

Facility Description

Location:

Duke University Medical Center
135 Jones Building
(919) 613-7831

Mailing Address:

PO Box 3010
Research Drive
Jones Room 135
Durham NC 27710

Email:

sequence@mc.duke.edu

Web Site:

<http://cancer.duke.edu/dna>

Personnel:

- Scott Langdon, Ph.D. Managing Director
- Charles Bullard, Research Technician
- Delira Hubbard, Research Technician

Equipment:

Two Applied Biosystems 3730 DNA Analyzers, one Applied Biosystems 3100 Genetic analyzer, several Applied Biosystems PCR thermocyclers plus various centrifuges, balances, etc. needed to prepare the DNA sequencing samples.

Process:

Premixed samples should be dropped off in 135 Jones 9AM - 4PM.

Turn-around Time:

Two business days. Unless there is a backlog or technical problems.

Policies:

- All samples are processed on a first come, first-served basis.

- Laboratories are charged for all reactions (including failed reactions) so long as our positive controls work.
- A valid Duke fund code or PO# is required for all DNA sequencing.
- The DNA Sequencing facility is unable to archive data. It is the responsibility of each customer to save their own data.

Fees:

Contact the DNA Sequencing Facility for current pricing.

“Peer-reviewed” Duke Cancer Center members are eligible for a discount.

Volume discounts are available for:

- -\$1.00 for ≥ 48 samples
- -\$2.00 for ≥ 96 samples
- -\$3.00 for ≥ 500 samples

Additional charges:

- \$2.00 manual data handling
- \$2.00 special reaction fee (Genomic samples - to cover addition reagent charges)

General Description:

We use the Perkin Elmer Dye Terminator Cycle Sequencing system with AmpliTaq DNA Polymerase combined with ABI 3730 and 3100 PRISM DNA Sequencing instruments in this facility. We use BigDye™v1.1 terminator sequencing chemistry. With dye terminator labeling, each of the four dideoxy terminators (ddNTPs) is tagged with a different fluorescent dye. This system has the advantages of 1) any unlabeled primer can be used, 2) all four sequencing reactions are performed in one tube and run in one lane on the gel, 3) false stops caused by anything other than incorporation of a ddNTP are not fluorescently labeled and thus not detected, and 4) sequencing difficulties caused by DNA conformation and base content are minimized by performing the PCR reaction at 60°C.

DNA Sequence:

We use the ABI PRISM DNA Sequencing Analysis software program to analyze the DNA sequencing gel and read the bases. The overall quality is, of course, very dependent on DNA quality and signal strength. A typical run generates about 800 bases with an accuracy of 98-99% for the first 600 bases. As you read past 600 bases the accuracy decreases. Readable sequence begins

about 50 bases from the primer. High quality samples often generate accurate sequence to 700 bases and beyond.