

# DNA Sequencing Help

Log on the Lims system at [dnatools.coh.org](http://dnatools.coh.org)

## Concentrations

- Template - PCR Product  
Concentration at least 3 ng per 100 base pairs per ul  
2.5 ul needed per reaction
- Template – BAC Clones  
1 ug/ ul concentration for BAC DNA
- Template – Plasmid  
Concentration at 100 ng/ul  
2.5 ul needed per reaction
- Custom Primers  
Concentration at 1-2 pmol/ul  
10 pmol/ul for BAC sequencing primers  
4 ul needed per reaction

**Entry Form** (After you have clicked Enter DNA Sequencing Requests, and entered the number of reactions)

- For City of Hope users, leave the PO\_Number field blank. This is for outside users only.
- Under the Grant Code Num. field please enter your grant code. If you do not have a grant code, enter your six digit account number here.
- The account number you enter must use the subaccount of 8028. Do not change this. Enter your entire account number (20/30 – 8028 – 6 digit account number)
- Enter the PI name “last name, first name” and in all capitals. Ex: SMITH, JOHN

## Chemistry

- BigDye\_V3.1 – For basic sequencing. Can read out to approximately 900 base pairs.
- BigDye\_V1.1 – Used only for short (less than 500 base pairs) PCR products when high resolution near the priming site is needed.
- BigDye\_3.0-dGTP – Used only for templates that are likely to form strong secondary structures, hairpin loops, or with high GC content (greater than 75%). When using this chemistry, please include the T<sub>m</sub> of your primer in the comments box.

Make sure after submitting the entry form, you print 2 copies of the table that appears. One of these copies must be placed in the inbox to inform us that the samples are ready to go. If we do not receive a copy of this form, your samples will never be sequenced. The other copy is for your reference.

Please label your samples with less than 8 characters if possible. Write the labels on the cap of the tubes you are submitting. Make sure that the labels on your tubes matches identically to the labels on the order form.

If you need a Chromatograph viewer, you can download one from Applied Biosystems for free at [www.appliedbiosystems.com/support/software/sequencescanner/](http://www.appliedbiosystems.com/support/software/sequencescanner/)