



OMICS RESOURCE CENTRE

Sample Submission Guidelines

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INTRODUCTION

Thank you for submitting to the Omics Resource Centre (ORC)!

ORC is a next-generation sequencing ("NGS") service laboratory and research support core operating within the Western College of Veterinary Medicine ("WCVM") at the University of Saskatchewan ("USask") in Saskatoon, Canada. The Omics Resource Centre is not a standalone legal entity but operates as a Fee-for-Service facility within WCVM and USask, aligning with university policies on research compliance, ethics, data stewardship, and infrastructure governance.

ORC's mission includes:

- (a) To serve as a permanent, modular, and sustainable omics infrastructure performing crude sample processing and nucleic acid extraction, NGS library preparation, and sequencing services.
- (b) To support genomics and multiomics research across veterinary medicine, animal science, environmental stewardship, and related disciplines with consultations, instrument access and training for highly qualified personnel (HQPs).
- (c) To function as an extension of the academic research environment, tailored to meet evolving scientific needs with precision, scalability, and professional-grade execution by offering custom standard operating procedure (SOP) development with and without the goal of automation.

Please contact the ORC before starting your sample collection to consult on sample handling options and review the following requirements before preparing samples, then proceed with the ORC LIMS Requester Module or proceed to the Critical Info & Checklist tab of the ORC Sample submission template ("SST").





Project submission confirms the acceptance of ORC's TERMS & CONDITIONS on the ORC website or the ORC LIMS Requester Module. If you have any questions, please email us at orc@usask.ca or contact us through ORC LIMS.

EXPERIMENTAL & SEQUENCING APPLICATION DESIGN

ORC offers free-of-charge consultations either in person (WCVM room 2209) or via MS Teams at a mutually convenient time. Please contact ORC at orc@usask.ca to schedule a meeting or use this link to book a virtual meeting (https://tinyurl.com/ORC-Consultations). Prior to the meeting, please provide any relevant papers and a short description of your project as well as any questions you may have. ORC also provides educational material on its website (https://wcvm.usask.ca/research/facilities/orc.php). Customers will find an assortment of informational guides, papers and links which are continuously updated. ORC highly recommend that Customers review 'ENCODE Experiment Guidelines' in the experiment planning stage (https://www.encodeproject.org/about/experiment-guidelines/).

PAYMENTS & SUBMISSION REQUESTS

The Customer is required to contact <u>orc@usask.ca</u> to request a Quote prior to receiving access to ORC's LIMS in order to place a service request and to be permitted to submit samples.

The Quote is valid for three months from date of issue, and samples must be received before quote expiration. The Customer must review their Quote for additional accounting information that may be required by their procurement department or financial manager. ORC may be registered as a vendor with the Customer's procurement department. The Customer must forward the service quotation to their finance department or contact their finance officer for assistance.

For Customers internal to USASK ("Internal Customer") the Quote will be exempt of Goods and Services Tax (GST). Internal Customers are required to provide a USASK fund number (i.e. CFOAPAL) and the contact information of their financial officer.

For Customers external to USASK ("External Customer") ORC requires institutional purchase orders (POs) for payment before starting to process samples. For External Customers the Quote will contain the GST. The External Customer is responsible for paying any tax, duty, custom or other fee of any nature imposed on the Services by any federal, provincial, local or foreign government authority other than the GST on ORC's income and in addition to the price for the Services quoted or invoiced.





SAMPLE LABELING

Carefully fill out the 'Critical and Sample Information' sections in the Customer portal of ORC LIMS or the SST. You must be a registered Customer with ORC LIMS and all required sample submission information tabs must be filled out before sample processing can begin.

All nucleic acid samples should be submitted in either in nuclease-free, sterile 1.5 ml low-binding DNA microcentrifuge tubes with Safe-Lock or Snap-Lock design (e.g. Eppendorf or Axygen) in a 9x9 cardboard freezer storage box, or in 0.2ml strip tubes (with attached caps only), or in 96-well PCR plates sealed with either an adhesive seal when stored in standard -20 freezers or fridges and thermos-sealed when stored at -80C or in liquid nitrogen. Please note that we do not accept submissions of samples in loose tubes or tubes of another format than described above.

Plated samples should be ordered by columns (A1-H1), not rows (A1-A12) and clustered in batches of 24. Let us know which samples should be processed together as a batch. Keep those samples clustered on the plate.

We require printed labels or legible handwriting that includes a running integer per sample, e.g. 1, 2, 3...n on the top and on the side of a tube in addition to the ORC Estimate ID on the tube box (e.g. JOHN_DOE_WCVM-ORC-25-0521-3) or a 96-well plate with only the ORC Estimate ID on the top and side that corresponds to information provided either through the ORC LIMS Requester Module or the Sample Information tab of the SST. Please all crucial sample information in the ORC LIMS Requester Module or the Sample Information tab of the SST. Please do not leave anything out. We reserve the right not to process your samples without this data.

CRUDE SAMPLE REQUIREMENTS

If you are <u>submitting crude samples</u> for nucleic acid extraction, please keep in mind that **ORC does not accept samples that contain <u>active</u> pathogens, biohazardous contaminations or samples in fixatives or solvents. All pathogens must be inactivated.** We request that customers provide all necessary sample information in the Critical Info & Checklist tab of the SST or in the requester module of <u>ORC LIMS</u>. ORC reserves the right not to process your samples without this data or untruthful information.

All crude samples for nucleic acid extraction should be submitted in either 96-deep-well plates (*e.g.* Cole-Parmer Spex® 2200-100 Deep-Well Microplate or Greiner Bio-One Masterblock® 96-Well Deep Well Microplates; FisherScientific, cat#07000118) or 2 ml collection tubes (*e.g.* Eppendorf™ DNA LoBind™ FisherSci cat#13-698-792).

If customers provide other samples storage containers (Falcon tubes, Ziplock bags etc.) the overall processing time will be delayed, and the customer will be charged extra for sample preparation time on their estimate.

Customers are asked to provide a few extra samples for testing along their actual sample set (highly recommended). For crude samples, pathogen removal is required. All samples including those with unknown pathogen status need to be treated similar to pathogen-infested samples. The customer remains financially liable for missing or failed decontamination procedures.





We highly recommend the Zymo Research DNA/RNA Shield™ reagent (Zymo Research, cat# R1100-50), as DNA and RNA stabilization solution in any biological sample. The recommended Zymo Research DNA/RNA Shield™ fixative reagent [cat# R1100-50] can be supplied by the ORC on request. This DNA and RNA stabilization solution preserves the genetic integrity and expression profiles of samples at ambient temperatures and completely inactivates infectious agents (viruses, bacteria, fungi, & parasites). Zymo Research DNA/RNA Shield™ reported no volatile organic compounds (VOCs) and as such samples preserved with this reagent can be handled without specific ventilation equipment (e.g. fume hood; https://zymoresearch.eu/collections/dna-rna-shield/products/dna-rna-shield). Samples treated with Zymo Research DNA/RNA Shield™ reagent [cat# R1100-50] as directed by the manufacturer can be submitted to the ORC at ambient temperature (i.e. 4-30C) and are stabilized for up to 30 days (up to 7 days for >35C). Refer to detailed guidelines on reagent use further below in the 'Sample collection information' section or follow the manufacturer protocol under https://zymoresearch.eu/collections/dna-rna-shield/products/dna-rna-shield.

Customers who do **use classical fixatives** (GA, FA, formalin, acetone, AA, TFA) and decontaminants with high VOC emission <u>MUST</u> remove the fixative/decontaminant by washing the samples twice with 70% ethanol and remove any ethanol <u>BEFORE</u> sample submission. Sample should be optimally submitted on ice packs to preserve DNA integrity.

Customers who request small or total RNA extraction from their decontaminated crude samples must store their samples at -80C and submit their samples in a deep-freeze state (i.e. either through local submission in an liquid nitrogen transfer vessel or shipped on dry ice).

CRUDE SAMPLE COLLECTION INFORMATION

| CRUDE SAMPLE TYPE | SAMPLE AMOUNT REQUIRED | TREATMENT | SAMPLE-TO-DNA/ RNA SHIELD™ RATIO |
|---|------------------------------|---|-------------------------------------|
| FFPE | 1 curl | A 96-well deep-well plate or 2ml microtubes containing a single 10 μm paraffin curl (~600-800mm2) or 2x 10 μm paraffin curl @ 400mm2 each or 3x 10 μm paraffin curl @ 200mm2 each. Note: Do not use the first 2-3 sections from the FFPE sample block. | N/A |
| Biological fluids (whole blood, semen, washes,) | 200ul or less | samples >200ul need to be aliquoted to 200ul. DNA/RNA Shield™ mandatory. | 1:3 volumes |
| Swabs (nasal, rectal, fecal,) | 1ml or less | DNA/RNA Shield™ mandatory. Swab isolation method (see 'ORC_SOP003'): Thoroughly rinse mouth out with water before isolating cells. Brush the inside of the cheek with a buccal swab (synthetic not cotton tipped) for 15 seconds (approximately 20 brushes), making sure to cover the entire area of the inner cheek. Rinse the brush into a 96-well plate or tube using DNA/RNA Shield™, swirl the swab a few times and remove swab. | submerged (or optionally rinsed) |





| Solid tissue (ear, tail, liver,) | 25mg or less | Users need to cut samples in pieces not larger than 5mm in each dimension! Can be submitted in a frozen state OR in DNA/RNA Shield™ OR classically fixated tissue. Classically fixated tissue must be washed with 70% ethanol and the ethanol must be removed prior to sample sumbission. | submerged (typically 200ul) |
|----------------------------------|---|---|---------------------------------------|
| Hair follicle | >20 follicles | Hair samples can be collected in coin bags and stored at RT for up to a week. 1) Grab a sufficient number of hairs (20+) close to the root and pull in one quick motion to remove the hairs including the follicles. 2) Visually inspect that follicles are present. 3) Keep the hair in a pinch under any circumstances and use standard single sided adhesive tape to wrap around the hair bundle approximately 2 cm above the follicles. 4) Trim the excess hair shaft from the side opposite the follicles behind the tape wrap. 5) Place the hair bundle with the tape wrap into a coin envelope and store at room temperature for up to 7 days. 6) In the lab, use forceps to move the hair out of the envelope. Position the forceps below the wrapping tape, pinch the hair bundle and cut off and dispose of the adhesive tape wrap. 7) Transfer the remaining hair bundle with the follicles into a fresh nuclease-free sterile 2 ml micro tube. Alternatively, samples can be directly stored in 2 ml tubes at 4C or -20C for long term storage. Prior to sample submission ALL hair samples are required to be clipped 1-2cm above the follicle and placed into 2ml microtubes. Hair samples can be submitted in a frozen/cooled state OR submerged in DNA/RNA Shield™. | Optional: submerged (typically 200ul) |
| Saliva | 200ul or less | DNA/RNA Shield™ mandatory | 1:1 volumes |
| Bacterial culture | <10 ⁸ cells pelleted or 2-3 bacterial colonies in DNA/RNA Shield™ | DNA/RNA Shield™ mandatory | 200ul |
| Feces | 50mg/100ul or less | DNA/RNA Shield™ mandatory | 1:9 volumes |
| Environmental (fungi, soil) | 50mg or less | Can be submitted in a frozen state OR in DNA/RNA Shield™ OR classically fixated tissue. Classically fixated tissue must be washed with 70% ethanol and the ethanol must be removed prior to sample submission. | 1:4 volumes |





NUCLEIC ACID SAMPLE REQUIREMENTS

If you are <u>submitting nucleic acid samples</u>, please carefully review the NUCLEIC ACID SAMPLE REQUIREMENTS section to understand the minimum quantity of material you'll need to submit for your desired application. Please use the chart below to review the nucleic acid mass and volume requirements for library preparation methods that we offer.

We request that customers strictly follow quality control guidelines to ensure successful projects. Samples should ideally be resuspended in molecular biology grade water (RNAse-free; not DEPC treated) or EB (10mM TRIS, pH=8.0-8.4, e.g. from Qiagen cat#19086) as buffers such as TE contain contaminants that can interfere with downstream enzymatic reactions leading to library prep failure and processing delays. Samples should be quantified using fluorometry (Qubit/Quant-iT) and tested for contamination using spectrometry. It is our standard of service to measure all incoming samples on our fluorometric instruments regardless of whether this info was provided by customer. Please provide 260/280 and 260/230 ratios in ORC LIMS Requester Module or on the Sample Information tab of the SST. All information requested in the ORC LIMS Requester Module or the Sample Information tab and Critical Info & Checklist tab of the SST is crucial. Please do not leave anything out. We reserve the right not to process your samples without this data.

For nucleic acid sample submissions, we highly recommend providing at least twice the minimum mass, concentration and volume indicated in the overview below to maximize the chances of successful NGS library preparation. We recommend submitting a >5 μ L aliquot of each sample for QC in order to avoid freeze/thaw of main samples.

Please also note that NanoDrop routinely overestimates concentration and as is only accepted to test for sample purity NOT as quantification method. Please use alternative fluorometric nucleic quantification procedures (Qubit, Microplate readers) or book a quantification service with ORC.

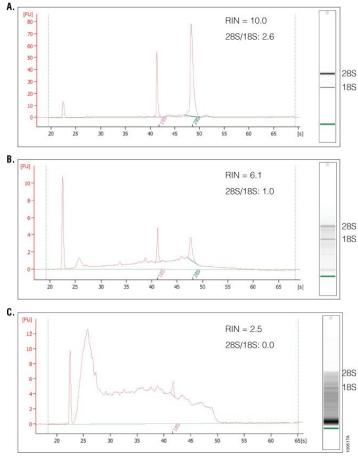
For **DNA** sample submissions we require gel images or BioAnalyzer/TapeStation traces. All DNA samples need to be <u>RNA-free</u>. For **RNA** samples, we <u>require DNase treatment</u> with a bead-based method and integrity assessment with BioAnalyzer/TapeStation. For DNA removal we highly recommend the bead-based Invitrogen DNA-free DNA Removal Kit (cat# AM1906), as on-column digestion methods such as Qiagen RNase-Free DNase Set may lead to poor library quality. Both DNase treatment and TapeStation services are offered at ORC. Please request this on your quote.

Samples of unknown quality (e.g. no Qubit or microplate reader data and/or gel images submitted, or with quality below our stated thresholds) are accepted, but on an "on risk" basis; the ORC cannot guarantee successful library preparation or sequencing of these "on risk" samples, and the customer remains financially liable for failed libraries. We additionally do not guarantee library performance of customer-prepared libraries, nor of challenging samples with high GC/AT content.

With any sample, when library preparation fails, we will make one attempt to re-prepare the failed sample. If the second preparation fails, this typically indicates an undetected problem with the submitted material; we can prepare again but will charge for an additional sample. We'll reach out to you to discuss your options.



| Process | min. mass (ng) | min. conc. (ng/μL) | 260/280 | 260/230 | RIN score |
|--|----------------|-----------------------|---------|---------|-----------|
| RNA: mRNA-Seq | 100 | 12 | >2.0 | 2.0-2.2 | >8.0 |
| RNA: 3' mRNA-Seq | 10 | 2 | >2.0 | 2.0-2.2 | ANY |
| RNA: total RNA-Seq | 10 | 2 | >2.0 | 2.0-2.2 | >8.0 |
| RNA: small RNA-Seq | 100 | 20 | >2.0 | 2.0-2.2 | >8.0 |
| RNA: FFPE-RNA-Seq | 10 | 2 | >2.0 | 2.0-2.2 | ANY |
| RNA: Ribodepletion | 100 | 10 | >2.0 | 2.0-2.2 | ANY |
| DNA: Shotgun sequencing | 10 | 0.25 | 1.8-2.0 | 2.0-2.2 | |
| DNA: Amplicon sequencing and 16s/its rRNA metagenomics | 10 | 5 | 1.8-2.0 | 2.0-2.2 | |
| DNA: whole-exome sequencing | | 100 | 1.8-2.0 | 2.0-2.2 | |
| Sample QC (TapeStation) | 5 (μl) | - | | | |
| Sample quantification (Synergy LX) | 5 (μl) | - | | | |
| Library pool | 45 (μl) | 1 (nM) | | | |



Example of RNA integrity number assessment (RIN score). Panel A. RNA isolated from HEK293 cells showing high integrity with RIN = 10. **Panel B.** RNA isolated from mouse heart tissue showing moderate integrity with RIN = 6.1.





Panel C. RNA isolated from HEK293 cells showing very low integrity with RIN = 2.5. The 28S/18S rRNA ratios are given. Gel images from the 2100 Bioanalyzer are shown at the right with the 28S and 18S rRNA bands indicated. Source: https://www.promega.ca/resources/pubhub/methods-of-rna-quality-assessment/

ORC SHIPPING ADDRESS

Either the processing request through the <u>ORC LIMS Requester Module</u> or the ORC Sample Submission Template must be received by the ORC at <u>orc@usask.ca</u> before sample processing can begin.

Samples can be dropped off in person at the ORC (WCVM Room 2209). If shipping, please be sure to include sufficient dry ice for the transit time. If shipping from outside of Canada, always ship on a Monday to allow for Customs clearance. Include orc@usask.ca on shipment information, including FedEx tracking notifications. International shipments must include 3 copies of a commercial invoice form. Ship to the following address:

University of Saskatchewan
Omics Resource Centre
Attention: Martin Mau
52 Campus Dr, Room WCVM2209
Saskatoon, SK S7N 5B4
306.966.7424 | orc@usask.ca

Once samples and the sample submission form or the request through <u>ORC LIMS Requester Module</u> have been received, you are financially liable for the costs of the requested services.

DATA RELEASE & STORAGE

When the Customer's project has finished sequencing, the Customer will receive a data release email with download instructions. ORC's standard process for all Internal Customers and most External Customers is data delivery through the **Globus platform** using Customer's institutional account and sign-on credentials.

For non-institutional External Customers ORC shares the data as a **Globus Guest Collection**. ORC creates a special folder on its Globus endpoint, grants the Customer specific read permissions, and sends the Customer a link to access that folder directly through the <u>Globus web app</u>. The Customer will then be prompted to authenticate using a temporary, one-time identity to download the files. If you encounter any problems downloading your data, please report the issue in a reply to the data release email.

ORC will typically deliver the sequencing order Results in FASTQ files (raw reads and quality scores), unless other formats are requested on the Quote and the Customer agrees to pay additional charges, if any.

Upon completion of the Service, data will be made available to the Customer through the Globus platform unless otherwise specified in the Quote.





Samples, libraries and sequencing data will be stored for a **maximum of two** (2) months ("ARCHIVAL PERIOD") from the release email. If the Customer wishes to claim their libraries or leftover crude samples and extracts (if available), please state this in a reply to the data release email. After the ARCHIVAL PERIOD unless otherwise arranged, the Customer's raw data of Results shall be destroyed, without further notice to the Customer, in accordance with these TERMS & CONDITIONS, as may be modified from time to time.

The Customer will inform ORC in case the standard data delivery option does not work for them. ORC may be able to deliver data directly to the Customer's AWS S3 bucket or, as a last resort, the Customer can choose to have data shipped on a hard drive purchased by ORC and invoiced to the Customer at a cost of CA\$300 per hard drive.

Omics Resource Centre 52 Campus Drive Saskatoon Sk S7N 5B4 Canada Telephone: 306-966-7424

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