D. A. Glaser

Form Approved
Budget Bureau No. 68-R

SECTION I

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: 01 June 1975
THROUGH: 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
M11 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
M11 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. "Per" signatures not acceptable.)	A. SIGNATURE OF PERSON NAMED IN ITEM 2A: Donald A. Glaser	DATE
	B. SIGNATURE(S) OF PERSON(S) NAMED IN ITEM 10	DATE

D. A. Glaser

SECTION 1

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

LEAVE BLANK

PROJECT NUMBER

RESEARCH OBJECTIVES

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]6, 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.

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Privileged Communication

Professional Personnel (continued)

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

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D. A. Glaser

DETAILED BUDGET FOR FIRST 12-MONTH PERIOD

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

DESCRIPTION (Itemize)		TIME OR EFFORT %/HRS.	SALARY	FRINGE BENEFITS	TOTAL
PERSONNEL					
NAME	TITLE OF POSITION				
	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 / ~~FOREIGN~~	One major trip to East Coast for professional persons to attend a major conference (Gordon, CSH ($2,000); attendance by professionals and grads at local conference (Arrowhead, Biophysics); confer with colleagues and equip. suppliers. ($1,000)				3,000
PATIENT COSTS (See instructions)					
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)

_______ % S&W*

34.2 % TDC*

DATE OF DHEW AGREEMENT: 5-23-73

☐ WAIVED
☐ UNDER NEGOTIATION WITH:

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

Privileged Communication

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

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

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Donald A. Glaser

Privileged Communication

SUPPLIES

Item	Amount	Total
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

Item	Amount	Total
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

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Donald A. Glaser

Privileged Communication

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

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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		
	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.

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, 255 (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

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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.)

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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)

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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.

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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

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.

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 10^8 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 of 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.

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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

Name	Position	Years
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

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, 2258 (1971)).