USDA, ARS, ERRC, Integrated Biomolecular Resources
DNA Sequencing Sample Request Form

Please complete and enclose the request form, a copy of your AD700 with the samples and separate pages listing the names of the cultures, and/or the concentration of each template and primers. 96-well plates are best submitted with Excel spreadsheets showing the well location of the templates and primers. A spreadsheet template is also available on the web site. E-mailing a copy of the spreadsheets (david.needleman@ars.usda.gov) will expedite the processing of all samples. Label tubes to exactly match the names of the templates and primers in your list. Names should be limited to 8 alphanumeric characters.

JOB NUMBER: _____ **Submission Date: _____**
(Assigned by IBR)

Submitted by: _____ CRIS **#:** _____

Phone/E-mail: _____ Location: _____

Approved by Lead Scientist: _____
(Signature)

Indicate the number of templates of each DNA type submitted in either plates or tubes

DNA Type	**Container**	
PCR Product	96-well Plates: _____	Tubes: _____
Plasmid	96-well Plates: _____	Tubes: _____
Cosmid	96-well Plates: _____	Tubes: _____

Number of custom primers submitted: _____ Store for future use (yes/no)? _____
(6 months maximum time)

If using standard primers maintained by the IBR, verify that the vector is compatible with our standard primer sequence.

Total number of DNA sequencing reactions requested: _____

Use this table to determine the concentration and volume of template and primer required for a DNA sequencing reaction. Templates and primers must be provided in DI water or 10mM Tris buffer, not TE.

Template type	Template Concentration	DNA Volume/Reaction	Custom Primer Conc.*	Custom Primer Volume/Reaction
PCR Product < 1kb	15-40 ng/µl	10 µl	3.2 µM*	10 µl
PCR Product 1-2kb	20-60 ng/µl	10 µl	3.2 µM*	10 µl
Plasmid	80–150 ng/µl	10 µl	3.2 µM*	10 µl
Cosmid	0.5-1.0 µg/µl	15 µl	10 µM*	15 µl

***pmol/µl**

General Information:

Copy number:

Most high copy number plasmids such as pUC vectors, pBluescript vectors, pGEM vectors, and pTZ vectors are fine

Host Strain:

The host strain can have an impact on the quality of the template DNA prepared even by the best methods. Strains that generally produce the best DNA are DH5alpha, DH1, C600, and HB101. Strains XL-1-Blue, JM109, and MV1190 are acceptable. Strains such as JM101, JM83, TG1, and TG2 are **not** recommended.

Culture media:

Luria broth (LB) is recommended as a broth medium. Avoid Terrific broth and other rich media.

Primers:

We will provide the following primers listed below:

M13 Forward (-20):	GTA AAA CGA CGG CCA GT
M13 Reverse:	CAG GAA ACA GCT ATG AC
T7 Primer:	TAA TAC GAC TCA CTA TAG GG
T3 Primer:	AAT TAA CCC TCA CTA AAG GG
SP6 Primer:	AAT TAG GTG ACA CTA TAG

Custom Primers:

Primers should be provided in DI water at the required concentration (see table above).
Sequencing primers should adhere to the following recommendations to be successful:

- A length of 18-25 bases.
- GC% content between 40 and 60%.
- A Tm (melting temperature) between 50° and 60°C
- No secondary priming sites
- Avoid primers that can self-hybridize
- Avoid long runs of identical nucleotides
- No significant hairpins (>3bp)
- Free of salts, EDTA, or other contaminants

For primers with a G+C content of less than 50%, it may be necessary to extend the primer sequence to keep the melting temperature above the lower limit of 50°C.

Template Preparation and Purification:

The success of automated sequencing critically depends on having high purity template in the correct concentration. Fluorescent sequencing is very sensitive to the presence of common contaminants such as salts, bacterial proteins, cell wall carbohydrates, residual detergents, silica fines carried over from template purification kits, and organic solvents (ethanol, isopropanol, etc.). Capillary electrophoresis is especially sensitive to residual salts in template preparations. The template should be provided in DI water or 10mM Tris buffer, **but not TE**. The EDTA will inhibit the sequencing reaction by chelating the magnesium required by the DNA polymerase.

Purification methods that are **not** recommended include:

1. Classical alkaline lysis
2. Boiling mini-preps
3. CsCl banding
4. Commercial glass bead/silica methods

The quantity of DNA template is also critical for success. The DNA concentration should be measured by a quantitative method such as:

1. Absorbance at 260 nm
2. Fluorescence in an agarose gel with standards
3. Fluorescence in a fluorometer

Please refer to the following page for instructions and shipping information. Thank you.

Shipping:

Send to:
ATTN: David Needleman
C/O USDA, ARS, ERRC
600 E. Mermaid Lane
Wyndmoor, PA 19038-8598

Important General Instructions:
Visibly mark on the outside of the box that it contains samples. Include your name and any instructions upon arrival, such as "Freeze, or Refrigerate, Upon Arrival". If samples are unmarked they will be left at room temperature or not delivered.

Ship all samples by overnight delivery
NO RECEIPT ON WEEKENDS OR HOLIDAYS (samples will be refused)

Sample Instructions:

96-well DNA Samples:

Use a rigid 96-well V-bottom or conical bottom plate. Avoid flat bottom plates. Cover the plate with a tight fitting rubber mat or thick aluminum tape making sure to seal around the wells. Ship the plate overnight, frozen on dry ice. We recommend wrapping your plate in bubble wrap or other packing material to protect the plates as the ice evaporates and the contents shift.

For a partial plate, less than 48 samples:

Fill the wells of the 96-well plate with your samples column by column, starting with A01, then B01, C01, etc.

Single-Tube DNA Samples:

Please submit samples in microcentrifuge tubes that are no smaller than 0.5 ml, *i.e.* **do not use 0.2 ml tubes**. Each tube should be sealed with parafilm and clearly labeled with the sample's identity, which matches the information provided to us on the request form. We recommend putting your tubes in a larger 50 ml centrifuge tube for added protection during shipment. Samples may be shipped either at room temperature, on ice, or lyophilized. Please protect the tubes by padding them or putting them in an additional container to prevent breakage.

Colonies:

Colonies in agar 96-well plates are preferred, but Petri plates may be used. Make sure the plates are dry, seal all edges with parafilm, and place in sealed plastic bag on an ice block. You may also wish to wrap the plates in bubble wrap for protection.

Glycerol Stocks

Glycerol stocks in 96-well plates should be sent on dry ice. Make sure the plates are dry, seal all edges with parafilm, and place in sealed bag. You may also wish to wrap the plates in bubble wrap for protection.