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Total Nucleic Acid Extraction and Collection of Protein Fraction from OCT-embedded Tissue using the Microlab Nimbus

I. Purpose

To develop an extraction protocol that can yield high quality total nucleic acid in sufficient quantities to generate libraries from the DNA and RNA as well as collect sufficient high quality protein to be processed and analyzed on a mass spectrophotometer.

II. Scope

All procedures are applicable to all users at the BCGSC.

III. Policy

This procedure will be controlled under the policies of the Genome Sciences Centre, as outlined in the Genome Sciences Centre High Throughput Production Quality Manual (QM.0001). Do not copy or alter this document. To obtain a copy see a Quality Systems associate.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Tech D and Library Construction Group Leaders to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Systems Team to audit this procedure for compliance and maintain control of this procedure.

V. References

Document Title	Document Number
Agencourt RNAdvance Tissue Protocol (Note: ALINE EvoPure RNA Isolation Kit uses the same protocol as Agencourt RNAdvance Tissue Protocol. ALINE kit doesn't provide a manual, therefore our SOP is based on the Agencourt protocol and ALINE reagents.)	000473v003

VI. Related Documents

Document Title	Document Number
Quantifying DNA Samples Using the Qubit™ 4 Fluorometer	LIBPR.0153
Guidelines for Handling Potentially BioHazardous Materials	LIBPR_WorkInst.0013

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VII. Safety

All Laboratory Safety procedures will be complied with during this procedure. The required personal protective equipment includes a laboratory coat and gloves. See the safety data sheet (SDS) for additional information.

VIII. Materials and Equipment

Name	Supplier	Number	Model or Catalogue #	
1.2mL Low Profile Plate, AB1127	Abgene	AB-1127		✓
1000µL Rainin tips	Rainin	RT-L1000F		✓
20µL Pipet-Lite	Rainin	L12-20	✓	
2.0mL 96 Deep Well Waste Plate	Axygen	P-2ML-SQ-C-S		✓
20µL Rainin tips	Rainin	RT-L10F		✓
200µL Pipet-Lite	Rainin	L12-200		✓
200µL Rainin tips	Rainin	RT-L200F		✓
EvoPure RNA Isolation Kit	ALINE	R-907-400-C5		✓
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohol	00023878		✓
ABGENE (AB1000/150) - 0.2mL Ultra 96-Well PCR plates	Thermo Fisher Scientific	SP5201150		✓
Bench Coat (Bench Protection Paper)	Fisher	12-007-186		✓
Black ink permanent marker pen	VWR	52877-310		✓
Ultrapure Water, Dnase/Rnase-free, distilled	Invitrogen	10977-023		✓
Basin, Pipettor Solution Basins, for 4-,8-,12-channel pipettors, 55mL, sterile	VWR	21007-972		✓
Distriman Repeater	Mandel	GF-3005		✓
Falcon pipettor, equ2420 PAF-2	VWR	357590	✓	
Safetouch gloves	Fisher	270-058-53		✓
Gilson distriman, Distri-2	Mandel	GF-F164001	✓	
Gilson P10 pipetman	Mandel	GF-44802		✓
Gilson P1000 pipetman	Mandel	GF-23602		✓
Gilson P2 pipetman	Mandel	GF-44801		✓
Gilson P20 pipetman	Mandel	GF23600		✓
Gilson P200 pipetman	Mandel	GF-23601		✓
Voyager Eight Channel Pipette, 50.0-1250.0µL	Mandel	TM-4124		✓
Tips, 1250.00µL Pipette Tip, 96 tips/rack, 480 tips/cs, Filter, Sterile	Mandel	TM-4445		✓
Hamilton Microlab Nimbus96	Hamilton		✓	
Ice bucket – Green	Fisher	11-676-36		✓
IPA (2-Propanol) 4L	Fisher	A464-4		✓
Large Kimwipes	Fisher	06-666-117		✓
Large Volume Magnet Plate	Alpaqua	96M-EX	✓	
Mandel P1000 DF1000 tips	Mandel	GF-F171703		✓

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Name	Supplier	Number	Model or Catalogue #	
Mandel P200 DF200 tips	Mandel	GF-F171503		✓
MicroAmp Clear Adhesive Film	ABI	4306311	✓	
Mini-centrifuge	Eppendorf	5417R		✓
MultiMACS Separation Unit, equ2250	Miltenyi Biotec	120-004-352		✓
Gel Imager	In House	N/A	N/A	N/A
Nimbus 1000µL CO-RE filter tips	Hamilton	235940		✓
Nimbus 50µL CO-RE filter tips	Hamilton	235979		✓
P200 Multichannel, 20-200µL, equ1325	Ranin	L-20-12	✓	
Peltier Heaters	In-house	N/A	N/A	N/A
Reservoir, divided, 12 COL, 252mL, V-bottom	Agilent Technologies	201256-100		✓
Plate, 384-Well reservoirs, diamond-bottom, Low-Profile	Ultident	24-RES-SW384-LP		✓
Rainin AutoRep E	Mettler Toledo Inc	AR-E1		✓
Rainin Encode Tip, sterile, 12.5mL	Mettler Toledo Inc	ENL-12MLS		✓
RNAse free 1.5 mL Eppendorf tube	Ambion	12400		✓
RNAse Zap	Ambion	9780		✓
Small Autoclave waste bags 10”X15”	Fisher	01-826-4		✓
VX-100 Vortex Mixer	Rose Scientific	S-0100		✓
Wet ice	In house	N/A	N/A	N/A
KingFisher Deepwell 96 Plate, V-bottom, polypropylene	ThermoFisher	95040450		✓
Reservoir 96 well Pyramid PP 287mL	Agilent Technologies	201244-100		✓
Autoclave waste bags 12" x 24"	ThermoFisher	01-826-5		✓
Proteinase K	Qiagen	19131		
Thermomixer R	Eppendorf			✓

IX. Procedure: Extraction of Total Nucleic Acids

Note: All version numbers for Nimbus protocols have been removed on this document. They are present when running the protocol. If you are unsure which version to use, consult your supervisor.

1. Reagent Preparation Guidelines

Because we will be extracting total nucleic acids including RNAs, we must follow the guidelines for handling RNA.

If extracting total nucleic acid manually, please follow the steps in Appendix B.

1.1. RNases are ubiquitous and general precautions should be followed in order to avoid the introduction of contaminating nucleases during the FFPE extraction process.

1.2. Always work with gloved hands and change gloves frequently.

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1.3. Use RNase free, filtered pipette tips for pipetting whenever possible.

1.4. Use dedicated RNase free equipment, e.g. pipettes, pipette tips, gels boxes, etc.

1.5. Avoid using reagents, consumables and equipment that are in common use for other general lab processes.

1.6. When available, work in a separate room, fume hood or lab space.

1.7. Use plastic, disposable consumables that are certified RNase free.

1.8. Purchase reagents, such as commonly used buffers and water, that are certified RNase free.

1.9. Wipe down work surfaces with RNAse Zap before starting.

2. Limitations

This protocol has been optimized to process four sections of 50µm slices of OCT-embedded tissue. The tissue is placed into lysis buffer immediately after sectioning. If the first step of extraction (rocking overnight at room temperature) will not begin that day, the tubes should be stored at -80°C until needed. (Non-OCT embedded) Frozen cell pellets have also been successfully extracted using this protocol.

3. Buffy Coat and Whole Blood Samples submitted

Please follow the guidelines for working with biohazardous and infectious materials.

3.1. Use 50µL buffy coat or whole blood and add 20µL of 20 mg/mL Proetinase K in the biological safety cabinet.

3.2. Incubate for 30 minutes at 25°C in a Thermomixer. Be sure to parafilm the tube.

3.3. Add 330µL of Buffer RLTplus with TCEP and nutate for a minimum of 30 minutes.

3.4. Proceed to step 6.

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4. Saliva Samples submitted

Please follow the guidelines for working with biohazardous and infectious materials.

4.1. Mix tubes by inverting 10 times.

4.2. Transfer 420µL of the sample into a 2mL and parafilm the tube.

4.3. Be sure to add HelaS3 cell as control alongside.

4.4. Incubate for 2 hours at 50°C on Eppendorf Thermomixer.

4.5. Proceed to step 6.

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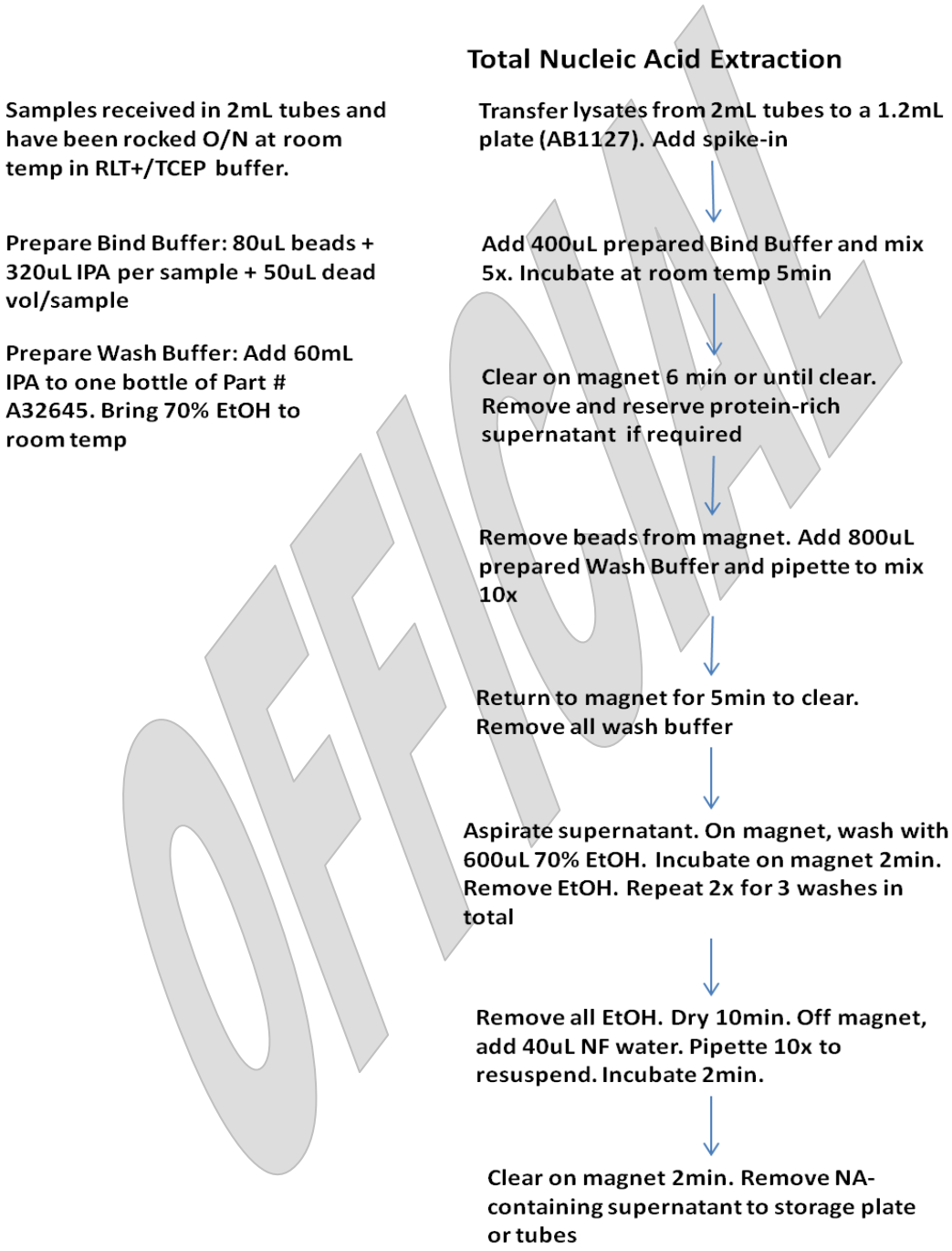
5. Workflow Overview



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6. Spike-in Addition to Samples

Please work in the fume hood. Even though the TCEP in the buffer is odourless, it is still harmful.

Work with a second person while adding spike-in and arranging your sample tubes to avoid errors.

6.1. The OCT sections have been rocked overnight. Please take them off the nutator and give them a quick spin at 14000rpm for 2 minutes.

6.2. Arrange the 2mL tubes in an 8x12 tube rack in the orientation provided by your supervisor.

6.3. Thaw out the spike-in needed for the samples being extracted. Your supervisor will provide this information.

6.4. By referencing the plate layout provided, add spike-in to an AB1127 plate that corresponds to the samples to be arrayed into column 1.

6.5. Once the spike-in has been added, open the sample tubes that will be added to column 1. Use the Viaflow pipette to aspirate the samples and then dispense into column 1. If cell debris is observed, use single channel pipette to transfer samples.

6.6. Continue in this way for the remaining samples: adding spike-in to a column on the AB1127 plate first and then adding the sample to be extracted.

7. Extraction of Total Nucleic Acids

7.1. Using the worksheet, OCT Extraction on Nimbus Worksheet, located in R:\Library Core\Work Sheets and Calculators\EvoPure Extractions on Nimbus, prepare and dispense Bind Buffer and Wash Buffer into the required plastic ware. Dispense 70% EtOH and nuclease-free dH_2O.

7.2. Log onto the Nimbus 1 or Nimbus 4 (5th floor north, inside the RNA area) and open the software (Hamilton Run Control).

7.3. In the Library Construction folder, open “OCT_Total_NA_Extraction” program. Press play to start the program. From this point forward you will be prompted with regards to any actions you will need to perform.

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7.4. If required, be sure to collect the protein fraction which is in the supernatant (the total nucleic acids are bound to the beads on the magnet). Store the protein fraction on ice, covered, until the next incubation step is running. During the incubation, move the protein plate to the -80°C freezer for storage.

7.5. Follow the workflow overview as an aide to keep track of the steps the Nimbus is performing.

7.6. Following elution of the total nucleic acids, remove and seal the destination plate. If you will be doing QC steps (Qubit) directly after extractions, seal with a clear tape seal. For long term storage, use foil tape. Store the total nucleic acid at -80°C.

7.7. Clear the deck of all plastic labware and discard. Discard any unused reagents including any residual Bind Buffer. The bead mixture cannot be saved for another day. Log off from the computer.

7.8. Wipe down the Nimbus along with the waste chute.

8. QC of Extracted Samples using Qubit

To avoid bead transfer during pooling or QC, put the sample plate on a magnet whenever taking aliquots.

8.1. After extraction, pool the same samples wells together. Have a second person watch this step.

8.2. Qubit the pooled sample as “neat” and a 1/5 dilution. Refer to LIBPR.0153 “Quantifying DNA Samples Using the Qubit™ 4 Fluorometer”.

8.3. Complete the OCT Extraction tracking sheet found in R:\Library Core\Work Sheets and Calculators\Library construction work instructions. Send the sheet along with the QC files to your supervisor.

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Appendix A: LIMS SOP

1. Spike_In_Tissue
2. A-Total Nucleic Acid Extraction
3. DNA QC

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Appendix B

Manual Extraction of Total Nucleic Acids using the ALINE EvoPure Kit

Note: This process is not suited for manual use due to the intensive bead wash steps. It WILL cause repetitive use injury if multiple samples are processed at once and therefore should only be attempted as a last resort and with caution.

I. Materials and Equipment

Name	Supplier	Number	Model or Catalogue #	
Bench Coat (Bench Protection Paper)	Fisher	12-007-186		✓
1.2mL Square Well Storage Plate, AB1127	Thermo Scientific	AB-1127		✓
ABGENE (AB1000/150) - 0.2mL Ultra 96-Well PCR plates	Thermo Fisher Scientific	SP5201150		✓
Large Volume Magnet Plate (Magnum FLX)	Alpaqua	96M-EX	✓	
Rainin 1200µL multi-channel pipettor	Rainin	L12-1200 XLS	✓	
1000µL Rainin tips	Rainin	RT-L1000F		✓
200µL Pipet-Lite	Rainin	L12-200	✓	
200µL Rainin tips	Rainin	RT-L200F		✓
20µL Pipet-Lite	Rainin	L12-20	✓	
20µL Rainin tips	Rainin	RT-L10F		✓
EvoPure RNA Extraction Kit	ALINE Biosciences	R-907-400-C5		✓
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohol	00023878		✓
Ultrapure Water, Dnase/Rnase-free, distilled	Invitrogen	10977-023		✓
IPA (2-Propanol) 4L	Fisher	A464-4		✓
Basin, Pipettor Solution Basins, for 4-,8-,12-channel pipettors, 55mL, sterile	VWR	21007-972		✓
Black ink permanent marker pen	VWR	52877-310		✓
Distriman Repeater	Mandel	GF-3005		✓
Falcon pipettor, equ2420 PAF-2	VWR	357590	✓	
Fisherbrand Textured Nitrile gloves - large	Fisher	270-058-53		✓
Gilson distriman, Distri-2	Mandel	GF-F164001	✓	
Gilson P10 pipetman	Mandel	GF-44802		✓
Gilson P1000 pipetman	Mandel	GF-23602		
Gilson P2 pipetman	Mandel	GF-44801		✓
Gilson P20 pipetman	Mandel	GF23600		✓
Gilson P200 pipetman	Mandel	GF-23601		✓
Voyager Eight Channel Pipette, 50.0-1250.0µL	Mandel	TM-4124		✓
Tips, 1250.00µL Pipette Tip, 96 tips/rack, 480 tips/cs, Filter, Sterile	Mandel	TM-4445		✓

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Name	Supplier	Number	Model or Catalogue #	
Ice bucket – Green	Fisher	11-676-36		✓
Large Kimwipes	Fisher	06-666-117		✓
P200 Multichannel, 20-200µL, equ1325	Ranin	L-20-12	✓	
Mandel P1000 DF1000 tips	Mandel	GF-F171703		✓
Mandel P200 DF200 tips	Mandel	GF-F171503		✓
MicroAmp Clear Adhesive Film	ABI	4306311		✓
Mini-centrifuge	Eppendorf	5417R		✓
Peltier Heaters	In-house	N/A	N/A	N/A
RNAse free 1.5mL eppendorf tube	Ambion	12400		✓
RNAse Zap	Ambion	9780		✓
DNA*AWAY*	Molecular BioProducts	7010		✓
Small Autoclave waste bags 10”X15”	Fisher	01-826-4		✓
VX-100 Vortex Mixer	Rose Scientific	S-0100	✓	
Wet ice	In house	N/A	N/A	N/A
KingFisher Deepwell 96 Plate, V-bottom, polypropylene	Thermo Scientific	95040450		✓
Reservoir, divided, 12 COL, 252mL, V-bottom, High Profile	Agilent Technologies	201256-100		
Reservoir 96 well Pyramid PP 287mL	Agilent Technologies	201244-100		✓

II. Procedure

1. Reagent preparation Guidelines

<u>General Remarks on Handling RNA</u>

1.1. RNases are ubiquitous and general precautions should be followed in order to avoid the introduction of contaminating nucleases during the FFPE extraction process.

1.2. Always work with gloved hands and change gloves frequently.

1.3. Use RNase-free, filtered pipette tips for pipetting whenever possible.

1.4. Use dedicated RNase-free equipment, e.g. pipettes, pipette tips, gels boxes, etc.

1.5. Avoid using reagents, consumables and equipment that are in common use for other general lab processes.

1.6. When available, work in a separate room, fume hood or lab space.

1.7. Use plastic, disposable consumables that are certified RNase-free.

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1.8. Purchase reagents, such as commonly used buffers and water, that are certified RNase free. Prepare small individual aliquots of such buffers to avoid repeated transfer out of stock buffers. This lowers the risk of contaminating the stock solution.

1.9. Wipe down work surfaces with RNaseZap before starting.

2. Limitations

This protocol has been optimized to process up to 4 sections of 50µm scrolls of OCT-embedded tissue.

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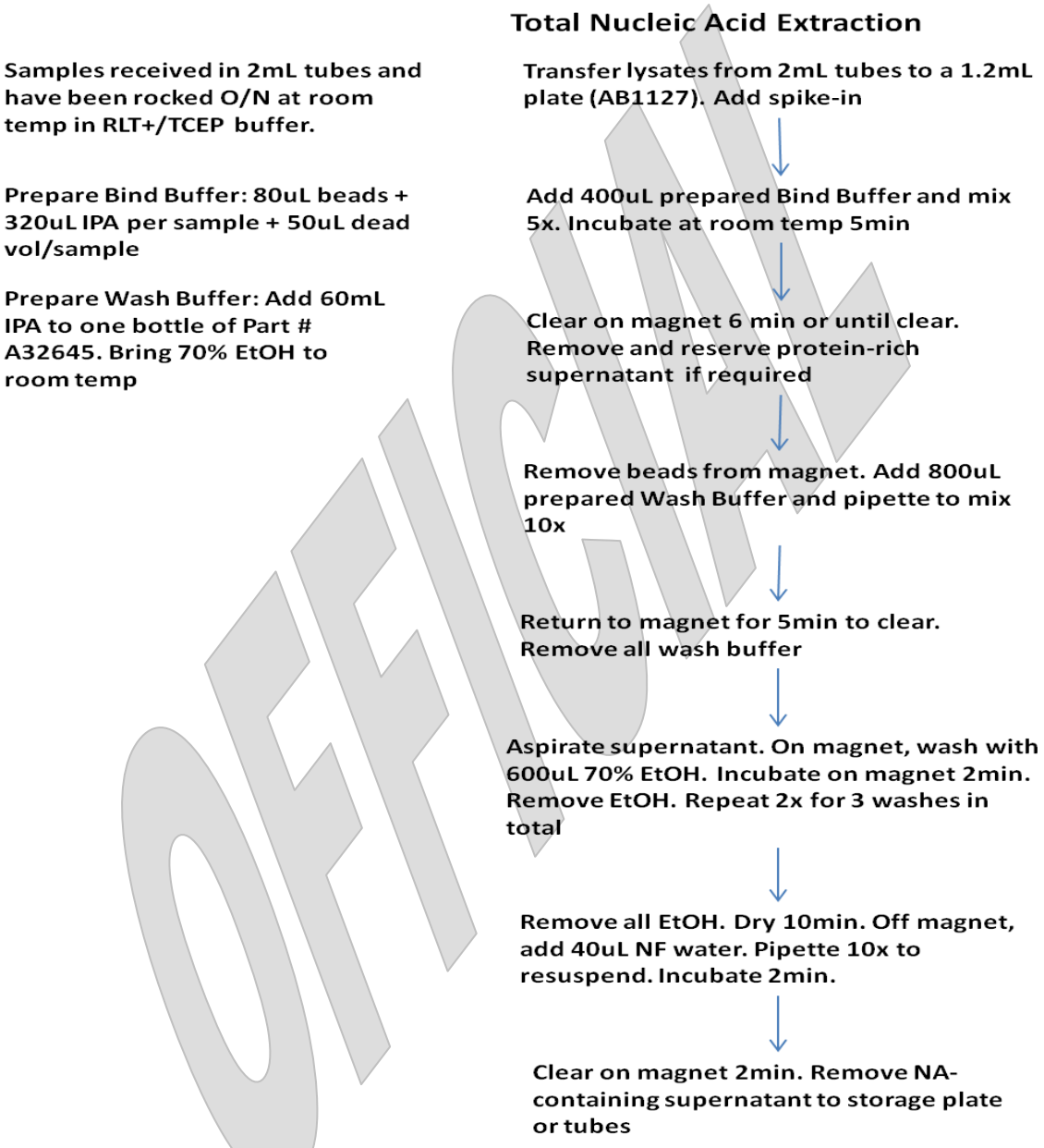
3. Workflow



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4. Buffy Coat and Whole Blood Samples submitted

Please follow the guidelines for working with biohazardous and infectious materials.

4.1. Use 50µL buffy coat or whole blood and add 20µL of 20mg/mL Proetinase K in the biological safety cabinet.

4.2. Incubate for 30 minutes at 25°C in a Thermomixer. Be sure to parafilm the tube.

4.3. Add 330µL of Buffer RLTplus with TCEP and nutate for a minimum of 30 minutes.

4.4. Proceed to step 6.

5. Saliva Samples submitted

Please follow the guidelines for working with biohazardous and infectious materials.

5.1. Mix tubes by inverting 10 times.

5.2. Transfer 420µL of the sample into a 2mL and parafilm the tube.

5.3. Be sure to add HelaS3 cell as control alongside.

5.4. Incubate for 2 hours at 50°C on Eppendorf Thermomixer.

5.5. Proceed to step 6.

6. Spike-in Addition to Samples

Please work in the fume hood. Even though the TCEP in the buffer is odourless, it is still harmful.

Work with a second person while adding spike-in and arranging your sample tubes to avoid errors.

6.1. The OCT sections have been rocked overnight, buffy coat has been rocked for 30 minutes and saliva has been incubated for 2 hours at 50°C. Please take them off the nutator and give them a quick spin at 14000rpm for 2 minutes.

6.2. Arrange the 2mL tubes in an 8x12 tube rack in the orientation provided by your supervisor.

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6.3. Thaw out the spike-in needed for the samples being extracted. Your supervisor will provide this information.

6.4. By referencing the plate layout provided, add spike-in to an AB1127 plate that corresponds to the samples to be arrayed into column 1.

6.5. Once the spike-in has been added, open the sample tubes that will be added to column 1. Use the Viaflow pipette to aspirate the samples and then dispense into column 1.

6.6. Continue in this way for the remaining samples: adding spike-in to a column first and then adding the sample to be extracted.

7. Extraction of Total Nucleic Acids

7.1. Before beginning the extraction protocol:

7.1.1. Prepare EvoPure RNA Bind Buffer following the calculator OCT Extraction on Nimbus Worksheet located in R:\Library Core\Work Sheets and Calculators\EvoPure Extractions on Nimbus. This buffer contains beads that will bind DNA and RNA molecules. Once the Total Nucleic Acids are purified, a DNase I step will be applied to a portion of the Total Nucleic Acids to purify the Total RNA.

7.1.2. Dispense 450µL of the prepared EvoPure Bind Buffer into the required wells of a new AB1127 plate.

7.1.3. Prepare the EvoPure RNA Wash I Buffer by adding 60mL of IPA to one bottle of Wash Buffer concentrate (Part # A32645, provided with the kit). Indicate on the bottle that the IPA has been added and record the date. Dispense 7mL per column into E & K deep 12 channel plate.

7.1.4. Retrieve sufficient 70% EtOH from 4°C to warm to room temperature. You will need 1.9mL/sample dispensed into a MagMax Express Deep 96-well plate.

7.2. Shake the prepared EvoPure Bind Buffer immediately before dispensing into a v-bottom sterile solution basin. Using a Rainin 1200µL multi-channel pipettor, dispense 400µL into each sample and mix 5 times at 80% total volume.

7.3. Incubate at room temperature for 5 minutes.

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7.4. Place the sample plate onto a Magnum FLX strong magnet plate and incubate for 6 minutes or until the supernatant has cleared. The protein fraction is in the supernatant, the Total Nucleic Acids is bound to the beads on magnet.

7.5. Using the multi-channel pipettor, remove the protein-rich supernatant from the beads and reserve it if required. Store the protein fraction on ice, covered, until the next incubation step is running. During the incubation, move the protein plate to the -80^{o}C freezer for storage.

7.6. Remove the sample plate from the magnet. Add 800µL of EvoPure RNA Wash I Buffer to each sample and pipette 10 times at 80% total volume to mix thoroughly.

7.7. Return the plate to the magnet for 5 minutes to clear. Remove the wash buffer.

7.8. Dispense 70% EtOH into a fresh v-bottomed sterile solution basin. With the plate on the magnet, add 600µL of 70% EtOH to each sample. Allow beads to resettle for 2 minutes. Remove all EtOH.

7.9. Repeat the EtOH wash 2 additional times, for a total of 3 EtOH washes.

7.10. With the plate on magnet, allow to dry for 10 minutes. During the incubation, dispense Ultrapure water into a fresh low profile plate. Remove the plate from the magnet and add 40µL of nuclease-free dH_2O. Pipette 10 times to mix thoroughly. Allow to elute for 2 minutes.

7.11. Move the plate back onto the magnet for 2 minutes. Remove the supernatant (containing the total nucleic acids) to an AB1000, the final destination plate. Label the plate with the tra and the date. Store at 4°C. Follow the LIMS protocol in the automated version of the protocol.

8. QC of Extracted Samples using Qubit

To avoid bead transfer during pooling or QC, put the sample plate on a magnet whenever taking aliquots.

8.1. After extraction, pool the same samples wells together. Have a second person watch this step.

8.2. Qubit the pooled sample as “neat” and a 1/5 dilution. Refer to LIBPR.0153 “Quantifying DNA Samples Using the Qubit™ 4 Fluorometer”.

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8.3. Complete the OCT Extraction tracking sheet found in R:\Library Core\Work Sheets and Calculators\Library construction work instructions. Send the sheet along with the QC files to your supervisor.

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