

Results From the DNA Sequencing Research Group's General Survey of Core Facilities, 2000

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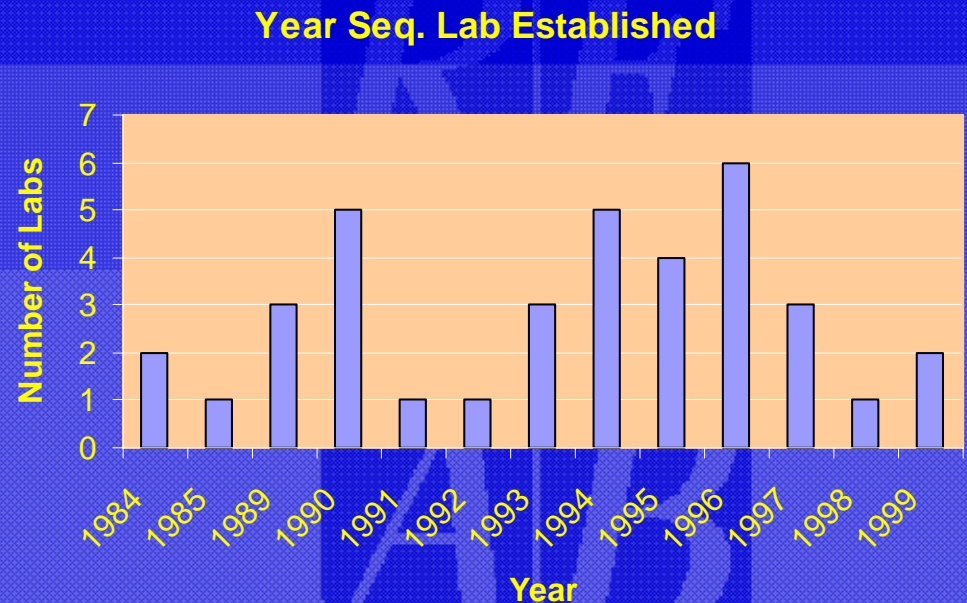
Survey Goals

- The goal of this survey was to come up with a detailed summary of the composition and configuration of the average DNA sequencing core laboratory.
- For this survey, we had 37 labs submit information.



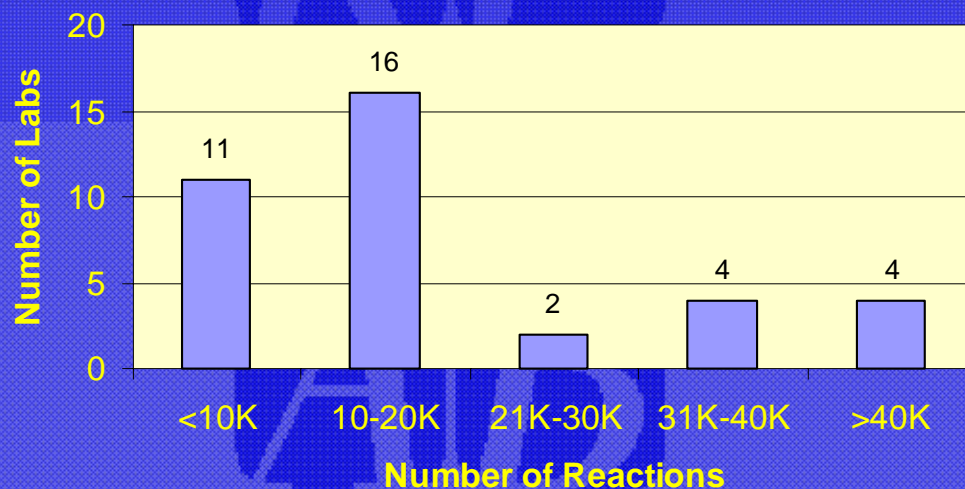
Year the Core Was Established

- Mean = 2.3 years
- Median = 6 years
- Range = 1-15 years



Number of Reactions Run in 1999

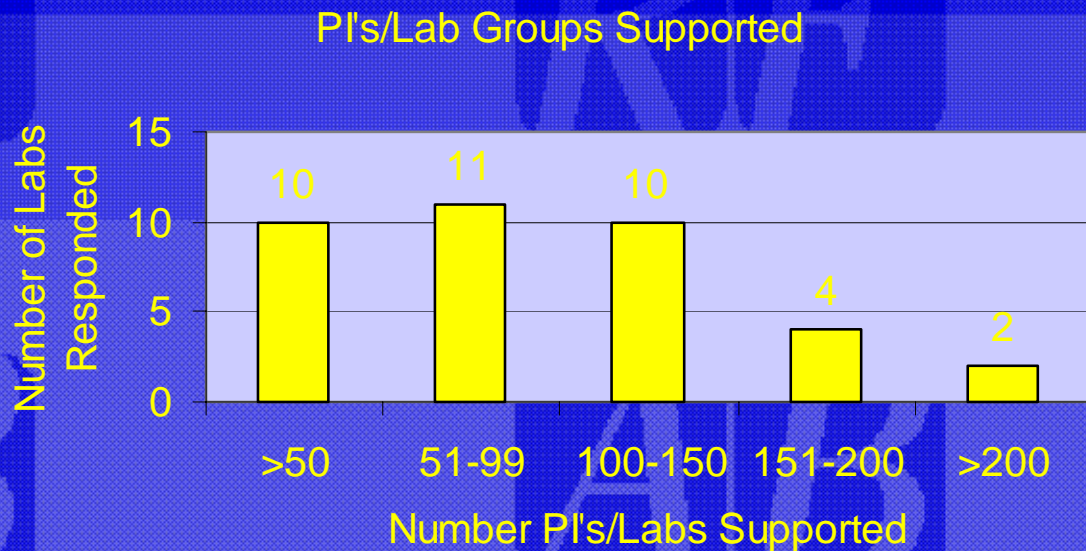
Number of Reactions in Last 12 Months



- Mean = 18,813
- Median = 13,000
- Range = 268 – 80,000

Principal Investigators/Lab Groups Supported

- Mean = 87
- Median = 80
- Range = 3 - 225

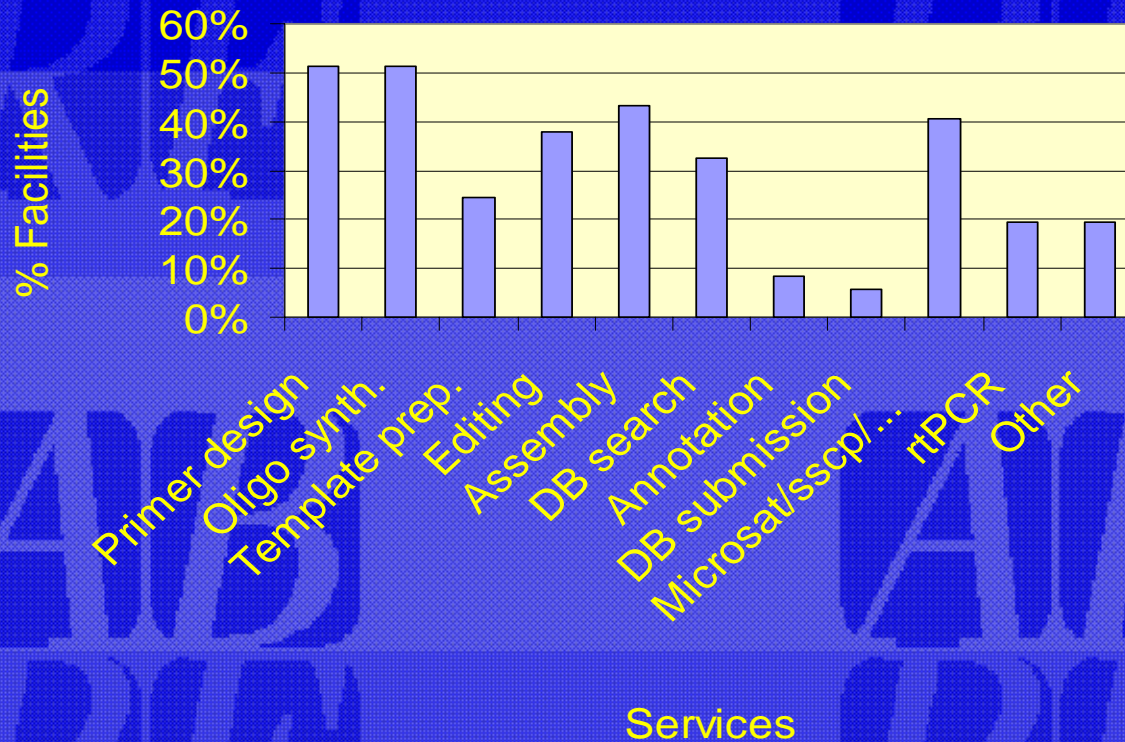


Turnaround Time

- Five of the respondents have a set cut-off time to help define the turnaround time for samples.
- Mean = 53 hours
- Median = 48 hours
- Range = 20 - 96 hours

Services Provided Besides DNA Sequencing

Services Other than DNA Sequencing



Physical Resources

Square feet of lab space:

- Mean = 545
- Median = 368
- Range = 28 - 5,000

Square feet of office space:

- Mean = 127
- Median = 90
- Range = 0 - 1,200

Subsidized vs. Self-Supporting

- Subsidized = 62%
Percent subsidized
Mean = 26%
Median = 27.5%
Range = 4 - 100%
- Self-supporting = 38%

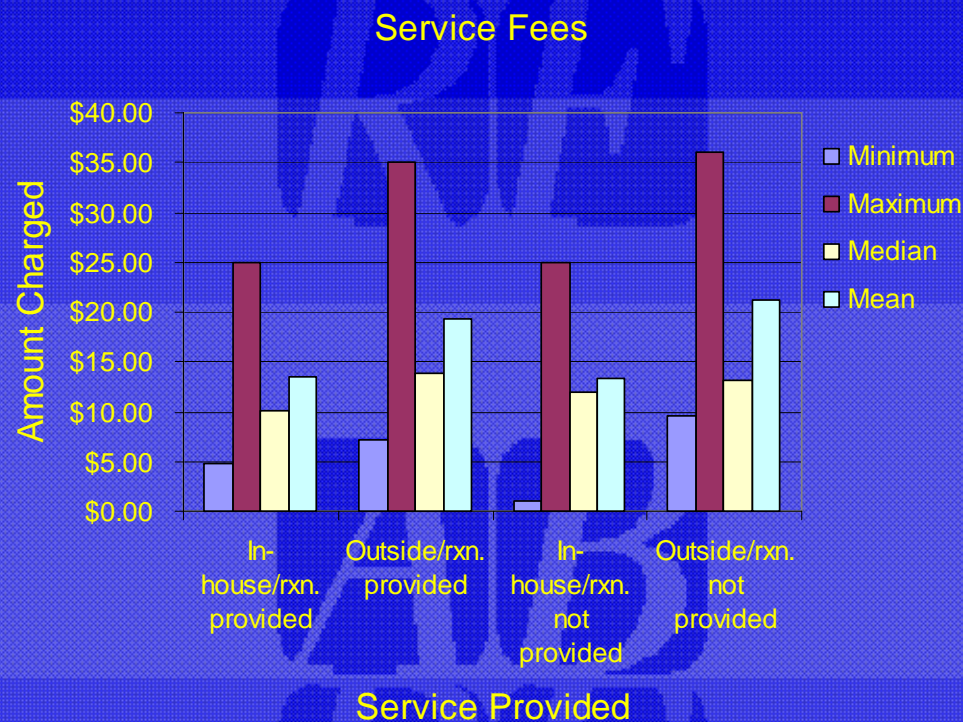
Service Fees

- Most facilities do not charge for editing or expedited service.

- In-house=customers at your institution/company
- Outside=customers not at your institution/company
- reaction provided=customer performed reaction
- reaction not provided=seq. reactions performed by lab

- Other charges:

- Printing
- Primers
- Fragment assembly
- Reactions that don't work



Director of Facility

- Mean = 6 years
- Median = 5 years
- Range = 1 - 12 years

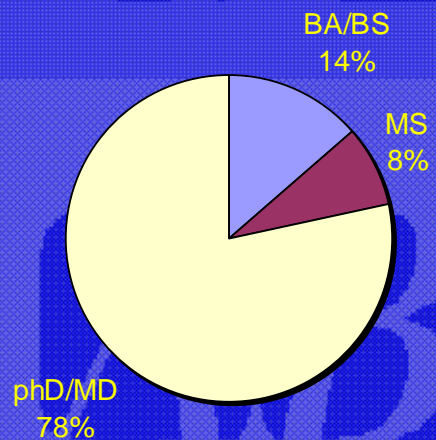
Scotch: 31 responses

- single malt = 52%
- blend = 16%
- Mother Theresa = 10%
- no pref = 23%

Only brand that had more than one vote (2)

- Glenfiddich

Facility Director Education



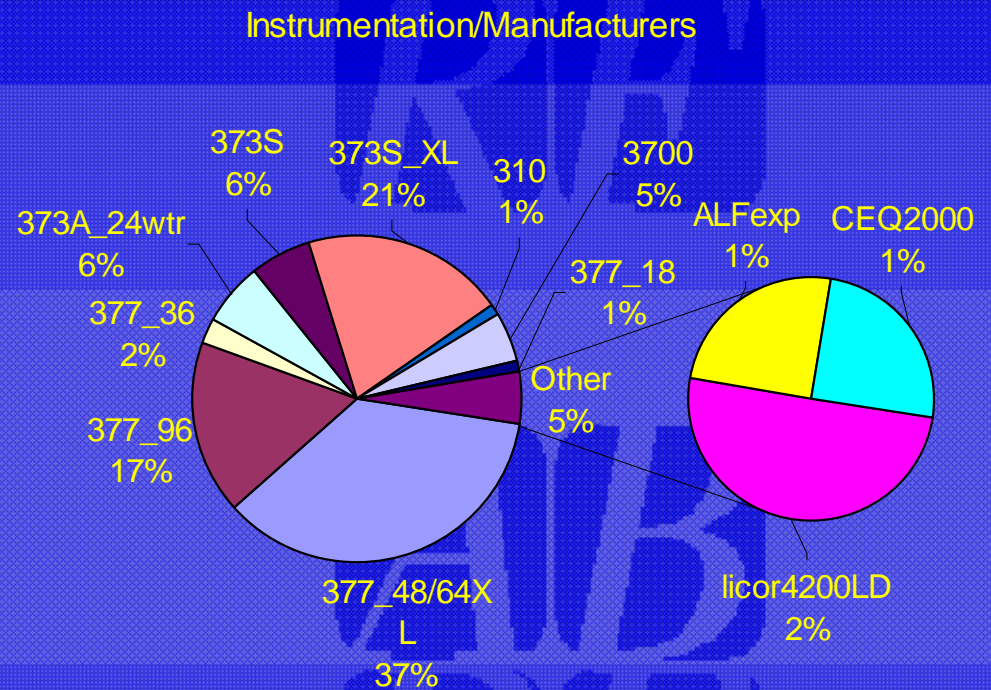
Staff

- FTE's working in the core:
 - Mean = 2
 - Median = 2
 - Range = 0.1 - 8
- Experience of staff:
 - Mean = 2 years
 - Median = 3 years
 - Range = 0 - 10 years

Instrumentation

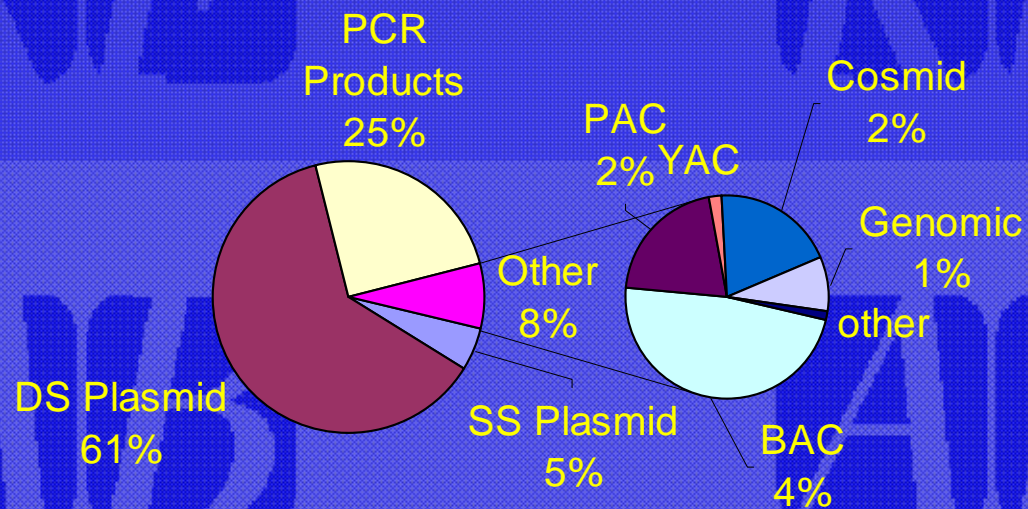
Number of sequencers in the core facility:

- Mean = 3
- Median = 2
- Range = 1 - 15



Templates

Type of Templates

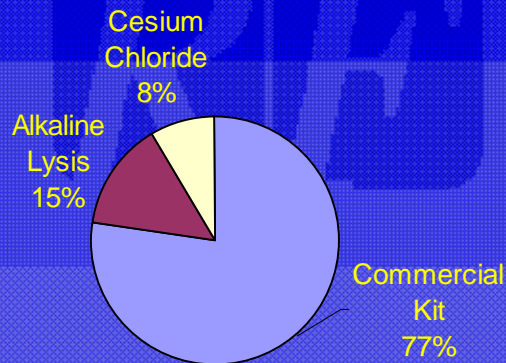


Robotics

- 3 Respondents use robotics
 - 2 Beckman BioMek 2000's
 - 1 Qiagen BioRobot 9500
- Determination of Quality/Quantity
 - Agarose gel
 - OD₂₆₀
 - Both Agarose gel and OD₂₆₀

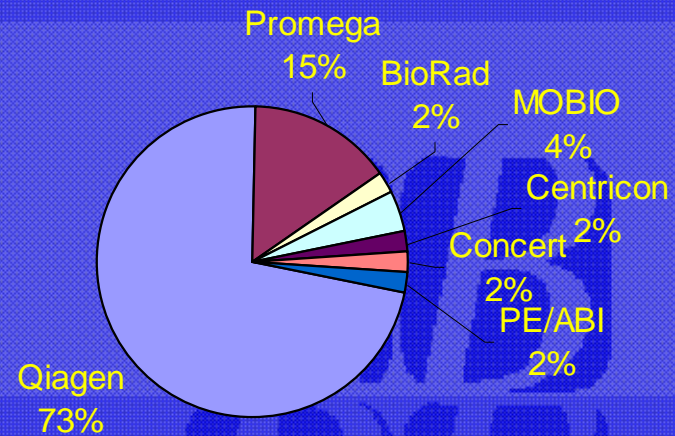
Template Preparation

Recommended Template Preparation



Most labs don't require documentation of template purity

Recommended Commercial Kits



Determination quantity/quality:

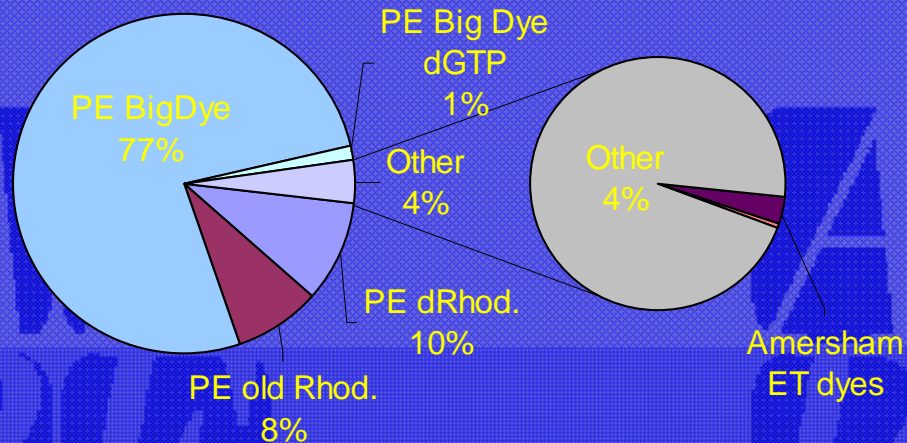
- OD260 = 47%
- Agarose gel = 42%
- Fluorescent assay = 8%
- Other = 3%

Sequencing Chemistry - Dyes

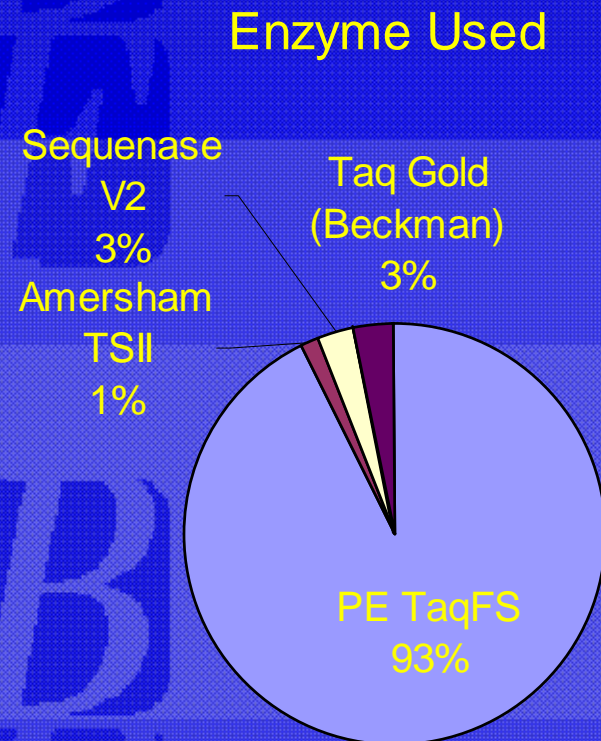
Dye Types

- Dye Terminator = 99.6%
- Dye Primer = 0.4%

Flourescent Dyes Used



Sequencing Chemistry - Enzyme

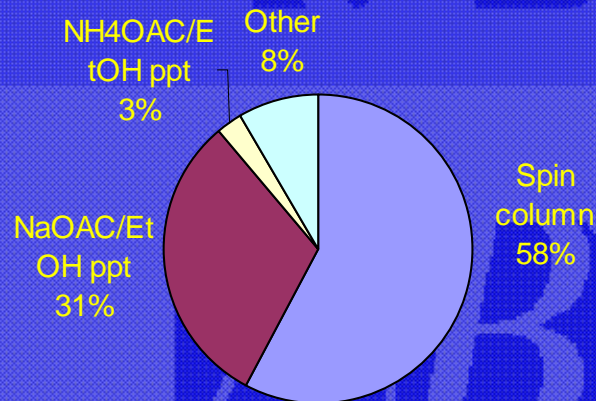


Reaction Cleanup

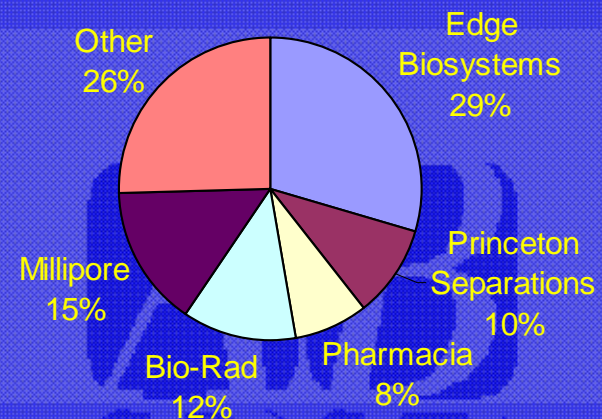
Format of post-PCR cleanup:

- Single tube = 64%
- 96-well plate = 25%
- Both = 11%

Type of Post-Reaction Cleanup

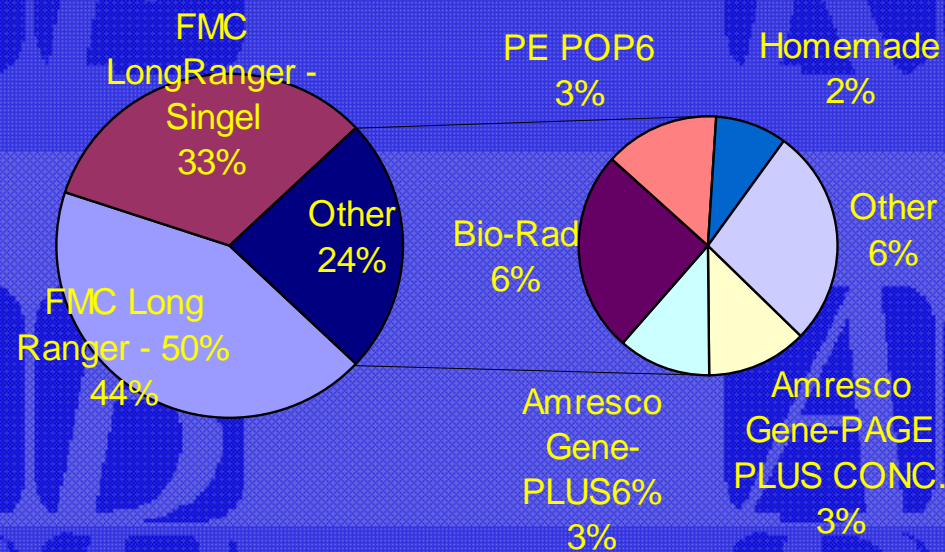


Spin Column Manufacturers

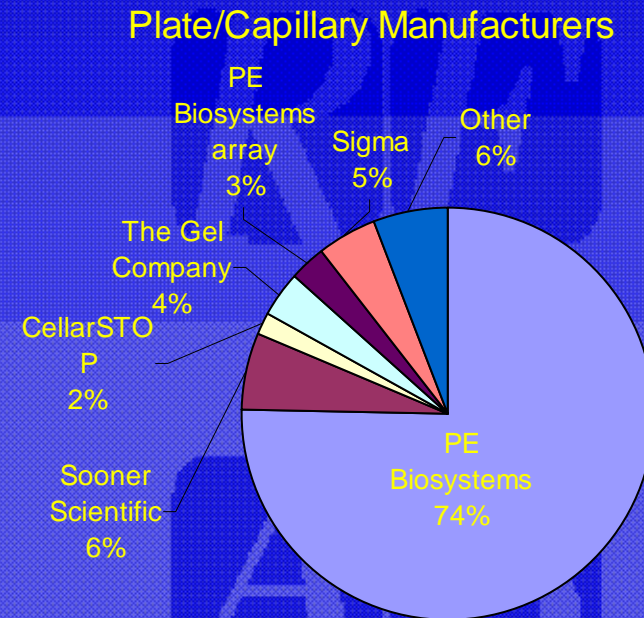
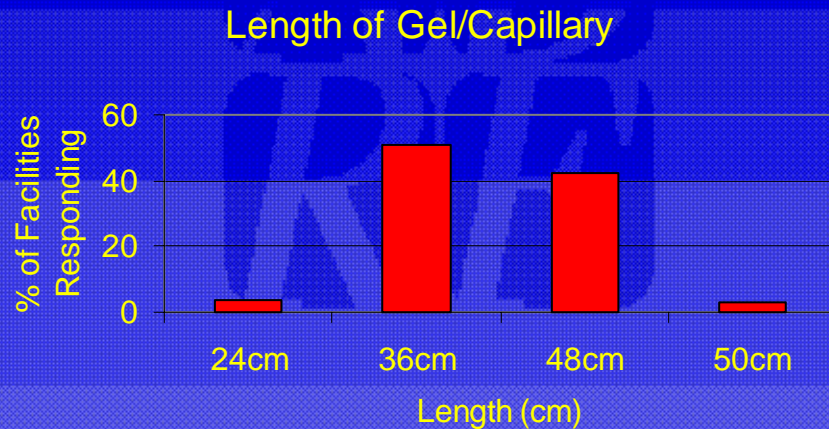


Gel Mixes/Capillary Matrices

Gel/Capillary Matrices Sources



Gel Information



- Routine standards are run:

Yes = 89%

No = 11%

Data Analysis/Basecaller

- By far the most prevalent data analysis software used is the PE Biosystems Version 3.3 (69%).
- The most used basecallers were ABI 100 (59%) and ABI 50 (19%).



Additional Software/Tools Used

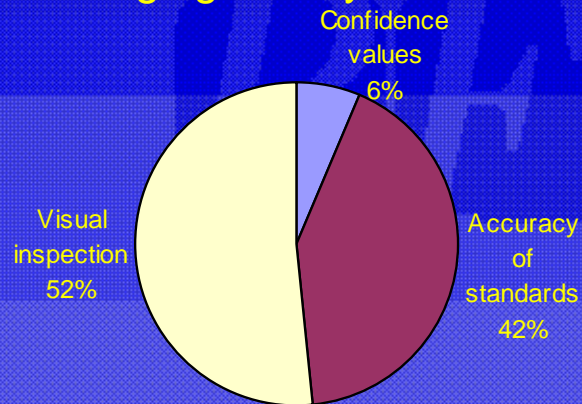
26 Responses (multiple answers per response):

- 19 use Sequencher (GeneCodes)
- 8 use The Wisconsin Package (Genetics Computer Group)
- 3 use Lasergene (DNASTAR)
- 2 use DNAsis (Hitachi Genetic Systems)
- 2 use AutoAssembler (PE Biosystems)



Data Management/Distribution

Judging Quality of Data

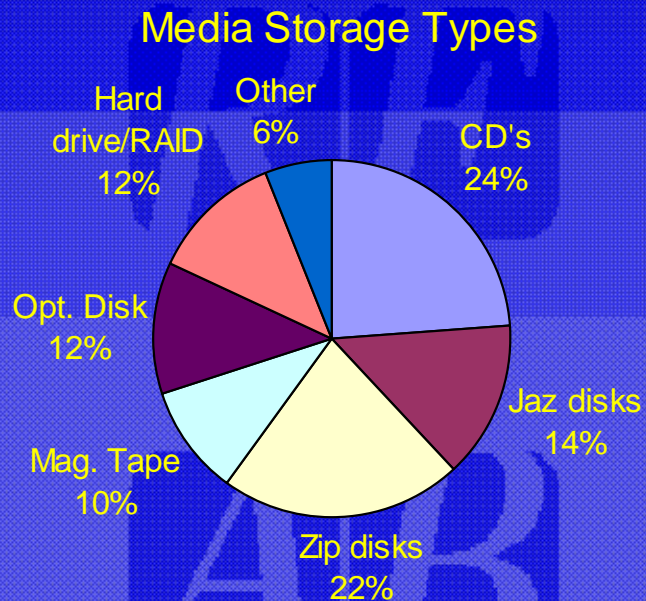


- Most labs check the quality of the data by two of the above methods (73%) and 11% check all three.

Results Distribution

- 21/37 Store results forever
- 16/37 Store results for 0.25-6 months. (Avg. 2 mo.)
 - Hard Copy (32/37)
 - Range 1-100%
 - Median 100%
 - Floppy/Zip disk (20/37)
 - Range 1-100%
 - Median 42.5%
 - Electronic (35/37)
 - Range 5-100%
 - Median 99%

Data Storage/Archiving



- Data Storage
 - 30/37 Store data forever
 - 7/37 Store data for 1-36 months
 - Mean = 11 months
 - Median = 6 months

Information Management Systems

- 28/33 use in-house designed/modified program or programs like Excel and Paradox for tracking/billing.
- 5/33 are moving or have recently moved to a commercial product for tracking/billing
 - All 5 commercial LIMS systems are from different vendors.

Future Directions of Core Facility

- Longer reads, better tracking, less expensive.

	Plans to add new instrumentation
6 months	4 PE Biosystems 3700
	2 PE Biosystems 377's
6-12 months	3 PE Biosystems 377's
	1 3700 or MegaBACE
	1 PE Biosystems 3700
	1 377 or 3700
12-18 months	2 PE Biosystems 3700
	1 PE Biosystems 377
	1 3700 or MegaBACE
	1 waiting for midsize capillary
18+months	1 PE Biosystems 3700
no plans	19

Suggestions for Future Studies

- Manual editing differences from lab to lab
- Stress levels of different labs (customer pressures)
- List of tips for different procedures in sequencing (PCR, P1 seq., repeats in sequence...)
- More 3700 information
- Length of Reads required by customer

Average Core Facility Profile

- The average core facility is 7 years old and runs ~40,000 sequencing reactions per year.
- They support 111 PI's/lab groups with a turnaround time of 38 hours.
- This lab offers at least 2 other services and has on average 2,500 sq. ft. of lab space and 600 sq. ft. of office space.



Average Facility Profile

Continued...

- The lab is subsidized 48% and usually does not charge extra for services such as editing and expedited samples.
- The director of the facility has 6 years of experience and likes single malt Glenfiddich scotch, the rest of the staff has 5 years of experience.

Instrumentation/Reactions

- Average of 7 sequencers (95% from PE Biosystems).
- Sequencing DS-DNA templates (61%), PCR products (21%) and SS-DNA (5%).
- Qiagen Template preparation, with quality checked by both agarose gel and OD₂₆₀.
- Dye Terminator using TaqFS, cleaned up with spin columns (58%, Edge) or EtOH pptn. (31%).



Gel Information

- FMC is the gel matrix of choice (83%), where read length is usually 36cm (51%) or 48cm (43%).
- PE Biosystem plates/arrays are used (77%) and standards are usually run (89%).
- The analysis software is PE Biosystems Version 3.3, where basecaller ABI100 is used.



Data Management/Distribution

- Visual inspection and quality of the reads of the standards are the way quality of a sequencing run is checked.
- Customers get hard copy and electronic copies of their data.
- Data is kept on disks (Jaz, Zip and Optical, 48%) forever (81%).

Other Tools

- Sequencher (GeneCodes) is the most prevalent tool used. (73%)
- An in-house designed or modified program is used to track samples and do billing.
- Another sequencer will be done in 6-12 months from PE Biosystems.
- Future directions are to do more for less.

Survey Differences

1998 vs. 2000

- Average age of lab was up 3 years.
- Number of reactions run, up by ~30,000.
- Number of PI's/lab groups supported doubled.
- Turnaround time was reduced by 10 hours.
- Outside service charges were 1/2 the price.
- Trend towards dye terminators. (88% vs. 99.6%)



1998 vs. 2000 Continued

- BigDyes replaced most Rhodamine use.
- For data analysis software, labs have moved from PE Biosystems Ver. 3.0 to Ver 3.3.
- Number of labs participating in survey was down from 59 to 37 which may be due to a shorter time available for entering the survey.

Acknowledgements

- All the labs who participated in the survey.
- Web site for this poster is :
 - <http://abrf.org/ABRF/ResearchCommittees/DSRG/DSRG2000/>

Note: This is case sensitive!

