Request for DNA Sequencing
Rancho Santa Ana Botanic Garden

Background information:

***The process*:**

DNA templates are sequenced using a 3130xl Genetic Analyzer (Applied Biosystems/ Life Technologies Corporation, Carlsbad, California, USA). Templates are amplified (cycle-sequenced) in a PCR reaction with 3'-dye labeled dideoxynucleotide terminators (4, each with a unique fluorescent compound attached; BigDye version 3.1 (Applied Biosystems), using AmpliTaq DNA polymerase and a single primer appropriate for the template strand. The reaction mixture is as follows:

1-2 µl template DNA (amount varied depending on concentration)
1 µl primer (at 1.6 pmol/µl)
1.5 µl BigDye 3.1 terminator mix (Applied Biosystems)
3.5 µl 5X Sequencing Buffer (Applied Biosystems)
12-13 µl dH2O (varies depending on DNA volume)
(you provide these components)

The PCR reaction involves 35 cycles under the following conditions:

Step	Temperature	Time
Denaturing	94º C	15 sec.
Annealing	50º C	15 sec.
Extension	60º C	4 min.

Following the cycle sequencing, samples are purified using G-50 Medium Sephadex (GE Healthcare, Piscataway, New Jersey, USA), packed in a 96-well filter plate from Phenix Research, transferred to a sample plate, and run on the 3130xl Genetic Analyzer within 12 hours. During electrophoresis, a laser scans each of 16 capillaries causing any dye particles in its path to fluoresce. The strength and the color of the fluorescence is recorded and subsequently analyzed to assign a base to each peak. The resulting data are presented in the form of a chromatogram, showing the heights of each dye peak throughout the length of the run, and the base identity assigned to each peak. Due to the length of the capillary array, the maximum template length that can be sequenced accurately is 750-850 bp (50 cm array; Pop-7). We run control DNA frequently to assess data quality. These data will be made available upon request.

***Sample preparation*:**

We assume that DNA template arrives prepared for cycle sequencing. That is, any required amplification has been done and templates have been cleaned, if necessary. Please provide samples in 600 µl PCR tubes, containing all necessary reaction components for cycle sequencing except the terminator mix and buffer (i.e., template, primer, and dH2O to a volume of 15 µl). We will add the terminator mix and buffer, cycle sequence and purify the samples, and run them on the Genetic Analyzer. If you wish to invest in the dye terminator mix and provide your samples with all of the reaction components included, we will run the cycle-sequencing reactions and the electrophoresis. Please call or write to negotiate reduced rates.

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Please provide clear, simple identification on your samples, such as your initials + sample number (e.g. LP01, LP02, LP03, etc.), **short** names, and **short** descriptors. We will attempt to include all this information in the sample file names.

***Data output options*:**
The results of a sequencing run are provided as digital files containing the analyzed chromatogram data. Files may be zipped for Pomona researchers, however those at other colleges will receive separate data files for each sample, or arrange for transfer of data through Dropbox or some other web-based tool. The file of analyzed data can only be read using certain software packages (e.g. Sequencher 4.9, DNAStar, etc.). If you do not have access to this type of software, we can provide a text file containing the sequence that can be opened with any word processing program. The chromatograms allow you to evaluate the quality of the sequence data by comparing the signal strengths of the bases called at each location. Please provide an email address to which files can be sent.

Cost and billing information:
Sequencing costs are currently $9.00 per sample. We submit invoices to departmental secretaries once each month; please provide whatever information your department needs in order for the charges to be billed to the appropriate source (e.g. account number or other funding source reference). Discounts also available for ½ and full plates (48 and 96 samples respectively). Call for special pricing.

***Turnaround time*:**
Submit samples by 4:00 p.m. on Monday for data by Wednesday at 9:00 a.m. unless Monday is a holiday. For Monday holidays, please have samples in by noon on Tuesday. Include a sample information sheet with a faculty member name, billing contact name, sample information and contact information (email, phone). If the above schedule is inconvenient, especially for class related projects, please contact us. Special arrangements may be made for alternative submission and processing dates.

Other information:

If you have any questions about any of this information, please call 625-8767 ext. 265 or email *lwashburn@rsabg.org*

Thanks!

Loraine K. Washburn

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Contact information:

Faculty Name _____ Date _____
Department _____ Phone _____
Institution _____ email _____
Billing contact _____ Phone _____
Billing reference (account number, etc.) _____

Sample information:

Total volume in tubes _____ µL
Primer(s) _____ concentration
Terminator mix _____ included _____ please add

Sample descriptions:

Number	Name	Description