Molecular Biology Department
Iranian Biological Resource Center

Sample Submission Guide

DNA Extraction Service

- Blood Samples

Blood Samples Collection Instructions:

1. Collect 2-5 ml of blood from the test specimen. (We need at least 2 ml of blood for extraction of DNA)
2. Immediately transfer the blood to the lavender (purple) topped tube (EDTA anti-coagulant), any size is acceptable; (do not overfill tubes).
3. Invert the tube several times to prevent clotting. Clotted blood cannot be used. Do not send clotted blood. If necessary, a new blood sample should be collected. Do not put tape over the cap of the tube.
4. Label the tubes with the sticky labels marked with specimens' name, date of collection and the name of research center.
5. Complete the Request Form with all the required information on the shipped samples.
6. **Please be concerned that the samples must be safe. The infected human and animals' samples will not be accepted in this lab.**

Packaging and Shipping Instructions:

1- Be prepared to ship the sample the same day as it is collected, if at all possible. If you must hold the sample for a while, make sure that it is refrigerated. Note: Do not freeze the sample at any time. Refrigeration (for no longer than 5 days) is recommended.

2- Samples may be shipped at ambient temperature (no refrigeration or ice necessary) if shipped immediately. Normally, it is preferred to ship by an overnight service and be sure that the samples will not arrive in laboratory in weekend.

Appropriate packaging requires:

- Blood tube inside a sealed plastic bag (or other sealed container).
- Absorbent material inside the plastic bag. One paper towel is sufficient to soak up any potential leakage. Please do not wrap towel around the blood vial.

- Cushioning wrap - bubble wrap sheets are best - wrap around the plastic bag loosely. Please do not tape or staple to the plastic bag. Do not use household insulation.

For Warm weather:

If the temperature at your location at the time of shipping is 80 degrees F (26ºC) or above, follows these additional instructions:

  - Obtain 2 freezable ice packs, available in hardware, drug and grocery stores.
  - Freeze them overnight. Do not substitute ice cubes, frozen food products or dry ice.
  - Obtain a Styrofoam box that fits into an outer box and is large enough to hold the frozen ice packs and the sample and toweling in a plastic bag and add cushioning material as needed.
  - Do not freeze the sample before shipping. It will not freeze when packed as described here.
  - Put the request form and check in a separate sealed bad to keep them dry.
  - Put in a sturdy container, either box or tube, with enough cushioning inside to prevent movement of the contents.
  - Enclose the request form in the container

- You will be notified via email when your sample arrives; if you do not use email, please enclose a self-addressed postcard for receipt notification.

- Cultured Cell

Requirements prior to collection:

  - Work at the aseptic conditions. All media, supplement and reagents must be sterile to prevent microbial growth in the cell culture.

  - Sample tubes should be labeled with sticky labels marked with Name of cell line, Species and tissue origin, and the date of collection

  - Only handle one cell line at a time. This will reduce the possibility of cross contamination by mislabeling and also will reduce the spread of bacteria and mycoplasma by the generation of aerosols across numerous opened media bottles and flasks in the cabinet etc.

Sample Preparation

- Adherent cultures should be collected when they are in the log phase, before they reach confluence.
- Cells may be in the form of a pellet or in growth media, freeze media or phosphate-buffered saline. Send one cryovial of each sample containing a minimum of 2 $x10^6$ cells/vial.

- The shipment should be carried out on dry ice at the beginning of a week to avoid complications of reception during the weekend.

Sample Packaging and Shipping Instructions:

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- Freeze them overnight. Do not substitute ice cubes, frozen food products or dry ice..
- Obtain a Styrofoam box that fits into an outer box and is large enough to hold the frozen ice packs and the sample and toweling in a plastic bag and add cushioning material as needed.
- Put the request form and check in a separate sealed bad to keep them dry.
- Put in a sturdy container, either box or tube, with enough cushioning inside to prevent movement of the contents.
- Enclose the request form in the container.
- Ship the package by overnight delivery service.
- You will be notified via email when your sample arrives; if you do not use email, please enclose a self-addressed postcard for receipt notification.

Note: DNA yields from tissue culture cell lines vary depending on the ploidy (number of chromosomes per cell) of the cell line. Raji cells (diploid) yield approximately 5–6 μg DNA per 1×10^6 cells. PC3 cells (near triploid) yield approximately 8 μg per 1×10^6 cells. HeLa cells (100% aneuploid, near tetraploid) yield over 15 μg of DNA per 1×10^6 cells.

- Bacterial & Archeal Cells

The Bacterial and Archeal samples should be prepared in the agar plate or glycerol stock (OD600: 1.5-5 or $3\text{-}4 \times 10^9$ cells/ml) forms. Please be concerned that the sample must be nonpathogenic. Place samples in a plastic bag and wrap with a bubble bag. Ship them at ambient temperature.

- Plant Tissue & Seed

Sample type	Required sample amount	Shipping condition
Plant leaf (Fresh/Frozen)	At least one gram of fresh or frozen sample	In dry ice or liquid nitrogen
Plant Leaf (dried/ lyophilized)	At least 100 mg of dried or lyophilized sample	Preferably in dry ice
Plant seed	2- 20 seeds according to the size of sample	Ambient temperature

- Above mentioned sample amounts are for a Mini prep DNA extraction.
- If you need high yield of Extracted DNA (Maxi prep), please send us large amount of samples
- After picking up the leaf samples put them separately into zipper bags (label each bag) and transfer them immediately into liquid nitrogen.
- Please take notice that leaf and seed samples must be clean and devoid of any dirt on them.
- Plant leaves mustn't be squeezed or destroyed and or sliced (should be entire leaves).
- For shipment, put samples into an appropriate Styrofoam with liquid nitrogen/dry ice and insulate it completely.

Bacteria & Archeae 16SrDNA Analysis Service

1) Template preparation

i) Bacterial & Archeal cells

The bacterial and archeal samples should be prepared in the agar plate or glycerol stock (OD600: 1.5-5 or 3-4 $\times$ 10^9 cells/ml) forms. Please be concerned that the sample must be nonpathogenic.

ii) Genomic DNA

There are many commercial kits available. Please submit DNA in deionized water. Do not use TE to dilute or re-suspend the DNA. Please provide gDNA in the appropriate concentration as table below.

iii) PCR fragments

The PCR amplification should be performed using *Taq* DNA polymerase. It is highly recommended that your PCR template is first observed on a gel to confirm that there is a specific product with the correct size. The Gel extraction kit or PCR cleanup kit can be used to remove all of the unwanted elements from your template. Please provide PCR product in the appropriate concentration as table below.

iv) Plasmid DNA

There are many commercial kits available. Please submit plasmid DNA in deionized water. Do not use TE to dilute or re-suspend the DNA because EDTA inhibits the cycle of sequencing reaction.

Please provide plasmid DNA in the appropriate concentration as table below. Extra amount of DNA ensures that we have enough samples for a re-sequencing in case the first reaction fails. If samples' concentrations do not fall within this range or if you fail to provide us enough templates to do the reaction, the experiment might be delayed.

2) Quantitation

Using UV absorbance to quantitate dilute DNA solutions tends to give widely inaccurate results. A good way to quantitate DNA is to run an aliquot on a minigel and compare the intensity to the control of a known concentration.
There are also concentration ladders that are commercially available. For each reaction, please provide appropriate concentration as table below **Please be advised that "Gel Electrophoresis rather than Nano-drop "is recommended.**

3) Primers preparation

Primer Considerations

Primers should be provided in DI water at the required concentration.
- High Purity
- Appropriate concentration
- No secondary priming sites
- No mismatches
- A length of 18-25 bases.
- GC% content between 40% and 60%.
- No significant hairpins (>3bp)
- Free of salts, EDTA, or other contaminants

Please supply primers at concentration of (10 pmole/µl =60 ng/µl) in deionized water at volume of greater than 20 µl.
Templates and primers must be provided in DI water or 10mM Tris buffer, not in TE

Template Type/Format	**Sample Requirements**
Bacteria & Archeae cells	* Agar Plate/Glycerol Stock
Genomic DNA	* 30-50ng/µl * Minimum volume of 20µl
PCR Product (Purified)	* 50 ng/µl * Minimum volume of 20µl
PCR Product (Unpurified)	* 100 ng/µl * Minimum volume of 20µl
Plasmid	* 100 ng/µl * Minimum volume of 20µl

Guidelines for packaging for safe transportation of samples for 16S rDNA analysis service:

1. Please prepare DNA samples in micro-centrifuge tube (preferably in 0.5mL tubes) or directly on 96-well plate sealed with strip-caps.
2. Label clearly on the side and top of tube with a permanent marker.
3. Please use simple labels on your tubes (e.g. initials and numbers) and avoid long sample names.
4. Ensure that the lid is tightly closed and seal the lid with parafilm.
5. Place samples in a plastic bag. Do not stick samples directly onto order forms, please attach order form to the plastic bag.
6. Wrap the plastic bag with a bubble bag and send in a padded envelope by overnight delivery either through courier. DNA samples can be sent at room temperature.
7. Please prepare bacterial samples in agar plate or glycerol stock, place them in a plastic bag and wrap with a bubble bag.
8. Please contact us through our office or notify IBRC via email and provide tracking number if any.

Bioinformatic Analysis of 16SrDNA Service

- Please contact us for giving more information about your project and more clarification of your requirement.

Plant & Animal Fingerprinting Service

- If you would want to send us Organisms for DNA extraction and downstream applications, then please refer to **above guidelines for DNA extraction service**.

- If you prefer to send us extracted DNA, then please prepare high quality DNA of your samples with an average concentration of 30-50 ng/ul and with minimum volume of 50ul (for an experiment with about 20 primers set).

- For preparation of Primer solutions, please follow the instruction of primer Synthesizer Company. Then send us primer sets with appropriate packaging.

Cloning of Eukaryotic Genes Service

- For sending of organisms and or Genomic DNA, please refer to above mentioned guidelines.

- Please contact us for giving more information about your project and more clarification of your requirement.

Bioinformatics Analysis of Nucleotide and Amino Acid data

- In purpose of nucleotide analysis please send notepad and chromatograph files of the desired nucleotide data in a CD with order form.

- In purpose of Amino Acid data analysis please send the complete sequence of the desired amino acid data or nucleotide data in a CD with order form.