Do Biotechnology Patent Attorneys Really need A Ph.D ?

A thesis in partial fulfillment of the requirements for the degree of Masters in Intellectual Property

Franklin Pierce Law Center
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By

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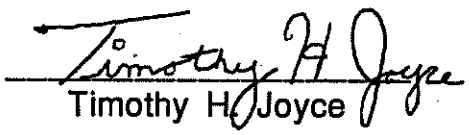

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Abstract

Do Biotechnology Patent Attorneys really need a Ph.D?

A study of the factors which influence biotechnology companies to choose a particular outside patent counsel and the technical expertise desired of such counsel.

The general trend in hiring in both law firms and biotechnology companies has been in favor of patent practitioners who possess both a well rounded technical background and legal expertise. Law firms hire biotechnology practitioners with graduate level technical training perhaps for marketing, client security and cost effective business reasons. Companies perhaps hire such advanced technical practitioners since such individuals provide added value to the company as well as a cost effective means for the company to avoid having to technically train its patent counsel.

A real question has puzzled both law firms and companies in regards to what level of technical expertise is necessary to adequately and cost effectively prosecute biotechnology patent applications. Have law firms gone overboard in marketing their services by hiring as many associates as they can which have both a law degree and a graduate level technical degree? Is such graduate level technical training really worth it or is it just another way for law firms to justify costly billing to clients?

This thesis hopes to answer these questions by focusing on the factors which influence companies decisions in regards to what they really want from a patent practitioner. In addition, the thesis hopes to clarify issues for small biotechnology patent firms and practitioners who wish to enter the field of biotechnology patent law or have not been able to make such an entry into such a dynamic field.

The initial pages of this thesis hope to provide a background and context of the technical training necessary to cost effectively and efficiently prosecute biotechnology patent applications. Initial models are developed and then applied to the biotechnology industry in general. The data has been derived from a small geographical area, but the author believes that the results of the survey could accurately be extrapolated to be representative of the national trends and industry in general. The author believes by focusing on what the companies actually desire for technical expertise will inevitably determine the future hiring and work trends of both law firms and companies alike.

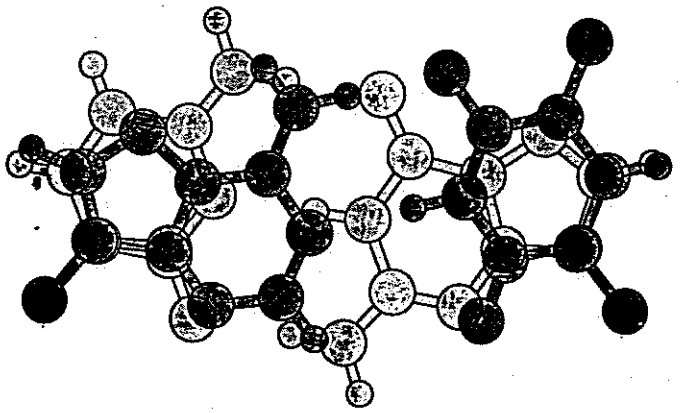
ACKNOWLEDGMENTS

I wish to state my appreciation to my faculty advisors William Murphy III, Thomas G. Field Jr. and Christopher Blank for their guidance, support and patience. I also wish to thank Karl Jorda for his advice and insight regarding technical training at large biotechnology companies.

I especially want to thank Gloria Doubleday of Commonwealth Bioventures Inc. in Worcester, Massachusetts. She gave me guidance and support in a similar market survey and this provided me with the necessary background to execute and administer this market study and paper.

My thanks also are extended to James Sherblom at TSI Corporation, Marc Goldberg of the Massachusetts Biotechnology Research Institute and the executives and corporate counsel who responded to this survey and were kind enough to give up some valuable time to assist in this research effort.

Finally, I wish to express my deep appreciation to my family and friends for their support and understanding during law school.



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Preface and Forward:

At first glance an an answer to the question "Do Biotechnology Patent Attorney's need a Ph.D." may be apparent. However, after some reflection and consideration of supply and demand economics, law firm competition and cost of training to biotechnology companies, an answer to such a question may not be as readily available. Inevitably, a quantitatively significant answer to such a question may not be possible. For instance, the answer to such a question could be different depending upon when it is asked and who it is asked to. However, the knowledge gained in the actual inquiry itself may provide insightful data or information to biotechnology professionals and scientists.

This paper is designed to clarify a series of general principles and trends with regards to biotechnology patent law. The knowledge or derived principles of this paper are quantified in such a way that the reader may generally apply the results of the survey to their own individual law firm, company or narrowly tailored situation. Massachusetts biotechnology companies were chosen since they appear to be representative of the biotechnology industry in general.

One may respond to the question "Do Biotechnology Patent Attorneys need a Ph.D.?" in a variety of ways. Categorically and philosophically speaking, however, one would generally answer the question in one of the four following ways:

1. "Yes", biotechnology patent attorneys need a Ph.D.

2. "No", biotechnology patent attorneys do not need a Ph.D.

3. Combination of 1 and 2 in which Ph.D.'s are not necessary, but may be useful or relevant in certain situations for patent practitioners.

4. Such an inquiry is vague or irrelevant.

The author would suggest that solution three is probably the most accurate and representative of Massachusetts biotechnology companies position. The author will attempt to show that answer

three is perhaps the best answer philosophically and pragmatically for law firms, biotechnology companies and the industry in general.

Purpose and Goals of the Present Survey:

The general purpose of the present survey is to provide an answer to the question "Do Biotechnology Patent Attorneys need a Ph.D.?". The present papers focus is to give an in depth analysis of the issue and determine the relative importance of graduate technical training to law firm and company competitiveness. Perhaps the question should be reworded to clarify the sub-issue of competitiveness and cost effectiveness to read "Do Biotechnology Patent Attorneys need a Ph.D. to cost effectively and competitively prosecute biotechnology patents". The present paper considers the relative temporal limitations of such an inquiry and, therefore, separates the inquiry into past, present and future. For instance, with the rapid change of the biotechnology industry and the dynamic nature of the technology an answer to such a question could be very different ten years ago from how such a question would be answered now. Therefore, this survey attempts to separate the trends of the past from the present. After an initial inquiry is made into past trends a market study was conducted to determine the present state of the industry. The information of past and present was then combined to build relative models which should hold promise for both law firms and companies to apply and plan with in the future.

The background information presented in this thesis will generally:

1. Provide a general background and perspective concerning the relative need for biotechnology patent attorneys.

2. Attempt to quantify the relative significance of a graduate level technical degree to a biotechnology patent attorney.

3. Attempt to determine and correlated supply and demand of scientists with supply and demand of biotechnology patent attorneys.

4. Assess the overall areas of scientific demand and areas of technical demand in regards to biotechnology patent attorneys.

5. Assess the overall supply and demand nationally for Ph.D.'s and their relative significance as patent practitioners.

6. Assess the hiring trends of law firms and hypothesize why their hiring has begun to follow particular patterns.

7. Provide a general background regarding problems in the United States Patent and Trademark Office and the models they have used and developed.

8. Provide information regarding the backlog of patent applications in the United States Patent and Trademark Office and show the importance of technical training to effectively and efficiently prosecute patent applications.

The market study incorporated in this thesis was conducted to determine the present state of the biotechnology industry and attempts to generally:

1. Provide an answer to the question of whether a biotechnology patent attorney could benefit from a Ph.D.

2. Provide an adequate cross-section of large, medium, and small size companies and how they would answer such a question.

3. Determine the importance of a Ph.D. to Massachusetts biotechnology companies.

4. Determine the relative importance of "other factors" and their influence on hiring outside legal counsel to perform legal services.

5. Clarify who makes decisions with regards to hiring outside patent counsel.

6. Clarify technical demands of the biotechnology industry and compare this with industry predictions of demand for various technical areas.

7. Compare biotechnology company perceptions of patent cost to other professional services.

8. Determine how biotechnology companies prefer to handle a particular problem they are confronted with and whether they would seek an individual or firm to aid them.

9. Determine a series of general factors or principles considered when selecting a patent firm.

10. Determine the important factors when biotechnology companies select an individual to handle their work.

11. Provide reasons for considering and not considering new patent counsel to handle a biotechnology companies work.

12. Quantify legal experience necessary for new legal practitioners (i.e. necessary number of patent applications written to even be considered as outside patent counsel).

13. Determine the three biggest problems for biotechnology companies regarding legal services.

The general background information and results of the market survey have then been combined to help aid companies and law firms in future planning and organization of their staffing needs and departments. The data has been extrapolated to generally provide:

1. Information which biotechnology companies and law firms can readily assimilate and utilize.

2. New models which will aid companies and law firms to cut costs in their training expenses.

3. Reasons why the traditional model of inventor equal to attorney may not work in the future.

4. Predict trends in the biotechnology industry and how staffing could be done for biotechnology companies and law firms.

5. Provide reasons why the cost of biotechnology patents will increase and how companies can prevent such increases.

Background and Introduction:

In August of 1988 the United States General Accounting Office was asked to conduct a study and examine the large backlog of biotechnology patent applications in the United States Patent and Trademark Office, an agency of the Department of Commerce. The findings of the survey generally indicated a basis for the demands of advanced technical training in such patent arts. For this reason a brief description of the survey resu
reader is provided with a general ba
and need for biotechnology patent
technical and legal training. The da
background information from which
regarding levels of technical trainin
practitioners should have.

The Office of General Account
level of technical scrutiny required t
biotechnology patent exceeds that re
most other areas of technology. The
biotechnology examiner because of t
complexity, its newness and its rapi

During fiscal year 1988, biotec
15 percent more time (an average of 19.3 hours versus time spent on all patent applications. This time represents the average time an examiner actually spends on a biotechnology application during the average 29-month pendency period. In addition, there has been an annual increase in the number of patent applications filed in the Patent Office (See Figure #1 below for an indication of the years 1983 to 1988).[2]

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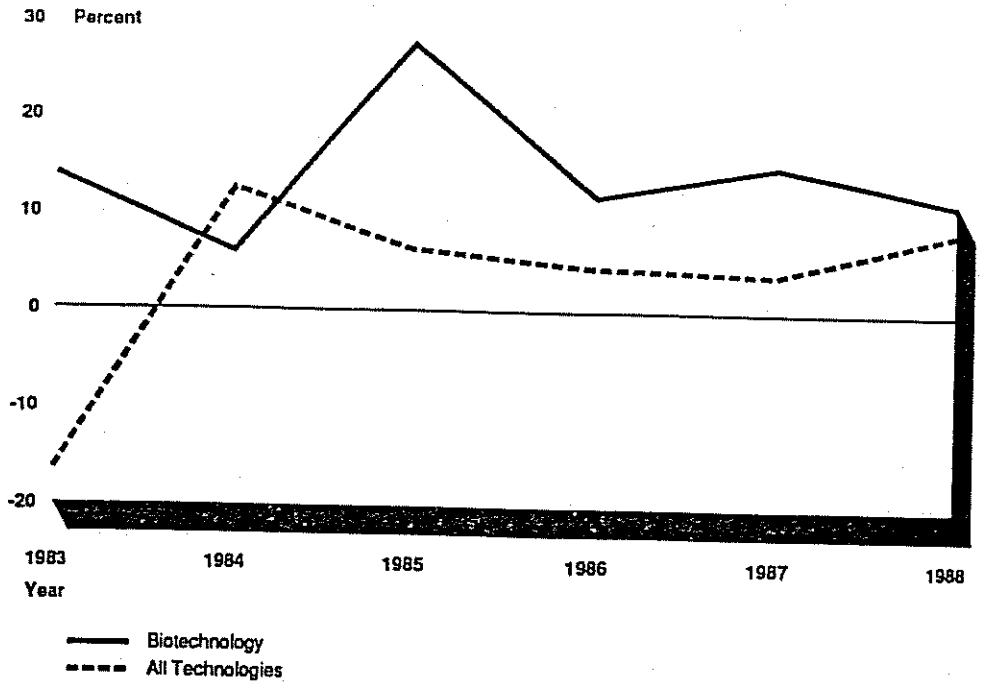
[1] Biotechnology: Backlog of Patent Applications, April 1989, Briefing Report to Congressional Requesters, Page 19.

[2] Biotechnology: Backlog of Patent Applications, April 1989, Briefing Report to Congressional Requesters, Page Page 12.

Figure #1 - Annual Percentage Increase in Number of Patent Applications, Fiscal Years 1983 through 1988



Source: U.S. Department of Commerce, U.S. Patent and Trademark Office, Hiring analysis.

The Patent Office reports that biotechnology patent applications differ little from other applications except for their complexity. According to the Patent Office, a long learning curve is necessary for the examiners of biotechnology applications to become proficient and reach peak productivity. The average new patent examiner needs 4 to 5 years to reach full productivity, while new examiners in the biotechnology area take approximately 20 percent longer--about 6 years.[3] Most biotechnology arts have a high degree of difficulty. As a result, biotechnology patent applications need to be directed to examiners possessing broad and in-depth backgrounds in a variety of areas ranging from genetics to microbiology. The rapid growth of information and the explosive development of biotechnology has also led to greater need to understand a variety of interrelated disciplines, such as genetics, immunology, cell biology, biochemistry, microbiology, and bioengineering. Almost two-thirds

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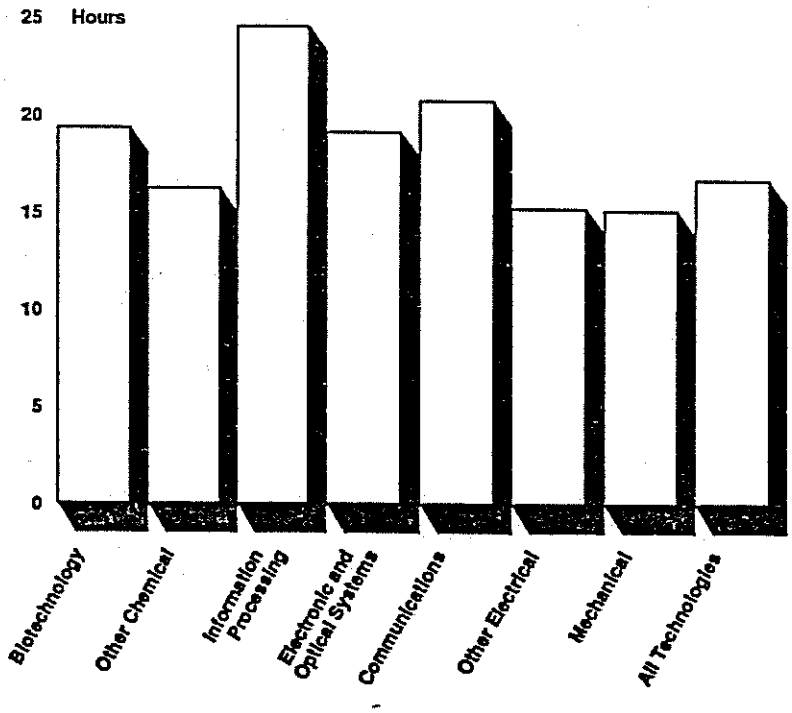
[3] Biotechnology: Backlog of Patent Applications, April 1989, Briefing Report to Congressional Requesters, Page 19.

of the biotechnology examiners hold post-graduate degrees in the field and over one-third hold doctoral degrees.[4]

Average
per pate

Du y examiners spent about
15 perce hours versus 16.7 hours)
on biote he average time spent on
all paten tent examining groups,
only the s) and communications
(20.7 ho echnology area required
more ex y examining group to
process

Figure #2 Application in Fiscal Year 1988



Source: U.S. Department of Commerce, U.S. Patent and Trademark Office, Hiring analysis.

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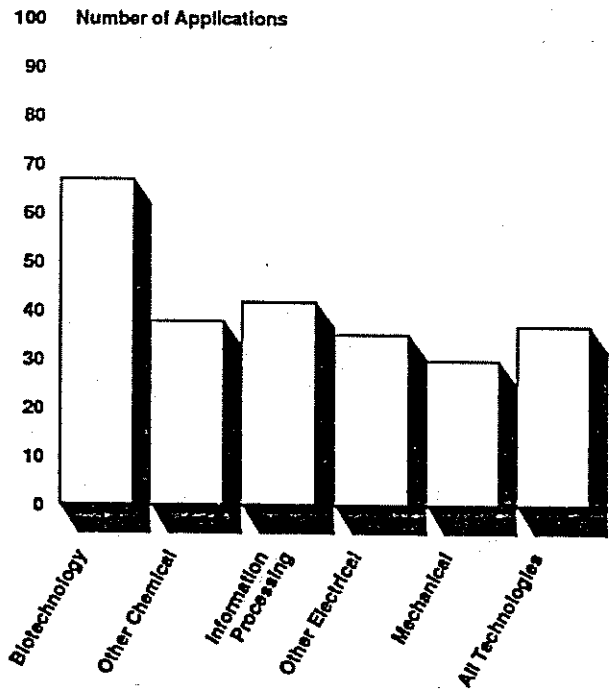
4 Biotechnology: Backlog of Patent Applications, April 1989, Briefing Report to Congressional Requesters, Page 19.

5 Biotechnology: Backlog of Patent Applications, April 1989, Briefing Report to Congressional Requesters, Page 20.

As of December 31, 1988 the biotechnology group had the highest average number of backlogged patent applications per examiner (66.7) of any technology group. In addition, the biotechnology group had the lowest ratio of experienced to total assigned examiners of all the examining groups. Only 35 of 91 biotechnology examiners (38 percent) had more than 4 years experience, whereas 827 of the total 1,382 Patent Office examiners (60 percent) had more than 4 years experience.[6]

Figure #3 - Average Number of Backlogged Patent Applications Per Assigned Examiner as of December 31, 1988



Source: U.S. Department of Commerce, U.S. Patent and Trademark Office, Hiring analysis.

Patent pendency periods for biotechnology and other technology applications:

The survey of the United States Patent and Trademark Office determined that the waiting period for biotechnology patent

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6 Biotechnology: Backlog of Patent Applications, April 1989, Briefing Report to Congressional Requesters, Page 19.

applications is longer than that for applications in any other technology. During the 9-month period of April through December 1988, the average time from the date of application to the date of issue was 29.4 months. This average remained constant throughout the period. The average waiting period for all patents issued by the Patent Office was 21 months. Only the information processing patent waiting period, with an average pendency of 28.1 months, was near the biotechnology average. A large backlog of applications not yet acted upon that were over 12 months old contributed to the long average waiting period for biotechnology patents issued in 1988. This condition caused long delays in making first actions. The biotechnology group had the longest waiting period for first actions of any examining group--14.5 months.[7]

Extent of delays in obtaining patents by type of biotechnology development:

The survey also concluded that longer-than-average delays were typical across the spectrum of biotechnology developments, but applications in certain areas, such as biotechnology equipment, proceeded more quickly than others. Size and age of the backlog contributed to lower pendency average for some biotechnology products. The average time from the date of application to the date of issue during the 9-month period of April through December 1988 for each of the biotechnology art units ranged from 25.5 to 39.2 months. The biotechnology art unit with the lowest pendency average was still 4.5 months above the overall Patent Office average.[8]

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[7] Biotechnology: Backlog of Patent Applications, April 1989, Briefing Report to Congressional Requesters, Page 25.

[8] Biotechnology: Backlog of Patent Applications, April 1989, Briefing Report to Congressional Requesters, Page 30.

Table #1 - Average Waiting Period From Application to Issue for Biotechnology Patents, April Through December 1988

Art unit/ description	Total patent issues	Average months
181/equipment	401	26.0
182/immunology	593	34.2
183/biochemicals	384	26.6
184/plants and animals	185	25.5
185/genetic engineering	36	39.2
186/biochemicals	0	[a]
Biotechnology total	1,599	29.4

Source: U.S. Department of Commerce, U.S. Patent and Trademark Office, Hiring analysis.

Within the biotechnology examining group, the two art units covering genetic engineering and immunology had the highest average waiting periods from application to patent issue during the period of April through December 1988. During this same period, the average pendency period for all biotechnology patents was 29.4 months.

Additionally, art unit 185 (genetic engineering) had the longest average period from application to first action for those biotechnology actions initiated during the period of April through December 1988.[9]

Table #2 - Average Waiting Period From Application to First Office Action for Biotechnology Patents, April Through December 1988

Art unit/ description	Total first actions	Average months
181/equipment	730	11.5
182/immunology	810	15.2
183/biochemicals	1,321	12.1
184/plants and animals	1,052	14.8
185/genetic engineering	920	19.9
186/biochemicals	223	13.0
Biotechnology total	5,056[a]	14.5

Source: U.S. Department of Commerce, U.S. Patent and Trademark Office, Hiring analysis.

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9 Biotechnology: Backlog of Patent Applications, April 1989, Briefing Report to Congressional Requesters, Page 30.

With these general trends and backlogs, it is no wonder why the United States Patent and Trademark Office, law firms and biotechnology companies have begun emphasizing the importance of advanced technical proficiency in this field.

Law Firm Trends:

A general literature search was conducted to determine the relative trends in hiring of patent attorneys who had a legal degree and some additional graduate level technical degree. Table #3 is a listing of Massachusetts intellectual property law firms and the general staffing trends of these firms for 1991 and 1992. The table generally summarizes the law firms which have staff members who have a legal degree In conjunction with an additional graduate level technical degree (including M.S., Ph.D. or M.B.A.). Firm listings were taken from the Martindale Hubble Law Directory. Firms which had no members with dual degrees were not included on Table #3. Sole practitioners were not included in the overall listing. Firms which listed members "of counsel" who had additional degrees were counted in the overall tabulations.

The general results of the brief literature search determined that Massachusetts intellectual property law firms favored advanced degrees from their associates and partners. Table #3 shows an overall increase in both M.S., M.B.A. and Ph.D. degrees from 1991 to 1992. It is believed that a large percentage of the new associates and partners maintaining dual degrees, practice in the biotechnology areas. It is believed that this general trend will increase in 1993, with an emphasis in hiring of new associates who have a legal degree and Ph.D. in a biological discipline.

Based on these results it appears that intellectual property firms have been hiring based on a trend which favors advanced degrees. This may be true due to some of the following reasons:

1) The biotechnology industry is developing and is at a stage which demands increased technical proficiency.

Table #3- Intellectual Property Law Firms Hiring Patterns

Taken from Martindale Hubbell Law Directory and includes law firms with associates, counsel, and partners having a law degree with an additional Ph.D, M.S., M.B.A or graduate technical degree. The table does not include sole practitioners or intellectual property firms without such individuals.

Intellectual Property Law Firm	1991	1992
Choate, Hall and Stewart	3 Ph.D's 1 M.S.	3 Ph.D's 4 M.S 1 M.B.A.
Dike, Bronstein, Roberts and Cushman	2 M.S.	4 M.S.
Fish & Richardson	11 M.S. 4 Ph.D's	11 M.S. 8 Ph.D's 1 M.B.A.
Foley, Hoag &Eliot	3 Ph.D's 2 M.S. 1 M.B.A.	4 Ph.D.'s 9 M.S. 3 M.B.A.
Lahive & Cockfield	6 M.S. 1 M.B.A.	7 M.S. 1 M.B.A.
Lappin, Rosen & Goldberg	1 M.B.A.	1 M.B.A.
Nutter, McClennan & Fish	3 M.S. 2 M.B.A 1 Ph.D.	Parts of firm separated into Cesari & Mckenna
Samuels, Gauthler, & Stevens	1 Ph.D	1 Ph.D.
Perkins, Smith & Cohen	0 0	2 M.S. 1 Ph.D.
Allegretti & Witcoff	0 0	1 M.S. 0
Schneider, Reilly, Zabin & Costello	1 M.S.	1 M.S.
Weingarten, Schurgin, Gagnebin & Hayes	3 M.S. 1 M.B.A.	3 M.S. 1 M.B.A.

Wolf, Greenfield & Sacks P.C.	3 M.S. 1 Ph.D.	3 M.S. 4 Ph.D's
International Law Collaborative	1 M.S.	1 M.S.
Hamilton, Brook, Smith & Reynolds	1 M.S 1 M.B.A 1 Sc.D.	1 M.S 1 M.B.A. 1 Sc.D.
Blodgett &Blodgett P.C.	2 M.S.	2 M.S.
Bromberg & Sunstein	1 Ph.D	2 Ph.D's 1 M.B.A.
Testa, Hurwitz & Thibeault	3 M.B.A.'s	3 M.B.A.'s 1 M.S. 1 Ph.D.

2) The time, complexity and backlog of patent applications in the Patent Office demands additional technical training so that patent applications in this art may be processed more quickly.

3) Law firms wish to provide added value to their clients and at the same time cut costs of training new associates.

4) The hiring of Ph.D.'s is a way in which intellectual property firms can distinguish or market their services over their competitors.

5) Added technical training may provide law firms with additional client security.

6) Inventors in the biotechnology field tend to generally be Ph.D's and they prefer to work with attorneys who have similar training and competence.

7) Clients are not capable of distinguishing good legal work from poor legal work and rely on the added training as a means to reduce risk of mistake.

Defining the Present Model used by Law Firms and biotechnology companies in which Inventor is said to Equal the Attorney

It appears that many law firms and biotechnology companies have begun a hiring and staffing trend which favors the hiring of lawyers who have a Ph.D. in a technical area. Many of the inventors in the biotechnology industry are Ph.D.'s and they prefer to work with individuals of similar competence. In other words, the attorney which they choose to work with would generally have a Ph.D. also in a technical discipline. The inventor is said to equal the attorney since they have similar technical backgrounds. Many law firms and biotechnology companies have sent Ph.D.'s to law school in hopes of developing strong biotechnology patent departments. However, the real question to be answered concerns whether this is really what biotechnology companies want from their law firm. Inevitably, since biotechnology companies provide the lively-hood of most intellectual property firms it seems appropriate that the answer to the question "Do Biotechnology Patent Attorneys need a Ph.D." come

from the biotechnology companies that farm work out to intellectual property firms. In reality if the biotechnology companies do not want such advanced technical training, law firms need not expend additional and unwanted expenses for such advanced technical practitioners.

Analysis of the Present State of the Industry

The question which remains to be answered concerns whether or not biotechnology companies really want Ph.D.'s or graduate level specialists to handle their work. For this reason the present market study was conducted to determine the level of technical and legal expertise that biotechnology companies really wanted from their patent attorney.

A. Survey Methodology and Results:

The survey questionnaire was designed to be completed and returned by a single individual within a company (See Figure #4 for a graphical depiction of the survey methodology used). Questionnaires were addressed to Chief Executive Officers, Vice Presidents of Research and Development, or Patent Counsel (i.e people who would be making decisions regarding the hiring of outside patent counsel). Company names and addresses were obtained through the Massachusetts Biotechnology Council Directory and the 1992 GEN Guide to Biotechnology Companies. The survey pool included 65 Massachusetts based biotechnology companies. Efforts were made to personalize the questionnaires as much as possible (See Appendix 4 for a copy of the cover letter used in the survey). Of the original 65 biotechnology companies which were included in the survey (See Table #4) 28 companies completed or partially completed the questionnaire and mailed it back in the self addressed stamped envelope. (See Table #5 and Table #6 for a listing of companies responding to the survey and not responding to the survey). This represents an overall survey response rate of approximately 43% of the originally defined universe of possible responding companies.

Table #4

Companies Included in Survey

1. A/G Technology
2. Advanced Magnetics
3. Alkermes
4. Alpha Beta Technology
5. Amicon Division, W.R. Grace and Co.
6. Applied Biotechnology
7. BASF Bioresearch Corporation
8. Betagen
9. BioRational Technologies Inc.
10. Genica Corporation
11. Biogen
12. Biomeasure Inc.
13. Biopure Corporation
14. Biosurface Technology Inc.
15. Biotransplant Inc.
16. BMA Labs
17. Boston Biotech Company
18. Cambridge Biotech Corporation
19. Cambridge Neuroscience Inc.
20. Cellcor Therapies Inc.
21. Charles River Laboratories
22. Commonwealth Bioventures Inc.
23. Consolidate Machine Corporation
24. Costar Corporation
25. Creative Biomolecules
26. Diacrin Inc.
27. Dupont
28. Easte Acre Biologicals
29. Endogen Inc.
30. Enzytech Inc.
31. Gene Trak Systems
32. Genetics Institute Inc.
33. Genzyme Corporation
34. Hemagen Diagnostics Inc.
35. Immulogic Pharmaceutical Corporation
36. ImmunoGen Inc.
37. Ingold Electrodes Inc.
38. Matritech Inc.
39. Mattek Corporation
40. Micron Separations Inc.
41. Millipore Corporation
42. New England Biolabs Inc.
43. OmniGene Inc.
44. Organogenesis
45. Polyfiltronics Inc.
46. Procept Inc.
47. Remediation Technologies Inc.
48 Repligen Corporation
49. Sepracor Inc.
50. Seragen Inc.
51. T Cell Sciences
52. Transkaryotic Theraples Inc
53. Tropix Inc.
54. Vertex Pharmaceuticals
55. Whitehead Institute
56. Worcester Foundation
57. Worcester Polytechnic Institute
58. Tonometrics
59. TSI Corporation
60. Stryker Biotech
61. Remediation Technology Inc.
62. Lab Pro Systems Inc.
63. RBI Inc.
64. IEC Inc.
65. E-Z-EM

Table #5

Companies Responding to Survey

Company	Position of responder	Name
1. Cellcor Inc.	Vice President of Finance	Harry Wilcox
2. Commonwealth Bioventures	Vice President of Operations	Gloria Doubleday
3. Lab Pro Systems Inc.	President	Bruce Fowler
4. Matritech	President	Stephen Chubb
5. RBI Inc.	Chairman	J.L. Neumeyer
6. OmniGene Inc.	Vice President of R&D	Keith Backman
7. Worcester Polytech.	Head of Biomedical Eng.	Robert Peura
8. BASF Bioresearch Corp.	Vice President of Finance	Joseph Michaels
9. Dupont	Manager	Gail Burnett
10. W.R. Grace and Co.	Vice President	Barry A. Solomon
11. Matritech Inc.	Vice President of R&D	Graham Lidgard
12. T Cell Sciences	Senior Corporate Attorney	Pamela A. Hay
13. Consolidated Machine Corp.	Vice President	G.H. Lowe
14. Immunotech Corp.	Scientist	Eric c. Sigillo
15. WhiteHead Institute	Associate Director	John Pratt
16. Cambridge Neuroscience	Patent Counsel	Greg B. Butler
17. Seragen	Manager of Intellectual Property	J. Guthrie
18. TSI Corporation	Senior Vice President of R&D	Paul Leibowitz
19. Biogen Inc.	Patent Counsel	Leon Yankowich
20. Remediation Tech. Inc.	Associate Principal	Alfred Leushner
21. Alpha-Beta Technology	Chief Finnancial Officer	David Easson
22. genzyme	General Counsel	Mark A. Hofer
23. Stryker Biotech	Director of Operations	Joseph Tyler
24. Immulogic Pharm. Corp.	Patent Counsel	Stacy Channing
25. BioRational Technology	President	Pamela Weathers
26. Polyfiltronics Inc.	Vice President of R&D	Roy Manns
27. Worcester Foundation	Director	Thoru Pederson
28. Sepracor Inc.	Executive Vice President	Robert Bratzler

Table #6

Companies not Responding to Survey

1. A/G Technology
2. Advanced Magnetics
3. Alkermes
4. Transkaryotic Therapies Inc
5. Tropix Inc.
6. Applied Biotechnology
7. Betagen
8. Genica Corporation
9. Tonometrics
10. Biomeasure Inc.
11. Biopure Corporation
12. Biosurface Technology Inc.
13. Biotransplant Inc.
14. BMA Labs
15. IEC Inc.
16. Boston Biotech Company
17. E-Z-EM
18. Cambridge Biotech Corporation
19. Charles River Laboratories
20. Costar Corporation
21. Creative Biomolecules
22. Diacrin Inc.
23. Easte Acre Biologicals
24. Endogen Inc.
25. Enzytech Inc.
26. Gene Trak Systems
27. Genetics Institute Inc.
28. Hemagen Diagnostics Inc.
29. Mattek Corporation
30. Micron Separations Inc.
31. Millipore Corporation
32. New England Biolabs Inc.
33. Organogenesis
34. Procept Inc.
35. Repligen Corporation
36. Dupont
37. Vertex Pharmaceuticals

B. Background of the Survey:

The first part of the survey was designed to clarify the survey objective which was to understand needs for biotechnology legal services and gather general background data concerning the responder and company (e.g. name, position, address, and telephone number). In addition, the responder was asked to list his or her educational degrees. This information was elicited to test whether there was any correlation between level of technical expertise of the biotechnology company decision-maker and level of technical expertise this person preferred to hire or utilize (i.e. Ph.D level decision-maker having a bias or tendency to hire technically similar level outside counsel patent practitioners).

C. Survey data Receipt:

Survey participants were mailed surveys on September 8, 1992 and asked to complete the questionnaire and mail it back in the self addressed stamped envelope by October 1, 1992 (approximately one month to answer the questionnaire).

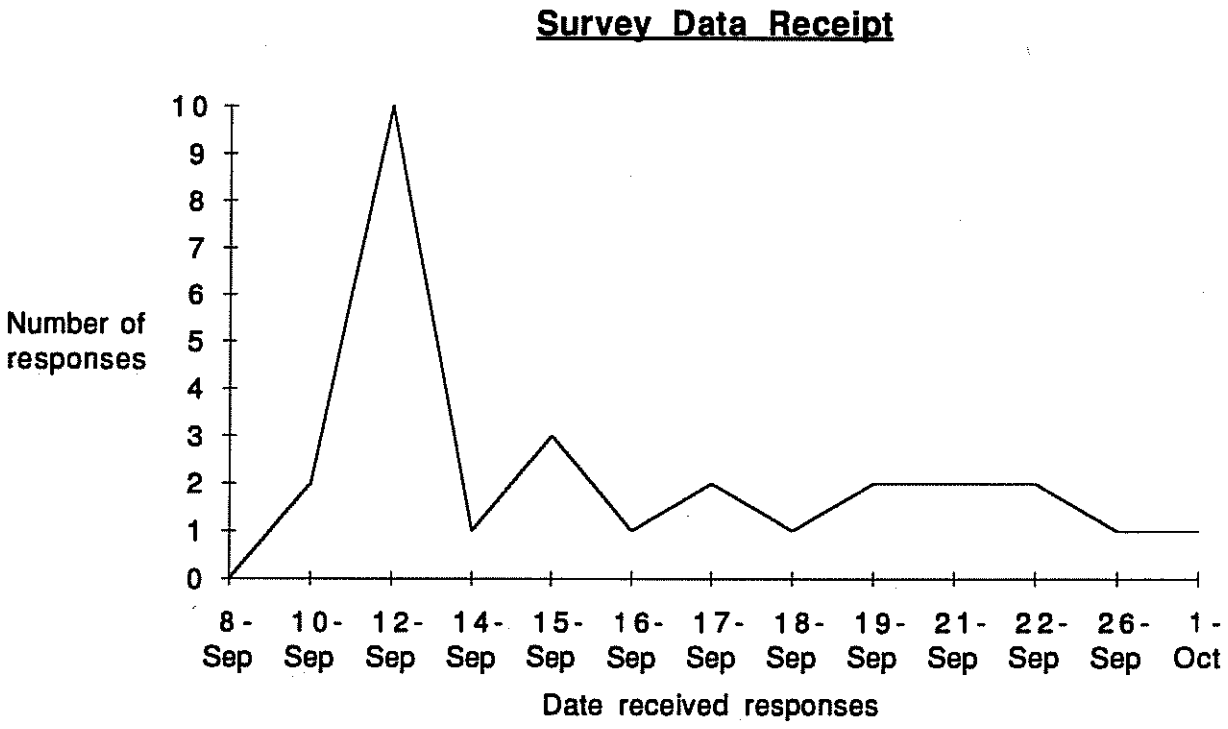
The highest return rate for the entire survey was approximately four days after the initial mailing date (See Figure #5). The remaining time for response remained generally at a constant of about two returned questionnaires per day for the remainder of the survey. The initial high rate of return may have been due to the survey execution and methodology used combined with the intense interest of practitioners in the industry to determine the necessary technical training of biotechnology patent practitioners.

D. Survey Methodology and Results:

Question 1A: Are you the individual responsible for making decisions or participate in decision making with regard to the selection of outside legal services?

Part A of question 1 was designed to determine whether the survey was addressed to the appropriate individual who would be making decisions with regard to the selection of outside legal services which would be used by the biotechnology company. The

FIGURE #5


Survey Data Receipt
Number of responses
10
9
8
7
6
5
4
3
2
1
0
8-Sep
10-Sep
12-Sep
14-Sep
15-Sep
16-Sep
17-Sep
18-Sep
19-Sep
21-Sep
22-Sep
26-Sep
1-Oct
Date received responses

question was worded expansive enough to include all persons who made decisions with regard to selection of outside legal counsel services or participated in someway with such a selection. Legal services were defined broadly and not just limited to patent legal services.

Question 1B: Do you use outside patent counsel services?

Part B of question 1 was designed to clarify what type of decision making power the responder had. The question was designed to specifically limit the universe defined in question 1A to decision-makers who made decisions with regards to outside patent counsel services. Part B of the questionnaire also provided a space for biotechnology companies to list what outside legal services they used and who provided such services. This was left as an option to the responder if he or she desired to provide such information.

The results of the survey indicated that all the responding biotechnology companies utilized outside patent counsel for some type of service. Companies generally had a variety of different reasons and approaches to use of outside legal counsel services including: litigation, patent prosecution, patentability opinions and interferences. It appeared that no generalization could be drawn from the activities and that each company used somewhat different and personalized strategies.

Question 2 (preface) : If you answered yes to the above, please answer the following; otherwise please forward this questionnaire to the appropriate person.

The preface of question 2 was designed so that if both the appropriate person did not receive the survey and the company did not utilize outside counsel legal services, then the responder was asked to terminate the survey and forward it to the appropriate person in the company who could successfully complete the survey. This methodology appeared to be effective in increasing the overall response rate of the survey.

Question 2A: Do you find that the cost for obtaining and maintaining patent protection is costly compared to other professional services?

Question 2A was designed to determine the patent decision makers perception of patent costs to other professional services which the company might utilize. The language "other professional services" was purposely left undefined so that each individual could base their opinion on a more personalized perception of what a professional service entailed for their company.

Approximately 20 of the 28 companies responding to the survey indicated that the cost of obtaining and maintaining patent protection was costly compared to other professional services. A very small percentage of companies indicated that the cost of obtaining patent services was not costly compared to other professional services (See Figure #6 for a graphical depiction of the perception of patent cost by the biotechnology companies to other professional services).

2B. When you have a particular problem which can not be handled by in-house counsel and you must utilize outside counsel, do you generally seek a particular firm or individual to handle your problem?

Question 2B was designed to determine whether biotechnology companies would prefer a law firm in general or a particular attorney at a law firm to handle a particular problem of the biotechnology company. Companies responding to the survey generally indicated that they preferred having an individual handle their problem (almost two to one favoring an individual to handle their particular problem over a law firm) (See Figure #7 for a graphical depiction).

PERCEPTION OF PATENT COST
TO OTHER PROFESSIONAL SERVICES

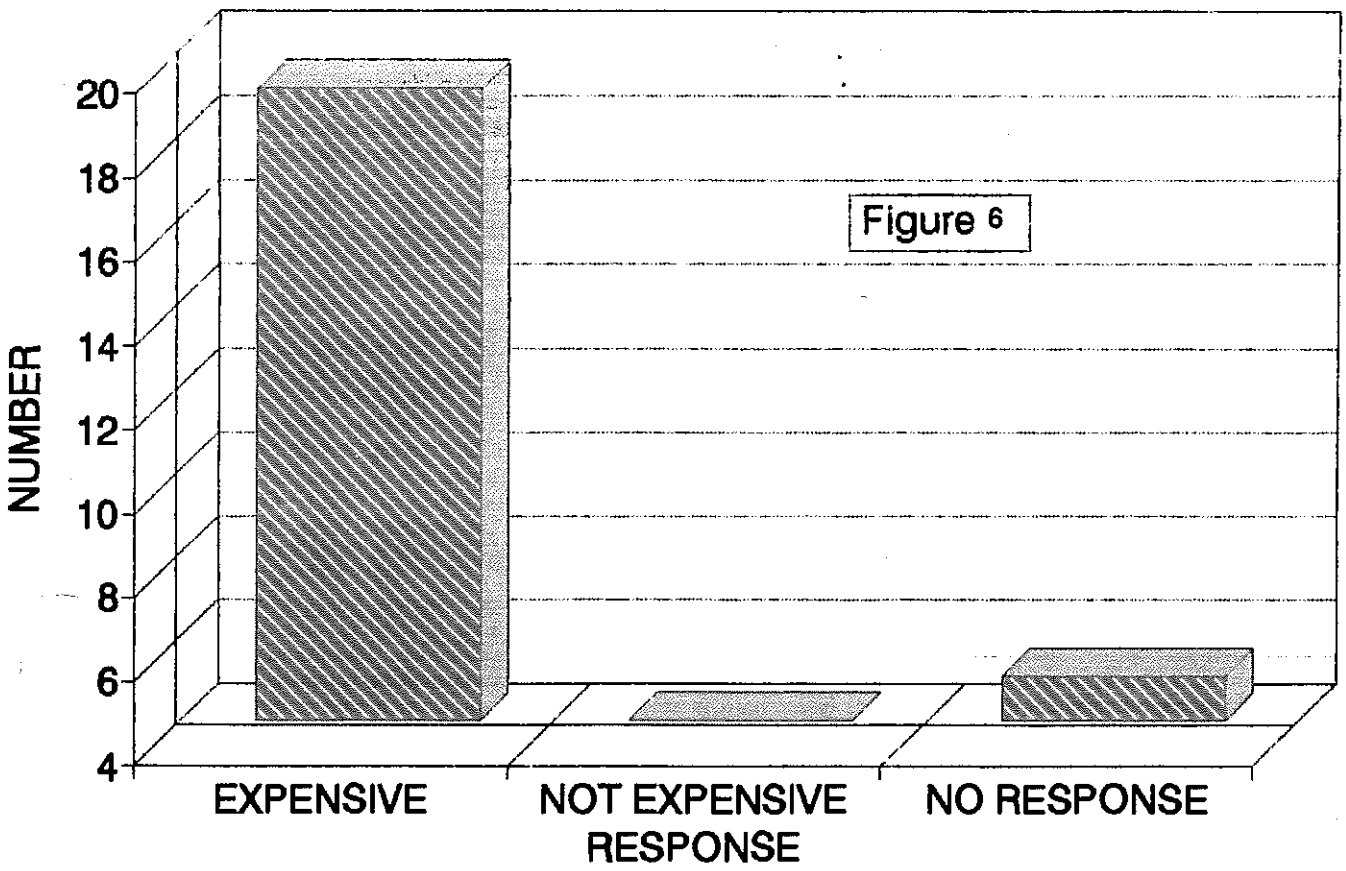


Figure 6

CHOICE BETWEEN FIRM OR INDIVIDUA
TO HANDLE A PARTICULAR PROBLEM

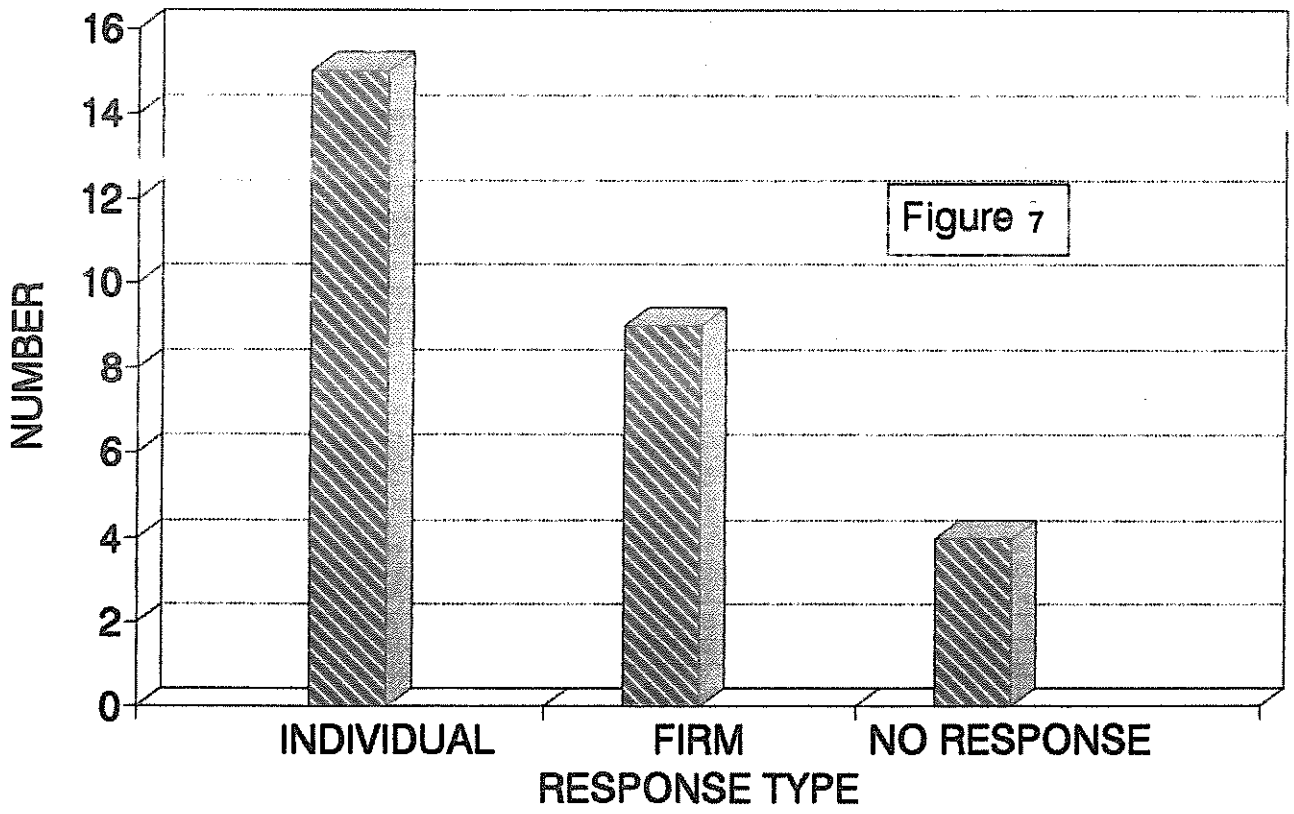


Figure 7

2C. Please list the two most important factors you consider when selecting an outside firm or individual to handle your patent prosecution work.

Question 2C was designed to elicit information regarding how firms and individuals are selected to perform patent work and factors influencing how these decisions are made.

The results of the survey generally fell into two categories. These categories included factors for selecting a patent firm and factors for selecting an individual to perform patent work. The results and influential factors for each category were somewhat different.

The factors of highest importance in selecting a patent firm included legal experience, area of technical training of the firm and overall reputation of the firm. Other factors having some relevance and ~~some~~ probative value included resources, cost, track record, turn around time, and whether the firm was previously used by the biotechnology company. (See Figure #8 for a more precise graphical quantification).

The ~~most~~ important factors for selecting an individual to perform patent work were somewhat different from the factors for selection of a patent firm. Experience in the field, technical proficiency and then legal proficiency ranked highest with regard to individual selection. Other less probative factors included intelligence of the practitioner, interest in the responders company, claim design, litigation record, cost, and dependability (See Figure #9).

2D. Please list two reasons why you would consider or would not consider utilizing patent counsel which you have never used before.

Question 2D was designed and worded similar to 2C, but included the additional limitation that the choice of patent counsel or individual who would perform the work was new or never used by the biotechnology company before. The question was particularly designed to further limit the factors involved in decision making with regard to selection of new patent counsel. The questionnaire

MOST IMPORTANT FACTORS
IN SELECTING A PATENT FIRM

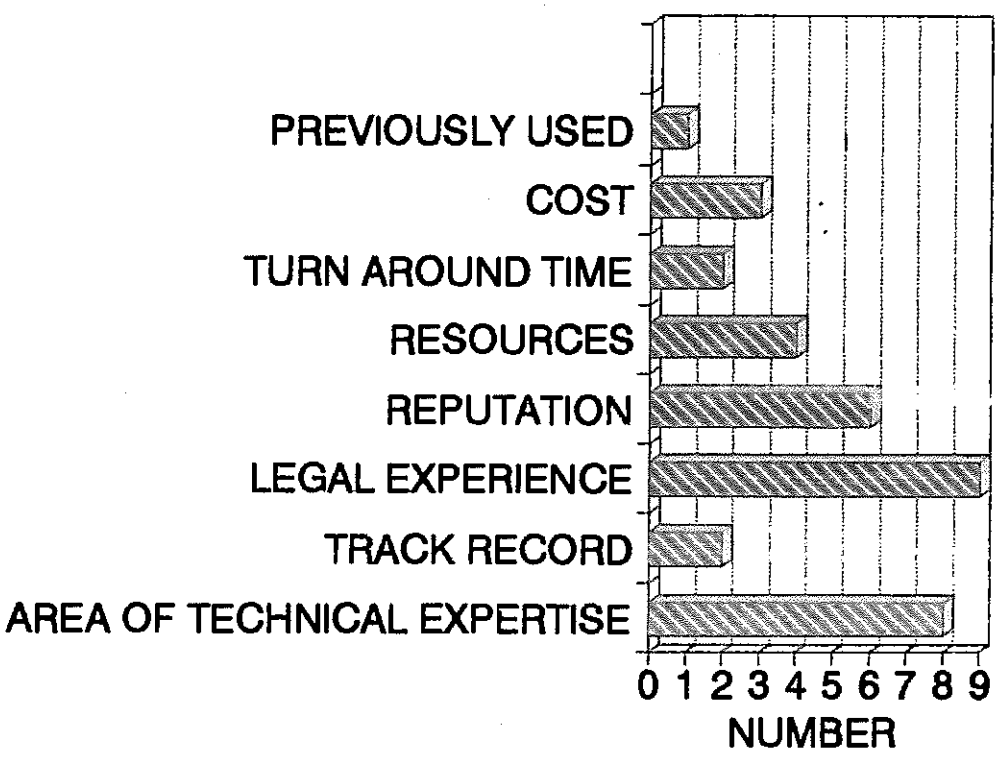


FIGURE 8

was designed so that the responder could fill in reasons for why they would or would not consider utilizing new patent counsel for their company.

The question was designed to mean patent counsel outside of the company as opposed hiring patent counsel to manage within the company.

The results could generally be classified into two separate categories of reasons a biotechnology company would consider using new outside patent counsel and reasons a biotechnology company would not consider using new outside patent counsel. Generally, biotechnology companies would consider new patent counsel if they were referred or recommended or had the necessary technical experience to handle the biotechnology companies work. Other less important reasons included new outlook by the patent counsel, good chemistry, the practitioner was not a "time-keeper" and if the present outside patent counsel for the biotechnology company was overloaded (See Figure #10 for a graphical depiction showing the quantitative importance of the reasons).

The reason new patent counsel would not be considered by a biotechnology company included lack of knowledge or experience, non-referral and non-responsive and lack of speed. Other less important reasons included small staff, cost, and if the patent counsel was a "time-keeper" (See Figure #11).

2E. Please check the area of scientific training which is in highest demand at your company(s). Check only ***one.***

Question 2E was designed to particularly measure the areas of highest technical demand of the responding biotechnology companies. Since the person answering the questionnaire was also a decision-maker with regard to patent strategy it was likely that their perception of technical demand may have been different from other company members and have been more focused toward technical demand regarding patent law.

The results generally indicated that molecular biology and biochemistry were in highest demand with needs for immunological technical training close behind. Chemical engineering was fourth in highest demand followed by organic chemistry. Other areas in less

IMPORTANT FACTORS IN SELECTING
INDIVIDUAL TO DO PATENT WORK

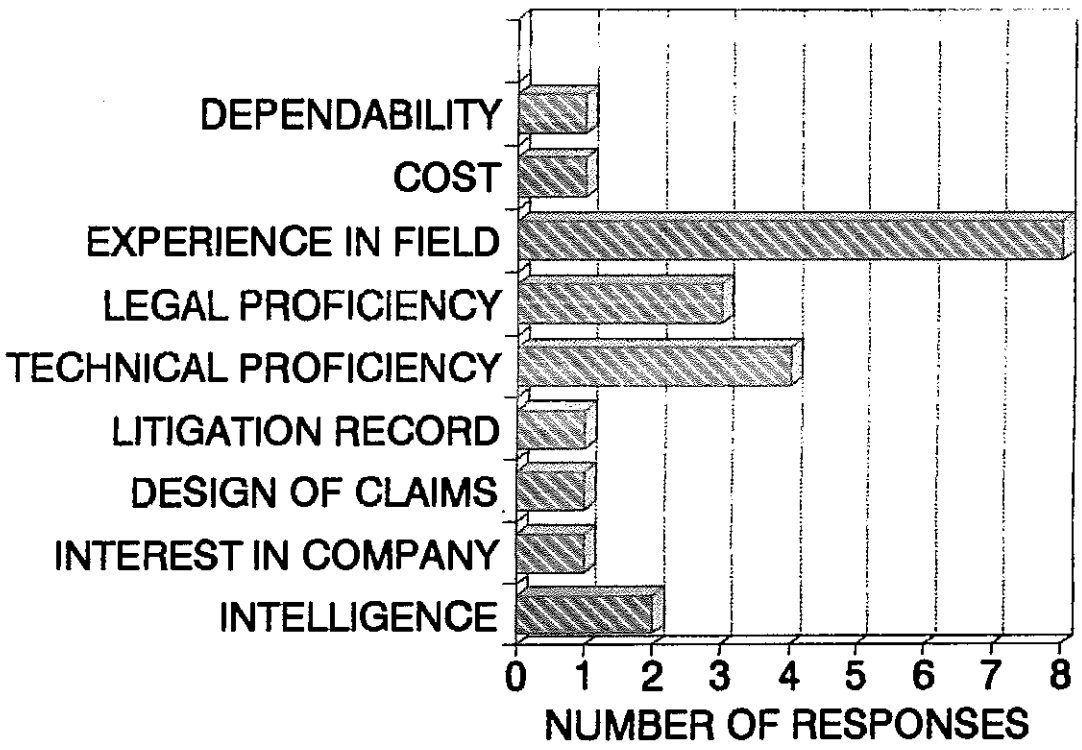


FIGURE 9

REASON WOULD CONSIDER
USING NEW PATENT COUNSEL

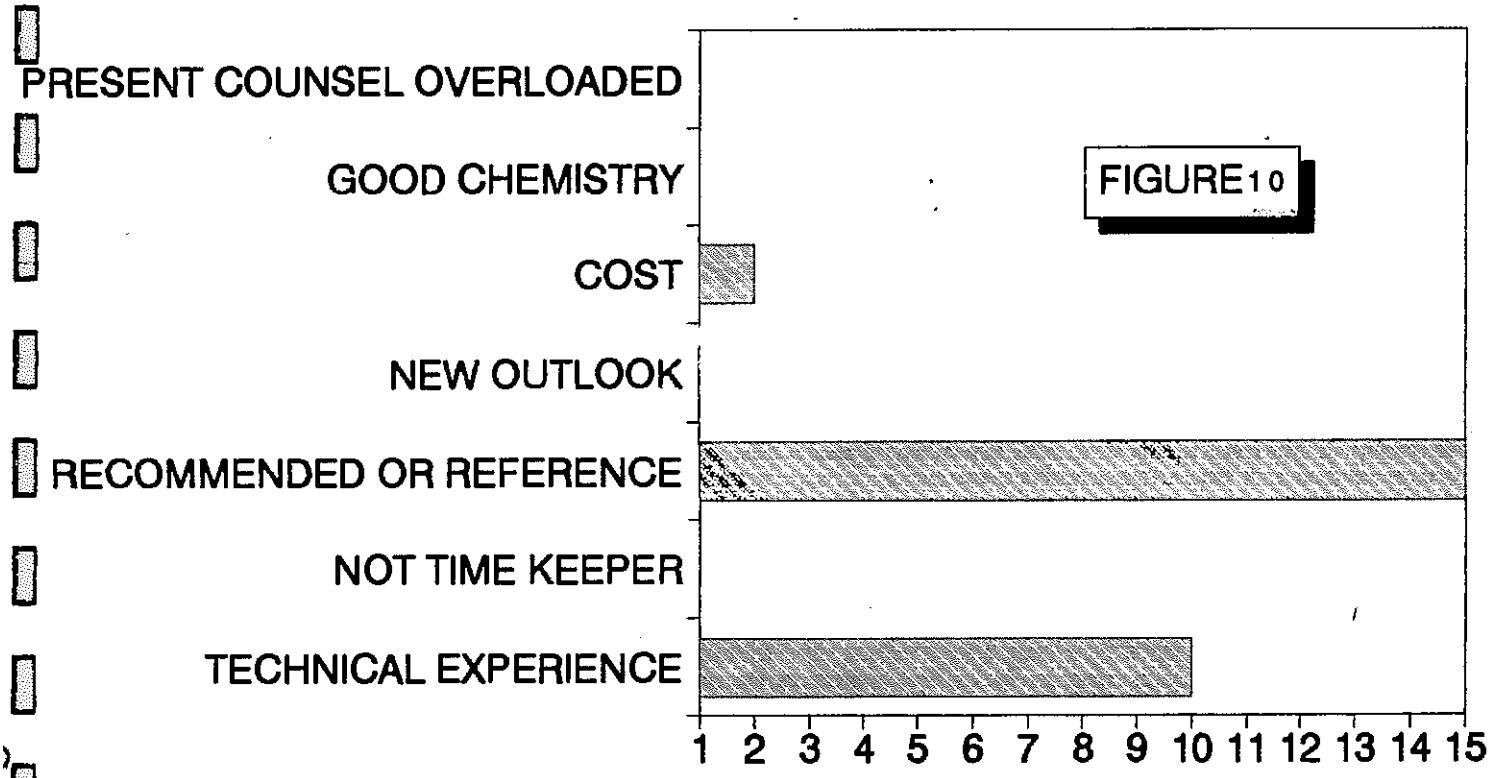
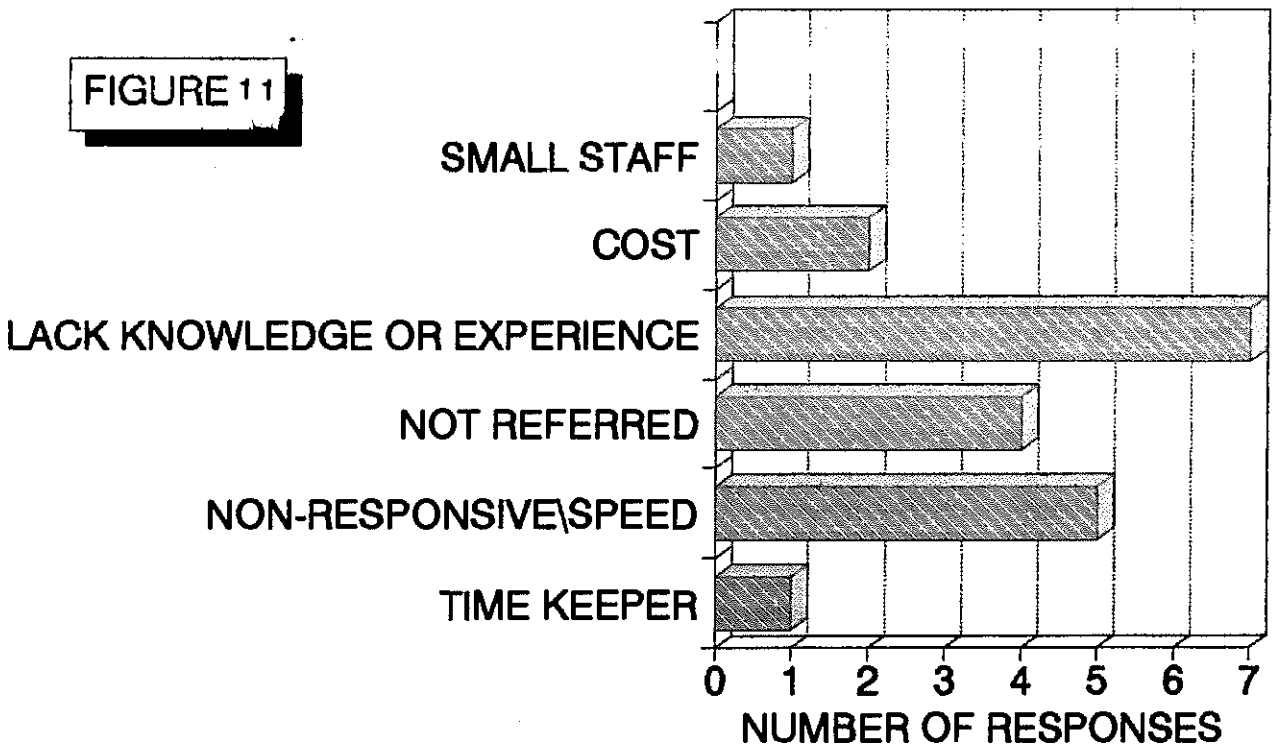


FIGURE 10

REASON WOULD NOT CONSIDER
NEW PATENT COUNSEL

FIGURE 11



demand included pharmaceutical training, biomedical engineering, plastics, generalist/engineering and computer science (See Figure #12). The results generally follow the national trends which predict molecular biologists and biochemists to be in high demand[10] and the Massachusetts trends which predict similar needs.[11]

2F. If you were required to hire a new patent attorney for your company and you were to evaluate the practitioner's ability solely on the number of patent applications he/she had written, how many patents would you require?

Question 2F was designed to measure the desired levels of legal training of patent attorneys hired by biotechnology companies. The question was drafted expansive enough to include situations in which the company would hire a new patent attorney as staff or as outside counsel.

The results generally show an expected bi-modal distribution based on this dichotomy. Responders to the questionnaire generally indicated less than 10 patent applications or somewhere between 25-50. This dichotomy was probably a result of responders interpreting the question somewhat differently. For instance, responders who chose less than 10 probably interpreted the question to mean the amount of applications necessary to hire a new patent attorney as staff to the company. While responders who indicated 25-50 patent applications probably interpreted the question to mean the hiring of new outside patent counsel. If this were true it would appear that companies set a higher standard for technical and legal training of their outside counsel than their inside counsel. This seems to be a logical exchange for the control that they would lose using outside counsel. (See Figure #13 for a graphical depiction).

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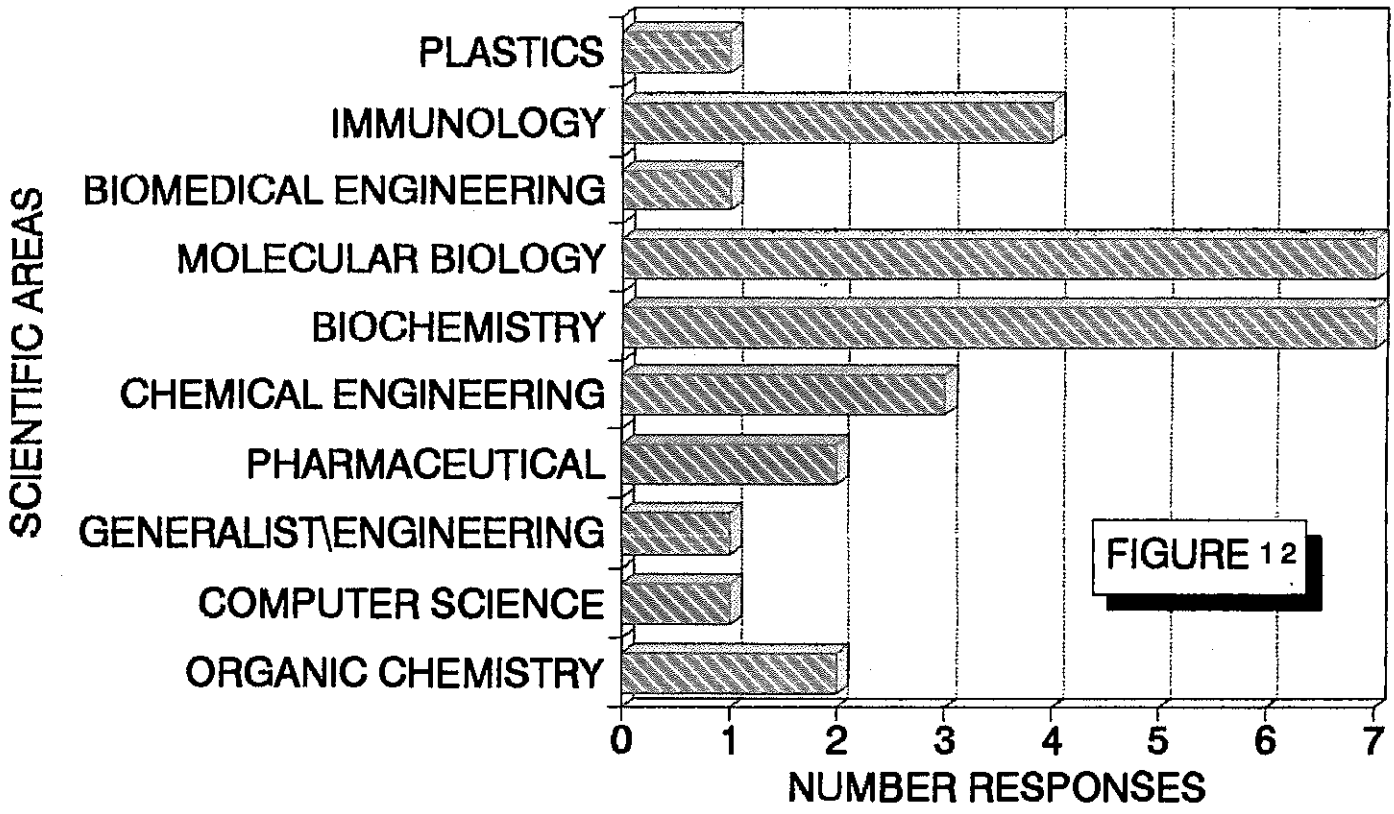
[10] New Developments in Biotechnology, Office of Technology Assessment Report, Page 136 (July, 1988).

[11] Report of the Massachusetts Higher Education Task Force on Biotechnology, Page 10 (March, 1990).

TECHNICAL AREAS IN HIGHEST DEMAND



DESIRED NUMBER OF PATENT APPLICATIONS WRITTEN

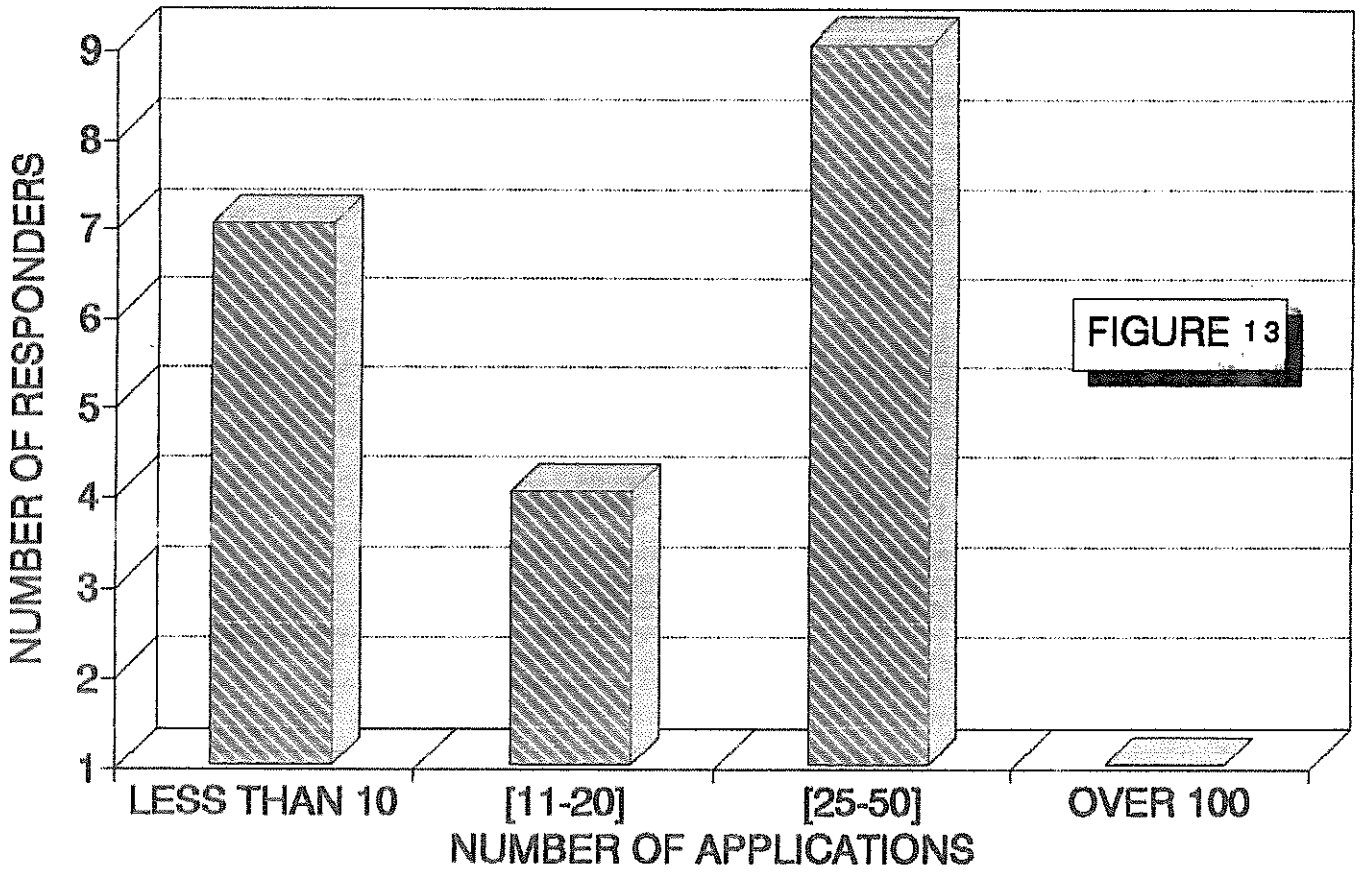


FIGURE 13

3. Qualities you like to see in your patent attorney (technical, legal and other).

Question 3 and much of the remainder of the survey questions used a scaling methodology so that each of the factors or qualities desired by biotechnology companies could be quantified by the survey responder. Factors were grouped into one of three areas on the questionnaire and included technical, legal and other qualities. Each of the factors in one of these grouping could then be labelled and scaled from 0 to 10. Anchors were used at 0 to indicate "not important", 5 to indicate "important" and 10 to indicate "essential".

Individuals were asked to mark an X on the scaling line. Some responders preferred to circle the numbers instead of marking an X on the line. In order to tabulate the results, marks between the numbers were treated as the previous base number plus .5. Circle or marked whole numbers were quantified as whole numbers. The anchors were then used to group the overall responses such that any numbers falling between 0 and 4.5 were treated as a mark for "not important", 5.0 to 9.5 was treated as the "important" range, and a circled 10.0 was treated as a mark for "essential". The tally marks in each range for each factor in each group were quantified and then graphed.

Figure #14 generally shows a graphical depiction and quantification of the technical training level desired of outside patent counsel by responding biotechnology companies. The graph generally shows an inverse relationship in which it was nearly "essential" to have a bachelors level degree in the area of biotechnology or biological sciences and nearly "not important" to have a Ph.D. in a technical field. Of the survey responders themselves who had a Ph.D. and answered the questions regarding technical training most indicated that a Ph.D. was not "essential" and was ranked numerically near "important" or between "important" and not important.

A masters level technical degree appeared to be the overall choice of biotechnology companies and included the largest numerical responders between "important" and "essential". Based on the results of this survey it appears that optimum training level for a patent attorney would be a masters level degree. Figure #14 generally shows the increase in importance of technical training

DESIRED GRADUATE LEVEL
TECHNICAL TRAINING

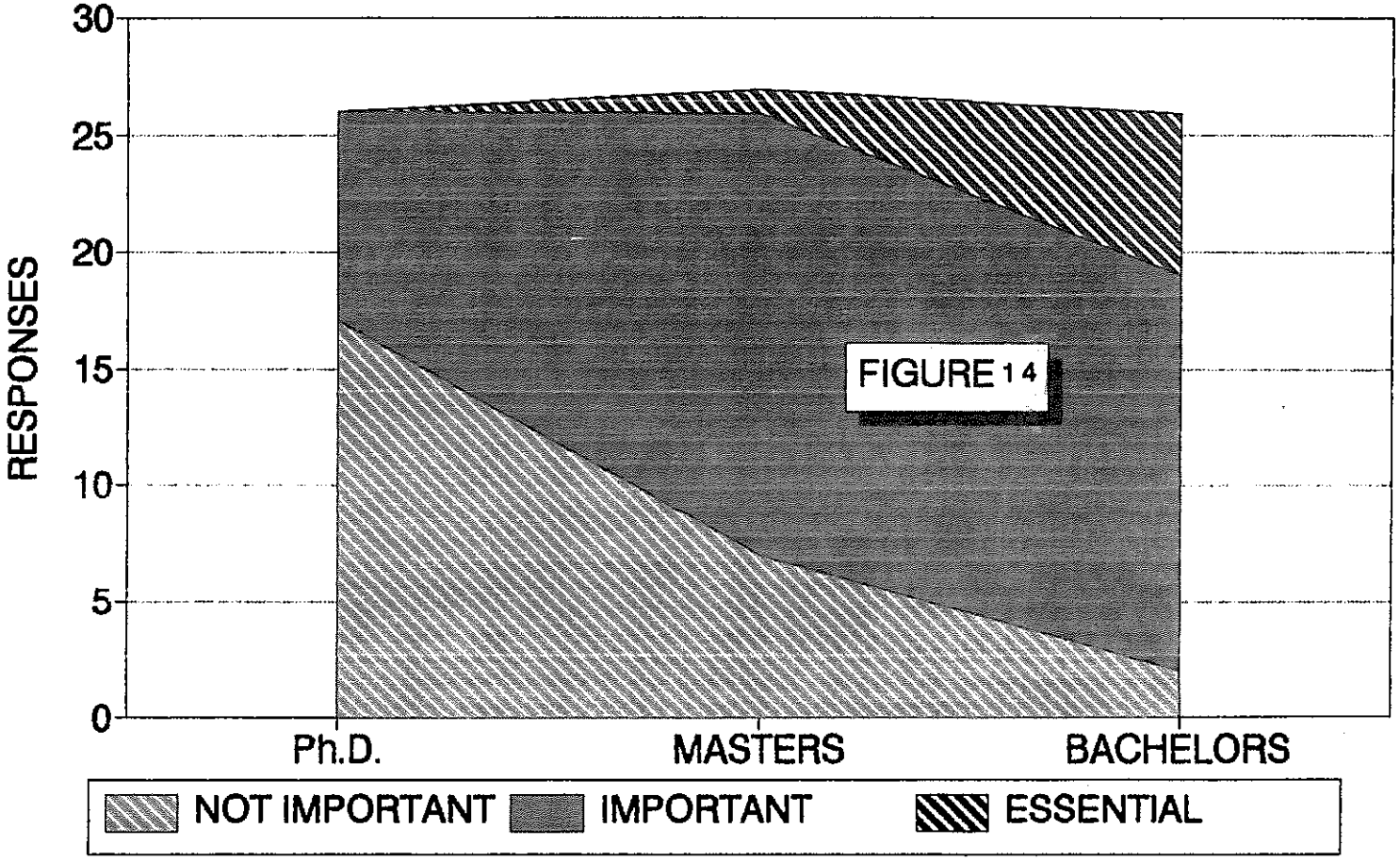


FIGURE 14

until a peak is reached at the masters level. After this point the technical training and/or necessary degrees desired by biotechnology companies substantially dwindles.

The second grouping included factors desirable in a patent attorney which were related to legal training and experience. Results for this part of question three were tabulated similarly to the first grouping concerning technical training. Figure #15 shows the ranking of each of the relative characteristics or factors. Reputation and experience ranked the highest and had the highest numerical quantification in the "essential" and "important" categories. Specialty in the biotechnology companies technology and the prosecution of technically significant patents ranked next highest. Cost to perform work and who the practitioner had worked for were ranked next. Firm resources had some importance but in most cases were "non-essential", while legal publications did have some value, but were largely ranked "not important". Attending a prestigious law school was largely considered "not important", but in some specific instances did have some importance. Advertising had a clear ranking as being "not important" (See Figure #15 for a graphical depiction).

4. What are your three biggest complaints with regards to legal services?

Question 4 was designed to measure general complaints about legal services. The question was designed open-ended so that responders could write-in exactly what they disliked. The question included both standard legal services and patent legal services. The question was worded expansively so that all areas of legal services could be probed and scrutinized.

The results generally indicated that cost was the biggest complaint followed by the slow speed of the retained practitioner and inattentiveness to the client. (See Figure #16 in which the grey bars indicated the 3 major areas of concern). Based on the person who responded to the question and the context of the survey it was understood that many of the responders might answer the question with regard to complaints about patent services (a more focused interpretation of the question). Such an interpretation by the responder was expected and welcomed. For instance, some survey

FIGURE #15

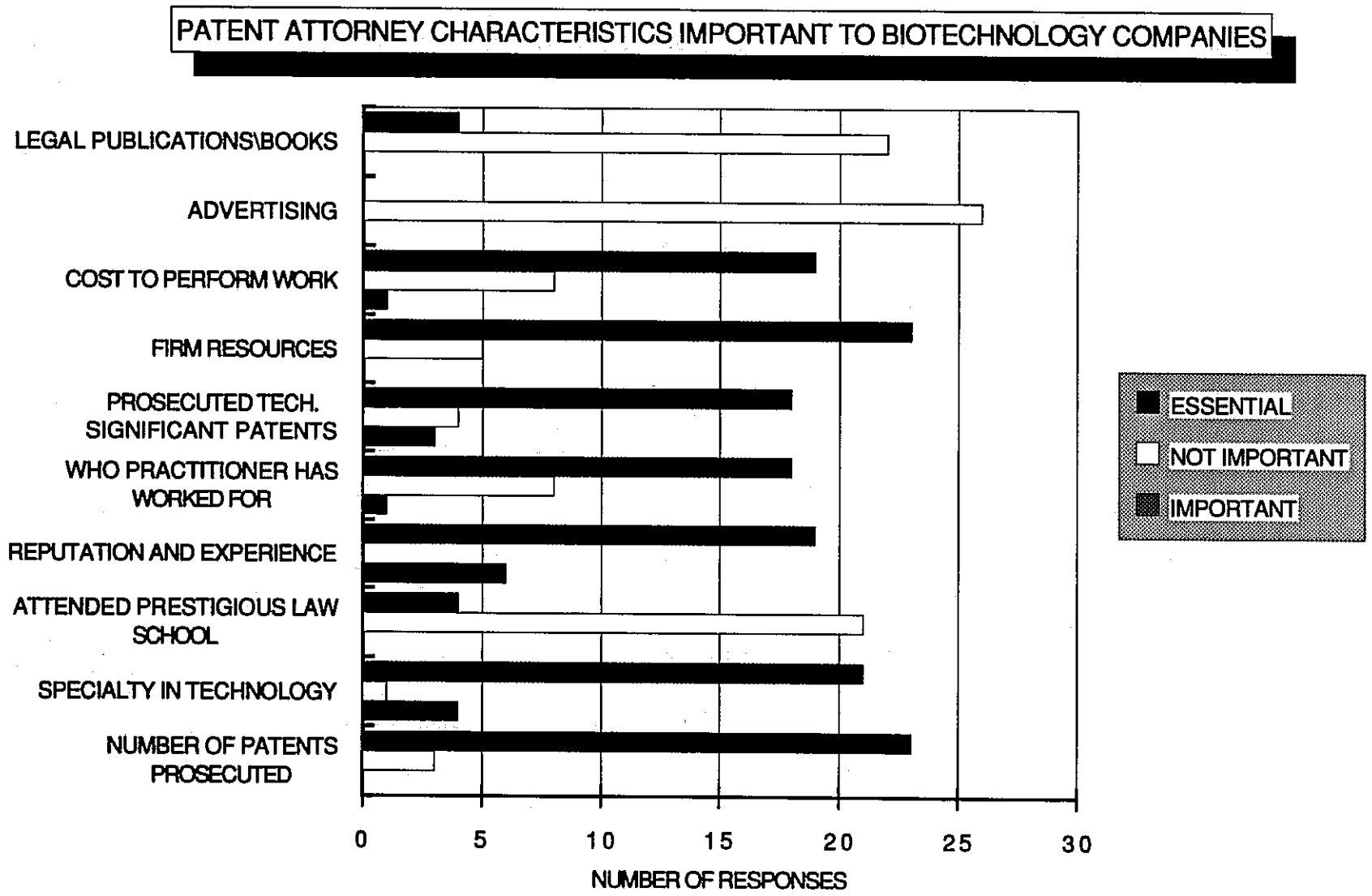
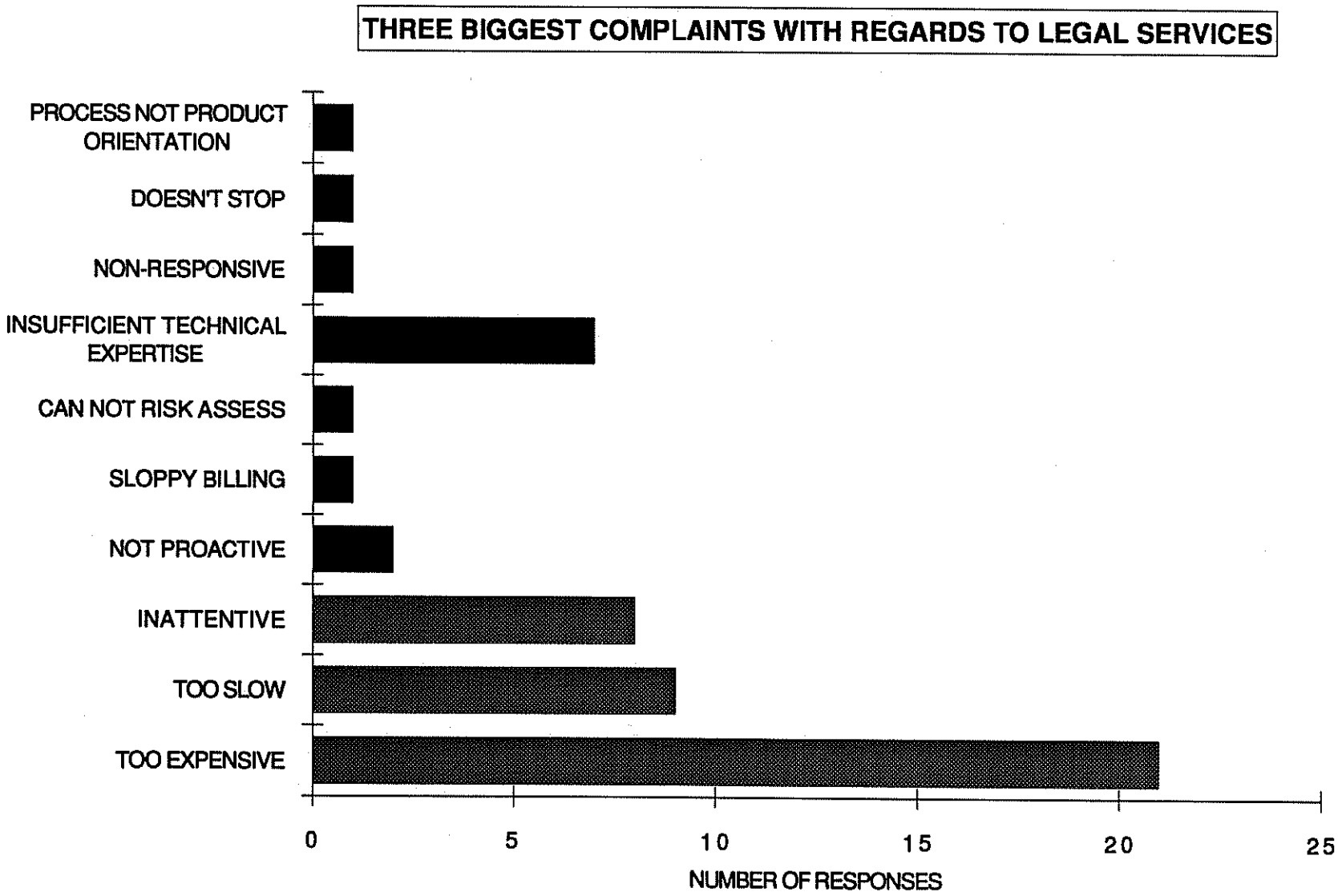

PATENT ATTORNEY CHARACTERISTICS IMPORTANT TO BIOTECHNOLOGY COMPANIES
LEGAL PUBLICATIONS\BOOKS
ADVERTISING
COST TO PERFORM WORK
FIRM RESOURCES
PROSECUTED TECH. SIGNIFICANT PATENTS
WHO PRACTITIONER HAS WORKED FOR
REPUTATION AND EXPERIENCE
ATTENDED PRESTIGIOUS LAW SCHOOL
SPECIALTY IN TECHNOLOGY
NUMBER OF PATENTS PROSECUTED
0
5
10
15
20
25
30
NUMBER OF RESPONSES
ESSENTIAL
NOT IMPORTANT
IMPORTANT

FIGURE #16


THREE BIGGEST COMPLAINTS WITH REGARDS TO LEGAL SERVICES
PROCESS NOT PRODUCT ORIENTATION
DOESN'T STOP
NON-RESPONSIVE
INSUFFICIENT TECHNICAL EXPERTISE
CAN NOT RISK ASSESS
SLOPPY BILLING
NOT PROACTIVE
INATTENTIVE
TOO SLOW
TOO EXPENSIVE
0
5
10
15
20
25
NUMBER OF RESPONSES

responders indicated that insufficient technical training was a high concern (almost as much as inattentiveness of the practitioner). Other areas of lower concern included: sloppy billing, non-proactive philosophy or legal service, inability to risk assess, non-responsiveness of the retained practitioner and the fact that the retained practitioner was process oriented and not product oriented.

Some reasons why the model of attorney equal to inventor may fail:

It is generally easier to predict supply and demand for scientists and engineers than it is to predict supply and demand for patent attorneys. Table #7 generally proposes a model for determining the overall supply and demand of patent attorneys. Supply and demand of patent attorneys is directly related to supply and demand for scientists and engineers. When the economy is strong and salaries are high, the majority of scientists and engineers will generally remain as scientists or engineers or "non-defectors". As the economy generally worsens, there are more "defectors" to patent law. It appears that a larger percentage of scientists and engineers will "defect" to patent law as opposed to patent attorneys "defecting" to science, engineering or some other profession. Table #7 shows the model of how supply and demand may be determined for patent attorneys. If one accepts this general model, one should also accept the proposition that fluctuations and changes to supply and demand of scientists and engineers will inevitably effect patent law. This would especially be true if there was a deficiency of scientists or engineers in a particular field.

On the otherside of the coin is the fact that although supply and demand for scientists and engineers can be predicted, such predictions often do not consider all factors and may not be representative of the truth. For instance, Robert Armstrong, Du Pont's manager of professional staffing in Wilmington, Delaware, describes a study that compared the percentage of high school students going into engineering with the average starting salary of engineers." The correlation is just beautiful," he says-when salaries rise more students go into the field. The supply of science and engineering students is really driven by students view of job

Table # 7 - Supply & Demand for Patent Attorneys

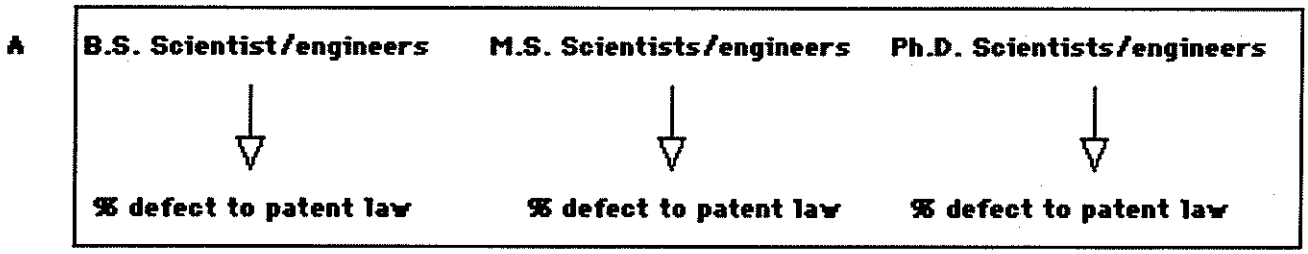
A proposed model in which the supply and demand for patent attorneys is directly proportional to the supply and demand for scientists and engineers. Defectors are defined as individuals who make career changes into or out of the patent law profession. Relative evidence strongly suggests that the majority of defectors go from science or engineering careers into patent law. Very few defectors will go the other way. This may be due to legal requirements economics and the barriers to entry into the patent profession.

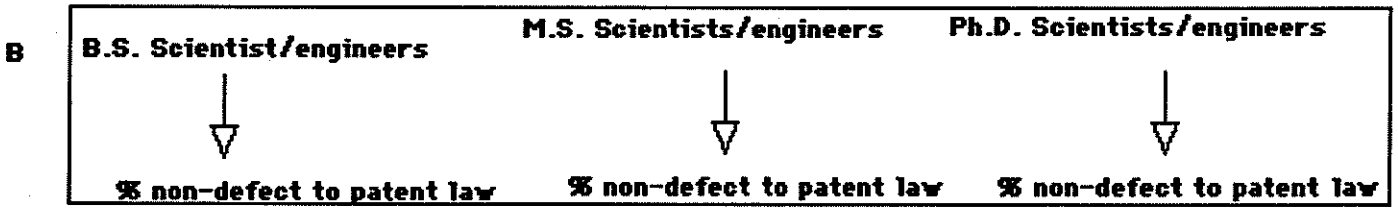
A+B-C= Supply of patent attorneys at any one time.

A = % of defectors to patent law from science or engineering fields.

B = % of non-defectors from science or engineering fields to patent law.

C = % defectors from patent law to science, business or other profession.





C — Patent attorneys defecting to other profession

Key: ↑ = increase, ↓ = decrease, ↕ = remains constant

Assumptions:

As Economy ↑ then B supply ↑ A supply ↓ C ↕

As Economy ↓ then B supply ↓ A supply ↑ C ↕

opportunities."[12]

This may provide some comfort for intellectual property law firms and biotechnology companies wishing to hire future attorneys with advanced degrees. However, recent data generally indicates that there may be a shortfall of biotechnology scientists and Ph.D.'s in the near future. If this is true the model of inventor equalling attorney may fail. Some reasons for the collapse of such a model include:

1) Biotechnology companies in the future may favor experts in law as opposed to technology as they shift modes from research and development to manufacturing.

2) As law related to Biotechnology and patent law evolve, it is likely that the general principles will become more predictable and additional technical training will be unnecessary.

3) By the year 2000 it is likely that the general salaries for scientist will increase and the overall supply of patent attorneys will decrease.

4) Ph.D.'s are trained in a very focused research area and generally do not provide substantial value over masters degree attorneys. (See Figure #17 for a graphical depiction expanding the results of the market study). Additionally, biotechnology companies generally favor the broad level training which masters level attorneys have.

5) As patent costs escalate biotechnology companies will begin to favor ways to reduce costs but maintain similar value.

6) It is likely that the overall importance of patents could decrease because of cost of court battles.[13]

7) Any additional added value which Ph.D. level attorneys bring to the table to fledgling biotechnology companies may not be necessary as companies evolve to maturity by the year 2000. (See

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[12] Who will do science in the 1990's, Science Magazine, April 1990 page 433.

[13] Ernst & Young Survey 1993, Pg 433.

Figure*17 - Relationship of value added to cost of utilizing a patent attorney with an advanced technical degree.

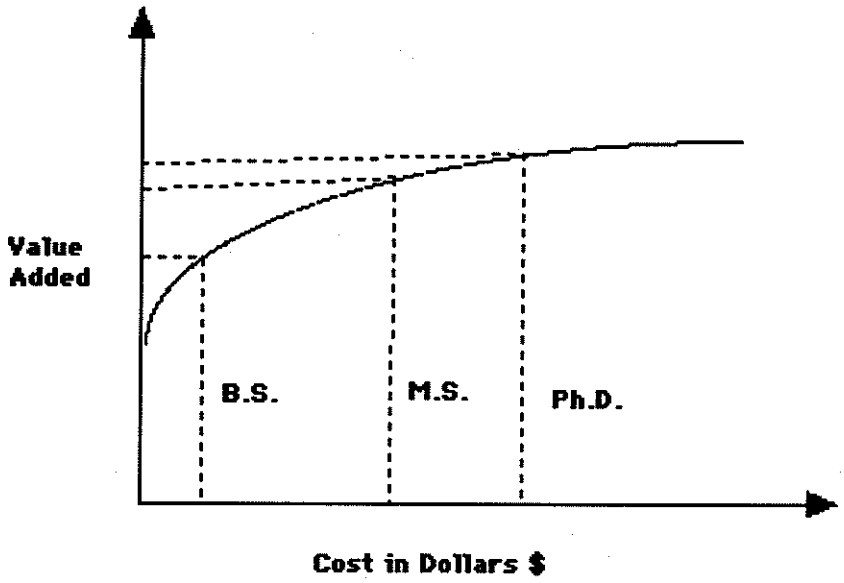
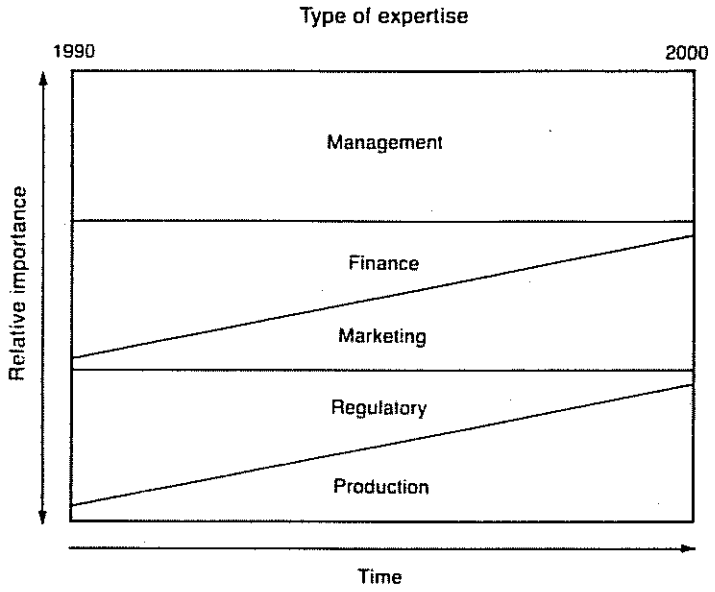


Figure 18 below)[14]

Figure #18 - Shifting Critical Success Factors



Source: Consulting Resources Corporation

Scientific and Business Trends are perhaps the strongest reason why the model of attorney equal to inventor will fail.

Perhaps the strongest reason why the model of inventor equal to attorney could fail is fluctuations in business trends and changes in supply and demand of scientists and engineers.

For instance, it has been estimated by the year 2000 that there will be a shortfall from the year 2000 to 2010 of approximately 7,500 Ph.D.'s per year in the United States. The overall demand per year will be approximately 18,000 Ph.D's per year. The supply to fill some of these positions will be due to 7,000 U.S. citizens and about 5,000 foreigners who stay in the U.S. [15] This general decline in science and engineering graduates will be mainly due to aging

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[14] Shifting critical success factors. (Source: Consulting Resources Corporation.)

[15] Information courtesy of Dr. Richard Atkinson, Chancellor, University of California, La Jolla, CA 92093. Chart deals with projections of years 2001-2010.

faculty and decrease in the college age population.[16] If one generally accepts the model that supply and demand of patent attorneys is directly related to amount of "defectors" from the scientific or engineering fields, it is likely that the increase in demand for scientists and engineers will have a proportional effect on supply and demand for patent attorneys (See Figure #19)

The Future:

After reviewing past data regarding the backlog of the United States Patent and Trademark Office and the present survey results we are able to reach the following conclusions:

1) As the needs for scientists increase the needs for patent attorneys in the biotechnology area will also increase.

2) The present model of attorney equal to inventor may fail due to the increased needs for scientist and engineers.

3) Costs for biotechnology patents could increase significantly within the next decade.

4) If law firms are to optimize their profit potential and biotechnology companies are to increase their patent portfolios, the present models must be re-worked.

A Suggested New Model:

After reviewing the past data and the relative needs and wants of the Massachusetts biotechnology industry, a new model is apparent which could perhaps solve the emerging problems which biotechnology companies and law firms may face. The new model to be applied to law firms and to biotechnology companies would generally follow the wants and needs of the biotechnology companies. The model would replace the existing Ph.D. level attorneys with master level attorneys in specialized areas including molecular biology, Chemical/therapeutic, agri/plant, biochemistry and immunology. Each masters level attorney would work directly

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16 Information courtesy of Dr. Richard Atkinson, Chancellor, University of California, La Jolla, CA 92093. Chart deals with projections of years 2001-2010.

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4) If law firms are to optimize their profit potential and biotechnology companies are to increase their patent portfolios, the present models must be re-worked.

A Suggested New Model:

After reviewing the past data and the relative needs and wants of the Massachusetts biotechnology industry, a new model is apparent which could perhaps solve the emerging problems which biotechnology companies and law firms may face. The new model to be applied to law firms and to biotechnology companies would generally follow the wants and needs of the biotechnology companies. The model would replace the existing Ph.D. level attorneys with master level attorneys in specialized areas including molecular biology, Chemical/therapeutic, agri/plant, biochemistry and immunology. Each masters level attorney would work directly

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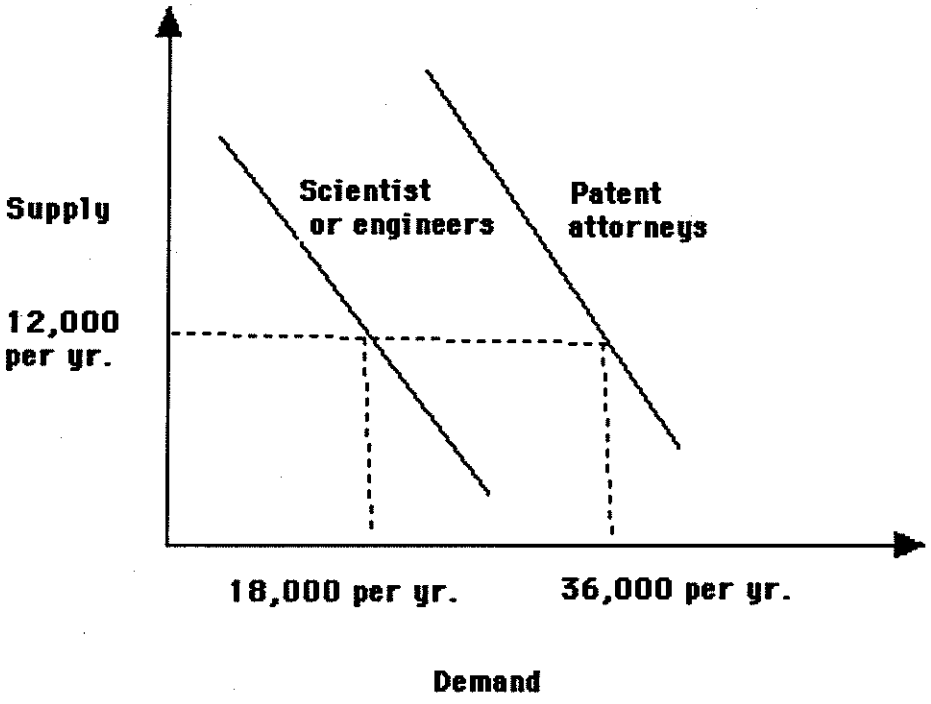
[16] Information courtesy of Dr. Richard Atkinson, Chancellor, University of California, La Jolla, CA 92093. Chart deals with projections of years 2001-2010.

Figure #19- Estimates of Supply and Demand for Patent attorneys.



with the client as opposed to the existing model in which Ph.D. level attorneys work directly with the scientists. Instead of each Ph.D. reporting directly to the partner, the model would entail the use of a single Ph.D. level attorney who would act as a generalist/middle man and work with the masters level attorneys on very technical inventions. This Ph.D. would report directly to the managing partner. The model would provide for increased specialization and retain the added value which the old model had provided since a Ph.D. would still be available for the client to work with.

The model could provide a more cost effective management structure for the client and law firm since the actual number of Ph.D's per company or law firm would be reduced. In other words, if there were more Ph.D.'s per company or law firm, the cost would generally be hirer for each client because of the additional costs of retaining more Ph.D. level attorneys. The model generally accounts for the shortfall of scientists and Ph.D.'s which is likely to occur by the year 2000 by substituting masters level attorneys for Ph.D. level attorneys. This model should generally provide maximum value and cost saving to both law firms and biotechnology companies.

The model is advantageous since it considers the customer or client as the next process. The next process concept is becoming familiar to not only managers but production line and indirect labor in major companies. As part of the training in quality improvement, these companies are telling test equipment operators that their customer is the packer who pack what was just tested. The message applies to all service people to which the item goes.[17] The new model follows the general operations management principles since the client will be treated as the next process. By focusing more and more on what biotechnology companies want, law firms will become increasingly attractive to new clients. Law firms will be able to maximize their profitability by utilizing such a model which favors **high quality, increased throughput time, lower costs, and flexibility** (See Figures #20, 21, and 22 for a comparison of the relative models and their differences.) Clients or customers will be handled on an individual basis. Additionally, this overall scheme could be applied directly by each of the biotechnology companies to streamline their own inhouse patent departments.

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[17] Operations Management, Serving the Customer, Schonberger and Knod Third Edition, Page 5.

Figure*20- Present Law Firm Model of Biotechnology Patent Department

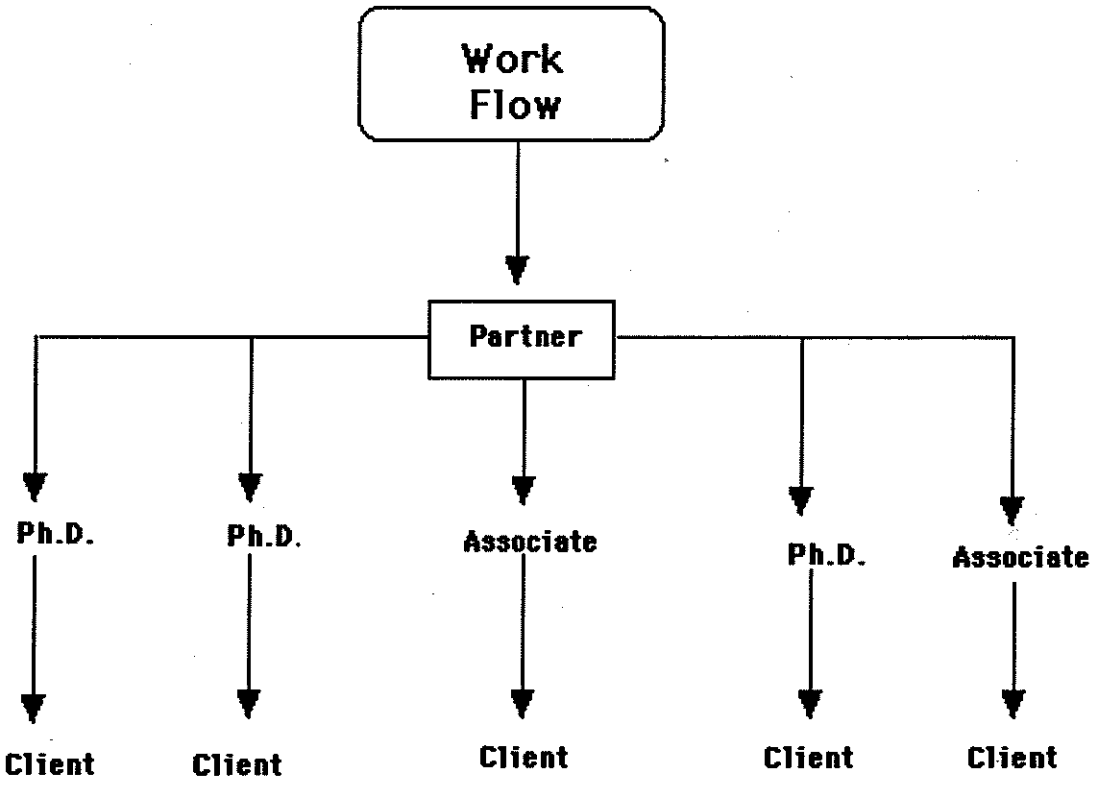


Figure*21 - In general, biotechnology companies want to deal with patent law firms which offer high quality, low costs, short lead times and flexibility. A well managed law firm must attempt to provide services that possess these four desired attributes. As law firm competitiveness increases into the year 2000 marketing and advertising will not be enough for law firms to stay competitive. They will have to take steps to streamline their operations management.

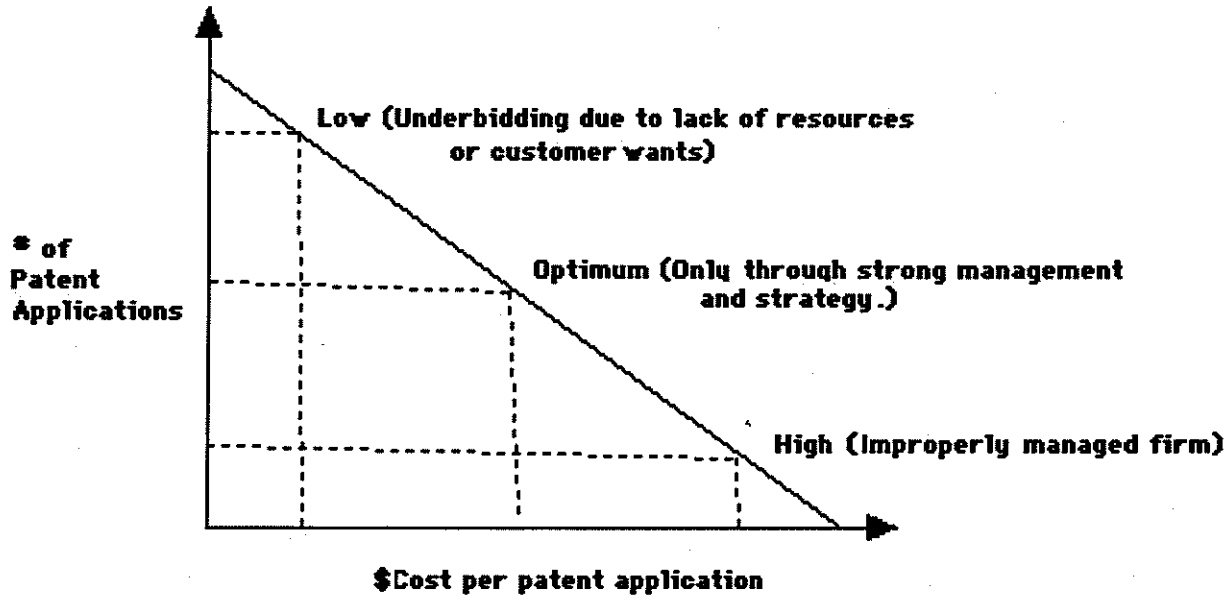
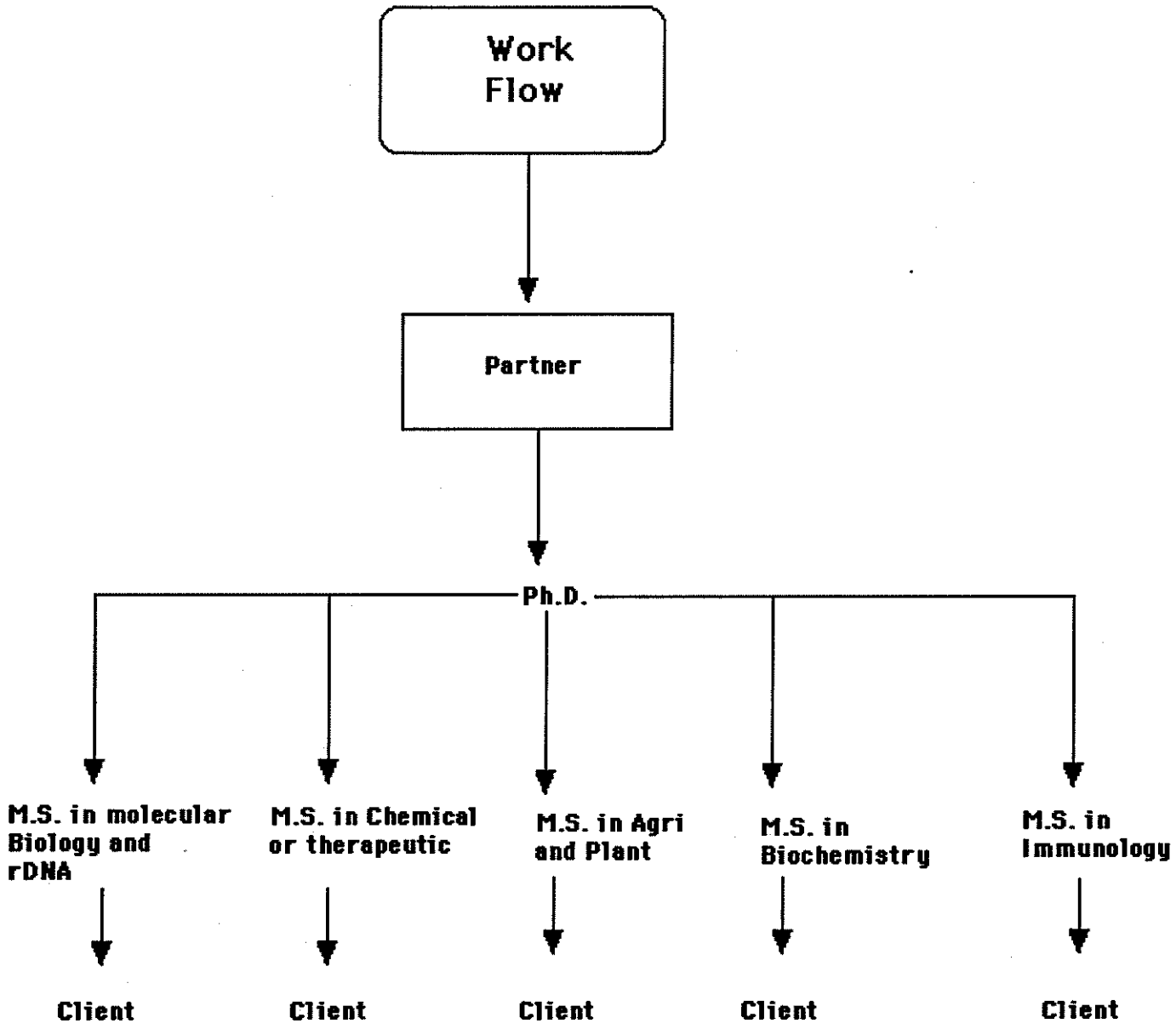


Figure #22 - Suggested Model for Law Firm Biotechnology Patent Department



Conclusions:

The author believes that the general data and results of this paper are representative of the biotechnology industry in general and may be representative of the national trends. The results of the market survey and proposed models indicate that one does **not** need a Ph.D. to prosecuted biotechnology patents. Additionally, law firms need not hire Ph.D. level patent attorneys to stay competitive since the majority of biotechnology companies would favor a masters level attorney. The suggested model provides a new perspective for law firms and biotechnology companies to handle some of the emerging problems which they could face into the year 2000. The conclusions of this paper do not reject that a Ph.D. for an attorney may be useful or even necessary in certain narrowly designed situations. The conclusions are generalizations drawn from what biotechnology companies desire and which is perceived to be an important perspective which should be considered by law firms and patent practitioners.

Appendix 1

Partial Listing of Massachusetts biotechnology companies responding to survey and description of strategic direction of companies. (Compiled by the Massachusetts Biotechnology Council).

Abbott Biotech, Inc.
119 Fourth Avenue
Needham Heights, MA 02194
(617) 449-6002

Abbott Biotech, Inc. develops, manufactures and markets biomedical products through the application of its proprietary technologies--the Cellular Enhancer technology and the ENCAPCEL system. The Company currently manufactures monoclonal antibodies and cardiovascular products at its Cell Culture Facility.

Advanced Magnetics, Inc.
61 Mooney Street
Cambridge, MA 02138
(617) 497-2070

Advanced Magnetics, Inc. is a world leader in the application of colloidal magnetic materials to health care. The Company's research *in vitro* products are sold to academic and industrial research laboratories around the world. Clinical *in vitro* products are manufactured on an OEM basis for leading immunoassay companies and the Company has successfully licensed proprietary *in vitro* products and technology to more than a half-dozen major companies. The Company has utilized its patented Biodegradable Superparamagnetic Particle Technology to establish a cutting edge diagnostics product portfolio.

Alkermes, Inc.
26 Landsdowne Street
Cambridge, MA 02139
(617) 494-0171

Alkermes, Inc. is a neuropharmaceutical company focused on the development and commercialization of therapeutics and diagnostic products for the treatment of disorders of the central nervous system.

Alpha-Beta Technology, Inc.
One Innovation Drive
Worcester, MA 01605
(508) 798-6900

Alpha-Beta Technology, Inc. is developing patentable carbohydrate-based products for the health care industry through the application of carbohydrate engineering. The Company's major products in development include Betafectin™ for the treatment and prevention of infections related to surgery and chemotherapy; Cholestran™ for the treatment and prevention of cardiovascular disease; and Fibercel™, a fat substitute.

Applied bioTechnology, Inc.
80 Rogers Street
Cambridge, MA 02142
(617) 492-7289

Applied bioTechnology, Inc. is developing and marketing a new generation of genetically engineered products for the treatment, prevention and diagnosis of infectious diseases and cancer.

BASF Bioresearch Corp.
195 Albany Street
Cambridge, MA 02139
(617) 868-5700

BASF Bioresearch Corporation has announced plans for a $100 million facility to be built at the Worcester Biotechnology Park. Ground is to be broken in the fall of 1990 with the project's completion date slated for early 1993. When it opens, the bioresearch facility will house some 250 researchers, including more than 50 PhD-level scientists whose mission will be to find cures and possibly preventions for cancer and diseases of the immune system, such as arthritis. In the fall of 1989, BASF Bioresearch Corp. opened a research laboratory in Cambridge, which now houses more than 50 researchers and staff.

Biogen, Inc.
14 Cambridge Center
Cambridge, MA 02142
(617) 864-8900

Biogen, Inc. is a leading biotechnology firm with a strategic focus on the development of human therapeutics. The firm was founded in 1978, and the first group of products based on its research is being sold by licensees throughout the world. The next generation of Biogen-developed products will be targeted for use in the AIDS therapeutics, inflammation, and selected cancer markets.

Biopure Corporation
68 Harrison Avenue
Boston, MA 02111
(617) 350-7800

Biopure Corporation is engaged in the identification, isolation, separation and purification of critical proteins, resulting in the industrial-scale production of ultra-pure products used in the pharmaceutical and health care markets. The Company is currently manufacturing a highly purified oxygen-carrying hemoglobin solution to be used as a temporary blood-substitute in humans and animals.

BioSurface Technology, Inc.
64 Sidney Street
Cambridge, MA 02139
(617) 494-8484

BioSurface Technology, Inc. is engaged in the commercialization of epithelial (skin) cell biology discoveries made by Prof. Howard Green of Harvard Medical School. The Company is currently generating revenues by providing cell culture services to patients in burn centers across the United States in order to provide a life-saving source of permanent skin replacement for these patients. The Company is also engaged in clinical trials of a novel wound-healing product based on cultured epithelial cells.

BioTransplant, Inc.
Building 96, 13th Street
Charlestown Navy Yard
Charlestown, MA 02129
(617) 242-4594

BioTransplant, Inc. is engaged in the discovery and development of novel immunologic approaches to organ transplantation. Committed to overcoming current problems of graft rejection, the Company's mission is to develop, manufacture and market products and services for patients requiring allograft and xenograft organ transplants. The systems to be sold to transplant surgeons will minimize systemic immunosuppression through the induction of specific immune tolerance. Dr. David H. Sachs, former chief of the immunology branch at the National Cancer Institute and now director of the Transplantation Biology Research Center at Mass. General Hospital, is BioTransplant's chief scientific advisor. Dr. Sachs has two decades of experience in the field of transplantation immunology. He will serve as Chairman of the Company's Scientific Advisory Board and lead BioTransplant's collaborative research efforts at the MGH.

Boston Biomedica, Inc.
375 West Street
West Bridgewater, MA 02379
(508) 580-1900

Boston Biomedica, Inc. is a diagnostic manufacturer and technical support laboratory that supplies a growing diagnostic industry with fully characterized serum and plasma-based components, research products and technical services, with particular emphasis given to HIV-1 (AIDS), HTLV-1, HIV-2 and Viral Hepatitis. In addition, the Company offers for research investigation 9 TRUE anti-HIV seroconversion panels that have been recognized worldwide as "gold standards" for determination of anti-HIV-1 test kit sensitivity.

Cambridge Biotech Corp.
365 Plantation Street
Biotechnology Research Park
Worcester, MA 01605
(508) 797-5777

Cambridge Biotech Corporation is using its advanced capabilities in engineering, protein and peptide chemistry, and immunology to develop diagnostic and vaccine products for AIDS, cancer, gastroenteritis, respiratory disease, feline leukemia, and other human and animal infectious diseases.

Cambridge NeuroScience, Inc.
One Kendall Sq. Bldg. 700
Cambridge, MA 02139
(617) 225-0600

Cambridge NeuroScience, Inc. employs advanced drug discovery technologies, such as electrophysiology, molecular biology, neuropharmacology and genetics, to develop novel medications for the treatment of severe neurological and psychiatric disorders, including stroke, Alzheimer's disease and schizophrenia.

Cellcor Therapies
200 Wells Ave.
Newton, MA 02159
(617) 332-2500

Cellcor Therapies, Inc. is a privately held health care company focused on the development and delivery of innovative treatment services based upon unique biotechnology cell processing. The company is also a leader in a newly emerging medical treatment industry known as biocare, a customized, patient-specific form of medical care based on new biotechnological processes. Since its establishment in 1987, Cellcor has concentrated on the development and delivery of a novel out-patient immunotherapy called autolymphocyte therapy (ALT), a procedure in which a patient's lymphocytes are removed, treated and reinfused to enhance the immune system.

Collaborative Research, Inc.
1365 Main Street
Waltham, MA 02154
(617) 275-0004

CRI, founded in 1961, is a leader in developing DNA Probe technology for the diagnosis of genetic diseases, cancer testing and personal identification. It is also a leading contractor with the U.S. government's Human Genome Initiative, having been awarded almost $9 million in grants and contracts in 1991 alone for projects to map chromosomes and perform DNA sequencing of important genes and chromosomes.

Costar Corporation
One Alewife Center
Cambridge, MA 02140
(617) 868-6200

Costar Corporation designs, develops, manufactures and markets a variety of plastic disposable products and other equipment used in life science laboratories worldwide. Costar's research and development activities cover a broad range of fields and technologies, including: mammalian cell biology and tissue culture, cellular and humoral immunology, biochemistry and molecular biology, microbiology, surface modification of polymers and polymer chemistry, membrane manufacture and fabrication, and biomaterials research.

Creative Biomolecules, Inc.
35 South Street
Hopkinton, MA 01748
(508) 435-9001

Creative Biomolecules, Inc. was founded in 1981 to develop, improve and commercialize protein-based products for human health care. Novel drugs are being produced using protein engineering production of a portfolio of products for stimulating the replacement and repair of soft and hard tissue; and a technology base has been developed for producing proteins for the targeted delivery of molecules for diagnosis and therapy. In addition, the Company has prepared a family of tPA analogs.

CytoMed, Inc.
840 Memorial Drive
Cambridge, MA 02139
(617) 661-3400

CytoMed is a biopharmaceutical company dedicated to the discovery, development and commercialization of novel therapies to block and neutralize the mediators involved in producing an inflammatory or immune response. The Company's products will address major, growing markets including autoimmune diseases and anti-inflammatory therapies.

Diacrin, Inc.
Bldg. 96, 13th Street
Charlestown Navy Yard
Charlestown, MA 02129
(617) 242-4594

Diacrin, Inc. is developing unique cell transplantation technology that will lead to cell therapies for treatment of diabetes, muscular dystrophy, and Parkinson's disease.

Diatech, Inc.
9 Delta Drive
Londonderry, NH 03053
(603) 437-8970

Diatech, Inc. was founded in 1990 to develop new pharmaceutical products using peptides. The lead investors are Medical Science Partners and Burr, Egan, Deleage & Co. The initial product under development is a peptide for diagnostic imaging of atherosclerotic plaque for which Diatech, Inc. has a worldwide exclusive license from the New England Deaconess Hospital. Other programs in progress include peptides for imaging clots, infections and cancer.

E.I. Dupont-
NEN Medical Products
331 Treble Cove Road
North Billerica, MA 01862
(508) 667-9531 (and)
549 Albany Street
Boston, MA 02210
(617) 350-9009

E.I. DuPont-NEN Medical Products is a leading manufacturer of radioactive and non-radioactive chemicals for life sciences research, radiopharmaceuticals for medical diagnosis, and clinical diagnostic systems. Scientists in Boston and at the Biomedical Research Lab in Billerica are conducting sophisticated research in the areas of immunology and molecular biology.

Endogen, Inc.
451 D Street
Boston, MA 02210
(617) 439-3250

Endogen, Inc. is a leading developer of high-quality lymphokine products for use in advanced immunological research, monitoring and diagnosis. The company manufactures and supports an evolving line of ELISAs, Ultrapurified Natural Lymphokines, Neutralizing Polyvalent Antibodies and Pre-Coupled Immunoaffinity Gels for Interleukin-1, Interleukin-2, Interleukin-6, Tumor Necrosis Factor, Lymphotoxin, GM-CSF and Interferon gamma. Endogen markets its products worldwide to researchers and clinicians investigating the roles of lymphokines in immune responses and disorders.

Enzytech, Inc.
64 Sidney Street
Cambridge, MA 02139
(617) 252-0001

Enzytech, Inc. is a biotechnology company developing and commercializing delivery systems for therapeutic proteins and value-added nutritional food ingredients.

Epigen, Inc.
148 Linden Street
Suite 207
Wellesley, MA 02181

Epigen, Inc. is a biopharmaceutical company dedicated to providing innnovative products to meet the medical needs of cancer patients. The company is utilizing proprietary Monoclonal Antibodies which are designed to monitor and image cancer patients. Its technology is also expected to be used in the treatment of these patients.

Genesis Pharmaceuticals, Inc.
840 Memorial Drive
Cambridge, MA 02139
(617) 354-8050

Genesis Pharmaceuticals, Inc. interacts with academic scientists and affiliate companies throughout the United States to facilitate drug discovery in multitherapeutic areas. Therapeutic targets presently address disorders of the immune system, such as AIDS and cancer, and diseases related to aging.

GENE-TRAK Systems
31 New York Ave.
Framingham, MA 01701
(508) 872-3113

GENE-TRAK Systems is a joint venture between Amoco Biotechnology Company and Genzyme Corporation and represents one of the largest groups in the world dedicated to the development and commercialization of tests for foodborne pathogens and infectious diseases using nucleic acid probe technology. Currently, GENE-TRAK Systems is selling diagnostic tests to detect *Salmonella, Listeria* and *E. Coli* to food processors and distributors. The Company also sells research assays for HIV-1 (AIDS virus) and Cytomegalovirus (CMV). The Company is developing a wide range of diagnostic tests for the clinical market, including tests for bacterial, viral and fungal pathogens responsible for several important infectious diseases.

Genetics Institute, Inc.
87 CambridgePark Drive
Cambridge, MA 02140
(617) 876-1170

Genetics Institute, Inc. is a leading biotechnology firm engaged in the discovery and development of human pharmaceuticals through recombinant DNA and other technologies. The Company has a diversified portfolio of licensed and proprietary pharmaceutical products. The Company is currently awaiting FDA approval for two major licensed products: erythropoietin (EPO) and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF). The Company has Factor VIII and tPA in human clinical trials. Genetics Institute's first proprietary product, Macrophage Colony Stimulating Factor (M-CSF) entered human clinical trials in 1989.

Genica Pharmaceuticals Corp.
Two Biotech Park
373 Plantation Street
Worcester, MA 01605

Genica Pharmaceutical's mission is to develop and commercialize diagnostics and therapeutics for neuromuscular and peripheral neurological diseases. Through strong academic affiliations and superior service, Genica provides neurologists with emerging, innovative technologies that improve the quality of health care for their patients in a cost-effective manner.

Genzyme Corporation
One Kendall Square
Cambridge, MA 02139
(617) 252-7500

Genzyme Corporation develops, manufactures and sells health care products including fine chemicals, diagnostics and therapeutics, to the pharmaceutical industry. The Company has production and research facilities in Cambridge, MA, and Maidstone and Haverhill, England.

Hybridon, Inc.
One Innovation Drive
Worcester, MA 01605
(508) 752-7000

Hybridon, Inc. is a biotechnology company that applies anti-sense technology to the development and commercialization of novel drug compounds for major diseases. The formation of the company followed twelve years of pioneering research by its scientific founder, Paul Zamecnik, M.D., who invented anti-sense technology, and his scientific staff at Harvard Medical School and Worcester Foundation for Experimental Biology. The company continues to collaborate with Dr. Zamecnik's laboratory, as well as with a prestigious network of researchers at the Harvard Medical School, the Massachusetts General Hospital, Mt. Sinai School of Medicine and the National Institutes of Health.

Hygeia Sciences, Inc.
330 Nevada Street
Newton, MA 02160
(617) 964-0200

Hygeia Sciences, Inc. develops and manufactures easy-to-use, non-instrumented immunodiagnostic assays for use in the home, laboratory or doctor's office. The tests are in the broad categories of reproductive biology, general health and infectious diseases.

ImmuLogic Pharmaceutical Corp.
One Kendall Sq., Bldg. 600
Cambridge, MA 02139
(617) 494-0060

ImmuLogic Pharmaceutical Corporation was founded in 1987 by Dr. Malcolm L. Gefter, professor of biology at the Massachusetts Institute of Technology. Dr. Gefter is a leading immunologist who has pioneered research on the fundamental mechanisms related to the establishment and control of the immune response. ImmuLogic has assembled a core group of outstanding scientists with expertise in immunology, molecular biology and biochemistry, and has initiated development projects in the areas of vaccines and therapeutics for allergies.

ImmunoGen, Inc.
148 Sidney Street
Cambridge, MA 02139
(617) 661-9312

ImmunoGen, Inc. is a leader in the commercialization of therapeutic monoclonal antibody-based pharmaceutical products. The Company is currently developing proprietary immunoconjugates for the treatment of especially lethal forms of cancer.

Interneuron Pharmaceuticals, Inc.
One Ledgemont Center
99 Hayden Ave., Suite 340
Lexington, MA 02173
(617) 861-8444

Interneuron Pharmaceuticals, Inc. (IPI) is a central nervous system pharmaceutical company specializing in the development of innovative products designed to treat neurological and psychiatric diseases. IPI's efforts are focused primarily around the technology of Dr. Richard Wurtman of M.I.T., who is chairman of the company's scientific advisory board.

Micron Separations, Inc.
PO Box 1046
135 Flanders Road
Westborough, MA 01581
(508) 366-8212

Micron Separations, Inc. (MSI) develops, manufactures and markets a comprehensive range of filters, filtration devices, hybridization membranes and diagnostic membranes to meet the needs of life science researchers, diagnostic kit manufacturers, biopharmaceutical companies, food and beverage processors, and electronic manufacturers throughout the world. MSI products are used for applications such as transfer and hybridization of DNA; disease detection; sterilization of pharmaceuticals; purification of wines, juices, and other beverages; and the removal of deleterious materials from biological solutions.

Millipore Corporation
80 Ashby Road
Bedford, MA 01730
(617) 275-9200

Millipore Corporation develops, manufactures and markets products for analysis and purification. Millipore's technology base includes membrane filtration, liquid chromatography, ion exchange, and peptide and DNA synthesis and sequencing. The Company markets its products to the pharmaceutical, electronics, and food and beverage industries, as well as to laboratories engaged in life science research and environmental testing, hospitals and clinics.

NemaPharm, Inc.
100 Inman Street
Cambridge, MA 02139
(617) 864-6830

NemaPharm applies innovative technologies utilizing well-studied model organisms, principally the soil nematode *Caenorhabditis elegans*, for the discovery of novel and useful chemicals. The product focus of NemaPharm includes new agricultural chemicals and animal health agents, as well as selected human pharmaceuticals.

New England Biolabs, Inc.
32 Tozer Road
Beverly, MA 01915
(508) 927-5054

New England BioLabs is a cooperative laboratory of experienced scientists dedicated to providing the highest purity research enzymes. Found in 1975, NEB has grown steadily and now provides more than 300 products used daily throughout the molecular biology community.

Nissin Molecular Biology Institute, Inc.
20 Overland Street
Boston, MA 02215
(617) 262-6899

Nissin Molecular Biology Institute was founded in May 1988 by Nissin Food Products Co., Ltd. of Japan as an independent laboratory to be based in Boston. The company is studying anti-viral agents for AIDS and other infectious diseases, as well as anti-cancer agents.

OmniGene, Inc.
85 Bolton Street
Cambridge, MA 02140
(617) 576-1966

OmniGene, Inc. is a biotechnology company founded in early 1991 by a group of managers and key scientists from BioTechnica International, Inc. (BTI) to acquire and operate the Cambridge-based non-agricultural businesses and research activities of BTI. The Company has assembled a select team of experienced managers and prominent scientists, including personnel who have pioneered the development of many key genetic engineering technologies.

Opta Food Ingredients, Inc.
64 Sidney Street
Cambridge, MA 02139
(617) 252-0005

Opta Food Ingredients, Inc. is dedicated to developing and marketing patentable, high value food ingredients used by food processors to create healthful, natural and convenient foods. The company combines basic expertise in food processing with the latest advances in biotechnology as it relates to food.

Organogenesis Inc.
83 Rogers Street
Cambridge, MA 02142
(617) 864-0640

Organogenesis Inc. is a fully integrated biotechnology company dedicated to the commercialization of products and services developed from the creation of tissue and organ equivalents for grafting in humans and for research and testing in government, academic and industrial laboratories. The TESTSKIN™ living skin equivalent is being sold as a model system for studies in product development and toxicity. GRAFTSKIN™, an allograft skin equivalent, is in Phase I clinical trials. Other organ equivalents are under study, both to replace diseased human organs and to serve as test organ systems.

OsteoArthritis Sciences, Inc.
One Kendall Square, Bldg. 200
Cambridge, MA 02139
(617) 252-6886

OsteoArthritis Sciences, Inc. was formed to develop and commercialize proprietary therapeutic drugs to treat osteoarthritis. Osteoarthritis (OA) is a very prevalent disorder second only to cardiovascular disease in its debilitating effects. Currently, $3.5 billion is spent annually in the United States in treating the symptoms of OA.

Procept, Inc.
840 Memorial Drive
Cambridge, MA 02139
(617) 491-1100

Procept, Inc. was formed to develop and market therapeutic compounds to enhance the clinical management of diseases of the immune system such as AIDS, allergy, autoimmune diseases, and disease related to organ and bone marrow transplantation. The Company's development efforts are based on its core technology, the manipulation of immune system receptors. Incorporated in 1985 to fund academic research in exchange for exclusive rights, Procept became an operating company in early 1989.

Protein Engineering Corp.
765 Concord Avenue
Cambridge, MA 02138
(617) 868-0868

Protein Engineering Corporation has developed a powerful, proprietary technology to engineer patentable novel proteins for specific pharmaceutical applications. The Company is directing its product development efforts towards important therapeutic areas including respiratory and inflammatory disorders and infectious disease.

Repligen Corporation
One Kendall Square, Bldg. 700
Cambridge, MA 02139
(617) 225-6000

Repligen Corporation is developing and manufacturing products for the health care market, primarily in the areas of AIDS, cancer and inflammation. The company's comprehensive AIDS program encompasses: vaccine and anti-infective development in collaboration with Merck & Co., Inc., therapeutic development in collaboration with Sandoz Ltd., and the manufacturing and marketing of recombinant antigenic fragments for HIV and HTLV-1 research and diagnostic applications. Platelet factor-4 is being developed as a potential therapeutic for cancer and other diseases. Repligen markets a line of recombinant Protein A products, primarily used to purify monoclonal antibodies. Repligen and Sandoz Ltd. formed a joint venture, Repligen Sandoz Research Corporation, to develop biotechnology-derived products for industrial and agricultural applications. Recently, Repligen licensed several patents that are key to its development of anti-inflammatory therapeutics.

Sepracor, Inc.
33 Locke Drive
Marlborough, MA 01752
(508) 481-6700

Sepracor, Inc. is a technology-driven separations company that develops, manufactures and markets products and processes based on highly selective films known as "smart" membranes. The company sells process-scale separations systems and disposable modules, focused at high value-added separations applications, directly to customers in the pharmaceutical, agrichemical and food/beverage industries. Sepracor plans to develop a family of medical devices based on bioactive membranes.

Seragen, Inc.
97 South Street
Hopkinton, MA 01748
(508) 435-2331

Seragen, Inc. is engaged in the design, creation and development of genetically engineered hybrid toxins. The Company has developed proprietary technologies for genetically fusing the toxic domains of the diptheria toxin gene with the targeting domains of naturally occurring polypeptides or hormones, creating novel therapeutic agents that are "programmed" to seek out and destroy specific disease-causing cells, leaving healthy cells unharmed.

Serono Laboratories, Inc.
76 Pacella Park Drive
Randolph, MA 02368
(617) 963-8154 (and)
Serono Diagnostics
100 Longwater Circle
Norwell, MA 02601
(617) 982-9000

Serono Laboratories, Inc. is a U.S. affiliate of The Ares-Serono Group, a worldwide developer and marketer of pharmaceutical and diagnostic products. Serono is responsible for the domestic marketing of certain pharmaceutical products, especially in the fields of human fertility and dermatology. The company is presently a manufacturer of recombinant human growth hormone for export.

T Cell Sciences, Inc.
38 Sidney Street
Cambridge, MA 02139
(617) 621-1400

Located in Cambridge, T Cell Sciences, Inc. is utilizing proprietary T cell receptor and soluble receptor technology to develop pharmaceutical products to treat heart disease, inflammatory diseases, autoimmune diseases and cancer. T Cell Diagnostics, Inc., a subsidiary of T Cell Sciences, develops, manufactures and markets products for monitoring immune system disorders. T Cell Diagnostics currently markets products to the medical research community and two products to the clinical diagnostic market.

Telor Ophthalmic Pharmaceuticals, Inc.
500 West Cummings Park
Suite 6950
Woburn, MA 01801
(617) 937-0393

Telor commenced operations in 1989 with the goal of building an ophthalmic pharmaceutical company to market innovative products for treatment of age-related, chronic eye diseases. Telor utilizes a unique network of leading researchers and clinicians in academic institutions to identify and research novel pharmacological and other approaches to treat the causes of eye disease. Lead developments include proprietary products for the treatment of glaucoma and increased intraocular pressure following surgery, with additional programs in the fields of cataracts, presbyopia, and macular degeneration.

Therion Biologics Corp.
76 Rogers Street
Cambridge, MA 02142
(617) 876-7779

Therion Biologics Corp. develops vaccines and immunotherapeutics for AIDS and other infectious and chronic diseases, including viral diseases and cancer.

Transkaryotic Therapies Inc.
195 Albany Street
Cambridge, MA 02139
(617) 491-7630

TKT INC. is devoted to the treatment and cure of human diseases utilizing recombinant DNA cell culture technology. One of the focuses of the Company is the development of a practical system of gene therapy - one that is applicable to a wide range of human diseases. In addition, TKT is developing novel protein delivery systems that will address many commonly encountered problems in clinical medicine.

TSI Corporation
One Innovation Drive
Worcester, MA 01605
(508) 755-0550

TSI is a life sciences company developing advanced toxicology tests and disease models based on genetic engineering and transgenic techniques to improve the efficiency of the pharmaceutical/chemical development process. TSI is also developing animal strains capable of producing commercially valuable pharmaceutical proteins, as well as strains with desirable growth and genetic characteristics.

Verax Corporation
124 Mt. Auburn Street
Suite 200
Cambridge, MA 02138

Verax's process technology is based on a patented microsphere utilized in the continuous culture of immobilized cells in a fluidized bed bioreactor. Verax offers a fully integrated proprietary system of hardware, consumables, applications support and contract manufacturing.

Vertex Pharmaceuticals Inc.
40 Allston Street
Cambridge, MA 02139
(617) 576-3111

Vertex is developing human therapeutics through the integrated application of structure-based rational drug design. Vertex is using the latest advances in chemistry, biology and physics to design unique molecules based on the structural features of proteins involved in the control of disease processes. Vertex's goal is to become a fully-integrated pharmaceutical firm by exploiting the advantages of rational drug design in the discovery and development of novel drugs.

Virus Research Institute
61 Moulton Street
Cambridge, MA 02138
(617) 864-6232

The Virus Research Institute is engaged in applied research and development of prophylactic and therapeutic products to combat infectious diseases.

W. R. Grace & Co.
One Ledgemont Center
128 Spring Street
Lexington, MA 02173
(617) 863-8720

W. R. Grace & Co. is an international corporation with extensive sales in specialty chemicals, energy products and services, and an emerging health care family of businesses. Grace's interest in biotechnology stems from its current sales of biological products, services and materials, including amino acids and chelating agents, semen to the dairy industry, filtration membranes, chromatography materials and cell culture equipment, dialysis services and materials, and generic pharmaceuticals. Grace's corporate research division supports these businesses with development projects related to membrane devices for medical therapeutics, diagnostics and artificial organs; hollow fiber mammalian cell bioreactor systems and media; and animal hormones and genetics.

Appendix 2

Examples of technically significant patents.

United States Patent [19]

Cohen et al.

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[54] BIOLOGICALLY FUNCTIONAL MOLECULAR CHIMERAS

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[57] ABSTRACT

Method and compositions are provided for replication and expression of exogenous genes in microorganisms. Plasmids or virus DNA are cleaved to provide linear DNA having ligatable termini, which are bound to a gene having complementary termini, to provide a biologically functional replicon with a desired phenotypical property. The replicon is inserted into a microorganism cell by transformation. Isolation of the transformants provides cells for replication and expression of the DNA molecules present in the modified plasmid. The method provides a convenient and efficient way to introduce genetic capability into microorganisms for the production of nucleic acids and proteins, such as medically or commercially useful enzymes, which may have direct usefulness, or may find expression in the production of drugs, such as hormones, antibiotics, or the like, fixation of nitrogen, fermentation, utilization of specific feedstocks, or the like.

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23 Claims, No Drawings

BIOLOGICALLY FUNCTIONAL MOLECULAR CHIMERAS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 959,288, filed Nov. 9, 1978, now U.S. Pat. No. 4,468,464; which is a continuation of application Ser. No. 687,430, filed May 17, 1976, now abandoned; which is a continuation-in-part of application Ser. No. 520,691, filed Nov. 4, 1974, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

Although transfer of plasmids among strains of *E. coli* and other Enterobacteriaceae has long been accomplished by conjugation and/or transduction, it has not been previously possible to selectively introduce particular species of plasmid DNA into these bacterial hosts or other microorganisms. Since microorganisms that have been transformed with plasmid DNA contain autonomously replicating extrachromosomal DNA species having the genetic and molecular characteristics of the parent plasmid, transformation has enabled the selective cloning and amplification of particular plasmid genes.

The ability of genes derived from totally different biological classes to replicate and be expressed in a particular microorganism permits the attainment of interspecies genetic recombination. Thus, it becomes practical to introduce into a particular microorganism, genes specifying such metabolic or synthetic functions as nitrogen fixation, photosynthesis, antibiotic production, hormone synthesis, protein synthesis, e.g. enzymes or antibodies, or the like—functions which are indigenous to other classes of organisms—by linking the foreign genes to a particular plasmid or viral replicon.

2. Brief Description of the Prior Art

References which relate to the subject invention are Cohen, et al., Proc. Nat. Acad. Sci., USA, 69, 2110 (1972); ibid, 70, 1293 (1973); ibid, 70, 3240 (1973); ibid, 71, 1030 (1974); Morrow, et al., Proc. Nat. Acad. Sci., 71, 1743 (1974); Novick, Bacteriological Rev., 33, 210 (1969); and Hershfeld, et al., Proc. Nat. Acad. Sci., in press; Jackson, et al., ibid, 69, 2904 (1972);

SUMMARY OF THE INVENTION

Methods and compositions are provided for genetically transforming microorganisms, particularly bacteria, to provide diverse genotypical capability and producing recombinant plasmids. A plasmid or viral DNA is modified to form a linear segment having ligatable termini which is joined to DNA having at least one intact gene and complementary ligatable termini. The termini are then covalently bound to form a "hybrid" plasmid molecule which is used to transform susceptible and compatible microorganisms. After transformation, the cells are grown and the transformants harvested. The newly functionalized microorganisms may then be employed to carry out their new function; for example, by producing proteins which are the desired end product, or metabolites of enzymic conversion, or be lysed and the desired nucleic acids or proteins recovered.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The process of this invention employs novel plasmids, which are formed by covalently inserting DNA having one or more intact genes into a plasmid in such a location as to permit retention of an intact replicator locus and systems (replicon) to provide a recombinant plasmid molecule. The recombinant plasmid molecule will be referred to as a "hybrid" plasmid or plasmid "chimera." The plasmid chimera contains genes that are capable of expressing at least one phenotypical property. The plasmid chimera is used to transform a susceptible and competent microorganism under conditions where transformation occurs. The microorganism is then grown under conditions which allow for separation and harvesting of transformants that contain the plasmid chimera.

The process of this invention will be divided into the following stages:

I. preparation of the recombinant plasmid or plasmid chimera;

II. transformation or preparation of transformants; and

III. replication and transcription of the recombinant plasmid in transformed bacteria.

I. PREPARATION OF PLASMID CHIMERA

In order to prepare the plasmid chimera, it is necessary to have a plasmid, which can be cleaved to provide an intact replicator locus and system (replicon), where the linear segment has ligatable termini or is capable of being modified to introduce ligatable termini. A small number of such plasmids presently exist. Of particular interest are those plasmids which have a phenotypical property, which allow for ready separation of transformants from the patent microorganism. The plasmid will be capable of replicating in a microorganism, particularly a bacterium, which is susceptible to transformation. Various unicellular microorganisms can be transformed, such as bacteria, fungii and algae. That is, those unicellular organisms which are capable of being grown in cultures or fermentation. Since bacteria are for the most part the most convenient organisms to work with, bacteria will be hereinafter referred to as exemplary of the other unicellular organisms. Bacteria, which are susceptible to transformation, include members of the Enterobacteriaceae, such as strains of *Escherichia coli;* Salmonella; Bacillaceae, such as *Bacillus subtilis;* Pneumococcus; Streptococcus, and *Haemophilus influenzae.*

A wide variety of plasmids may be employed of greatly varying molecular weight. Normally, the plasmids employed will have molecular weights in the range of about 1×10^6 to 50×10^6d, more usually from about 1 to 20×10^6d, and preferably, from about 1 to 10×10^6d, the desirable plasmid size is determined by a number for factors. First, the plasmid must be able to accommodate a replicator locus and one or more genes that are capable of allowing replication of the plasmid. Secondly, the plasmid should be of a size which provides for a reasonable probability of recircularization with the foreign gene(s) to form the recombinant plasmid chimera. Desirably, a restriction enzyme should be available, which will cleave the plasmid without inactivating the replicator locus and system associated with the replicator locus. Also, means must be provided for providing ligatable termini for the plasmid, which are

complementary to the termini of the foreign gene(s) to allow fusion of the two DNA segments.

Another consideration for the recombinant plasmid is that it be compatible with the bacterium to be transformed. Therefore, the original plasmid will usually be derived from a member of the family to which the bacterium belongs.

The original plasmid should desirably have a phenotypical property which allows for the separation of transformant bacteria from parent bacteria. Particularly useful is a gene, which provides for survival selection. Survival selection can be achieved by providing resistance to a growth inhibiting substance or providing a growth factor capability to a bacterium deficient in such capability.

Conveniently, genes are available, which provide for antibiotic or heavy metal resistance or polypeptide resistance, e.g. colicin. Therefore, by growing the bacteria on a medium containing a bacteriostatic or bacteriocidal substance, such as an antibiotic, only the transformants having the antibiotic resistance will survive. Illustrative antibiotics include tetracycline, streptomycin, sulfa drugs, such as sulfonamide, kanamycin, neomycin, penicillin, chloramphenicol, or the like.

Growth factors include the synthesis of amino acids, the isomerization of substrates to forms which can be metabolized or the like. By growing the bacteria on a medium which lacks the appropriate growth factor, only the bacteria which have been transformed and have the growth factor capability will clone.

One plasmid of interest derived from *E. coli* is referred to as pSC101 and is described in Cohen, et al., Proc. Nat. Acad. Sci., USA, 70, 1293 (1972), (referred to in that article as Tc6-5). Further description of this particular plasmid and its use is found in the other articles previously referred to.

The plasmid pSC101 has a molecular weight of about 5.8×10^6d and provides tetracycline resistance.

Another plasmid of interest is colicinogenic factor EI (ColEI), which has a molecular weight of 4.2×10^6d, and is also derived from *E. coli*. The plasmid has a single EcoRI substrate site and provides immunity to colicin E1.

In preparing the plasmid for ligation with the exogenous gene, a wide variety of techniques can be provided, including the formation of or introduction of cohesive termini. Flush ends can be joined. Alternatively, the plasmid and gene may be cleaved in such a manner that the two chains are cleaved at different sites to leave extensions at each and which serve as cohesive termini. Cohesive termini may also be introduced by removing nucleic acids from the opposite ends of the two chains or alternatively, introducing nucleic acids at opposite ends of the two chains.

To illustrate, a plasmid can be cleaved with a restriction endonuclease or other DNA cleaving enzyme. The restriction enzyme can provide square ends, which are then modified to provide cohesive termini or can cleave at different, but adjacent, sites on the two strands, so as to provide cohesive termini directly.

Where square ends are formed such as, for example, by HIN (*Haemophilus influenzae* RII) or pancreatic DNAse, one can ligate the square ends or alternatively one can modify the square ends by chewing back, adding particular nucleic acids, or a combination of the two. For example, one can employ appropriate transferases to add a nucleic acid to the 5' and 3' ends of the DNA. Alternatively, one can chew back with an enzyme, such as a λ-exonuclease, and it is found that there is a high probability that cohesive termini will be achieved in this manner.

An alternative way to achieve a linear segment of the plasmid with cohesive termini is to employ an endonuclease such as EcoRI. The endonuclease cleaves the two strands at different adjacent sites providing cohesive termini directly.

With flush ended molecules, a T_4 ligase may be employed for linking the termini. See, for example, Scaramella and Khorana, J. Mol. Biol. 72: 427–444(1972) and Scaramella, DNAS 69: 3389(1972), whose disclosure is incorporated herein by reference.

Another way to provide ligatable termini is to cleave employing DNAse and Mn^{++} as reported by Lai and Nathans, J. Mol. Biol. 89: 179(1975).

The plasmid, which has the replicator locus, and serves as the vehicle for introduction of a foreign gene into the bacterial cell, will hereafter be referred to as "the plasmid vehicle."

It is not necessary to use plasmid, but any molecule capable of replication in bacteria can be employed. Therefore, instead of plasmid, viruses may be employed, which will be treated in substantially the same manner as the plasmid, to provide the ligatable termini for joining to the foreign gene.

If the production of cohesive termini is by restriction endonuclease cleavage, the DNA containing the foreign gene(s) to be bound to the plasmid vehicle will be cleaved in the same manner as the plasmid vehicle. If the cohesive termini are produced by a different technique, an analogous technique will normally be employed with the foreign gene. (By foreign gene is intended a gene derived from a source other than the transformant strain.) In this way, the foreign gene(s) will have ligatable termini, so as to be able to be covalently bonded to the termini of the plasmid vehicle. One can carry out the cleavage or digest of the plasmids together in the same medium or separately, combine the plasmids and recircularize the plasmids to form the plasmid chimera in the absence of active restriction enzyme capable of cleaving the plasmids.

Descriptions of methods of cleavage with restriction enzymes may be found in the following articles: Greene, et al., *Methods in Molecular Biology*, Vol. 9, ed. Wickner, R. B., (Marcel Dekker, Inc., New York), "DNA Replication and Biosynthesis"; Mertz and Davis, 69, Proc. Nat. Acad. Sci., USA, 69, 3370 (1972);

The cleavage and non-covalent joining of the plasmid vehicle and the foreign DNA can be readily carried out with a restriction endonuclease, with the plasmid vehicle and foreign DNA in the same or different vessels. Depending on the number of fragments, which are obtained from the DNA endonuclease digestion, as well as the genetic properties of the various fragments, digestion of the foreign DNA may be carried out separately and the fragments separated by centrifugation in an appropriate gradient. Where the desired DNA fragment has a phenotypical property, which allows for the ready isolation of its transformant, a separation step can usually be avoided.

Endonuclease digestion will normally be carried out at moderate temperatures, normally in the range of 10 to 40° C. in an appropriately buffered aqueous medium, usually at a pH of about 6.5 to 8.5. Weight percent of total DNA in the reaction mixture will generally be about 1 to 20 weight percent. Time for the reaction will vary, generally being from 0.1 to 2 hours. The amount

of endonuclease employed is normally in excess of that required, normally being from about 1 to 5 units per 10 μg of DNA.

Where cleavage into a plurality of DNA fragments results, the course of the reaction can be readily followed by electrophoresis. Once the digestion has gone to the desired degree, the endonuclease is inactivated by heating above about 60° C. for five minutes. The digestion mixture may then be worked up by dialysis, gradient separation, or the like, or used directly.

The plasmid vehicle and foreign DNA fragments are then allowed to combine to form hydrogen bonds and recircularize. This process is referred to as annealing and DNA ligation. An appropriate buffered medium is employed containing the DNA fragments, DNA ligase, and appropriate cofactors. The temperature employed initially for annealing will be about −5 to 15° C. When DNA segments hydrogen bond, the DNA ligase will be able to introduce a covalent bond between the two segments. Where the two ends of each of the segments are hydrogen bonded to one another, they may be ligated to form a circularized recombinant plasmid. The mole ratio of the two segments will generally be in the range of 1–5:5–1. The particular temperature for annealing will depend upon the binding strength of the cohesive termini. While 0.5 to 2 or more days have been employed for annealing, it is believed that only a short period of 0.5 to 6 hours may be sufficient, since annealing and ligation can occur under ligating conditions. The time employed for the annealing will vary with the temperature employed, the nature of the salt solution, as well as the nature of the sticky ends or cohesive termini.

The foreign DNA can be derived from a wide variety of sources. The DNA may be derived from eukaryotic or prokaryotic cells, viruses, and bacteriophage. The fragments employed will generally have molecular weights in the range of about 0.5 to 20×10^6d, usually in the range of 1 to 10×10^6d. The DNA fragment may include one or more genes or one or more operons.

Desirably, if the plasmid vehicle does not have a phenotypical property which allows for isolation of the transformants, the foreign DNA fragment should have such property.

The covalent joining can be achieved in conventional ways employing a DNA ligase. Ligation is conveniently carried out in an aqueous solution (pH, 6–8) at temperatures in the range of 5° to 40° C. The concentration of the DNA will generally be from about 10 to 100 μg/ml. A sufficient amount of the DNA ligase or other ligating agent, e.g. T_4 ligase, is employed to provide a convenient rate of reaction, generally ranging from 5 to 50 U/ml. Small amounts of a protein, e.g. albumin, may be added at concentrations of 10 to 200 μg/ml. The ligation with DNA ligase is carried out in the presence of Mg^{++} at about 1–10 mM.

At the completion of the ligation, the solution may be chilled and is ready for use in transformation.

In accordance with the subject invention, plasmids may be prepared which have replicons and genes which could be present in bacteria as a result of normal mating of bacteria. However, the subject invention provides a technique, whereby a replicon and gene can coexist in a plasmid, which is capable of being introduced into a unicellular organism, which could not exist in nature. The first type of plasmid which cannot exist in nature is a plasmid which derives its replicon from one organism and the exogenous gene from another organism, where the two organisms do not exchange genetic information. In this situation, the two organisms will either be eukaryotic or prokaryotic. Those organisms which are able to exchange genetic information by mating are well known. Thus, prior to this invention, plasmids having a replicon and one or more genes from two sources which do not exchange genetic information would not have existed in nature. This is true, even in the event of mutations, and induced combinations of genes from different strains of the same species. For the natural formation of plasmids formed from a replicon and genes from different microorganisms it is necessary that the microorganisms be capable of mating and exchanging genetic information.

In the situation, where the replicon comes from a eukaryotic or prokaryotic cell, and at least one gene comes from the other type of cell, this plasmid heretofore could not have existed in nature. Thus, the subject invention provides new plasmids which cannot naturally occur and can be used for transformation of unicellular organisms to introduce genes from other unicellular organisms, where the replicon and gene could not previously naturally coexist in a plasmid.

Besides naturally occurring genes, it is feasible to provide synthetic genes, where fragments of DNA may be joined by various techniques known in the art. Thus, the exogenous gene may be obtained from natural sources or from synthetic sources.

The plasmid chimera contains a replicon which is compatible with a bacterium susceptible of transformation and at least one foreign gene which is directly or indirectly bonded through deoxynucleotides to the replicon to form the circularized plasmid structure. As indicated previously, the foreign gene normally provides a phenotypical property, which is absent in the parent bacterium. The foreign gene may come from another bacterial strain, species or family, or from a plant or animal cell. The original plasmid chimera will have been formed by in vitro covalent bonding between the replicon and foreign gene. Once the originally formed plasmid chimera has been used to prepare transformants, the plasmid chimera will be replicated by the bacterial cell and cloned in vitro by growing the bacteria in an appropriate growth medium. The bacterial cells may be lysed and the DNA isolated by conventional means or the bacteria continually reproduced and allowed to express the genotypical property of the foreign DNA.

Once a bacterium has been transformed, it is no longer necessary to repeat the in vitro preparation of the plasmid chimera or isolate the plasmid chimera from the transformant progeny. Bacterial cells can be repeatedly multiplied which will express the genotypical property of the foreign gene.

One method of distinguishing between a plasmid which originates in vivo from a plasmid chimera which originates in vitro is the formation of homoduplexes between an in vitro prepared plasmid chimera and the plasmid formed in vivo. It will be an extremely rear event where a plasmid which originates in vivo will be the same as a plasmid chimera and will form homoduplexes with plasmid chimeras. For a discussion of homoduplexes, see Sharp, Cohen and Davidson, J. Mol. Biol., 75, 235 (1973), and Sharp, et al, ibid, 71, 471, (1972).

The plasmid derived from molecular cloning need not homoduplex with the in vitro plasmid originally employed for transformation of the bacterium. The bacterium may carry out modification processes, which

will not affect the portion of the replicon introduced which is necessary for replication nor the portion of the exogenous DNA which contains the gene providing the genotypical trait. Thus, nucleotides may be introduced or excised and, in accordance with naturally occurring mating and transduction, additional genes may be introduced. In addition, for one or more reasons, the plasmids may be modified in vitro by techniques which are known in the art. However, the plasmids obtained by molecular cloning will homoduplex as to those parts which relate to the original replicon and the exogenous gene.

II. TRANSFORMATION

After the recombinant plasmid or plasmid chimera has been prepared, it may then be used for the transformation of bacteria. It should be noted that the annealing and ligation process not only results in the formation of the recombinant plasmid, but also in the recircularization of the plasmid vehicle. Therefore, a mixture is obtained of the original plasmid, the recombinant plasmid, and the foreign DNA. Only the original plasmid and the DNA chimera consisting of the plasmid vehicle and linked foreign DNA will normally be capable of replication. When the mixture is employed for transformation of the bacteria, replication of both the plasmid vehicle genotype and the foreign genotype will occur with both genotypes being replicated in those cells having the recombinant plasmid.

Various techniques exist for transformation of a bacterial cell with plasmid DNA. A technique, which is particularly useful with *Escherichia coli*, is described in Cohen, et al., ibid, 69, 2110 (1972). The bacterial cells are grown in an appropriate medium to a predetermined optical density. For example, with *E. coli* strain C600, the optical density was 0.85 at 590 nm. The cells are concentrated by chilling, sedimentation and washing with a dilute salt solution. After centrifugation, the cells are resuspended in a calcium chloride solution at reduced temperatures (approx. 5°–15° C.), sedimented, resuspended in a smaller volume of a calcium chloride solution and the cells combined with the DNA in an appropriately buffered calcium chloride solution and incubated at reduced temperatures. The concentration of Ca^{++} will generally be about 0.01 to 0.1M. After a sufficient incubation period, generally from about 0.5–3.0 hours, the bacteria are subjected to a heat pulse generally in the range of 35° to 45° C. for a short period of time; namely from about 0.5 to 5 minutes. The transformed cells are then chilled and may be transferred to a growth medium, whereby the transformed cells having the foreign genotype may be isolated.

An alternative transformation technique may be found in Lederberg and Cohen, J. Bacteriol., 119, 1072 (1974), whose disclosure is incorporated herein by reference.

III. REPLICATION AND TRANSCRIPTION OF THE PLASMID

The bacterial cells, which are employed, will be of such species as to allow replication of the plasmid vehicle. A number of different bacteria which can be employed, have been indicated previously. Strains which lack indigenous modification and restriction enzymes are particularly desirable for the cloning of DNA derived from foreign sources.

The transformation of the bacterial cells will result in a mixture of bacterial cells, the dominant proportion of which will not be transformed. Of the function of cells which are transformed, some significant proportion, but normally a minor proportion, will have been transformed by the recombinant plasmid. Therefore, only a very small fraction of the total number of cells which are present will have the desired phenotypical characteristics.

In order to enhance the ability to separate the desired bacterial clones, the bacterial cells, which have beeen subjected to transformation, will first be grown in a solution medium, so as to amplify the absolute number of the desired cells. The bacterial cells may then be harvested and streaked on an appropriate agar medium. Where the recombinant plasmid has a phenotype, which allows for ready separation of the transformed cells from the parent cells, this will aid in the ready separation of the two types of cells. As previously indicated, where the genotype provides resistance to a growth inhibiting material, such as an antibiotic or heavy metal, the cells can be grown on an agar medium containing the growth inhibiting substance. Only available cells having the resistant genotype will survive. If the foreign gene does not provide a phenotypical property, which allows for distinction between the cells transformed by the plasmid vehicle and the cells transformed by the plasmid chimera, a further step is necessary to isolate the replicated plasmid chimera from the replicated plasmid vehicle. The steps include lysing of the cells and isolation and separation of the DNA by conventional means or random selection of transformed bacteria and characterization of DNA from such transformants to determine which cells contain molecular chimeras. This is accomplished by physically characterizing the DNA by electrophoresis, gradient centrifugation or electron microscopy.

Cells from various clones may be harvested and the plasmid DNA isolated from these transformants. The plasmid DNA may then be analyzed in a variety of ways. One way is to treat the plasmid with an appropriate restriction enzyme and analyze the resulting fragments for the presence of the foreign gene. Other techniques have been indicated above.

Once the recombinant plasmid has been replicated in a cell and isolated, the cells may be grown and multiplied and the recombinant plasmid employed for transformation of the same or different bacterial strain.

The subject process provides a technique for introducing into a bacterial strain a foreign capability which is genetically mediated. A wide variety of genes may be employed as the foreign genes from a wide variety of sources. Any intact gene may be employed which can be bonded to the plasmid vehicle. The source of the gene can be other bacterial cells, mammalian cells, plant cells, etc. The process is generally applicable to bacterial cells capable of transformation. A plasmid must be available, which can be cleaved to provide a linear segment having ligatable termini, and an intact replicator locus and system, preferably a system including a gene which provides a phenotypical property which allows for easy separation of the transformants. The linear segment may then be annealed with a linear segment of DNA having one or more genes and the resulting recombinant plasmid employed for transformation of the bacteria.

By introducing one or more exogenous genes into a unicellular organism, the organism will be able to produce polypeptides and proteins ("poly(amino acids)") which the organism could not previously produce. In

some instances the poly(amino acids) will have utility in themselves, while in other situations, particularly with enzymes, the enzymatic product(s) will either be useful in itself or useful to produce a desirable product.

One group of poly(amino acids) which are directly useful as hormones. Illustrative hormones includes parathyroid hormone, growth hormone, gonadotropins (FSH, luteinizing hormone, chroionogonadatropin, and glycoproteins), insulin, ACTH, somatostatin, prolactin, placental lactogen, melanocyte stimulating hormone, thyrotropin, parathyroid hormone, calcitonin, enkephalin, and angiotensin.

Other poly(amino acids) of interest include serum proteins, fibrinogin, prothrombin, thromboplastin, globulin e.g. gamma-globulins or antibodies, heparin, antihemophilia protein, oxytocin, albumins, actin, myosin, hemoglobin, ferritin, cytochrome, myoglobin, lactoglobulin, histones, avidin, thyroglobulin, interferon, kinins and transcortin.

Where the gene or genes produce one or more enzymes, the enzymes may be used for fulfilling a wide variety of functions. Included in these functions are nitrogen fixation, production of amino acids e.g. polyiodothyronine, particularly thyroxine, vitamins, both water and fat soluble vitamins, antimicrobial drigs, chemotherapeutic agents e.g. antitumor drugs, polypeptides and proteins e.g. enzymes from apoenzymes and hormones from prohormones, diagnostic reagents, energy producing combinations e.g. photosynthesis and hydrogen production, prostaglandins, steroids, cardiac glycosides, coenzymes, and the like.

The enzymes may be individually useful as agents separate from the cell for commercial applications, e.g. in detergents, synthetic transformations, diagnostic agents and the like. Enzymes are classified by the I.U.B. under the classification as I. Oxidoreductases; II. Transferases; III. Hydrolases; IV. Lyases; V. Isomerases; and VI. Ligases.

EXPERIMENTAL

In order to demonstrate the subject invention, the following experiments were carried out with a variety of foreign genes.

(All temperatures not otherwise indicated are Centrigrade. All percents not otherwise indicated are percents by weight.)

EXAMPLE A

A. Preparation of pSC101 Plasmid

Covalently closed DNA was sheared with a Virtis stainless steel microshaft blade in a one milliliter cup. The DNA was sheared at 2,000 r.p.m. for 30 minutes in TEN buffer solution (0.02M Tris-HCl (pH 8.0)-1mM EDTA (pH 8.0)-0.02M NaCl), while chilled at 0°–4°.

The sheared DNA sample was subjected to sucrose gradient sedimentation at 39,500 r.p.m. in a Spinco SW 50.1 rotor at 20°. A 0.12 ml fraction was collected on a 2.3 cm diameter circle of Whatman No. 3 filter paper, dried for 20 minutes and precipitated by immersion of the disc in cold 5% trichloroacetic acid, containing 100 μg/ml thymidine. The precipitate was filtered and then washed once with 5% trichloroacetic acid, twice with 99% ethanol and dried.

B. Generalized Transformation Procedure

E. coli strain C600 was grown at 37° in H1 medium to an optical density of 0.85 at 590 nm. At this point the cells were chilled quickly, sedimented and washed once in 0.5 volume 10mM NaCl. After centrifugation. the bacteria were resuspended in half the original volume of chilled 0.03M calcium chloride. kept at 0° for 20 minutes, sedimented, and then resuspended in 0.1 of the original volume of 0.03M of calcium chloride solution. Chilled DNA samples in TEN buffer were supplemented with 0.1M calcium chloride to a final concentration of 0.03M.

0.2 ml of competent cells treated with calcium chloride was added to 0.1 ml of DNA solution with chilled pipets and an additional incubation was done for 60 minutes at 0°. The bacteria were then subjected to a heat pulse at 42° for two minutes, chilled, and then either plated directly onto nutrient agar containing appropriate antibiotics or, where indicated, diluted 10 times in L-broth and incubated at 37° before plating. The cell survival is greater than 50% after calcium chloride treatment and heat pulse. Drug resistance was assayed on nutrient agar plates with the antibiotics indicated in specific experiments.

EXAMPLE I

Construction of Biologically Functional Bacterial Plasmids in vitro

A. Covalently closed R6-5 plasmid DNA was cleaved by incubation at 37° for 15 minutes in a 0.2 ml reaction mixture containing DNA (40 μg/ml, 100mM Tris.HCl (pH 7.4)), 5mM $MgCl_2$, 50mM NaCl, and excess (2U) EcoRI endonuclease in 1 μl volume. An additional incubation at 60° for 5 minutes was employed to inactivate the endonuclease.

The resulting mixture of plasmid fragments was employed for transformation of *E. coli* strain C600 in accordance with the procedure previously described. A single clone was examined further which was selected for resistance to kanamycin and was also found to carry resistance to neomycin and sulfonamide, but not to tetracycline, chloramphenicol, or streptomycin after transformation of *E. coli* by EcoRI generated DNA fragments of R6-5. Closed circular DNA obtained from this isolate (plasmid designation pSC102) by CsCl-ethidium bromide gradient centrifugation has an S value of 39.5 in neutral sucrose gradients.

Treatment of pSC102 plasmid DNA with EcoRI restriction endonuclease in accordance with the above-described procudure resulted in the formation of 3 fragments that were separable by electrophoresis in agarose gels. Intact pSC102 plasmid DNA and pSC101 plasmid DNA a 27S species having a calculated molecular weight of $5.8 \times 10.^6$d), which had been separately purified by dye-buoyant density centrifugation, were treated with EcoRI endonuclease followed by annealing at 0°–2° for about six hours. The mixture was then subjected to ligation with pSC101 and pSC102 in a ratio of 1:1 respectively, by ligating for 6 hours at 14° in 0.2 ml reaction mixtures containing 5mM $MgCl_2$, 0.1mM NAD, 100 μg/ml of bovine-serum albumin (BSA), 10mM ammonium sulphate (pH 7.0), and 18 U/ml of DNA ligase. (J. Mertz and Davis, Proc. Nat. Acad. Sci., USA, 69, 3370 (1972); and Modrich, et al., J. Biol. Chem., 248, 7495 (1973). Ligated mixtures were incubated at 37° for 5 minutes and then chilled in ice water. Aliquots containing 3.3–6.5 μg/ml of total DNA were used directly for transformation.

Transformation of *E. coli* strain C600 was carried out as previously described. For comparison purposes, transformation was also carried out with a mixture of

pSC101 and pSC102 plasmid DNA, which had been subjected to EcoRI endonuclease, but not DNA ligase. The antibiotics used for selection were tetracycline (10 μg/ml) and kanamycin (25 μg/ml). The results are reported as transformants per microgram of DNA. The following table indicates the results.

TABLE I

Transformation of *E. coli* C600 by a mixture of pSC101 and pSC102 DNA

Treatment of DNA	Transformation frequency for antibiotic resistance markers: Tetracycline	Kanamycin	Tetracycline + kanamycin
None	2×10^5	1×10^5	2×10^2
EcoRI	1×10^6	1.1×10^3	7×10^1
EcoRI + DNA ligase	1.2×10^6	1.3×10^3	5.7×10^2

Kanamycin resistance in the R65 plasmid is a result of the presence of the enzyme kanamycin monophosphotransferase. The enzyme can be isolated from the bacteria by known procedures and employed in an assay for kanamycin in accordance with the procedure described in Smith, et al., New England J. Medicine, 286, 583 (1972).

In the preparation for the enzyme extracts, the *E. coli* are grown in ML-broth and harvested in a late logarithm phase of growth. The cells are osmotically shocked (see Nossal, et al., J. Biol. Chem., 241, 3055 (1966), washed twice at room temperature with 10 ml 0.01M Tris and 0.03M NaCl, pH 7.3, and the pellet suspended in 10 ml 20% sucrose, 3×10^{-3}M EDTA and 0.033M Tris (pH 7.5), stirred for 10 minutes at room temperature and centrifuged at 16,000 g for 5 minutes. The pellet is then suspended in 2 ml of cold 5×10^{-4}M $MgCl_2$, stirred for 10 minutes at 2° and centrifuged at 26,000 g for 10 minutes to yield a supernatant fluid referred to as the osmotic shockate. The solution should be stored at −20° or lower. (See Benveneste, et al., FEBS Letters, 14 293 (1971)).

The osmotic shockate may then be used in accordance with the procedure of Smith, et al, supra.

EXAMPLE II

Genome Construction between Bacterial Species in vitro: Replication and Expression of Staphylococcus Plasmid Genes in *E. coli*

S. aureus strain 8325 contains the plasmid pI258, which expresses resistance to penicillin, erythromycin, cadmium and mercury. (Lindberg, et al, J. Bacteriol., 115, 139 (1973)). Covalently closed circular pSC101 and pI258 plasmid DNA were separately cleaved by incubation at 37° for 15 minutes in 0.2 ml reaction mixtures by EcoRI endonuclease in accordance with the procedure described previously. Aliquots of the two cleaved species were mixed in a ratio of 3 μg of pI258:1 μg of pSC101 and annealed at 2°–4° for 48 hours. Subsequent ligation was carried out for six hours at 14° as described previously and aliquots containing 3.3–6.5 μg/ml of total DNA were used directly in the transformation as described previously.

Other transformations were carried out employing the two plasmids independently and a mixture of the two plasmids. Selection of transformants was carried out at antibiotic concentrations for tetracycline (Tc, 25 μg/ml) or pencillin (Pc, 250 U/ml). The transformation was carried out with *E. coli* strain C600 $r_K^- m_K^-$. The following table indicates the results.

TABLE II

Transformation of C600 $r_K^- m_K^-$ by pSC101 and pI258 Plasmid DNA

DNA	Transformants/μg DNA: Tc	Pc
PSC101 closed circular	1×10^6	<3
pI258 closed circular	<3.6	<3.6
pSC101 + pI258 untreated	9.1×10^3	<5
pSC101 + pI258 EcoRI-treated	4.7×10^3	10

The above table demonstrates that bacteria can be formed which have both tetracycline resistance and penicillin resistance. Thus, one can provide the phenotypical property penicillin resistance in bacteria from DNA, which is indigenous to another biological organism. One can thus use *E. coli* for the production of the enzyme, which imparts penicillin resistance to bacteria, and assay for penicillin in a manner similar to that employed for kanamycin. Penicillinase is used for destroying penicillin in blood serum of patients treated with penicillin in order to determine whether pathogenic organisms whose growth is inhibited by penicillin may be present.

EXAMPLE III

Replication and Transcription of Eukaryotic DNA in *E. coli*

The amplified ribosomal DNA (rDNA) coding for 18S and 28S ribsomal RNA of the South African toad, *Xenopus laevis* was used as a source of eukaryotic DNA for these experiments. Dawid, et al., J. Mol. Biol., 51, 341 (1970). *E. coli-X. laevis* recombinant plasmids were constructed in vitro as follows:

The reaction mixture (60 μl) contained 100mM Tris HCl (pH 7.5) 50mM NaCl, 5mM $MgCl_2$, 1.0 μg of pSC101 plasmid DNA and 2.5 μg of *X.laevis* rDNA, and excess EcoRI restriction endonuclease (1 μl, 2 U). After a 15 minute incubation at 37°, the reaction mixture was placed at 63° for 5 minutes to inactivate EcoRI endonuclease. The product was then refrigerated at 0.5° for 24 hours, to allow association of the short cohesive termini.

The reaction mixture for ligation of phosphodiester bonds was adjusted to a total volume of 100 μl and contained in addition to the components of the endonuclease reaction, 30mM Tris.HCl (pH 8.1), 1mM sodium EDTA, 5mM $MgCl_2$, 3.2nM NAD, 10mM ammonium sulphate, 5 μg BSA, and 9U of *E. coli* DNA ligase. All components were chilled to 5° before their addition to the reaction mixture. The ligase reaction mixture was incubated at 14° for 45 minutes, and then at 0.5° for 48 hours. Additional NAD and ligase were added and the mixture incubated at 15° for 30 minutes and then for 15 minutes at 3720 . The ligated DNA was used directly in the plasmid transformation procedure previously described. The DNA was used to transform *E. coli* strain C600 $r_K^- m_K^-$ and tetracycline resistant transformants (3.3×10^3/μg of pSC101 DNA) were selected and numbered consecutively CD1, CD2, etc. Plasmid DNA was isolated from a number of the transformants.

^{32}P-labeled 18S and 28S *X. laevis* rRNA were hybridized with DNA obtained from the plasmids CD4, CD18, CD30, and CD42. CD4 DNA annealed almost equally with both the 18S and 28S rRNA species. CD18 plasmid hybridized principally with 28S *X. laevis*

rRNA, while the DNA of plasmids CD30 and CD42 annealed primarily with 18S rRNA. These data indicate that portions of the *X. laevis* rDNA were, in fact, incorporated into a plasmid recombinant with pSC101, which was capable of transforming *E. coli*, so as to be capable of replicating *X. laevis* rDNA.

Transcription of *X. laevis* DNA was also carried out in *E. coli* minicells. The minicell producing *E. coli* strain P678-54 was transformed with plasmid DNA isolated from *E. coli* strain C600 $r_K^-m_K^-$ containing CD4, CD18, or CD42. Many cells containing the plasmids were isolated and incubated with [^{3}H] uridine; RNA purified from such minicells was hybridized with *X. laevis* rDNA immobilized on nitrocellulose membranes in order to determine whether the *X. laevis* rDNA linked to the pSC101 replicon is transcibed in *E. coli*. The results in the following table show the RNA species capable of annealing with purified *X. laevis* rDNA are synthesized in *E. coli* minicells carrying the recombinant plasmids CD4, CD18, and CD42, but not by minicells carrying the pSC101 plasmid alone.

Minicells containing plasmids were isolated as described by Cohen, et al., Nature New Biol., 231, 249 (1971). They were incubated with [^{3}H] uridine (50 μCi/ml, 30 Ci/mol) as described by Roozen, et al., J. Bacteriol., 107, 21 (1971) for 10 minutes at 37°. Minicells collected by centrifugation were resuspended in Tris.HCl (20mM, pH 7.5)-5mM $MgCl_2$-1mM EDTA pH 8.0 and rapidly frozen and thawed 3 times. RNA was extracted as described in Cohen, et al., J. Mol. Biol., 37, 387 (1968). Hybridization assays were carried out in nitrocellulose membranes as described in Cohen, et al., ibid, at saturating levels of pSC101 DNA. Hybridizations involving *X. laevis* DNA were not performed at DNA excess. Counts bound to blank filters (5–10 c.p.m.) were subtracted from experimentally determined values. ^{3}H count eluted from filters containing *X. laevis* DNA were rendered acid soluble by ribonuclease A 20 μg/ml, 0.30M NaCl-0.030M sodium citrate, 1 hour, 37°. The following table indicates the results.

TABLE III

	[^{3}H] RNA synthesized by *E. coli* minicells			
Plasmid		[^{3}H] RNA counts hybridized to		
carried by	Input	*X. laevis* rDNA		pSC101 DNA
minicells	cpm	0.2 μg	0.4 μg	18 μg
CD42	4810	905 (19%)	1436 (30%)	961 (20%)
CD18	3780	389 (10%)	—	1277 (34%)
CD4	3220	789 (15%)	—	1015 (19%)
pSC101	4170	0 (0%)	—	1500 (36%)

EXAMPLE IV

Plasmid ColE1 as a Molecular Vehicle for Cloning and Amplification of Trp Operon

In a volume of 200 μl (100mM Tris.HCl (pH 7.5)-5mM $MgCl_2$-50mM NaCl), 5.7 μg of ColE1 (*E. coli* JC411Thy$^-$/ColEl) (Clewell, et al., Proc. Nat. Acad. Sci., USA, 62, 1159 (1969) and 6.0 μg DNA from bacteriophage φ80pt190 (Deeb, et al., Virology, 31, 289 (1967) were digested to completion with homogeneously purified EcoRI endonuclease, monitoring the digestion by electrophoresis of the fragments in an agarose gel. The endonuclease was inactivated by heating at 65° for 5 minutes, the digest dialyzed overnight against 5mM Tris.HCl, pH 7.6, and the sample concentrated to 50 μl. The fragments were ligated as described in Dugaiczyk, et al., Biochemistry, 13, 503 (1974) at a concentration of 75 pmoles/ml of fragments.

Transformation was carried out as previously described except that the cells were grown to $A_{590}=0.600$ and following exposure to DNA were incubated in L-broth for 90 minutes. The cells were collected and resuspended in 10mM NaCl before plating. Cells employed as recipients for the transformations were *E. coli* strains C600 trpR', ΔtrpE5(MV1), C600 trpR$^-$ trpE 10220 recA(MV2), C600 ΔtrpE5(MV10) and C600 ΔtrpE5 recA(MV12). (trpR$^-$ is the structural gene for the trp repressor and ΔtrpE5 is a trp operon deletion entirely within trpE and removing most of the gene.) Approximately 2 μg of the DNA was used to transform the cells.

Cultures were plated on Vogel-Bonner agar supplemented with 50 μg/ml of the non-selective amino acids, 0.2% glucose and 5 μg/ml of required vitamins. Transformants to colicin immunity were initially selected on a lawn of a culture of a mutant strain carrying ColE1. Clones were then selected for their ability to grow in the absence of tryptophan. Cells capable of producing tryptophan were isolated, which could be used for the production of exogenous tryptophan. The subject example demonstrates the introduction of a complete operon from foreign DNA to provide a transformant capable of replicating the operon and transcribing and translating to produce enzymes capable of producing an aromatic amino acid.

It is evident from the above results, that both DNA from a eukaryotic source and RNA transcribed from the eukaryotic DNA can be formed in a bacterial cell and isolated. Thus, the subject process provides a simple technique for producing large amounts of eukaryotic DNA and/or RNA without requiring the reproduction and maintenance of the eukaryotic organism or cells. The employment of DNA for production of ribosomal RNA is merely illustrative of using a genome from a eukaryotic cell for formation of a recombinant plasmid for replication in a bacteria. Genomes from a eukaryotic cell for formation of genotypical properites, such as the production of enzymes, could have equivalently been used. As evidenced by the transformation with DNA from a bacteriophage, an entire operon can be introduced into a bacterial cell and the cell becomes capable of its transcription, translation, and production of a functional gene product. Thus, a wide variety of auxotrophic properites can be introduced into a bacterial cell.

In accordance with the subject invention, DNA vehicles are provided, which are covalently closed circular extrachromosomal replicons or genetic elements, including plasmids and viral DNA. THe vehicles generally will have molecular weights in the range of about 1 to 20×10^6 and are characterized by having an intact replicon, which includes a replicator locus and gene. The vehicle is capable of cleavage by a restriction enzyme to provide a linear segment having an intact replicon and cohesive termini, which may be directly obtained by the cleavage or by subsequent modification of the termini of the linear segment. The vehicle will be capable of transforming a bacterial cell and to that extent is compatible with the cell which will provide replication and translation. Preferably, the vehicle will have a phenotypical property which will allow for segregation of the transformant cells. Phenotypical properites include resistance to growth inhibiting materials, such as antibiotics, peptides and heavy metals,

morphological properites, color, or the like, and production of growth factors, e.g. amino acids.

The vehicle is combined with DNA indigenous to a biological organism other than the cell which provides replication and provides a genotypical or phenotypical property which is alien to the cell. The source of the DNA can be prokaryotic or eukaryotic, thus including bacteria, fungi, vertebrates, e.g. mammals and the like.

The plasmid vehicle and the alien DNA having complementary cohesive termini can be annealed together and covalently linked to provide a recombinant plasmid, which is capable of transforming a bacterial cell, so as to be capable of replication, transcription, and translation. As a result, a wide variety of unique capabilities can be readily introduced into bacteria, so as to provide convenient ways to obtain nucleic acids and to study nucleic acids from a foreign host. Thus, the method provides the ability to obtain large amounts of a foreign nucleic acid from bacteria in order to be able to study the function and nature of the nucleic acid. In addition, the subject method provides means for preparing enzymes and enzymic products from bacteria where the natural host is not as convenient or efficient a source of such product. Particularly, bacteria may allow for more ready isolation of particular enzymes, uncontaminated by undesirble contaminants, which are present in the original host. In addition, the products of the enzymic reactions may be more readily isolated and more efficiently produced by a transformant than by the original host. Besides enzymes, other proteins can be produced such as antibodies, antigens, albumins, globulins, glycoproteins, polysaccharides, and the like.

Although the foregoing invention has been described in some detail by way of illustration and example for purpsoes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

What is claimed is:

1. A method for replicating biologically functional circular recombinant DNA molecules in transformed host cells, which comprises:

(1) preparing biologically functional circular recombinant DNA molecules by: joining first and second DNA linear segments, said first and second DNA segments having termini of a predetermined character at least in part complementary, at least one of said first and second DNA linear segments having a gene for a phenotypical trait, said first DNA linear segment having an intact extrachromosomal replicon recognized by said host cell, and said second DNA linear segment having foreign DNA derived from a source which does not exchange genetic information with said host cells;

(2) transforming said host cells with said biologically functional recombinant DNA molecules;

(3) growing said host cells under appropriate nutrient conditions; and

(4) isolating said transformant cells with said biologically functional recombinant DNA molecules by means of said phenotypical trait imparted by said biologically functional recombinant DNA molecules.

2. A method according to claim 1 wherein said host cells are unicellular organisms.

3. A method according to claim 1, wherein said host cells are prokaryotic organisms.

4. A method according to claims 1, 2 or 3 wherein said foreign DNA of said segment includes an intact gene capable of expression in said host cells, and including the additional step of growing said isolated transformant cells in an appropriate medium, whereby said intact gene is expressed and protein produced.

5. A method for producing foreign RNA from biologically functional circular recombinant DNA molecules in transformed host cells, which comprises:

(1) preparing biologically functional circular recombinant DNA molecules by: joining first and second DNA linear segments, said first and second DNA segments having termini of a predetermined character at least in part complementary, at least one of, said first and second linear DNA segments having a gene for a phenotypical trait, said first DNA linear segment having an intact extrachromsomal replicon recognized by said host cells, and said second DNA linear segment having foreign DNA derived from a source which does not exchange genetic information with said host cells;

(2) transforming said host cells with said biologically functional recombinant DNA molecules;

(3) growing said host cells under appropriate nutrient conditions; and

(4) isolating said RNA containing transformant cells with said biologically functional recombinant DNA molecules by means of said phenotypical trait imparted by said biologically functional recombinant DNA molecules.

6. A method for replicating biologically functional circular recombinant DNA molecules in transformed host cells, which comprises:

(1) preparing biologically functional circular recombinant DNA molecules by: joining first and second DNa linear segments, said first and second DNA segments having termini of a predetermined character at least in part complementary, at least one of said first and second DNA linear segments having a gene for a phenotypical trait, said first DNA linear segment having an intact replicon recognized by said host cells from plasmid or viral DNA, and said second DNA linear segment having foreign DNA derived from a source which does not exchange genetic information with said host cells;

(2) transforming said host cells with said biologically functional recombinant DNA molecules;

(3) growing said host cells under appropriate nutrient conditions; and

(4) isolating said transformant cells with said biologically functional recombinant DNA molecules by means of said phenotypical trait imparted by said biologically functional recombinant DNA molecules.

7. A method according to claim 6, wherein said host cells are unicellular organisms.

8. A method according to claim 6, wherein said host cells are prokaryotic organisms.

9. As an article of manufacture, a cloned biologically functional circular recombinant DNA molecule capable of selection and replication in a host cell comprising: a first DNA segment containing an intact extrachromsomal replicon recognized by said host cell, said first segment being joined to a second DNA segment having foreign DNA derived from a source which does not exchange genetic information with said host cell.

10. An article of manufacture according to claim 9, wherein said host cell is a unicellular organism.

11. An article of manufacture according to claim 9, wherein said host cell is a prokaryotic organism.

12. As an article of manufacture, a cloned biologically functional circular recombinant DNA molecule capable of selection and replication in a host cell comprising: a first DNA segment containing an intact replicon recognized by said host cell from plasmid or viral DNA joined to a second DNA segment comprising a foreign gene capable of expression in said host cell wherein said foreign gene is derived from a source which does not exchange genetic information with said host cell.

13. An article of manufacture according to claim 12, wherein said host cell is a unicellular organism.

14. An article of manufacture according to claim 12, wherein said host cell is a prokaryotic organism.

15. An article of manufacture according to claims 12, 13, or 14, wherein said gene has as a phenotypical property expression of an enzyme.

16. As an article of manufacture, a cloned biologically functional circular recombinant DNA molecule capable of selection and replication in a host cell comprising: cloned copies of a first DNA segment containing an intact extrachromsomal replicon recognized by said host cell from a plasmid or viral DNA joined to a second DNA segment comprising a foreign gene capable of expression in said host cell, wherein said foreign gene is derived from a source which does not exchange genetic information with said host cell.

17. A transformant cell comprising a biologically functional circular recombinant DNA molecule capable of selection and replication in said cell, said DNA molecule comprising: a first DNA segment containing an intact extrachromsomal replicon recognized by said cell, said first segment being joined to a second DNA segment having foreign DNA, wherein said foreign DNA is derived from a source which does not exchange genetic information with said host cell.

18. A transformant cell according to claim 17, wherein said cell is a unicellular organism.

19. A transformant cell according to claim 17, wherein said cell is a prokaryotic organism.

20. A transformant cell comprising a biologically functional circular recombinant DNA molecule capable of selection and replication in said cell, said DNA molecule comprising: a first DNA segment containing an intact extrachromsomal replicon recognized by said cell from plasmid or viral DNA, said first segment being joined to a second DNA segment comprising a foreign gene capable of expression in said cell wherein said foreign gene is derived from a source which does not exchange genetic information with said host cell.

21. A transformant cell according to claim 20, wherein said cell is a unicellular organism.

22. A transformant cell according to claim 20, wherein said cell is a prokaryotic organism.

23. A transformant cell according to claims 20, 21, or 22 wherein said cell includes the expression product of said foreign gene.

* * * * *

United States Patent [19]

Leder et al.

[11] Patent Number: 4,736,866

[45] Date of Patent: Apr. 12, 1988

[54] TRANSGENIC NON-HUMAN MAMMALS

[75] Inventors: Philip Leder, Chestnut Hill, Mass.; Timothy A. Stewart, San Francisco, Calif.

[73] Assignee: President and Fellows of Harvard College, Cambridge, Mass.

[21] Appl. No.: 623,774

[22] Filed: Jun. 22, 1984

[51] Int. Cl.[4] C12N 1/00; C12Q 1/68; C12N 15/00; C12N 5/00

[52] U.S. Cl. 800/1; 435/6; 435/172.3; 435/240.1; 435/240.2; 435/320; 435/317.1; 935/32; 935/59; 935/70; 935/76; 935/111

[58] Field of Search 435/6, 172.3, 240, 317, 435/320, 240.1, 240.2; 935/70, 76, 59, 111, 32; 800/1

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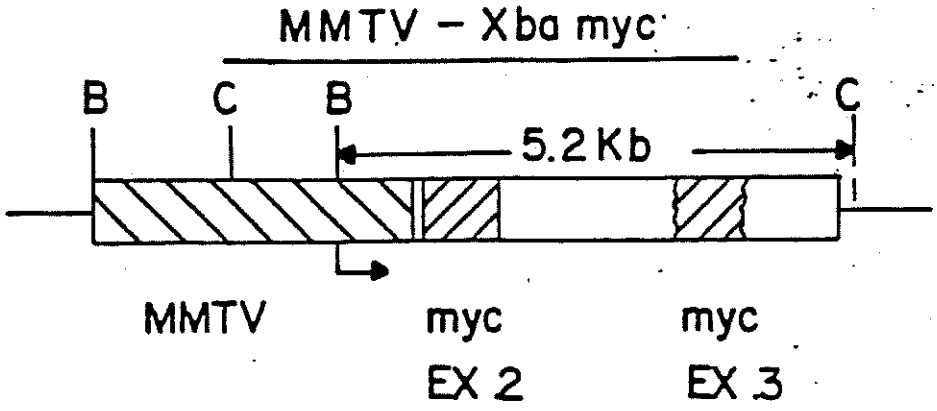
Primary Examiner—Alvin E. Tanenholtz
Attorney, Agent, or Firm—Paul T. Clark

[57] ABSTRACT

A transgenic non-human eukaryotic animal whose germ cells and somatic cells contain an activated oncogene sequence introduced into the animal, or an ancestor of the animal, at an embryonic stage.

12 Claims, 2 Drawing Sheets



16. A process according to claims 10, 11 or 12 wherein the antigenic substance is selected from the group consisting of IgE, hepatitis A, hepatitis B, hepatitis Non A/Non B, alphafetoprotein, carcinoembryonic antigen, insulin and human thyroid stimulating hormone.

17. A process according to claims 10, 11 or 12 wherein the labelled antibody is labelled with a member selected from the group consisting of a radioactive isotope, an enzyme and a fluorogenic material and said examination is by means selected from the group consisting of radiometric means, enzymatic means and fluorometric means.

18. A process according to claim 17 wherein said label is the radioactive isotope ^{125}I.

19. In an immunometric assay to determine the presence or concentration of an antigenic substance in a sample of a fluid comprising forming a ternary complex of a first labelled antibody, said antigenic substance, and a second antibody said second antibody being bound to a solid carrier insoluble in said fluid wherein the presence of the antigenic substance in the samples is determined by measuring either the amount of labelled antibody bound to the solid carrier or the amount of unreacted labelled antibody, the improvement comprising employing monoclonal antibodies having an affinity for the antigenic substance of at least about 10^8 liters/mole for each of said labelled antibody and said antibody bound to a solid carrier.

20. A process according to claim 19 wherein the fluid sample is first contacted with the second monoclonal antibody to form a binary complex of the antigenic substance and said second monoclonal antibody insoluble in the fluid and then contacted with said first labelled monoclonal antibody to form the ternary complex.

21. A process according to claim 19 wherein the fluid sample is first contacted with the second monoclonal antibody to form a binary complex of the antigenic substance and said second monoclonal antibody insoluble in the fluid; the sample separated from the solid carrier and the solid carrier contacted with a solution of said first labelled monoclonal antibody to form said ternary complex.

22. A process according to claim 21 wherein said solid carrier after formation of the ternary complex is washed to separate the fluid sample from the carrier.

23. A process according to claim 22 wherein the solid carrier is washed with phosphate buffered saline.

24. A process according to claims 19, 20 or 21 wherein said first monoclonal antibody is the product of a different cell line than said second monoclonal antibody.

25. A process according to claims 19, 20 or 21 wherein said antigen has at least two identical binding sites and said first and second monoclonal antibodies are the product of the same cell line.

26. A process according to claims 19, 20 or 21 wherein the affinity is at least about 10^9 liters/mole.

27. A process according to claims 19, 20 or 21 wherein the antigen is selected from the group consisting of IgE, hepatitis A, hepatitis B, hepatitis None A/Non B, alphafetoprotein, carcinoembryonic antigen, insulin and human thyroid stimulating hormone.

28. A process according to claims 19, 20 or 21 wherein the labelled antibody is labelled with a member selected from the group consisting of a radioactive isotope, an enzyme and a fluorogenic material.

29. A process according to claim 28 wherein said label is the radioactive isotope ^{125}I.

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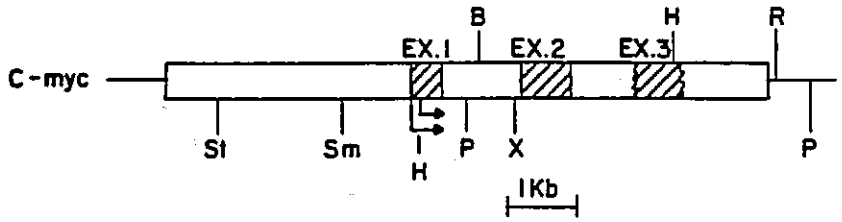

FIG 1

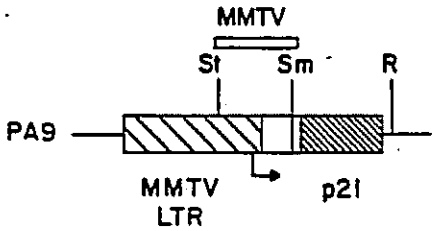

FIG 2

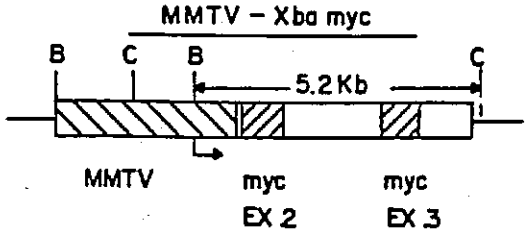

FIG 3

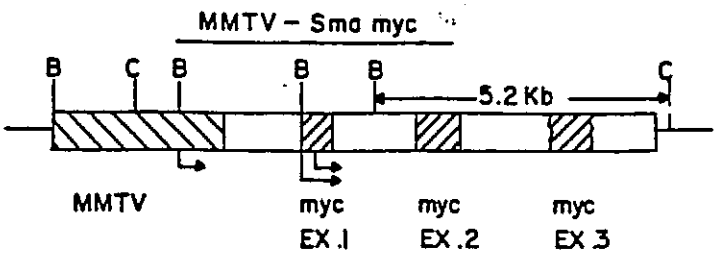

FIG 4

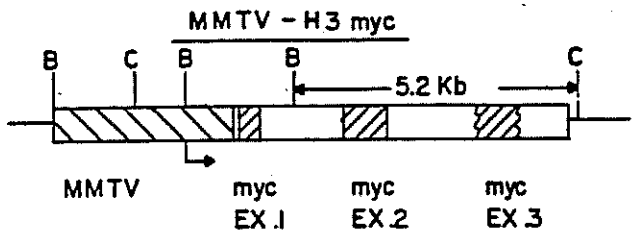

FIG 5

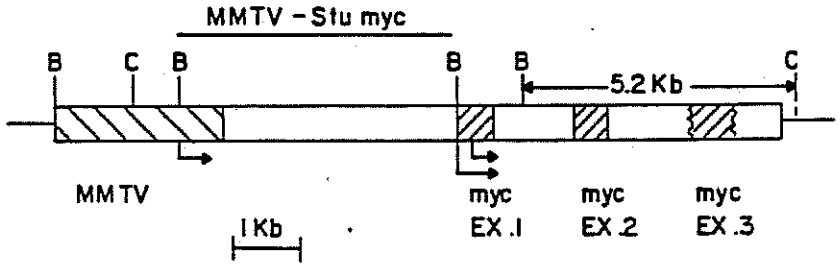

FIG 6

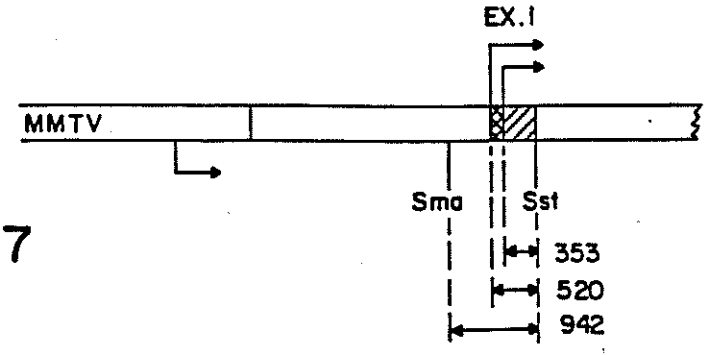

FIG 7

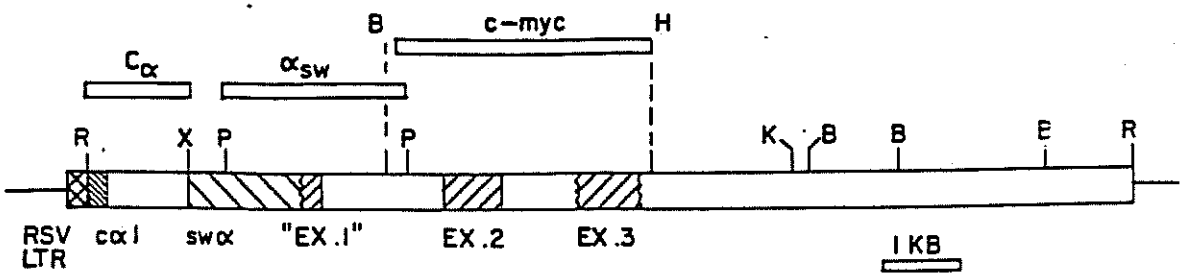

FIG 8

TRANSGENIC NON-HUMAN MAMMALS

BACKGROUND OF THE INVENTION

This invention relates to transgenic animals.

Transgenic animals carry a gene which has been introduced into the germline of the animal, or an ancestor of the animal, at an early (usually one-cell) developmental stage. Wagner et al. (1981) *P.N.A.S. U.S.A.* 78, 5016; and Stewart et al. (1982) *Science* 217, 1046 describe transgenic mice containing human globin genes. Constantini et al. (1981) *Nature* 294, 92; and Lacy et al. (1983) *Cell* 34, 343 describe transgenic mice containing rabbit globin genes. McKnight et al. (1983) *Cell* 34, 335 describes transgenic mice containing the chicken transferrin gene. Brinster et al. (1983) *Nature* 306, 332 describes transgenic mice containing a functionally rearranged immunoglobulin gene. Palmiter et al. (1982) *Nature* 300, 611 describes transgenic mice containing the rat growth hormone gene fused to a heavy metal-inducible metalothionein promoter sequence. Palmiter et al. (1982) *Cell* 29, 701 describes transgenic mice containing a thymidine kinase gene fused to a metalothionein promoter sequence. Palmiter et al. (1983) *Science* 222, 809 describes transgenic mice containing the human growth hormone gene fused to a metalothionein promoter sequence.

SUMMARY OF THE INVENTION

In general, the invention features a transgenic non-human eukaryotic animal (preferably a rodent such as a mouse) whose germ cells and somatic cells contain an activated oncogene sequence introduced into the animal, or an ancestor of the animal, at an embryonic stage (preferably the one-cell, or fertilized oocyte, stage, and generally not later than about the 8-cell stage). An activated oncogene sequence, as the term is used herein, means an oncogene which, when incorporated into the genome of the animal, increases the probability of the development of neoplasms (particularly malignant tumors) in the animal. There are several means by which an oncogene can be introduced into an animal embryo so as to be chromosomally incorporated in an activated state. One method is to transfect the embryo with the gene as it occurs naturally, and select transgenic animals in which the gene has integrated into the chromosome at a locus which results in activation. Other activation methods involve modifying the oncogene or its control sequences prior to introduction into the embryo. One such method is to transfect the embryo using a vector containing an already translocated oncogene. Other methods are to use an oncogene whose transcription is under the control of a synthetic or viral activating promoter, or to use an oncogene activated by one or more base pair substitutions, deletions, or additions.

In a preferred embodiment, the chromosome of the transgenic animal includes an endogenous coding sequence (most preferably the c-myc gene, hereinafter the myc gene), which is substantially the same as the oncogene sequence, and transcription of the oncogene sequence is under the control of a promoter sequence different from the promoter sequence controlling transcription of the endogenous coding sequence. The oncogene sequence can also be under the control of a synthetic promoter sequence. Preferably, the promoter sequence controlling transcription of the oncogene sequence is inducible.

Introduction of the oncogene sequence at the fertilized oocyte stage ensures that the oncogene sequence will be present in all of the germ cells and somatic cells of the transgenic animal. The presence of the oncogene sequence in the germ cells of the transgenic "founder" animal in turn means that all of the founder animal's descendants will carry the activated oncogene sequence in all of their germ cells and somatic cells. Introduction of the oncogene sequence at a later embryonic stage might result in the oncogene's absence from some somatic cells of the founder animal, but the descendants of such an animal that inherit the gene will carry the activated oncogene in all of their germ cells and somatic cells.

Any oncogene or effective sequence thereof can be used to produce the transgenic mice of the invention. Table 1, below, lists some known viral and cellular oncogenes, many of which are homologous to DNA sequences endogenous to mice and/or humans, as indicated. The term "oncogene" encompasses both the viral sequences and the homologous endogenous sequences.

TABLE 1

Abbreviation	Virus
src	Rous Sarcoma Virus (Chicken)
yes	Y73 Sarcoma Virus (Chicken)
fps	Fujinami (St Feline) Sarcoma Virus (Chicken, Cat)
abl	Abelson Marine Leukemia Virus (Mouse)
ros	Rochester-2 Sarcoma Virus (Chicken)
fgr	Gardner-Rasheed Feline Sarcoma Virus (Cat)
erbB	Avian Erythroblastosis Virus (Chicken)
fms	McDonough Feline Sarcoma Virus (Cat)
mos	Moloney Murine Sarcoma Virus (Mouse)
raf	3611 Murine Sarcoma$^+$ Virus (Mouse)
Ha-ras-1	Harvey Murine Sarcoma Virus (Rat)
Ki-ras 2	(Balb/c mouse; 2 loci) Kirsten Murine Sarcoma Virus (Rat)
Ki-ras 1	Kirsten Murine Sarcoma Virus (Rat)
myc	Avian MC29 Myelocytomatosis Virus (Chicken)
myt	Avian Myelo Blastomas (Chicken)
fos	FBJ Osteosarcoma Virus (Mouse)
ski	Avian SKV T10 Virus (Chicken)
rel	Reticuloendotheliosis Virus (Turkey)
sis	Simian Sarcoma Virus (Woolly Monkey)
N-myc	Neuroblastomas (Human)
N-ras	Neuroblastoma, Leukemia Sarcoma Virus (Human)
Blym	Bursal Lymphomas (Chicken)
mam	Mammary Carcionoma (Human)
neu	Neuro, Glioblastoma (Rat)
ertAl	Chicken AEV (Chicken)
ra-ras	Rasheed Sarcoma Virus

TABLE 1-continued

Abbreviation	Virus
	(Rat)
mnt-myc	Carcinoma Virus MH2 (Chicken)
myc	Myelocytomatosis OK10 (Chicken)
myb-ets	Avian myeloblastosis/ erythroblastosis Virus E26 (Chicken)
raf-2	3611-MSV (Mouse)
raf-1	3611-MSV (Mouse)
Ha-ras-2	Ki-MSV (Rat)
erbB	Erythroblastosis virus (Chicken)

The animals of the invention can be used to test a material suspected of being a carcinogen, by exposing the animal to the material and determining neoplastic growth as an indicator of carcinogenicity. This test can be extremely sensitive because of the propensity of the transgenic animals to develop tumors. This sensitivity will permit suspect materials to be tested in much smaller amounts than the amounts used in current animal carcinogenicity studies, and thus will minimize one source of criticism of current methods, that their validity is questionable because the amounts of the tested material used is greatly in excess of amounts to which humans are likley to be exposed. Furthermore, the animals will be expected to develop tumors much sooner because they already contain an activated oncogene. The animals are also preferable, as a test system, to bacteria (used, e.g., in the Ames test) because they, like humans, are vertebrates, and because carcinogenicity, rather than mutogenicity, is measured.

The animals of the invention can also be used as tester animals for materials, e.g. antioxidants such as beta-carotine or Vitamin E, thought to confer protection against the development of neoplasms. An animal is treated with the material, and a reduced incidence of neoplasm developmcot, compared to untreated animals, is detected as an indication of protection. The method can further include exposing treated and untreated animals to a carcinogen prior to, after, or simultaneously with treatment with the protective material.

The animals of the invention can also be used as a source of cells for cell culture. Cells from the animals may advantageously exhibit desirable properties of both normal and transformed cultured cells; i.e., they will be normal or nearly normal morphologically and physiologically, but can, like cells such as NIH 3T3 cells, be cultured for long, and perhaps indefinite, periods of time. Further, where the promoter sequence controlling transcription of the oncogene sequence is inducible, cell growth rate and other culture characteristics can be controlled by adding or eliminating the inducing factor.

Other features and advantages of the invention will be apparent from the description of the preferred embodiments, and from the claims.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The drawings will first briefly be described.

DRAWINGS

FIG. 1 is a diagrammatic representation of a region of a plasmid bearing the mouse myc gene and flanking regions.

FIG. 2 is a diagrammatic represenation of a region of a plasmid, pA9, bearing the mouse mammary tumor virus long terminal repeat (MMTV LTR) sequences.

FIGS. 3–6 and 8 are diagrammatic representations of activated oncogene fusions.

FIG. 7 is a diagrammatic representation of a probe useful for detecting activated myc fusions.

MMTV-MYC FUSED GENES

Gene fusions were made using the mouse myc gene and the MMTV LTR. The myc gene is known to be an activatable oncogene. (For example, Leder et al. (1983) *Science* 222, 765 explains how chromosomal translocations that characterize Burkitt's Lymphoma and mouse plasmacytomas result in a juxtaposition of the myc gene and one of the immunoglobulin constant regions; amplification of the myc gene has also been observed in transformed cell lines.) FIG. 1 illustrates the subclone of the mouse myc gene which provided the myc regions.

The required MMTV functions were provided by the pA9 plasmid (FIG. 2) that demonstrated hormone inducibility of the p21 protein; this plasmid is described in Huang et al. (1981) *Cell* 27, 245. The MMTV functions on pA9 include the region required for glucocorticoid control, the MMTV promoter, and the cap site.

The above plasmids were used to construct the four fusion gene contructions illustrated in FIGS. 3–6. The constructions were made by deleting from pA9 the Sma-EcoRI region that included the p21 protein coding sequences, and replacing it with the four myc regions shown in the Figures. Procedures were the conventional techniques described in Maniatis et al. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory). The restriction sites shown in FIG. 1 are StuI (St), SmaI (Sm), EcoRI (R), HindIII (H), PvuI (P), BamHI (B), XbaI (X), and ClaI (C). The solid arrows below the constructions represent the promoter in the MMTV LTR and in the myc gene. The size (in Kb) of the major fragment, produced by digestion with BamHI and ClaI, that will hybridize to the myc probe, is shown for each construction.

MMTV-H3 myc (FIG. 5) was constructed in two steps: Firstly, the 4.7 Kb Hind III myc fragment which contains most of the myc sequences was made blunt with Klenow polymerase and ligated to the pA9 SmaI-EcoRI vector that had been similarly treated. This construction is missing the normal 3' end of the myc gene. In order to introduce the 3' end of the myc gene, the PvuI-PvuI fragment extending from the middle of the first myc intron to the pBR322 PvuI site in the truncated MMTV-H3 myc was replaced by the related PvuI-PvuI fragment from the mouse myc subclone.

The MMTV-Xba myc construction (FIG. 3) was produced by first digesting the MMTV-Sma myc plasmid with SmaI and XbaI. The XbaI end was then made blunt with Klenow polymerase and the linear molecule recircularized with T4 DNA ligase. The MMTV-Stu myc (FIG. 6) and the MMTV-Sma myc (FIG. 4) constructions were formed by replacing the p21 protein coding sequences with, respectively, the StuI-EcoRI or SmaI-EcoRI myc fragments (the EcoRI site is within the pBR322 sequences of the myc subclone). As shown in FIG. 1, there is only one StuI site within the myc gene. As there is more than one SmaI site within the myc gene (FIG. 4), a partial SmaI digestion was carried out to generate a number of MMTV-Sma myc plasmids; the plasmid illustrated in FIG. 4 was selected as not showing rearrangements and also including a suffi-

ciently long region 5' of the myc promoter (approximately 1 Kb) to include myc proximal controlling regions.

The constructions of FIGS. 4 and 6 contain the two promoters naturally preceding the unactivated myc gene. The contruction of FIG. 5 has lost both myc promoters but retains the cap site of the shorter transcript. The construction of FIG. 3 does not include the first myc exon but does include the entire protein coding sequence. The 3' end of the myc sequence in all of the illustrated constructions is located at the HindIII site approximately 1 Kb 3' to the myc polyA addition site.

These constructions were all checked by multiple restriction enzyme digestions and were free of detectable rearrangements.

PRODUCTION OF TRANSGENIC MICE CONTAINING MMTV-MYC FUSIONS

The above MMTV-myc plasmids were digested with SalI and EcoRI (each of which cleaves once within the pBR322 sequence) and separately injected into the male pronuclei of fertilized one-cell mouse eggs; this resulted in about 500 copies of linearized plasmid per pronucleus. The injected eggs were then transferred to pseudo-pregnant foster females as described in Wagner et al. (1981) *P.N.A.S. U.S.A.* 78, 5016. The eggs were derived from a CD-1 X C57Bl/6J mating. Mice were obtained from the Charles River Laboratories (CD^{R}-1-Ha/Icr (CD-1), an albino outbred mouse) and Jackson Laboratories (C57Bl/6J), and were housed in an environmentally controlled facility maintained on a 10 hour dark: 14 hour light cycle. The eggs in the foster females were allowed to develop to term.

ANALYSIS OF TRANSGENIC MICE

At four weeks of age, each pup born was analyzed using DNA taken from the tail in a Southern hybridization, using a ^{32}P DNA probe (labeled by nick-translation). In each case, DNA from the tail was digested with BamHI and ClaI and probed with the ^{32}P-labeled BamHI/HindIII probe from the normal myc gene (FIG. 1).

The DNA for analysis was extracted from 0.1–1.5 cm sections of tail, by the method described in Davis et al. (1980) in Methods in Enzymology, Grossman et al., eds., 65, 404, except that one chloroform extraction was performed prior to ethanol precipitation. The resulting nucleic acid pellet was washed once in 80% ethanol, dried, and resuspended in 300 μl of 1.0 mM Tris, pH 7.4, 0.1 mM EDTA.

Ten μl of the tail DNA preparation (approximately 10 μg DNA) were digested to completion, electrophoresed through 0.8% agarose gels, and transferred to nitrocellulose, as described in Southern (1975) *J. Mol. Biol.* 98, 503. Filters were hybridized overnight to probes in the presence of 10% dextran sulfate and washed twice in 2 X SSC, 0.1% SDS at room temperature and four times in 0.1 X SSC, 0.1% SDS at 64° C.

The Southern hybridizations indicated that ten founder mice had retained an injected MMTV-myc fusion. Two founder animals had integrated the myc gene at two different loci, yielding two genetically distinct lines of transgenic mice. Another mouse yielded two polymorphic forms of the integrated myc gene and thus yielded two genetically distinct offspring, each of which carried a different polymorphic form of the gene. Thus, the 10 founder animals yielded 13 lines of transgenic offspring.

The founder animals were mated to uninjected animals and DNA of the resulting thirteen lines of transgenic offspring analyzed; this analysis indicated that in every case the injected genes were transmitted through the germline. Eleven of the thirteen lines also expressed the newly acquired MMTV-myc genes in at least one somatic tissue; the tissue in which expression was most prevelant was salivary gland.

Transcription of the newly acquired genes in tissues was determined by extracting RNA from the tissues and assaying the RNA in an Sl nuclease protection procedure, as follows. The excised tissue was rinsed in 5.0 ml cold Hank's buffered saline and total RNA was isolated by the method of Chrigwin et al. (1979) *Biochemistry* 18, 5294, using the CsCl gradient modification. RNA pellets were washed twice by reprecipitation in ethanol and quantitated by absorbance at 260 nm. An appropriate single stranded, uniformly labeled DNA probe was prepared as described by Ley et al. (1982) *PNAS USA* 79, 4775. To test for transcription of the MMTV-Stu myc fusion of FIG. 6, for example, the probe illustrated in FIG. 7 was used. This probe extends from a SmaI site 5' to the first myc exon to an SstI site at the 3' end of the first myc exon. Transcription from the endogenous myc promoters will produce RNA that will protect fragments of the probe 353 and 520 base pairs long; transcription from the MMTV promoter will completely protect the probe and be revealed as a band 942 base pairs long, in the following hybridization procedure.

Labelled, single-stranded probe fragments were isolated on 8M urea 5% acrylamide gels, electroeluted, and hybridized to total RNA in a modification of the procedure of Berk et al. (1977) *Cell* 12, 721. The hybridization mixture contained 50,000 cpm to 100,000 cpm of probe ($SA = 10^8$ cpm/μg), 10 μg total cellular RNA, 75% formamide, 500 mM NaCl, 20 mM Tris pH 7.5, 1 mM EDTA, as described in Battey et al. (1983) *Cell* 34, 779. Hybridization temperatures were varied according to the GC content in the region of the probe expected to hybridize to mRNA. The hybridizations were terminated by the addition of 1500 units of Sl nuclease (Boehringer Mannheim). Sl nuclease digestions were carried out at 37° C. for 1 hour. The samples were then ethanol-precipitated and electrophoresed on thin 8M urea 5% acrylamide gels.

Northern hybridization analysis was also carried out, as follows. Total RNA was electrophoresed through 1% formaldehyde 0.8% agarose gels, blotted to nitrocellulose filters (Lehrach et al. (1979) *Biochemistry* 16, 4743), and hybridized to nick-translated probes as described in Taub et al. (1982) *PNAS USA* 79, 7837. The tissues analyzed were thymus, pancreas, spleen, kidney, testes, liver, heart, lung, skeletal muscle, brain, salivary gland, and preputial gland.

Both lines of mice which had integrated and were transmitting to the next generation the MMTV-Stu myc fusion (FIG. 6) exhibited transcription of the fusion in salivary gland, but in no other tissue.

One of two lines of mice found to carry the MMTV-Sma myc fusion (FIG. 4) expressed the gene fusion in all tissues examined, with the level of expression being particularly high in salivary gland. The other line expressed the gene fusion only in salivary gland, spleen, testes, lung, brain, and preputial gland.

Four lines of mice carried the MMTV-H3 myc fusion (FIG. 5). In one, the fusion was transcribed in testes,

lung, salivary gland, and brain, in a second, the fusion was transcribed only in salivary gland, in a third, the fusion was transcribed in some of the somatic tissues tested, and in a fourth, the fusion was transcribed in salivary gland and intestinal tissue.

In two mouse lines found to carry the MMTV-Xba myc fusion, the fusion was transcribed in testes and salivary gland.

RSV-MYC FUSED GENES

Referring to FIG. 8, the plasmid designated RSV-S107 was generated by inserting the EcoRI fragment of the S107 plasmacytoma myc gene (Kuroh et al. (1981) *Nature* 293, 332) into a derivative of the Rous Sarcoma Virus (RSV) enhancer-containing plasmid (pRSVcat) described in Gorman et al. (1982) *PNAS USA* 79, 6777, at the EcoRI site 3' to the RSV enhancer sequence, using standard recombinant DNA techniques. All chloramphenicol acetyl transferase and SV40 sequences are replaced in this vector by the myc gene; the RSV promoter sequence is deleted when the EcoRI fragments are replaced, leaving the RSV enhancer otherwise intact. The original translocation of the myc gene in the S107 plasmacytoma deleted the two normal myc promoters as well as a major portion of the untranslated first myc exon, and juxtaposed, 5' to 5', the truncated myc gene next to the a immunoglobulin heavy chain switch sequence.

The illustrated (FIG. 8) regions of plasmid RSV-S107 are: crosshatched, RSV sequences; fine-hatched, alpha 1 coding sequences; left-hatched, immunoglobulin alpha switch sequences; right-hatched, myc exons. The thin lines flanking the RSV-S107 myc exon represent pBR322 sequences. The marked restriction enzyme sites are: R, EcoRI; X, XbaI; P, Pst I; K, Kpn I; H, HindIII; B, BamHI. The sequences used for three probes used in assays described herein (C-a, a-sw and c-myc) are marked.

PRODUCTION OF TRANSGENIC MICE

Approximately 500 copies of the RSV-S107 myc plasmid (linearized at the unique Kpn-I site 3' to the myc gene) were injected into the male pronucleus of eggs derived from a C57BL/6J x CD-1 mating. Mice were obtained from Charles River Laboratories (CD-1, an albino outbred mouse) and from Jackson Laboratories (C57BL, 6J). These injected eggs were transferred into pseudopregnant foster females, allowed to develop to term, and at four weeks of age the animals born were tested for retention of the injected sequences by Southern blot analysis of DNA extracted from the tail, as described above. Of 28 mice analyzed, two males were found to have retained the new genes and both subsequently transmitted these sequences through the germline in a ratio consistent with Mendelian inheritance of a single locus.

First generation transgenic offspring of each of these founder mice were analyzed for expression of the rearranged myc genes by assaying RNA extracted from the major internal tissues and organs in an S1 nuclease protection assay, as described above. The hearts of the offspring of one line showed aberrant myc expression, the other 13 tissues did not.

Backcrossing to C57BL/6J and inbreeding matings produced some transgenic mice which did not demonstrate the same restriction site patterns on Southern blot analysis as either their transgenic siblings or their parents. In the first generation progeny derived from a mating between the founder male and C57BL/6J females, 34 F1 animals were analyzed and of these, 19 inherited the newly introduced gene, a result consistent with the founder being a heterozygote at one locus. However, of the 19 transgenic mice analyzed, there were three qualitatively different patterns with respect to the more minor myc hybridizing fragments.

In order to test the possibility that these heterogenous genotypes arose as a consequence of multiple insertions and/or germline mosaicism in the founder, two F1 mice (one carrying the 7.8 and 12 Kb BamHI bands and the other carrying only the 7.8 Kb BamHI band) were mated and the F2 animals analyzed. One male born to the mating of these two appeared to have sufficient copies of the RSV-S107 myc gene to be considered as a candidate for having inherited the two alleles; this male was backcrossed with a wild-type female. All 23 of 23 backcross offspring analyzed inherited the RSV-S107 myc genes, strongly suggesting that the F2 male mouse had inherited two alleles at one locus. Further, as expected, the high molecular weight fragment (12 Kb) segregated as a single allele.

To determine whether, in addition to the polymorphisms arising at the DNA level, the level of aberrant myc expression was also altered, heart mRNA was analyzed in eight animals derived from the mating of the above double heterozygote to a wild-type female. All eight exhibited elevated myc mRNA, with the amount appearing to vary between animals, the lower levels of expression segregated with the presence of the 12 Kb myc hybridizing band. The level of myc mRNA in the hearts of transgenic mice in a second backcross generation also varied. An F1 female was backcrossed to a C57BL/6J male to produce a litter of seven pups, six of which inherited the RSV-S107 myc genes. All seven of these mice were analyzed for expression. Three of the six transgenic mice had elevated levels of myc mRNA in the hearts whereas in the other three the level of myc mRNA in the hearts was indistinguishable from the one mouse that did not carry the RSV-S107 myc gene. This result suggests that in addition to the one polymorphic RSV-S107 myc locus from which high levels of heart-restricted myc mRNA were transcribed, there may have been another segregating RSV-S107 myc locus that was transcriptionally silent.

CARCINOGENICITY TESTING

The animals of the invention can be used to test a material suspected of being a carcinogen, as follows. If the animals are to be used to test materials thought to be only weakly carcinogenic, the transgenic mice most susceptible of developing tumors are selected, by exposing the mice to a low dosage of a known carcinogen and selecting those which first develop tumors. The selected animals and their descendants are used as test animals by exposing them to the material suspected of being a carcinogen and determining neoplastic growth as an indicator of carcinogenicity. Less sensitive animals are used to test more strongly carcinogenic materials. Animals of the desired sensitivity can be selected by varying the type and concentration of known carcinogen used in the selection process. When extreme sensitivity is desired, the selected test mice can consist of those which spontaneously develop tumors.

TESTING FOR CANCER PROTECTION

The animals of the invention can be used to test materials for the ability to confer protection against the

development of neoplasms. An animal is treated with the material, in parallel with an untreated control transgenic animal. A comparatively lower incidence of neoplasm development in the treated animal is detected as an indication of protection.

TISSUE CULTURE

The transgenic animals of the invention can be used as a source of cells for cell culture. Tissues of transgenic mice are analyzed for the presence of the activated oncogene, either by directly analyzing DNA or RNA, or by assaying the tissue for the protein expressed by the gene. Cells of tissues carrying the gene can be cultured, using standard tissue culture techniques, and used, e.g., to study the functioning of cells from normally difficult to culture tissues such as heart tissue.

DEPOSITS

Plasmids bearing the fusion genes shown in FIGS. 3, 4, 5, 6, and 8 have been deposited in the American Type Culture Collection, Rockville, Md., and given, respectively, ATCC Accession Nos. 39745, 39746, 39747, 39748, and 39749.

OTHER EMBODIMENTS

Other embodiments are within the following claims. For example, any species of transgenic animal can be employed. In some circumstances, for instance, it may be desirable to use a species, e.g., a primate such as the rhesus monkey, which is evolutionarily closer to humans than mice.

We claim:

1. A transgenic non-human mammal all of whose germ cells and somatic cells contain a recombinant activated oncogene sequence introduced into said mammal, or an ancestor of said mammal, at an embryonic stage.

2. The mammal of claim 1, a chromosome of said mammal including an endogenous coding sequence substantially the same as a coding sequence of said oncogene sequence.

3. The mammal of claim 2, said oncogene sequence being integrated into a chromosome of said mammal at a site different from the location of said endogenous coding sequence.

4. The mammal of claim 2 wherein transcription of said oncogene sequence is under the control of a promoter sequence different from the promoter sequence controlling the transcription of said endogenous coding sequence.

5. The mammal of claim 4 wherein said promoter sequence controlling transcription of said oncogene sequence is inducible.

6. The mammal of claim 1 wherein said oncogene sequence comprises a coding sequence of a c-myc gene.

7. The mammal of claim 1 wherein transcription of said oncogene sequence is under the control of a viral promoter sequence.

8. The mammal of claim 7 wherein said viral promoter sequence comprises a sequence of an MMTV promoter.

9. The mammal of claim 7 wherein said viral promoter sequence comprises a sequence of an RSV promoter.

10. The mammal of claim 1 wherein transcription of said oncogene sequence is under the control of a synthetic promoter sequence.

11. The mammal of claim 1, said mammal being a rodent.

12. The mammal of claim 11, said rodent being a mouse.

* * * * *

United States Patent [19]

David et al.

[11] **4,376,110**

[45] **Mar. 8, 1983**

[54] **IMMUNOMETRIC ASSAYS USING MONOCLONAL ANTIBODIES**

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[73] Assignee: **Hybritech, Incorporated, La Jolla, Calif.**

[21] Appl. No.: **175,133**

[22] Filed: **Aug. 4, 1980**

[51] Int. Cl.³ G01N 33/54; G01N 33/56

[52] U.S. Cl. 436/513; 435/7; 436/548; 436/529; 436/540

[58] Field of Search ..... 23/230 B; 424/12, 1, 424/8; 435/7

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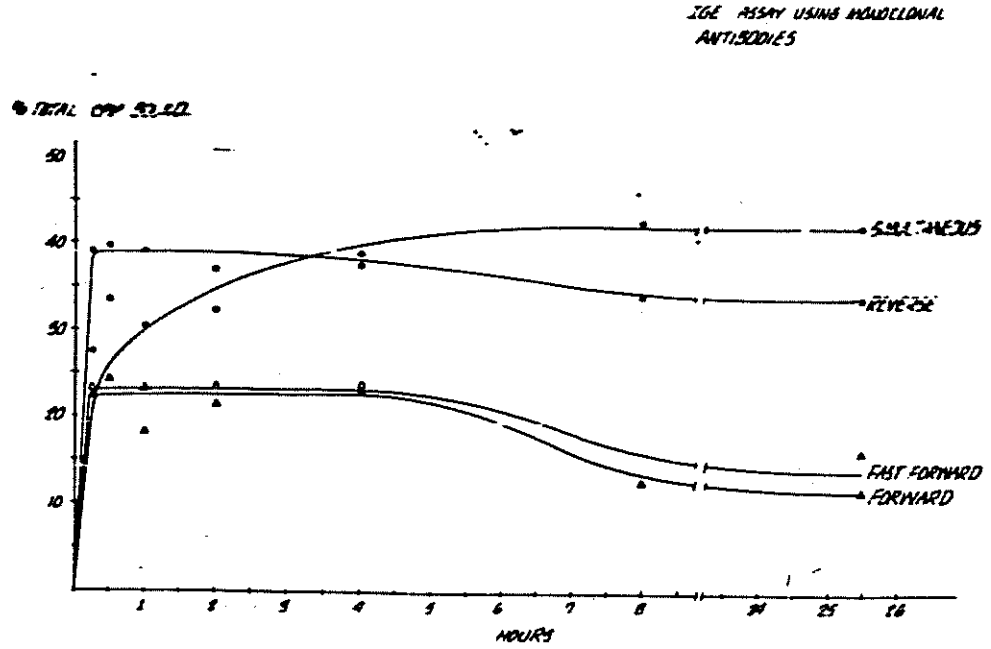
Primary Examiner—Sidney Marantz
Attorney, Agent, or Firm—Lyon & Lyon

[57] **ABSTRACT**

"Two-site" or "sandwich" immunometric assay techniques for determination of the presence and/or concentration of antigenic substances in fluids using monoclonal antibodies. One monoclonal antibody is presented in a soluble labeled form and a second monoclonal antibody is presented bound to a solid carrier; the soluble and bound monoclonal antibodies may be the products of either the same or different cell lines. Each monoclonal antibody has an affinity for the antigenic substances of at least about 10^8 liters/mole.

29 Claims, 2 Drawing Figures



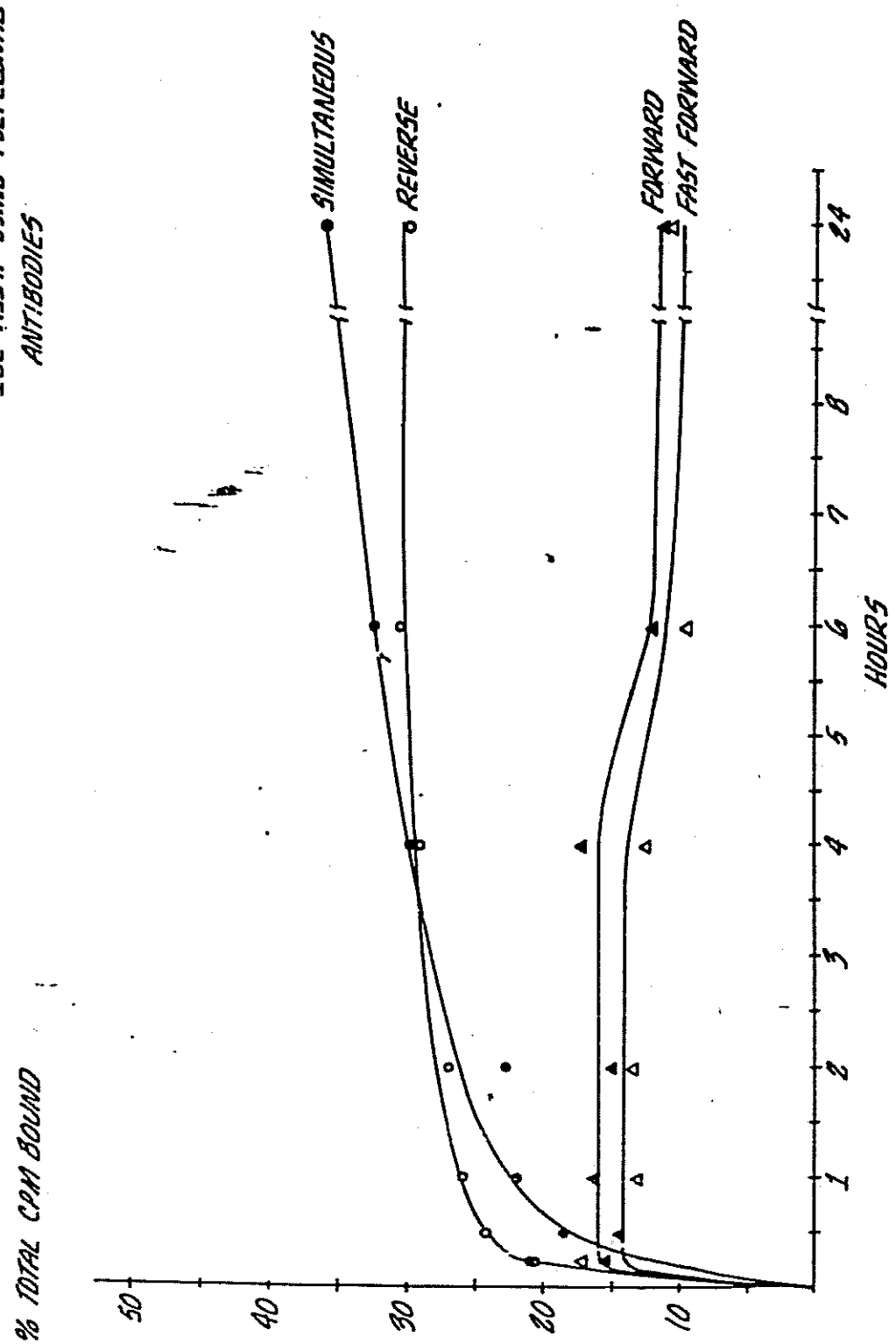
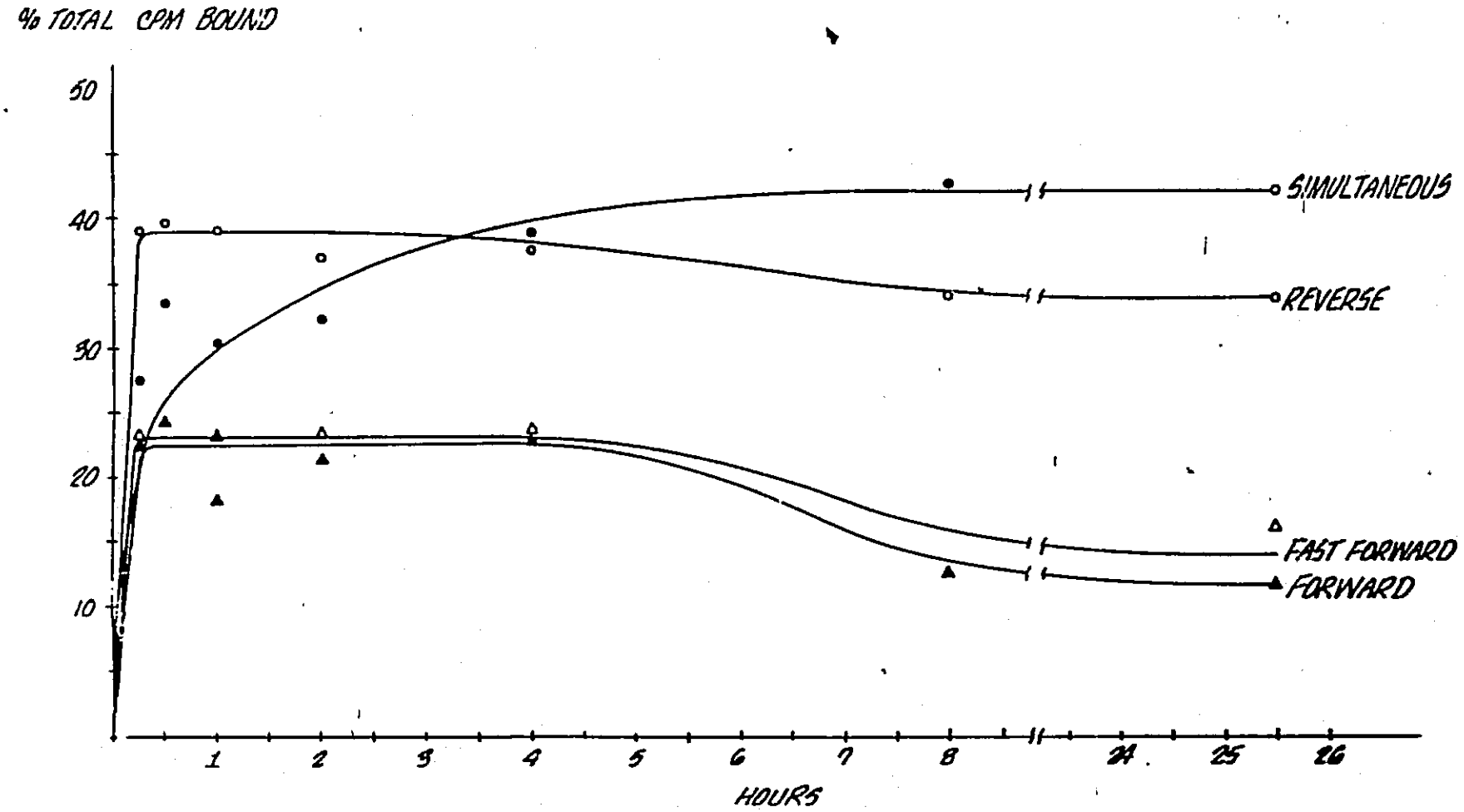

IGE ASSAY USING POLYCLONAL ANTIBODIES
% TOTAL CPM BOUND
50
40
30
20
10
1
2
3
4
5
6
7
8
24
HOURS
SIMULTANEOUS
REVERSE
FORWARD
FAST FORWARD

FIG. 1

FIG. 2.

IGE ASSAY USING MONOCLONAL ANTIBODIES



IMMUNOMETRIC ASSAYS USING MONOCLONAL ANTIBODIES

FIELD OF THE INVENTION

This invention relates to methods for detecting and/or determining the concentration of antigenic substances in fluids such as serum. In another aspect it relates to immunometric assay techniques. In yet another aspect it relates to monoclonal antibodies.

BACKGROUND

The determination of the presence or concentration of antigenic substances, for example, those associated with a wide variety of physiological disorders, in serum or other body fluids relies increasingly upon immunoassay techniques. These techniques are based upon formation of a complex between the antigenic substance being assayed and an antibody or antibodies in which one or the other member of the complex may be labelled, for example, by a radioactive element such as I^{125}, which permits its detection and/or quantitative analysis after separation of the complexed labelled antigen or antibody from uncomplexed labelled antigen or antibody.

In the case of a competition immunoassay technique, the antigenic substance in a sample of fluid being tested for its presence competes with a known quantity of labelled antigen for a limited quantity of antibody binding sites. Thus, the amount of labelled antigen bound to the antibody is inversely proportional to the amount of antigen in the sample. By contrast, immunometric assays employ a labelled antibody. In such an assay, the amount of labelled antibody associated with the complex is directly proportional to the amount of antigenic substance in the fluid sample.

Immunometric assays have been found to be particularly well suited for the detection of polyvalent antigens, i.e., antigenic substances that are able to complex with two or more antibodies at the same time. Such assays employ a quantity of unlabelled antibody bound to a solid support that is insoluble in the fluid being tested and a quantity of soluble antibody bearing a label such as a radioactive isotope that permits detection and/or a quantitative estimate of the amount of the ternary complex formed between solid phase antibody, antigen, and labelled antibody.

In immunometric assays known to the prior art, typically a "forward" assay, in which the antibody bound to the solid phase is first contacted with the sample being tested to extract the antigen from the sample by formation of a binary solid phase antibody:antigen complex, is employed. After a suitable incubation period, the solid support is washed to remove the residue of the fluid sample, including unreacted antigen if any, and then contacted with a solution containing a known quantity of labelled antibody.

After a second incubation period to permit the labelled antibody to complex with the antigen bound to the solid support through the unlabelled antibody, the solid support is washed a second time to remove the unreacted labelled antibody. In a simple "yes/no" assay to determine whether the antigen is present in the sample being tested, the washed solid support is tested to detect the presence of labelled antibody, for example, by measuring emitted radiation if the label is a radioactive element. The amount of labelled antibody detected is compared to that for a negative control sample known to be free of the antigen. Detection of labelled antibody in amounts substantially above the background levels indicated by the negative control is interpreted to indicate the presence of the suspect antigen. Quantitative determinations can be made by comparing the measure of labelled antibody with that obtained for standard samples containing known quantities of the antigen.

This kind of assay is frequently referred to as a "two-site" or "sandwich" assay since the antigen has two antibodies bonded to its surface at different locations. This and related techniques are described by Wide at pp. 199–206 of "Radiommunoassay Methods", Edited by Kirkham and Hunter, E. & S. Livingstone, Edinburgh, 1970. An assay based on this technique for the detection of the antigen associated with serum hepatitis using an 125_I labelled antibody is described in U.S. Pat. No. 3,867,517.

Despite their great utility, the prior art immunometric assays have been recognized to be slow procedures, in part because two washing steps are required and because lengthy incubation periods are required to reach equilibrium, i.e., the point at which the amount of complex formed does not change with increasing time.

To eliminate at least one of the washing steps associated with this procedure, so-called "simultaneous" and "reverse" assays have been proposed. A simultaneous assay involves a single incubation step as the antibody bound to the solid support and the labelled antibody are both added to the sample being tested at the same time. After the incubation is completed, the solid support is washed to remove the residue of fluid sample and uncomplexed labelled antibody. The presence of labelled antibody associated with the solid support is then determined as it would be in a conventional "forward" sandwich assay.

A reverse assay involves the stepwise addition first of a solution of labelled antibody to the fluid sample followed by the addition of unlabelled antibody bound to a solid support after a suitable incubation period. After a second incubation, the solid phase is washed in conventional fashion to free it of the residue of the sample being tested and the solution of unreacted labelled antibody. The determination of labelled antibody associated with the solid support is then determined as in the simultaneous and forward assays.

Both the simultaneous and reverse assay techniques require a sufficient excess amount of solid phase antibody to bind most or all of the antigen present to avoid a high dose hook effect where artificially negative or low quantitation of antigen is observed at extremely high concentrations of antigen. For this reason, the forward assay has been the approach preferred by the prior art. That is because large amounts of highly purified, active antibody specific to the antigen of interest for preparing a solid phase with sufficient antigen binding capacity is difficult to obtain from the "polyclonal" antibodies used in prior art processes. Methods for affinity purifying such antibodies have generally been time consuming and resulted in low yields and loss of high affinity antibodies. When an immunogenic substance is introduced into a living body, the body's immune system reacts by generating antibodies to every site on the immunogen it recognizes. A large immunogenic protein molecule may have dozens of sites and a foreign cell may have hundreds. Thus, while each antibody-producing cell produces antibody specific for a single antigenic site the immune system has generated a specie of spe-

cific antibody producing cells for each immunogenic site recognized. In addition, the body has produced relatively large quantities of antibodies to antigens other than the one of interest such that most of the antibody in the polyclonal mixture is not specific for the antigen of interest. Accordingly, the antibodies used in prior immunometric assays are necessarily "polyclonal" in nature since the antibodies are derived from antisera raised in a conventional manner in animals and their purification is difficult.

When employing conventional polyclonal antibody mixtures in the reverse and simultaneous assays, the formation of a "sandwich" comprising the antigen complexed by two or more labelled antibodies which complex with the antigen at different sites is possible. These complexes could remain soluble in the sample being tested, be removed by subsequent washing steps, and not "counted" when the solid phase is analyzed for solid phase bound labelled antibody. If this happens to a significant extent, sensitivity of the assay is reduced and erroneous results may arise. However, if the unlabelled bound antibody is added to the sample first as in the forward sandwich assay, steric considerations prevent formation of a sandwich comprising the antigen complexed to two or more unlabelled antibodies where labelled antibody is excluded from also binding to the antigen. Accordingly, the antigen is free to react with a labelled antibody molecule. Nevertheless, it has been proposed to use a simultaneous assay for human thyroid stimulating hormone (HTSH) by employing a large excess of the unlabelled antibody bound to a solid phase to minimize formation by soluble labelled antibodies. See Jeong et al., "Comparison to Radioimmunoassay (RIA) with a Unique, Single-Incubation Two-Site Immunoradiometric Assay (IRMA) as Applied to the Determination of Human Thyroid Stimulating Hormone (HTSH)", Bio-Rad Laboratories, 1979.

It has also been proposed to use a reverse assay for HTSH, hepatitis associated antigen (HAA) and carcinoembryonic antigen (CEA) by employing a quantity of labelled antibody sufficient to assure a labelled antibody:antigen complex but insufficient to form a "sandwich" of all the antigen present in a sample. See U.S. Pat. No. 4,098,876.

Since all three of the procedures known to the prior art use a polyclonal mixture of antibodies, the potential for cross-reaction with other materials in serum or other fluid than the antigen for which the test is intended is increased. The occurrence of cross-reactivity with other antigens also reduces the sensitivity of the test for the suspect antigen and increases the prospect of a "false-positive" assay. Furthermore, the use of polyclonal antibodies in a simultaneous or reverse assay requires a careful consideration of the amount of labelled antibody used relative to the amount of solid phase antibody and/or antigen present.

In view of these shortcomings, the limitations to the immunometric procedures known to the prior art are readily apparent. The conventional forward assay is time consuming; the simultaneous and reverse assays are accomplished with fewer steps but require large quantities of solid-phase specific antibody and are not well suited to determination of small concentrations of antigen since formation of a sandwich of the antigen with a multiple number of labelled antibody molecules competes with formation of the sandwich comprising bound antibody:antigen:labelled antibody; and all are subject to misinterpretation of false-positives due to the polyclonal nature of the antibody.

Accordingly, one object of the present invention is to provide an improved process for the immunometric assay for antigenic substances.

More specifically, an object of the present invention is to provide more rapid immunometric assay techniques.

Another object of the present invention is to provide more sensitive immunometric assay techniques.

Yet another object of the present invention is to provide improved "simultaneous" and "reverse" immunometric assays.

The manner in which these and other objects are realized by the present invention will be apparent from the summary and detailed description set forth below.

SUMMARY OF THE INVENTION

According to the present invention, the polyclonal antibody used in an immunometric assay as the unlabelled antibody bound to a solid support and the antibody used as the soluble labelled antibody are replaced by at least one and usually two or more different monoclonal antibodies, i.e., each antibody specific to a single antigenic site and separately produced by clones derived from unique cell lines. In a preferred embodiment of the invention, the monoclonal antibody used as the antibody bound to the solid support is the product of a different cell line than is the monoclonal antibody used for the labelled antibody and the two monoclonal antibodies are selected to bind the antigenic substance at sites remote from each other so as to not interfere with the others binding to the antigen. The advantages of the present invention, particularly in simultaneous and reverse assays, over prior art methods will become clear after consideration of the accompanying drawings and the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph illustrating the results obtained using polyclonal antibodies in four types of immunometric assay for human IgE.

FIG. 2 is a similar graph illustrating the difference in results obtained using monoclonal antibodies in the same four types of immunometric assay for human IgE.

DETAILED DESCRIPTION OF THE INVENTION

As indicated above, according to the present invention, the polyclonal antibody used in an immunometric assay for an antigenic substance is replaced by a monoclonal antibody. The present invention is useful for the determination of the presence or concentration of a wide variety of polyvalent antigenic substances. Accordingly, as used herein, the term antigen or antigenic substance refers broadly to substances to which antibodies can be produced. Among such substances may be mentioned haptens, hormones such as insulin and human thyroid stimulating hormone (HTSH), gamma globulins, allergens, viruses, virus subunits, bacteria, toxins such as those associated with tetanus and with animal venoms, and even some drugs. Among the specific antigens which may be assayed by the process of the present invention may be mentioned carcinoembryonic antigen (CEA), hepatitis A and B, hepatitis Non A/Non B, IgE and alphafetoprotein.

The monoclonal antibodies useful in the present invention are obtained by the process discussed by Mil-

stein and Kohler and reported in Nature 256 495–497, 1975. The details of this process are well known and will not be repeated here. However, basically it involves injecting a mouse, or other suitable animal, with an immunogen. The mouse is subsequently sacrificed and cells taken from its spleen are fused with myeloma cells. The result is a hybrid cell, referred to as a "hybridoma", that reproduces in vitro. The population of hybridomas are screened to isolate individual clones each of which secrete a single antibody species to the antigen. The individual antibody species obtained in this way are each the product of a single B cell from the immune animal generated in response to a specific antigenic site recognized on the immunogenic substance.

When an immunogenic substance is introduced into a living host, the host's immune system responds by producing antibodies to all the recognizable sites on the substance. This "shotgun" approach to producing antibodies to combat the invader results in the production of antibodies of differing affinities and specificities for the immunogenic substance. Accordingly, after the different hybridoma cell lines are screened to identify those that produce antibody to the desired antigen, the antibodies produced by the individual hybridoma cell lines are preferably screened to identify those having the highest affinity for the immunogenic substance stimulating their original production before selection for use in the present invention. Selection based on this criterion is believed to help provide the increased sensitivity in the immunometric assay of the present invention using monoclonal antibody compared to the polyclonal antibody used in the prior art which, at best, has an affinity for the antigen which is roughly the average of the affinities of all antibodies produced by the immune system. Preferably, the monoclonal antibody selected will have an affinity of at least 10^8 liters/mole and, more preferably, an affinity of at least about 10^9 liters/mole.

Furthermore, those monoclonal antibodies having the highest affinities can be further screened by running a simulated assay on specimens known to give false positive results with processes employing conventional polyclonal antibodies to identify those monoclonal antibodies which do not cross-react and give false positive results.

Because the two-site immunometric assay relies upon formation of an antibody:antigen:antibody sandwich, usually two different monoclonal antibodies which do not interfere with the binding of each other to the antigen are selected to be the bound antibody and the soluble labelled antibody. Since both are necessary to complete the sandwich, reverse and simultaneous assays can be conducted without concern that a complex of labelled antibody:antigen:labelled antibody will form which will preclude formation of a complex between the antigen and the antibody bound to the solid phase and therein lies a particular advantage of the present invention. Furthermore, a forward assay can be accomplished without the intermediate washing step since the two antibodies bind to different sites. We refer to such a process as a "fast forward" assay.

However, particularly in the case of a forward assay, the same monoclonal antibody can be used for both the labelled antibody and the antibody bound to the solid support when the antigenic substance possesses identical antibody binding sites sufficiently remote from each other to allow more than one antibody molecule to be bound at the same time. In such a system the addition first of the bound antibody to the sample precludes formation of a sandwich because of steric considerations. When the labelled monoclonal antibody is subsequently added, it is also able to complex with the antigen bound to unlabelled antibody on the solid phase.

The unlabelled monoclonal antibody used in the presence of the present invention to extract the antigenic substance from the sample being tested may be immobilized on any of the common supports used in immunometric assays. Among these may be mentioned filter paper, plastic beads or test tubes made from polyethylene, polystyrene, polypropylene or other suitable material. Also useful are particulate materials such as agarose, crosslinked dextran, and other polysaccharides. The techniques for such bonding are well known to those skilled in the art. For example, antibodies may be bound to polysaccharide polymers using the process described in U.S. Pat. No. 3,645,852.

The labelled monoclonal antibody used in the present invention may be provided with the same labels used in prior art immunometric assays. Among these may be mentioned fluorogenic labels for detection by fluorimetry as described in U.S. Pat. No. 3,940,475 and enzymatic markers as described in U.S. Pat. No. 3,645,090. It is presently preferred to label the antibody with a radioisotope such as I^{125} using, for example, the procedure of Hunter and Greenwood, *Nature*, 144 (1962), page 945 or that of David et al., Biochemistry, Vol. 13, pp. 1014–1021, 1974.

In a typical assay, the amount of labelled antibody associated with the insoluble sandwich complex is determined by examination of the insoluble carrier material by suitable means. However, it is also possible to relate the presence or absence of antigen in the fluid sample being assayed to the amount of labelled antibody which does not react during the assay and remains in a soluble form.

The advantages of the present invention in which monoclonal antibodies are used in immunometric assays as compared to polyclonal antibodies are seen by reference to the following example. In this example, four comparative assays, a simultaneous assay, a reverse assay, a forward assay, and a "fast" forward assay, were run using both monoclonal antibody and polyclonal antibody using a standard serum containing 100 IU/ml of human IgE as the positive sample. Normal horse serum containing no IgE was used as a negative control.

The polyclonal antibody to IgE used, as the labelled antibody in the example was obtained from Pharmacia Diagnostics of Piscataway, New Jersey. The polyclonal antibody bound to the solid support was obtained from Tago, Inc. of Burlingame, California.

Monoclonal antibody to IgE was obtained using the method of Milstein and Kohler discussed above. The two antibodies selected each exhibited an affinity for IgE of greater than 10^9 liters/mole and did not interfere with the others binding to IgE.

The assays were run using unlabelled antibody bound to agarose by the process of U.S. Pat. No. 3,645,852. Labelling of antibody was by ^{125}I according to the process of David et al. referred to above. Phosphate buffered saline, pH 7.4, was used to wash all samples.

EXAMPLE

(1) Simultaneous Assay Method

Duplicate samples were run in which 100 μl of a suspension of antibody immobilized on agarose particles is mixed with 100 μl of specimen (serum) and 100 μl of soluble ^{125}I-labelled antibody. This mixture as incu-

bated for the specified times shown in Table I (for polyclonal antibody) and Table II (for monoclonal antibody) set forth below, plus 30 minutes. The extra 30 minutes incubation period was added to equalize this assay method with the other assay methods which required an additional 30 minute incubation time for a second added reagent. Following the incubation periods the agarose particles were washed by addition of buffer and centrifuged. After removal of the washing liquid by aspiration, the resulting pellet of agarose particles was then counted for bound ^{125}I-labelled antibody. The counts obtained for each of the complexes after the specified incubation time are set forth in Tables I and II.

(2) Reverse Assay Method

Duplicate samples were run in which 100 μl of specimen (serum) is mixed with 100 μl of ^{125}I-labelled soluble antibody and incubated for the specified times shown in Tables I and II. 100 μl of a suspension of antibody immobilized on agarose particles is then added and the mixture was allowed to incubate for another 30 minutes. The agarose particles were then washed and counted as in the simultaneous assay method. The counts are reported in Tables I and II.

(3) Forward Assay Method

Duplicate samples were run in which 100 μl of specimen (serum) is mixed with 100 μl of a suspension of antibody immobilized on agarose particles and incubated for the specified times shown in Tables I and II. The agarose particles were then washed once by the addition of 2.5–3.0 ml of buffer which, after mixing, was centrifuged, and the liquid removed by aspiration. 100 μl of ^{125}I-labelled soluble antibody was then added and the mixture incubated an additional 30 minutes. The agarose particles were then washed and counted as in the simultaneous assay method. The counts were reported in Tables I and II.

(4) Fast Forward Assay Method

The assay was performed, in duplicate, in a similar manner to the forward assay method except that the wash step between the initial incubation of specimen with antibody immobilized on agarose particles and the addition of soluble ^{125}I-labelled antibody was omitted.

The counts/minute for the duplicate controls and the duplicate assays of the samples containing IgE using polyclonal antibody and monoclonal antibody are shown in Tables I and II, respectively. These data were used to prepare FIGS. 1 and II in the following way. The average of the counts/minute for a control for a given incubation period was subtracted from the average of the counts for the corresponding IgE assay. The difference was calculated as a percentage of the total counts/minute of labelled antibody added to the sample and is plotted on the Y axis as the percentage of total counts/minute of antibody bound to the solid phase. The incubation time is plotted on the X axis.

A comparison of the plots shown in FIG. 2 displaying the results of assays using monoclonal antibody with those of FIG. 1 of assays using polyclonal antibody shows that in each kind of assay, simultaneous, reverse, forward, and fast forward, the assay using monoclonal antibody was more sensitive as indicated by the higher percentage of total counts bound to the solid phase with 100 IU IgE/ml specimen. Unexpectedly, in the case of the simultaneous and reverse assays, we have found that those run with monoclonal antibody reach equilibrium more rapidly than does the corresponding assay using polyclonal antibody. Therefore, by using a monoclonal antibody in these procedures, the time for the assay can be reduced significantly beyond the time saving achieved by merely eliminating a washing step. In that regard, the reverse assay with monoclonal antibody reached equilibrium in less than one hour. The same assay run with polyclonal assay did not reach equilibrium until after 4 hours. Similarly, in the case of simultaneous assays, the assay using monoclonal antibody reached equilibrium within 8 hours whereas the assay with polyclonal antibody did not reach equilibrium within 24 hours. Accordingly, the present invention substantially provides more rapid and sensitive simultaneous and reverse assays than the prior art processes and eliminates the concern that formation of a soluble "sandwich" complex will compete with formation of the desired insoluble complex.

The examples described above using monoclonal antibody to assay for IgE is merely one exemplar of the use of the present invention. That variations in the actual processes described in the example will be useful will be apparent to those skilled in the art. Therefore, the present invention is to be considered limited only by the appended claims.

TABLE I

Assay Results Using Polyclonal Antibody

	Simultaneous Assay		Reverse Assay		Forward Assay		Fast Forward Assay	
Incubation Time (Hrs)	Control Samples	IgE Samples	Control Samples	IgE Samples	Control Samples	IgE Samples	Control Samples	IgE Samples
0.25	372,314	[illegible]	302,243	2568,2581	357,326	2092,2077	396,293	2271,2238
0.50	348,265	2391,2366	294,262	2958,2999	258,233	1905,1817	—,—	—,—
1.00	315,277	2793,2708	309,277	3154,3218	355,424	2157,2255	304,284	1789,1706
2.00	342,356	2897,2887	290,[illegible]	1377,3212	302,314	1946,2019	288,312	1728,1867
4.00	421,385	3696,3746	28,280	3411,3651	274,255	2019,2392	283,292	1720,1683
6.00	447,436	4028,4101	296,281	3762,3641	241,267	1750,1452	301,257	1283,1424
24.00	526,577	4564,4628	233,263	3651,3346	320,277	1553,1604	273,256	1450,1470

TABLE II

Assay Results Using Monoclonal Antibody

	Simultaneous Assay		Reverse Assay		Forward Assay		Fast Forward Assay	
Incubation Time (Hrs)	Control Samples	IgE Samples	Control Samples	IgE Samples	Control Samples	IgE Samples	Control Samples	IgE Samples
0.25	135,132	5610,5803	388,594	8407,8358	210,205	4618,4894	194,183	[illegible]
0.50	558,459	7472,7115	240,231	8238,8271	223,228	4987,5273	198,197	[illegible]
1.00	268,265	6289,6529	325,265	8010,8377	230,187	3454,4308	215,192	4887,[illegible]

TABLE II-continued

Assay II- ulin Using Monoclonal Antibody

Incubation Time (Hrs)	Simultaneous Assay: Control Samples	Simultaneous Assay: IgE Samples	Reverse Assay: Control Samples	Reverse Assay: IgE Samples	Forward Assay: Control Samples	Forward Assay: IgE Samples	Fast Forward Assay: Control Samples	Fast Forward Assay: IgE Samples
2.00	282,275	6787,6784	255,382	7956,7644	226,197	4834,4268	192,210	4837,6944
4.00	308,272	8158,8155	343,385	8817,7788	231,216	5269,4420	218,187	4929,5071
8.00	549,667	8884,9494	698,850	7870,7356	226,741	2886,3631	405,343	3833,3713
23.95	426,423	8669,9844	497,323	7037,7359	194,201	2465,2586	346,233	3965,2943

We claim:

1. A process for the determination of the presence of concentration of an antigenic substance in a fluid comprising the steps:

(a) contacting a sample of the fluid with a measured amount of a soluble first monoclonal antibody to the antigenic substance in order to form a soluble complex of the antibody and antigenic substance present in said sample, said first monoclonal antibody being labelled;

(b) contacting the soluble complex with a second monoclonal antibody to the antigenic substance, said second monoclonal antibody being bound to a solid carrier, said solid carrier being insoluble in said fluid, in order to form an insoluble complex of said first monoclonal antibody, said antigenic substance and said second monoclonal antibody bound to said solid carrier;

(c) separating said solid carrier from the fluid sample and unreacted labelled antibody;

(d) measuring either the amount of labelled antibody associated with the solid carrier or the amount of unreacted labelled antibody; and

(e) relating the amount of labelled antibody measured with the amount of labelled antibody measured for a control sample prepared in accordance with steps (a)–(d), said control sample being known to be free of said antigenic substance, to determine the presence of antigenic substance in said fluid sample, or relating the amount of labelled antibody measured with the amount of labelled antibody measured for samples containing known amounts of antigenic substance prepared in accordance with steps (a)–(d) to determine the concentration of antigenic substance in said fluid sample, the first and second monoclonal antibodies having an affinity for the antigenic substance of at least about 10^8 liters/-mole.

2. A process according to claim 1 wherein said first monoclonal antibody is the product of a different cell line than said second monoclonal antibody.

3. A process according to claim 1 wherein said antigen has at least two identical binding sites and said first and second monoclonal antibodies are the product of the same cell line.

4. A process according to claims 1, 2 or 3 wherein the affinity is at least about 10^9 liters/mole.

5. A process according to any of claims 1, 2 or 3 wherein said solid carrier resulting from step (d) is washed to separate the fluid sample from the carrier.

6. A process according to claim 5 wherein the solid carrier is washed with phosphate buffered saline.

7. A process according to claims 1, 2 or 3 wherein the antigenic substance is selected from the group consisting of IgE, hepatitis A, hepatitis B, hepatitis Non A/Non B, alphafetoprotein, carcinoembryonic antigen, insulin and human thyroid stimulating hormone.

8. A process according to claims 1, 2 or 3 wherein the labelled antibody is labelled with a member selected from the group consisting of a radioactive isotope, an enzyme and a fluorogenic material and said examination is by means selected from the group consisting of radiometric means, enzymatic means and fluorometric means.

9. A process according to claim 8 wherein said label is the radioactive isotope ^{125}I.

10. A process for the determination of the presence of an antigenic substance in a fluid comprising the steps:

(a) simultaneously contacting a sample of the fluid with first and second monoclonal antibodies to said antigenic substance, each monoclonal antibody having an affinity for the antigenic substance of at least about 10^8 liters/mole, said first monoclonal antibody being labelled and soluble in said fluid and being provided for in a measured amount and said second monoclonal antibody being bound to a solid carrier insoluble in said fluid, in order to form an insoluble complex of said first monoclonal antibody, said antigenic substance and said second antibody;

(b) separating said solid carrier from the fluid sample and unreacted labelled antibody;

(c) measuring either the amount of labelled antibody associated with the solid carrier or the amount of unreacted labelled antibody; and

(d) relating the amount of labelled antibody measured with the amount of labelled antibody measured for a control sample prepared in accordance with steps (a)–(c), said control sample being known to be free of said antigenic substance, to determine the presence of antigenic substance in said fluid sample, or relating the amount of labelled antibody measured with the amount of labelled antibody measured for samples containing known amounts of antigenic substance prepared in accordance with steps (a)–(d) to determine the concentration of antigenic substance in said fluid sample.

11. A process according to claim 10 wherein said first monoclonal antibody is the product of a different cell line than said second monoclonal antibody.

12. A process according to claim 10 wherein said antigenic substance has at least two identical binding sites and said first and second monoclonal antibodies are the product of the same cell line.

13. A process according to claims 10, 11 or 12 wherein the affinity is at least about 10^9 liters/mole.

14. A process according to any of claims 10, 11 or 12 wherein said solid carrier resulting from step (b) is washed to separate the fluid sample from the carrier.

15. A process according to claim 14 wherein the solid carrier is washed with phosphate buffered saline.

16. A process according to claims 10, 11 or 12 wherein the antigenic substance is selected from the group consisting of IgE, hepatitis A, hepatitis B, hepatitis Non A/Non B, alphafetoprotein, carcinoembryonic antigen, insulin and human thyroid stimulating hormone.

17. A process according to claims 10, 11 or 12 wherein the labelled antibody is labelled with a member selected from the group consisting of a radioactive isotope, an enzyme and a fluorogenic material and said examination is by means selected from the group consisting of radiometric means, enzymatic means and fluorometric means.

18. A process according to claim 17 wherein said label is the radioactive isotope ^{125}I.

19. In an immunometric assay to determine the presence or concentration of an antigenic substance in a sample of a fluid comprising forming a ternary complex of a first labelled antibody, said antigenic substance, and a second antibody said second antibody being bound to a solid carrier insoluble in said fluid wherein the presence of the antigenic substance in the samples is determined by measuring either the amount of labelled antibody bound to the solid carrier or the amount of unreacted labelled antibody, the improvement comprising employing monoclonal antibodies having an affinity for the antigenic substance of at least about 10^8 liters/mole for each of said labelled antibody and said antibody bound to a solid carrier.

20. A process according to claim 19 wherein the fluid sample is first contacted with the second monoclonal antibody to form a binary complex of the antigenic substance and said second monoclonal antibody insoluble in the fluid and then contacted with said first labelled monoclonal antibody to form the ternary complex.

21. A process according to claim 19 wherein the fluid sample is first contacted with the second monoclonal antibody to form a binary complex of the antigenic substance and said second monoclonal antibody insoluble in the fluid; the sample separated from the solid carrier and the solid carrier contacted with a solution of said first labelled monoclonal antibody to form said ternary complex.

22. A process according to claim 21 wherein said solid carrier after formation of the ternary complex is washed to separate the fluid sample from the carrier.

23. A process according to claim 22 wherein the solid carrier is washed with phosphate buffered saline.

24. A process according to claims 19, 20 or 21 wherein said first monoclonal antibody is the product of a different cell line than said second monoclonal antibody.

25. A process according to claims 19, 20 or 21 wherein said antigen has at least two identical binding sites and said first and second monoclonal antibodies are the product of the same cell line.

26. A process according to claims 19, 20 or 21 wherein the affinity is at least about 10^9 liters/mole.

27. A process according to claims 19, 20 or 21 wherein the antigen is selected from the group consisting of IgE, hepatitis A, hepatitis B, hepatitis None A/Non B, alphafetoprotein, carcinoembryonic antigen, insulin and human thyroid stimulating hormone.

28. A process according to claims 19, 20 or 21 wherein the labelled antibody is labelled with a member selected from the group consisting of a radioactive isotope, an enzyme and a fluorogenic material.

29. A process according to claim 28 wherein said label is the radioactive isotope ^{125}I.

* * * * *

Appendix 3

Survey questionnaire used in market survey.

Biotechnology Patent Law Survey

Objective: To understand needs for biotechnology legal services.

BACKGROUND INFORMATION:

Name _____ Telephone # _____

Company _____

Position _____

Degree(s) _____

SELECTION OF LEGAL SERVICES:

1. A. Are you the individual responsible for making decisions or participate in decision making with regards to selection of outside legal services?

yes _____ no _____

B. Do you use outside patent legal counsel services?

yes _____ no _____

If you answered yes to the above question please explain for what services you use outside patent counsel. (Feel free to list the firm or person you use for these services).

_____

_____

_____

2. If you answered **yes** to the above, please answer the following; otherwise, please forward this questionnaire to the appropriate person.

A. Do you find that the cost for obtaining and maintaining patent protection is costly compared to other professional services ?

yes _____ no _____

3. Qualities you like to see in your patent attorney. Please mark an **X** on the lines below.

Technical	Not important				Important						Essential
	^				^						^
Ph.D	0	1	2	3	4	5	6	7	8	9	10
Masters degree	0	1	2	3	4	5	6	7	8	9	10
B.S or B.A in related science	0	1	2	3	4	5	6	7	8	9	10

Other please specify

_____

_____

Legal training:	Not important				Important						Essential
	^				^						^
Number of patent applications prosecuted	0	1	2	3	4	5	6	7	8	9	10
Specialty in your technology	0	1	2	3	4	5	6	7	8	9	10
Prestigious law school (e.g. Harvard, Yale, Stanford)	0	1	2	3	4	5	6	7	8	9	10
Reputation and experience	0	1	2	3	4	5	6	7	8	9	10

Other please specify

_____

_____

Other features:	Not important				Important						Essential
	^				^						^
Who the practitioner has previously worked for	0	1	2	3	4	5	6	7	8	9	10
Technical significance of patents prosecuted	0	1	2	3	4	5	6	7	8	9	10
Firm resources	0	1	2	3	4	5	6	7	8	9	10
Cost to perform the work	0	1	2	3	4	5	6	7	8	9	10

Appendix 4

Cover letter used in market survey.

Franklin Pierce Law Center

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30 Fort Meadow Dr.
Hudson, MA 01749
(508)562-2880

Dear Business Executive:

I am a graduate student at the Franklin Pierce Law Center and am conducting a survey to gather data for use in my Masters in Intellectual Property thesis (an advanced and specialized legal degree usually requiring two or more years of study after successful completion of law school).

The survey is being conducted to determine the necessary level of technical expertise of personnel who prosecute patents for biotechnology companies. It appears that biotechnology companies presently prefer to hire or utilize patent legal counsel who have both a law degree and a graduate degree in a technical discipline. However, it is not very common for legal practitioners to possess such dual degrees. This may present a problem to Massachusetts Biotechnology Companies who may desire to hire their own in-house attorneys with such technical expertise. Therefore, the focus of this survey is to determine whether it is really necessary that patent practitioners possess such dual degrees and whether there are other effective means to obtain quality patent legal services without such technical training. **The survey is designed so that you may complete it in less than 2 minutes.**

The results of the survey will provide the necessary data for me to complete my Master's in Intellectual Property degree from the Franklin Pierce Law Center in Concord, New Hampshire.

A copy of the tabulate results will be sent to you If you fill out the questionnaire and mail it back to me by **October 1,1992** in the self addressed stamped envelope.

Sincerely,

Timothy H. Joyce

Timothy H. Joyce, J.D.'92

Faculty Advisors:
Karl Jorda, Ex-Patent Counsel for Ciba-Geigy Corp.
Robert Rines, Faculty, Massachusetts Institute of Technology
William Murphy III, Ph.D. Harvard Business School

Appendix 5

Biotechnology Patent Practitioners in Massachusetts and Boston (as of 1990).

Blodgett, Gerry A., (Mr.); (617) 753-5533; registered patent attorney #26090; patent attorney, Blodgett & Blodgett, P.C. (3 lawyers, 3 intellectual property lawyers, 2 patent agents); 43 Highland St., Worcester, Massachusetts 01609; *born* August 22, 1947; *education* Worcester Polytechnic Institute (M.S. chemical engineering, 1972), Suffolk Univ. Law School (J.D., 1976), George Washington Univ. National Law Center (LL.M. patent law, trade regulation, 1980); *admitted to practice* Massachusetts, 1977; District of Columbia, 1978; *practice is* 100% intellectual property; *intellectual property portion of practice is* 70% patents, 10% trademarks, 10% copyrights, 5% unfair trade, 5% trade secrets; *intellectual property portion of practice is* 20% counseling clients, 15% litigating, 40% writing or prosecuting applications, 15% contracts and transactions, 10% supervising lawyers; *type of intellectual property work* firm works in all technologies, with special emphasis on machine design and control, plastic processing, material science, computer systems and biotechnology; also, deeply involved in trademark and copyright issues especially concerning technology marketing programs; author, "Relative Significance—A Concept in Chemical Structural Obviousness", Journal of Patent Office Society, 1981; *background* member, American Intellectual Property Law Association, since 1976; technical advisor, U.S. Court of Customs and Patent Appeals, 1976-78; research associate, Prof. B. Sand, Worcester Polytechnic Institute, E.P.A. Grant, 1972-74.

Barakat, Barbara A., (Ms.); (617) 227-0700, *fax* (617) 723-4609; registered patent attorney #32190; associate, Lorusso & Loud (7 lawyers, 7 intellectual property lawyers), *with firm since* 1985; 440 Commercial St., Boston, Massachusetts 02109; *born* September 6, 1957; *education* College of the Holy Cross (B.A. chemistry, 1979), Boston College Law School (J.D., 1982); *admitted to practice* Massachusetts, 1983; New York, 1983; *practice is* 100% intellectual property; *type of intellectual property work* firm has expertise in all technological fields including electronic hardware, computers, computer software, robotics and complex machinery and is particularly proud of its achievements in the field of biotechnology, medical technology and health care related biotechnology; *background* member, Boston Patent Law Association, since 1985; member, American Intellectual Property Law Association, since 1985; member, American Bar Association, since 1985; member, Massachusetts Bar Association, since 1983.

Bresnick, Sidney R., (Mr.); (617) 720-3500; registered patent attorney #24094; of counsel, Wolf, Greenfield & Sacks, P.C. (27 lawyers, 25 intellectual property lawyers), *with firm since* 1989; Federal Reserve Plaza, 600 Atlantic Ave., Boston, Massachusetts 02210; *born* March 25, 1932; *education* Univ. of Massachusetts (B.S. chemistry, microbiology, 1953), Massachusetts Institute of Technology (S.M. food technology, 1955), Boston College Law School (LL.B., 1965); *admitted to practice* New York, 1966; *practice is* 100% intellectual property; *intellectual property portion of practice is* 80% patents, 20% trademarks; *intellectual property portion of practice is* 25% counseling clients, 75% litigating; *type of intellectual property work* litigation and counseling, including infringement and validity studies, involving patents and trade secrets; author, "Patents and the Food Industry", Food Technology, 1987; *background* member, New York Patent Law Association, since 1971; member, American Intellectual Property Law Association, since 1987; member, Pennie & Edmonds, 1974-83; member, Fitzpatrick, Cella, Harper & Scinto, 1984-89.

Byrne, Sarah L., (Ms.); (617) 227-0700; associate, Lorusso & Loud (7 lawyers, 7 intellectual property lawyers), *with firm since* 1987; 440 Commercial St., Boston, Massachusetts 02109; *born* August 19, 1961; *education* Union College (B.S., 1983), Boston Univ. School of Law (J.D., 1986); *admitted to practice* Connecticut, 1986; Massachusetts, 1987; *practice is* 100% intellectual property; *type of intellectual property work* firm has expertise in all technological fields including electronic hardware, computers, computer software, robotics and complex machinery and is particularly proud of its achievements in the field of biotechnology, medical technology and health care related biotechnology; *background* member, Massachusetts Bar Association, since 1981.

Clark, Paul T., (Mr.); (617) 542-5070; registered patent attorney #30162; partner, Fish & Richardson (38 lawyers, 38 intellectual property lawyers, one patent agent), *with firm since* 1979; One Financial Center, 25th Floor, Boston, Massachusetts 02111-2658; *born* October 7, 1947; *education* Case Western Reserve Univ. (A.B. English, 1969), Northeastern Univ. (M.S. biology, 1976), Northeastern Univ. School of Law (J.D., 1979); *admitted to practice* Massachusetts, 1979; *practice is* 100% intellectual property; *intellectual property portion of practice is* 95% patents, 5% trademarks; *intellectual property portion of practice is* 30% counseling clients, 20% litigating, 20% writing or prosecuting applications, 10% contracts and transactions, 20% supervising lawyers; *type of intellectual property work* biotechnology patent law; co-author, "Advanced Topics in Intellectual Property Management", Massachusetts Continuing Legal Education, 1987-89; co-author, "Fundamentally Ancient Statutes Take on Space-Age Biotechnology", National Law Journal, 1986.

Conlin, David G., (Mr.); (617) 523-3400; registered patent attorney #27026; partner, Dike, Bronstein, Roberts, Cushman & Pfund (16 lawyers, 15 intellectual property lawyers), *with firm since* 1973; 130 Water St., Boston, Massachusetts 02109; *born* August 31, 1943; *education* State Univ. of New York at Buffalo (B.S. chemical engineering, 1965), Georgetown Univ. Law Center (J.D., 1971); *able to conduct business in* German; *admitted to practice* Virginia, 1971; Massachusetts, 1974; *practice is* 100% intellectual property; *intellectual property portion of practice is* 70% patents, 15% trademarks, 5% copyrights, 5% unfair trade, 5% trade secrets; *intellectual property portion of practice is* 10% counseling clients, 60% litigating, 20% writing or prosecuting applications, 10% contracts and transactions; co-author, Patent Practice, Patent Resources Institute, 1975-89; co-author, Intellectual Property Rights in Biotechnology Worldwide, Macmillen, 1987; *background* president, Boston Patent Law Association, 1982-83; chairman awards committee, American Intellectual Property Law Association, 1978-79; technical advisor, U.S. Court of Customs and Patent Appeals, 1971-73; examiner, U.S. Patent and Trademark Office, 1971-73.

Davis, Sherman Gilbert, (Dr.); (508) 771-2241; registered patent agent #28146; 39 Emerson Way, Centerville, Massachusetts 02632; *born* January 26, 1920; *education* Univ. of Massachusetts (B.S. chemistry, microbiology, 1941), Univ. of Massachusetts (M.S., 1942), Univ. of Massachusetts (Ph.D., 1948); *practice is* 100% intellectual property; *intellectual property portion of practice is* 100% patents; *intellectual property portion of practice is* 30% counseling clients, 70% writing or prosecuting applications; *type of intellectual property work* patents for medical, pharmaceutical and related devices.

Desrosier, Thomas J., (Mr.); (617) 879-3775; registered patent attorney #30168; E.I. du Pont de Nemours & Co., Inc. (186 lawyers, 72 intellectual property lawyers, 3 patent agents), *with firm since* 1980; Eight Gerald St., Framingham, Massachusetts 01701; *born* January 19, 1955; *education* Univ. of Vermont (B.S. chemistry, biology, 1977), Wake Forest Univ. School of Law (J.D., 1980); *admitted to practice* New Hampshire, 1980; *practice is* 100% intellectual property; *intellectual property portion of practice is* 80% patents, 10% trademarks, 10% trade secrets; *intellectual property portion of practice is* 20% counseling clients, 20% litigating, 30% writing or prosecuting applications, 30% contracts and transactions; *type of intellectual property work* chemical, biochemical, biotechnology; *background* member, American Intellectual Property Law Association, since 1980.

Campbell, Paula A., (617) 861-6240; registered patent attorney #32503; associate attorney, Hamilton, Brook, Smith & Reynolds (13 lawyers, 13 intellectual property lawyers), *with firm since* 1987; Two Militia Drive, Lexington, Massachusetts 02173; *born* April 19, 1955; *education* Purdue Univ. (B.S. biology, 1978), Suffolk Univ. Law School (J.D., 1985); *admitted to practice* Massachusetts, 1985; *practice is* 100% intellectual property; *intellectual property portion of practice is* 90% patents, 5% trademarks, 5% copyrights; *intellectual property portion of practice is* 15% counseling clients, 80% writing or prosecuting applications, 5% contracts and transactions; *type of intellectual property work* biotechnology and chemical patents; *background* member, Boston Patent Law Association, since 1988.

Channing, Stacey L., (Ms.); (617) 861-6600; registered patent attorney #31095; patent attorney, W.R. Grace & Co.—Conn. (50 lawyers, 23 intellectual property lawyers), *with firm since* 1983; 55 Hayden Ave., Lexington, Massachusetts 02173; *born* November 29, 1957; *education* Brown Univ. (A.B. biology, political science, 1978), Boston Univ. School of Law (J.D., 1981); *admitted to practice* Massachusetts, 1981; *practice is* 100% intellectual property; *intellectual property portion of practice is* 65% patents, 25% trademarks, 3% copyrights, 2% unfair trade, 5% trade secrets; *intellectual property portion of practice is* 30% counseling clients, 10% litigating, 30% writing or prosecuting

Deconti, Giulio A., (Mr.); (617) 861-6240; registered patent attorney #31503; associate, Hamilton, Brook, Smith & Reynolds (13 lawyers, 13 intellectual property lawyers); Two Militia Drive, Lexington, Massachusetts 02173; *born* August 24, 1953; *education* Brown Univ. (Sc.B. biology, 1975), Brown Univ. (M.S., 1979), Georgetown Univ. Law Center (J.D., 1982); *admitted to practice* District of Columbia, 1982; *practice is* 100% intellectual property; *type of intellectual property work* specializes in biotechnology; *background* member, American Intellectual Property Law Association; member, Boston Patent Law Association, since 1983.

Craig, Anne I., (Ms.); (617) 227-0700; registered patent attorney #32976; associate, Lorusso & Loud (7 lawyers, 7 intellectual property lawyers), *with firm since* 1987; 440 Commercial St., Boston, Massachusetts 02109; *born* December 16, 1953; *education* Univ. of Massachusetts (B.S. zoology, 1976), New England School of Law (J.D., 1983); *admitted to practice* Massachusetts, 1983; *practice is* 100% intellectual property; *type of intellectual property work* firm has expertise in all technological fields including electronic hardware, computers, computer software, robotics and complex machinery and is particularly proud of its achievements in the field of biotechnology, medical technology and health care related biotechnology; *background* co-chairperson, Programs Committee, High Technology Subcommittee of Boston Law Section of Massachusetts Bar Association, since 1988; associate, Ardiff & Morse, 1985-87; title examiner, Massachusetts Land Court, 1984; systems analyst, Bank of Boston, 1980-83.

Dacey, G. Eugene, (Mr.); (617) 523-3515, (617) 262-2021; registered patent attorney #19122; partner, Morse, Altman, Dacey & Benson (3 lawyers, 3 intellectual property lawyers), *with firm since* 1979; One Exeter Plaza, Boston, Massachusetts 02116; *born* July 19, 1928; *education* Columbia Univ. (B.S., 1952), Harvard Law School (LL.B., 1957); *able to conduct business in* Hungarian, German; *admitted to practice* Massachusetts, 1957; *practice is* 100% intellectual property; *intellectual property portion of practice is* 60% patents, 15% trademarks, 15% copyrights, 5% unfair trade, 5% trade secrets; *intellectual property portion of practice is* 25% counseling clients, 15% litigating, 45% writing or prosecuting applications, 10% contracts and transactions, 5% supervising lawyers; *type of intellectual property work* medical, surgical and scientific instruments; electrical and electromechanical instruments; biotechnology devices and methods; chemical processes and products; mining instruments and mechanical devices; *background* member, Boston Patent Law Association, since 1958; patent counsel, Instrumentation Laboratory, Inc., 1974-79; patent attorney, E G & G, Inc., 1960-65; associate, Kenway, Jenney, Witter & Hildreth, 1957-58.

Fasse, J. Peter, (Mr.); (617) 542-5070; registered patent attorney #32983; associate, Fish & Richardson (38 lawyers, 38 intellectual property lawyers, one patent agent), *with firm since* 1987; One Financial Center, 25th Floor, Boston, Massachusetts 02111-2658; *born* April 6, 1961; *education* Massachusetts Institute of Technology (B.S. bioelectrical engineering, 1983), Massachusetts Institute of Technology (B.S. life sciences, 1983), George Washington Univ. National Law Center (J.D., 1987); *able to conduct business in* German; *admitted to practice* Massachusetts, 1987; *practice is* 100% intellectual property; *intellectual property portion of practice is* 75% patents, 10% trademarks, 10% unfair trade, 5% trade secrets; *intellectual property portion of practice is* 5% counseling clients, 80% litigating, 15% writing or prosecuting applications; *type of intellectual property work* patent litigation in all areas and patent prosecution primarily in the biotechnology and biomedical areas; *background* patent examiner, Genetic Engineering Art Unit of U.S. Patent and Trademark Office, 1984.

Fraser, Janet K., (617) 542-5070; Fish & Richardson (38 lawyers, 38 intellectual property lawyers, one patent agent); One Financial Center, 25th Floor, Boston, Massachusetts 02111-2658.

Freeman, John W., (Mr.); (617) 542-5070; registered patent attorney #29066; partner, Fish & Richardson (38 lawyers, 38 intellectual property lawyers, one patent agent), *with firm since* 1979; One Financial Center, 25th Floor, Boston, Massachusetts 02111-2658; *born* March 27, 1947; *education* Williams College (B.A. chemistry, 1969), Univ. of Pennsylvania Law School (J.D., 1972); *admitted to practice* Pennsylvania, 1974; Massachusetts, 1980; *practice is* 100% intellectual property; *type of intellectual property work* biotechnology patent prosecution and licensing, specializing in technology transfer with universities; *background* chairman, Chemical Practice Committee of Boston Patent Law Association, since 1980; member, American Intellectual Property Law Association, since 1985.

Furlong, Robert W., (Mr.); (617) 542-5070; registered patent attorney #15640; Fish & Richardson (38 lawyers, 38 intellectual property lawyers, one patent agent), *with firm since* 1952; One Financial Center, 25th Floor, Boston, Massachusetts 02111-2658; *born* May 20, 1916; *education* Harvard Univ. (A.B. chemistry, 1937), Univ. of Akron, McDowell Law Center (LL.B., 1944); *able to conduct business in* German; *admitted to practice* Ohio, 1944; Massachusetts, 1952;

Gates, Edward R., (Mr.); (617) 426-6131; registered patent attorney #31616; associate, Wolf, Greenfield & Sacks, P.C. (27 lawyers, 25 intellectual property lawyers), *with firm since* 1984; Federal Reserve Plaza, 600 Atlantic Ave., Boston, Massachusetts 02210; *born* June 25, 1954; *education* Yale Univ. (B.S. biology, 1977), Boston Univ. School of Law (J.D., 1984); *admitted to practice* Massachusetts, 1985; *practice is* 100% intellectual property; *intellectual property portion of practice is* 60% patents, 30% trademarks, 10% trade secrets; *intellectual property portion of practice is* 25% counseling clients, 25% litigating, 25% writing or prosecuting applications, 25% contracts and transactions; *type of intellectual property work* intellectual property in microbiology, biotechnology and medical arts; *background* member, Boston Patent Law Association, since 1986; research associate, Tufts Medical School Cancer Research Center, 1978-80.

Hillman, Robert E., (Mr.); (617) 542-5070; registered patent attorney #22837; partner, Fish & Richardson (38 lawyers, 38 intellectual property lawyers, one patent agent), *with firm since* 1963; One Financial Center, 25th Floor, Boston, Massachusetts 02111-2658; *born* June 25, 1937; *education* Massachusetts Institute of Technology (S.B. engineering, 1959), Harvard Law School (LL.B., 1962); *admitted to practice* Massachusetts, 1963; *practice is* 100% intellectual property; *intellectual property portion of practice is* 75% patents, 20% trademarks, 5% not divisible by subtype; *intellectual property portion of practice is* 10% counseling clients, 60% litigating, 30% supervising lawyers; *type of intellectual property work* general intellectual property practice with emphasis on litigation; author, "Patent Law", The National Law Journal, 1986; author, "Patents and Biotechnology: The Falkow-Moseley Story", ASM News, 1986; *background* member, American Intellectual Property Law Association; member, Boston Patent Law Association.

Hofer, Mark A., (Mr.); (508) 872-8400; registered patent attorney #30068; chief patent counsel, Integrated Genetics (one lawyer), *with firm since* 1987; One Mountain Road, Framingham, Massachusetts 01701; *born* April 12, 1953; *education* Rutgers Univ. (A.B. microbiology, 1975), Rutgers Univ. (M.S. bio-medical engineering, 1977), New York Law School (J.D., 1980); *able to conduct business in* French; *admitted to practice* New Jersey, 1981; New York, 1981; *practice is* 90% intellectual property, 10% corporate; *type of intellectual property work* handles all of company's intellectual property and licensing needs as well as their contract needs and most of their corporate law and management needs; author, "Section 102 Defenses", Second Annual Joint Patent Law Association, 1986; author, "Deposit Requirements", Biotech 85, 1985; *background* member, American Intellectual Property Law Association, since 1981; member, New Jersey Patent Law Association, since 1981; member, Boston Patent Law Association, 1988; patent attorney, Johnson & Johnson, 1981-87.

Jacobs, Bruce F., (Mr.); (617) 576-5766; registered patent attorney #26184; Jacobs Patent Offices (one lawyer, one intellectual property lawyer), *with firm since* 1987; 124 Mount Auburn St., Ste. 200, Cambridge, Massachusetts 02138; *born* July 4, 1946; *education* Tufts Univ. (B.S. chemical engineering, 1967), Georgetown Univ. Law Center (J.D., 1971); *admitted to practice* District of Columbia, 1972; New York, 1974; *practice is* 100% intellectual property; *intellectual property portion of practice is* 90% patents, 10% trade secrets; *intellectual property portion of practice is* 25% counseling clients, 60% writing or prosecuting applications, 15% contracts and transactions; *type of intellectual property work* chemical and biochemical patent law in the areas of polymer chemistry, materials science, agricultural chemicals and biotechnology; licensing; *background* of counsel, Perman & Green, 1986-88; patent counsel, Engelhard Corp., 1981-85; senior attorney, American Cyanamid Co., 1977-81; associate, Arthur, Dry & Kalish, P.C., 1973-77.

Lorusso, Anthony M., (Mr.); (617) 227-0700, *fax* (617) 223-4609; registered patent attorney #25059; partner, Lorusso & Loud (7 lawyers, 7 intellectual property lawyers); 440 Commercial St., Boston, Massachusetts 02109; *born* 1940; *education* Boston College (B.S., 1962), Suffolk Univ. Law School (J.D., 1967); *admitted to practice* Massachusetts, 1967; District of Columbia, 1969; *practice is* 100% intellectual property; *type of intellectual property work* firm has expertise in all technological fields including electronic hardware, computers, computer software, robotics and complex machinery and is particularly proud of its achievements in the field of biotechnology, medical technology and health care related biotechnology; *background* member, Boston Patent Law Association; member, American Intellectual Property Law Association; member, Federation Internationale des Conseils en Propriete Industrielle; member, U.S. Trademark Association.

Oliverio, Michael L., (617) 843-3612; registered patent attorney #30915; attorney, Wolf, Greenfield & Sacks, P.C. (27 lawyers, 25 intellectual property lawyers), *with firm since* 1986; Federal Reserve Plaza, 600 Atlantic Ave., Boston, Massachusetts 02210; *born* May 13, 1952; *education* Univ. of Massachusetts (B.S. chemistry, 1974), Yale Univ. (M.S. organic/polymer chemistry, 1975), Boston College Law School (J.D., 1980); *admitted to practice* Massachusetts, 1980; New York, 1981; *practice is* 80% intellectual property, 10% corporate, 10% criminal law litigation; *intellectual property portion of practice is* 50% patents, 30% trademarks, 5% copyrights, 5% unfair trade, 10% trade secrets; *type of intellectual property work* biotechnology, polymer chemistry, immumology, computer software and hardware, mechanical textile spinning, mechanical x-ray diagnostic equipment; *background* attorney, Kenyon & Kenyon, New York City, New York, 1979-84; attorney, Cohen and Burg, Boston, MA.

Ruoff, Carl F., (Mr.); (617) 227-0700; associate, Lorusso & Loud (7 lawyers, 7 intellectual property lawyers), *with firm since* 1987; 440 Commercial St., Boston, Massachusetts 02109; *born* April 11, 1957; *education* Massachusetts Institute of Technology (B.S. chemical engineering, 1979), Suffolk Univ. Law School (J.D. patents, 1986); *admitted to practice* Massachusetts, 1987; *practice is* 100% intellectual property; *type of intellectual property work* firm has expertise in all technological fields including electronic hardware, computers, computer software, robotics and complex machinery and is particularly proud of its achievements in the field of biotechnology, medical technology and health care related biotechnology; author, "Continued Development of Peat Biogasification Process", Patent Energy Alternative Symposium Resources and Conservation, 1980, 1983; *background* member, American Institute of Chemical Engineers, since 1979; member, Boston Patent Law Association, since 1987; member, American Institute of Chemical Engineers, since 1979.

Smith, Arthur A., Jr., (Mr.); (617) 227-0700; registered patent attorney #24178; associate, Lorusso & Loud (7 lawyers, 7 intellectual property lawyers), *with firm since* 1987; 440 Commercial St., Boston, Massachusetts 02109; *born* June 19, 1935; *education* Boston College (B.A., 1956), Suffolk Univ. Law School (LL.B., 1965); *admitted to practice* Massachusetts, 1965; *practice is* 100% intellectual property; *type of intellectual property work* firm has expertise in all technological fields including electronic hardware, computers, computer software, robotics and complex machinery and is particularly proud of its achievements in the field of biotechnology, medical technology and health care related biotechnology; author, "Implications of the Uniform Patent Legislation to Colleges and Universities", Journal of College and University Law, 1981-82; *background* member, American Bar Association; member, Society of University Patent Administrators; member, National Council of University Attorneys.

Sunstein, Bruce D., (Mr.); (617) 426-6464; registered patent attorney #27234; partner, Bromberg & Sunstein (10 lawyers, 10 intellectual property lawyers), *with firm since* 1979; 10 West St., 7th Floor, Boston, Massachusetts 02111-1212; *born* May 27, 1944; *education* Massachusetts Institute of Technology (B.S. humanities, science, 1965), Indiana Univ. (M.A. English, 1969), Univ. of California at Berkeley School of Law (J.D., 1973); *admitted to practice* California, 1973; Massachusetts, 1977; *practice is* 80% intellectual property, 20% general business; *intellectual property portion of practice is* 60% patents, 20% trademarks, 15% copyrights, 2% unfair trade, 3% trade secrets; *intellectual property portion of practice is* 25% counseling clients, 10% litigating, 25% writing or prosecuting applications, 15% contracts and transactions, 25% supervising lawyers; *type of intellectual property work* licensing, prosecution, and litigation in areas including computers and electronics, publishing, advertising, footwear, biomedical devices, mechanical systems and devices; *background* member, Licensing Executives Society, since 1975; member, American Intellectual Property Law Association, since 1975; member, Patent, Trademark and Copyright Section of the American Bar Association, since 1975.

Tendler, Robert Kanof, (Mr.); (617) 723-7268; registered patent attorney #24581; 33 Broad St., Boston, Massachusetts 02109; *born* March 6, 1942; *education* Amherst College (B.A. physics, math, 1964), George Washington Univ. National Law Center (J.D., 1967); *able to conduct business in* French; *admitted to practice* Massachusetts, 1969; *practice is* 100% intellectual property; *intellectual property portion of practice is* 70% patents, 20% trademarks, 5% copyrights, 2% unfair trade, 3% trade secrets; *intellectual property portion of practice is* 10% counseling clients, 10% litigating, 70% writing or prosecuting applications, 10% contracts and transactions; *type of intellectual property work* electronics, physics, optics, computers, biotechnology, medical robotics and machine control, communications, semiconductor technology, mechanical systems; patent prosecution, licensing and litigation; author, "A Patent's Value Goes Beyond Royalties", New Hampshire Business Review, 1985; *background* member, American Intellectual Property Law Association, since 1967; member, Boston Patent Law Association, since 1967.

Warburg, Richard Jeremy, (Dr.); (617) 542-5070; registered patent agent #32327; employee, Fish & Richardson (38 lawyers, 38 intellectual property lawyers, one patent agent), *with firm since* 1985; One Financial Center, 25th Floor, Boston, Massachusetts 02111-2658; *born* April 12, 1957; *education* Birmingham Univ. (B.Sc. genetics, 1978), Birmingham Univ. (Ph.D. microbiology, genetics, 1981), Suffolk Univ. Law School (J.D.); *practice is* 100% intellectual property; *intellectual property portion of practice is* 100% patents; *intellectual property portion of practice is* 20% counseling clients, 80% writing or prosecuting applications; *type of intellectual property work* mainly biotechnology, microbiology, genetics; co-author, "Patents and Biotechnology—The Aids Controversy", American Society for Microbiology, 1987; co-author, "Patents and Biotechnology—The Falkow-Moseley Story", American Society for Microbiology, 1986; *background* member, Boston Patent Law Association, 1987.

Williams, Stephan P., (Mr.); (617) 723-1300; registered patent attorney #28546; associate, Ares-Serona, Inc. (3 lawyers, one intellectual property lawyer), *with firm since* 1988; Exchange Place, 37th Floor, Boston, Massachusetts 02109; *born* September 10, 1949; *education* Worcester Polytechnic Institute (B.S. chemistry, 1971), Suffolk Univ. Law School (J.D., 1979); *admitted to practice* Massachusetts, 1979; *practice is* 100% intellectual property; *type of intellectual property work* chemical, biotechnology; *background* member, Boston Patent Law Association; member, American Intellectual Property Law Association.