

DNA Sequencing Help

Log on to the Lims system at: <http://dnatools.coh.org/index.html>

- If you are new to the system and don't have an account please create one at dnatools see link above.
- Once you have your user name and password, please follow directions as to placing your order on the lims system. Tutorials are provided on the lims system to assist you on how to input your order.

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.
- An **iLab** order **MUST** be placed in order to sequence your sample requests.

Concentrations:

- Template - PCR Product
Concentration at least 0.5 ng per 100 base pairs for fragments less than 500 bp.
- **Dilute PCR sample to 1 ng/ul if PCR fragment is less than 500 bp.
If your PCR sample is greater than 500bp then dilute PCR to 3ng/ 100 base pairs in 2 ul volume.
For example: If your PCR size is 600 base pairs, multiply 600 X 0.03 which will give you the total amount of DNA necessary (18ng) for sequencing. Then dilute your PCR sample to the correct working concentration of 9ng/ul in 2ul total volume.**
- Template – BAC Clones
1 ug/ ul concentration for BAC DNA
- Template – Plasmid
Concentration at 100 ng/ul
2.0 ul needed per reaction
- Custom Primers
Concentration at 1pmol/ul
4ul needed per reaction
10 pmol/ul for BAC sequencing primers
4ul needed per reaction

Chemistries:

- 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%) or bisulfite-treated samples. When using this chemistry, please include the Tm 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 the lab personnel that samples have been placed in the PI box located in the freezer. 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/