The automation of DNA sequencing has revolutionized the speed and accuracy with which whole genomes are now sequenced. Less than 20 years ago, the first automated DNA sequencer was introduced, combining four-color fluorescently labeled primers and real-time data collection to significantly increase simultaneous sample processing. Since then, there have been many advances made in this area. The development of fluorescently labeled dideoxynucleotides allowed more flexibility by eliminating the need for time-consuming synthesis of costly, labeled primers. Introduction of cycle sequencing lowered the requirements for starting material, and modification of thermostable DNA polymerases permitted better incorporation of dye-labeled terminators. Newer fluorescent dyes were developed to increase signal strength and provide more uniform peak intensity patterns, leading to greater sensitivity and better base-calling. In instrumentation, the launch of capillary sequencers provided higher throughput and required less manual intervention. Early sequencers used polycrylamide slab gels and provided sequence reads of 350–400 for only 24 samples in about 13 h, whereas current capillary-based platforms now boast...
reads greater than 700 bases for as many as 384 samples in less than 2 h.

Unfortunately, the cost of the automated DNA sequencers and the speed at which they are replaced with newer technology keeps them out of reach for most individual laboratories. This has led to the need for DNA sequencing facilities as shared resources within both academic and commercial locations. In 1998, the first DNA Sequencing Research Committee (DSRG) survey was launched to characterize a typical core DNA sequencing facility. Since then, the DSRG conducts similar surveys every 2–3 years to maintain an up-to-date profile and to establish what influences, if any, recent technological advances have had on the composition of the average DNA sequencing laboratory.

METHODS
This year, the DSRG prepared a detailed survey that consisted of 58 questions, designed to look at staffing, funding, instrumentation, services, and customer relations within each participating DNA sequencing laboratory. The survey was launched in December of 2002, and the questionnaires were completed by January 15, 2003. The survey announcements were posted to several web servers, including the ABRF list server, to encourage participation. In all, 45 facilities requested surveys and 30 surveys were completed and submitted for final analysis. To ensure anonymity, all surveys were submitted and tracked using a unique four-digit number. The results from the survey were collected and analyzed, and preliminary results were presented at the ABRF 2003 meeting in Denver, Colorado. A more in-depth look at the findings is presented here.

RESULTS AND DISCUSSION
Based on responses to our survey, the average DNA sequencing resource laboratory has been in existence for 8 years. Most of these operate as part of a larger facility offering multiple services that may also include the following: DNA template preparation, fragment analysis, real-time PCR, oligonucleotide synthesis, protein sequencing, peptide synthesis, microarray analysis, microscopy, freezer programs, and bioinformatics. Over the last year, the average facility received approximately 47,000 DNA sequencing requests from 108 laboratories.

Staffing
The majority of DNA sequencing core facilities (84%) are overseen by an external faculty member or an advisory committee. Within the facility itself, there is usually a director or laboratory manager and four technical staff. Most of the directors have PhDs (64%); 13% have master’s degrees and 11% have bachelor’s degrees. On average, a director has seven years of experience, and the salary range is $36,000 to $100,000, with a mean of $58,000. Technical staff generally remain in their positions for 3 years. Full-time staff earn salaries ranging from $23,000 to $46,000; the mean salary is $32,500.

Funding and Sequencing Charges
This survey shows that many of the resource facilities (68%) are subsidized to some extent. Of these, 7% are fully subsidized, and are not required to charge users for individual sample submissions. The partially funded laboratories receive supplemental funding from a variety of sources that include research grants, institutional funds, departmental monies, or philanthropy. When asked whether funding is adequate, only 51% of respondents felt that funding for instruments was sufficient, whereas 63% felt that salaries were well supported.

Sequencing reaction prices vary widely from one institution to another (Figure 1). Charges range from $6 to $23 (US$) per reaction. The average amount charged for an in-house sequencing reaction and run is $13. Reduced pricing is also provided for large projects (40%), departmental work (30%), cancer center members (20%), and center directors (10%).

Factors determining the cost for sequencing reactions include whether the facility was fully subsidized or completely self-sufficient. Many facilities rely on the sequencing charges to fully cover their operational costs (41%), while others only require partial cost recovery (37%). The extent to which the charges are used to offset various expenses is shown in Table 1.

Seven percent of the respondents mentioned that they use the ABRF survey information to determine their pricing. An overwhelming 87% of respondents indicated that users of their respective facilities are satisfied with the cost of sequencing.

Instrumentation
Each facility that participated in the survey has between one and five sequencing instruments, with an average of two instruments per laboratory. Figure 2 shows the current distribution of instrument types. Important to note is that capillary models now comprise 49% of the instruments in core sequencing facilities, and that many laboratories (40%) have completely switched to capillary models. Those laboratories that have both types of instruments (23%) tend to dedicate one instrument to sequencing and the other to genotyping, or may use the high-throughput capillary sequencer for large-scale projects.
Chemistry

Respondents to our survey indicated that dye terminator chemistry is predominant, although this is distributed among nine different types of DNA sequencing kits. The complete breakdown is shown in Figure 3. The two most popular chemistries are BigDye version 3.0 (30%) and BigDye version 3.1 (14%) (Applied Biosystems). Sixty-four percent of facilities regularly use additives in sequencing reactions. The most popular additives are dimethylsulphoxide (DMSO) (69%), glycerol (21%), magnesium chloride (5%), and betaine (5%).

Template Preparations and Reactions

Most sequencing facilities (87%) do not have strict guidelines concerning DNA template preparation and none had requirements concerning the source of sequencing primer. Only 33% of all facilities offer template purification as a service. There is even distribution of facilities that assembled reactions in tubes, 96 well plates, or both. After cycle sequencing excess dye terminators are removed from the sequencing products using chromatography columns (62%),

### Table 1

<table>
<thead>
<tr>
<th>Use of DNA Sequencing Fees^a</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent costs</td>
<td>100%</td>
</tr>
<tr>
<td>Service contracts</td>
<td>90%</td>
</tr>
<tr>
<td>Technician salaries</td>
<td>72%</td>
</tr>
<tr>
<td>Lab consumables</td>
<td>69%</td>
</tr>
<tr>
<td>Upgrades</td>
<td>66%</td>
</tr>
<tr>
<td>Director salaries</td>
<td>52%</td>
</tr>
<tr>
<td>New instruments</td>
<td>48%</td>
</tr>
<tr>
<td>Full operating costs</td>
<td>38%</td>
</tr>
</tbody>
</table>

^aShown is the percentage of facilities whose income from DNA sequencing is used to support expenditures within the different categories.
ethanol precipitation (32%), or magnetic beads (7%). Following a failure, customer reactions are repeated by 83% of the sequencing labs, but most of these are done only upon request. Forty-four percent of facilities do not charge for these repeats, 35% charge for the sample if it failed twice, and 22% charge for the repeat only if it is successful.

The plasmid pGEM is used as a quality control template in greater than 90% of core facilities. Other templates used are pBluescript, M13 phage and pGEX. The control sequences may be reviewed internally (40%), shown to customers on request (33%), or posted for general viewing (7%).

Consultation and Training
In addition to the expected duties within a sequencing facility, our study shows that a substantial amount of time is being spent in consultation and informal training of customers. An average of 5 h per week per facility, and as much as 30 h per week is dedicated solely to this purpose. Customer complaints are taken very seriously, and 57% of respondents indicated that the facility manager or director is responsible for dealing with them. Most of these are dealt with on an individual basis, and possible solutions are offered that include running reactions at no charge or referring customers to trouble-shooting handouts or web pages. Some laboratories also regularly use anonymous questionnaires to evaluate potential problem areas, and correct areas of concern. Only 43% of laboratories offer formal training sessions, and these are offered at no charge.

The number one complaint received by 83% of sequencing facilities is that customers express concern when their sequencing results are less than optimal. Thirty-one percent also mentioned turn-around time
as a common complaint, and only 7% mentioned that customers argue that the prices are too high. On a positive note, laboratory members also receive positive comments, as shown in Figure 4.

Stress relief

Unlike traditional research laboratories, core laboratories have the added pressure of dealing with customers. As in all service professions, this can be associated with a certain amount of stress. From our survey, it was determined that 68% of respondents undertake measures to help technicians within their laboratories deal with stress and customer pressures. Formal training in customer relations is offered within 14% of laboratories, and another 11% offer informal training within regular lab meetings. In many facilities (18%) the director/manager uses offerings of food and drink to keep spirits high. Unfortunately for technicians, only 4% mentioned that they keep their staff happy with regular raises.

Past and Present

Since the 1998 DSRG general survey, the average number of sequencing instruments per core facility has not increased.14 However, what has changed since then is the type of instrument. In recent years many new instruments, primarily capillary-based sequencers, have entered the market place. These sequencers, such as, Applied Biosystems’ 3100, 3730, and 3730XL; Amersham Biosciences’ MegaBACE 500 and 1000; and Spectrumerix’ 961C are displacing slab gel models within core facilities. The DSRG 1998 study showed that, at that time, only 5% of sequencers in use were capillary models,14 and a modest increase to 7% was reported in the DSRG 2000 study.15 Our current study shows that that number has now jumped to nearly half of all instruments in core facilities. Likewise, the Applied Biosystems 377—the former workhorse—has dropped from 57% (2000)15 to only 38% of all instruments within sequencing labs.

As the throughput capabilities of capillary sequencing instruments is so much higher, it is perhaps not surprising that the number of samples processed annually has jumped almost 4-fold in the last 5 years. Current throughput has been determined at an average of 47,000 sequences, which is a significant increase from the 19,000 requests received by an average core facility in 2000,15 or the 12,000 samples reported in 1998.14

Despite the higher demand, sequencing prices remain constant—we do not report a change in price per in-house reaction compared with what was reported in the DSRG 2000 general survey.15 We suggest that the increasing costs of instruments, kits, salaries, etc. have kept this in check—in fact, most laboratories are only able to keep prices as low as they are through various subsidies. However, this does not appear to be a concern, for the vast majority of core laboratories reported that customers are satisfied with prices. Perhaps it is the strong dedication to customer service that keeps them coming back.

ACKNOWLEDGMENTS

The members of the DSRG would like to thank everyone who participated in the survey. We would also like to thank new committee members Heather Lin and David Needleman for their help with the preparation of this manuscript.

REFERENCES

EDMAN SEQUENCING RESEARCH GROUP

The Edman Sequencing Research Group encourages all ABRF members involved in Edman sequencing to participate in this year’s study. The ESRG 2004 sample will be the 16th study conducted by the ESRG, offering a unique, yet informative challenge, distinct from previous studies. The ESRG was pleased with the participation in the ESRG 2003 sample study and has posted the results at the ABRF website in the form of the Denver meeting poster and the ESRG study presentation given by Nancy Denslow. The current ESRG members are Dan Brune, Richard Cook, Myron Crawford, Nancy Denslow, Ryuji Kobayashi, Ben Madden, John Neveu, and EB liaison, Laurey Steinke.

FRAGMENT ANALYSIS RESEARCH GROUP

Within the past three years a variety of new instruments and new chemistries have emerged and have found their way into core facilities. Some of these have replaced older platforms to become the major players for running fragment analysis applications. Because researchers who wish to collaborate on genotyping projects may use different equipment and reagents to process their samples, it is desirable to update our knowledge on the characteristics of the different methods and instruments currently in use in the core facilities. Based on such a need, the Fragment Analysis Research Group (FARG) has designed the current FARG study to continue exploring the range of variation in data that may be due to the methods for collection and analysis.

Designed as a mock “crime scene investigation,” the 2004 FARG study involves running, analyzing, and reporting data from a set of 11 samples supplied to the participants who are invited to solve the mystery and name the suspects that match the sample found at the “crime scene.” The results will be collected using an online datasheet, and FARG will report the percentage of entries correctly identifying the “perpetrator.” The size data for the seven loci reported for two of the samples will be analyzed, and FARG will investigate whether size variation for each allele can be correlated with the components used to produce the data (e.g., instrumentation, chemistries, internal lane standard). Laboratories wishing to participate in this study should contact Caprice Rosato (rosatoc@science.oregonstate.edu).

A manuscript outlining the results from the 2003 FARG Study/Survey (Current Trends in Fragment Analysis) is in progress for submission to JBT. Two tutorials from the 2003 ABRF meeting on the topic of Mutation Detection by Single Base Extension methods are being finalized for posting on the website, and a manuscript describing the FARG 2002 study on Multiplex PCR is also near completion.

THE NUCLEIC ACIDS RESEARCH GROUP

The NARG completed their 2003 study in which they examined (1) whether dual-labeled Taqman probes are as easy and economical to make as regular oligonucleotides, and (2) whether crude unpurified dual-labeled probes can perform well in most qPCR experiments. Final results of this study will be published shortly, but a summary of the results is posted on the NARG page at the ABRF web site. The study demonstrated that it is possible to make high quality FRET probes without purification that perform well in qPCR assays, and quality control can be as simple as a PAGE gel.

The NARG is currently developing a general survey to profile laboratories performing real time PCR. In addition, NARG will conduct a study on primer design to allow participants an educational opportunity to test their primer design skills and demonstrate some of the important factors for this important facet of real time PCR.

Watch for the opening of these studies on the ABRF Roundtable.

ABRF 2004 in Portland, OR will offer many opportunities to share information about real time PCR. There will be a scientific session featuring Dr. Stephen A. Bustin, University of London and Dr. David Ginzinger, UCSF. In addition, a tutorial on Molecular Beacons by Fred Russell Kramer, Public Health Research Institute will be presented. The NARG will share the findings of their general survey and their primer design study and there will be a roundtable/troubleshooting session. We hope that you will join us.

The NARG is happy to welcome Yongde Bao, University of Virginia School of Medicine and Deborah Grove, Pennsylvania State University to the Nucleic Acids Research Group.
CURRENT MEMBERSHIP
Pamela Scott Adams (Chair)—Trudeau Institute
Yongde Bao—University of Virginia School of Medicine
Deborah S. Grove—Pennsylvania State University
Brian Holloway—Centers for Disease Control
Steven Scaringe—Dharmacon Research, Inc.
Dr. Gregory L. Shipley—UT Health Science Center–Houston
Dr. Anthony T Yeung—Fox Chase Cancer Center
Dr. Susan H Hardin (Ad hoc, EB liaison)—University of Houston

PROTEOMICS RESEARCH GROUP

The Proteomics Research Group (PRG) has had a busy summer. After a successful and enlightening study for ABRF ’03 we decided to do another study, again designed to help individual labs evaluate their own capabilities in the field of proteomics.

This year we have decided to look at sample preparation—as it is performed in the laboratories as well as identification of closely related proteins and the differences between them. After much consideration, the study was mapped and the pilot distributed to the PRG committee for evaluation. Once satisfied that the sample was reasonable and could be processed by many laboratories at different levels, large quantities were prepared and again checked. The samples, having been carefully prepared, are now ready to send out to anyone wishing to participate in the study.

To participate in this study you can request a sample by sending your name, address, and membership status to ABRFPRG04@wistar.upenn.edu. The deadline for sample requests is September 26, 2003. This study is open to both members and nonmembers of the ABRF. As always, the resulting data will be returned via a third party to protect the anonymity of the respondents.