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May 15 - May 19, 1985

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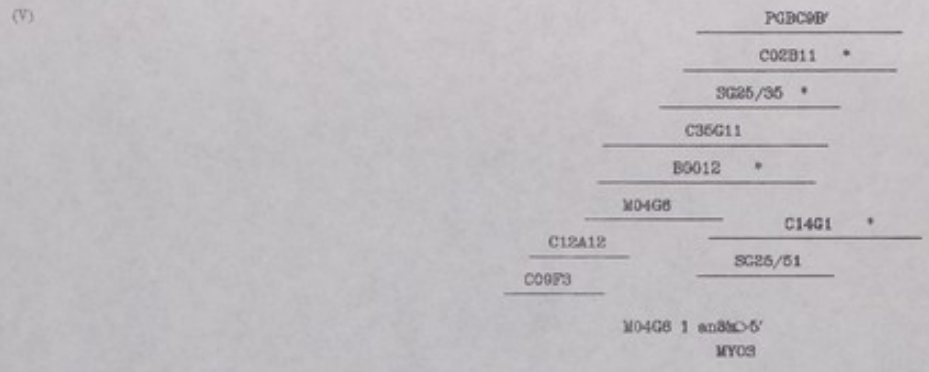
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PGBC9B'
C02B11 *
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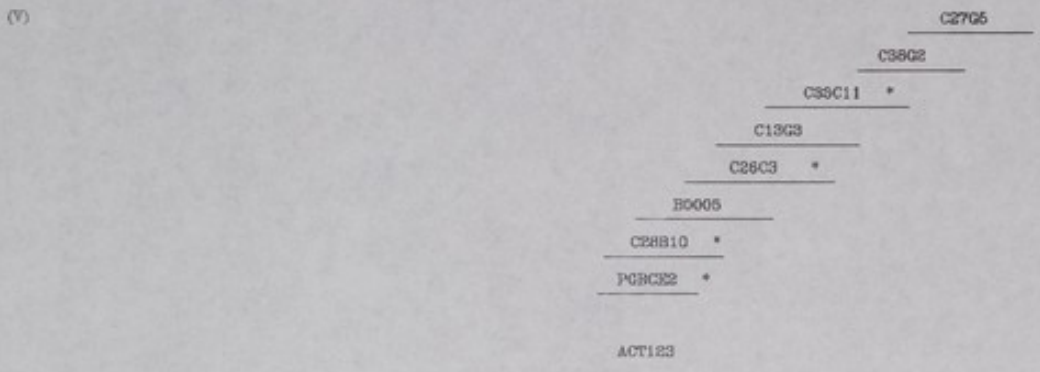
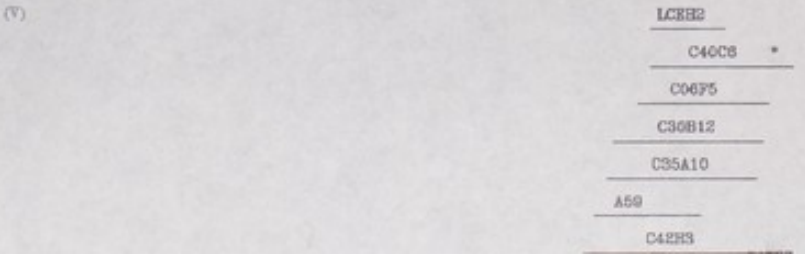
M04G6
C14G1 *
C12A12
SG25/51
C09F3
M04G6 1 an8hC>5'
MYO3


(V)
C27G5
C38G2
C33C11 *
C13G3
C26C3 *
B0005
C2HB10 *
PGBCE2 *
ACT123


(V)
LCEH2
C40C8 *
C06F5
C30B12
C35A10
A59
C42H3

C06F5

C30B12

C35A10

A59

C42H3

C45E2

D2042

an.cm.in C42H3
HISTONE

(V)

C04A5

C17F5

DB11 *

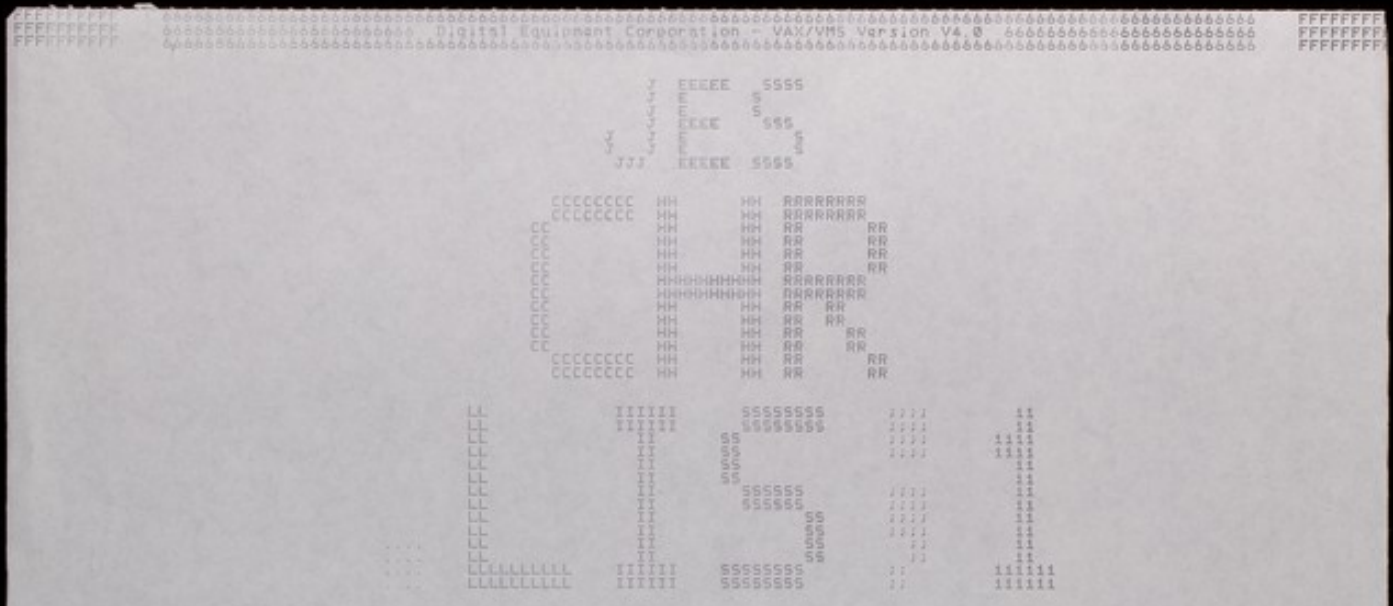
WK10

COL?

(V)

RW2070-2 *

53



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_LPA0: on 13-MAY-1985 15:49 from queue LPA0

Digital Equipment Corporation - VAX/VMS Version V4.0

(1)

PCE7 *

RE13B-

RE6A

B0467 *

C27H11 *

RIB

(1)

C05F4 *

B0642

SG25/3

PGBC8-10

C03C9 *

SG23/3

SG25/38 *

SG25/3

PGBC8-10

C03C9 *

SG23/3

SG25/36 *

C13G1 *

centre<>end
UNC54

(1)

C12E8

SG25/28

C33F11 *

PGBC9D'

C12C7 *

C47H4 *

B0332 *

C33F11 odd; NG rep
MY01

(1)

C26G11

COL7

C15A11

C05B12 *

C45D6 *

COL7

Aim of 'nutty' project with J.S. to completely map C. elegans genome – ultimate goal – Also foster inter lab communication and provide people with information about flanking sequences (1)

INTRO.

Silly numbers

	b.p./genome.	seq. time. (50 kb p person/yr)
coli	4×10^6	80 myr
Yeast	2×10^7	400
nematode	10^8	2000
human	2×10^9	40000.

100 x rate to sequence nematode.

What to do to give us handles on these genomes, esp. when large numbers of people ~~involved?~~ interested.

~~Obviously clone, but then 'once and for all' work.~~

Mapping = Bank of overlapping set of clones aligned to genetic map.

Allowing easy access to regions of interest for specific projects and, long term, as a prelude to general sequencing.

Nematode : ~ 2500 cosmid clones.
Say library of 6000 with reasonable overlap.

HISTORICAL

One approach – Olson / yeast –
- precisely run agarose gels of λ clone digests.
- scanning & computer matching & assembly of overlaps.
- resolution of agarose gels may not be good enough for larger genomes.

Sydney & Karn basic idea (SLIDE 1.)
Not restriction map, but 'fingerprints' to allow construct map. by a 'shotgun' approach. re. sequencing strategy

METHOD in ~~DETAIL~~.

Main bank (cosmid) – 2x sized pSauA in Bam site of pJB8.
(Importance of sizing.
(Insert size by agarose gel ~35 kb).
Microtitre gridding of 7000 cosmids.
Hedgehogging onto amp. plates for colony growth prior to minipreps. (Easier than λ's).
f/p as slide.
Gels – denat. 4%
minimisation of distortion (Wacker metal, late)

Marker digest ~) Sau3A SLIDE 2.
frag. sizes

- Digitisation (~~currently using sonic dig~~.
  - sonic digitiser
  - ref. to standard grid for correction of local distortion and variation.

Comparison with data base — PRINTOUT
  - probability of matches due to random chance
  - probability cut off.

Assembly of data into 'contigs'. — film marking, interactive adjustment, V.D.U. ~ explain
~~Visual inspection of acceptability of matches~~
— insufficient accuracy for computer to do this.

Overhead simple contig. or SLIDE 3
- shuffling interactive on V.D.U.
- length
- 'hidden clones'
- notes on chromos. allocn.
- joining.

- Overhead more complex contig. or SLIDE 4
genetic / in situ — Donna'
(1-2000 kb).

Incorporation of markers & clones from other sources (cosmid, λ's, plasmids if enough HIII sites).

Overhead, SB4 example? — few HindIII sites but confirmed by M-Gilb.

Statistics to date — Overhead CEZOG ?? or write up?

Slides graphs 5 6

- 2 phase? — hotspots + underlying steady growth of new data but presumably some holes.

Might hope different banks would tend to extend map faster.

Analysis of other banks

MIT MBO
OUR MBO
OUR RI

Overlap indexes.

Conclusions re. other banks. - all pretty much the same

Rate of progress

~200 clones / week

Currently = ~ 800kb (?) growth of map, so still worth proceeding with random approach
Specific walks overtaken by random.
Use in walking by probing. (SLIDE 7)
No 2ndary screen.
Families. Ones that don't belong to walk useful anyway.
- so not altogether an 'all or nothing' method.

CONCLUSIONS:

Working for ~~a~~ nematode-sized genome.
~~Human - divide into chromosomes or orthogonal digest~~
~~End random approach~~
~~Leaping / walking / negative screening?~~
Show gene list & explain.

When end random approach?
Then Leaping?
walking?
negative screening?

C. elegans genome ~ 10^8 b.p. BOARD

i.e. ~ 20 x coli

1/20 human.

So 2500 cosmid clones to cover with no overlap.

Say ~ 6000 with reasonable overlap.

Shotgun fingerprinting scheme based on idea of Brenner & Kaan.

SLIDE 1.

Explanation.

Construction of main bank.

– Carefully sized ~~frags. in~~ pH. Sau3A frags in pJB8.

Microtitre plate gridding of 4000 cosmids.

Hedgehogged for colony growth prior to f.p. on denaturing 4% ~~thin~~ acrylamide gels.

SLIDE 2.

Marker ~~digests~~ lanes of λ Sau3A

Separation of frags from ~ 40 bp — ~ 2 Kb; 20-30 bands/clone

Minimisation of distortion by wackering metal plate.

Following exposure, digitisation by sonic digitiser, using a standard grid based on the marker lanes of an early gel to correct local ~~distortion~~ expansion and contraction from gel to gel.

Digitised data ~~comp~~ compared at a given tolerance with the data base as a whole.

Printout indicates possible matches, and ~~is~~ the probability of such matches being due to random chance.

Visual assessment of matches & assembly into 'contigs' by interactive adjustment on VDU

(Inability of) computer to construct contigs due to insufficient accuracy of data (loss of resolution by sonic digitisn, partial products etc.) compounded with size of genome).

SLIDE (3)

- interactive adjustment on VDU
- length of lines =
- hidden clones.
- ~~incorp^n of marker genes cosmids/λ's/plasmids~~ chromosom. allocn.; genetic in situ.
- notes.
- joining.

SLIDE (4).

Incorp^n of walk λ's.

Allocations

Confirmation of dubious assignments, esp: other people clones if not enough Hind III sites - i.e. plasmids, λ's — by probing different enzyme digests eg. Bgl Maxam/Gilbert on apparently matching bands.

Statistics to date. - includes analysis of other banks - more to say later.
— WRITE UP.

Progress curves — SLIDE 5?B.
Rates of progress — ~~SLIDE 6~~.

2000 clones:	1300 kb/100
4500	560 " "
7000	240 " "

SLIDE 6.

One might hope different banks would lead to faster aquisition of new data.
Other banks:- Mbo in ~~p35~~ ? pJ59.
Mbo in pJ58.
R1 in pJ58
Mbo in LORISTB.

Varying numbers of clones from each bank fl'd.
As a general assesment, we have a program to reduce ~~to~~ the contribution of any given set of clones to a histogram.

SLIDE (7).

The height of each line represents the number of bands in the set occuring a given number of times in the database as a whole. Normalised so that the total height of all the lines is the same for a set of any size.

So the more new ~~dat~~ coverage a set provides, the more the histogram will be skewed to the left.

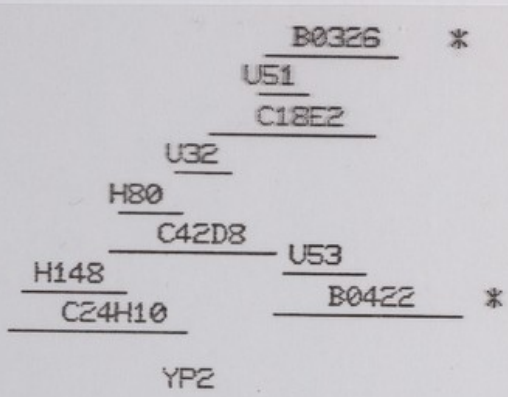
SLIDE 8.

- similarity of SanJA, R1, Mbo.
- encouragement of LORISTB but small sample a different bug. (E08467 v 1046).

SLIDE 9.

Explanation RAN / on DB.

$\frac{8 \times 10^{7+4}}{35 \times 10^{8}}$ $35 \overline{)180,000}$.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U51
C18E2
U32
H80
C42D8
U53
H148
B0422 *
C24H10
YP2

```
46-D2062   (36b, 248)
 clone name/control (H gets help) =
```

D2062 *

C07D6

C15G1

806-C0L2 (17b, 572)

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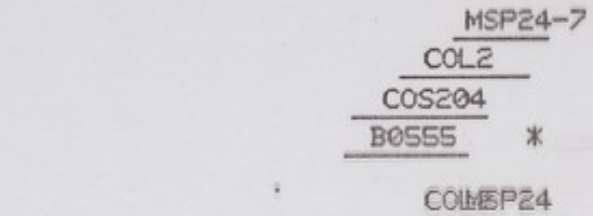
next clone for current contig/contig control=

(IV)



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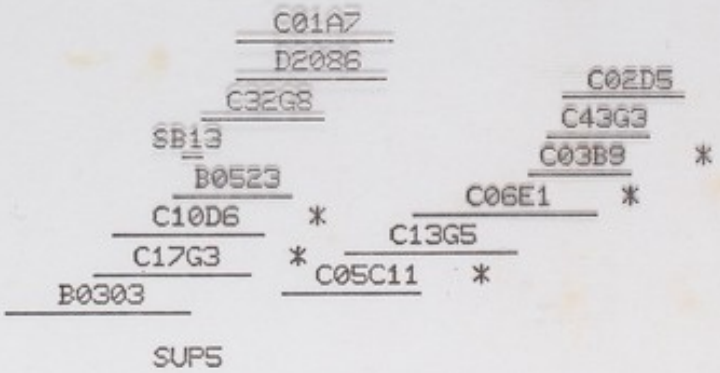
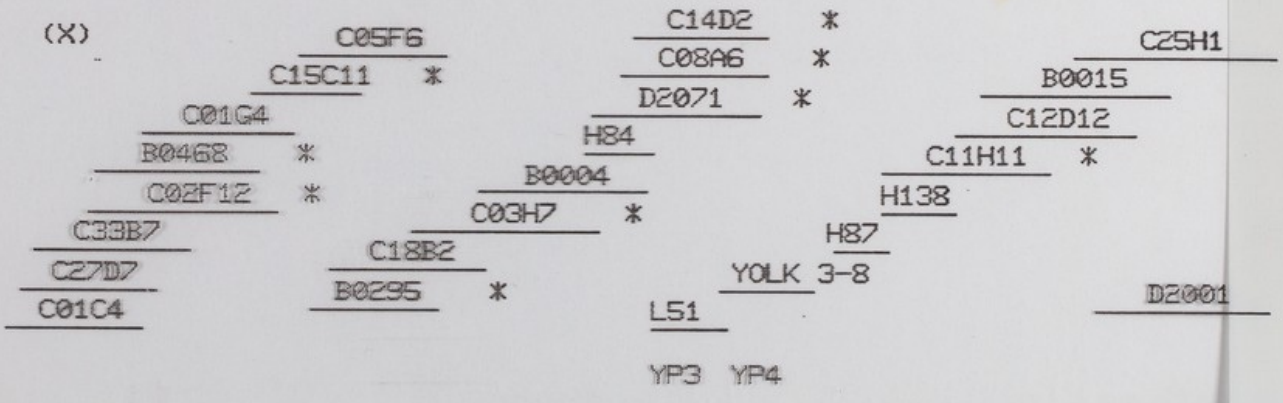


807-L51 (8b, 37)
clone name/control (H gets help) =



clone	gene		genetic%	in situ%	
PCE7	RIB	(I)	99.9	0 - 0	60kb
U32	VIT2		0.0	0 - 0	127kb
YOLK 3-8	VIT3,4	(X)	0.0	11 - 26	282kb
SG25/36	UNC54	(I)	97.0	0 - 0	58kb
COL3	COL3	(IV)	84.2	0 - 0	191kb
SG25/35	MYO3	(V)	0.0	45 - 65	93kb
C05F1	EP7	(III)	68.6	60 - 74	215kb
EEG4	LIN14	(X)	58.0	0 - 0	134kb
HK5	PMYO		0.0	0 - 0	19kb
COL6	COL6	(II)	0.0	0 - 0	109kb
PGBC6S	MYO2	(X)	0.0	52 - 74	75kb
C15G3	C15G3	(X)	0.0	50 - 70	134kb
C14H8	TC1A	(X)	57.8	0 - 0	53kb
ACT13	ACT4	(X)	0.0	17 - 29	64kb
C05C9	TC1G	(X)	57.9	0 - 0	92kb
SG25/26	MYO1	(I)	0.0	40 - 53	84kb
C10F8	TC1K	(X)	57.8	0 - 0	110kb
MSP113-1	MSP113	(IV)	0.0	32 - 49	47kb
IG10A	LIN12	(III)	68.3	0 - 0	240kb
HSL151	MHSA	(IV)	98.4	0 - 0	75kb
SB1	tRNA-W4		0.0	0 - 0	60kb
L122	L122	(IV)	88.8	0 - 0	57kb
C04F6	VIT5		0.0	0 - 0	58kb
COL7	COL7	(I)	0.0	0 - 0	43kb
PGBCE2	ACT123	(V)	51.5	35 - 60	98kb
YOLK 2-3	VIT1	(X)	0.0	0 - 0	162kb
COL2	COL2	(IV)	84.9	0 - 0	116kb
MSP24-7	MSP247	(IV)	84.9	0 - 0	116kb
IICOL9	COL9	(X)	0.0	0 - 0	61kb
LDM17	UNC22	(IV)	89.7	0 - 0	70kb
LCEH2	HISTONE	(V)	0.0	30 - 45	79kb
L121	L121	(IV)	89.9	0 - 0	33kb
SB15	tRNA-W1	(IV)	90.3	0 - 0	113kb
TP3	tRNA-W2		0.0	0 - 0	103kb
RW7	tRNA-W3		0.0	0 - 0	49kb
SB2	SUP7	(X)	41.4	0 - 0	51kb
SB13	SUP5	(III)	68.1	0 - 0	147kb
D2406	CALMOD	(IV)	0.0	52 - 68	47kb
MSP2-4	MSP24		0.0	0 - 0	135kb
CGBP1	CGBP1	(IV)	88.3	0 - 0	30kb
L138	L138	(IV)	87.9	0 - 0	14kb
L136	L136	(IV)	87.6	0 - 0	58kb
C33C3	UNC86TC1	(III)	67.5	0 - 0	85kb
COL8	COL8	(III)	0.0	0 - 0	58kb
WK10	COL?	(V)	0.0	0 - 0	53kb
RW2070-2	5S	(V)	0.0	70 - 80	7kb
RH7	GAPDH		0.0	0 - 0	28kb
MSP24-2	MSP242	(IV)	84.9	0 - 0	116kb
MSP2-13	MSP213		0.0	0 - 0	135kb
MA7	MSP/2		0.0	0 - 0	47kb
RUSSB7	16KHS		0.0	0 - 0	46kb
L5.1	L5.1	(II)	50.5	0 - 0	35kb
C01D10	MSP7G		0.0	0 - 0	147kb
C55B8	MSP/1		0.0	0 - 0	88kb

HARVARD CLUB OF NEW YORK CITY
27 WEST 44TH STREET
NEW YORK, N.Y. 10036

March 18, 1965

Dear Al,

Excuse my not answering sooner.

I will love to see you when you come to the States.

Though it would be difficult to put you up at the house, (Mom's recuperating from a heart attack) I should like to put you up at the Harvard Club which is quite nice or a hotel around the corner if you wish to be closer.

How was Peggy's leaving party? Send my love to the lab and particularly to Peggy.

Please write if I should reserve May 20 + 21.

Yours,

George Pieczenik

P.S. If there are reprints that tell what's been happening at the lab for the last 5 years please bring.

Mut-M Paul.
-send.

May 19/20

Graham Hatfull
129 York St., Apt. 2E
New Haven, Connecticut 06511

U.S.
0101

519 (H) 203-787-4166

Work: [~~203 436 2163~~ unassigned.]

~~785-4584~~ Info: ~~436 4771~~ 203-785-4584

Keith

~~Home~~ 301-897-8546

Lab 301-496-9106

Lab Mol. Gen.

NINCDS/NIH

Blg 36

Bethesda MD 20205

Dr Goodie Rosenwink
HARVARD CLUB OF NEW YORK CITY
27 WEST 44th STREET
NEW YORK, N.Y. 10036 10023

61 West 62nd St Apt 11G

NEW YORK, NY 100
PM
22 MAR
1985

Lillian M. Gilbreth
USA 40c

Carl Schurz
4c
USA

Dr. Alan Coulson
M.R.C. Laboratory of Molecular
Biology
Hills Road
Cambridge
England

VIA AIRMAIL

6Y
E1

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

Dr. Coulson:

Dr.Tieczenik called with the following message. He has booked a room for you at the Harvard Club, 44th St. between 5th and 6th, New York city, for the nights of the 20th and 21st. He'd like you to be his guest. They'll charge $77.00 per night.

Please call him and leave a message as to whether you have enough information.

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

Dr. Coulson

COLD SPRING HARBOR LABORATORY
P. O. BOX 100
COLD SPRING HARBOR, NEW YORK 11724

SUMMER PROGRAM OFFICE

Dr. Alan R. Coulson
MRC Laboratory of Mol. Biology
Hills Road
Cambridge CB2 2QH ENGLAND

INVOICE NO M 29855

DATE 1985

C.S.H. ACCT. NO.
401-00-683

C. ELEGANS Meeting, May 15 - 19, 1985

Registration	146
Room and Board	244
TOTAL PAID (Fees waived by organizers)	390

FEES WAIVED

PART I

PLEASE INCLUDE OUR INVOICE NUMBER WHEN MAKING PAYMENT

COLD SPRING HARBOR LABORATORY
P. O. BOX 100
COLD SPRING HARBOR, NEW YORK 11724

SUMMER PROGRAM OFFICE

Alan Coulson
MRC Lab of Mol Biology
Hils Road
Cambridge ENGLAND

INVOICE NO M 29019

DATE 1985

C.S.H. ACCT. NO.
401-00-683

C. ELEGANS Meeting, May 15 - 19, 1985

Registration	146
Room and Board	244
TOTAL	390
Deposit	112
BALANCE DUE	278

PART III

PLEASE INCLUDE OUR INVOICE NUMBER WHEN MAKING PAYMENT

COLD SPRING HARBOR LABORATORY
Cold Spring Harbor, N.Y. 11724

(516) 367-8346 from 9 a.m. - 12 p.m.

REGISTRATION:

All participants must register at Blackford Hall (3rd building on your right) upon arrival.

PARKING:

If you drive to the Laboratory, DO NOT PARK in any of the Laboratory parking lots as ALL SPACES are reserved for employees. You will be informed at registration where your car should be parked.

MEALS are served at Blackford Hall:

Breakfast	7:30 - 9:00 a.m.
Lunch	11:30 - 1:30 p.m.
Dinner	5:30 - 7:00 p.m.

HOW TO REACH THE COLD SPRING HARBOR LABORATORY:

BY CAR:

Using Long Island Expressway, Exit 41 North (Rts 106-107). Stay on 106-107 and bear right at fork (106-Oyster Bay). Take Route 106 to intersection of Routes 106-25A (East Norwich). Make right turn onto 25A; go about 5 miles to top of steep hill. At foot of hill, to the left, a three-story, gold Victorian house marks the Lab entrance. If you pass the Fish Hatchery on right-hand side, you have gone too far.

BY PLANE:

Kennedy and LaGuardia Airports are most convenient to the laboratory. Transportation to the Laboratory from either airport is provided by several limousine services; the most frequently used are Long Island Airports Limousine Service and Transport Limousine of Long Island. It is important to note that the limousine will deliver and/or pick you up at the Laboratory at Blackford Hall, if you ask the driver to do so. Limousines may be reserved by using the free direct phones available in most of the terminal buildings or inquire at any Information counter.

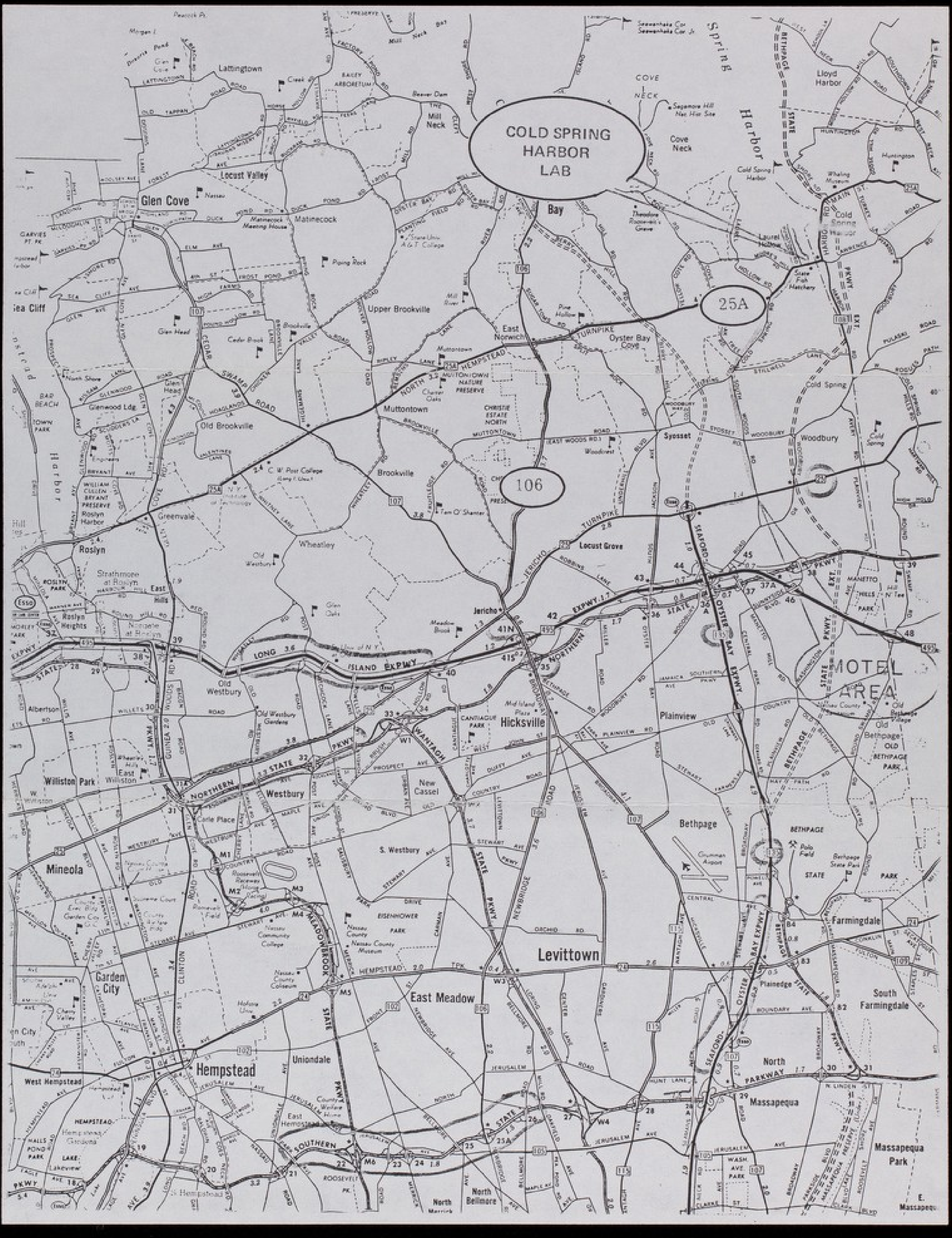
BY TRAIN FROM MANHATTAN:

Alternatively one may use the Long Island Railroad which is located on the lower level of the Pennsylvania Station (34th Street and 7th Avenue.). Take the PORT JEFFERSON BRANCH train to SYOSSET.

NOTE: All passengers using the Port Jefferson Branch might possibly change at Jamaica Station.

Take taxi from Syosset to the Laboratory (about 4 miles). Taxis usually meet arriving trains, but in the event that none are available call:

Orange and White Taxi - 271-3600
Syosset Taxi Service - 921-2141


COLD SPRING
HARBOR
LAB
25A
106
MOTEL
AREA
Lattingtown
Locust Valley
Glen Cove
Matinecock
Mill Neck
Cove Neck
Lloyd Harbor
Cold Spring Harbor
Laurel Hollow
Upper Brookville
East Norwich
Oyster Bay Cove
Muttontown
Old Brookville
Glenwood Ldg.
Sea Cliff
Glen Head
Brookville
Greenvale
Roslyn Harbor
Roslyn
Roslyn Heights
Wheatley
East Hills
Jericho
Locust Grove
Syosset
Woodbury
Cold Spring
Old Westbury
Westbury
Hicksville
Plainview
Albertson
Williston Park
East Williston
Carle Place
New Cassel
Mineola
S. Westbury
Bethpage
Old Bethpage
Garden City
Levittown
Farmingdale
East Meadow
Plainedge
South Farmingdale
Hempstead
Uniondale
West Hempstead
North Massapequa
Massapequa
Massapequa Park
North Bellmore
North Merrick
Lakeview
S Hempstead
LONG ISLAND EXPWY
NORTHERN STATE PKWY
SOUTHERN STATE PKWY
WANTAGH STATE PKWY
SEAFORD OYSTER BAY EXPWY
BETHPAGE STATE PKWY
JERICHO TURNPIKE
HEMPSTEAD TPK
Oyster Bay
Cold Spring Harbor

Cold Spring Harbor Laboratory
P.O. Box 100, Cold Spring Harbor, New York 11724

C. elegans Meeting
May 15-19, 1985

PROGRAM:

The first session begins at 7:30 p.m. Wednesday, May 15, and the last session begins at 9 a.m. Sunday, May 19, 1985. You will receive program information upon arrival at the Laboratory; we do not give it out beforehand.

ACCOMMODATIONS:

If (coming from the West Coast or overseas) you find that you must arrive earlier than the first day of the meeting, please advise us well in advance so that your accommodations will be ready. There is also a $60 housing/food charge for each day prior to or beyond the meeting itself.

RETURN FLIGHT AND LIMOUSINE ARRANGEMENTS:

It will expedite your departure if you sign up upon arrival at the Laboratory - giving us the airport, flight time and airline - we will write in the departure time from the laboratory. Generally, the limousine companies plan on a two-hour trip to the airports because of the congestion on the Long Island Expressway. Signup sheets for airport limousines will be posted in the lower level of Blackford Hall by the Meetings Office.

The last session begins at 9 a.m. on Sunday, May 19, and will probably end between 12 - 1 p.m., followed by lunch; therefore you should plan your return flight after 4 p.m. if you are leaving on the last day.

CANCELLATION:

If you must cancel out or if someone else will be replacing you, please let us know immediately.

Gladys Kist
Meetings Coordinator
(516) 367 8345

Enclosure