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Vanderbilt Sequencing Information Sheet

- Ordering:** To place a sequencing order, download the Microsoft Excel application form at <http://www.GenHunter.com/VUsequencing/SeqOrderForm.xls> and send it back to GenHunter by email at sequencing@genhunter.com (preferred) or fax at [832-9461](tel:832-9461). Include a small purchase order number or credit card number on the application. An account will be set up for your lab if you do not have one already and we will invoice Vanderbilt once a month using the purchase order/credit card number given.
- Sample Pickup:** After receiving your order, a GenHunter representative will come to your laboratory to pick up the samples. If you submit your order by 10am, they will be picked up the same day (generally between 10-11:45am). When the order is submitted, the samples must be ready for pickup; we will not call beforehand and can not wait for samples to be prepared. Generally customers place their samples in order in a rack or box labeled "GenHunter Sequencing" in a common freezer or refrigerator.
- Sample Guidelines:** Plasmids, PCR products*, and BACs can be sequenced. We can accept plasmids purified with Qiagen's or other high quality mini-prep systems at a concentration of 100-200 ng/μL (plasmids larger than 10 kb, should be at 200-300 ng/μL). PCR products need to be at a concentration of 20-40 ng/μL, confirmed by gel electrophoresis, and purified using Qiagen's QiaQuick PCR Purification kit or other high quality purification. BACs should be supplied at 500-1000 ng/μL.
*If your PCR products are amplified from genomic DNA, "nested" are recommended, but not required.
- Primer Guidelines:** GenHunter supplies SP6, T7, T3, M13F, M13R, CMV-For, BghRev, Lseq, Rseq, Lgh, Rgh, L-AP, and R-AP. For other primers, please supply them with the templates at a concentration of 2μM**. All primers must be a minimum of 17 base pairs long and the concentrations given in μM.
**If you need to convert the primer concentration from ng/μL to μM, use this conversion:
$$\mu\text{M} = [(\text{ng}/\mu\text{L}) / (\text{Molecular Weight})] \times 1000$$
$$\text{Molecular Weight} \cong (\# \text{ of bases in primer}) \times 320$$
- Additional Guidelines:**
 - Separate Tubes:** We request separate tubes for each reaction in which templates and primers are used to simplify setup and eliminate potential pipetting errors. **Example:** If the same template is used in 4 reactions, please supply 4 separate tubes, each labeled identically on the cap. If the sample is used more than 8 times, you only need to provide 8 tubes but the volume will need to be increased (see below).
 - Volumes:** A minimum volume of 8μL per reaction in which templates or primers are used is requested. **Example:** If the same primer is used 6 times, supply 6 tubes each containing at least 8μL. If the sample is used more than 8 times, you only need to provide 8 tubes, each with 8μL per time used (e.g. If the same primer is used 16 times, we need 8 tubes with 16μL per tube).
 - Tubes:** Samples must be submitted in flip-top, 1.5mL tubes with the template/primer name clearly written on the lid and matching the application. Screw-top or other volume tubes are not acceptable. If you can label primers in different colored ink (or different colored tubes) than the templates, that is helpful.
- Results:** The results are emailed to the address given as soon as completed, in 1 to 3 working days. We will supply the sequencing text file and/or the ABI .ab1 file, whichever is requested. Many free programs to view .ab1 files are available including ABI's "Sequence Scanner v1.0" (www.appliedbiosystems.com - PC only), Chromas Lite (www.technelysium.com.au/chromas_lite.html - PC only), and FinchTV (www.geospiza.com/finchtv - Mac or PC). A copy of the electropherogram will be delivered to you, usually the next business day, or mailed to your address.

Please contact us if you need any further information or have any questions.

Additional information can be found at: <http://www.GenHunter.com/VUsequencing>