equipment

The materials and equipment listed below are needed to perform this method.

4.1. Equipment

- Water incubator or air incubator
- Centrifuge
- Vortex
- QIASymphony
- pipette P1250

4.2. Consumables

- 15ml sterile centrifuge tubes

- PT-L2P
- 70% v/v ethanol
- 1.5mL or 1.7mL of micro/Eppendorf tubes
- sterile 2ml microtubes (Sarstedt Cat#72.694)
- Absolute ethanol
- TE buffer (Tris-EDTA buffer pH8.0 Sigma SKU 93283-100ML)
- P1250 tips (1250uL filter tips)
- QIASymphony DSP DNA Midi Kit (QIAGEN® catalogue number: 937255)
- Filter-Tips, 1500ul for use with QIAsymphony instruments (QIAGEN® catalogue number: 997024)
- Filter-Tips, 200ul for use with QIAsymphony instruments (QIAGEN® catalogue number: 990332)

5. Safety Requirements

5.1. Clean back-opening gowns, eye protection and powderless gloves must be worn during all operations in this standard operating procedure.

5.2. All samples must be treated as potential infection risks and must be handled according to good laboratory procedures under PC2 requirements and methods to prevent occupational exposure.

5.3. All biohazardous material and chemical waste must be disposed in accordance with the UNSW Laboratory Hazardous Waste Disposal Guideline

5.4. All work surfaces should be clean prior to commencing.

6. Method

6.1. Saliva to DNA (manual)

6.1.1. The saliva sample is stored at 4°C until processed. Estimate the volume of saliva provided by the donor. The amount of saliva collected is directly proportional to the amount of DNA recovered. The average weight of an OG-500 empty kit is 6.81g.

6.1.2. Register all participants in OpenSpecimen following the *Biospecimen Services SOP - Registering a Participant and Recording Consent in OpenSpecimen* listed in *Section 9 - Related Documents*

6.1.3. Book the specimens into OpenSpecimen following the instructions in each individual collection protocol and complete the e-processing form (Appendix A). Concentration, volume and total amount should be recorded in the e-processing form at the end of this Method.

6.1.4. Mix the sample in the DNA Genotek kit by inversion and gentle shaking for a few seconds.

6.1.5. Incubate the sample at 50°C in a water incubator for a minimum of 60 min or in an air incubator for a minimum of 2 hours. This must be done in the original collection tube. The sample may be incubated at 50°C overnight if more convenient.

6.1.6. Transfer the entire sample to a 15 ml centrifuge tube, in a biological safety cabinet. Transfer can be carried out either by pouring or by pipetting with a P1250 pipette. Add 1/25th volume PT-L2P and mix by vortexing for a few seconds.

6.1.7. Incubate the 15 ml disposable centrifuge tube containing the sample on ice for 10 min.

6.1.8. Centrifuge the 15 ml disposable centrifuge tube containing the sample for 10 min at >4000 rpm. A longer period of centrifugation (up to 20 min) can be carried out when reducing the turbidity of the final DNA solution is important.

6.1.9. Carefully transfer the majority of the clear supernatant with a P1250 pipette to a new sterile 15 ml disposable centrifuge tube. Leave a small volume of the supernatant behind to avoid disturbing the pellet. Discard the pellet.

6.1.10. Add 1.2x volume of room temperature absolute ethanol to the clear supernatant. Mix gently by inversion 10 times. Stand the sample at room temperature for 10 min to allow the DNA to fully precipitate.

6.1.11. Centrifuge the 15 ml disposable centrifuge tube containing the sample for 10 min at >4000 rpm. Carefully remove the supernatant with a P1250 pipette and discard it.

6.1.12. Add 1 ml of 70% v/v ethanol to the tube without disturbing the smear or the pellet. Let stand at room temperature for 1 min. Gently swirl and completely remove the 70% ethanol. Should the pellet detach, centrifuge the 15 ml disposable centrifuge tube containing the sample for 5 min at 4000 rpm.

6.1.13. Rehydrate the DNA by adding 0.2-1 ml of TE buffer and by vortexing the sample for 30 sec. To ensure maximal DNA recovery, the sample must be vortexed after the addition of TE. Excessive drying of the pellet (>10 min) and using less than 500 ul of TE can make it difficult to rehydrate the DNA.

6.1.14. Ensure complete rehydration of the DNA by performing the following steps: vigorous pipetting and vortexing; incubation at 50°C for 60 min with occasional vortexing; or incubation at room temperature for 1-2 days.

6.1.15. Centrifuge the rehydrated DNA (in 15 ml disposable centrifuge tube) at room temperature for 15 min at >4000 rpm. Transfer supernatant with a P1250 pipette to a fresh 1.5/1.8 ml micro tube without disturbing the pellet. Quantitate DNA using QIAxpert. The expected concentration of the fully rehydrated DNA is 20-200 ng/uL.

6.1.16. Label the tubes and store DNA tubes at -80°C. See *Section 6.3 - Labelling and Storage* below.

6.2. Saliva to DNA (QIASymphony)

6.2.1. DNA was extracted from a 1 mL aliquot of each Oragene saliva sample on the QIAsymphony SP using the QIAsymphony DSP DNA Midi Kit (QIAGEN® catalogue number: 937255 or 937236) and protocol Oragene_ID372_V2.xml.

6.2.2. This protocol was specifically developed for use with Oragene saliva samples and includes a heated elution (37°C) into 60 µL of elution buffer, among other customized parameters. The setup of the QIAsymphony SP was conducted according to the procedures contained in the QIAsymphony Handbook.

6.3. Labelling & Storage

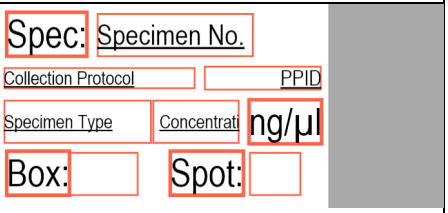
6.3.1. To assign a storage location, when booking in the specimen, select the next available box for the collection protocol in OpenSpecimen and the storage location will be automatically assigned within that box.

6.3.2. Specimens should be labelled with, at a minimum, specimen number, PPID, specimen type, box/spot location, Collection Protocol. See *Section 9 – Related Documents - Biospecimen Services SOP – Biospecimen Storage and Retrieval* for more information.

6.3.3. Labels should be printed and not handwritten.

6.3.4. Use the following label template:

Label template



7. Monitoring of compliance to this SOP is ongoing.

7.1. The Biospecimen Services Manager or their delegate is responsible for ongoing monitoring of biobank operations to verify compliance with this SOP.

7.2. The Biospecimen Services Manager or their delegate is responsible for obtaining annual updates to this SOP and for communicating these changes to all personnel.

8. Definitions

Term or Abbreviation	Definition
OG-500	Saliva sample collection kit used for DNA extraction.
PT-L2P	prepIT.L2P is a reagent used to ensures maximum recovery of high quality DNA from Oragene and ORAcollect saliva samples
TE Buffer (TE)	The most common buffer used for dilution and storage of DNA. TE buffer is made of Tris-Chloride (10 mM) EDTA (1 mM) and has pH8.0.
PPID	Unique code or number assigned to the participant.
Specimen Number	Unique code or number assigned to individual specimens.
Collection Protocol	A Collection Protocol is an ongoing accrual and storage of specimens, undertaken by a Principal Investigator (PI) in association with Biospecimen Services. A Collection Protocol in OpenSpecimen has defined specimen collection groups, time points and a predetermined specimen type and processing protocol.

9. Related Documents

Document	Description
Biospecimen Services SOP – Biospecimen Storage and Retrieval, Version 3, 12/05/2023	SOP that describes how to store, retrieve and ship biospecimens.
Biospecimen Services SOP - Registering a Participant and Recording Consent in OpenSpecimen, Version 1, 26/04/2023	SOP that describes the data entry procedure to follow when registering participants and recording consent in Open Specimen.
NSW/CTRNet Required Operational Practice 9: Biospecimen Collection and Processing	ROP that describes the key principles regarding biospecimen collection and processing that should be adhered to, to meet the current best practice standards.

NSW/CTRNet Required Operational Practice 10: Biospecimen Storage and Retrieval	ROP that describes the key principles regarding biospecimen storage and retrieval that should be adhered to, to meet the current best practice standards.
NSW/CTRNet Required Operational Practice 13: Safety and Waste Disposal	ROP that describes the key principles regarding safety and waste disposal that should be adhered to, to meet the current best practice standards.
UNSW Laboratory Hazardous Waste Disposal	UNSW Guideline for disposing of hazardous waste in laboratories.
UNSW Biosafety Policy	UNSW Procedure for identifying biohazardous material and meeting legislative and regulatory requirements.
UNSW Personal Protective Equipment Guideline	UNSW Guideline for selecting, using and maintaining PPE.

10. Appendices

Appendix A: e-Processing Form

11. References, Regulations & Guidelines

Documents	Description
Oragene DNA (OG-500) data sheet	Oragene DNA saliva kit product details including how to use it for saliva collection.
QIAsymphony_DSP_DNA_Handbook	Introduce QIAsymphony DSP DNA Mini/Midi kits in detail including Material, Principles, handling, storage and Procedure, etc.
Laboratory protocol for manual purification of DNA from whole sample	Protocol for purify saliva to DNA manually provided by dnagenotek
Automated extraction of gDNA from Oragene saliva samples using the QIAGEN QIAsymphony SP	The company dnagenotek provides the Processing Protocols for its product Oragene OG500 DNA saliva kit.

12. Version History & Authorisation

Version	Date	Author	Summary of Changes	Authorised By:
1	March 2020	Anusha Hettiaratchi	Original	Anusha Hettiaratchi
2	17/05/2023	Pearl Zhu/ Ussha Pillai	Updated to new SOP format	Manager, Biospecimen Services: Anusha Hettiaratchi

Appendix A: e-Processing Form

Collection Protocol: _____

DATE	PPID	LAST NAME	First Name	Parent LABEL	Derived TYPE	Derived LABEL	Location BOX/SPOT		FinalVol (µl)	Conc. (µg/µl)	Total Amt (µg)
dd-mm-yyyy	HSA1234	L	F	XXXXXX	DNA	XXXXXX	XXXX	XX			

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