Micromon
GENOMICS, BIOTECHNOLOGY AND DIAGNOSTICS

Phone (03) 9905 8592
Fax (03) 9905 4803

MicromonGenomics@monash.edu
http://DNA.med.monash.edu.au

Next-Generation Sequencing: Sample Submission Guidelines

SAMPLE SUBMISSION FORMS

Please complete a sample submission form before submitting your samples: enter your details directly into the form, save it, and email it to **MicromonGenomics@monash.edu**. If you encounter problems when saving the form, please use the latest Adobe Acrobat/Reader software; some older versions, and the default Mac PDF reader, do not conform to Adobe PDF standards when saving fillable forms (they appear blank after saving). Please also include a single-column spreadsheet containing your sample names, exactly as they are marked on your tubes. For plate submissions, include a plate coordinate column, or arrange sample names in grid format. Please note that you must include a Monash cost centre and fund code for Monash payments, or a purchase order number for all other payments. We cannot commence your projects until we the form is complete.

SAMPLE QC AT MICROMON

All samples delivered to Micormon Genomics will undergo QC at no charge for the first measurement. This will include either Agilent Bioanalyzer 2100 microfluidic separation or Fragment Analyzer 5200 capillary separation for sizing, and either DNA- or RNA-specific Invitrogen Qubit or DeNovix DS11 fluorimetry for quantitation. If samples require resubmission for any reason, or if samples from a project are replaced due to initial sample failure, the QC steps are billed at our standard stand-alone QC Service rate (contact us for further information).

Although we will carry out QC measurements of your samples, we advise that you carry out some preliminary investigations of your samples before submitting them if you are able to do so, in order to streamline your sample processing and avoid resubmission of samples that don't pass our QC requirements. If you carry out any QC on your samples, please submit these data along with your sample submission forms.

SPATIAL TRANSCRIPTOMICS

Our spatial transcriptomics services are based around the 10X Genomics Visium and MGITech Steromics systems. Please contact Micromon Genomics to arrange a meeting to discuss sample preparation requirements and logistics for spatial transcriptomics projects.

SINGLE CELL (10X GENOMICS CHROMIUM)

Our single-cell analysis methods are based around the 10X Chromium platform. Please contact Micromon Genomics to arrange a meeting to discuss sample preparation requirements and logistics for single-cell sequencing projects using the Chromium system (including transcriptomics, immune profiling, cell surface protein assay, ATAC-seq, multi-omics, and hash-tagging).

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DNA SEQUENCING

Samples for DNA sequencing should be purified using a method that can deliver high-quality DNA that is as intact as possible. Most commercial column-based purification kits should suffice. The DNA should be of sufficient quality for use in general molecular biology techniques such as PCR, restriction enzyme digestion, *etc*.

Sample purity should be assayed spectrophotometrically by measuring the ratio of absorbance at 260nm & 280nm. The ratio should be 1.8 to 2.0. Samples should be free of substances that inhibit enzymatic action (*e.g.* haem, EDTA, lithium salts, humic acids, detergents, chaotropic salts, *etc.*) and molecular carriers (*e.g.* tRNA, acrylamide, glycogen, 'pellet paint', *etc.*)

If possible, sample concentration should be measured using a DNA-specific measurement technique such as fluorometry (*i.e.* Invitrogen Qubit, or similar) as this provides a much more accurate determination of the true DNA concentration.

Micromon will advise you of the library preparation technology that will be used for your samples. Table 1 lists the *minimum recommended* mass and volume per sample. If your samples are more concentrated than indicated, please do not dilute them. Smaller quantities of DNA may be provided after approval by a Micromon staff member.

Library Type	DNA Mass	Concentration
Nextera XT	≥ 2ng	≥ 0.2 ng/µL
Nextera	≥ 75ng	≥ 10 ng/µL
TruSeq	≥ 1.5ug	≥ 20 ng/µL
MGI DNA FS	≥ 20ng	≥ 5 ng/µL

Table 1: Masses and volumes of DNA required, per sample, for each library preparation technology.

The DNA should be dissolved in 10 mM Tris-HCl (pH 8.5), ultrapure water or another buffer that is free of enzyme inhibitors and carrier molecules (*e.g.* EDTA, acrylamide, etc.). Alternatively, DNA may be delivered dry. Samples should be delivered on ice (aqueous samples) or at room temperature (dry samples) to the address listed in the 'Sample Delivery' section of this document.

CHiP - SEQUENCING

A total of 20ng of DNA is required for immunoprecipitated DNA samples. The DNA should be dissolved in a volume no larger than 50µL of 10 mM Tris-HCl (pH 8.5), TE or a suitable buffer such as your purification kit's elution buffer (i.e. Qiagen EB), or similar.

We recommend that you very carefully determine the concentration of your precipitated DNA using a sensitive technique such as quantitative real-time PCR or a fluorometric measurement such as a PicoGreen assay (we recommend the Invitrogen Qubit fluorimeter and QuantIT/Qubit kits, or similar).

Samples should be delivered on ice to the address listed in the 'Sample Delivery' section of this document.

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SINGLE–TUBE LONG FRAGMENT DNA SEQUENCING (stLFR)

The stLFR method depends on the presence of very long DNA molecules in your sample (high molecular-weight, or HMW, gDNA). If you are not familiar with handling HMW gDNA, please contact Micromon for additional handling tips.

Samples should be purified using a method that can produce high-quality DNA that is both as pure and as intact as possible. It should score 8+ using an Agilent Fragment Analyzer genomic DNA QC (we will perform this QC for you). You should use a commercial kit specifically designed to produce high molecular weight DNA. We recommend Circulomics NanoBind, Agilent Recoverase or Qiagen MagAttract. Please read the associated literature with these kits, particularly with regard to methods for preventing the accidental shearing of your DNA. You can also use traditional phenol/chloroform with ethanol precipitation and spooling of the DNA, or density gradient purification, but you should measure your sample spectrophotometrically to ensure that all phenol, chloroform, alcohol and gradient substrate has been removed. Please note that most standard commercial purification kits will not produce DNA of a sufficiently high molecular weight. Regardless of the purification method, please avoid using precipitation carriers and markers.

Sample purity should be assayed spectrophotometrically by measuring the ratio of absorbance at 260nm & 280nm. The ratio should be 1.8 to 2.0. Samples should be free of substances that inhibit enzymatic action (*e.g.* haem, EDTA, lithium salts, humic acids, *etc.*) and molecular carriers (*e.g.* tRNA, acrylamide, glycogen, Pellet Paint, *etc.*)

If possible, sample concentration should be measured using a DNA-specific measurement technique such as fluorometry (*i.e.* Invitrogen Qubit, or similar). This provides a much more accurate determination of the true DNA concentration.

The minimum mass of DNA is 50ng in no more than 35uL, and no less than 10uL. The DNA should be dissolved in 10 mM Tris-HCl (pH 8.5), ultrapure water or another buffer that is free of enzyme inhibitors.

A fresh sample should be prepared specifically for this library submission. If required, store the samples at 4 degrees, but do not freeze and thaw.

Samples should be delivered on wet or blue ice to the address listed in the 'Sample Delivery' section of this document.

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RNA SEQUENCING

Please note: If you are submitting cDNA, then the requirements in terms of quantity and purity are the same as those for 'DNA Sequencing', listed above. The following section applies only to the submission of RNA samples such as total RNA, small RNA or mRNA.

All samples for RNA sequencing, profiling and transcriptomics (including small RNA) should be purified using any method that can deliver high-quality, intact RNA. We recommend the Qiagen range of RNA purification kits and columns, although other similar kits should also be sufficient. If you are planning a smallRNA/microRNA sequencing project, please ensure that your RNA purification kit recovers these RNA species (many kits don't collect RNA less shorter than 100b). Please avoid using precipitation carriers and markers such as tRNA, 'pellet paint', lithium chloride and so forth, as they may reduce the quality of library preparation and/or sequencing. Samples should be dissolved in DEPC treated, or certified RNase free water. Table 1 indicates the *minimum recommended* mass and volume of RNA required per sample; Micromon will advise you on the RNA library type. Smaller quantities of RNA may be provided after approval by a Micromon staff member. If your RNA is more concentrated than indicated, do not dilute it.

Library Type	RNA Mass	Concentration
Tecan/Nugen Ovation SoLo starting from cells	Contact Micromon	Contact Micromon
Tecan Nugen Ovation SoLo starting from RNA	≥ 20pg	≥ 2 pg/µL
MGIEasy RNA Directional (poly-A mRNA)	≥ 200ng	≥ 20 ng/µL
MGIEasy RNA Directional (ribodepletion)	≥ 100ng	≥ 10 ng/µL
MGIEasy Small RNA	≥ 100ng	≥ 10 ng/µL
Perkin Elmer NEXTFlex Small RNA	≥ 10ng	≥ 1ng/uL

Table 1: Masses and volumes of RNA required, per sample, for each library preparation technology.

Samples should be delivered frozen on dry ice to the address listed in the 'Sample Delivery' section of this document.

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MICROBIAL COMMUNITY PROFILING and METAGENOMICS

Samples for 16s/18S/ITS sequencing, microbial community profiling and metagenomics should be purified using a method that can deliver high-quality DNA that is as intact as possible and free from environmental contaminants. We recommend MOBIO/Qiagen products. Please avoid using precipitation carriers and markers such as tRNA, 'pellet paint', lithium chloride and so forth, as they may reduce the quality of library preparation and/or sequencing.

Sample purity should be assayed spectrophotometrically by measuring the ratio of absorbance at 260nm and 280nm. The ratio should be between 1.8 and 2.0. Samples should be free of substances that inhibit enzymatic action or compete with DNA (*e.g.* haem, humic acids, EDTA, lithium salts, *etc.*) and molecular carriers (*e.g.* tRNA, acrylamide, *etc.*)

If possible, sample concentration should be measured using a DNA specific measurement technique such as fluorometry (*i.e.* Invitrogen Qubit, or similar). This provides a much more accurate determination of the true DNA concentration.

We require 10ng or genomic DNA per sample at a concentration of at least 0.2ng/µL. Smaller quantities of DNA may be provided after approval by a Micromon staff member.

The DNA should be dissolved in 10 mM Tris-HCl (pH 8.5), ultrapure water or another buffer that is free of enzyme inhibitors and carrier molecules (*e.g.* EDTA, acrylamide, tRNA, etc.). Alternatively, DNA may be delivered dry.

Samples should be delivered on ice (aqueous samples) or at room temperature (dry samples) to the address listed in the 'Sample Delivery' section of this document.

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SAMPLE DELIVERY

Please contact us to discuss your project and to obtain a quote before submitting your samples. We cannot accept samples without prior approval and without a completed and electronically delivered (by email) sample submission form. In addition to your sample submission form, please email a spreadsheet or text file containing your sample names. Please be aware that you will receive files with names including your sample names. Avoid names that are long, complex, contain whitespace or characters that do not appear on a QUERTY keyboard, underscores, or any characters that cannot appear in a file name. Any such characters will be replaced with a single hyphen character.

Samples should be contained in 1.5mL or 2.0mL screw-capped microcentrifuge tubes to minimise evaporation, or in 96-well trays sealed with plate seals (heat sealed, or storage seals; do not use low-adhesive PCR seals). Do not use Parafilm (or equivalent) to seal your tubes. If you are not delivering your samples in person, please place these tubes inside a 50mL screw cap centrifuge tube, or similar, packed with tissue or other space filling material to prevent them moving during transport. Place these tubes in a shipping package suitable for the temperatures at which your sample will be stored. The samples should be shipped on wet ice for aqueous DNA, dry ice for aqueous RNA, or room temperature for dry samples and RNA in ethanol/sodium acetate.

Label the tubes clearly using a permanent marker directly on the tubes, or on tube labels that can withstand the temperatures at which you will be shipping your samples. Your tube labels **must correspond exactly** to the electronically provided sample names. Tubes should not display any other markings, labels, dates or concentrations. If you wish to communicate additional details to Micromon staff, please include these details in the 'Notes' section of the sample submission form or enclose additional paperwork.

Samples should be delivered to the following address either by post, courier or in person (during business hours of 8am - 5pm, Monday to Friday):

Micromon Genomics
35 Rainforest Walk
Monash University
Wellington Road, Clayton
Victoria, 3168
Australia.

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TERMS AND CONDITIONS OF THE SEQUENCING SERVICE

1. The research services and materials provided by Monash to You are subject to the following terms and conditions. 'You' or 'yours' refers to you the client; and 'us', 'we' or 'ours' refers to Monash University, Micromon and the High Throughput Sequencing Service (**MHTSS**). In these terms and conditions unless expressed or implied to the contrary:
   a. **Fee** means the amount payable for the provision of the Services as specified in the Quotation.
   b. **GST** means GST as defined in the A New Tax System (Goods and Services Tax) Act 1999 as amended (GST Act) or any replacement or other relevant legislation and regulations.
   c. **Quotation** means the quotation annexed to and forming part of these Terms and Conditions.
   d. **Services** mean the sequencing services specified in the Quotation.
   e. **Your Instructions** mean the instructions from You to Us as specified in the Quotation in relation to the provision of the Services.
2. We will perform the Services taking into account Your Instructions, if any.
3. We will supply all personnel, equipment, materials and other things necessary to perform the Services excepting only those items expressed in the Quotation to be supplied by You (**Your Items**). We will return or destroy (at Our election) all of Your Items upon completion of the Services or termination of Our agreement constituted by these Terms and Conditions. You must cooperate with Us and must not interfere with or obstruct the proper performance of the Services.
4. In consideration of the performance of the Services, You will pay to us the Fee detailed in the Quotation (or as otherwise provided) within 30 days of receipt of a tax invoice from us. The total Fee for materials and or Services, unless otherwise stated, excludes GST and freight.
5. Raw and processed data will be retained by Us for a period of not less than 6 years from the date of receipt of the data by You. You may request the raw data and in such circumstances it will be provided on a FAT32 (Microsoft Windows) pre-formatted hard disk drive, at a price to be agreed.
6. The risk in the materials ordered passes to you at the time the materials are ordered. Title in the materials ordered passes to you at the time of payment in full of the Fee.
7. You may not alter or cancel an order for Services without our prior written consent. If we agree to alter or cancel the order, you indemnify us against any loss, damage and expense incurred by us in relation to the alteration or cancellation of that order, including the cost of return freight, return shipping to factory of origin, items purchased from third parties for inclusion in the materials and all labour and engineering costs incurred by us in the execution or part execution of the materials and including compensation payable to any of our suppliers and loss of profit. In any event, we may alter the sample requirements, the methods used to prepare the samples and to perform the Services without notice.
8. Ownership of rights in any pre-existing intellectual property not created as part of the Services which are contributed by a party for the purpose of carrying out the Services, will remain with the contributing party. Where the pre-existing intellectual property is contributed by You, We are licensed to use, modify or adapt that intellectual property for provision of the Services.
9. Subject to clause 8, the intellectual property rights attaching to all material created or prepared by Us in connection with performance of the Services shall vest in You. We will assign all right, title and interest in the materials created or derived from performance of the Services to You.
10. Subject to payment of all outstanding Fees (if any), We will execute all and any documents and do all acts and things necessary to effect the assignment under clause 9, including after completion or termination of the Services.
11. You acknowledge that research work is by its nature uncertain and that outcomes cannot be assured. Whilst We will exercise reasonable care and diligence in carrying out the Services, We specifically exclude any warranty as to the outcomes of the Services or that the Services will be completed within certain time-frames.
12. All statutory or implied conditions and warranties are excluded to the extent permitted by law. To the extent permitted by law, liability under any condition or warranty which cannot legally be excluded is limited to:
    a. in the case of materials, the repair the Materials or supply replacement materials; and
    b. in the case of research services, supplying the research services again or paying the cost of having the research services supplied again.
13. You acknowledge and agree that, to the extent permitted by law, we have no liability in contract, tort (including negligence or breach of statutory duty), by statute or otherwise for loss or damage (whether direct or indirect) of profits, opportunity, revenue, goodwill, bargain, production, contracts, business or anticipated savings, corruption or destruction of data or for any indirect, special or consequential loss or damage whatsoever.
14. Our total liability under any contract and these Terms and Conditions shall not exceed the total dollar amount of the materials purchased or research services purchased by you under each contract.
15. You will defend, indemnify, and hold us harmless from and against any and all actions, judgements, liabilities, loses, damages, expenses, claims, suits and demands of whatever nature (**Losses**), arising from or connected with the provision of materials or research services pursuant to these Terms and Conditions, except to the extent that the Losses arise due to negligence or wilful misconduct by us.
16. The arrangements constituted by these Terms and Conditions may be terminated by either party at any time on the provision of 14 days written notice of intention to terminate.
17. If the arrangements constituted by these Terms and Conditions are terminated at your instigation you will still be required to pay for any milestones already reached as set out in the description of services and a pro-rata amount for any milestones partially achieved as advised by us acting reasonably.
18. These Terms and Conditions will be governed by and construed in accordance with the laws of Victoria and the Courts of Victoria will have jurisdiction to entertain any action in respect of, or arising out of, these Terms and Conditions.
19. These Terms and Conditions constitute the entire agreement between you and us and supersedes all communications, negotiations, arrangements and agreements, whether oral or written, between you and us with respect to the subject matter of these Terms and Conditions.
20. All Quotations are strictly confidential and the price, terms and conditions must not be disclosed to any third party.
21. Quotations are only valid for 30 days from the date of issue.