FRAGMENT ANALYSIS; A CHANGING FIELD AND A NEW COMMITTEE

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Abstract:

As sequencing of human and other genomes reveals genetic differences between individuals, the next logical step has been to analyze these allelic variations, known as polymorphisms. This information is already commonly used to establish identity in forensic and paternity testing as well as tissue typing. It also enables specific localization of genetic disorders to particular chromosomes by statistically linking mapped polymorphisms to a disease in a family or population. Automated sequencing has led the way to automated fragment analysis--a progression from radioactively labeled PCR product analyzed singly to multi-color fluorescently labeled product multiplexed by both size and color. Recognizing the increasing number of resource facilities offering automated DNA fragment analysis, the following information was collected by an on-line survey. This information was then tabulated and analyzed to help build a profile of the average ABRF member facility performing fragment analysis.

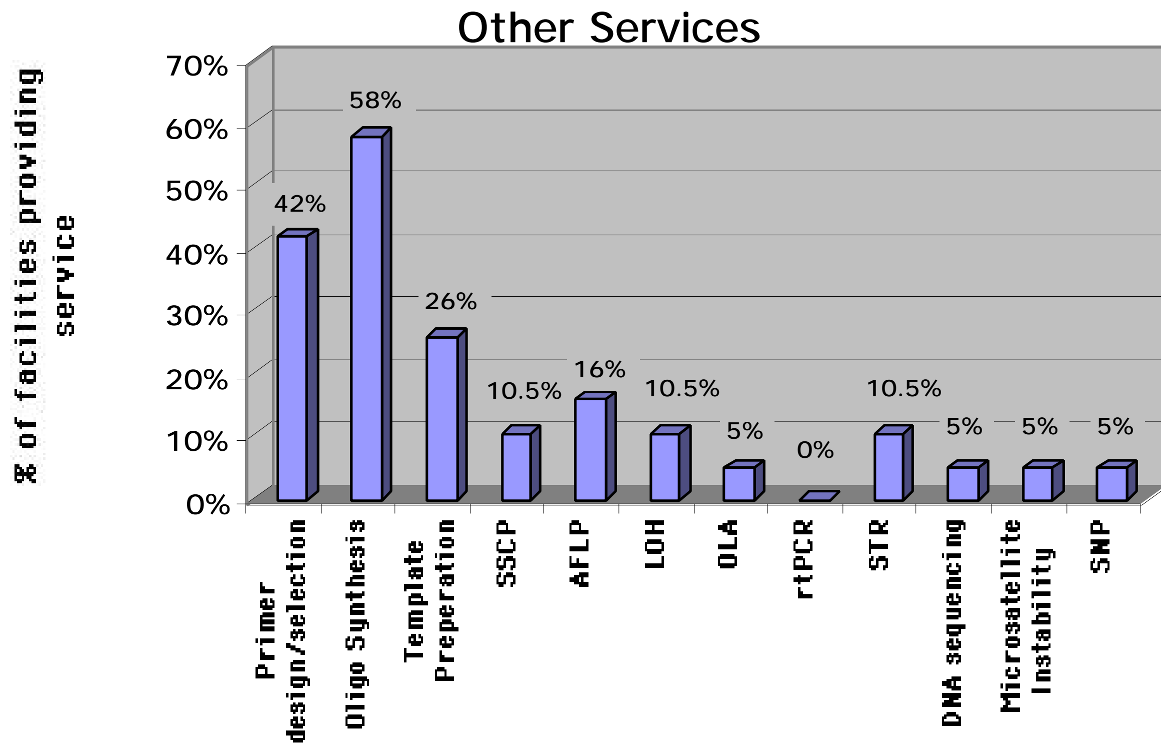


Figure 1 Facilities that offer fragment analysis provide a wide range of services in addition to genotyping.

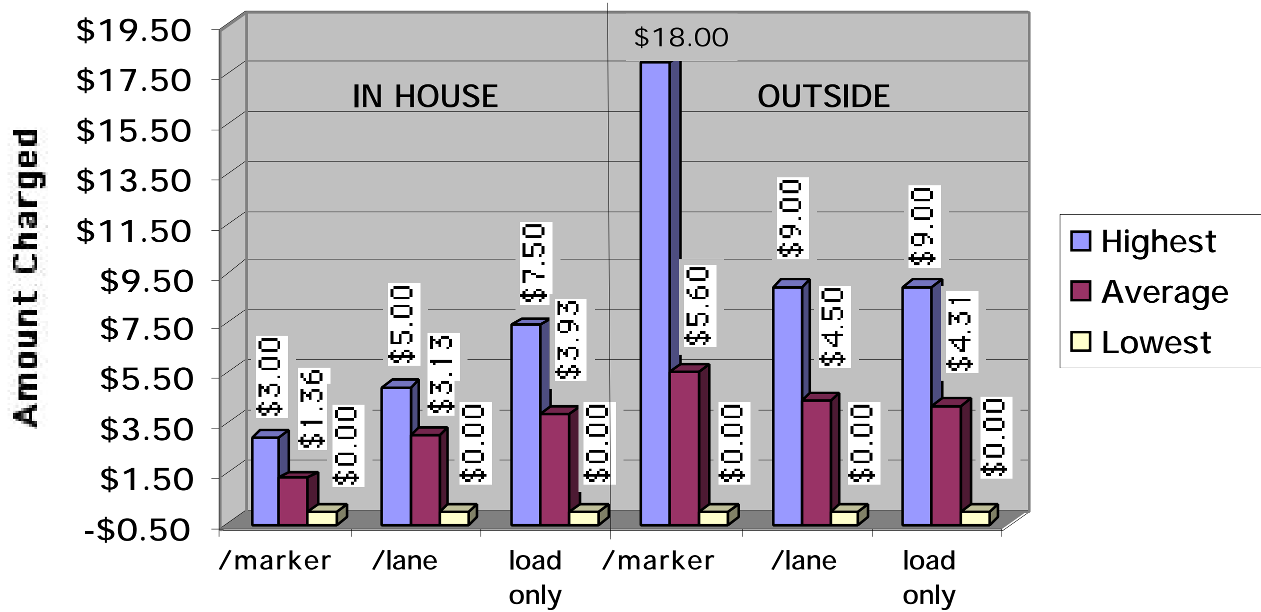


Figure 2 This figure provides information on current charges for providing fragment analysis both within a facilities organization and outside the organization. The three main pricing schemes of per marker, per lane and loading a user prepared sample are represented.

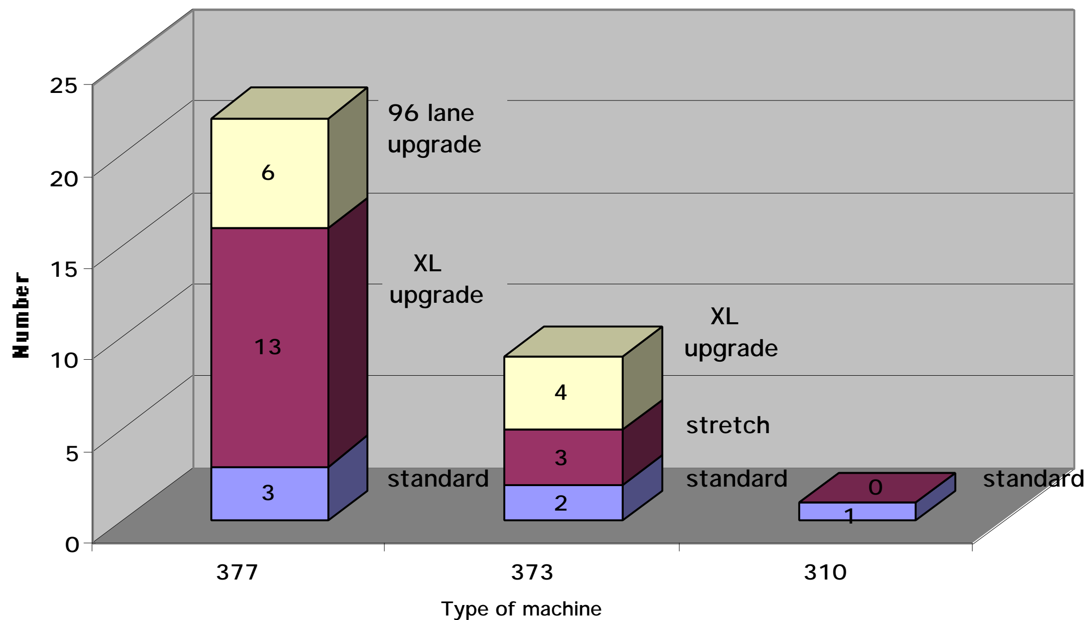
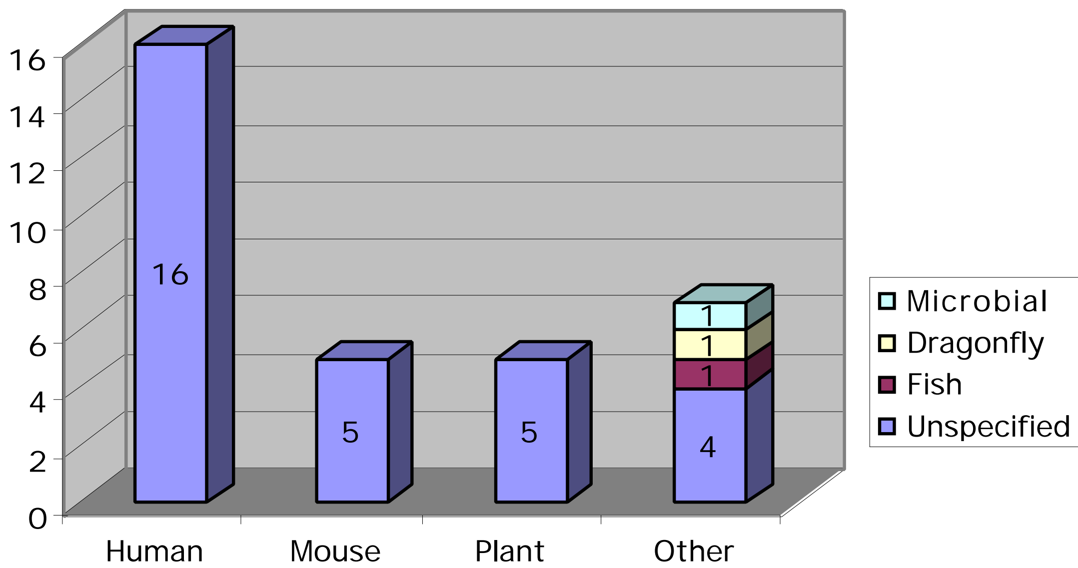


Figure 3 Because the majority of respondents to the survey use ABD* equipment we asked for information on the particular configuration being used on the machine.

*ABD=Applied Biosystems Division/Perkin Elmer, or ABI (Applied Biosystems Inc.), or PE Biosystems

TYPES OF DNA ANALYZED



Human only	Mouse only	Plant only	Two species	Three or more species
37%	5%	5%	32%	21%

Figure 4 Three main types of DNA templates are currently being tested in the surveyed facilities. More than half the labs tested two or more types.

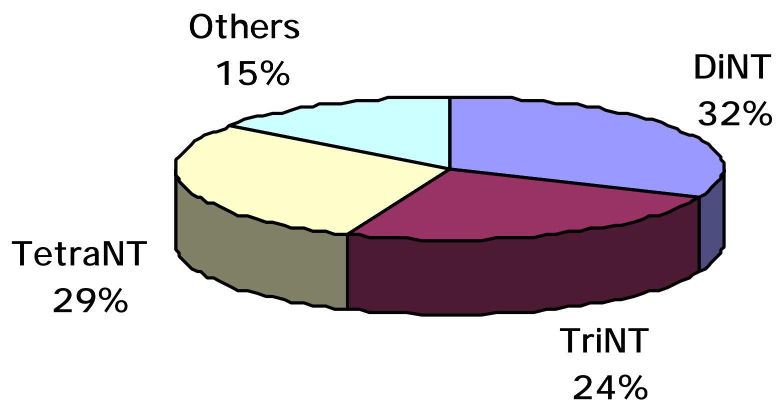
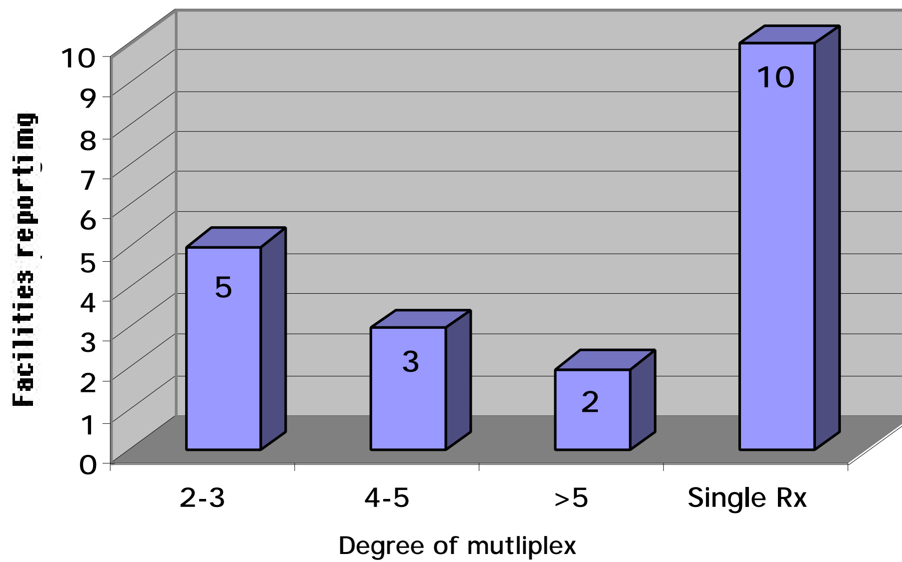


Figure 5 Most facilities use variable number tandem repeats (VNTRs) as markers and this figure provides information on the specific type(length) of repeat used. The three main types of repeats shown are dinucleotide, trinucleotide, and tetranucleotide. The other types of markers were not specified.



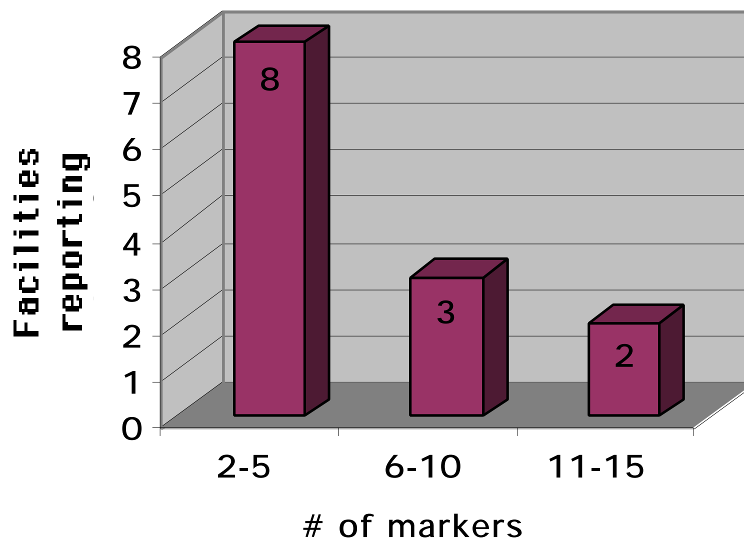
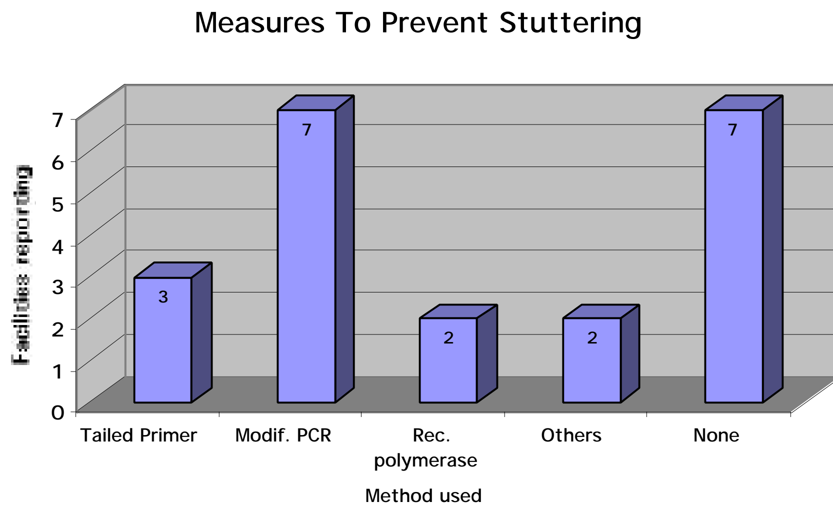


Figure 6 Because of the utilization of multiple dye labels, multiple marker products can be run in a single lane and still be distinguished from each other. The ability to multiplex at the PCR level(multiple primer sets and a single template in one reaction tube) is possible when special care is taken in the primer design.



<u>Figure 7</u> A frequent problem seen in amplification and subsequent analysis of repeat regions is stuttering or addition of a base to the end of a product. In order to try and eliminate this occurence methods such as modified primers(tailed), modification of PCR conditions or recombinent DNA polymerases are utilized.

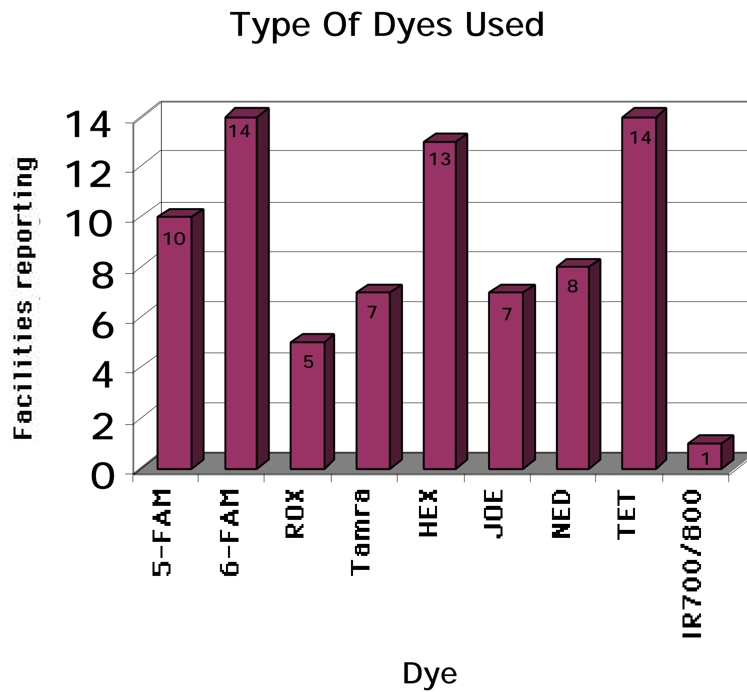


Figure 8 This figure shows the usage frequency of different labels that are used when a product is generated for visualization and analysis.

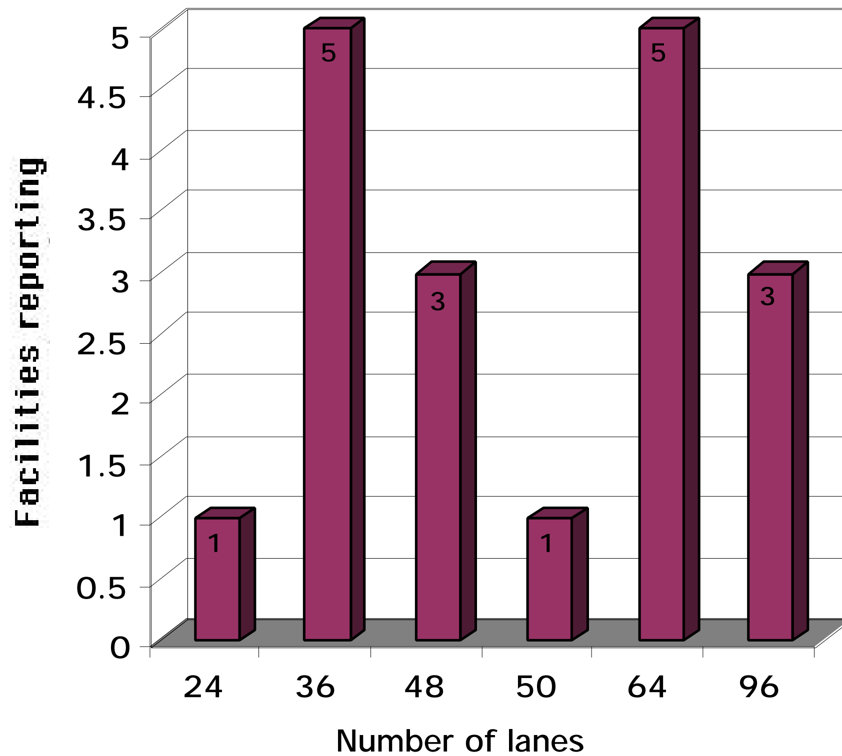


Figure 9 Many different options are available for the number of lanes that can be run on a single gel. This figure shows the frequency of use of the more common gel comb configurations.

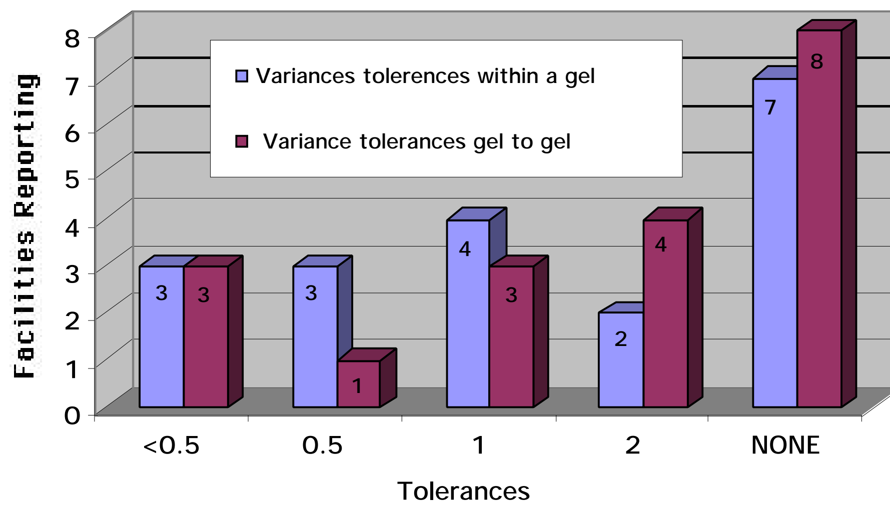


Figure 10 Fragment analysis is done through the use of product size and this is generally a nucleotide base count. Because a typical study is looking for variances in size between multiple samples, it is important to have little variance in samples with like sizes from lane to lane and even gel to gel. This figure shows the acceptable levels tolerated by the reporting facilities.

Data Storage

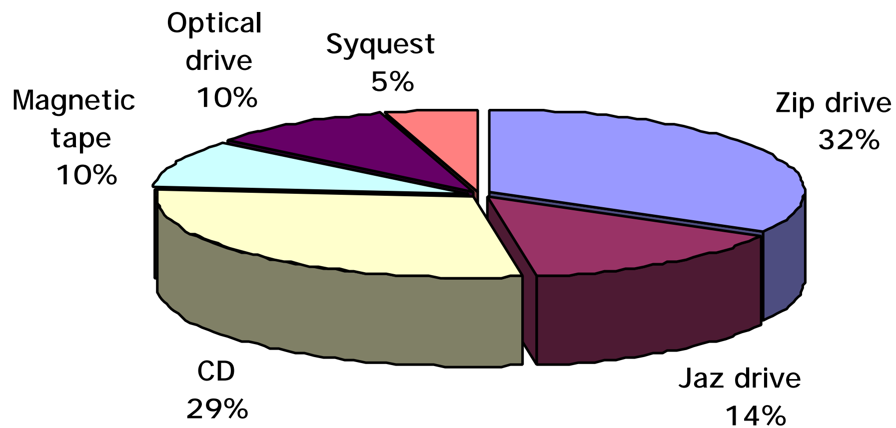
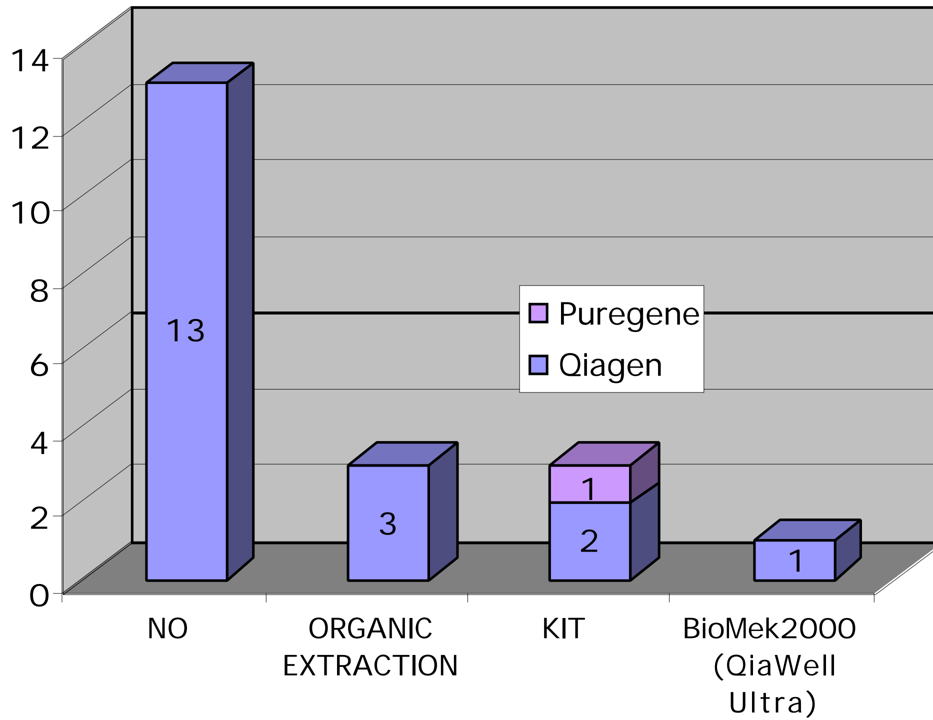


Figure 11 Many projects that are carried out using fragment analysis use a large number of samples to achieve statistical significance. The data produced by these studies must be cataloged and archived for analysis. This figure shows the major storage options used.



<u>Figure 12</u> The preponderance of the surveyed facilities do not provide template preparation service. Those that do relied mainly on organic extraction and kits.

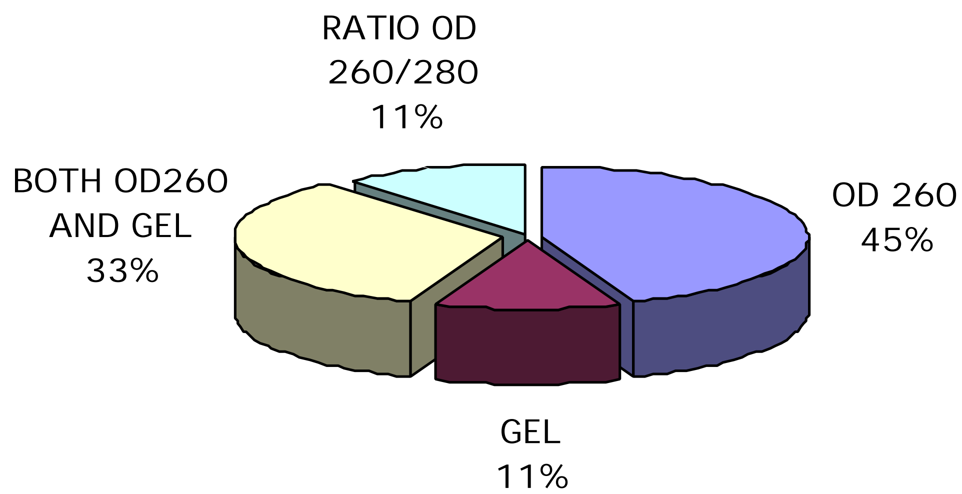


Figure 13 The preferred methods of checking quality and quantity are OD 260 or OD260 and gel.

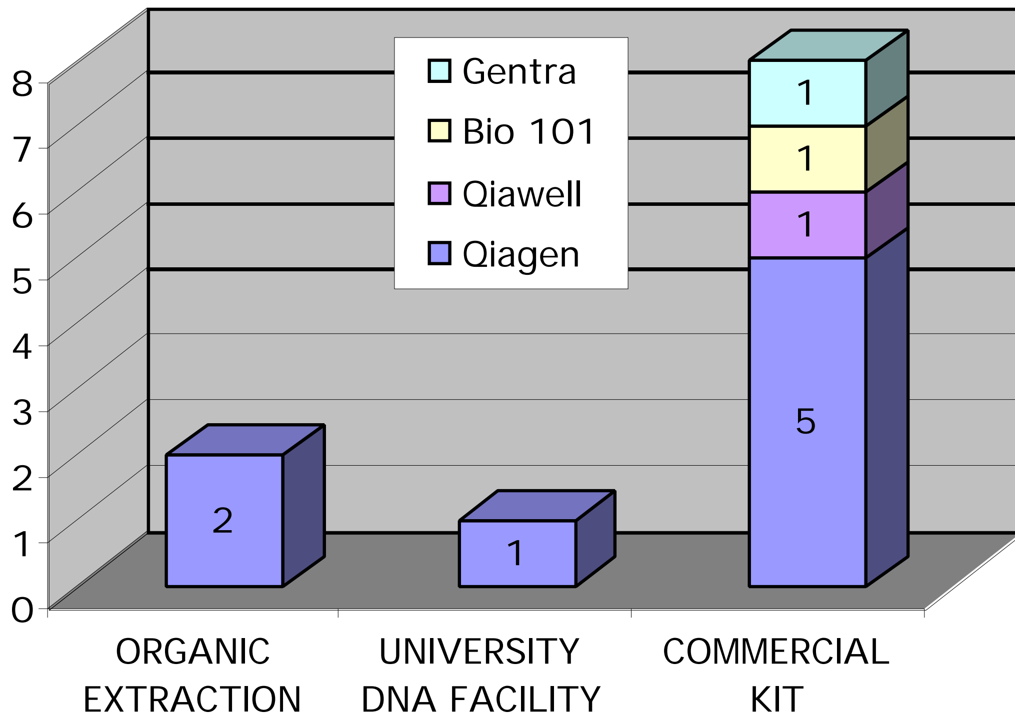


Figure 14 Most of the surveyed facilities recommended commercial kits to users for DNA preparation.

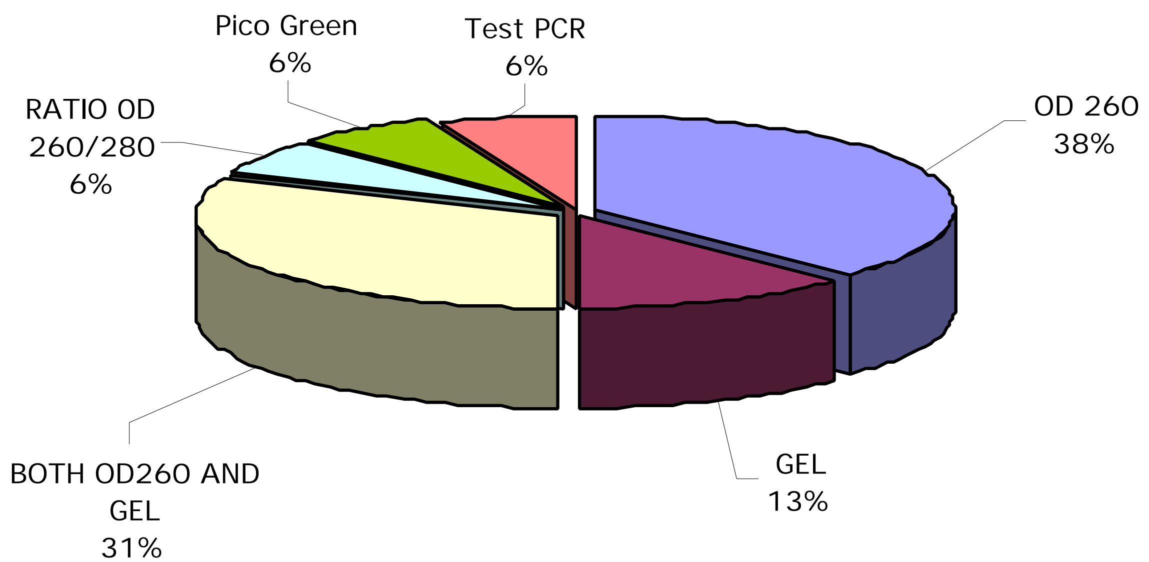


Figure 15 For the most part, the same methods used by the facilities (OD260 or OD260 and gel) were recommeded to users. 88% of responding labs did not require documentation. 12% required gel, OD260 or both.

Conclusion:

The survey data has led to the following profile of the average facility performing fragment analysis. The 20 laboratories responding to the survey were varied in their resources and responses. We feel preliminary analysis results can be drawn from this cross-section of laboratories.

Average Facility Profile:

A. Facility and Equipment
-Established 1998
-Use four color fluorescence as label
-Do not use manual analysis to verify results
-Run about 5000 lanes/year
-Perform services for about 13 principle investigators or lab groups
-Have a turnaround of 72 hours from sample drop-off to analyzed data
-Provide oligo synthesis as well as template preparation
-Budget is not subsidized
-In-house charge/lane $3.13
-In-house charge/marker $1.36
-In-house charge/load only lanes $3.93
-Have 1.9 full-time equivalents with 2.5 years experience
-Run ABD 377 with XL upgrade

B. Template
-Human DNA template
-recommend DNA quality to be determined by 260/280

C. Chemistry
-Use dinucleotide markers
-Do not multiplex at PCR level
-Runs 2-5 markers/lane
-Uses PCR condition modification to prevent stuttering
-Do not clean up PCR reactions
-Run 36cm WTR gels at .2mM thickness
-Use ABD/PE sharkstooth comb for running XL gels 48 samples or above
-Use ABD/PE glass plates
-Normally run a control on every gel
-Using the latest versions of analysis and base calling software

D. Data
-No variance tolerances from gel to gel or within a gel
-Data is distributed as a hard copy
-Hewlett Packard printers are used to print out results
-Data is archived on a Zip Drive

Acknowledgments:

We would like to thank all of the facilities that participated in this survey and for anyone else who would like to submit data so that we can expand the study further the address is: http://128.220.137.80/FARCintro.html

We would also like to thank the DNA Sequencing Research Committee for allowing us to use a previous survey they performed as a template for ours.