RTL GENOMICS

A division of MicroGen Diagnostics

Sample Submission Guidelines

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Shipping Address

RTL Genomics 5901 63rd St., Suite B Lubbock, Texas 79424

Contact: Rocío Navarro Phone: (806) 771-1134

*** Please make sure to contact us via email before shipping your samples. ***

A Note Regarding These Guidelines

Our guidelines are flexible and attempt to answer many of the questions we are asked. Keep in mind that we are here to facilitate your research and we will work with you to obtain the best results at a reasonable cost. If you have any questions regarding these guidelines, or anything else, please do not hesitate to contact us at <u>info@rtlgenomics.com</u>.

Guidelines for Packaging Samples

This section details the guidelines for shipping samples. If you wish to send samples in a container not listed in this section, please contact us at <u>info@rtlgenomics.com</u> for guidelines pertaining to your exact needs. We are always willing to assist in this process.

Please keep in mind that we would prefer you send your DNA samples in 1.5-mL microcentrifuge tubes. All sample types should be accompanied by an RTL Genomics submission form.



Shipping Samples in 1.5-mL Microcentrifuge Tubes

Clients using the standard DNA sequencing service should submit their DNA samples in 1.5-mL microcentrifuge tubes.

Packaging Guidelines for the Primary Container (Sample Container):

- 1. For DNA send a volume of at least 20 μ L per assay requested.
 - a. While we are able to work with many concentrations, best results occur when your samples have a concentration of 20 ng/ μ L and a quality ratio of 1.8.
- 2. If you expect shipping time will take longer than one day, please wrap the lid of each sample tube with parafilm to protect against evaporation during transit.
- 3. Place sample tubes within a hard, protective container (*e.g.* 50-mL centrifuge tube or used pipet tip box). Secure the sample using packing materials (packing peanuts, paper, etc.).
 - a. Using a protective container will reduce the risk of your sample tubes being crushed during transport or individual samples being lost within the packing materials (packing peanuts, paper, etc.) when they are received.
- 4. Reinforce screw caps with parafilm, use a metal crimp seal or skirted stopper for metal and glass.
- 5. Affix or write with permanent marker a proper label on primary container (*e.g.* vial) containing a sample to identify the contents and relate them to the submission form, described below.

Packaging Guidelines for the Secondary Container:

- 1. Use a watertight/leak proof container and reinforce the container using parafilm as necessary to contain the individual vials contents (*e.g.* zip-lock type bag).
- 2. Surround each primary container (*e.g.* vial) with sufficient absorbent packing material to completely absorb the contents should the primary container break during transit.
- 3. Include a submission form with a complete list of the contents including the sample name, label and the volume in µL for liquids for each vial/tube. Place this submission form in a separate (zip-lock bag or other watertight package) to prevent it from becoming wet and unreadable. Please email a digital copy (Excel format) of this form to RTL Genomics Laboratory to info@rtlgenomics.com.



Packaging Guidelines for the Outer Container:

- 1. Use an outer container (shipping box) of sufficient strength to protect the specimen. We discourage the use of flat shipping envelopes designed for shipping paper documents.
- 2. Include on the packing slip an accurate address label with the name, complete address and phone number for both the shipper and the recipient.
 - a. Note: Only list the primary investigator or scientist so the RTL Genomics scientists and personnel can get you your final analyses without delays. Please avoid using anyone not directly related to your research, such as your secretary, as your contact when possible.
- 3. Affix the "double up arrows" sticker (example sticker shown below in the section titled "Example Labels") if orientation is important during transit and handling.
- 4. Clients can typically ship purified DNA samples overnight (next day delivery) at room temperature.
 - a. RTL Genomics does not require using dry ice or liquid nitrogen to ship DNA samples due to cost. However, if you wish to refrigerate your samples during shipping, we recommend using ice packs or dry ice.
 - b. If you plan refrigerate your samples during shipping then please read the section *"Guidelines for Shipping Refrigerated Samples"*, found on page 8, as well as following any instructions provided to you by your carrier.

Shipping Samples in 96-Well Plates

Clients that wish to submit their samples in 96-well plates should submit at least 20 μ L of sample per well (per assay desired). We recommend that clients prepare their plates for shipping using the following methods:

Packaging Guidelines for the Primary Container (Sample Container):

- For DNA send a volume of at least 20 µL (per assay required) of sample per well. Please see the recommended sample arrangement for sample submission in 96well plates at the end of this document. We suggest organizing the samples in a column-wise manner (*i.e.* starting with A1 and going down the column) and document the order of your samples in the submission form.
- If you are shipping samples unrefrigerated then carefully and securely seal your plates with strip caps and ship the samples at an ambient temperature. However if you prefer to send your samples frozen then good quality foil tape is also acceptable. Do not use clear tape because it pulls loose easily under freezing conditions.



- 3. Place your plate inside a pipet tip box or similar box to prevent the plate from flexing during shipping as flexing may cause the tape to pull loose. Another option is to pack your plates inside a small box then pack the small box inside a second larger box.
- 4. If you are mailing custom primers in micro tubes, place them inside of a 50-mL polypropylene tube or other form of protective container. Next, secure them with packing materials. Do not tape primer tubes to the outside of your template plate as this could lead to the tube being smashed, broken, or otherwise damaged during transit.
- 5. Affix or write with permanent marker a proper label on primary container (*e.g.* Plate 1) containing samples to identify the contents and relate them to the submission form, described below.

Packaging Guidelines for the Secondary Container:

- 1. Use a watertight/leak proof container and reinforce the container using parafilm as necessary to contain the sample contents (*e.g.* zip-lock type bag).
- 2. Surround each primary container (*e.g.* plate) with sufficient absorbent packing material to completely absorb the contents should the primary container break during transit.
- 3. Include a submission form with a complete list of the contents including the sample name, label and the volume in µL for liquids for each vial/tube. Place this form in a separate zip-lock bag or other watertight package to prevent it from becoming wet and unreadable. Please email a digital copy of this list to RTL Genomics to info@rtlgenomics.com to ensure safe arrival.

Packaging Guidelines for the Outer Container:

- 1. Use an outer container (shipping box) of sufficient strength to protect the specimen. We discourage the use of flat shipping envelopes designed for shipping paper documents.
- 2. Include on the packing slip an accurate address label with the name, complete address and phone number for both the shipper and the recipient.
 - Note: Only list the primary investigator or scientist so the RTL Genomics scientists and personnel can get you your final analyses without delays. Please avoid using anyone not directly related to your research, such as your secretary, as your contact when possible.
- 3. Affix the "double up arrows" sticker (example sticker shown below in the section titled "Example Labels") if orientation is important during transit and handling.
- 4. If you plan to ship your samples frozen then please package your samples in accordance with the guidelines provided in the section *"Guidelines for Shipping Refrigerated Samples"*, found on page 8. You must also check with your carrier



and follow any guidelines provided to you by them regarding the shipment of refrigerated samples.

Guidelines for Shipping Refrigerated Samples

The following guidelines are for clients shipping samples to RTL Genomics using dry ice, ice packs or other means of refrigeration. Due to continuing changes in state and federal regulations, clients should always check with their shipping department to ensure regulatory compliance.

Guidelines for shipping refrigerated samples to RTL Genomics are as follows:

- 1. Place samples in a Styrofoam cooler with cold packs/blue ice or dry ice. **Please do <u>not</u> use wet ice to ship your samples**.
- 2. Place enough cushioning material (*e.g.* paper towels) around your samples to prevent movement within the Styrofoam cooler. Failure to provide cushioning material could result in damage or breakage of your sample tubes due to bouncing around of ice packs or dry ice.
- 3. Place the Styrofoam cooler inside a cardboard box to ensure acceptance by carrier.
 - a. A new outer box without any other labels or excess of tape works best.
 - b. If you must re-use a box, then make sure to either remove or black out any other preexisting labels on the shipping box.
- 4. Packages shipped with dry ice must permit the release of carbon dioxide gas. If you are submitting samples with dry ice, the outer cardboard box must have the following:
 - a. A Miscellaneous Dangerous goods #9 hazard label fixed to the outside of package.
 - b. A label indicating "Carbon Dioxide, solid UN1845" on the outside of the package.
 - c. A label with the net weight of dry ice on the outside of the package.
 - d. An air waybill with the following information:
 - i. Classification (*i.e.* Carbon Dioxide, solid, 9, UN1845),
 - ii. The number of packages, and
 - iii. The net quantity of dry ice per package.



DNA, RNA and Un-extracted Sample Preparation

DNA Samples

Higher quality DNA will result in higher quality results. We cannot guarantee good results when low quality DNA is submitted to the laboratory. RTL Genomics offers DNA clean-up and normalization services. If you require these services but do not wish to pay for them, please perform them prior to sending your DNA or amplicons.

Be aware of potential hazards that may contaminate your DNA or amplicons:

- Do not extract or manipulate or open the tubes of your DNA in environments where amplicons of any kind are handled.
- Always use strict aseptic techniques and high quality molecular grade materials and reagents.
- Any non-molecular grade reagent has the potential for carrying background genetic material which may be detected by our sensitive methods.

Genomic DNA Samples

Many customers send us samples that have already been extracted. **Obviously**, not all DNA is of the same quality. We encourage customers to use high quality DNA extraction kits for isolating their DNA. Please inform us of difficult DNA.

- 1. Qiagen has good extraction kits for a wide variety of sample types. Great tissue, stool kits and soil kits. (www.qiagen.com)
- 2. Mpbio (www.mpbio.com)
- 3. Promega (www.promega.com)
- 4. Invitrogen (www.invitrogen.com)
- 5. Epibio (www.epibio.com)

Regardless of extraction technique we request that, if possible, you determine the concentration and A260/280 ratio to determine if the quality is appropriate. If you determine your DNA quality is low, we suggest using a DNA clean-up kit (*e.g.* Qiagen) to purify your DNA. If you can barely get your own samples to amplify then they will most likely fail our quality control of amplification. We provide one PCR amplification attempt in the quoted cost per sample. If a significant number of your samples do not amplify, and we determine your DNA quality is low, we will offer to clean-up your DNA, for a small fee. We have extensive experience troubleshooting amplification of DNA from many environmental sources. Please inform your RTL Genomics lab scientist if you would like them to troubleshoot your samples for an additional fee.



When shipping your DNA or RNA we ask that you provide the volume (uL) and concentration (ng/ μ L) of each sample on the submission form. In general, we request at least 20 μ L volume of your DNA

sample (per assay required) with a concentration normalized around 20 ng/µL. If this is a problem just let us know, we can still process your samples but again, the quality going into our process reflects on the quality output.

We know that it is not always possible to send that much DNA and this is not a requirement, however pre-normalized samples are generally processed more quickly than nonnormalized samples. You can send your samples in any appropriate storage container, but we prefer samples to come in either 1.5 uL flip-top tubes or 96-well plates. We suggest all samples should be sealed with parafilm to reduce evaporation. Making sure your samples are safely packaged is your responsibility. RTL Genomics has QC procedures for checking in samples, but we might not always catch that 2 or 3 samples out of 500 leaked or evaporated during transit.

PCR Products

With difficult samples (*e.g.* extreme environments) it may be best to send us purified PCR products. We will need to perform a nested PCR to add linkers and tags. Please communicate with us at <u>info@rtlgenomics.com</u> so we can coordinate assays.

When shipping your PCR amplicon, we ask that you specify the volume and concentration $(ng/\mu L)$ on the submission form. In general, we request at least 20 μL volume of your sample with a concentration normalized around 20 $ng/\mu L$. If this is a problem just let us know, we can still process your samples but again, the quality going into our process reflects on the quality output.

We know that it is not always possible to send that much DNA and this is not a requirement, however pre-normalized samples are generally processed more quickly than non-normalized samples. You can send your samples in any appropriate storage container, but we prefer samples to come in either 1.5 uL flip-top tubes (n<8), strip PCR tubes ($8 \ge n \le 32$) or 96-well plates (n>32). We suggest all samples should be sealed with parafilm to reduce evaporation. Making sure your samples are safely packaged is your responsibility. RTL Genomics has QC procedures for checking in samples, but we might not always catch that 2 or 3 samples out of 500 leaked or evaporated during transit.



RNA Samples

For RNA-seq (Illumina), RNA needs to be a minimum of 1ug with an A260/280 of \ge 2.0 in a volume of 50 uL. If requiring rRNA depletion, please contact our scientists at info@rtlgenomics.com to discuss options. RNA needs to be eluted in Rnase-free water and to be shipped on dry ice. If you do not wish to ship on dry ice, the RNA needs to be in a stabilizing buffer (*e.g.* RNAlater, RNAshield, etc.) to prevent degradation. If samples are degraded, this will directly impact the results of library preparation and sequencing. Please ship the samples in the appropriate packaging which is outlined in "*Guidelines for Shipping Refrigerated Samples*".

Samples for Extraction

If your sample type is not listed in the following section, please contact us at <u>info@rtlgenomics.com</u> so that our Extraction Team can begin developing an extraction protocol to best suit your needs. This is often a crucial step in improving DNA recovery for specific sample types. The Extraction Team may still reach out if we encounter any questions about your samples even if they are listed below.

Our scientists at RTL Genomics are able to provide extraction from a wide range of sample types. In most cases we require small amounts of sample to perform DNA extraction (< 1g), being the preferred primary container 2 mL screw-top tubes (if the sample size allows it). As the best shipment conditions vary based on sample type, we do not have one comprehensive shipping method for extraction samples. We believe you know the best storage method for your samples, however if you are unsure which method to use then we suggest going with dry ice. Also, please keep the following advice in mind:

- Please do not suspend samples in buffer, ethanol, media, etc. It is best to send the sample in a sterile centrifuge tube. This is not a strict requirement but many buffers influence the success of DNA extractions.
- The goal when sending us samples is to send the most representative sample possible. For many heterogeneous samples (*e.g.* soil, feces, water), you will want to handle everything aseptically as contamination will be detected by our instruments as part of your sample.
- Homogenizing the sample you would like us to analyze is a good way to gain a representative analysis of larger samples. You can then take a few small subsamples, put these together in a safe sterile container (usually 15 or 50 mL screw-top polypropylene), and then ship it to us.
- Feel free to contact us at <u>info@rtlgenomics.com</u> with any questions you may have.



Advice for International Customers (Outside the United States)

- Please only ship purified DNA suspended in molecular grade water or appropriate elution buffer.
- If you need to ship a sample to be extracted, please consult with us and allow us to obtain any necessary permits. Purified DNA typically is no problem. Refer to section "*DNA Samples*" on page 9 for instructions on how to get the best DNA possible from your samples.
- For more detailed information regarding shipping samples to RTL Genomics from international sources, please see our information packet titled "*Instructions for Shipping Material from Outside the United States*".
- Feel free to contact us at <u>info@rtlgenomics.com</u> with any questions you may have.

Feces/Rumen and Other GI Content Samples

- International customers: Please consult with RTL Genomics before shipping fecal or other GI material.
- In most cases, fecal material should be homogenized and subsampled prior to shipping. In this way, the representative nature of the sample is in the hands of primary investigators.
- It is vital that samples are adequately contained and packaged for shipping. We
 prefer to receive fecal samples in screw-top 50mL polypropylene tubes (or other
 safe, non-fragile containers) to prevent tubes from coming open or breaking during
 shipment.
- Care should be taken to ensure that samples do not break. Breakage of one sample risks contamination of the rest of the containers. RTL Genomics will screen for damage at arrival. If we do find evidence of issues with samples, we will notify the primary contact listed in the submission form.
- Additional cost may be incurred if extensive decontamination is required due to improperly packaged samples.

Water Samples

- International customers: Please consult with RTL Genomics before shipping water samples.
- Our standard water extraction protocol uses 100 mL of starting material for best results, however a minimum of 50 mL sample can be used. For shipping water samples, we recommend homogenizing samples and aliquoting into 50mL screwtop tubes or appropriate water collection screw-top bottles. It is important to leave at least 1/10th of the container empty to allow for expansion caused by freezing (we will store samples in our freezer upon arrival).



Whole Organism Samples

- International customers: No international shipping of bacterial isolates without consultation. Contact us at <u>info@rtlgenomics.com</u> to allow RTL Genomics scientists and staff to obtain necessary permits.
- Send a minimum of 10⁹ cells frozen (dry ice) in an appropriately sized tube (for small volumes, 2 mL screw-top tubes are preferred).
- Careful consideration should be given to shipping potentially pathogenic organisms. Permits may be required and RTL Genomics staff (<u>info@rtlgenomics.com</u>) should be included in consultation before shipping cultures or isolates that are potentially pathogenic.

Tissue Samples

- International customers: No international shipping of tissue without consultation. Contact us at <u>info@rtlgenomics.com</u> to allow RTL Genomics scientists and staff to obtain necessary permits.
- The amount of tissue needed for extraction will vary based on tissue type and assay/sequencing service desired. Generally, a sample about the size of a peanut will provide enough sample material to perform a successful extraction, however, we realize this is not always possible based on different tissue types and sample source. Consultation with RTL Genomics is always recommended if unsure of extraction amount needed for your project.
- Clinical samples that are potentially pathogenic should be shipped using biological substance cat B shipping standards and must include a UN3373 (biological substance cat B) label (example label shown below in the section titled "Example Labels").

Soil Samples

- International customers: Soil samples must have the required USDA Soil Import Permit Label. Please contact us at <u>info@rtlgenomics.com</u> so we can provide the required documents for shipping.
- We suggest sending soil in a screw-top 50 mL tube (screw-top prevents the tubes from coming open in shipment). Generally, we only need 1/3 of the volume this kind of tubes of soil to perform the extraction.

Other Sample Types

• If your sample type is not listed above and you are unsure of how to send it, please contact us at <u>info@rtlgenomics.com</u>. Our extraction team will reach out to offer suggestions and ask for additional sample information if necessary.



Sample Submission Guidelines

Example Labels



Example UN3373 (Biological Substance Cat B) Sticker



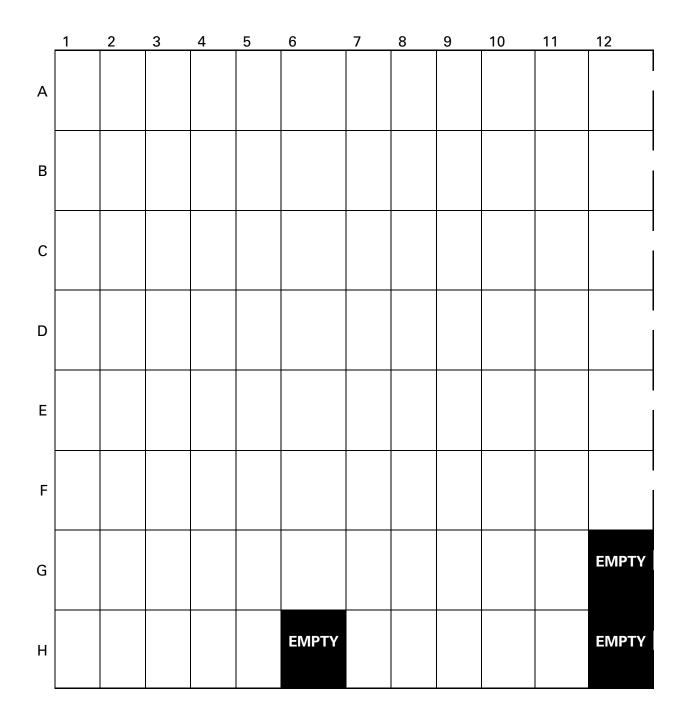
Example of a Double Up Arrows Sticker



Example Dry Ice Label



Recommended Sample Arrangement for Sample Submission in 96-well Plates





Pre-Payment & Credit Policy

- RTL Genomics is willing to accept pre-payments if this document is signed and returned during the initial quoting period.
- RTL Genomics will only accept pre-payments of existing quotes and invoices.
- The quote number must be referenced in the notes of the bank transfer, this is the best way to ensure the pre-payment is properly credited to the corresponding account.
 - If funds are sent to RTL Genomics without referencing the quote number, the client must reach out directly to inform us of the transfer and assist in identifying it by providing a reference or bank transfer number/ID.
- RTL Genomics will hold pre-payments for 18 months without the need of communication from the client. After a year has passed since receipt of the credit, we will begin sending monthly statements for 5 months; after the 5 months we will send bi-weekly statements for 3 months. If the funds still have not been claimed at the end of the 18 months, they will be forfeited.
- Refunds can be requested by any customer at any time. If any services have been
 rendered on the order, the client will receive the original value of the credit less the
 value of services rendered, materials used, and any bank fees associated with the
 fund transfer. Per sample rate may be adjusted according to the number of samples
 received.
- Quotes are valid for 8 months from the issue date. RTL Genomics reserves the right to uphold quotes on a case-by-case basis but will most often require a new quote.



- If samples and payment are received but RTL Genomics is unable to carry out the necessary work due to a lack of client communication, RTL Genomics will hold them for 1 month and try to contact the client weekly. After this, RTL Genomics will continue to store those samples while trying to contact the client monthly for a period not to exceed 8 months; at this point samples will be destroyed, and a refund will be issued according to the aforementioned payment refund guidelines.
- If samples are received without a quote or submission form, RTL Genomics will hold them for 1 month and try to contact the client weekly before they are discarded. If the client has a previous agreement with RTL Genomics for sample storage, that agreement will take precedence.
- The client is responsible for any associated transfer fees; this cost will be indicated in the quote as "Payment Service Fee".

I have read and understood the RTL Genomics Pre-Payment & Credit Policy and by signing this document I agree and accept its terms and conditions.

Signature: _____

Date:	