University of California, Davis c/o Evolution and Ecology 0208 Storer Hall Davis, CA 95616

UCDNA SEQUENCING FACILITY

Introductory Information

About us

- We are the College of Biological Sciences ucDNA Sequencing Facility. If you have questions about our facility, please contact Sheryl Bernauer slbernauer@ucdavis.edu, or Shelley Williams scwilliams@ucdavis.edu, or both of us at DNADivas@ucdavis.edu. You may also reach us at (530)754-9259. **For more information, and to access our online submission system, please visit our website at:** http://dnaseq.ucdavis.edu.

Services

- We can sequence double-stranded and single-stranded DNA from plasmids, BACs, cosmids, and PCR products. We run two ABI 3730 Capillary Electrophoresis Genetic Analyzers which are each capable of processing approximately twelve 96-well plates in a 24-hour period. We use ABI BigDye Terminator v3.1 sequencing chemistry and frequently get read lengths of 850+bp.
- We also have a run only fragment analysis service using our ABI 3130XL Capillary Electrophoresis Genetic Analyzer. Please contact one of our staff for more information.

Business Hours

- The facility is open from 8:00AM to 4:00PM Monday-Friday (excluding holidays). Samples are sequenced on a first-come first-serve basis so **turnaround time** is typically 1-2 days but is variable according to current workload and what time you drop off your samples. **The earlier in the day you bring your sequences, the faster you can expect to get results. Larger orders may take longer.** Please call to get a more accurate turnaround time.

Location/Sample Drop-off

- We are located in the basement of Storer Hall in room 0208 (go right off the elevator and around the corner). Once you have submitted your online form at http://dnaseq.ucdavis.edu/orders, bring your samples to one of our three drop-off locations:
- 0208 Storer Hall -- Main lab on UC Davis campus
- VM3B Store Room 1240 -- NE corner of VM3B on UC Davis campus
- 1106 Research I – UC Davis Medical Center in Sacramento *

  * (keycard access required for access to Research I see our website for more information)

  Due to the small amount of freezer space in the facility, DNA and primers are not stored for long once the sequencing reaction is successfully completed. **If you would like us to save your DNA for your prompt pick-up, please indicate this on your request form.

Pricing

- ***Check out our low prices!!*** Our pricing is as follows: For our UC customers we charge **$5.50/sequence** for 1-47 reactions and **$4.00/sequence** for 48+ reactions. Billing is done on a weekly basis.

Data

- All users will be provided with the analyzed sequence data as it comes from the 3730 Analyzer. ABI specifications are for 500bp of sequence with 98% accuracy but we are well over this standard. However, this means that even for the best sequence, up to 2% of your data could be miscalls. ***Please do not rely solely on your text file!*** Accurate sequence data can ***only*** be determined from an ***electropherogram***. Manual editing is the responsibility of the user but we are available for tips and trouble-shooting. You have the option of receiving your data via e-mail or we can copy your files onto flash drive that you provide.

Software

- You can download the appropriate sequence viewing software for your needs from our website at: http://dnaseq.ucdavis.edu/SoftwareDownloads.html

Sample Submission

- We need template DNA and primers, each in well-labeled, 0.5mL tubes. Please **label the tubes on the top with your initials and a number or a simple, short name.** Make sure that the names on the tubes match the names on your request form. If you will be submitting 48 or more samples, you may submit them in a 96-well plate. Please be sure that you orient the samples as follows: samples 1-8 in wells A1-H1, samples 9-16 in wells A2-H2, etc.

- Please provide us with **6µL of purified template DNA *per reaction*** at the appropriate concentration *(see DNA concentration guidelines below)*. **Remember: One sequencing reaction is one template with one primer.** Please supply **4µL of primer per reaction in a 0.5mL tube** at **20ng/µL or 3µM.** The following universal primers are provided by the facility at no extra cost: **SP6**, **T3**, **T7**, **T7 Term, M13-21**, **M13-R, CMV-F, CMV-R, BGH-R, pBAD-F, pBAD-R, pGEX5' and pGEX3'**. Primer sequences are available at **http://dnaseq.ucdavis.edu/Primers.html**.

Cloned products

- From our many years of experience, it is CRITICALLY important that the DNA template be clean and free of contaminants! Qiagen™ preps are one of the most consistent methods of template preparation but you can also try BioRad or Clonetech. The success of your sequencing reaction depends on many things, most importantly on the purity and cleanliness of your DNA. ***Note:*** *Please be sure that your DNA is NOT re-suspended in TE since the EDTA in TE inhibits the cycle sequencing reaction. We suggest that you re-suspend your DNA in either ddH2O or Qiagen's EB.*

PCR products

- The DNA should be purified by running through a spin column such as Centricon 100 or QIAquick™ to remove excess primers and dNTP's. This step is very important to the quality of your sequencing results. You might also consider running your products out on an agarose gel to make sure you only have a single product. If you have more than one product, you will get mixed sequence which translates to unusable data.

Template DNA Concentration Guidelines: ***(critically important!!):***

Cloned products:	**Size: (plasmid + insert)**	**Target Concentrations:**
	Double stranded template (3kb)	**100-200ng/µL**
	Double stranded template (4kb)	**200-250ng/µL**
	Double stranded template (5kb)	**250-300ng/µL**
	Double stranded template (6kb)	**300-400ng/µL**
	Double stranded template (7kb)	**400-500ng/µL**
	Double stranded template (8kb)	**500-700ng/µL**
	Double stranded template (9kb)	**700-800ng/µL**
	Double stranded template (10kb)	**800ng/µL-1µg/µL**
	Double stranded template (>10kb)	**>>1µg/µL**

PCR products: **2ng/µL per 100bp of PCR product**
(Ex: A 700bp PCR product would be submitted at 14ng/µL)

Please be advised that DNA templates over 10kb or PCR products less than 100bp may yield inconsistent results depending on your DNA and primer quality.

Check your concentrations carefully. Agarose gels are not always accurate. The most accurate way to check concentrations is to use a spectrophotometer. When using a spec please be sure to check for purity as well as concentration. Protein and RNA do not sequence well!

Thank you and happy sequencing!!