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REPORT OF WORK PERFORMED UNDER UNIDO CONTRACT NO 88/99
UNIDO PROJECT NO GE/GLO/86/001
(Activity code: G03301)
FOR THE PROVISION OF SERVICES RELATING TO THE ICGEB PRACTICAL LABORATORY COURSE ON GENETIC MANIPULATION OF STREPTOMYCES, IN WUHAN, PEOPLE'S REPUBLIC OF CHINA

BY STAFF OF THE JOHN INNES INSTITUTE

NORWICH NR4 7UH

Professor D A Hopwood (Team Leader)

Professor K F Chater

Dr M J Bibb

Dr T Kieser

Mrs H M Kieser

Miss C J Bruton

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INTRODUCTION

This course was run in the Central Laboratory building of the Huazhong (Central China) Agricultural University, Wuhan, PRC. This university has recently been designated as the premier agricultural university in China for research and teaching in certain designated areas of biology, one of them being microbiology. The Central Laboratory has been equipped with facilities and large items of equipment to make it suitable for research in genetic engineering by members of the various departments of the university. Currently, three groups also have their own research laboratories in this building. One of them is the Streptomyces groups, headed by Prof Zhou Qi. Its other staff members include Prof Liang Rongfan, Dr Deng Zixin and Ms Zhou Xuifen, together with several graduate students. Dr Deng received four years of doctoral and post-doctoral training in genetic manipulation of Streptomyces at the John Innes Institute in Norwich and Ms Zhou one year. This group is the most advanced and best equipped for research in Streptomyces molecular genetics in China. The practical course was run in the research laboratory of Prof Zhou's group (which could just accommodate 18 students plus teaching staff), with lectures being held in a nearby seminar room in the Central Laboratory building. There was also a large, comfortable lobby area for social interactions, refreshments, etc. Together with excellent residential accommodation and catering facilities for visiting "foreign experts", and overseas and Chinese students, all located on an attractive campus, the venue was ideal for the course. We are greatly indebted to the President of the University, Prof Sun Jizhong, for making all relevant facilities available and for his strong support of the course in both material and administrative dimensions.

PUBLICITY, AND SELECTION OF STUDENTS

The course was announced by means of a leaflet and application form (Appendix A) which were sent to: the contact organisations designated by UNIDO in India, Thailand, Vietnam, Mauritius and Bhutan; the Permanent Mission of Afghanistan in Vienna; and to UNIDO itself for transmission by them to the Permanent Missions for Pakistan, Indonesia and Iran in Vienna. For China, a corresponding leaflet and application form (Appendix B) were distributed to all potentially interested laboratories by the staff of Huazhong Agricultural University, in liaison with the China National Center for Biotechnology Development in Beijing.

Eleven applications were received in Norwich from non-Chinese applicants, six from India, three from Thailand and two from Vietnam. This total is certainly not a true indication of potential demand for the course, since there is no doubt that information about it did not reach all relevant centres in the UNIDO member countries. However, it was not difficult for the Norwich staff to select, from the 11 applicants, five well-qualified, students - the maximum number whose travel and subsistence costs could be supported from the UNIDO grant.

Some 25 applications were received by Prof Zhou Qi from Chinese laboratories. This number certainly very significantly under-represents the total demand, since each institution was asked to make its own selection of its most worthy student or (for the larger institutions) two students. From the 25 applications, 13 were selected by the Chinese selection committed consisting of Prof Zhou Qi (Huazhong Agricultural University, Wuhan), Prof Xue Yugu (Institute of Microbiology, Beijing), Prof Zheng Youxia (Institute of Plant Physiology, Shanghai) and Dr Liu Yonghui (China National Center for Biotechnology Development).

The names and addresses of the 18 students are in Appendix C. Most of them were graduate students or young post-doctorals, with a few more senior people.

THE COURSE

The course ran from 2 p.m. on Sunday 9 April to 9 p.m. on Friday 21 April, with one and a half days of social activities on Saturday and Sunday 15 and 16 April. On most days, experimental work and associated discussions occupied the periods 8.00 to 11.30 a.m. and 1.30 to 5.30 p.m., with lectures each day from 7.30 - 9.30 p.m. On some days, short seminars by student participants ran from 1.30 - 2.30 p.m. and on Saturday 15 April they filled the morning.

On Saturday and Sunday 22 and 23 April a symposium on Streptomyces Genetics and Genetic Engineering, organised by the Microbial Genetics Section of the Chinese Society of Genetics, was attended by course participants and teachers as well as scientists from many Chinese laboratories engaged in research in this field. It ended with a round table discussion on "Challenges and promise of Streptomyces genetics in China".

Experiments

A list of experiments and protocols for them are in Appendix D.

Details of practical techniques are described in the publication "Genetic Manipulation of Streptomyces: A Laboratory Manual", John Innes Foundation, 1985, a copy of which was supplied to each participant. The Chinese participants were also supplied with a copy of the recent

Chinese translation of the Manual (Hunan Scientific Press, 1988).

In general, the experiments were very well performed and gave good results, providing excellent material for acquiring practical skills and leading to good discussions of theoretical concepts. It became absolutely clear that the course would not have been successful without the participation of all six of the John Innes Institute staff, with their complementary coverage of different aspects of a very wide ranging subject, as well as the staff of the Huazhong Agricultural University. In addition, we were fortunate to have the assistance in the laboratory of Prof Wang Yiguang, of the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijing. Her knowledge of the subject, and her ability to explain points in Chinese where necessary, greatly aided the smooth running of the course.

Lectures

A list of the lecture topics is in Appendix E.

Discussion of points of practical technique

A total of two hours was devoted to discussions of points of practical techniques (led by Dr Tobias Kieser) including the following: avoiding contamination - autoclaving, laboratory hygiene; use of microwave oven; achieving good sporulation; alternative regeneration media; plasmid isolation - effects of various chemicals on transformation efficiency; "star" activity of restriction enzymes; effect of PEG on ligation; use of PEG for DNA size fractionation; ethanol precipitation of DNA.

Seminars by participants

A list of seminars is in Appendix F.

Course evaluation

An evaluation from (Appendix G) was filled in anonymously by each student. In general, the course was extremely well received. The overall assessment was as follows:

Excellent	5
Excellent/very good	1
Very good	8
Good	4
Satisfactory	0
Poor	0
Very poor	0

With o points for "excellent", 5 for "very good", ... etc the average overall score was a very gratifying 5.1 out of a possible 6.0.

In addition, a few useful suggestions were made for further improvements to such a course.

FINANCIAL SUPPORT AND BUDGET

The staff of Huazhong Agricultural University and the six members of the John Innes Institute spent many man-months of time preparing for the course and teaching it. This staff time - which was provided free of charge by the two institutions - represents a very considerable financial input to the course. This included not only the teaching staff, but also a large committment of support staff time in Wuhan. In addition, Huazhong Agricultural University paid for travel within China for four John Innes Institute staff and the

entire costs of accommodation, subsistence and recreation for all six John Innes Institute staff.

UNIDO undertook to provide up to £19,000 for direct expenses, broken down as follows:

Travel of four teaching staff from UK	£3,500	
Travel and/or subsistence for trainees from ICGEB member countries	£5,000	(contingency)
Minor equipment for host laboratory	£2,000	
Expendable equipment	£8,000	
Miscellaneous (printing/distribution of laboratory manuals and/or teaching aids for students)	500	
	£19,000	

In addition, since it was abundantly clear that the course could not be run satisfactorily without all six of the designated staff members from John Innes Institute (this was strongly confirmed during the course itself), extra funds had to be sought for the international travel of the two remaining staff members. For this, a successful application was made to the Royal Society of London for Professor Hopwood and Dr Bibb to travel to China under the Exchange Agreement which exists between the Royal Society and the China Association for Science and Technology (CAST): this therefore represents a significant input to the finances of the course from the Royal Society (£1572) for international travel and from CAST (for expenses within China).

A statement of expenditure under the UNIDO grant is in Appendix H.

FUTURE PROSPECTS

There is no doubt that the course will have an extremely beneficial effect on the development of *Streptomyces* genetic manipulation in China. Moreover, its likely benefits will extend well outside *Streptomyces* since, in many respects, this organism can act as a model, and a training ground, for work in other groups of microorganisms. (Sales of the Chinese edition of the *Streptomyces* Practical Manual already confirm this.)

A particularly exciting development occurred during the final discussion at the Symposium on Molecular Genetics and Genetic Engineeering of *Streptomyces* on Sunday 23 April. This was the formulation and signing by all those present of a Declaration (Appendix I) with two paragraphs. One refers to the free distribution of strains, plasmids and vectors between research scientists. The second is a proposal to set up an "open laboratory", if possible with international support, for work on *Streptomyces* molecular genetics and genetic engineering at the Huazhong Agricultural University in Wuhan. The concept of "open laboratories" is well established in China. They exist to allow scientists interested in a specific field to have access to equipment and expertise that is not yet widely available. The unanimous support for these developments on the part of the participants at the Symposium was extremely gratifying. The John Innes team will now be actively supporting efforts to establish such a laboratory for *Streptomyces* work. The endeavour has the whole-hearted support of the President of Huazhong Agricultural University, Professor Sun Jizhong.

D A HOPWOOD Norwich, 24 May 1989

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY

"GENETIC MANIPULATION OF *STREPTOMYCES*"

AN ICGEB LABORATORY COURSE TO BE HELD AT

HUAZHONG AGRICULTURAL UNIVERSITY, WUHAN, PEOPLE'S REPUBLIC OF CHINA

9-24 APRIL 1989

INSTRUCTORS WILL INCLUDE: M.J. Bibb, C.J. Bruton, K.F. Chater, Deng Zixin, D.A. Hopwood, H.M. Kieser, T. Kieser, Zhou Qi, Zhou Xiufen

COURSE CONTENTS: The course will be aimed primarily at research workers at late post-graduate and post-doctoral level who wish to work on *Streptomyces* genetics and cloning, either to study problems intrinsic to *Streptomyces* such as differentiation, antibiotic synthesis or resistance, transposition, amplified DNA, etc. or to use streptomycetes as hosts for the production of foreign and amplified gene products.

The working language of the course will be English.

We expect to devote about 60% of the time to practical work, and the remainder to lectures, seminars and discussion of the experimental results. Experiments will be carried out in some or all of the following areas: (1) genetic mapping by plasmid-mediated conjugation and by protoplast fusion; (2) isolation, physical characterisation and transformation of *Streptomyces* plasmids; (3) isolation of new *Streptomyces* phages from soil, physical characterisation and transfection; (4) cloning of a selection of *Streptomyces* genes (antibiotic resistances, melanin production, catabolic functions, antibiotic biosynthesis) on plasmid and phage vectors; (5) use of promoter probes to study gene expression in *Streptomyces* and between *Streptomyces* and *E. coli*; there may also be the possibility for participants to do some experiments with their own organism, phage or plasmid.

ACCOMMODATION AND MEALS: Accommodation (single room with bath and toilet) and meals will be on the campus of Huazhong Agricultural University. The cost of accommodation and meals for the duration of the course for applicants outside the People's Republic of China will be covered by the organizers.

TRAVEL: The costs of travel to and from Wuhan will be the responsibility of each participant. However, it may be possible to provide a travel grant to contribute towards these costs for applicants who would otherwise have difficulty in covering them. **See application form.**

APPLICATIONS: The number of places on the course will be limited to 18. **Applications from UNIDO member nations other than the People's Republic of China must be made on the attached form, which should be sent as soon as possible to Professor D A Hopwood, John Innes Institute, Colney Lane, Norwich NR4 7UH, England.** Applications from those resident in the People's Republic of China should be sent to Professor Zhou Qi, Department of Agricultural Chemistry and Soil Science, Huazhong Agricultural University, Wuhan, Hubei, China. **The final deadline for receipt of applications will be 1 January 1989.** Applicants will be notified about acceptance as soon as possible after that date.

TIMETABLE: Students will be expected to arrive by the evening of Saturday 8 April, 1989. On Sunday 9 April there will be introductory lectures and outlines of the experimental work, which will begin on 10 April and continue until 24 April. On most evenings there will be discussions and short seminars by students on the course on topics of direct or indirect relevance to *Streptomyces* genetics and molecular biology. Over the weekend of 22-23 April it is expected that there will be a symposium on modern developments in *Streptomyces* genetics attended by course participants and others. It is hoped that all students will stay until Monday 24 April.

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HUAZHONG AGRICULTURAL UNIVERSITY, WUHAN, PEOPLE'S REPUBLIC OF CHINA

9-24 APRIL 1989

APPLICATION FORM
Non-Chinese Applicants

Full name: Age on 1/4/89:

Professional address: Sex:

Nationality:

Telephone number:
Telex number:

Details of university education (subjects studied, etc.) and/or research experience:

Current position:

Publications: (or attach a separate list)

Current and future research interests:

Why do you wish to attend this course?

If you are a graduate student, please attach a letter of support from your supervisor

If you need to apply for a grant towards the cost of travel, please justify your claim and give details of costs on a separate sheet.

Signature: Date:

Send completed form by **1 January 1989** to:

Professor D A Hopwood
John Innes Institute
Colney Lane
Norwich NR4 7UH
England
Phone No. (0603) 52571
Fax No. (0603) 56844
Telex No. 975122 (JIINOR) G

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY

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ACCOMMODATION, MEALS AND TRAVEL: Accommodation and meals will be on the campus of Huazhong Agricultural University. The cost of accommodation and meals, as well as travel expenses, for students from the People's Republic of China are the responsibility of individual students.

APPLICATIONS: The number of places on the course will be limited to 18. Applications from UNIDO member nations other than the People's Republic of China must be made on the attached form, which should be sent as soon as possible to Professor D A Hopwood, John Innes Institute, Colney Lane, Norwich NR4 7UH, England. **Applications from those resident in the People's Republic of China should be sent to Professor Zhou Qi, Department of Agricultural Chemistry and Soil Science, Huazhong Agricultural University, Wuhan, Hubei, China. The final deadline for receipt of applications will be 1 January 1989.** Applicants will be notified about acceptance as soon as possible after that date.

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AN ICGEB LABORATORY COURSE TO BE HELD AT
HUAZHONG AGRICULTURAL UNIVERSITY, WUHAN, PEOPLE'S REPUBLIC OF CHINA

9-24 APRIL 1989

APPLICATION FORM
Chinese Applicants

Full name: Age on 1/4/89:
Name in Chinese characters:

Professional address: Sex:

Telephone number:
Telex number:

Details of university education (subjects studied, etc.) and/or research experience:

Current position:

Publications: (or attach a separate list)

Current and future research interests:

Why do you wish to attend this course?

If you are a graduate student, please attach a letter of support from your supervisor

Signature: Date:

Send completed form by **1 January 1989** to:

Professor Zhou Qi
Department of Agricultural Chemistry
and Soil Science
Huazhong Agricultural University
Wuhan, Hubei
People's Republic of China

UNIDO/ICGEB/JOHN INNES INSTITUTE COURSE ON
"GENETIC MANIPULATION OF STREPTOMYCES"
HUAZHONG AGRICULTURAL UNIVERSITY, WUHAN, 9-21 APRIL, 1989

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UNIDO COURSE ON GENETIC MANIPULATION OF *STREPTOMYCES*

WUHAN, APRIL 1989

List of Experiments

1(a) Recombination analysis in *Streptomyces coelicolor* by mating
(b) Recombination analysis in *Streptomyces coelicolor* by protoplast fusion

2 Pocks caused by plasmid in *Streptomyces lividans*

3(a) Isolation of phages from soil
(b) Analysis of phage DNA

4 Lysogeny

5. Mutational cloning with a øC31 vector

6. Cloning in *Streptomyces* on plasmid vectors

7. "Strong incompatibility" between derivatives of the *Streptomyces* plasmid pIJ101

8. Plasmid isolation by alkaline lysis and gel electrophoresis of linear and circular DNA

* * * * * * *

Responsibility for:

Experiments 1 and 2:	David Hopwood and Helen Kieser
" 3,4 and 5:	Keith Chater and Celia Bruton
" 6:	Mervyn Bibb and Tobias Kieser
" 7 and 8:	Deng Zixin

Pages of the manual for methodology

Recombination Analysis in Streptomyces coelicolor by Mating

The strains form complementary pairs differing in 6 markers. In crosses (b) and (c) one parent contains the SCP2* plasmid whereas in cross (a) both parents lack any known autonomous sex plasmid. Thus we should be able to demonstrate the enhancement of recombination promoted by a sex plasmid. The results will also be compared with those of a protoplast fusion between the two plasmid-free strains (Experiment 1B).

The crosses are: (a) M130 (hisA1 uraA1 strA1) X M124 (proA1 argA1 cysD18)

(b) M110 (hisA1 uraA1 strA1 SCP2*) X M124

(c) M130 X M107 (proA1 argA1 cysD18 SCP2*).

Day -4 Make the crosses on slants of R2YE (containing added arginine, cystine, histidine and uracil; proline is of course already present in R2YE). **(This will be done by the instructors and the crosses will be provided to you on Day 1.)**

pp.3-6, p.45

Day 1 Harvest the spores from the crosses (each group should do cross (a) and **either** cross (b) or cross (c)) and plate at suitable dilutions on MM containing arginine, cystine, proline, uracil and streptomycin (a selective medium recovering

recombinants inheriting his^{+} and str) e.g. at 10^{0} and 10^{-1} for cross (a) and 10^{0}, 10^{-1} and 10^{-2} for cross (b) or cross (c). Also plate both crosses on MM + histidine, uracil and streptomycin and on MM + arginine, cystine and proline (to recover the parental genotypes) at (say) 10^{-4} and 10^{-5}. (NB: we regard the original suspension as 10^{0}, so the first tenfold dilution is "10^{-1}", etc).

pp.46-49
pp.50-51

Day 5 Count the colonies on at least one plate of each medium. Calculate the "recombination frequency" for comparison with the results of the protoplast fusion (Experiment 1B). Make a master plate of 50 colonies from the medium that selected recombinants, from cross (b) or cross (c).

Day 9 Replicate the master plate to the set of 5 diagnostic media.

pp.58-59

Day 11 Classify the genotypes of the recombinants. Pool the results with those of other groups. Analyse the patterns of crossing-over in the various map intervals and compare with the corresponding data from the protoplast fusion of M130 x M124 (Experiment 1B).

Pages of the manual for methodology	Recombination analysis in *Streptomyces coelicolor* by protoplast fusion

For this experiment we are using the same pair of plasmid-free strains as we used in the "control" cross in Experiment 1A. The results of the fusion will be compared with those of the matings.

pp.12-14

Day 0 Inoculate a flask containing 25 ml of YEME (containing 34% sucrose, 5 mM $MgCl_2$ and 0.5% glycine) with strain M130 and another with strain M124. Incubate on the shaker at 30-35°.

pp.64-67

Day 2 Prepare protoplasts from the 2 strains. Fuse them and plate out on R2YE plates (containing added arginine, cystine, histidine and uracil: proline is of course already present in R2YE) at (say) 10^{0}, 10^{-1} and 10^{-2} dilutions.

Day 8 Harvest spores from a plate with good confluent growth and plate out, at (say) 10^{-3}, 10^{-4} and 10^{-5} on the plate selecting recombinants and at 10^{-5} and 10^{-6} on the two "parental" media (see **Day 1** of Experiment 1A).

Day 11 Count the colonies on at least one plate of each medium. Calculate the "recombination frequency". Compare it with those obtained in the crosses (Experiment 1A).

pp.46-49	Day 5	**Using the plates of colonies already prepared and provided to you,** make a master plate of 50 colonies from the medium that selected recombinants (MM + arginine, cystine, proline, uracil and streptomycin).
	Day 9	Replicate the master plate to the set of 5 diagnostic media.
pp.58-59	Day 11	Classify the genotypes of the recombinants. Pool the results with those of other groups. Analyse the patterns of crossing-over in the various map intervals and compare with the corresponding data from the mating (Experiment 1A).

Pocks Caused by Plasmids in Streptomyces lividans

In this experiment we shall examine the ability of various Streptomyces plasmids to give rise to pocks when spores of a plasmid-carrying strain are plated in a "lawn" of a plasmid-free strain. This pock-formation is called the Ltz^{+} phenotype. We shall be using plasmids carrying a selectable marker (tsr for thiostrepton resistance), and the plasmid-carrying strain has a chromosomal mutation (str, for streptomycin resistance). We shall therefore be able to follow the transfer of the plasmid, and of the chromosome of the donor strain, within the mycelium of the recipient strain by replica plating the pocks to a selective medium containing thiostrepton (to follow the plasmid) or streptomycin (to follow the donor chromosome).

The donor strains are as follows:

TK24	(plasmid-free control, with chromosomal marker str-6)
TK24/pIJ303	(pIJ101 derivative, Ltz^{+}, tsr)
TK24/pIJ643	(pIJ101 derivative, Ltz$^{+/-}$, tsr)
TK24/pIJ702	(pIJ101 derivative, Ltz^{-}, tsr, mel)
TK24/pIJ922	(SCP2* derivative, Ltz^{+}, tsr)

The recipient strain is TK21 (plasmid-free, with no chromosomal markers).

Day 1 Take 6 plates of R2YE and place a drop of a dense spore suspension of TK21 on each. To 5 of the plates, add a drop of a suitable dilution of a suspension of one of the 5 donor strains. Keep the sixth plate as the "recipient only" control. Using a separate spreader, spread the spore suspension evenly over each plate. (It may be useful to use

2 plates of R2YE for each donor strain and to plate it at two different dilutions to make sure that suitable numbers of pocks are obtained.) Also spread the same quantity of spores of each donor strain on plate of R2YE without adding spores of the recipient, using the same dilutions of the donor spores as were used to detect pocks. These "donor only" plates will reveal the number of viable spores (colony-forming units) in each spore suspension for comparison with the number of pocks on the recipient lawns.

Days 3,4,5 — Examine the recipient lawns at intervals. Look for the presence and appearance of pocks. Count the pocks. Count colonies on the "donor only" plates and compare the colony counts with the numbers of pocks on the recipient lawns. Return the lawn plates to the incubator so that the pocks will develop further.

Day 8 — Replicate the lawn plates to MM containing streptomycin and to MM containing thiostrepton.

Day 10 — Examine the replica plates and compare the distribution of thiostrepton-containing growth (which monitors the presence of the plasmid) and streptomycin-containing growth (which monitors the presence of the chromosome of the donor strain).

(a) Isolation of phages from soil; (b) analysis of phage DNA

The aims of this section are (a) to isolate phages from soil samples provided by participants, according to the protocols on pages 15-22, using as hosts S. lividans (or strains provided, as densely sporulating plates or spore suspensions, by participants); and (b) to propagate examples of the phages obtained and extract DNA (see pages 99-102), and carry out a preliminary restriction analysis (pages 131-145).

Day 1 p.m. Experiment 3(a). Set up overnight enrichment cultures containing soil samples (see p.22 procedure II, steps 1 and 2).

Day 2 a.m. Experiment 3(a). Extract soil sample with DNB (see p.21, procedure I, steps 1-4) and process the overnight enrichment cultures up to step 4 (p.22).

p.m. Experiment 3(a). Complete steps 5 and 6 (p.22) for both procedures, using, as appropriate, S. lividans or the strain provided by the participant in the top layers.

Day 3 a.m. Experiment 3(a). Restreak from promising plaques (see p.18).

Day 4 a.m. Experiment 3(a). Soak out single plaques.

Day 8 p.m. Experiment 3(b). Set up 3 large Petri plates for each phage (see p.100, step 1).

Day 9 a.m. to p.m. Experiment 3(b), p. 100, steps 2-9b.

Day 10 a.m. Experiment 3(b). Complete DNA isolation. See p.101, steps 10b-14b.

Day 11 a.m. Experiment 3(b). Digest 5 ul aliquots of the DNAs with EcoRl, BamHI, SalI, SstII, each in a total volume of 20 ul (see p.131-132).

p.m. Run samples on gel. Stain and photograph gel.

EXPERIMENT 4

Lysogeny

The aims of this section are to isolate ⌀C31 lysogens (pages 23 - 25) of a strain which supports ⌀C31 plaque formation (S. lividans), using procedure A, page 24, and of a strain which does not (S. coelicolor), using procedure B, page 25.

Day 4 a.m.	Spread 1 R2YE plate with 0.05 ml dense spore suspension of S. lividans 66 and 1 R2YE plate with 0.05 ml S. coelicolor A3(2) spore suspension, allow plates to day. Using a sterile pipette tip, spot 20 ul ⌀C31 (w.t.) on to centre of S. lividans plate, allow to dry (see p. 23-24 Procedure A. Steps 1-3). Spot 20 ul ⌀C31 KC301 (c^{+} att^{+}::tsr) on to centre of S. coelicolor plate and allow to dry. Assay both phages on both hosts (see p. 15).
Day 5 a.m.	Check titres of phages assayed.
Day 8 p.m.	S. lividans plate, harvest spores within spot, wash in pyrophosphate, filter, harvest and plate out appropriate dilutions on R2YE plates (see p.24, Procedure A, Steps 4-10). S. coelicolor plate, replicate to MM plate containing thiostrepton (see p.25, Procedure B, Step 4).
Day 11 a.m. OR p.m.	S. lividans: replicate for phage release (see p.24, procedure A, step 11). S. coelicolor: check thiostrepton plate for $thio^{R}$ colonies.
Day 12 a.m.	S. lividans: identify lysogens as colonies which have released phages.

EXPERIMENT 5

Mutational cloning with a øC31 vector

One of several ways of using øC31 as a vector exploits the (sometimes mutagenic) integration of the phage into the host genome by homologous recombination involving a cloned segment (see p.169-171). We shall carry out mutational cloning with a segment of DNA from the glycerol operon (*gyl*) of *Streptomyces coelicolor*. The segment of *gyl* DNA will be provided and inserted into the *BglII* site of the *att*-deleted vector øC31 KC515 (p.319). The resulting chimaeric molecules will be recognised by their ability to transduce *S. coelicolor* to thiostrepton resistance. The necessary lysogens are made by recombination between the cloned *gyl* DNA and the recipient's chromosome. If the cloned *gyl* DNA is entirely internal to a transcription unit, the resistant transductants should have a Gyl^- phenotype.

If the recipient is a øC31 lysogen, thiostrepton resistance can arise by integration of the vector with or without inserts into the resident prophage. In this case the *gyl* operon of the recipient will not be inactivated.

Materials

Those required for preparation and transfection of *S. lividans* protoplasts (see p.12-13 and p.115-116), ligation of DNA (see p.156) and agarose gel electrophoresis of DNA (see p.143).

In addition:

Strains

J1507: *S. coelicolor* *hisA1* *uraA1* *strA1* Pgl^- NF (Dag^-) spore suspension

J1507/øC31 lysogen spore suspension

DNA

Vector: øC31 KC515 DNA (predigested with BglII)

Donor: isolated BglII fragment of gyl DNA

Transfection control: øC31 cl DNA (10 ug/ml)

Media

R2YE plates containing histidine and uracil

MM plates (containing $(NH_4)_2SO_4$ - see p.233 Note) containing histidine and uracil and thiostrepton (50 ug/ml) with, as carbon source, glucose or glycerol (at 0.5%). (2 with each carbon source.)

Other materials

3 M sodium acetate; glass spreaders; replicating velvets and replicating block

Procedure and timetable

Day 3 14.00 Mix vector DNA (30 ul containing 1 ug DNA) and gyl DNA (30 ul containing 100 ng DNA), add 8 ul 3 M sodium acetate and precipitate DNA by adding 200 ul absolute ethanol. Stand at -20° for c. 1 h. Spin in Eppendorf centrifuge, 10 min, remove all the ethanol, and redissolve in 10 ul X1 ligase buffer containing ATP (p.156). Remove 2 ul to store at -20°, add 1 ul DNA ligase to remainder and incubate overnight at 12°.

Day 4 09.00 Prepare S. lividans 66 protoplasts (see p.12-13 Steps 5-10).

14.00 Transfect the prepared protoplasts using Procedure II (see p.115-117, 1-7), with (a) the ligated DNA (plate out 2 x 0.1 ml of each dilution at 10°, 10^{-1}, 10^{-2}, 10^{-3}); (b) the sample of unligated DNA stored at -20° (plate out 1 x 0.1 ml of each dilution at 10°, 10^{-1}); (c) 5 ul of øC31 cl DNA (plate out 0.1 ml of each dilution at 10°, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} using small Petri plates).

Day 5 a.m. or p.m. Check plaques. Spread 2 R2YE plates containing histidine and uracil with 0.05 ml J1507 spore suspension, and 2 with a mixture of J1507 and J1507 ØC31 lysogen (0.05 ml of each). Dry the plates. Replicate transfection plates containing c. 100 plaques on to the spread plates. Incubate 30° for 4 days.

Day 9 (or later depending on sporulation)

a.m. or p.m. Replicate the J1507 and J1507 + J1507/ØC31 plates to MM plates containing histidine, uracil and thiostrepton and glucose or glycerol.

Days 11, 12 Check the thiostrepton plates for resistant colonies corresponding to the original plaques.

EXPERIMENT 6

Introduction

The purpose of this experiment is to demonstrate the methodology employed in cloning genes from chromosomal DNA into *Streptomyces* using a plasmid vector. Attempts will be made to isolate genes involved in histidine and uracil biosynthesis from *Streptomyces coelicolor* A3(2) and to isolate a streptomycin-resistance gene from *Streptomyces glaucescens*. A purified DNA fragment containing the erythromycin resistance gene of *Saccharopolyspora erythrea* (formerly *Streptomyces erythraeus*) will be added to the cleaved samples of chromosomal DNA to serve as an internal standard for the efficiency of the cloning procedure. The recipient strains will be either *S. coelicolor* J1501 (*hisA1* *uraA1* *strA1* *pgl* NF SCP2$^-$) or *Streptomyces lividans* 1326.

The chromosomal DNA samples will be cleaved partially with *MboI* and subjected to size fractionation or sucrose gradients and fragments in the size range 8-20 kb used for ligation.

The vector provided is pIJ4083 which is a *xylE* promoter-probe plasmid. Fragments will be cloned into the unique *BamHI* site located in a polylinker that precedes the coding sequence of the promoter-less *xylE* gene. This gene encodes a catechol-2,3-dioxygenase that converts catechol to the yellow compound 2-hydroxymuconic semialdehyde. Insertion of a promoter-active fragment in the appropriate orientation at the *BamHI* site will yield yellow colonies when sprayed with catechol.

Because of the slow growth and development of streptomycetes, and to ensure that you carry out all aspects of the procedure, the experiment is divided into two parts. Experiment 6A starts with the transformation of *Streptomyces* protoplasts with ligated DNA samples. Both the ligation mixtures and the protoplasts will have been prepared in advance of the course. The aim of this experiment is to find specific clones and to asses the quality of the gene banks. Experiment 6B commences with the isolation of total DNA and continues with its partial digestion with *MboI* and size-fractionation. Its major purpose is to demonstrate these techniques. If time permits you will also analyse transformants obtained from this experiment.

Please note: If in experiment 6A you transform *S. coelicolor* J1501 with the ligation mix containing *S. coelicolor* DNA, please ensure that in experiment 6B you transform *S. lividans* 1326 with DNA derived from *S. glaucescens* (and vice-versa).

EXPERIMENT 6A

Cloning of Chromosomal Genes into Plasmid Vectors - Transformation and Analysis of Clones

Day 1 (Monday)

1. Transformation of S. coelicolor J1501 (hisA1, uraA1, strA1, pgl NF SCP2$^-$) or S. lividans 1326 protoplasts with already prepared ligation mixes (Manual p. 110).

a. Thaw protoplasts, transfer 1 ml to two Sterilin tubes (labelled 1 and 2), fill tubes with P buffer (make sure that it is complete with TES, $CaCl_2$ and K_2HPO_4 as described in the Manual p. 245).

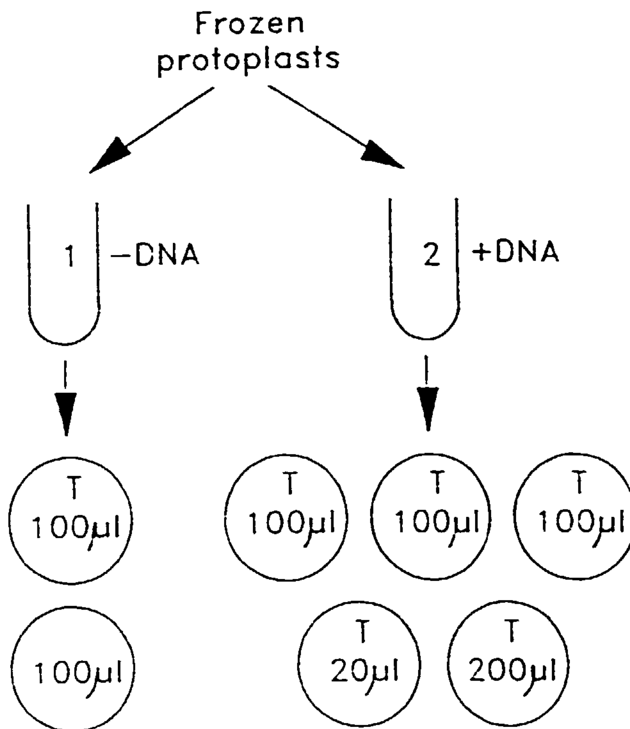
b. Spin 5 min at setting 3.

c. While samples are spinning label the 7 R2YEhu plates with Group identification, type of DNA, amount plated and "T" (for thiostrepton overlay) where applicable.

d. Decant supernatant from **b.** and resuspend protoplast pellet in the c. 100 µl buffer left in the tube.

e. Add 500 µl T buffer (containing 25% PEG; Manual p. 246) to tube No. 1, mix by pipetting and spread 100 µl aliquots onto two R2YEhu plates ("No DNA control").

f. For tube No. 2 mix in 20 µl of "ligation mix" (DNA solution) and then add 500 µl T buffer. Mix by pipetting and spread aliquots (see diagram) onto five R2YEhu plates (add 80 µl P buffer to the plate which gets only 20 µl of protoplast suspension).



g. It is recommended to incubate the plates labelled "T", which will be overlayed with thiostrepton, separately from the others.

Day 2 (Tuesday)

2. Overlay all of the regeneration plates except one of the "No DNA control" plates. For each overlay use 1 ml of a suspension of 300 μg/ml thiostrepton in water (add 60 μl stock solution (50 mg/ml in DMSO) to 10 ml water and shake before use).

Day 5 (Friday)

3. Count colonies.
4. Spray one regeneration plate with a 0.5M aqueous solution of catechol (IN FUME CUPBOARD). Score yellow colonies.

Day 8 (Monday)

5. Replicate sporulated regeneration plates to selective media and a non-selective control in the following order:

S. coelicolor J1501	*S. lividans* 1326
MM his thio	MM str thio
MM ura thio	MM ery thio
MM his ura thio ery	MM thio
MM his ura thio	

Day 10 (Wednesday)

6. Score replica plates. Spray non-selective replicas with a 0.5M aqueous solution of catechol. Score yellow colonies.

EXPERIMENT 6B

Cloning of Chromosomal Genes into Plasmid Vectors - Preparation, size fractionation and ligation of chromosomal DNA into pIJ4083

Day 1 (Monday)

1. Prepare sucrose gradients (Manual p. 152)
2. Isolate total DNA (S. coelicolor M145 or S. glaucescens) from the frozen mycelium provided. The following is an adaptation of procedure 3 (Manual p. 77) for use with 1.5 ml Eppendorf tubes. Use 4 tubes of mycelium.

a. To each of the 4 tubes containing 200 µl of cell suspension add 100 µl lysozyme solution (20 mg/ml) containing RNase (50 µg/ml).

b. Incubate 20 min at 37°C.

c. Add 400 µl 2x Kirby mixture (Manual p.77) and vortex for 1 min (make sure that the vortexing is effective).

2x Kirby mixture

2 g	Tris-isopropylnaphthalene sulphonate
12 g	Sodium-4-amino-salicylate
5 ml	2M Tris-HCl, pH 8
6 g	phenol (Analar)
6 mg	8-hydroxyquinoline
90 ml	water

d. Add 200 µl phenol/chloroform and vortex for 15 sec (start timing when milky).

e. Spin for 3 min.

f. Remove upper phase to new tube.

g. Repeat steps d - f with 50 μl phenol/chloroform until there is only a very small interphase left (usually two or three times).

h. Add 0.1 volume 3M sodium acetate and 1 volume isopropanol; mix thoroughly by inversion; leave at room temperature for at least 2 min.

i. Spool DNA onto sealed Pasteur pipette. If several tubes with the same mycelium are prepared at the same time, all the DNA can be spooled onto a single pipette.

j. Wash spooled DNA in 3 x 1 ml aliquots of 70% ethanol. Leave the DNA for at least 2 min in each wash.

k. Dry DNA in air for about 1 min.

l. Re-dissolve in 50 μl TE (more if the DNA comes from more than one tube). Agitate the tubes gently (do not vortex) from time to time to help the DNA to dissolve. (Test whether all the DNA has been removed from the Pasteur pipette by dipping the pipette into 100% ethanol - any remaining DNA is rapidly dehydrated and appears white.)

3. Run gel to determine approximate concentrations of DNA samples (use 1 x, 5 x and 25 x dilutions). Each gel must contain standards of known concentration.

4. Digest total DNA partially with <u>Mbo</u>I. Label Eppendorf tubes 1 - 5. Consult the following figure for the next steps.

a. Dilute c. 300 μg total DNA in tube No. 5 to a total volume of 600 μl with <u>Mbo</u>I 1 x buffer.

b. Add 5 u <u>Mbo</u>I to tube No. 1.

c. Transfer 200 μl of DNA solution from tube No. 5 to tube No.

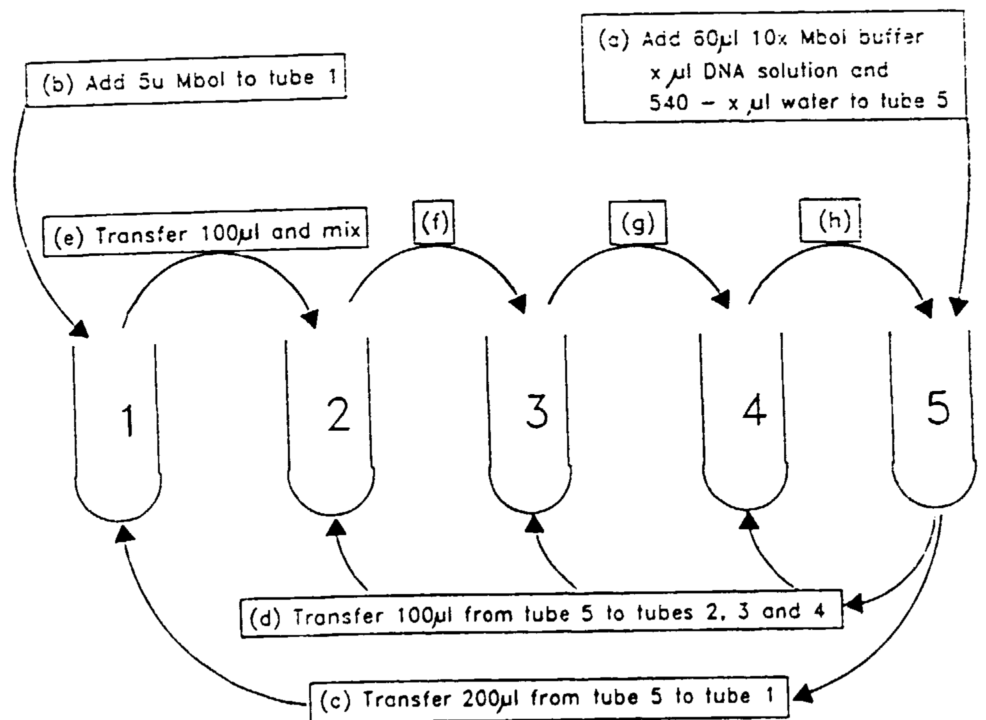
1. Mix by drawing up and down a cut pipette tip.

d. Transfer 100 µl DNA solution . tube No. 5 to tubes No. 2, 3 and 4.

e. Transfer 100 µl DNA + MboI from tube No. 1 to tube No. 2, mix.

f. Transfer 100 µl from tube No. 2 to tube No. 3.

g.h. Proceed in the same way to tube 5. (Tube No. 5 will end up with 200 µl solution.) Incubate samples for 1 h at 37°C, add 2 u CIAP to each tube and incubate for another 15-30 min. Heat samples for 10 min at 70°C; store the samples on ice until step 7.



5. Digest 1.2 µg pIJ4083 with 2 units of BamHI in a total volume of 50 µl for 1 h at 37°C.

6. Run aliquots of the total DNA digests (5 µl from tubes 1 to 5) and 10 µl of the digested vector on an agarose gel. **Remember** to include non-digested vector, total DNA and size standards as controls.

7. Pool appropriate samples; add 0.5 volume phenol/chloroform and vortex for 15 sec; spin for 3 min.

8. Remove upper phase to new tube; add 0.1 volume 3 M sodium acetate and 1 volume isopropanol; mix thoroughly by inversion; leave at room temperature for at least 2 min.

9. Spin 3 min; discard supernatant and redissolve pellet in 100 µl TE buffer.

10. Load sample on to sucrose gradient and run overnight.

11. Prepare protoplasts of either S. coelicolor J1501 or S. lividans 1326 from frozen mycelium (Manual p. 12) together with Experiment 1B and freeze for transformation on Day 4 (Thursday).

Day 3 (Wednesday)

12. Harvest sucrose gradient by collecting 250 µl fractions.

13. Run 10 µl aliquots from each sucrose gradient fraction on a gel (**remember** standards).

14. Pool appropriate fractions from sucrose gradient and concentrate (Manual p. 153; step 8). (Sucrose solutions need to be diluted two-fold with TE buffer before the DNA can be precipitated. Add tRNA to a final concentration of 20 µg/ml.)

15. Set up ligation (Manual p. 154; make sure to add 0.5 mM ATP). Aim for a final DNA concentration of c. 10 µg/ml (1 µg

vector, 5 µg target DNA and 10 ng of "*ermE* fragment" in a total volume of 600 µl). (Remove 60 µl of the mixture before adding the ligase to run on a gel later in parallel with the ligated sample (step 17).

Day 4
(Thursday)

16. Precipitate ligation mix and non-ligated control; add tRNA to a final concentration of 20 µg/ml as carrier. Redissolve pellets in 10 µl TE (control) and 20 µl TE (ligation).
17. Check ligation on gel (run all of control sample and 2 µl of ligation).
18. Transform protoplasts of either *S. coelicolor* J1501 or *S. lividans* 1326 (Manual p. 110). In addition to the ligation mix, use 20 µl TE as a "No DNA control" and 10 ng pIJ4803 as a control for transformation frequency. Plate out as indicated below.

No DNA	Vector	Ligated DNA	
100µl	T 25µl	T 5µl	T 25µl
T 100µl	T 125µl	T 125µl	T Rest

T – Overlaid with thiostrepton
Rest – spin down remaining protoplasts and plate out in c. 100µl

Day 5
(Thursday)

19. Overlay transformation plates with thiostrepton (see Exp. 7A step 2).

Day 8 (Monday)

20. Count colonies.

Day 10 (Wednesday)

21. Replicate sporulated regeneration plates to selective media and to non-selective control plates in the following order:

S. coelicolor J1501	S. lividans 1326
MM his thio	MM str thio
MM ura thio	MM ery thio
MM his ura thio ery	MM thio
MM his ura thio	

Day 12 (Friday)

22. Score replica plates from step 19.

23. Spray non-selective plates with a 0.5M aqueous solution of catechol (IN FUME HOOD). Score yellow colonies.

"Strong Incompatibility" Between Derivatives of the Streptomyces Plasmid pIJ101

The aim of this experiment is to demonstrate a phenomenon called "strong incompatibility" between derivatives of pIJ101, a Streptomyces multi-copy plasmid. The DNA sequence which causes this "strong incompatibility" is named sti You will be provided with a sti^- plasmid, pIJ424 (a plasmid with the minimal replication region of pIJ101 but without the sti sequence), a Sti^+ plasmid, pIJ2740 (pIJ424 containing sti in the functional orientation) and a sti^r plasmid, pIJ2741 (pIJ424 with sti in the reverse orientation, not functional). You will try to introduce these plasmids respectively into S. lividans strain TK24 containing pIJ58 (Sti^-, aph on plasmid) or pIJ649 (Sti^+, vph on plasmid). At the same time, you will transform another plasmid, pWOR120, which is unrelated to pIJ101 to see the relative transformation frequency of the strains, and set up a "No-DNA" control.

Each group will be provided with TK64 protoplasts with a resident plasmid, pIJ58 or pIJ649, but transform with only one of the DNA preparations (Group 1 - TE only, (no DNA control); Group 2 - pIJ424; Group 3 - pIJ2740; Group 4 - pIJ2741; Group 5 - 2 x concentrated pIJ2741; Group 6 - pWOR120). The combination of the transformation experiments from all six groups will give an overall picture of "strong incompatibility"; the sporulated transformation plates will be replicated onto MM+thio+neo; MM+thio+vio; and MM+thio as a control. (Please note that proline needs to be added as supplement for the MM medium.)

Procedures

(prepared in advance)

1 S. lividans TK64 protoplasts containing pIJ58 or pIJ649 respectively, stored at -70°C.

2 pIJ424, pIJ2740, pIJ2741, pWOR120.

Day 1 3 Use 5 ul (c. 0.1 - 0.3 ug) pIJ486, pIJ2740, pIJ2741 and pWOR120 respectively to transform S. lividans TK64 protoplasts containing pIJ58 or pIJ649. Set up "No-DNA" control. Incubate at 28°C for c. 20 h.

Day 2 4 Overlay plates with 1 - 1.5 ml water containing 300 ug of thiostrepton. Incubate for another 4 to 5 days.

Day 7 5 Estimate relative frequency for each transformation experiment.

Day 8 6 Replicate sporulated plates onto MM+thio+neo; MM+thio+vio; and MM+thio.

Day 11 7 Analyse the results.

Plasmid Isolation by Alkaline Lysis and Gel Electrophoresis of Linear and Circular DNA

The purpose of this experiment is to demonstrate a rapid plasmid isolation procedure and to give an introduction to the interpretation of agarose gel pictures containing linear and circular DNA species.

Each group of three students prepares a lysate of 5 strains according to the procedure in the English manual, page 86. We only perform steps 1 to 5 of the procedure and load the samples directly onto agarose gels.

Each tube contains 100 ul of cell suspension which had been stored frozen. Add 400 ul of the blue lysozyme/RNase solution to each tube to give a total volume of 500 ul. Then proceed with step 2, page 86. Remember to mix NaOH/SDS before use.

The tubes with black lids are different for each group and should be used to investigate what happens when one parameter in the procedure is changed. This experiment can be done in parallel with the standard procedure. See below for instructions.

The preparations are first checked on a 0.7% agarose gel in Tris-borate (TBE) buffer containing Ethidium bormide (careful!) run for c. 2 h at high voltage. Arrange the samples according to the sizes of the plasmid beginning with the largest one. They can be loaded onto the gels with a single yellow tip. Do not forget to add two concentrations (0.1 and 0.5 ug) linear size standards (lambda <u>Hind</u>III) to each gel. Details about agarose-gel electrophoresis are in the English manual, page 136 ff. The linear standards are on pages 140 and 141.

Please write the order of the samples down carefully (prepared sheets are available) so that we can discuss the results. After the first gel a set of good preparations is to be selected for running under different gel conditions. If time is limiting, this may be done by the instructors.

Special experiments — each group does only one of these (tubes with black tops).

A Produce denatured forms of all plasmids by adding double strength (0.6 M) alkaline SDS in step 3 of the procedure. Run the sample next to the normal preparations on the gels.

B Test how critical the volume of alkaline SDS is. Use 100, 200, 250, 300, 400 and 500 ul for the isolation of one plasmid.

C Make total lysates of all strains as for the determination of plasmid copy number. dilute the samples 10X and run them next to the normal plasmid preparations.

Method: lysozyme treatment as usual. Lyse with 250 ul 2% (neutral) SDS (mix before use). Extract with 200 ul phenol/chloroform. Vortex at least 60 sec. to reduce the viscosity of the samples.

D Test how critical it is to get the total volume of cell suspension in lysozyme solution right. Prepare tubes with 400, 450, 500, 550 and 600 ul suspension by removing from some tubes and adding to others.

E Determine the optimal amount of phenol/chloroform for step 5 of the procedure. Use 20, 40, 80, 160 and 320 ul of acid phenol/chloroform. When loading these samples onto the gels it is best to use separate tips for the 20 and 40 ul samples.

F Incubate for 0, 5, 10, 20 or 40 min at 70°C in step 4 of the procedure.

Plasmids for DNA isolation and gel electrophoresis

Strain designation	Plasmid	Size (kb)	Copy number	Markers
K132	pIJ386	3.3	50	aphI
M386	pIJ486	6.2	100	neo, tsr
DX49	pIJ2745	6.8	1000	neo, tsr
TK129	pIJ355	11.8	300	vph
ED8767	pHZ507	8.5		tsr, amp

UNIDO COURSE ON GENETIC MANIPULATION OF STREPTOMYCES
WUHAN, APRIL 1989

List of Lectures

1. The biology of actinomycetes (Keith Chater)
2. *Streptomyces* phages and vectors derived from them (Keith Chater)
3. *In vivo* genetic analysis of *Streptomyces* by conjugation and protoplast fusion (David Hopwood)
4. Analysis of gene expression in *Streptomyces*. I. transcription (Mervyn Bibb)
5. Analysis of gene expression in *Streptomyces*. II. translation. Expression and export of foreign proteins (Mervyn Bibb)
6. Methods of cloning and manipulating antibiotic biosynthetic genes. (David Hopwood)
7. Methods of analysing differentiation in *Streptomyces* (Keith Chater)
8. DNA sequencing and sequence analysis (Mervyn Bibb)
9. *Streptomyces* plasmids and vectors derived from them (Tobias Kieser)
10. Genome mapping by pulse field gel electrophoresis and related methods (Helen Kieser)
11. Genome rearrangements and mobile genetic elements in *Streptomyces* (Tobias Kieser)

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STUDENT SEMINARS

Student	Title of Seminar
Qin Zhongjun	Attempts to clone genes for agriculturally important antibiotic production from *Streptomyces hygroscopicus* 5102
S Kumaravel	Amplified DNA in *Streptomyces lividans*
Tang Li	Genetic manipulation of the producers of medicamycin and spiramycin
Pairoh Pinphanichakarn	Genetic manipulation of glucose isomerase production by *Streptomyces*
Huan Liandong	Cloning and expression of *Streptomyces* promoters
Manjula Reddy	Genetics of rifamycin production by *Nocardia mediterranea*
He Wen	Cloning and expression of a lincomycin resistance gene of *Streptomyces lincolnesis* in *S. lividans*
Cheng Han	Genetic manipulation of glucose isomerase production by *Streptomyces*
Feng Gang	Genetic manipulation of glucose isomerase production by *Streptomyces*
Yan Zhifen	Cloning and expression of promoters from *Streptomyces aureofaciens* in *S. lividans*
Jiang Hao	Attempts to clone antibiotic resistance genes from *Streptomyces mycarofaciens*

UNIDO/ICGEB/JOHN INNES INSTITUTE COURSE ON
"GENETIC MANIPULATION OF STREPTOMYCES",
HUAZHONG AGRICULTURAL UNIVERSITY, WUHAN, 9-21 APRIL 1989

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1. In general, did the course meet your expectations? YES/NO
   (a) In what ways was it deficient?

   (b) In what ways was it better than you expected?

2. In the laboratory, was the amount of work:

Too much?	About right?	Too little?

   How could the experiments have been improved?

3. In general, were the lectures useful? YES/NO
   (a) What other lecture topics would you have liked to be included?

   (b) Should any of the topics (which?) have been omitted?

4. Was there enough opportunity for informal discussion with the course teachers? YES/NO

5. Please make any additional comments here.

6. What is your overall assessment of the course?

Excellent	Good	Poor
Very good	Satisfactory	Very poor

Final Financial Statement (£ sterling)

Travel and subsistence for trainees from ICGEB member countries

Item			
Travel for:			
M. Reddy (India)		620.91	
S. Kumaravel (India)		860.43	
P. Pinphanichakarn (Thailand)		385.00	
L. G. Hy and L. D. Pham (Vietnam)			
Cost of International Money Order for $2,100	1202.60		
Refund of above I.M.O.	1262.39 Cr		
Cost of reconverting a returned I.M.O. for $300 to sterling	5.32	54.47 Cr	
Accommodation and subsistence for 5 trainees		1005.00	2,816.87

Travel of teaching staff from U v.

Item		
International air fares	3221.00	
Travel insurance	210.00	
Chinese visas	116.00	
Rail fares, taxis and incidental expenses Norwich-London Heathrow and back	167.35	3,714.35

Minor equipment for host laboratory

Item		
Microcentrifuge	674.48	
Bench top centrifuges (2)	1052.89	
Automatic pipettes	371.45	
Chest freezer	229.00	2,327.82

Expendable materials, equipment and shipping costs

Item		
Purchased by John Innes Institute and shipped to Wuhan	5384.83	
Purchased and received by Huazhong Agricultural University, 2,050.00 + bank charge 9.00	2059.00	7,443.83

Miscellaneous

Item		
Laboratory manuals	450.00	
Photocopying and postage	50.00	
Producing draft and final reports	85.00	585.00
TOTAL		£ 16,887.87

由联合国工业发展组织国际遗传工程和生物技术中心和英国John Innes Institute联合举办的"链霉菌遗传操作"实验训练班和由中国遗传学会微生物遗传专业委员会组织召开的全国链霉菌分子遗传和基因工程学术讨论会于1989年4月9日至23日在中国武汉华中农业大学先后举行。实验操作训练班和学术讨论会获得圆满成功。参加会议全体人员一致建议：

1. 在今后研究工作中，相互交流有关链霉菌菌种和质粒载体等。除非该菌种或质粒有商业价值或属有关合同或专利。被接受菌种和质粒的单位需在今后结果和论文中提及来源。

2. 为了推动中国链霉菌分子遗传和基因工程的发展，加强国际合作交流，拟在中国武汉华中农业大学设立一个链霉菌分子遗传和基因工程国际开放性实验室。建议英国John Innes Institute的D. A. Hopwood教授为该开放实验室的指导教师，K. Chater教授、M. Bibb博士、T. Kieser博士为顾问。请国际组织予以资助。

(Translation)

At Huazhong Agricultural University, Wuhan, China, two meetings were held from 9-23 April 1989. One meeting, the course on Streptomyces gene manipulation, was sponsored and organized jointly by John Innes Institute of England, The Biotechnology Centre of China and the Genetic Engineering Section under the United Nations Industrial Development Organization. The other meeting, the Symposium of Streptomyces Molecular Genetics and Genetic Engineering, was organized by the Microbial Genetics Section of the Chinese Society of Genetics. Both meetings have been conducted very successfully, and, consequently, all the participants of the two meetings have unanimously agreed to propose:

1. From now on, all Streptomyces strains, plasmids and vectors (except those that have commercial value and/or have been patented or under contract) should be offered freely among scientists to meet their research requirements. Those who have received the gifts must acknowledge in their papers the source of the offer;

2. In order to promote the current study of Streptomyces molecular genetics and genetic engineering in China, and also to strengthen international exchange, it has been proposed that in Huazhong Agricultural University an open laboratory of Streptomyces molecular genetics and genetic engineering should be established. It has also been proposed that Professor D A Hopwood of John Innes Institute be the supervisor of the open laboratory, and Professor K F Chater, Dr M J Bibb and Dr T Kieser the laboratory's advisors. The wish was expressed to seek financial support from international organizations.

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