D. A. Glaser

SECTION I

Form Approved
Budget Bureau No. 68-R

DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

GRANT APPLICATION

LEAVE BLANK		
TYPE	PROGRAM	NUMBER
REVIEW GROUP		FORMERLY
COUNCIL (Month, Year)		DATE RECEIVED

TO BE COMPLETED BY PRINCIPAL INVESTIGATOR (Items 1 through 7 and 15A)

1. TITLE OF PROPOSAL (Do not exceed 53 typewriter spaces)

Scanner-Computer Investigations of Biological Systems

2. PRINCIPAL INVESTIGATOR

2A. NAME (Last, First, Initial)

Glaser, Donald A.

2B. TITLE OF POSITION

Professor of Physics and Molecular Biology

2C. MAILING ADDRESS (Street, City, State, Zip Code)

Department of Molecular Biology
University of California
Berkeley, California 94720

2D. DEGREE

Ph.D.

2E. SOCIAL SECURITY NO.

[redacted]

2F. TELEPHONE DATA — Area Code: 415 — TELEPHONE NUMBER AND EXTENSION: 642-4310

2G. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT (See Instructions)

Virus Laboratory

2H. MAJOR SUBDIVISION (See Instructions)

College of Letters and Science

3. DATES OF ENTIRE PROPOSED PROJECT PERIOD (This applicat

FROM	THROUGH
01 June 1975	31 May 1980

4. TOTAL DIRECT COSTS REQUESTED FOR PERIOD IN ITEM 3

6,124,938

5. DIRECT COSTS REQUESTED FOR FIRST 12-MONTH PERI

1,646,655

6. PERFORMANCE SITE(S) (See Instructions)

University of California
Berkeley, California 94720

7. Research Involving Human Subjects (See Instructions)

A. [X] NO B. [ ] YES Approved: _____ Date

C. [ ] YES – Pending Review

8. Inventions (Renewal Applicants Only - See Instructions)

A. [ ] NO B. [ ] YES – Not previously reported

C. [X] YES – Previously reported

TO BE COMPLETED BY RESPONSIBLE ADMINISTRATIVE AUTHORITY (Items 8 through 13 and 15B)

9. APPLICANT ORGANIZATION(S) (See Instructions)

The Regents of the University of California
c/o Campus Research Office
Mll Wheeler
University of California
Berkeley, California 94720

10. NAME, TITLE, AND TELEPHONE NUMBER OF OFFICIAL(S) SIGNING FOR APPLICANT ORGANIZATION(S)

Telephone Number (s) _____

11. TYPE OF ORGANIZATION (Check applicable item)

[ ] FEDERAL [X] STATE [ ] LOCAL [ ] OTHER (Specify)

12. NAME, TITLE, ADDRESS, AND TELEPHONE NUMBER OF OFFICIAL IN BUSINESS OFFICE WHO SHOULD ALSO BE NOTIFIED IF AN AWARD IS MADE

Mr. August G. Manza, Manager
Campus Research Office
Mll Wheeler, University of California
Berkeley, California

Telephone Number (415) 642-0120

13. IDENTIFY ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR INSTITUTIONAL GRANT PURPOSES (See Instructions)

Biomedical Sciences Support Grant

14. ENTITY NUMBER (Formerly PHS Account Number)

451470

15. CERTIFICATION AND ACCEPTANCE. We, the undersigned, certify that the statements herein are true and complete to the best of our knowledge and accept, as to any grant awarded, the obligation to comply with Public Health Service terms and conditions in effect at the time of the award.

SIGNATURES (Signatures required on original copy only. Use ink, "/s/" signatures not accepted.)	A. SIGNATURE OF PERSON NAMED IN ITEM 2A	DATE
	B. SIGNATURE(S) OF PERSON(S) NAMED IN ITEM 10	DATE

SECTION 1

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

RESEARCH OBJECTIVES

LEAVE BLANK
PROJECT NUMBER

NAME AND ADDRESS OF APPLICANT ORGANIZATION

University of California
Berkeley, California 94720

NAME, SOCIAL SECURITY NUMBER, OFFICIAL TITLE, AND DEPARTMENT OF ALL PROFESSIONAL PERSONNEL ENGAGED ON PROJECT, BEGINNING WITH PRINCIPAL INVESTIGATOR

Donald A. Glaser, [redacted], Professor of Physics and Molecular Biology, Virus Lab.
Ronald Baker, [redacted] Assoc. Development Engrg., Virus Laboratory
John Bercovitz, [redacted], Asst. Development Engrg., Virus Laboratory
James Berk, [redacted] Assoc. Development Engrg., Virus Laboratory
Fraser Bonnell, [redacted], Principal Programmer, Virus Laboratory
John Couch, [redacted] Post-Grad. Research Biophysicist, Virus Laboratory
Ted Fujita, [redacted], Asst. Develop. Engrg., Virus Laboratory
Robert Henry, [redacted], Sr. Development Engrg., Virus Laboratory
Leif Hansen, [redacted], Principal Development Engrg., Virus Laboratory (cont. on p. 2a

TITLE OF PROJECT

Scanner-Computer Investigations of Biological Systems

USE THIS SPACE TO ABSTRACT YOUR PROPOSED RESEARCH. OUTLINE OBJECTIVES AND METHODS. UNDERSCORE THE KEY WORDS (NOT TO EXCEED 10) IN YOUR ABSTRACT.

Large scale genetic and physiological studies of bacteria, yeasts, and animal cells grown in tissue culture will be carried out using recently constructed automated equipment and computer-directed pattern recognition techniques. By automatic examination of up to 10^8 colonies in a batch, rare mutants will be isolated and partially characterized, mutagenic effects of chemical and physical agents will be measured even at low doses, and genetic recombination frequencies measured accurately for mapping purposes. Mutants for detailed studies of DNA synthesis in E. coli and B. subtilis will be isolated and partially characterized. Mutants of E. coli, S. typhimurium and Saccharomyces cerevisiae will be isolated for study of biosynthetic and degradative pathways and for analysis of the mechanisms of genetic recombination. Genetic maps of E. coli, Saccharomyces cerevisiae, and some mammalian cells will be enlarged.

Feasibility studies of automatic recognition of bacterial and fungal pathogens in medical and public health applications will be extended. Mutagenic effects of food additives and other environmental chemicals will be tested in several bacterial, yeast, and animal cell systems. Carcinogenic effects of chemical and physical agents including ionizing radiation will be measured using animal cells. Interactions of hormones and other agents with tumor cells grown in tissue culture will be examined to investigate the biochemical mechanism of the interactions and to test possible anti-neoplastic effects of a variety of substances. Mutant tumor cells sensitive to some agents and resistant to others will be isolated for further study. Screening programs may be undertaken when feasible for mutagens, carcinogens, anti-neoplastic agents, and effects of low doses of mutagens and ionizing radiation.

Additional instrumentation will be constructed as needed.

Privileged Communication [redacted]

Professional Personnel (continued)

Larry Johnson, [redacted], Assoc. Devel. Engrg., Virus Laboratory
Alex Para, [redacted] Asst. Devel. Engrg., Virus Laboratory

DETAILED BUDGET FOR FIRST 12-MONTH PERIOD

FROM: June 1, 1975 THROUGH: May 31, 1980

DESCRIPTION (Itemize)		TIME OR EFFORT %/HRS.	AMOUNT REQUESTED (Omit cents)		
PERSONNEL					
NAME	TITLE OF POSITION		SALARY	FRINGE BENEFITS	TOTAL
	PRINCIPAL INVESTIGATOR				
(See details on attached p. 3a)					
					505,872
CONSULTANT COSTS					
Occasional engineering consultants for special technology					1,500
EQUIPMENT					
(see details on attached p. 3b)					828,200
SUPPLIES					
(See details on attached p. 3c)					204,632
TRAVEL — DOMESTIC	One major trip to East Coast for professional persons to attend a major conference (Gordon, CSH ($2,000); attendance				
TRAVEL — ~~FOREIGN~~	by professionals and grads at local conference (Arrowhead, Biophysics); confer with colleagues and equip. suppliers.				3,000
PATIENT COSTS (See instructions)	($1,000)				
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES (Itemize)					
(See details on attached p. 3c)					103,451
TOTAL DIRECT COST (Enter on Page 1, Item 5) →					1,646,655

INDIRECT COST (See Instructions)

DATE OF DHEW AGREEMENT:

_____ % S&W*

34.2 % TDC* 5-23-73

☐ WAIVED

☐ UNDER NEGOTIATION WITH: _____

*IF THIS IS A SPECIAL RATE (e.g. off-site), SO INDICATE.

Donald A. Glaser

Privileged Communication [redacted]

PERSONNEL Name	Title of Position	%Time	Salary	Fringe Benefits	Total
Donald A. Glaser	Principal Inves.	2 mos.	8,652	1,296	9,948
Mechanical Engineers					
Leif Hansen	Prin.Dev.Engrg	100	25,224	3,026	28,250
John Bercovitz	Asst. Dev.Engrg.II	100	14,748	1,770	16,518
Larry Johnson	Assoc.Dev.Engrg.III	100	18,792	2,255	21,047
Larry Henderson	Draftsman I	100	8,628	1,035	9,663
Shop and Maintenance					
Walter Debold	Prin.Lab.Mech.V	100	16,236	1,948	18,184
Lloyd Davis	Prin.Lab.Mech.V.	100	16,236	1,948	18,184
James Munger	Lab.Asst.II	100	8,196	983	9,179
X	Lab.Asst.I	100	7,560	907	8,467
XX (2)	Lab.Helpers	100	12,624	1,514	14,138
Computer Programming					
Fraser Bonnell	Prin.Progr.V	100	24,612	2,953	27,565
X	Sen.Progr.	100	16,656	1,999	18,655
X	Programmer	100	12,444	1,493	13,937
Computer Operations					
Robert Henry	Sr.Dev.Engrg.V	100	24,012	2,881	26,893
XX(2)	Computer Operator	100	19,906	2,388	22,294
Instrumentation					
Ronald Baker	Assoc.Dev.Engrg.V	100	20,748	2,489	23,237
James Berk	Assoc.Dev.Engrg.I	100	17,052	2,046	19,098
Ted Fujita	Asst.Dev.Engrg.V	100	17,052	2,046	19,098
Alex Para	Asst.Dev.Engrg.I	100	14,040	1,685	15,725
Pat Donahoo	Lab.Asst.I	100	7,560	907	8,467
Biological Operations					
John Couch	Asst.Res.Biophysicist	100	15,290	2,294	17,584
Philip Spielman	Staff.Res.Assoc.II	100	14,040	1,685	15,725
Marilynn Brookm	Staff.Res.Assoc.II	100	13,044	1,565	14,609
Carol Greiner	Staff.Res.Assoc.I	100	12,144	1,457	13,601
Eva Bennett	Lab.Asst.II	100	8,208	985	9,193
James Colby	Lab.Asst.I	100	7,272	872	8,144
XXX (3)	Post-Doctorals	100	35,568	5,335	40,903
XXXX (4)	Grad.Students 50% - 9 mo 100% - 3 mo		23,292	2,796	26,088
Administration and Procurement					
Madeline Moore	Adm.Asst.II	100	10,248	1,230	11,478
	Total Salaries		450,084	55,788	505,872

Privileged Communication [redacted]

EQUIPMENT

PDP 10-I System		Unit Cost	Total
KI-10	Processor	240,000	240,000
MF-10	Memory(2)64K words	80,000	160,000
RP-10	Disk Control	26,000	26,000
RP-03	Disk Drives(3)	20,000	60,000
DF-10	Data Control for Disk and MagTape(2)	14,000	28,000
TM-10B	Mag TapeDrive Control	20,000	20,000
TU-40	Mag.Tape Drive (2)	25,000	50,000
TD-10	DEC Tape Control	15,300	15,300
TU-56	DEC Tape Drives(2)	4,700	9,400
DC-10A	DataLine Scanner	10,000	10,000
DC-10B	Data Line Group, 8 lines	5,500	5,500
LF-10F	Line printer, 1250 lpm	47,500	47,500
VB-10C	Graphic display	35,000	35,000
PDP-11	Controller for flying-spot scanner, (18 bits)	30,000	30,000
		Sub-total (PDP 10-I)	736,700
PDP 10-I (Software package/p.a.)			5,000
Laser			
4 watt tunable blue laser (Spectrophysics Model 164)			9,500
Flying-Spot Scanner--speed up			
PDP-11 - controller			30,000
New faster yoke and A/D converters			10,000
PDP 10 to PDP 11 direct memory bus			37,000
		Total Equipment	828,200

Privileged Communication [redacted]

SUPPLIES

Software notebook updates ($300); software updates subscription ($1,100/yr); teletype paper, printer paper, Calcomp plotter paper ($1,300); Mag tapes ($300); DEC tapes ($400).	3,400
Petri dishes at $25/case (500 dishes per case), 2 cases per week x 50 wks = 100 cases x $25.	2,500
Agar at $1.30/liter; 0.030 liters per dish; 1000 dishes per week x 0.03 liters/dish x 50 weeks x $1.30	1,950
Agar for Cyclops trays--20 trays/wk x 1.5 liters/ tray x 50 weeks x $1.30	1,950
Agar for Dumbwaiter--2 experiments/wk x 256 trays x 1.5 liters x 50 weeks x $1.30 liter	49,920
Miscellaneous drugs, chemicals, nutrients and glassware	5,000
Film and Development $0.18/ft for 35 mm film Cyclops--20 trays/wk x 50 wks x 32 squares/tray x 6 photos/square x $0.18 ÷ 8 photos/ft.	4,320
Dumbwaiter--256 trays/expt. x 100 expts/yr x 32 squares/tray x 6 photos/square x $0.18 ÷ 8 photos/ft	110,592
Miscellaneous small electronic, mechanical and optical parts for constructing laser selector-inoculator electronic controls, and new cell manipulation devices	25,000
TOTAL SUPPLIES	$204,632

OTHER EXPENSES

Computer maintenance ($2,948/mo.--see p. 3d)	35,376
EE Machine Shop (2,000 hrs. at $11/hr)	22,000
LBL Machine Shop	20,000
Machine shops (special jobs on and off campus)	10,250
LBL Supplies	9,600
Phones	2,700
Xerox	1,800
Page charges, 15 pp at $75/page	1,125
Publications - professional journals	500
Mail	100
TOTAL OTHER	$103,451

Donald A. Glaser

Privileged Communication

[redacted]

Computer Maintenance (continued from p. 3)

	Maintenance per mo
KI-10 Processor	550.00
MF-10	888.00
RP-10	79.00
RP-03	510.00
DF-10	134.00
TM-10B	43.00
TU-40	316.00
TD-10	20.00
TU-56	68.00
DC-10A	19.00
DC-10B	18.00
LP-10F	153.00
VB-10C	150.00
Total per mo	2,948.00

BUDGET ESTIMATES FOR ALL YEARS OF SUPPORT REQUESTED FROM PUBLIC HEALTH SERVICE
DIRECT COSTS ONLY (Omit Cents)

DESCRIPTION	1ST PERIOD (SAME AS DETAILED BUDGET)	ADDITIONAL YEARS SUPPORT REQUESTED (This application only)					
		2ND YEAR	3RD YEAR	4TH YEAR	5TH YEAR	6TH YEAR	7TH YEAR
PERSONNEL *** COSTS	505,872*	556,459**	612,104	673,314	740,645		
CONSULTANT COSTS *(Include fees, travel, etc.)*	1,500	1,650	1,815	1,997	2,197		
EQUIPMENT	828,200	75,000	75,000	75,000	75,000		
SUPPLIES	204,632	225,095	247,604	272,364	299,600		
TRAVEL — DOMESTIC	3,000	3,300	3,630	3,993	4,392		
TRAVEL — FOREIGN							
PATIENT COSTS							
ALTERATIONS AND RENOVATIONS							
OTHER EXPENSES	103,451	113,796	125,175	137,692	151,461		
TOTAL DIRECT COSTS	1,646,655	975,300	1,065,328	1,164,360	1,273,295		
TOTAL FOR ENTIRE PROPOSED PROJECT PERIOD *(Enter on Page 1, Item 4)* ⟶					$ 6,124,938		

REMARKS: *Justify all costs for the first year for which the need may not be obvious. For future years, justify equipment costs, as well as any significant increases in any other category. If a recurring annual increase in personnel costs is requested, give percentage. (Use continuation page if needed.)*

* Cost of living figured at 10% by June 1975.

** Each consecutive year figured at 10% increase for cost-of-living increase and inflation.

*** Employee benefits figured at 12% for non-academic; 15% for academic salaries.

SECTION II – PRIVILEGED COMMUNICATION

BIOGRAPHICAL SKETCH

(Give the following information for all professional personnel listed on page 3, beginning with the Principal Investigator. Use continuation pages and follow the same general format for each person.)

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Donald A. Glaser	Professor of Physics and Molecular Biology	9/21/26
PLACE OF BIRTH (City, State, Country)	**PRESENT NATIONALITY (If non-U.S. citizen, indicate kind of visa and expiration date)**	**SEX**
Cleveland, Ohio, USA	USA	☒ Male ☐ Female

EDUCATION *(Begin with baccalaureate training and include postdoctoral)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	SCIENTIFIC FIELD
Case Institute ofTechnology	BS	1946	Mathematics and Physi
California Institute of Technology	PhD	1949	" "
Case Institute of Technology	Sc.D.	1959	" "

HONORS Henry Russel Award, 1955; Charles Vernon Boys Prize, 1959; American Physical Society Prize, 1959; D. Sc., Case Institute of Technology, 1959; Nobel Prize (Physics), 1960; Ellio Cresson Model (Franklin Institute) 1961; Alumni Distinguished Service Award (Cal.Tech.) 1967; Gold Medal Award (Case Institute of Technology) 1967.

MAJOR RESEARCH INTEREST	ROLE IN PROPOSED PROJECT
Cell genetics and control mechanisms	Principal Investigator

RESEARCH SUPPORT *(See instructions)*

NIH Grant GM 19439	6/1/73 - 5/31/74	Genetic Control of Cell Physiology and Structure	$42,262
NIH Grant GM 13244 09	6/1/73 - 5/31/74	Scanner-computer investigations of biological systems	$405,698

RESEARCH AND/OR PROFESSIONAL EXPERIENCE *(Starting with present position, list training and experience relevant to area of project. List all or most representative publications. Do not exceed 3 pages for each individual.)*

Visiting Professor of Biology MIT 1961-62
Miller Professorship UC Berkeley 1962-64
Consultant Brookhaven National Laboratories, Argonne National Laboratory, and a variety of other laboratories and agencies on scientific and instrumentation problems in physics and biology.

1. C. B. Ward, M. W. Hane, and D. A. Glaser, "Synchronous re-initiation of chromosome replication in E. coli B/r after nalidixic acid treatment," PNAS 66, 365 (1970).
2. C. B. Ward and D. A. Glaser, "Control of initiation of DNA synthesis in E. coli B/r," PNAS 67, 855 (1970).
3. C. B. Ward and D. A. Glaser, "Correlation between rate of cell growth and rate of DNA synthesis in Escherichia coli B/r," PNAS 68, 1061 (1971).
4. D. A. Glaser and C. B. Ward, "Computer identification of bacteria by colony morphology", Frontiers of Pattern Recognition, Acad. Press, N. Y. (1972).
5. J. Couch, J. Berk, D. A. Glaser, J. Raymond, and T. Wehr, "Automated recognition of bacterial strains by analysis of colony morphology", Proceedings of the 13th International Congress of Genetics, Berkeley, California, August 1973.
6. J. Raymond, J. Couch, D. A. Glaser, and C. T. Wehr, "Automatic selection of conditionally defective mutants of microorganisms," Proceedings of the 13th

(continued)

7. C. T. Wehr, L. Waskell and D. A. Glaser, "Isolation and characterization of cold-sensitive DNA mutants of Escherichia coli K12", Proceedings of the 13th International Congress of Genetics, Berkeley, California, August 1973.

8. R. M. Burger and D. A. Glaser, "Effect of nalidixic acid on DNA replication by toluene-treated Escherichia coli", Proc. Nat. Acad. Sci. 70, 1955 (1973).

9. D. L. Parker and D. A. Glaser, "Chromosomal sites of DNA-membrane attachment in Escherichia coli", submitted to J. Mol. Biol. September 1973.

10. D. L. Parker and D. A. Glaser, "Effect of growth conditions in DNA-membrane attachment in Escherichia coli," in preparation.

11. A. H. Dougan and D. A. Glaser, "Rates of chain elongation of ribosomal RNA molecules in Escherichia coli", submitted to J. Mol. Biol., 1973.

12. L. Waskell and D. A. Glaser, "The isolation and partial characterization of mutants of E. coli with cold-sensitive synthesis of DNA", in preparation.

13. D. A. Glaser, "Some effects of ionizing radiation on the formation of bubbles in liquids", Phys. Rev. 87, 665 (1952).

14. D. A. Glaser, "Bubble chamber tracks of penetrating cosmic ray particles", Phys. Rev. 91, 762 (1953).

15. D. A. Glaser, "Progress report on the development of bubble chambers", Nuovo Cimento 2, suppl. 2, 361 (1954).

16. D. A. Glaser and D. C. Rahm, "Characteristics of bubble chambers", Phys. Rev. 97, 474 (1955).

17. D. A. Glaser, "The Bubble Chamber", Scientific American 1955.

18. J. L. Brown, D. A. Glaser, and M. L. Perl, "Liquid xenon bubble chamber", Phys. Rev. 102, 586 (1957).

19. D. A. Glaser, D. C. Rahm, and C. Dodd, "Bubble counting for the determination of the velocities of charged particles in bubble chambers", Phys. Rev. 102, 6, 1653 (1956).

20. D. A. Glaser, Decays of strange particles, Kiev Conference, 1959.

21. D. A. Glaser, et al., "The neutral branching ratios of K^{o} particles", Phys. Rev. Letters.

22. D. A. Glaser and L. O. Roellig, "Elastic $\pm$p and p-p scattering at 1.23 Bev/c." Phys. Rev. 116, 1001 (1959).

23. D. A. Glaser et al, "Direct proof of θ_1^o neutral decay", Phys. Rev. Letters 3 51 (1959).

24. D. A. Glaser and W. H. Wattenberg, "An automated system for the growth and analysis of large numbers of bacterial colonies using an environmental chamber

and a computer-controlled flying-spot scanner", Ann. N. Y. Acad. Sci. 139, 243 (1966).

25. D. A. Glaser, "Biological objectives and strategy for the design of a space vehicle to be landed on Mars." Chap. 18, Biology and the Exploration of Mars, Nat. Acad. Sci. Nat. Res. Council publication, 1966.

26. D. A. Glaser, J. McCarthy and M. Minsky, "The automated biological laboratory, Chap. 19, Ibid. (1966).

27. B. Wolf, A. Newman, and D. A. Glaser, "On the origin and direction of replication of the E. coli K12 chromosome", J. Mol. Biol. 32, 611 (1968).

28. M. L. Pato and D. A. Glaser, "The origin and direction of replication of the chromosome of Escherichia coli B/r. Proc. Nat. Acad. Sci. 50, 1268 (1968).

29. C. B. Ward and D. A. Glaser, "The origin and direction of DNA synthesis in E. coli B/r. Proc. Nat. Acad. Sci. 62, 681 (1969).

30. C. B. Ward and D. A. Glaser, "Evidence for multiple growing points on the genome of rapidly growing E. coli B/r. Proc. Nat. Acad. Sci. 63, 800 (1969).

31. C. B. Ward and D. A. Glaser, "Analysis of the chloramphenicol sensitive and resistant steps in the initiation of DNA synthesis in E. coli B/r. Proc. Nat. Acad. Sci. 64, 905 (1969).

(See beginning of list for more recent publications.)

Ronald Baker - Associate Development Engineer. b. 2/16/29 in Fulham, London, England. U. S. A. Ruislip Manor Secondary School, First Year National. Instrument Maker, Ingersole Ltd., England, 1951-54; Tool and Instrument Maker, G. E. Research Labs., England, 1954-57; Mechanical Designer, Physics Department, U. of Michigan, 1957-59; Mechanical Designer. Lawrence Radiation Laboratory, 1959-65.

John Bercovitz - Assistant Development Engineer. b. 9/3/45 , Baltimore, Md. Cal. Poly, Pomona, BSME, 1972. Design Engineer, Riverside 1969-1973.

James Berk - Associate Development Engineer, b. 9/17/42, New London, Wisconsin. UCLA, BA Physics, 1965; UCLA, MS Physics, 1967; UCLA, PhD Physics, 1969. Research Biochemist, UCBerkeley, 1970-73. National Science Foundation Fellow 1966-67.

Fraser Bonnell - Principal Programmer. b. 7/28/35, Port Chester, N. Y., USA. UCLA, BA, 1957; UC Berkeley, MA, 1958; Teaching Assistant, Department of Mathematics, UC Berkeley, 1959-61; Computer Programmer, Lawrence Radiation Laboratory, Livermore, 1961-65; Instructor, UC Extension Division, various semesters since 1961.

John Couch - Research Associate. b. 5/6/41, Hartford, Arkansas. MIT, SB Physics, 1963; Stanford, PhD Biophysics, 1970; Acting Instructor in Biophysics, Stanford, 1971.
W. R. Fair, J. L. Couch, N. Wehner, Biochemical Medicine 8 (329-339), Purification and Assay of the Prostatic Antibacterial Factor (PAF); Nakayama, H. and Couch, J. L., "Thymineless death in Escherichia coli in various assay systems: viability determined in liquid medium", J. Bacteriol. 114, 228 (1973); J. L. Couch and P. C. Hanawalt, "DNA repair replication in temperature-sensitive DNA synthesis deficient bacteria", Biochem. Biophys. Res. Commun. 29, 779 (1967); J. L. Couch and P. C. Hanawalt, "Analysis of s-bromouracil distribution in partially substituted deoxyribonucleic acids", Anal. Biochem. 41, 51 (1971); P. C. Hanawalt, D. F. Pettijohn, E. C. Pauling, C. F. Brunk, D. W. Smith, L. C. Kanner, and J. L. Couch, "Repair replication of DNA in vivo," Cold Spring Harbor Symposia on Quantitative Biology, Vol. XXXIII (1968), p. 187.

Ted Fujita - Assistant Development Engineer. b. 9/19/43, Topaz, Utah. UC Berkeley, BS, 1964; UC Berkeley, MS, 1965. Project Engineer, Berkeley Scientific Labs, 1965-69.

Robert Henry - Senior Development Engineer. b. 6/8/36, Winfield, Kansas. U. Kansas, BS, 1959; UC Berkeley, MS, 1965; Boeing Aircraft-Electrical draftsman 1957-58 (summers); Western Electric Co., Engineer, 1959-60; RCA, Engineer, 1960-64.

Leif Hansen - Principal Development Engineer. 5/29/27, Copenhagen, Denmark. USA citizenship. Technical University of Denmark MSME, 1954; Senior Design Engineer, General Dynamics-Astronautics, 1957-62; Senior Engineer, Lawrence Radiation Laboratory, Berkeley, 1962-65. (Mechanical Engineer, RDAF 1954-56).

(continued)

Larry Johnson - Associate Development Engineer. b. 2/28/37, Sioux Falls, South Dakota. SD School of Mines, BSME, 1959. Sperry, Project Engineer, 1959-65; Boeing, Design Engineer, 1965-66; FMC, Sr. Design Engineer, 1966-70; Thermidex, Sr. Project Engineer.

Alex Para - Assistant Development Engineer. b. 2/22/50, Buenos Aires, Argentina. Citizen of Argentina. Chabot College, AA, 1968; UC Berkeley, BS, 1971; Engineer's Aid, UC Berkeley, 1969-70; Sr. Engineers Aid, UC Berkeley, 1970-71. Sr. Development Engineer, UC Berkeley 1971-72.

Table of Contents

Privileged Communication

Justification of first 12-month period

Personnel

During the first 12-month period we plan to maintain the engineering staffs at their present size, because we expect there to be extensive debugging, modification, and minor additions made to the machine as we gain experience in its use. Those who designed the machine will be the most effective at understanding its shortcomings and making necessary improvements. As time goes on the shop activities will shift from construction of new equipment to maintenance of the existing equipment at probably the same level of manpower as required during the construction phases of the project. The instrumentation and electrical engineering group will similarly be engaged in debugging, modifications, and minor additions to the equipment.

In order to operate the computer facility around the clock, we will need to have two full-time computer operators, but no other major expense is contemplated. For biological operations, a Senior Biologist with considerable experience in computer programming and instrumentation is being proposed and the budget also provides for the salaries of three postdoctoral researchers and four graduate students since training grants for these categories of people are no longer available.

As the experimental program gains momentum, we will need to add two relatively junior programmers to help biologists formulate protocols and write programs to carry out the necessary operations.

Equipment

PDP10-I System to replace our PDP-6 System. By the time this proposed program begins in June 1975, we will have owned and operated our present PDP-6 system for 10 years at an enormous saving in the cost of leasing the same equipment. Lease rates are usually computed to amortize the equipment in about 40 months and we will have operated the equipment for 120 months at the same cost. Several years ago the PDP-6 computer became essentially obsolete when it was replaced by the PDP-10, and then by the PDP10-I system. Probably by June 1975 there will be a yet newer replacement of the PDP10-I system. At the present time (October 1973) there is only one operating PDP-6 computer left in the United States at the Rand Corporation who are planning to get rid of it in the next few months. There may also be another highly modified PDP-6 computer at M.I.T. not maintained by D.E.C. (Digital Equipment Corporation) and perhaps used for special experimentation in computer science. D.E.C. no longer maintains the software for the PDP-6 and it is costly and difficult for us to modify the constant improvements in PDP-10 software so they are useable on the PDP-6. New software, beginning to be issued by D.E.C., is not suitable at all for the PDP-6 computer and we will soon be unable to take advantage of the "community knowledge" and library of programs available for PDP-10 applications.

It is not practical for us to maintain the computer ourselves and D.E.C. maintains only one trained maintenance person who, in fact, can only be trained at our own computer by his immediate predecessor. We absolutely then depend on this one person because ours is the only computer of its kind still maintained by D.E.C.

The change to the new PDP 10 system most recently available in June 1975 is expected to give us a speed increase of at least a factor of 4 in analyzing photographs from the Dumbwaiter and Cyclops. Since these instruments take photographs at the rate of 1 per second and our present rate of analyzing pictures is about 1 per 10 seconds to 1 per 20 seconds, we have an extremely unfavorable ratio of analysis time to production time for these photographs. With this additional

Privileged Communication [redacted]

Justification of first 12-month period (continued)

factor of 4 or more available in the PDP-10 system, the ability of the computer to analyze data will be nicely matched to the rate of production by the biological machines. For all these reasons, the switch to the new system is extremely desirable.

Laser. This laser is needed to measure the light scattering of droplets of cell culture formed in the high-speed dripper-inoculator in order to determine whether a droplet contains a cell and the kind of cell contained therein. By rejecting empty droplets and droplets containing multiple cells as described in the Biological Plans part of the proposal, we will increase the effective size of the Cyclops and Dumbwaiter by a factor of 3 and be able to carry out critical sorting operations for experiments on animal cells.

Flying-Spot Scanner--speed up. To further increase the speed at which photographs can be analyzed, we propose to update the Flying Spot Scanner to current technology by the substitution of the PDP-11 computer to serve as a controller for the scanner in place of the home-made circuit that does the job now. In addition we will substitute new, improved versions of the deflection yoke system for the precision cathode ray tube and faster A/D converters. Finally, we would add a PDP-10 to PDP-11 direct memory access-dump for bringing scanner information directly into the PDP-10 memory without going through the slower I/O Bus.

Supplies--The cost of supplies is based on the assumption that the Cyclops will continue to operate for small-scale experiments and for "second-pass" experimental material produced by large Dumbwaiter experiments. It will operate with petri dishes or with glass trays at a modest level as described in the budget figures themselves. The budget for Dumbwaiter supplies is based on the expectation that we will be able to carry out 2 batches per week for 50 weeks per year which seems at this time a reasonable average level of activity.

Travel--On the average of one major trip to the East Coast for professional persons to attend a major conference such as the Gordon Conference and the Cold Spring Harbor Conference, as well as attendance by professionals and graduate students at local conferences. Also conferences with colleagues and equipment suppliers.

Other--Computer maintenance contracts are based on present cost estimates by the manufacturer who carries out the maintenance. Machine-shop time and other campus shops is required from time to time when our own single machinist is overworked or when special facilities and large machines are required for a particular job. The budget is based on one man year of work for this purpose.

The Equipment Budget for subsequent years provides for new accessories bound to be required as the experimental program expands, including for instance, a television-scanner system for on-line real time analysis of growing colonies to eliminate the photography step and provide for the possibility of intervention in the experiment in real time and very rapid read-out necessary for particular applications. For study of animal cells it will probably be necessary to design a camera that photographs a small area of agar at a time through a low-power microscope for studying very small clones of animal cells. Other requirements of these kinds are bound to arise. We will justify this budget item on a year-to-year basis.

Privileged Communication [redacted]

Research Plan

A. Introduction

1. Objectives. When this program-project began in July 1965, the overall goal was to automate many of the procedures of petri dish technique on a large scale using computer-directed machinery and pattern recognition techniques in a flexible way so that a wide variety of biomedical problems could be attacked. Now, in November 1973, after successful operation of several prototypes, much of the equipment is in operation and all of the major equipment will be in full operation at the end of the current grant period in June 1975.

In its short period of operation the machinery has successfully aided in the isolation of cold sensitive mutants of E. coli K12 unable to synthesize DNA at 20°C. It has also performed highly accurate automatic recognition of growing colonies of 10 species of bacterial pathogens important in medical diagnosis as a demonstration of its abilities in many health-related applications. In the next few months we will begin new experiments in genetic mapping, mutant isolation and physiological characterization with E. coli, Salmonella typhimurium, Bacillus subtilis, Saccharomyces cerevisiae, and animal cells grown in tissue culture. Many of these projects will be done in collaboration with scientific investigators who have on-going projects in these areas.

During the next five-year grant period beginning in June 1975, we propose to extend these projects and add others involving the construction of genetic maps, the isolation of important mutants, and the characterization of mutants and strains. Some of these projects will be chosen to aid in critical steps of the productive work of a number of independent scientific investigators already working in these fields, and some will be important parts of our own biological programs. In addition, we propose to examine the feasibility of health related projects including screening of environmental chemicals, including food additives for their mutagenic effects on bacteria, yeast and animal cells, the potential carcinogenic and anti-neoplastic effects of various agents on animal cells, and the effects of very low levels of ionizing radiation on various cells. If large-scale screening projects appear feasible and desirable, special funds will be sought to carry them out if necessary. Finally, a modest instrumentation program will be continued to add new capabilities to the machinery as they become necessary. Scientists from many laboratories are expected to take advantage of this facility.

2. Background. Since this program includes a number of different biological projects, the biological background, rationale, aims, and methods will be discussed project by project in subsequent sections of this proposal. What brings them together in this program is a similarity in the technical manipulations and the common requirement for large-scale experiments too tedious, slow and costly to carry out by hand. In some cases quantitative measurements on growing colonies are extremely difficult without the automatic pattern recognition facility.

Except for small labor-saving devices, techniques for growing colonies on solid media have changed little since they were invented. Many projects in the contemporary biology of clonable cells are severely limited by the difficulty of isolating particular mutants, characterizing them, and locating them on the genetic map of the organism. Numerous health-related programs including medical bacteriology; contamination monitoring; mass screening programs for mutagens, carcinogens, and anti-neoplastic agents; and industrial strain-improvement programs utilize similar techniques. It is hoped that this program-project will be useful in all these fields as well as in work in fundamental biology.

3. Rationale of this automation. To carry out experiments requiring study of large numbers of colonies we are constructing a machine (the "Dumbwaiter") in which 256 40cm x 80cm agar-filled glass trays circulate in an incubator past stations where various operations can be performed. An inoculation device deposits single cells carried in microdroplets in regular rows and columns for maximum uniform packing and easy subsequent manipulation. During incubation, time-lapse photographs of the colonies are made using up to 5 different colors of light. A flying-spot scanner (similar to a television camera) under control of a computer (PDP-6) examines the photographs, finds all the colonies, and records their size, appearance, and growth rate. The computer then computes the frequency of various classes of colonies for measuring mutation rates, map distances, recombination frequencies, and other required biological results. In addition the computer can direct a colony "picker" to retrieve part of a colony for replica plating, suspension in liquid, restreaking, or delivery to a test tube or small petri dish for further manual work in the laboratory. Alternatively the computer can direct the spraying of some or all colonies with nutrients or drugs on some predetermined schedule or according to the actual performance of each particular colony. Thus the computer can intervene in on-going experiments. Irradiation, genetic crossing on the agar, and similar operations can also be performed as the trays move through the Dumbwaiter. Design of the DW (Dumbwaiter) and associated equipment has been done to allow a wide variety of accessories to be added to carry out special manipulations as they are required for particular experiments. If colonies are placed 1 mm apart, the DW can hold almost 10^8 colonies per load of 256 trays. Several loads can be processed each day for many types of non-interfering experiments.

What does this kind of large-scale automation have to contribute to biomedical science? Solution of many biological problems depends on the ability to isolate a particular kind of mutant, to measure the rate of a particular genetic recombinational event, or to measure responses of growing cells to specific chemical, biological and physical conditions. Automation allows highly reproducible experiments to be performed with large numbers of organisms so rare events can be observed and more common events measured with high statistical accuracy. Computer-directed pattern recognition allows quantitative aspects of growth to be explored for regularities that would escape qualitative visual examination.

None of this increased statistical and quantitative power reduces the need for thoughtful study of the biological system in advance of large-scale experiments and of careful analysis of the results. Nor is this kind of automation likely to reduce the number or quality of people involved in a given research area. Rather the same people will be able to accomplish tasks impossible without the machinery and to do many more conventional experiments with much reduced tedium.

In medical, public health, and industrial applications, large scale screening, contamination surveys and diagnostic assays, and other similar tasks can be done with the unbiased reliability of automation and the economies of large-scale. It is expected that these machines or adaptations of them will be cost-effective and quality-effective for a variety of immediate health-related applications.

4. Comprehensive Progress Report.

(a) Period covered by this report: June 1970 to November 1973.

(b) Summary. Equipment has been built for inoculating up to 100 40 cm x 80 cm agar-filled glass trays with single cells in regular rows and columns of adjustable spacing, incubating the trays under tightly controlled conditions, photographing them periodically, and analyzing the photographs with a scanner-computer system. Frequencies of various colony types are recorded by the computer which can also direct the

automatic picking, replica plating, and restreaking of colonies it is instructed to select. Nutrients, drugs, viruses, and other agents can be delivered to whole trays or selected colonies under computer control.

Design and construction of a fully-automated system able to carry 10^8 colonies on 256 trays is near completion.

With the presently operating system we have isolated cold-sensitive mutants of E. coli K12 unable to synthesize DNA at 20°C using 1/5 as much agar and much less labor than a parallel project using hand methods. Nine bacterial species isolated from human urine and a laboratory strain have been studied with the automated system. Using newly developed programs, the computer can correctly identify unknown colonies of these ten types with accuracies better than 98%.

A. 4(c) Detailed Report

1) Biological Projects. Although the goals and budget of this program-project were directed principally toward development of the automation system, several biomedical projects have been carried out to demonstrate the abilities of the system and to speed the work on biological projects in our laboratory supported by NIH as GM 19439 (replacing GM 12524). The NIH preferred funding the instrumentation and biology programs separately so that they would be reviewed separately by appropriate panels.

(a) Finding, Counting and Sizing Colonies. Computer programs have been written for scanning photographs of 100-mm petri dishes prepared by hand for finding, counting, and sizing the colonies correctly in spite of overlaps of colonies and wide variation in colony sizes. The counting algorithm has an accuracy of better than 99% on dishes containing up to about 400 colonies and requires about 10 seconds per dish. It is thus greatly superior to any commercial colony counter, but is not used currently because simpler and faster programs are effective with regular array-inoculated dishes and trays.

(b) Isolation of Cold-Sensitive Mutants (by a method widely applicable to mutant hunting). Investigations of DNA synthesis in E. coli, its control, and its connection with cell division, require isolation and genetic mapping of conditionally lethal DNA mutants. Work on our laboratory and many others has uncovered 7 or 8 classes of heat sensitive mutants normal at 30°C or 37°C but unable to synthesize DNA at about 41°C. These classes map at 7 or 8 distinct sites, but probably DNA synthesis is even more complex and additional sites defining more structural or control genes remain to be discovered. We are searching for new classes among cold-sensitive mutants unable to synthesize DNA at 20°C by taking time lapse photographs of colonies grown from mutagenized cultures and shifted between 20°C (restrictive temperature) and 37°C (permissive temperature). Imposing conditions on colony diameters and growth rates leads to efficient selection of cold-sensitive mutants by the scanner in a way that saves much labor and materials when compared with a competitive hand experiment run in our laboratory. Cold sensitive mutants mapping at a known site (C class) have been found and 3 new mutants may represent a new class not yet precisely mapped. Since the colony picker is not yet in operation, the scanner aids in locating mutant colonies by displaying a map of each dish on the display scope. Holding the dish against the screen, mutant colonies are picked wherever the computer has drawn an X.

This method (later using the picker to eliminate hand labor) can be used for any mutant selection based on colony size or appearance. We have tested it successfully with known leucine auxotrophs by growing mixed prototrophic and auxotrophic cultures in limiting leucine and then spraying the agar with additional leucine, taking photographs during the incubation intervals.

(c) Automated Recognition of Bacterial Strains by Analysis of Colony Morphology. To test the ability of the system to identify bacterial pathogens for medical and public health applications, we photographed 24-hour colonies of nine species isolated from human urinary infections plus one Bacillus subtilis strain. Using methods of colony morphology analysis described below, the system "learned" to recognize the 10 test species by examining about 1000 colonies of each. Upon scanning an additional 1000 colonies presented in mixtures or in pure cultures, the program makes two decisions: 1) whether to attempt an identification (answered "no" if the "colony" is not round, is actually a piece of dirt, an imperfection in the agar, etc.), and 2) to what species does the colony belong (if 1) is answered "yes"). Results were as follows:

	% Attempted	% Correct
Aerobacter aerogenes	83	100
Bacillus subtilis	83	100
Escherichia coli	83	100
Herellea vaginicola	77	100
Klebsiella pneumoniae	81	98
Proteus morganii	86	100
Pseudomonas putida	83	100
Salmonella typhimurium	89	100
Serratia marcescens	86	100
Staphylococcus aureus	89	100

2) Technical Progress. When completed in January 1975, our automated system will prepare minimal agar medium in 400-liter batches, dispense it with a programmable variety of additives into 256 40cm x 80cm presterilized glass trays, and circulate the trays inside a precision incubator past stations for inoculation, time-lapse photography, colony picking and replica plating or restreaking, and treatment with chemicals, radiation, viruses, etc. In January 1973, the prototype test version came into operation and is now carrying out almost all of the operations of the final system semi-automatically on a reduced scale (about 100 trays maximum capacity). Photographs are examined by a flying-spot scanner (similar in operation to a television camera) connected to a medium-sized computer. The computer finds all the colonies, measures their diameter, characterizes their appearance (using up to about 100 parameters), and issues commands for colony picking, nutrient spray, mutant purification by colony restreaking and replica plating, according to a protocol written by a biologist.

By June 1975, when the presently proposed program is due to begin, the system should be in full operation. Technical aspects of this work have been reported in two published papers: D. A. Glaser and W. H. Wattenburg, An automated system for the growth and analysis of large numbers of bacterial colonies using an environmental chamber and a computer-controlled flying-spot scanner, Ann. N. Y. Acad. Sci. 139, 243 (1966); D. A. Glaser and C. B. Ward, Computer identification of bacteria by colony morphology, Frontiers of Pattern Recognition, Academic Press, N. Y. (1972),

a large number of oral reports, and Progress Reports to the NIGMS. Detailed publications will be prepared after the full system is completely operational. We have seen no reports of similar systems in operation elsewhere.

(a) Objectives and General Description. Many of the biological objectives of this program require the ability to examine about 10^6 fairly well isolated colonies (about one colony per square centimeter). For other studies up to 10^8 colonies need to be examined but they may be crowded into a smaller space (about 100 colonies per square centimeter). The machine must therefore have a capacity of about 10^6 cm^2 of solid growth medium (agar, silica gel, or other medium). To provide the required area of agar in the smallest possible volume, the machine uses stacks of horizontal agar-covered trays spaced one inch apart. These trays are made of inexpensive window glass with metal frames and can be washed and sterilized very easily by reasonably standard techniques. They also provide a very uniform growth surface of high optical quality so that good photographs of growing colonies can be made. Ordinary plastic or glass petri dishes made by hand in the laboratory in small batches in the conventional way can be laid on the trays for incubation, photography, and manipulation in the machine. Alternatively, large-scale experiments can be carried out by pouring a sheet of agar directly on the tray. A design has been chosen which makes it possible to intermix these two modes so that the petri dishes made by hand can be analyzed at the same time as large-scale experiments prepared automatically by the machine The entire machine is fully automated to perform large-scale microbiological experiments in conjunction with a sophisticated data gathering and processing system. Because the stacks of trays are moved up and down by mechanical devices, we have called the machine "A Dumbwaiter".

The design concept of the Dumbwaiter is very simple. Glass trays carried in aluminum frames are stacked directly on top of each other in two stacks about 25' apart. Cross-ducts are provided to transfer trays from the top of one stack to the top of the other, and from the bottom of one stack to the bottom of the other. The trays then circulate in a rectangular path moving up through one stack across to the top of the other, down through the second stack, and across from the bottom of the second stack back to the bottom of the first stack. This over-all design plan can be seen in the attached figure. On the cross-ducts for moving the trays horizontally will be mounted cameras for photographing the trays and special accessores for inoculating the agar with organisms; administering drugs and nutrients, irradiating with ultra-violet light or other radiation, picking, restreaking and replica plating colonies and other manipulations. Trays are handled singly only in the cross-ducts. In every other part of the Dumbwaiter and auxiliary equipment, the trays will be handled in stacks of 64. The stacks and transfer paths are enclosed in housings in which a sterile growth environment is maintained.

Mixing, sterilization, and pouring of agar is carried out outside of the Dumbwaiter. Accessory stations will also be provided for washing the trays for re-use, for sterilizing them, and for special incubation and cold-storage of trays of colonies which do not need to be photographed very frequently. Four moveable magazines will be provided for storing stacks of trays and transporting them from the Dumbwaiter to and from various auxiliary stations where these special operations will be carried out. The separation of these necessary functions to a number of specialized stations was found to be the best way to provide rapid, reliable and economical operation of the system. On the following pages we will give the details, status, and characteristics of the Dumbwaiter and its auxiliary equipment.

(b) Operational prototype (Cyclops). As design and testing of Dumbwaiter components proceeds, we often need to construct temporary devices for testing design principles and mechanical devices that will be used in the Dumbwaiter. At the same time, we were anxious to begin carrying out biological experiments before the Dumbwaiter comes into full operation. We have, therefore, constructed a machine called "Cyclops" consisting of a Dumbwaiter camera mounted on an x-y motion capable of handling one or two Dumbwaiter trays in the same way that will be done in one of the horizontal cross-ducts of the completed Dumbwaiter. Cyclops is capable of photographing agar-laden glass trays or trays carrying conventional plastic petri dishes, of inoculating sterile agar with organisms to be grown, of spraying drugs, nutrients and other substances, of picking and restreaking colonies and carrying out most of the mechanical and optical operations of the Dumbwaiter. It is not able to incubate and circulate trays, however, and at the present time requires the trays to be transported by hand. Nearly all of the other ancillary facilities of the Dumbwaiter are being used routinely for experiments done on the Cyclops as will be described below.

(c) Moveable magazines. The moveable magazines serve many purposes. Their main function is to transport and protect the 64-tray stacks. Each stack rests on a dolly on rails on the bottom of this magazine. When the moveable magazine is engaged to a fixed magazine for transferring a stack in or out of the Dumbwaiter, the rails in the moveable magazine mate with corresponding rails in the fixed magazine. The moveable magazine and the fixed magazine both have doors facing each other. The space left between the doors after engagement is accomplished will be sterilized by UV radiation. The doors will then be coupled together and simultaneously lifted up into an enclosed UV irradiated container above the fixed magazine. The lifting of the doors is performed by an air cylinder. The stack transfer can now be executed using a hand-driven transport screw located in the moveable magazine.

Whenever actual stack transfer is not taking place, the dolly is locked in a fixed horizontal position and the stack is secured in vertical compression by hand-operated screws in the magazine top cover. This will prevent unwanted movement of trays in the stack during transport and handling of the magazine. The vertical compression will be especially important to keep all trays parallel to each other during the agar-pouring and annealing process. The agar-pouring will be done while the moveable magazine is resting on levelling jacks in a combination sterilization, pouring, and annealing oven. The stack dolly is equipped with mercury levels (permitting 180°C dry sterilization) to assure accurate levelling of the stack before the agar pouring. The agar-pouring probes enter the moveable magazine through two automatically sealing vertical slots in a side wall. The moveable magazines have no thermal insulation and they do not have any temperature control system. The moveable magazines will be transported on an air-cushioned transport pad of adjustable height.

All four of the portable magazines for the final Dumbwaiter system have now been completed and one test model dubbed "Oddball" has been made for experimenting with control of temperature and humidity in the portable magazines and for use in semi-automatic operation together with the Cyclops. Oddball is in constant use in connection with Cyclops and is performing well.

(d) Agar-mixing plant and pouring devices. Agar is mixed in stainless-steel tanks and fed to a pouring device by a metering arrangement. The pouring device attaches to the side of the moveable magazines and consists of a vertical

ball-screw with attached nut. The nut carries two agar-pouring probes which go through the slots in the magazine and oscillate in and out through the ventilatic holes in the trays. Both the mixing plant and pouring device can be automated easily using a PDP-8E Computer as the main control element. The agar plant is a rather elaborate system for mixing 400 liters of agar in a batch and delivering it sterile into the Dumbwaiter. It is complete and has been used several times for pouring test plates which showed no contamination, gelled perfectly, and showed good optical clarity. It produces 400 liter batches of neutral agar containing a minimum addition of salts. The agar will be dispensed through a hole in the roof of the sterilizing oven into the tray-filled magazines, through a manifold which will allow the addition of specific nutrients, carbon sources and other additives under computer control. In this way it will be possible to prepare a full load of trays for the Dumbwaiter containing a variety of different agars for the simultaneous performance of several experiments at one time. It is not economical to use the agar plant on the reduced scale experiments being carried out at the present time.

(e) Sterilizing Oven. A large oven necessary for sterilizing glass trays in their moveable magazines for the use of the Dumbwaiter has been completed and is routinely in use. It holds a temperature of 175°C for eight hours for dry sterilization of stacks of glass trays held in the Oddball moveable magazine as it is used now with the Cyclops system.

(f) Automatic agar dispensing system. Under the control of the PDP-8E computer, the automatic agar-dispensing system can dispense enough agar to fill a glass tray in 56 seconds, without splashing, using only four electrically-controlled valves. Also under control of the PDP-8E computer is a mechanical system using a vertical ball screw stepping motor combination that indexes a pair of nozzles vertically from one pair of trays to the next through the stack to fill them in order. After all the trays have been filled with agar, the temperature in the sterilizing oven is gradually lowered so that all trays have the same annealing experience. This is important to provide uniform and reproducible agar surfaces to all of the organisms in the subsequent batch experiment.

(g) Constant temperature rooms. Four rooms have been constructed at one end of the laboratory to accommodate moveable magazines from the Dumbwaiter that carry the equivalent of 5,000 petri dishes each. The rooms have been tested and are able to hold a temperature in the range of 0° to 50°C with an accuracy of ± 0.1°C or better. This arrangement permits incubation of dishes at a variety of temperatures as well as cold-storage of those dishes that must be held before the next step of processing. The rooms are in use for small-scale experiments involving the Cyclops system and the Oddball magazine, as well as for other experiments carried out by hand. Precision controls and recording apparatus for the constant temperature rooms allow the experimenter to know the exact status of his incubating culture at all times.

(h) Photography. Colonies are illuminated from below by a very stable source of very parallel light provided by a high-pressure xenon arc lamp and a 7" off-axis paraboloid mirror. The camera is provided with an automatic focusing device which changes the focusing distance up and down to compensate for changes in the thickness of agar, in the placement of dishes and trays and other sources of mechanical error that could make slight changes in the distances from the camera to the agar surface. The camera is kept in focus with an accuracy of ± 0.005" in spite of these sources of variation. It uses electronically-driven shutters

under the control of an integrating light meter designed to guarantee reproducible exposures from one picture to the next. To correct for slight errors in the system as well as small uncertainties in the uniformity of the original photographic materials as well as the subsequent developing process, a "step wedge" of variable optical density is photographed in the corner of each picture so that subsequent measurements on the film of the image of this grey wedge will allow all the data to be reduced to standard exposure conditions. A color wheel carrying five filters of various colors operates in the illuminating light beam so that black and white photographs can be taken under computer control of any choice of five colors. Finally, the camera has built into it a data board carrying an array of binary coded lights which record the frame number, the date, and a variety of other data necessary in the interpretation of the pictures.

(i) Inoculation. Inoculation of large agar-filled glass trays or of trays loaded with 100 mm petri dishes is now accomplished by the use of a vibrating nozzle which generates a stream of very fine droplets containing about 10^{-9} liters of bacterial suspension per droplet. Under computer control this dispenser can deposit the droplets at any desired distance thus "planting" the agar with droplets containing bacteria in regular rows and columns which grow into regularly spaced colonies. This development increases the capacity of Cyclops and will do the same for the final Dumbwaiter as well as making the finding and scanning of colonies by computer much more rapid and economical. With colonies 1 mm apart as planted by this microdispenser, the capacity of the Dumbwaiter will be about 10^5 colonies per batch. If accurate rates of colony growth and precise observation of colony morphology are necessary, the colonies must be kept 3 to 5 mm apart depending on the circumstances. By electrostatically charging the droplets as they are formed, it is possible to deflect them in desired patterns and on this basis we now have in operation, a "swath dripper" which speeds up the planting of the trays by a factor of 5 or more. The usefulness of this dispenser will be increased enormously in the future when laser light scattered from the individual forming droplets gives a signal that can be used to throw away empty droplets and droplets containing more than one bacteria by the electrostatic deflection system. It should then be possible to deposit one and only one cell on every site. Contamination problems are also minimized by the use of this microdispenser because any colony growing at an "illegal" site on the agar is automatically rejected as not part of the deliberately inoculated system.

(j) Automatic Film Processor. An automatic film processor has been installed and modified to develop our film to a gamma of one in a very reproducible and economical way so that only minor corrections need to be made to give reliable optical density measurements of images on the film.

(k) A large capacity air sterilizer has been built, tested and is providing sterile air required for a number of pieces or equipment for Cyclops.

(l) Washing glass trays is now accomplished by the use of an old commercial laboratory glassware washing machine renovated and modified for use with the Cyclops project.

(m) Colony picker. Almost completed is a device for picking and recovering colonies of particular interest. The picker is a 4" square aluminum plate carrying 400 one-millimeter diameter quartz rods at right angles to the plane of the plate. These rods can be activated one at a time under computer control to pick some cells from any colony of interest. Using instructions from the

PDP-6 film scanner, the PDP-8 computer positions the colonies of interest under the Picker which picks up the first 400 colonies of interest. These can then be replicated on fresh agars of various composition or can be streaked out for mutant purification by moving one row of 20 quarts rods at a time across the surface of fresh agar. After rapid heat sterilization, another 400 colonies can be picked. The new Picker and its computer controls and programs is expected to be in operation in December 1973.

(n) Tray-Washing Machine. About half the size of the Dumbwaiter itself, the tray-washing machine accepts stacks of 64 trays from a moveable magazine, strips off the agar of one frame at a time, washes the frame and returns it to the bottom of the stack. Construction of the tray-washing machine is under way and is expected to be completed during January 1974 except for plumbing and electrical interconnections with the rest of the system.

(o) Dumbwaiter. The design of the Dumbwaiter itself is well under way and the construction of the machine is expected to be complete by January 1975. Since all of the more sophisticated portions of the Dumbwaiter design have already been tested on the Cyclops system we expect to have the Dumbwaiter system fully operational well before June 1975 including all of the ancillary facilities many of which are in constant use already.

3. Data Analysis.

(a) Flying-spot scanner. The function of the flying-spot scanner is analogous to that of a television camera but is based on the use of a precision cathode ray tube on which a tiny spot of light (less than 0.001 inches in diameter) can be instructed to appear on any part of the face of the cathode ray tube with an accuracy of 1 part in 8,000 along both x and y axes. A high-quality lens throws an image of this small spot of light on a frame of 35 mm plus-x film and a photomultiplier behind the film measures the amount of light that passes through the film at that particular point. Under computer control the spot can carry out an orderly raster scan of the image with a resolution of 8,000 lines or it can carry out particular geometric strategies to outline the boundaries of a particular object. The scanner is under direct control of the PDP-6 computer with high speed interfaces constructed in our laboratory. It is able to scan the entire picture with a resolution of 8,000 lines and measure the optical density of the film with a precision of 1 part in 64. The first step in analyzing the picture is to locate all the objects; determine which of them are round enough to be considered single colonies; and compute the center and the diameter of each colony for use in calculating growth rates of colonies (diameter as a function of time of growth). For experiments requiring recognition of colony morphology, the computer next instructs the flying spot to pass slowly across the diameter of the colony, making an optical density measurement at every step. This results in 300 or 400 optical density measurements being made per diameter. The operation is repeated for four diameters and averaged to give a diametral optical density profile for the colony. This profile is then used to determine the diameter, the highest peak height, and Fourier analysis of the shape is carried out with as many as 15 Fourier coefficients being recorded. To include features such as pigmentation, surface iridescence and sheen, turbidity due to small optical inhomogenieties, and other subtle but visually observable properties, we often take as many as five different black and white photographs of each field of view using five different color filters. The blue-red difference is often dramatic because blue light is

scattered more by small turbid regions than red light even though the colony doesn't have any obvious coloration. In our most recent experiments we extracted all together about 85 parameters per colony and can carry this total operation out in about 20 seconds for a dish containing 20 to 50 colonies. The scanner system is set up to analyze photographs of standard 100 mm diameter plastic petri dishes or of 100 mm square areas of agar on large glass trays. In one very recent experiment these 85 parameters were used to identify colonies of ten species of bacteria important in clinical-medical bacteriology with accuracies exceeding 98%. Measurements of colony growth rate under various temperature conditions have also been used recently to isolate cold-sensitive mutants of E. coli K12 from which cold-sensitive DNA mutants have been selected. These results will be discussed further in the biological section below. Demonstration experiments have been carried out to show that this method is able to detect auxotrophs by allowing growth of a mutagenized population in an agar medium containing a small amount of tryptone or other complete medium. After using the available tryptone, auxotrophs will cease growing and make small colonies compared with a wild type that continues to grow. By taking several time-lapse photographs, spraying the agar with nutrients, and then photographing again after a suitable interval of incubation, it is possible to learn which of a number of possible nutrients is the required substance.

(b) Computer programs. Extensive computer programming has been done to drive the flying-spot scanner in the most efficient and rapid mode to locate objects, to determine their circularity, their diameters if circular, and their optical density profiles as described above. Library routines for storing large amounts of such data and comparing it for analysis of time-lapse sequences of photographs are available. Since each photograph now has the image of an optical gray wedge in it, the scanner is able to measure the densities of the images of these steps on the 35 mm film and make corrections if necessary to its optical density scale and determine the optical density profiles on the various colonies. Other special programs have been used to drive plotters and cathode ray display tubes for recording and studying the results of the various data analysis strategies. Finally, the PDP-6 prepares tapes containing the addresses of colonies of interest. By January 1974 the PDP-6 will also write instructions that will direct the Picker in physically recovering these colonies and carrying out manipulations with them. Tapes carrying all these instructions will then be mounted on the PDP-8 computer that operates the Cyclops device so that trays can be further photographed as necessary or colonies can be picked, replicated, restreaked or printed upon agar in plastic petri dishes for further study and manipulation by hand in the laboratory.

(c) Dumbwaiter controls. The Dumbwaiter will be under control of a new PDP-11 computer which will coordinate the photography, film advance, color wheel program and inoculation, spraying and picker operations as well as monitoring the temperature, humidity and gaseous environment at a variety of sensors. In addition to this, the PDP-11 will be programmed to carry out all the necessary motions of the x-y stages in the top and bottom cross-ducts. Communications programs between the PDP-6 and PDP-11 will be written during the next year to coordinate the output of the scanner with operations in the Dumbwaiter. It may be desirable in future to connect the PDP-6 directly with the PDP-8 driving the Cyclops and the PDP-11 driving the Dumbwaiter to eliminate the need for physical transfer of magnetic tapes.

A. 4D. Publications .

Although much detailed technical work, engineering design, and testing has been carried out, publication of a description of the hardware and software systems will be delayed until the whole system is in operation so its actual operating parameters can be reported.

The following reports supported by this program have been published:

1. C. B. Ward and M. W. Hane, and D. A. Glaser, "Synchronous re-initiation of chromosome replication in E. coli B/r after nalidixic acid treatment", Proc. Nat. Acad. Sci. 66, 365-369 (1970).

2. C. B. Ward and D. A. Glaser, "Control of initiation of DNA synthesis in E. coli B/r," Proc. Nat. Acad. Sci. 67, 255-262 (1970).

3. C. B. Ward and D. A. Glaser, "Correlation between rate of cell growth and rate of DNA synthesis in Escherichia coli B/r," Proc. Nat. Acad. Sci. 68, 1061-1064 (1971).

4. D. A. Glaser and C. B. Ward, "Computer identification of bacteria by colony morphology", Frontiers of Pattern Recognition, Acad. Press, N. Y. (1972).

5. J. Couch, J. Berk, D. A. Glaser, J. Raymond, and T. Wehr, "Automated recognition of bacterial strains by analysis of colony morphology", Proceedings of the 13th International Congress of Genetics, Berkeley, California, August 1973. (Abstract)

6. J. Raymond, J. Couch, D. A. Glaser, and C. T. Wehr, "Automatic selection of conditionally defective mutants of microorganisms," Proceedings of the 13th International Congress of Genetics, Berkeley, California, August 1973. (Abstract).

7. C. T. Wehr, L. Waskell and D. A. Glaser, "Isolation and characterization of cold-sensitive DNA mutants of Escherichia coli K12", Proceedings of the 13th International Congress of Genetics, Berkeley, California, August 1973. (Abstract)

In addition, biological work supported by GM 12524 (now GM 19439) that has been reported in the same period, and will motivate some of the first applications of the automation system are:

1. P. Scotti, "The behavior of temperature-sensitive T4 DNA polymerase mutants in temperature shift experiments", Virology 43, 366 (1970).

2. M. Hane, "Some effects of nalidixic acid on conjugation in Escherichia coli K12", J. Bact. 105, 45-56 (1971).

3. C. B. Ward and D. A. Glaser, "Inhibition of initiation of DNA synthesis by low concentrations of penicillin",

4. R. M. Burger, "Kinetics of labeling of fast-renaturing DNA in Bacillus subtilis", J. Mol. Biol. 56, 199-201 (1971).

5. R. M. Burger, "Toluene-treated Escherichia coli replicate only that DNA which was about to be replicated in vivo." Proc. Nat. Acad. Sci. 68, 2124 (1971).

6. R. M. Burger and D. A. Glaser, "Effect of nalidixic acid on DNA replication by toluene-treated Escherichia coli", Proc. Nat. Acad. Sci. 70, 1955 (1973).

7. D. L. Parker and D. A. Glaser, "Chromosomal sites of DNA-membrane attachment in Escherichia coli", submitted to J. Mol. Biol. September 1973

8. D. L. Parker and D. A. Glaser, "Effect of growth conditions in DNA-membrane attachment in Escherichia coli," in preparation.

9. A. H. Dougan and D. A. Glaser, "Rates of chain elongation of ribosomal RNA molecules in Escherichia coli", submitted to J. Mol. Biol. 1973.

10. L. Waskell and D. A. Glaser, "The isolation and partial characterization of mutants of E. coli with cold-sensitive synthesis of DNA", in preparation.

A. 4e. Staffing

W. Keith Hadley	Assistant Professor of Clinical Pathology and Laboratory Medicine, UC Medical Center.	1970-71
Calvin Ward	Postdoctoral Fellow	1967-69
	Assistant Research Biologist	1969-71
Beverly Wolf	Assistant Research Biologist	1965-72
Ronald Baker	Associate Development Engrg.	1964-present
John Bercovitz	Assistant Development Engrg.	2/73-present
James Berk	Associate Development Engrg.	3/73-present
Fraser Bonnell	Principal Programmer	1965-present
John Couch	Research Associate	1971-present
Ted Fujita	Assistant Development Engrg.	1969-present
Robert Henry	Senior Development Engrg.	1964-present
Leif Hansen	Principal Development Engrg.	1965-present
Larry Johnson	Associate Development Engrg.	2/73-present
Alex Para	Assistant Development Engrg.	9/72-present

Glaser, Donald A.

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B) Specific Aims; C) Methods of Procedure; and D) Significance

Since this program project is a collection of different biological projects, we will devote a section of this proposal to the aims, methods, and significance of each project separately. These projects have in common the need to isolate and characterize mutants or recombinants difficult to find by hand methods because they are rare and have no easy biological or chemical selection technique, but can be defined by growth rate or colonial morphology under particular growth conditions. In some cases the events are not rare but their frequency must be known with high accuracy so that large numbers of colonies must be examined. The isolation procedures involve inoculation with single cells, incubation, time-lapse photography, replica plating, colony picking, colony restreaking, growth rate or morphology analysis, and other operations that our system is designed to carry out on a large scale. Some of these projects are already under way; some will be begun soon; others will require preliminary feasibility studies; and still others will be added later. They represent a sampling of projects proposed in conversations with a number of scientists and involve a range of clonable cells from bacteria to mammalian cells. They include fundamental studies of molecular evolution and biochemical pathways as well as applied studies of mutagenic effects of environmental chemicals and efficacy of proposed antineoplastic agents. With each project title is listed the scientific investigator(s) who proposed and will guide the work. In some cases a true collaboration with our laboratory is expected to develop; in others the effort will be to help provide mutants for independent and on-going research done in other laboratories; in still others a feasibility study or actual screening effort with direct health-related goals will be undertaken.

1) Isolate, map, and characterize temperature sensitive mutants of E. coli unable to synthesize DNA at 20°C or at 41°C.
Donald A. Glaser, Professor of Physics and Molecular Biology, University of California, Berkeley.

Method: Automated replica plating and incubation at the permissive and restrictive temperatures followed by photography and computer matching of replicas is a straightforward method that will soon be possible. In current use is a series of time-lapse photographs taken of single primary colonies incubated at permissive, non-permissive, and permissive temperatures on a time schedule that allows the computer to impose limits on the colony size to define the mutant class selected. Less agar and fewer manipulations are required for the time-lapse method, but some mutants may be killed at the restrictive temperature so different classes of mutants may be produced by the two methods. Mapping is done by interrupted mating or episomal complementation followed by measurement of co-transduction frequency. Results are obtained by automated colony counting on selective media. Characterization of mutants will be done mainly by conventional methods.

Significance: Knowing the number and location of genes involved in DNA synthesis and its initiation in E. coli is the first step in the genetic and biochemical dissection of this all-important cellular process. Mutants obtained in this study will be shared with other laboratories engaged in enzymological analysis to speed the overall progress in understanding DNA synthesis. (Dr. William Wickner in Professor Arthur Kornberg's laboratory, Biochemistry Department, Stanford University, is studying one of our cold-sensitive mutants that may represent a new DNA gene). An understanding of this most complex and central process in bacteria is bound to be important for understanding the analogous processes in cells of higher organisms, including proliferating animal cells. Alternatively antibiotics that function by perturbing DNA synthesis may be understood or

rationally sought if vulnerable features of DNA synthesis in pathogens is understood.

2) Measure anomalous DNA synthesis events for temperature-sensitive mutants, for UV sensitive and UV resistant mutants, and for recombination-deficient mutants, including gene duplications, deletions, point mutations, other chromosomal changes.
D. A. Glaser

Methods: Changes in proteins involved in DNA synthesis may produce detectable changes in the rates of occurrence of various mutational events including point mutations, deletions, and duplications. The rate of point mutations can be estimated from the rate of revertable auxotrophs. Deletions can be scored as non-reverting auxotrophs, and duplications can be scored by assays for certain enzymes. In particular colonies are able to grow on lactobionate as sole carbon source only if there is a duplication in the lactose operon. Chlorate resistance is being used as a selective condition for deletion of chlorate genes whenever a nearby site for some other function is also affected. These and other assays will be used to study the roles of various DNA synthesis-related genes known to affect UV sensitivity, recombination, or any of the genetically-defined class of temperature sensitive DNA mutants, whether enzymatically characterized or not.

Significance: In evolutionary changes to optimize survival, certain changes in the chromosome must be advantageous in pruning away unnecessary DNA, duplicating genes required to produce large amounts of product, providing surplus duplicate genes for future mutational experiments, and enlarging the chromosome to provide scope for greater complexity. The probability of these changes must be affected by the structure of DNA synthesis-related proteins. An understanding of these effects is critical for understanding evolution at the chromosome level and also necessary to understand diseases of higher animals that may result from slight perturbations of the DNA synthesizing machinery. Rational searches for antibiotics against bacterial pathogens may be possible if this class of perturbations in their DNA synthesis can be understood.

3) Intensive mapping of the E. coli chromosome and measurement of changes in size of the chromosome as frequencies of various mutational events are changed.
D. A. Glaser

Methods:
i. Temperature sensitive lesions can be readily introduced into the bacterial chromosome and mapped by P1 transduction using the already fairly densely placed well established markers in E. coli (or using P22 in Salmonella). Thus the map can be densely filled with temperature sensitive relatively well localized mutants.

ii. Temperature sensitive mutants which densely cover small local regions of the map can be prepared by mutagenizing P22 transducing phage in Salmonella (or P1 transducing phage in E. coli) and transducing in a wild type gene for a known lesion on the recipient strain. Transductants for this particular marker gene will then carry a number of lesions in the neighboring region (around 1% of the chromosome) corresponding to the size of the transducing phage (this method has been developed very successfully in recent work (Hong, J., Smith, G., and Ames, B. N., PNAS 68, 3253 (1971)).

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iii. Ordering of mutants within the close neighborhood of each other can be done by two and three factor crosses by generalized transduction and also by a new episome complementation method developed and described by Robert N. Reeves and John R. Roth, JMB 56, 523 (1971). Use of automatic techniques will allow the enormous labor required to make an intensive map to be done easily using transduction, mating, and other techniques that can be carried out on agar. The establishment of a large library of temperature sensitive and more completely characterized mutants covering the chromosome map thoroughly would have very many applications in the study of bacteria and especially of yeasts and higher organisms. We propose to begin the work with bacteria for which the techniques seem straightforward and extend it later to higher organisms.

Significance:

i. By periodic measurement of map distances by cotransduction or interrupted mating one can monitor increases and reductions of the chromosome by the net effect of gene doubling, recombination, deletion and other processes that may affect its size. With a large number of standard markers and standard procedures the machine can keep a steady picture of the state of integration or autonomy of various plasmids, of the chromosome number, if that is subject to change, and of the size of the chromosome. It seems more likely that the size of the chromosome is not an accurately conserved quantity but there will be variations in the population and it is hoped that methods of measurement will be sensitive enough to make some description of this distribution and how it changes when the parent strain of the population contains various mutations especially affecting DNA replication and repair.

ii. There may be regions of the map for which no temperature sensitive mutants or other conditionally lethals can be found. It is of great interest to know how much of the DNA specifies no function and is functionless except for its role in evolution of new genes to carry out new functions or for structural functions at the DNA or RNA level.

iii. When the whole map or at least regions of it are densely filled with markers it may be possible to discern overall patterns of placement and organization of the genes according to their function or evolutionary history and thus to understand better evolutionary or physiological demands that led to this particular pattern or structure. The operon concept is the most obviously important fact of this type but there may be others as yet unrecognized.

iv. It will be possible to supply large numbers of densely located temperature sensitive and other kinds of mutants in particular regions of the map for intensive further study of particular problems in this and other laboratories. We intend to use the method immediately for trying to generate large numbers of mutants in the neighborhood of known sites for DNA regulatory mutations hoping to discover other DNA regulatory mutants in the same neighborhood. As the techniques develop we will probably be able to supply other laboratories with large numbers of mutants important to their particular interests.

If automation makes it possible to follow changes in a densely-mapped bacterial chromosome subjected to a variety of mutational situations, the resulting insights into chromosome mechanics will be extremely valuable. Extension of this approach to chromosomes of higher organisms has important consequences for understanding a wide range of cellular malfunctions for cells in which good biological mechanisms of genetic recombination are available.

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4) Genetic characterization of the chromosome terminus and the regulation of cell division in E. coli.
David R. Zusman, Assistant Professor of Bacteriology, University of California, Berkeley.

Methods:

Recent evidence from gene frequency measurements (1-3) autoradiography (4) and biochemical analysis (5) demonstrate bidirectional chromosome replication in E. coli. The origin of replication appears to map at about 75 map min while the terminus has been mapped at about 30 min (6). The termination of chromosome replication appears to be necessary for chromosome segregation and subsequent septum cross wall formation (7-9).

It has been suggested that chromosome termination may trigger division by the transcription of division related genes, located at the chromosome terminus, at the time of their replication.(7,10-15). This hypothesis has recently received some experimental support: (a) studies of cell division following DNA, RNA, and protein inhibition at the time of chromosome termination in synchronous cultures (16-18) indicate that the specific replication of the last 0.5% of the chromosome (0.45 map min) is required for subsequent cell division; blocking protein synthesis during this replication will block the subsequent cell division. (b) Several filament forming septation mutants have been obtained which map near 30 min, the chromosome terminus (15, 19-20).

Unfortunately, the region of the genetic map around 30 min is one of the most poorly understood areas (21). Very few markers have been identified; a stable F' has never been isolated for this region (22). We therefore propose to study this region of the E. coli map in great detail using the automated techniques now available. Hopefully the study of this region will help us understand the nature of the link between chromosome termination and cell division.

We have isolated a man$^-$ mutant (30.5 map units) that is non-reverting. We propose to use the transducing phage P_1 to cotransduce mutagenized markers (23) from a man$^+$ strain to our man$^-$ strain. Transductants grown on mannose minimal medium will be plated out using Dr. Glaser's automation equipment, replica plated at different temperatures, and temperature sensitive colonies obtained. These colonies will be characterized for nutritional defects or division defects. The nutritional mutants will be saved to help us map this region of the chromosome. The division-membrane mutants will be studied more carefully to determine possible relationships with chromosome structure and/or regulation of division.

Complementation studies should indicate the specific number of division related genes localized in this region of the chromosome and the possible existence of a division operon. Double mutants will be prepared so that the in vivo interaction (epistasis) of known mutants of different phenotypes can be studied (15). This approach can lead to the sequencing of related gene functions and is the first step necessary to determine the ordered pathway for septation in a manner similar to the study of T-even phage development and other self assembly systems (24).

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Significance: Detailed understanding of the relationship between DNA synthesis and cell division in E. coli may give important insights into the same relationship for proliferating animal cells, which generally do not synthesize DNA except in preparation for cell division.

5) Studies in biochemical evolution in E. coli and B. subtilis.
Joshua Lederberg, Professor of Genetics and Biology and Chairman of the Genetics Department, School of Medicine, Stanford University, Stanford, California.

We wish to observe alterations in polypeptide products resulting from mutations in synthetic genes (generally synthetic homopolymer sequences) which have been inserted into the genomes of E. coli and B. subtilis bacteria. Immunochemical methods will be used for detecting these alterations by examining large numbers of small colonies for which no biological selection condition is known. By observing evolution of a polypeptide, much can be learned about the genetic code and about rates of various kinds of mutations in different nucleotide environments.

6) Genetic organization of the E. coli chromosome: mutation rate versus map position of the translocated lactose operon.
Gordon Edlin, Associate Professor of Genetics, University of California, Davis, California.

The purpose of these experiments is to probe the genetic organization of the E. coli chromosome. Ultimately we would like to understand why genes are located at particular sites in the chromosome. One approach to this question is to measure frequency of mutations in a gene (or genes) which have been translocated to a number of different sites in the chromosome. A model system for these experiments is provided by the lactose operon. A set of strains exist which are genetically uniform except that the lactose genes have been translocated to a

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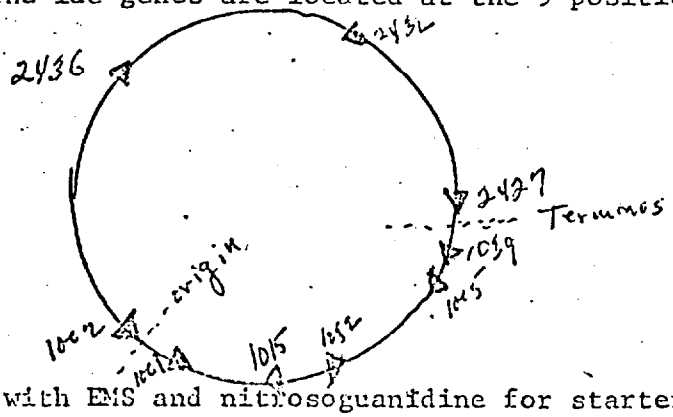
number of different sites in the chromosome.

These strains will be mutagenized with a variety of mutagens (nitrosoguanidine, ethylmethane sulfonate, U.V. light, etc.) and the frequency of $lac^+ \rightarrow lac^-$ cells will be measured. Preliminary studies have shown that the frequency of mutations in the lactose genes are a function of chromosomal location.

After analysis of the lactose genes, the same analysis can be applied to other genetic systems such as an amino acid biosynthetic pathway, ribosomal protein, etc. Genetic techniques for constructing the appropriate bacterial strains already exist.

"Enclosed is a brief statement for your grant. We would like to go ahead on this as soon as possible since it is all worked out and is basically a matter of cranking out the data. The diagram shows the nine strains we want to test. The lac genes are located at the 9 positions



We will mutagenize with EMS and nitrosoguanidine for starters. We can measure the mutagenesis here by measuring the number of valine resistant colonies. That gives us a number to use to normalize the mutagenic effectiveness. We would then bring down the mutagenized culture to be sprayed onto trays. We want to test the number of lac^- cells. I think the easiest way to do this is to place them on EMB lactose agar. Lac^+ are red and Lac^- are white. We probably need to photograph at 2 or 3 times to reliably distinguish the 2 types and probably have to set some limits in the computer as to what it calls white and what it calls red so we probably need a dry run. Once that is determined we can run them as fast as time allows. I presume we will work with Phil on this. Let me know how and when you want to proceed."

7) Recombination deficient mutants of E. coli.
Alvin J. Clark, Professor of Molecular Biology and Bacteriology and Immunology, University of California, Berkeley.

Method: "Our work in large measure stems from the discovery of recombination deficient mutants of various recombination proficient strains of E. coli. In doing the necessary mutant hunts the present bottle-neck is the picking of colonies of survivors of mutagenic treatment and patching them in geometric array. I am very interested in testing the dripper you have invented as a means of depositing cells in geometric array prior to testing their clones for recombination deficiency. It is very possible this may facilitate many experiments we have been sitting on because of the labor involved in picking and patching."

8) New Salmonella typhimurium tester strains for detecting mutagens and carcinogens among environmental chemicals.
Bruce Ames, Professor of Biochemistry, University of California, Berkeley

Method: This work is an extension of work already published to special cases for which the labor of mutant isolation and characterization is limiting.

1. Ames, N. B., Lee, F. D., and Durston, W. E., Proc. Nat. Acad. Sci. U. S., 70, 782 (1973).

2. Ames, N. B., Durston, W. E., Yamasaki, E., and Lee, F. D., Proc. Nat. Acad. Sci., 70, 2281 (1973).

9) Fine structure mapping in the histidine operon.
Bruce Ames.

Method: This work is an extension of work already published to special cases for which the labor of mutant isolation and characterization is limiting.

1. Ames, N. B., Lee, F. D., and Durston, W. E., Proc. Nat. Acad. Sci. U. S., 70, 782 (1973).

2. Ames, N. B., Durston, W. E., Yamasaki, E., and Lee, F. D., Proc. Nat. Acad. Sic., 70, 2281 (1973).

10) Metal ion mutagenesis and plasmid curing in Salmonella typhimurium.
Peter Flessel, Assistant Professor of Biology, University of San Francisco, San Francisco, California

Method: "I have been looking at the interactions of metals with bacteria using two assay systems. First, I have been studying metal ion mutagenesis and second, plasmid curing by metal salts. The decision to focus on metals was based on the near presence of a colleague in the chemistry department who had been studying metal carcinogenesis for fifteen years and was eager for some company.

"The work to date has been basically an extension of Bruce Ames' scheme applied to metals. So far I have shown that $MnCl_2$ and $NiCl_2$ are mutagens in S. tymphimurium. Our search for new metal mutagens is continuing and I suspect we will find others in the next few months. The mechanism of metal mutagenesis has not been thoroughly explored. It is not known, for example, whether metal ion penetration of the cell membrane is a prerequisite to mutagenesis. To find out, I would like to select for mutants which are temperature sensitive for resistance to metals. The assumption is that resistance would be a reflection of the failure to take up the metal. I would select for growth at 42 (permease denatured) and no growth at 37 (permease functional) in the presence of the metal. Having obtained such mutants I would test them for susceptibility to metal mutagenesis at both temperatures. I realize my proposal is perfectly straightforward. If I carry it out with the time and resources at my disposal, it is at least a year's work. With the "dumbwaiter" I think I could have the first mutants in several weeks."

11) Genes affecting virulence in Salmonella and isolation of mutants suitable for a live vaccine.
Bruce Stocker, M.D., Professor and Acting Chairman, Department of Medical

Microbiology, School of Medicine, Stanford University, Stanford, California.

For experiments on the mapping of genes affecting the virulence of Salmonella species, it is expedient to obtain genetically marked sublines in particular strains. For example, in lines of S. typhimurium which differ from the available genetically marked stocks of strain LT2 by their high virulence for the mouse, on intraperitoneal inoculation. It has been the experience of several workers that auxotrophic mutants obtained by mutagen treatment of virulent strains of Salmonella commonly have unwanted additional mutations causing reduced virulence, by unknown mechanisms. Therefore, in theory, the best method of procedure would be to introduce chosen negative alleles, determining nutritional requirements or inability to ferment particular substrates, by transduction. To do this by ordinarily available methods, even with the aid of penicillin enrichment, is hardly practicable, because of the amount of labor required to detect the rare transductants, which cannot be selected for. Dr. Glaser's apparatus should make possible the detection and isolation of the desired transductants by an automated procedure. A second problem, in the same general field, is the isolation of particular classes of auxotrophic, etc. mutant in mouse-virulent strains, for possible use as live vaccines, stably non-virulent because of, for instance, growth factor requirement, but otherwise unaltered. Mutants blocked in the synthesis of the diaminopimelic acid component of the bacterial cell wall should be unable to multiply in the tissues of a mammalian host because of absence of this substance, a component of bacterial but not of eukaryotic organisms. Heavy mutagen treatment of the bacterial strain to be used would be likely to cause additional, unwanted mutations: furthermore, it is unlikely that such mutants can be selected for by the penicillin enrichment technique. Probably the only way to isolate such mutants is by direct examination of a bacterial population for cells able to produce small colonies on defined medium supplemented with a small amount of diaminopimelic acid and able to resume growth on provision of additional diaminopimelic acid. Dr. Glaser's methods and apparatus should make this feasible, whereas it is hardly so by other methods.

12) Proline non-utilizing mutants of Salmonella typhimurium.
John R. Roth, Associate Professor of Molecular Biology, University of California, Berkeley

We've been analysing the proline degradative pathway. It involves an operon containing three genes, two genes for degradative enzymes and one permease. Permease mutants can be obtained by positive solution. The other two classes are more difficult to obtain. Because even wild type cells grow rather slowly on proline, the standard penicillin enrichment works very poorly. Screening of mutagenized cells with your apparatus should permit mutant isolation. These will be strains which fail to grow on proline as sole N. source but can use either NH_3 or glutamate as a nitrogen source."

13) Isolate mutants of Bacillus subtilis deficient in DNA synthesis at high or at low temperatures.

14) Isolate mutants of Bacillus subtilis resistant to certain phage and to drugs like p. hydroxyphenylazouracil for studies on DNA synthesis.
A. T. Ganesan, Professor of Genetics, Stanford University, School of Medicine, Stanford, California.

Our research project involves the study of the mechanism of DNA replication and

its genetic control in Bacillus subtilis, a transformable bacteria. We have isolated several temperature sensitive mutants that are defective in DNA synthesis The thermosensitive protein has been studied in a few cases. There are about 9 groups of genes that control DNA synthesis. There may be even more. We isolate these mutants routinely by conventional, slow and laborious procedures. The automated petri dish machine would be ideal for the above project. We are specifically interested in both low and high temperature sensitive mutants, and mutants that are resistant to drugs like p. hydroxyphenylazouracil. This drug specifically inhibits DNA polymerase III in Bacillus subtilis. Polymerase III is directl involved in DNA synthesis. Resistant mutants would help to locate the position of the gene for the enzyme. The system is also adaptable to test phage mutants which are currently studied. The instrument is a very valuable and unique tool for our projects. We would very much like to collaborate with Dr. Glaser in obtaining several important mutants and adapting the machine for other related projects in cell biology.

15) Screening for possible mutagens among environmental chemicals by mutations affecting sporulation in Bacillus subtilis.
Lawrence E. Sacks, Research Microbiologist and
James T. MacGregor, Research Pharmacologist
United States Department of Agriculture, Agricultural Research Service,
Western Regional Laboratory, Berkeley, California.

Thousands of chemicals, whose biological effects are little understood, have been disseminated into our environment and the food we eat by modern technological society. Most frightening of these chemicals are the mutagens, with their potential for teratogenic effects, cancer, and unknown long-term effects of alteration of germ-cells.

In screening for possible mutagenic chemicals, microorganism systems offer the advantages of speed, simplicity, and economy over animal systems. A disadvantage of microbial systems now in use is that they test only for mutations occurring in one or a few genes. A bacterial system sensitive to mutations on many genes, scattered throughout the chromosome, would seem to offer important advantages over currently used systems (1). We believe such a system is that governing sporulation in the genus Bacillus. Sporulation is a very complex process requiring the participation of a minimum of 28 operons for the sporulation process alone(2). Other systems (e.g. TCA cycle) are required for successful sporulation. Eight hundred genes have been estimated to be required for successful sporulation (3).

Selection of asporogenic mutants is simplified by their characteristic white color easily distinguished from the wild-type brown colonies, colored by formation of a pigment late in the sporulation of B. subtilis, Marburg strain. Using a highly transformable strain of this organism, and a wide variety of mutagenic agents, many sporulation genes have been mapped (4) in programs designed to unravel the genetic control of sporulation. We propose only to invert this procedure, and to use sporulation mutants to identify new mutagenic agents.

Dr. Glaser's instrument, capable of identifying single mutants in huge populations, will be of great value in identifying mutagenic activity at very low concentration levels. This combined use of a bacterial system involving over a hundred genes with scanning by an instrument capable of identifying mutation rates below 10^{-7} should result in a very rapid, extremely sensitive method for identifying mutagenic chemicals.

We summarize below some advantages of the proposed system:

1. It is based on forward mutation, the most general type of detection system. Any type of mutation which inactiviates or substantially alters a gene essential for sporulation will be detected.

2. A large number of genes are involved in sporulation (2, 3). Some mutagens are specific for particular regions of the DNA. The more genes surveyed, the less chance of excluding mutagenic "hot-spots".

3. Sporulation mutants are often characterized by a block at a particular stage in their morphological development. The frequency of occurrence of particular stages of arrest will permit an assessment of the randomness (or specificity) of each mutagen.

4. The B. subtilis system is well-suited to genetic studies. Highly transformable strains exist, and many genes have already been mapped (4). Dr. Glaser's scanning system, however, is not limited to the pigmented B. subtilis colonies. Other well-studied species (e.g. B. megaterium, B. cereus) may also be employed.

References

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2. Piggot, P. J. (1973). "Mapping of asporogenous mutations of Bacillus subtilis: A minimum estimate of the number of sporulation operons", J. Bacteriol. 114:1241-53.

3. Balassa, G. (1971). "The genetic control of spore formation in Bacilli", Current Topics in Microbiology and Immunology 56:100-92.

4. Young, F. E. and Wilson, G. A. (1972). "Genetics of Bacillus subtilis and other gram-positive sporulating Bacilli." in Spores V (H. O. Halvorsen, R. Hanson, L. L. Campbell, Eds.) pp. 77-106.

16) Proline degradation mutants in yeast.
John R. Roth, Associate Professor of Molecular Biology, University of California, Berkeley.

We've started looking at proline degradation in yeast. Here the available mutant enrichment techniques generally work poorly. Most people seek yeast mutants in a fairly "brute-force" sort of way. A large set of proline-non-utilizing mutants would be very useful to us. This hunt would need to follow the Salmonella hunt and probably should follow preliminary work (in progress) on the few available mutants. In this way the most advantageous conditions can be determined.

17) Saturation mapping of one yeast chromosome.
John R. Roth

Yeast has roughly 4-5 times as much DNA as bacteria. Roughly one hundred genes have been located. The spacing of these genes are wide enough to make it difficult to map new markers. A saturation map (or even a very sub-saturation one) would give new markers for mapping and a minimum estimate of the gene density.

Strains will be obtained through Robert Mortimer which are monosomic for a small chromosome. Other strains will be obtained which are monosomic for a large but poorly marked chromosome. These strains are diploid for all but one chromosome.

A hunt for temperature-sensitive mutants should yield mutants carrying lesions in the chromosome for which these strains are haploid. These can then be analysed. Determining the number of genes involves is fairly easy because of the simplicity of yeast complementation tests. I'd like to try this in a year or so after I've gotten back from sabbatical leave. (I'll be doing yeast genetics during that time.)

18) Genetic mapping in Saccharomyces cerevisiae
Robert K. Mortimer, Professor and Chairman of Medical Physics Department, University of California, Berkeley.

The availability of detailed genetic maps is an important component in determining the suitability of an organism for genetic and molecular studies. For a number of years, we have carried on a program of genetic mapping in the yeast Saccharomyces cerevisiae as an adjunct to our other studies. These mapping studies have resulted in a genetic map which establishes the location of more than 150 genes on 17 chromosomes. However, because of the large number of chromosomes and the high frequency of genetic recombination in yeast, very few heavily mapped regions are available. Such regions are important for studies we wish to carry out on gene conversion and its relation to mechanisms of genetic recombination. We believe the instrument developed by Professor Glaser could help to speed up the further development of genetic maps in this organism. The approach we propose to use is based on the random spore technique described in our recent mapping paper (Mortimer and Hawthorne, Genetics 74: 33-54 (1973). A series of strains that each carry one of a set of ochre suppressors in combination with a suppressible canavanine resistance gene and an additional selection of nutritional genes will be crossed to a large series of temperature sensitive lethals. The resultant crosses will be sporulated, and the asci will then be treated with glusulase followed by sonication. The sonicated suspension will be inoculated onto complete medium containing canavanine. Only spores lacking the suppressor and carrying the resistance gene will grow. These can then be transferred by replica plating to a series of "drop-out" plates to score the nutritional genes and to a "36°" environment to score the conditional genes. The patterns of growth: nongrowth on these various media can then be recorded automatically by the scanner and the resultant data analyzed for linkage by a suitable computer program. In this procedure it will be necessary to inoculate at a concentration that reduces to a low level the probability of clones developing from more than a single spore. The instrument should greatly facilitate random spore analysis both by permitting larger samples to be analyzed and by automatically recording and analyzing the results.

19) Gene conversion and recombination in unselected mitotic yeast cells.
Seymour Fogel, Professor and Chairman of Genetics Department, University of California, Berkeley.

20) Post-meiotic segregation and heteroduplex DNA.
Seymour Fogel.

Two rather specific proposals for use of the automated microbiological equipment are presented. These focus on fundamental yet health related issues. Thus, the proposals concerning mitotic recombination in unselected cells and post-meiotic segregation in yeast could provide, as a model system, a framework essential to understanding higher eucaryotic systems. With only minor changes the procedures

strategies and overall rationale may be carried over to address such seemingly unrelated though central problems as chromosome nondisjunction, or screening mutagens, carcinogens, fungicides and antibiotics for their genetic effects.

I. Gene conversion and recombination in unselected mitotic yeast cells

Our current understanding of intragenic recombination in cells committed to a mitotic cycle emerges from data generated by selective methods. In effect, these depend on appropriate signal devices that lead to the detection and recovery of only wild type or prototrophic recombinants. However, we have recently demonstrated the occurrence of mitotic co-conversion in hybrids marked by three of four heterozygous sites in a single structural gene, and it must be emphasized that multisite conversions do not typically generate wild type recombinants. Thus, though co-conversions might represent the most frequent event class, they remain undetected and unscored in conventional selective procedures. By analogy to our studies on unselected complete meiotic tetrads, we propose to analyze (in the same hybrids) an unselected population of mitotic cells for all conversional events falling within a defined genetic region.

Mitotic gene conversion in yeast occurs with an average frequency of the order 10^{-4} to 10^{-5}. Accordingly, collecting a sample of 10^2 or 10^3 unselected conversional events involves screening a total population of 10^6 - 10^8 cells, or a sample beyond the capability of routine microbiological methods. Automated microbiology equipment, however, augurs well for the successful completion of this and similar studies.

Our analytical strategy requires a) automated single cell innocula; b) replica-plating the derivative clones; c) irradiating the replica prints (UV or X-ray); d) detecting, locating, and retrieving sectored clones; e) finally, complete genetic diagnosis of each sectored clone by random spore or tetrad analysis of each segment.

II. Post-meiotic segregation and heteroduplex DNA.

Common to all molecular models seeking to account for genetic recombination are enzymatically mediated steps that eventuate in heteroduplex or hybrid DNA production. At the in vivo genetic level, the presence of unresolved heteroduplex DNA is detected by post-meiotic segregation (PMS). PMS is comparatively frequent among the total aberrant octads of Ascobolus or Sordaria. However, technical difficulties with these forms, including a paucity of genetic markers, preclude total and critical analysis. With automated microbiological procedures adapted to random spore or tetrad analysis based on diploid yeasts suitably marked with 7-10 heterozygous sites (i.e., loci and alleles of known meiotic conversion frequencies), we could readily assess the frequency, extent and distribution of heteroduplex DNA in the yeast genome on a statistically reliable base. Sectored ascosporal clones, otherwise concordant for all segregating markers will be considered as PMS events.

Also, from the distribution of PMS events among spores produced by heteroallelic diploids of the type ++/12 or 1+/+2 (notation as before) where the mutant allele pairs may be chosen from extensive fine structure maps to represent a range of genetic distances, the regularities and basic attributes of heteroduplex DNA relative to gene conversion and recombination could be inferred.

Donald A. Glaser [redacted]

Privileged Communication

20a) Mammalian somatic cell genetics.

Frank Ruddle, Professor of Biology and Human Genetics, Department of Biology, Yale University, New Haven, Connecticut.

I believe that your machine has particular possibilities with regard to the recovery of conditional temperature sensitive mutants in tissue culture populations. As we have previously discussed, it would seem possible to establish colonies in the machine and then to shift to higher temperature and examine the colonies for retardation in the rate of increase of colony size. It would be possible to maintain the cultures at 34°C as a permissive condition and then to increase the temperature to 38.5° for 3 hr. periods out of a total period of 24 hrs. and carry this regimen forward for a period of one or two weeks. It would seem to me that this would not kill the temperature sensitive mutants but would result in a decided difference in their colony size which could be easily monitored by your photographic equipment. The isolation and characterization of temperature sensitive mutants will, I believe, be one of the most important aspects of somatic cell genetic work in the next decade. It should be possible by this means to obtain mutants which affect the biosynthesis of cell membranes, nucleic acid, and protein. It is also possible to pick up mutants which specifically affect the ability of mammalian cells to progress orderly through the cell cycle. All of these mutants can be analyzed by genetic complementation tests involving cell hybridization and chromosome segregation. For this purpose it would be best to make use of Chinese hamster cells or mouse cells as the population in which the mutants are recovered.

It seemed to me that your machine could be adapted also for recovery of mutants in differentiated cells. Quite a number of tissue culture cell lines which express specific differentiated traits are now available. For example, we are growing hepatoma cell lines which produce albumin. The albumin is secreted into the medium at high levels. It would seem to be possible to maintain colonies and then test the individual colonies for albumin production perhaps using a fluorescent reagent. One can then examine a large number of colonies for cells which fail to produce albumin. This would represent an excellent method for picking up non-producers. These cannot at the present time be enriched by selection techniques. One could also test for reversion to capacity to produce the differentiated product using the non-producing mutant as the base population. This kind of procedure could be adapted to cell lines which produce hemoglobin, myocin, nerve specific protein, etc.

When your machine is sufficiently developed to make use of mammalian cell populations, I would very much like to be in touch with you with regard to these possibilities. If you are interested in pursuing these possibilities I'd be more than happy to come out to Berkeley and spend a month or so in this connection.

Privileged Communication [redacted]

21) Isolate and characterize a large number of steroid- and cyclic AMP-resistant clones of mouse lymphoma cells.
Gordon M. Tomkins, Professor of Biochemistry, University of California, San Francisco.

General Objectives: For some years our laboratory has been studying biological regulatory mechanisms in cultured mammalian cells. We have concentrated primarily on the action of the steroid hormones but more recently have become interested in the cyclic nucleotides as well. The bulk of our work heretofor has been a biochemical analysis of the molecular mechanisms of cell-hormone interaction. Quite recently, however, we have begun to explore genetic techniques to pursue our objectives. For this purpose we have been using cultured mouse lymphoma cells which are killed on prolonged exposure to either the adrenal glucocorticoids or to cyclic AMP. This response occurs at physiological levels of the effector molecules and presumably reflects the well known immunosupressive action of the glucocorticoids and of agents which elicit cyclic nucleotide synthesis. In any event, we have been able to select variant lymphoma cells resistant to the killing actions of the steroids, cyclic AMP or both agents. Our results to date indicate that the transitions from effector-sensitive to effector-resistant occur at random at a rate, in the case of the steroids, of 3×10^{-6} per cell per generation and for cyclic AMP, of approximately 1×10^{-7} per cell per generation. Various mutagens increase the frequency of steroid resistant cells. Biochemical analysis of the phenotypes of steroid- and cyclic AMP-resistance had indicated that in the former case, three types of variants can be isolated: those lacking the normal cytoplasmic steroid binding activity; those where binding takes place, but in which the receptor-steroid complex is not translocated to the nucleus; and finally those in which binding and translocation occur but cell death does not result.

Preliminary investigations suggest that various phenotypes also give rise to cyclic AMP resistance. To date we have studied only cells in which the cyclic nucleotide binding protein and its associated kinase are deficient.

Specific Aims:
1. To isolate a large number of steroid- and cyclic AMP-resistant clones of lymphoma cells.
2. To determine the frequency of their occurrence and the effects of a variety of natural and artificial mutagens on the transition from sensitivity to resistance.
3. To determine the biochemical bases of cell killing.
4. To characterize the phenotypes in terms of various known steps in hormone action.
5. To carry out complementation analyses using cell hybridization techniques to determine the number of biochemical steps involved in cell-hormone interaction.
6. To determine whether the transitions result from genetic or other types of stochastic, heritable variations such, for example, as might occur during the differentiation process.
7. To investigate possible relationships between resistance to the steroids and to the cyclic nucleotides.
8. To apply similar methods to circulating malignant cells in patients with lymphoma or leukemia in an attempt to design more rational therapies for these diseases.

Significance: The projected studies bear on many aspects of cell biology and

clinical medicine. The glucocorticoids are major therapeutic agents in leukemia and in other malignancies. Their effectiveness is limited only by the emergence of hormone-resistant cell populations. Our observations with cultured cells can therefore serve as a useful model for studying how it might be averted. The finding that certain mutagens, in particular alkylating agents, enhance the conversion from steroid-sensitivity to steroid-resistance already indicates that therapeutic regimes which employ alkylating agents together with steroids might be redesigned to avoid the possibility that steroid-resistant cells are produced in the course of therapy.

These studies also suggest that new classes of agents, such as the cyclic nucleotides or compounds which elicit their production, might be used in tumor chemotherapy. The apparently lower frequency of resistance to cyclic nucleotides holds out the hope that these agents could be more effective therapeutically than the steroids.

From a theoretical point of view, these experiments could provide novel approaches to investigations of drug and hormone action by combining genetics, with cell biology and biochemistry. It should, for example, be possible to isolate cyclic AMP-resistant variants in which adenyl cyclase or various specific membrane receptors are deleted making it possible to study the interrelation between the elements in this important regulatory circuit. The same considerations hold true for the steroid hormones and studies on their mechanism of action.

Steroid and cyclic AMP-resistance are the result of changes in structure of the receptors. Since these molecules have been identified, and to some extent, purified, the generation of resistant mutants can be correlated with altered molecules. Therefore a more complete genetic analysis can be carried out than if the selective marker (e.g. drug resistance) were not correlated with a known protein.

22) Linkage analysis in mammals by somatic cell genetics.
Theodore T. Puck, Director, Institute for Cancer Research; Professor of Biophysics and Genetics, Eleanor Roosevelt Institute for Cancer Research, University of Colorado Medical Center, Denver, Colorado.

Preliminary discussion of this project has indicated the great labor of isolating mutants and establishing linkage. Feasibility studies need to be carried out before real research plans can be made. Because the genetic exchange system is so inconvenient compared with E. coli, the automation may be even more valuable for animal cells than for bacteria.

23) Sensitive detection of mutagenesis by changes in colony morphology-extension to additional bacterial and eukaryotic cells.
D. A. Glaser.

Method: Since colony morphology is a highly polygenic characteristic, it should be a very sensitive detector of mutagenesis. Extremely uniform reproducible culture conditions are required to guarantee reproducible colony morphology even in the absence of mutations. For measuring gross mutagenic effects down to very low "exposures", we plan to explore the limits of colony reproducibility for a variety of organisms.

Significance: If successful, colony morphology changes provide a method of assessing mutagenic effects on a wide variety of clonable cells, even if little

or no genetic information is available. Screening of chemical and physical mutagens is an obvious application.

24) Transformation and mutation of Mammalian Cells in vitro by low doses of mutagens and ionizing radiation.
D. A. Glaser

"Transformation of Mammalian Cells in vitro by Low Doses of X-rays", C. Borek and E. J. Hall, Nature 243, 450-453 (1973). Embryos of golden hamsters were minced and separated into individual cells growing on agar. The cells were irradiated with 1 to 600 rads of X-rays, incubated, stained, and the colonies formed (probably about 2 mm in size) examined for forms made by transformed cells.

Table of Effect of Transformed Cells

Dose	Clones Examined	Cells Transformed
0	36,000	0
1	17,900	3
10	10,200	4
25	5,500	8

Clearly large numbers of clones were examined for the infrequent event. The ability to use larger numbers of cells and examine the clones formed from them would make the numbers found more precise and allow better description of the dose response curve at low doses.

25) Behavioral Mutants of Motile Organisms
D. A. Glaser

In the original proposal for construction of the DW and scanner system, we described possible behavioral studies of motile organisms of standard or "instinctive" behavior as well as adaptive or "learned" behavior. The following is quoted as an example of the type of study we would like to pursue sometime during the next few years.

"Chemotaxis by the Nematode Caenorhabditis elegans: Identification of Attractants and Analysis of the Response by Use of Mutants", S. Ward, PNAS 70, 817-821 (1973). Known behavior mutants of this nematode were put onto gradients of an attractant on agar plates covered with agarose beads or sephadex beads. The patterns resulting differed between the wild type and the mutants. Some studies were done to understand the chemotaxis. The hunt for more mutants was proposed. Clearly, in hunting for mutants, the more worms to be examined the better. The worms are small enough to be inoculated in 0.05 ml of liquid from an Eppendorf pipette. The patterns are formed quickly and photograph well. Analysis of the path can be done by computer in the same way Berg follows the three-dimensional path of E. coli.

26) Further Automation Instrumentation Development.

Although the Dumbwaiter and all of its ancillary equipment is expected to be in full operation when this program-project would begin in June 1975, a number of specialized accessory instruments will probably be needed as the biological program develops. A sample of such instrumentation projects that we envision at this time follows:

(a) Optical Cell Sorter--At the present time the vibrating nozzle inoculator is used for laying down regular rays of droplets containing bacterial suspension. In the future it will be used to deposit yeast cells and animal cells as well. If the concentration of cells is adjusted so that each droplet contains on the average 1 cell, then 1/e of the droplets will be empty, 1/e of the droplets will contain 1 cell, and the rest of the droplets will contain more than 1 cell. For most measurements the only really useful results come from colonies descended from a single cell and the empty droplets are of obviously no use for most measurements. By illuminating the droplet at the time of its formation laser light, it is possible to make dark field measurements of light scattering, color, and fluorescence, which signal the presence of a cell and give some information about it. Such instruments work well with animal cells, but require further development to detect bacteria, which are much smaller.

1. W. A. Bonner, H. R. Hulett, R. G. Sweet, and L. A. Herzenberg, "Fluorescence activated cell sorting", Rev. Sci. Instruments 43, 404 (1972).

2. M. J. Fulwyler, R. B. Glascock, R. D. Hiebert, and N. M. Johnson, "Device which separates minute particles according to electronically sensed volume", Rev. Sci. Instruments 40, 42 (1969).

None of the existing systems seems capable of detecting bacteria and we hope to build such a system sensitive to bacteria as well as to larger cells.

(b) Increase Film-Scanning and Computing Speed--Since the Dumbwaiter can easily take one photograph per second and since the film-scanning time ranges from 10 to 20 seconds per picture depending on the experiment, the film-scanning and computing operations will be rate-limiting steps in the output of the entire system. We are, therefore, very anxious to cut the analysis time by installation of the PDP-11, PDP-10 scanner computer system as well as by some software improvements.

(c) Install Television System--For some future experiments it will undoubtedly be useful to analyze biological systems in real time and to intervene in the experiments without having to wait for the several-hour delay of taking pictures, developing, and analyzing them. For this purpose we plan to install a television system connected directly to the computer which will eliminate photography. In addition to allowing real-time intervention, it will be a considerable saving in the cost of photographic materials. On the other hand, the television system does not have the reliability of experiments recorded on film, nor do television cameras have as high resolution as our present flying-spot scanner. We imagine, therefore, that we will use both systems depending on the needs of the experiment.

(d) Irradiation Facility--We plan to provide a facility in the Dumbwaiter for irradiating cells with ultraviolet or infrared light and also with ionizing radiation on some schedule as required by the experiments.

(e) Semi-micro Photography--For study of very small colonies we will need to provide a semi-micro photographic system which will photograph a 1-cm or even 3-mm square on the agar instead of the present 100-mm square. There is a trade-off between the time and cost of photography and the size of agar area covered. Optimizing the trade-off will require different magnifications for different experiments.

(f) New Cell Manipulation Devices--Our present plans are to use colony pickers, restreakers, and replicating devices suitable for colonies of E. coli and other cells that have similar physical properties. We can well imagine that other clonable cells will require somewhat different techniques for manipulation and special cell manipulation devices will be required from time to time.

D. Significance

In the discussions above of the particular biological research projects, the significance of each one was pointed out. In general, these applications of modern automation technology coupled with computer-directed pattern recognition and analysis of data offer a powerful new tool for accelerating research in a wide variety of fields of molecular biology and cell biology. They reduce enormously the labor, time, and materials required to isolate rate mutants critical at a number of stages in research as well as to measure with high accuracy frequencies of genetic and mutational events which must be known for the genetic dissection of important biological processes.

In addition to the great gains expected in the speed of research in fundamental biology, the same large-scale automation techniques offer great promise for a variety of bio-assay applications, including the screening of environmental chemicals for their potential mutational and carcinogenic effects; the testing of proposed antibiotics, antineoplastic agents, and cell regulatory substances. In additional to the possibility of large scale testing of chemical agents, it seems possible to make highly accurate measurements of the effects of ionizing, as well as non-ionizing, radiation on a variety of clonable cells. The information resulting is important to studies in fundamental biology as well as the difficult problem of setting safe standards for allowable exposure to ionizing radiation among the general population and among workers in industries involving the presence of radioactive substances. With these large scale methods, it may be possible to extend the dose-effect relationship down to very low exposures and so to discover in an over-all sense whether there is a threshhold or minimum dose below which repair mechanisms prevent any detectable genetic damage at the single-cell level.

Finally, the success of these applications of the cutting-edge of modern technology serves as a demonstration which may stimulate similar applications in industrial as well as medical and research sectors. We already know of several projects for strain improvement of antibiotic producing organisms that have been directly stimulated by this work. Representatives of a very large number of pharmaceutical manufacturing firms, instrumentation manufacturers, and chemical companies have visited our facilities. Suppliers of agar for medical and research purposes have also visited our facilities and have discussed with us their problems in maintaining uniform reproducible quality in their product. Variability is a source of considerable difficulty in both medical and research applications and we have agreed in a general way to measure batch to batch variations by its effect on colony morphology and growth rates in an effort to help them improve the quality of their product.

The five-year period of this proposed program-project should be ample time to carry through successfully a number of the projects we are proposing as well as to test the feasibility of a number of others and evaluate the usefulness of this kind or technology to biomedical science and industry.

E. Facilities Available

Virus Laboratory - Molecular Biology Department.

Many of the biological experiments described here will be developed, at least to the pilot stage, in the Molecular Biology Department and Virus Laboratory as has been done in the past. All of the usual common research facilities of these laboratories will be available as necessary. In addition, a small, well-equipped machine shop is at our disposal.

Lawrence Berkeley Laboratory

From time to time we may call upon special shops and consult with experts from the Lawrence Berkeley Laboratory to help us with problems which they may have already encountered in their High Energy Physics and other programs. In addition we can often obtain electronic and other specialized supplies at very attractive prices and with immediate availability from the excellent stockroom facilities of the laboratory. We are very fortunate to be able to take advantage of the superb technical facilities and talent available at the Lawrence Berkeley Laboratory.

Electrical Engineering Department

The large-scale automatic equipment including the computer and flying-spot scanner are located in specially remodelled space provided for that purpose in the basement of the Electrical Engineering Building, Cory Hall. Members of the faculty and graduate students in Electrical Engineering have been taking an effective role in the development and use of this system. Thus collaboration with the Electrical Engineering Department and the Engineering Research Laboratories offers excellent special facilities for work of the type we are undertaking, as well as a unique opportunity for those members of the faculty and graduate students in Electrical Engineering interested in applying their special skills and knowledge to biomedical engineering.

Campus Computer Center

Only modest funds have been budgeted for use of the Campus Computer Center since, until now, we have been able to carry out all the computations associated with our work on our own computing system. When our own system is saturated, we may be able to reorganize our programs so that some of the pure computation can be put on magnetic tape and carried out at the Campus Computer Center which offers general facilities for large-scale computational work.

Physics Department

Laboratory space in the Physics Department is available for this work if needed and the excellent resources of the Machine Shop and Glassblowing Shop can be used from time to time as necessary.

Extra-fabrication Space

For fabrication of much of the sheet metal and welding work required for the construction and maintenance of the large-scale automated equipment, we have been granted the use of a corrugated metal building located in the parking lot of Cory Hall convenient to all of our other operations. Commonly called the "Ore House", this building was previously used for storing ores obtained from a nearby practice mining shaft.

F. Collaborative Arrangements

We have had extensive conversation and in some cases correspondence with all of the scientific investigators who have proposed projects using our equipment and who have visited our facilities. Since the Cyclops has been running only a short time, we have not encouraged active work in our laboratory until very recently and the precise relationship that will develop between these scientific investigators and members of our own laboratory remain to be defined. In every case the scientific

investigators proposing projects listed here have independent support for carrying out these projects in their own laboratories and we intend to provide use of our facilities and necessary supplies without any formal arrangements or exchange of funds. If scheduling of experiments and assigning of priorities becomes difficult, we will probably invite some of the scientific investigators to join us and constitute an Advisory Committee to help plan the work schedule. It is too early to foresee accurately how all of these relationships will develop so no formal administrative structure for collaboration is being planned at this time.

In addition to the scientific investigators named above, Professor Herbert B. Baskin and Professor Martin Graham of the Department of Electrical Engineering and Computer Science, University of California, Berkeley have been very helpful in giving advice concerning computer hardware and software. They generously agreed to continue this relationship and perhaps play a more active role in this program in coming years.

G. Principal Investigator Assurance.

The undersigned agrees to accept responsibility for the scientific and technical conduct of the research project and for provision of required progress reports if a grant is awarded as the result of this application

9 November 1973
Date

Donald A. Glaser
Donald A. Glaser
Principal Investigator