

SAMPLE PREPARATION & SHIPPING INSTRUCTIONS v517

This document outlines guidelines for preparing, quantifying, and submitting samples to Otogenetics. Investigators may submit DNA or RNA samples. Tissues or body fluid samples are usually accepted with additional handling and preparation fees. It is essential that samples submitted conform to the requirements outlined here to maximize the chances of success for your projects.

Table I: Recommended Input Quantity, Buffer and Quality Control (QC) Methods

	Application	Input quantity	Buffer	QC Method
DNA	Exome Sequencing	Genomic DNA** 20-500 ng/µl (total: 2 µg; Minimum* 200 ng)	TE Buffer	UV spectrophotometry
DNA	Sequencing of Targeted Genes	Genomic DNA 20-500 ng/µl (total: 2 µg; Minimum* 200 ng)	TE Buffer	UV spectrophotometry
DNA	Genome-wide 5 hmC profiling	Genomic DNA** >40 ng/µ (>10 ug total)	TE Buffer	UV spectrophotometry
DNA	PCR DNA Products Sequencing	Amplicon DNA 50-500 ng/µl (total: 2 µg; minimum*: 200 ng)	TE Buffer	UV spectrophotometry
DNA	ChIP sequencing	Purified DNA from ChIP 2 ng/ µl (20 ng minimum)	TE Buffer	PicoGreen assay Agilent Bioanalyzer
DNA	cDNA Sequencing	20-500 ng/µl (total 2 µg; minimum* 200 ng)	TE Buffer	UV spectrophotometry
DNA	Mitochondrial DNA Sequencing	20-500 ng/µl Whole DNA*** (total 1 µg; minimum*: 200ng)	TE Buffer	UV spectrophotometry
RNA****	Various workflow available for normal input	See RNA Sample Requirements	options listed in RNA Sample Requirements	UV spectrophotometry, plus gel electrophoresis or Agilent 2100 BioAnalyzer
RNA****	Options for FFPE and ultra low input available	See RNA Sample Requirements	options listed in RNA Sample Requirements	UV spectrophotometry, plus gel electrophoresis or Agilent 2100 BioAnalyzer

* Minimum requirement; Contact support@otogenetics.com if less than minimum amounts listed.

** Compatible genomic DNA purification: Qiagen Dneasy Blood & Tissue Mini Kit (Cat# 69506) for body fluid, frozen or fresh tissues; Beckman FormPureDNA (B89230) or Qiagen DNA FFPE Tissue Kit (Cat# 56404) for FFPE samples. **MUST include RNase-treatment step during genomic DNA preparation for exome and epigenetic modification profiling.** If you have limited amounts of DNA, a minimum of 200 ng of gDNA may be submitted.

*** We recommend extracting genomic DNA containing mtDNA (whole DNA) with Qiagen Dneasy Blood & Tissue Kit (Cat#69506) or DNA Blood Mini Kit (Cat#: 51104).

**** RNA sample types and requirements are provided in a separate file of RNA Sample Requirements .

I. SAMPLE REQUIREMENTS

1. Sample requirements for genomic DNA

- (1) Not degraded (agarose gel electrophoresis), unamplified genomic DNA, treated with RNase to eliminate residual RNA (Fig. 1).
- (2) Optimal concentration: 20 ng/μl to 500 ng/μl in TE (pH8.0).
- (3) Samples with an OD ratio A260/A280 ≥ 1.8 and A260/A230 ≥ 1.9 are preferred. The ratio but not necessarily indicative of DNA quality.
- (4) Genomic DNA should be stored in TE at 4°C (to avoid shearing forces during freezing/thawing). For long term storage, the samples can be stored at -20oC or -80°C.
- (5) DNase- and RNase-free screw-cap microcentrifuge tubes should be used for storage and shipping.

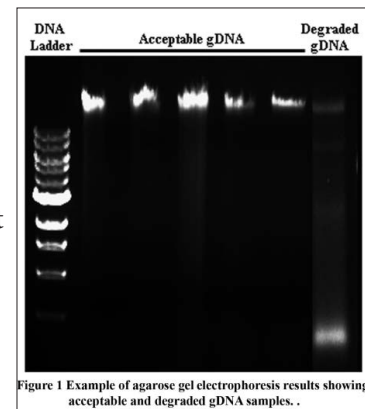


Figure 1 Example of agarose gel electrophoresis results showing acceptable and degraded gDNA samples.

Note: Due to the fragmentation associated with formalin fixation and paraffin embedding, DNA isolated from FFPE samples is always degraded, and the extent or nature of that degradation varies among samples. Therefore, the integrity of DNA isolated from FFPE samples determined by the methods described here only serves the purpose of comparing samples, the results of that analysis will not appear as described and will not otherwise be easily interpreted.

2. Sample requirements for total RNA* for RNA Sequencing (RNA-Seq) Applications

(*If you wish to send existing cDNA samples, please contact support@otogenetics.com to assess whether your samples are compatible with Illumina Sequencing)

- (1) Suspend total RNA in RNase-free H₂O (see our Recommended RNA Isolation Methods). **DO NOT** use DEPC-treated H₂O.
- (2) Quantify total RNA samples using an UV spectrophotometric method such as the NanoDrop system.

The samples should have an OD_{260/280} ratio between 1.8 and 2.0, and an OD_{260/230} ratio greater than 1.7. FFPE RNA or low quality RNA may also generate good RNA-Seq data based on size distributions contact Otogenetics (support@otogenetics.com).

- (3) Evaluate RNA integrity using a 1% denaturing RNA gel (Fig. 2) or an Agilent Technologies 2100 Bioanalyzer (Fig. 3). Please submit your gel image or Agilent Bioanalyzer trace for your samples. High-quality total RNA samples should show a 28S rRNA band at 4.5 kb and an 18S rRNA band at 1.9 Kb (Fig. 2). The intensity of the 28S band should be twice as that of the 18S rRNA band. Messenger RNA appears as a faint smear from 0.1 to 12 kb. If Agilent Technologies 2100 Bioanalyzer is used, high quality total RNA samples should give two distinct peaks (Fig. 3) and yield an RNA Integrity Number (RIN) value greater than 8.
- (4) Keep total RNA at -80oC in a DNase- and RNase-free 1.5–2.0 ml screw-cap microcentrifuge tube sealed with Parafilm.
- (5) Total RNA samples must be packaged with dry ice sufficient for the next day delivery.

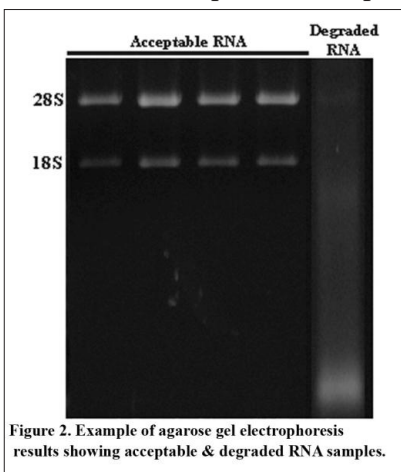


Figure 2. Example of agarose gel electrophoresis results showing acceptable & degraded RNA samples.

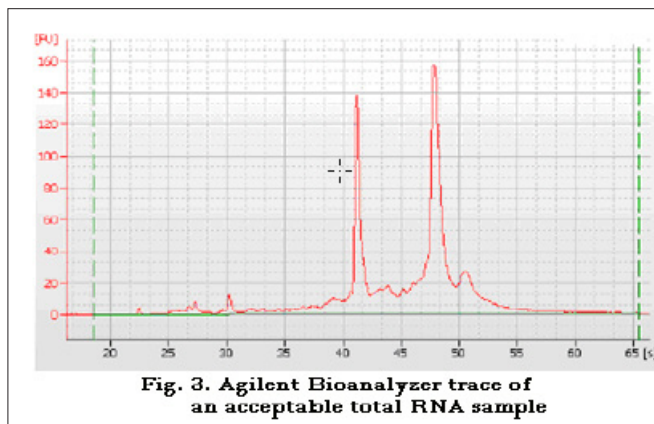


Fig. 3. Agilent Bioanalyzer trace of an acceptable total RNA sample

I. SAMPLE REQUIREMENTS (Cont.)

3. Sample Requirements for Body Fluid or Solid Tissues

○ Blood should be collected in EDTA tube or citric acetate tube with a minimal volume of 2-5 cc, stored at 20°C to 8°C (**DO NOT FREEZE!**) until shipped. A refrigerated specimen is viable for 2 weeks. The samples can be shipped at room temperature using 2-day or faster delivery methods.

○ Saliva samples should be collected with the Norgen Biotek Individual Collection container (Cat# 35700) or equal device and stored accordingly. Saliva and FFPE samples can be shipped at room temperature using 2-day delivery.

○ Solid tissues should be frozen on dry ice or liquid nitrogen. The samples should be packaged with dry ice sufficient for next day delivery.

II. SAMPLE IDENTIFICATION/LABEL RECOMMENDATIONS

○ It is important to prevent the sample labels from being dissolved by solvents and the labels from falling off the tubes. Do not use marker pen to mark directly on the tube wall or lid. It is better to write the sample information on a paper/plastic label, stick the label to the tube wall, and secure the label to the tube by wrapping the Scotch tape around the tube for one circle.

○ Please fill out and attach the sample information sheet provided by Otogenetics before shipping the samples. Please make sure that the sample information on the Sample Information Sheet matches the labels on the tubes.

III. SAMPLE PACKING RECOMMENDATIONS

(1) For DNA and RNA samples, we recommend 1.5ml or 2 ml screw-cap DNase- and RNase-free microcentrifuge tubes. Please use Parafilm to seal each tube before package. We do not recommend shipping samples dissolved in organic solvents (such as absolute ethanol or isopropanol) because the samples in organic solvents may leak and even cause cross contamination. If it is unavoidable to ship samples in organic solvents, please use screw-cap tubes and seal the opening of the tube with at least 10 layers of Parafilm.

(2) In order to avoid crushing during shipping, we highly recommend putting the sample tubes in a sustainer such as a 50 ml tube or a box with interior racks/holders. Cotton and absorbent papers can be used to keep tubes in the sustainer from moving.

(3) RNA and solid tissue samples should be kept in dry ice during shipment. Genomic DNA samples should be kept in blue ice during shipment. Blood samples and saliva samples should be shipped at room temperature.

IV. COMPLETING THE SAMPLE SUBMISSION MANIFEST

A Sample Form, a Billing Form, and signed Quote Form must be submitted for each sequencing service project. The Sample and Billing forms can be downloaded from our website (www.otogenetics.com). All information on the forms should be filled out. Please submit the completed forms via email to support@otogenetics.com before shipping samples to Otogenetics. Please also include a printed copy of each form in the shipment.

V. SHIPPING SAMPLES TO OTOGENETICS

Disclaimer: The information below constitutes only a recommendation for shipping samples classified as "non-regulated materials" to our facility. Due to continuing changes in regulations, clients should always check with their safety office and/or shipping department to ensure regulatory compliance. To date, gDNA is not defined as diagnostic specimen in the International Air Transport Association (IATA) packing instruction and therefore no special packaging requirements have to be filled.

Domestic (US and Canada) Shipping:

(1) Make sure that all samples are prepared and packaged according to the guideline given above.

(2) Select reliable couriers. We recommend FedEx (<http://fedex.com/>), UPS (www.ups.com) or DHL (<http://www.dhl.com/>), and express next-day service for shipments within the United States. Saliva samples may be shipped via 2-day service or regular mails. Please use priority international shipping for international shipment and confirm that the carrier can facilitate the importation of nucleic acid samples into the United States. Following the federal guidelines for shipping lab specimens.

(3) Ship the samples to:

Sample Receiving

Otogenetics Corporation

4553 Winters Chapel Rd. Suite 100

Atlanta, GA 30360 USA

Toll free: 1-855-686-4363 (1-855-otogene)

(4) Email support@otogenetics.com the tracking information once the package has left your facility, using Sample Tracking Quote# XXX as the subject for the email.

International Shipping:

1. Please arrange for the shipment so that it arrives at Otogenetics Corp during weekdays (Mon – Fri).

2. Use express services (the fastest delivery offered) from a courier, such as FedEx, DHL, World Courier, UPS or USPS.

3. Contact your local international courier and complete an INVOICE (commercial invoice, customs invoice or pro-forma invoice) which is requested for customs, and include it with the shipment.

Please fill the INVOICE as below:

- RNA or DNA Samples for Research Use Only
- Non-Dangerous, Non-Infectious
- No Commercial Value, Value for Customs Only
- Declare the value of the goods for customs [i.e. \$1.00 (USD) or €1.00 (EUR)]
- Number of samples and volumes [the # of samples, and the estimated volume]
- Type of container

4. You may be also required by the courier to sign a USDA Statement or Toxic Substances control Act Certification (declaration for regulations). Please refer to the information above or check "I certify that all the chemical substances in this shipment are not subject to TSCA".

5. Package the samples with (1) a completed Detailed Sample Information Form; (2) a completed and signed Project and Billing Information Form; (3) a signed Quote; and (4) include any QC data for the samples if you have them. Pack DNA samples in blue ice, and RNA samples and frozen tissues with sufficient dry ice. Send the package to:

Sample Receiving

Otogenetics Corporation

4553 Winters Chapel Rd. Suite 100

Atlanta, GA 30360 USA

Toll free: 1-855-686-4363 (1-855-otogene)

6. Email to support@otogenetics.com using Sample Tracking Quote# xxx as the subject with tracking information (including courier name and tracking number) to help ensure that the samples arrive safely and without any delay.

Priority for sequencing service projects is assigned on a first-come, first-serve basis. Return shipment of leftover DNA or RNA samples following analysis can be arranged for an additional shipping fee. Contact Otogenetics to set up return shipment after receiving your data. Otogenetics will keep RNA and DNA samples under frozen conditions for a maximum of 8 weeks following data release.