Automated DNA Sequencing Guidelines:

General Information about Templates:
The quality of your sequencing results will be directly proportional to the quality of your starting DNA. All templates are expected to be checked for quantity and quality before analysis by the core facility.

Criteria for cycle sequencing are more stringent than manual sequencing. The success of sequencing reactions depends critically on having high purity template in the correct amount. The most common contaminants are salts, bacterial proteins, cell wall carbohydrates, and organic solvents (ethanol, propanol, etc.)

DNA Isolation:
All DNA's must be submitted as clean plasmid or PCR products. A number of commercial kits (Qiagen, Promega, etc) are available for generating high quality plasmid preparations for sequencing. If Cesium Chloride preps are used, make sure there is no Cesium or EDTA present in the final sample. An extra ethanol precipitation is recommended if preparing plasmid with $CsCl_2$ preps. For PCR products, make sure **ALL** traces of the original PCR primers are removed!!! Depending on the cleanliness of the PCR reaction, a few options are available for preparing the DNA. If the PCR product is exceptionally pure (a single clear band), an ultrafiltration device such as the Qiagen PCR purification Kit (Cat#28104) will suffice in separating the PCR products from the primers. Or, if multiple bands are seen after PCR, either repeat PCR to optimize the reaction or run the product on an agarose gel and excise the band of interest. Please note that PCR products must be very pure in order to obtain good sequencing data. Low intensity extraneous bands could easily cause the sequence run to fail.

Recommendations for checking concentration and purity of DNA template:
Please measure your DNA with a spectrophotometer at absorbance of 260, and 280. Nucleic acids have their peak absorbance at 260, with a 260/280 ratio of 1.8 for DNA, 2.0 for RNA. Calculate the concentration of your DNA from OD_{260}: conc (ug/ml) = OD_{260} X dilution factor X 50. The OD_{260} of your DNA can be attributed to contaminants so it is very important to run some of your DNA on an agarose gel with a quantitative standard. Please run 1.0 ul of your plasmid DNA or 3-5 ul of "purified" PCR products, along with a known standard on an agarose gel. Compare the OD_{260} reading and the intensities of the template from the gel for any discrepancies. Prepare appropriate amounts of DNA template for sequencing according to the following table:

Recommended DNA Template and Primer Quantities

DNA Type	**Template** Concentration	**Primer** Concentration
PCR product:		
100 – 200 bp	20 ng/μl	3.2 pmol/μl
200 – 500 bp	20 ng/μl	3.2 pmol/μl
500-1000 bp	20 ng/μl	3.2 pmol/μl
1000 – 2000 bp	20 ng/μl	3.2 pmol/μl
>2000 bp	20 ng/μl	3.2 pmol/μl
Single – stranded (i.e. M13)	100 ng/μl	3.2 pmol/μl
Double – stranded (plasmids <15kb)	200 ng/μl	3.2 pmol/μl
Large DNA (plasmids over ~20 kb, cosmids, lambda clones)	500 ng/μl	25 pmol/μl
Large DNA (P1 clones, BACs)	800 ng/μl	25 pmol/μl

For each sequencing reaction, please send 10 μl of template and 10 μl of primer. Please send your DNA in a 1.5 mL microfuge tube, labeled with the following information: (1) the template name and date of your order. (2) Required amounts of DNA in dH_2O. Do not use TE or other EDTA containing buffer and avoid adding any divalent cations (i.e. Ca, Mn, Mg).

One of the most common problems encountered in automated DNA sequencing is that clients often try to measure the concentration of their mini-prepped plasmids or PCR products using a spectrophotometer. Some of you may be able to do so, but *most of you will not get reliable readings*. You need to be very cautious with your results. Thus, when sending samples for sequencing, please include a picture of your agarose gel containing your samples and a known size standard, especially if you are submitting PCR fragments (PCR and gel eluted fragments often do not produce enough DNA to reliably measure spectrophotometrically.).

The following sequencing primers can be supplied at no extra charge:

M13 Universal primer: 5’-GTA AAA CGA CGG CCA G
M13 Reverse primer: 5’-CAG GAA ACA GCT ATG AC
T7 Sequencing primer: 5’-TAA TAC GAC TCA CTA TAG GG
T3 Sequencing primer: 5’-ATT AAC CCT CAC TAA AGG GA
SP6 Sequencing primer: 5’-GAT TTA GGT GAC ACT ATA G
BGH reverse primer: 5’-TAG AAG GCA CAG TCG AGG

If other primers are needed, please supply them in the above mentioned concentration in dH_2O. The tubes containing the primers must be clearly labeled.

DNA sequences will be available in 2-4 days after they are submitted.

The Genomics Core Facility has impeded a new price structure for sequencing reactions, effective April 1, 2005. The cost of DNA sequencing is $21.15 per reaction. The cost is reduced to $10.61 per reaction if it's performed by the customer and purified prior to submitting to the Genomics Core Facility. **This price is subsidized by the LCRC for Stanley S Scott Cancer members so that the net price will be $14.10 per reaction and $7.08 per reaction if it's performed by the customer.** If you are currently not members of the SSSCC, please consider joining so that you can enjoy the benefit of the lower cost.

For information on becoming a member of the Stanley S. Scott Cancer Center, please contact Genny Melillo (gmelil@lsuhsc.edu).

Sequencing reactions will be rerun at no extra charge if a mistake was made by the Genomics Core Facility. If, however, reactions need to be rerun due to poor template quality or poorly designed primers, the customers will be charged the full cost of the reaction.

CONTACT PERSON: For all questions please call San-San Ng at 504-568-2200 or email: sng@lsuhsc.edu.