

Sample submission guidelines for NGS Grade Nucleic Acid Extraction: NGS-1000 series

Sample Type/Origin	For gDNA extraction	For RNA extraction
Animal Tissue	<p>Option 1: Tissue</p> <ul style="list-style-type: none">• Excise target tissue from animal• Rinse tissue with 1X PBS to remove all traces of blood• Weight: 50 - 100 mg of sample• Snap freeze sample in liquid nitrogen for 10 minutes• Store sample in -80 °C freezer• No. of replicates: 3• Shipping condition: Dry ice <p>Optional: Preserve sample in commercial DNA/RNA tissue stabilization solution, preserve and store sample according to stabilization reagent guide</p> <p>Option 2: Tissue in fine powder</p> <ul style="list-style-type: none">• Excise target tissue from animal• Rinse tissue with 1X PBS to remove all traces of blood• Grind tissue into fine powder with liquid nitrogen• Weight: 50 - 100 mg of sample• Store sample in -80 °C freezer• No. of replicates: 3• Shipping condition: Dry ice	
Cultured Cells	<p>Cell pellet</p> <ul style="list-style-type: none">• Cells up to 1 x 10⁷ (1 to 2 quantity of T25 flask, at 70% confluency)• Collect pellet from culture, wash cell pellet with PBS and remove PBS completely• Snap freeze pellet in liquid nitrogen for 10 minutes• Store sample in -80 °C freezer• No. of replicates: 3• Shipping condition: Dry ice <p>Optional: Preserve sample in commercial DNA/RNA stabilization solution, preserve and store sample according to stabilization reagent guide</p>	
Bacteria	<p>Bacterial pellet</p> <ul style="list-style-type: none">• Collect pellet from 10 - 30 ml overnight culture, remove culture medium completely• Weight: 200 - 500 mg of sample• Snap freeze pellet in liquid nitrogen for 10 minutes• Store sample in -80 °C freezer• No. of replicates: 3• Shipping condition: Dry ice	<p>Bacterial pellet</p> <ul style="list-style-type: none">• Dilute overnight culture to 1:50 ratio with culture media, continue grow for 3 - 5 hours• Collect the pellet from culture, remove culture medium completely• Weight: 200 - 500 mg of sample• Snap freeze pellet in liquid nitrogen for 10 minutes• Store sample in -80 °C freezer• No. of replicates: 3• Shipping condition: Dry ice
Yeast	<p>Yeast pellet</p> <ul style="list-style-type: none">• Collect pellet from culture, remove culture medium completely• Weight: 200 - 500 mg of sample• Snap freeze pellet in liquid nitrogen for 10 minutes• Store sample in -80 °C freezer• No. of replicates: 3• Shipping condition: Dry ice	<p>Yeast pellet</p> <ul style="list-style-type: none">• Dilute overnight culture to 1:50 ratio with culture media, continue grow for 4 - 5 hours• Collect the pellet from culture, remove culture medium completely• Weight: 200 - 500 mg of sample• Snap freeze pellet in liquid nitrogen for 10 minutes• Store sample in -80 °C freezer• No. of replicates: 3• Shipping condition: Dry ice
Fungi	<p>Fungal pellet</p> <ul style="list-style-type: none">• Collect pellet from culture, remove culture medium completely• Weight: 0.5 - 1 g of sample• Snap freeze pellet in liquid nitrogen for 10 minutes• Store sample in -80 °C freezer• No. of replicates: 3• Shipping condition: Dry ice	
Algae	<p>Microalgae pellet</p> <ul style="list-style-type: none">• Collect pellet from culture, remove culture medium completely• Weight: 0.5 - 1 g of sample• Snap freeze pellet in liquid nitrogen for 10 minutes• Store sample in -80 °C freezer• No. of replicates: 3• Shipping condition: Dry ice	
Blood	<p>Option 1: Buffy Coat</p> <ul style="list-style-type: none">• Collect up to 3 ml blood in EDTA tube, centrifuge the blood with 2,500 x <i>g</i> for 10 minutes using swing bucket centrifuge• Remove the plasma with 1 ml syringe, 21G needle• Collect the buffy coat in 1.5/2 ml tube• Store buffy coat in -80 °C freezer• Shipping condition: Dry ice <p>Option 2: Whole Blood, *Malaysia Customer only</p> <ul style="list-style-type: none">• Volume: 1 - 3 ml freshly collected blood in EDTA Tube• Blood samples to be submitted immediately after withdrawal.• Results will not be guaranteed for blood samples that are more than <u>3 days old</u>• Shipping condition: i) 4°C or Blue ice (reach within 3 days) ii) Dry ice (reach > 3 days)	<p>Option 1: White Blood Cell pellet</p> <ul style="list-style-type: none">• Collect up to 3 ml blood in EDTA tube• Transfer up to 1 ml whole blood into 2 ml microcentrifuge tube• Pretreat the whole blood sample with RBC lysis buffer (1st BASE, K.BUF-9101-100ml) <p><i>Protocol refer to: PrimeWay Total RNA Extraction Kit (1st BASE, KIT-9021), Protocol G – White Blood Cell (Leukocytes), Step 3A or 3B</i></p> <ul style="list-style-type: none">• Store WBC pellet in -80 °C freezer• No. of replicates: 3• Shipping condition: Dry ice <p>Option 2: Whole Blood in Tempus Blood RNA Tube</p> <ul style="list-style-type: none">• Collect up to 3 ml blood in Tempus Blood RNA Tube• Shake vigorously for 10 seconds to mix sample with stabilizing reagent in the Tempus Tube• Store sample in 4 °C fridge• Shipping condition: i) 4°C or Blue ice (reach within 5 days) ii) Dry ice (reach > 5 days)

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Insect	<p>Option 1: Insect</p> <ul style="list-style-type: none"> Immerse the live insect in absolute ethanol until the insect stops moving Blot dry the insect Weight: 50 - 100 mg of sample Snap freeze insect in liquid nitrogen for 10 minutes Store sample in -80 °C freezer No. of replicates: 3 Shipping condition: Dry ice <p>Optional: Preserve sample in commercial DNA/RNA tissue stabilization solution, preserve and store sample according to stabilization reagent guide</p> <p>Option 2: Insect in fine powder</p> <ul style="list-style-type: none"> Immerse the live insect in absolute ethanol until the insect stops moving Blot dry the insect Grind the insect into fine powder with liquid nitrogen Weight: 50 - 100 mg of sample Store sample in -80 °C freezer No. of replicates: 3 Shipping condition: Dry ice 	
Plant	<p>Fresh leaf/needle/stem/root, prefer young leaf/needle</p> <ul style="list-style-type: none"> Rinse with clean water to remove dirt attached to the plant sample Cut sample into small pieces (1 cm x 1 cm), keep in 50 ml centrifuge tube Weight: 2 - 5 g wet weight/ liquid nitrogen ground fine powder Snap freeze in liquid nitrogen for 10 minutes Store sample in -80 °C freezer No. of replicates: 3 Shipping condition: Dry ice <p><!-- Avoid pooling different plant parts/organs into the same tube, as lysis procedure is different for each part.</p>	
Soil/Sludge	<p>For Amplicon Sequencing application</p> <ul style="list-style-type: none"> Weight: 0.5 - 1 g of soil/sludge sample in screw cap tube Store sample in -80 °C freezer No. of replicates: 3 Shipping condition: Dry ice <p>For Shotgun Metagenomics application, we recommend to collect soil sample in soil preservation solution, e.g. Qiagen LifeGuard Soil Preservation solution.</p>	<ul style="list-style-type: none"> Collect soil sample in soil preservation solution, e.g. Qiagen LifeGuard Soil Preservation solution. Weigh 2 g of soil/sludge sample in 15 ml screw cap tube Add 5 ml of soil/sludge preservation solution Vortex or invert tube by hand until the entire soil/sludge sample and preservation solution are mixed well. <p><i>Excess preservation solution should be sitting on top of the soil sample.</i></p> <ul style="list-style-type: none"> Store sample in 4°C for overnight, transfer to -20°C freezer on the next day. No. of replicates: 2 Shipping condition: Dry ice <p>*Depends on soil types, additional sample replicates of 4 to 8 are possibly required in order to get sufficient RNA/DNA amount for downstream applications.</p>
Stool	<ul style="list-style-type: none"> Weight: 200 - 500 mg of sample in screw cap tube Label sample with printed labelling Store sample in -80 °C freezer No. of replicates: 3 Shipping condition: Dry ice 	-
Manure	<ul style="list-style-type: none"> Weight: 200 - 500 mg of sample in screw cap tube Label sample with printed labelling Store sample in -80 °C freezer No. of replicates: 3 Shipping condition: Dry ice 	-
Water	<p>Option 1: Filtered content on 0.22 µm membrane filter</p> <ul style="list-style-type: none"> Prefilter water sample with larger pore size cell strainer to remove large size debris Vacuum filtration of 300 - 500 ml* water sample with 0.22 µm pore size, 25/47 mm membrane filter <p>*If the sample has low turbidity (contained less filtered content), please continue to filter to reach volume of 1.5 L in total</p> <ul style="list-style-type: none"> Use a sterile scalpel blade to remove the membrane filter Insert the membrane filter into a new tube by rolling the membrane, with the side containing trapped sample facing inward of the tube Store membrane filter in -80 °C freezer No. of replicates: 2 Shipping condition: Dry ice <p>Option 2: Water sample</p> <ul style="list-style-type: none"> Volume: 500 ml* <p>*If the sample has low turbidity, please submit at least triplicates, i.e. 500 ml x 3</p> <ul style="list-style-type: none"> Store sample in dark in 4°C in a screw cap bottle Shipping condition: 4°C or Blue ice 	-

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Food	Food (Solid) <ul style="list-style-type: none"> • Weight: 2 - 5 g sample in screw cap tube/container/zip lock bag • Shipping condition: follow the food storage condition, as long as the food is not rotten during shipment a) If it was stored in 4°C, send in blue ice b) If it was stored in freezer, send in dry ice Food (Liquid) <ul style="list-style-type: none"> • Weight: 20 - 50 ml sample in screw cap tube • Shipping condition: follow the food storage condition, as long as the food is not rotten during shipment a) If it was stored in 4°C, send in blue ice b) If it was stored in freezer, send in dry ice	-
Swab sample (Buccal swab/ enviromental swab)	<ul style="list-style-type: none"> • Collect sample using swab or commercial swab sample collection kit • (Optional) Store swab in 1X PBS or swab collection kit provided stabilizer • Storage condtion: -80°C or follow the guide from the commercial kit • No. of replicates: 2 - 3 • Shipping condition: Dry ice 	Please enquire. Suggestion to follow commercially available collection kit guidelines for sample collection, preservation, storage and shipping.
Others	Please enquire.	

How to snap freeze sample?

- Place the sample into a sterile empty tube (1.5/2 ml tube with safety lock cap or 15/50 ml screw cap centrifuge tube)
- Close the tube
- Immediately submerge the tube into liquid nitrogen bath
- The sample should be froze for at least 10 minutes
- Store sample in -80 °C freezer